Synthesis, Characterisation, and Study of Biological Activity of Some Sulphur Heterocycles

A Dissertation
Submitted to The University of Dhaka
in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy (Ph D) in Chemistry

by

GOURANGA CHANDRA DAS

Registration No. **49**
Session: **2012-2013**

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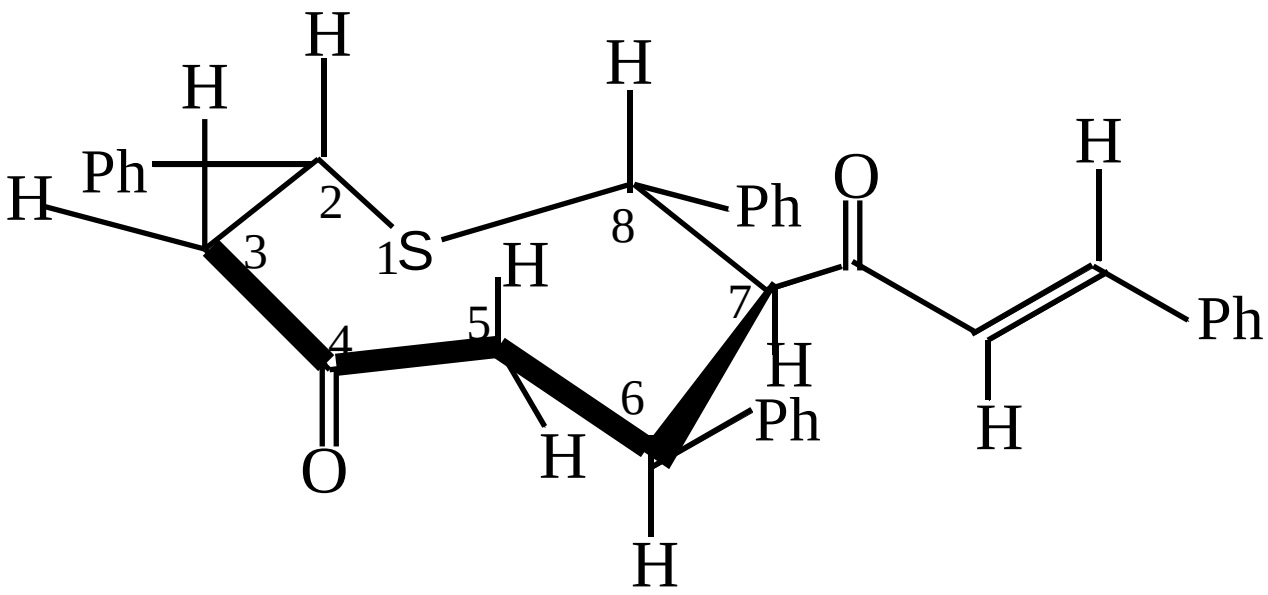
November 2016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Dedicated to

My late parents
and
my late wife Dr Minati Rani Singha

D E C L A R A T I O N

I do hereby declare that the work incorporated in this thesis entitled, **"Synthesis, Characterisation, and Study of Biological Activity of Some Sulphur Heterocycles"** in partial fulfillment for the requirement of the degree of Doctor of Philosophy (PhD) in Chemistry, Department of Chemistry, University of Dhaka, Dhaka 1000, Bangladesh is carried out by myself. I also declare that neither of this work nor a part of it was submitted by me elsewhere for any other degree or diploma.

Gouranga Chandra Das

C E R T I F I C A T I O N

THESIS APPROVAL SHEET

The thesis entitled, **"Synthesis, Characterisation, and Study of Biological Activity of Some Sulphur Heterocycles"**, submitted by Gounranga Chandra Das, Registration No **49**, session: 2012 -13 is approved for the degree of DOCTOR OF PHILOSOPHY (Ph D) in Chemistry.

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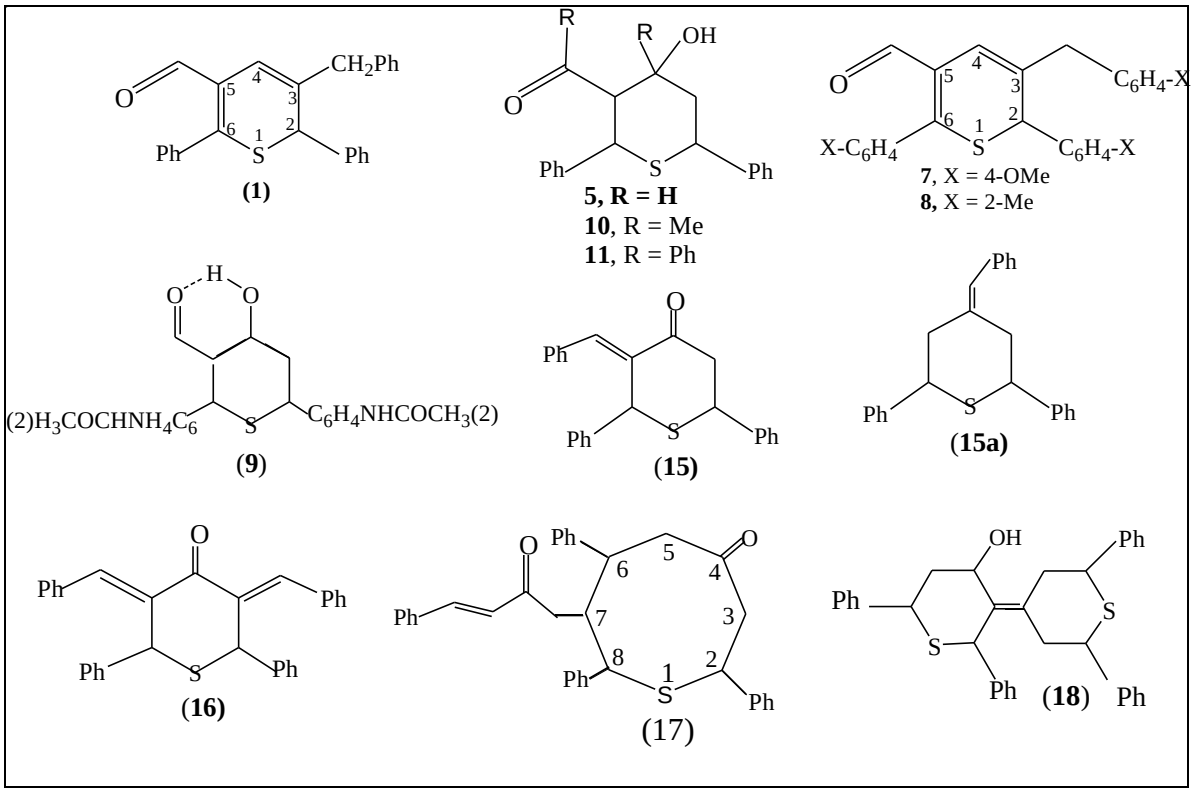
A B S T R A C T

Long back in 1959, Latif and co-workers, department of Chemistry, Rajshahi University , Bangladesh in an attempt to prepare dithiobenzoic acid [Ph-(C=S)-SH] from a mixture of benzaldehyde and ethanol in presence of aqueous sodium sulphide ended up with structure (1), 3-benzyl -2,6-diphenyl-2H-thiopyran-5-carboxaldehyde. The structure of (1) was later confirmed by a number of workers who also proposed mechanism for its formation. But none of them could either isolate or gave any evidence in support of the intermediates proposed in the mechanism. Keeping this view in mind , we took this project to justify their mechanism by using some of the intermediates and some more compounds which could be transformed into those intermediates *in situ* and ended up with compound (**1**) , compounds analogous to (1) are (**7**), (**10**) , and some other products not analogous to (1) are (**9**), (**15**), (**15a**), (**16**), (**17**), and (**18**). The compound (**9**) and (**10**) are analogous to the intermediate (5).

One of the products (**17**) was isolated for the first time is an interesting noble compound having sulphur in an eight membered ring (7-cinnamoyl-2,6,8-triphenyl-thiocane-4-one) .



The bioassay of some of our synthesised compounds against four selective gram-positive bacteria such as *Bacillus cereus, Bacillus subtilis, Bacillus megatorium* and *Straphylococcus aureus* and five gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Shigella flexneri, Shigella boydii, Vibrio cholera* was carried out. Some of which were very promising and fewer moderate. The bioassay results against two fungi such as *Candida albicans* and *Saccharomyces cerevisiae* were not very promising.

C O N T E N T S

Part I

LIST OF FIGURES

GENERAL REMARKS

1. All recorded temperatures (in degree Celsius) are uncorrected.

   T_m - melting temperature /^{0} C, T_b - boiling temperature /^{0} C

2. All the references are put continuously throughout the whole thesis.

3. The structures are numbered in Arabic letters and are only applicable to a particular chapter.

4. Most of the figures are placed together before references in the following sequences:

   i) UV-visible, ii) IR, iii) ^{1}H-nmr iv) ^{13}C-nmr, v) Mass, etc.

5. The UV-visible spectra were recorded on a SHIMADZU UV-160A spectrophotometer in ethanol or chloroform and the following notations were used in different spectra:

   λ - wave length in nm

6. The IR-spectra were recorded on a SHIMADZU IR-470 spectrophotometer in KBr pellets and as thin liquid films and the following notations were used in different spectra:

   ν - frequency of vibration in cm^{-1}, s – strong, sh – shoulder, b – broad, w – weak, etc

7. The NMR-spectra were recorded on a BRUKER AVANCE 400/100 MHz spectrophotometer in $CDCl_3$, MeOH (*d*). The notations used in the case of nmr were as follows:

   δ - chemical shift in ppm, *s* - singlet, *d* - doublet, *dd* - doublet of a doublet, *dt* - doublet of a triplet, *t* -triplet, *m* - multiplet
   J - coupling constant in hertz (Hz) .

8. Tlc analyses were carried out on glass plates coated with tlc grade silica-gel (E Merck). Iodine vapour was used to locate the spots of compounds.
   R_f - retardation factor (tlc)

9. Column chromatography was carried out on glass columns using column- - grade silica gel (60-120 mesh size).

10. Anhydrous potassium sulphate, sodium sulphate, sodium carbonate, potassium carbonates, etc were used as drying agents in organic solvents.

11. Ethoxyethane was used as extraction solvent.

Chapter 1

INTRODUCTION

PART I

This section is a preliminary one which mainly contains

- A general discussion on heterocyclic compounds specially thiopyran compounds and its derivatives
- A concise review on six membered sulphur heterocycle thiopyrans and its derivatives
- Aim of the Present Investigation
- Present Investigation
- Scope of the Present Investigation.

1.1 GENERAL

Organic chemistry is a subdivision within chemistry involving the scientific study of the structure, properties, composition, reactions and preparation (by synthesis or by other means) of carbon-based compounds and their derivatives. These compounds may contain any number of other elements, including nitrogen, oxygen, halogens as well as phosphorus, silicon and sulfur. Organic compounds are structurally diverse. The range of application of organic compounds is enormous. They either form the basis of, or are important constituents of, including foods, dyes, petrochemicals, plastics, explosives, paints etc. Most of the medicines that help us to cure diseases and relieve sufferings are also organic compounds. They form the basis of almost all earthly life processes (with very few exceptions).

The process of making one compound from another is called synthesis. The synthesis of organic compound is traditionally an important part of the training of organic chemists. Until about 1850 it was believed that organic compounds must have their origin in living organisms, and consequently could never be synthesised from inorganic materials[1]. Today, although many compounds of carbon are still most conveniently isolated from plant and animal sources, most of them are synthesised.

By undertaking the preparation of a varied range of compounds, and using a representative selection of reaction processes and practical techniques, the prospective organic chemist become familiar with the chemical and physical properties of organic substances and begins to understand more clearly the factors which governs their reactivity. The synthesis of quite simple compounds is of considerable educational value particularly if the reactions involved are of a general nature, which may be applied, with suitable modifications if necessary, to more complex systems[2].

Synthesis is carried out for many reasons. We may need a particular compound to investigate some hypothesis about how a certain organism metabolites the compound. In planning synthesis, we are required to think backward from relatively complex molecules to simpler ones that can act as the precursor for our target molecules. We carry out what is called a retro synthetic analysis and we represent this reasoning process in the following way:

Target molecules→ precursors

The open arrow is a symbol that relates the target molecule to its most immediate precursors, it signifies a "retro" or "backward step". In most instances more than one steps is required to bring about a synthesis. We then repeat the analytical process. The precursors become our target molecules and we find reason backward to another level of precursors and so on until the level of compounds that we are available as starting materials.

Target molecule=>1^{st} precursor =>2^{nd} precursor=> starting compound

In planning a synthesis we often have to consider four interrelated aspects:

1. Construction of the carbon skeleton
2. Functional group interconversion
3. Control of radiochemistry
4. Control of stereochemistry

1.2 HETEROCYCLIC COMPOUNDS

A heterocyclic compound (Greek; heteros means other) is one that contains a ring in which one or more of the ring carbons are replaced by another atom.

The non-carbon atoms in such rings are referred to as "*hetero-atoms*".
The common hetero atoms are nitrogen, oxygen, and sulphur; but other atoms such as boron, phosphorous or silicon can also be the members of heterocyclic rings.

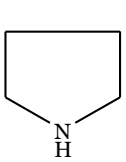
A number of substances which are heterocyclic compounds according to the above definition such as ethylene oxide, succinic anhydride, lactones, cyclic carbohydrates, etc are readily formed from open-chain substances and are easily converted into open-chain derivatives, due to the relative instability of their ring systems and lack of aromatic character, cannot be treated as heterocyclic compounds. Therefore, the heterocyclic compounds which we are to consider here, are much more stable and most of them possess aromatic properties with some exceptions (for example, tetrahydrothiopyrans, tetrahydropyrans, piperidines, pyrrolidine compounds, etc).

A number of vitamins (pyrodoxyn or vitamin $B_{6,}$ nicotinic acid or niacin) are heterocyclic compounds; without these compounds, many important metabolic processes could not take place. For example, importance of the pyrydinium group in the vitamin NAD^+ and pyridoxal phosphate.The nucleic acids, which carry and transmit genetic information in the cell, contain purine and pyrimidine rings in combined form. Heterocyclic compounds are involved in some of the colours of nature that have intrigued humankind from the earliest times. The colour of blood is due to an iron complex of "*heme"*, a heterocyclc composed of pyrrole units. This type of heterocycle is called a

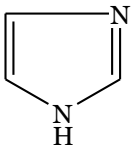
"*porphyrin*" ring. The green colour of plants is caused by chlorophyll, a class of compounds closely related to the porphyrins.

Other interesting compounds containing a pyridine ring are α- and β- eucaines (local anaesthetics), coramine (respiratory stimulants), demerol (analgesic), iso-niazid (antitubercular drug), 2,2-dipyridyl (analytical reagent), etc.

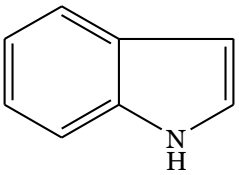
Nitrogen heterocycles occur widely in nature (e.g. alkaloids such as coniine from hemlock, nicotine from tobacco, piperine from black pepper); many of which contain heterocyclic ring systems. The naturally occurring amino acids, proline, histidine and tryptophan which contain a pyrrolidine, imidazole, and indole ring respectively.



Pyrrolidine



Imidazole



Indole

Oxygen heterocycles also occur in nature to some extent in combination or alone forms. e.g., glucose, fructose, sucrose, lactose, etc.

But sulphur heterocycles particularly thiopyran compounds are not found in nature generally and most of them are mainly of synthetic origin.

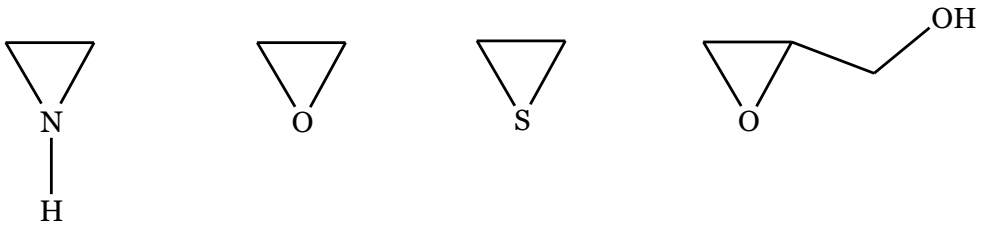
1.3 CLASSIFICATION

A variety of heterocyclic compounds of different ring sizes are known, but usually have five- or six-membered ring. Such ring may be single (mononuclear), or fused to other ring or rings.

Heterocyclic Compounds Having One or More Heteroatoms in One Ring

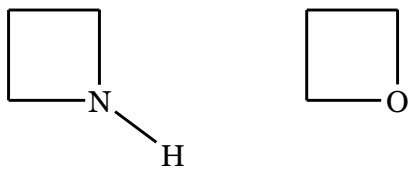
1.3.1 3-Membered Ring

Heterocyclic with three atoms in the ring are more reactive because of ring strain. Those containing one heteroatoms are generally stable. Those with two heteroatoms are more likely to occur as reactive intermediates. Some of the common 3-membered heterocycles are:



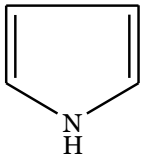
Aziridine Oxirane Thiirane Glycidol

1.3.2 4-Membered Rings

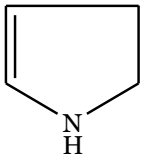


Azetidine Oxetane

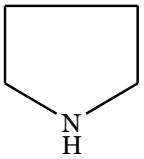
1.3.3 5-Membered Rings



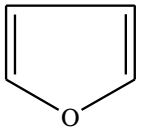
Pyrrole



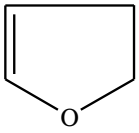
Dihydropyrrole



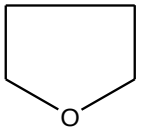
Tetrahydopyrrole



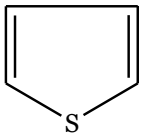
Furan



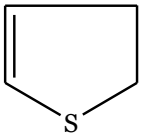
Dihydrofuran



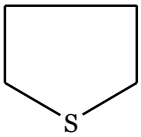
Tetrahydrofuran



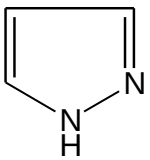
Thiophene



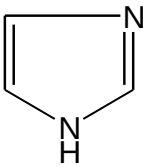
Dihydrothiophene



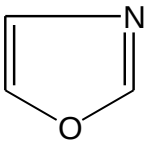
Tetrahydrothiophene



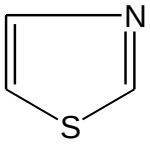
Pyrazole



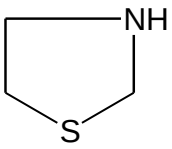
Imidazole



Oxazole

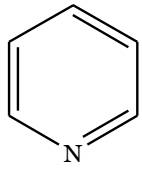


Thiazole

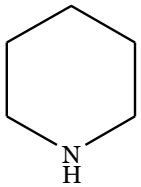


Thaizolidine

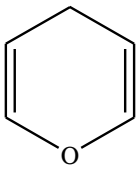
1.3.4 6-Membered Rings



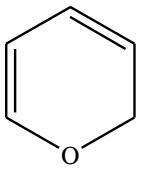
Pyridine



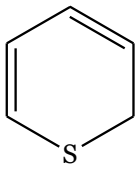
Piperidene



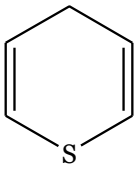
4H-Pyran



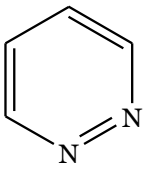
2H-Pyran



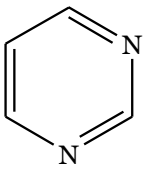
2H-Thiopyran



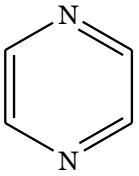
4H-Thiopyran



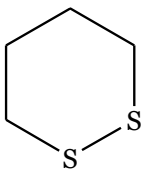
Pyridazine



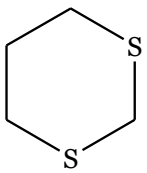
Pyrimidine



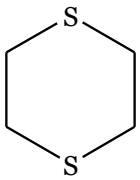
Pyrazine



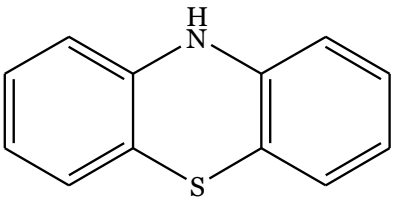
1,2-Dithiane



1,3-Dithiane



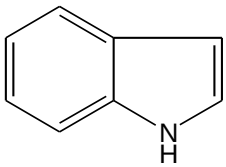
1,4-Dithiane



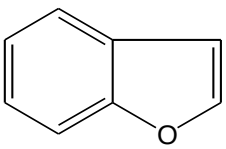
Phenothiazine

1.3.5 Condensed Herterocycles

They may consist of two or more fused rings which may be partly carbocyclic and partly heterocyclic, e.g.,

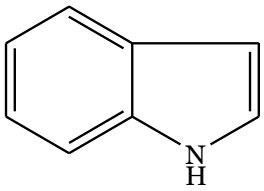


Indole

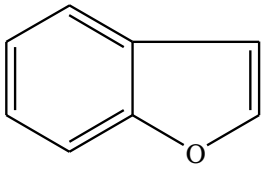


Benzofuran

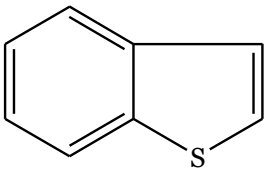
Heterocyclic Compounds Having One or More Heteroatoms in Two Rings



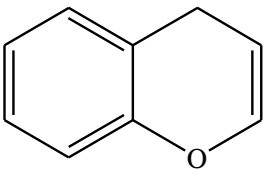
Indole



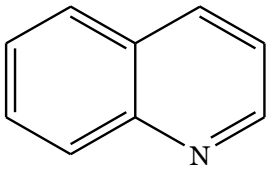
Benzofuran



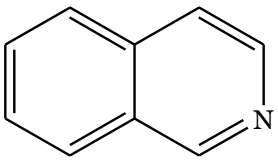
Benzothiophene



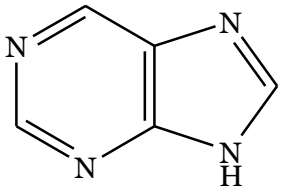
Benzopyran



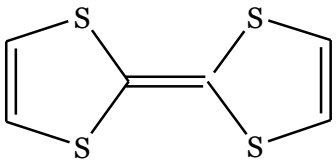
Benzopyridine
(Quinoline)



Isoquinoline

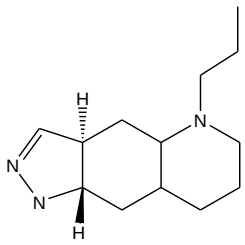


Purine

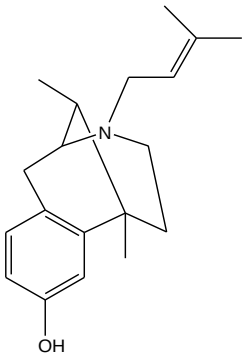


Tetrathiafulvalene

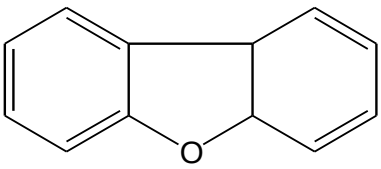
Heterocyclic Compounds Having One or More Heteroatoms in Three Rings



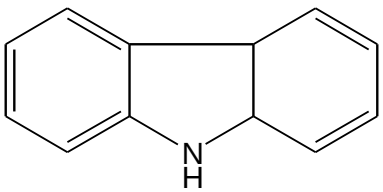
Quinpirole



Azocines

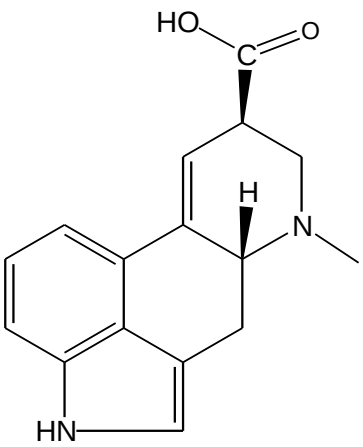


Dibenzofuran

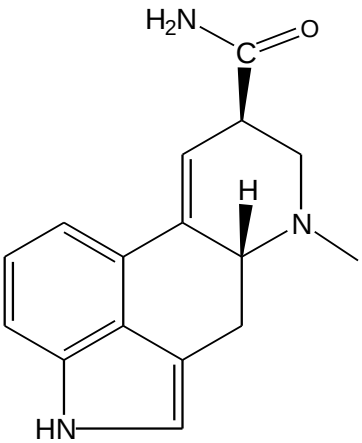


Carbazole

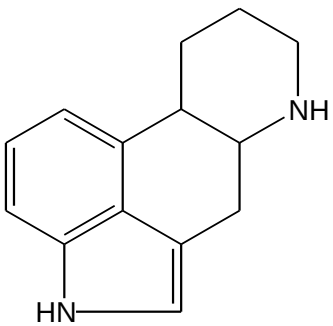
Heterocyclic Compounds Having One or More Heteroatoms in Four or More Rings



D-Lysergic acid

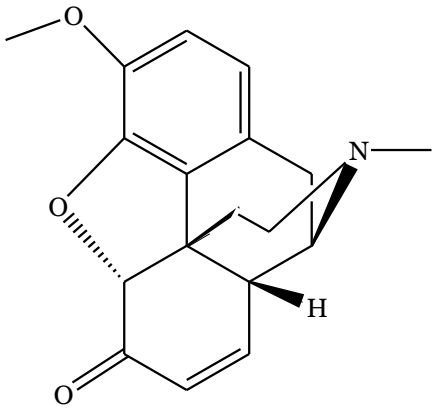


Ergine

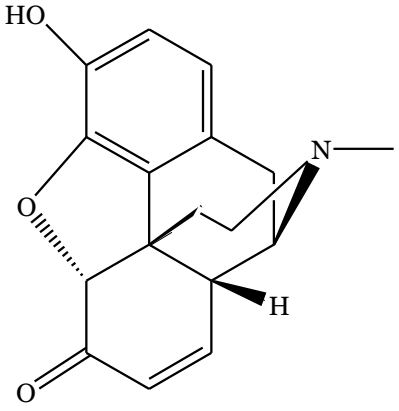


Ergoline

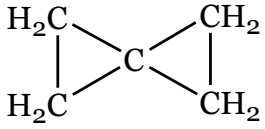
Heterocyclic Compounds Having Bridged-Rings



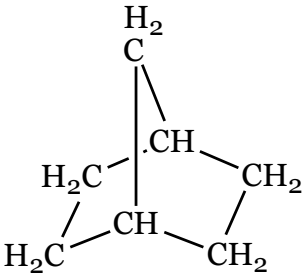
Codeinone



Morphinone



Spiro[2,2]pentane



Bicylo[2,2,1]heptane

A recent classification is based on their properties and dives them into

(i) Heteroparaffins i.e., heterocyclic compounds resembling paraffins. e.g., THF, pyrrolidine, piperidine etc and

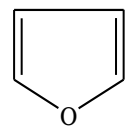
(ii) Heteroaromatics i.e.,heterocyclics resembling aromatic compounds. e.g., pyrrole, furans, thiophene, pyridine, indole etc

1.4 NOMENCLATURE AND NUMBERING[3]

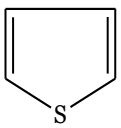
Heterocyclic compounds are generally known by their common or trivial names. The structure and names of some of the heterocyclics have been discussed under classification. Even the IUPAC systems has accepted most of these trivial names as much. However, the systematic IUPAC name of heterocyclic is obtained by combining the following prefixes and suffixes along with di-,tri-, etc., as desired.

Order of seniority	**Prefixes**	**Suffixes**
1	*oxa*- for Oxygen	---*ole* (5-membered ring)
2	*thia*- for Sulphur	---*ine* (6-membered ring)
3	*aza*- for Nitrogen	---*epine* (7- membered ring)
4	*phospha*-for Phosphorus	
5	*Bora*- for Boron	

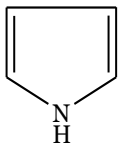
The terminal "*a*" of the prefixes is usually dropped when combining prefixes and suffixes.



Furan/Oxole

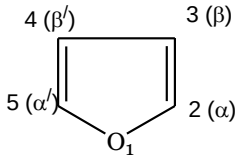


Thiophene/Thiole

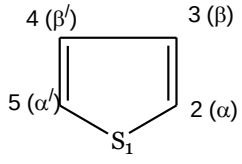


Pyrole/Azole

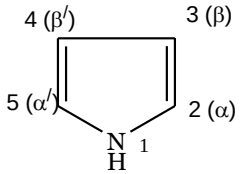
The ring positions are designated by numerals or Greek letters. The simple heterocyclics containing one hetero-atom are numbered in such a way that the hetero-atom gets the number 1 (or lowest number) and the numbering is continued in anti-clockwise direction. When Greek letters are used the position next to hetero-atom is designated as α-followed by β- and so on. For example:



Furan

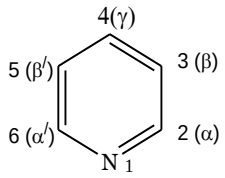


Thiophene

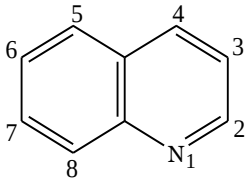


Pyrrole

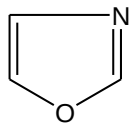
If a compound has two or more Oxazole different hetero-atoms, the priority is given to the higher group and higher position in the same group in the periodic table. i.e., O > S > N > P.



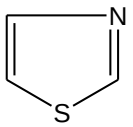
Pyridine



Quinoline



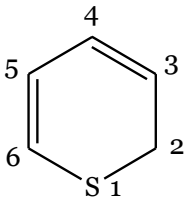
Oxazole



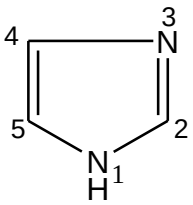
Thiazole

In the case of same heteroatoms, the priority is given to the atom with greater saturation (i.e., >N—H > =N—) and the numbering proceeds round the ring in order of precedence in the IUPAC system.

The state of hydrogenation is indicated by the symbol H preceded by a number showing the position of saturation[4]



2H-Thiopyran

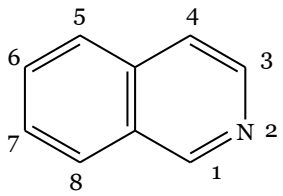


Imidazole
[1,3-Diazole]

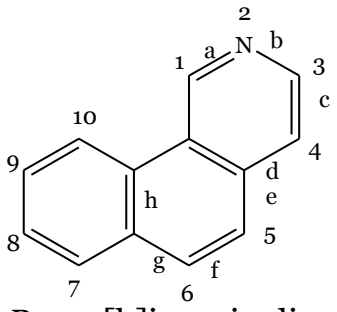
Fused Heyerocyclic Systems[5]

When heterocyclic ring is present, this is chosen as the parent compound. If more than one heterocyclic ring are present, the order of preference is given to the nitrogen containing ring. For a component containing a heteroatom other than nitrogen, the order of preference is that mentioned earlier (i.e. O before S etc). When the parent compound has been chosen, its name is prefixed by the name of the fused ring attached, e.g., benz(o), naphth(o). Also, the parent compound chosen is the component containing the largest number of rings and has a simple name. For the purpose of

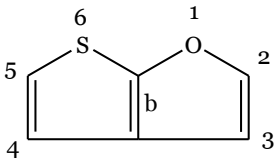
numbering, the structure is written with the greatest number of rings in a horizontal position and a maximum number of rings above and to the right of the horizontal row. Numbering is then carried out (usually) in a clockwise direction starting with the uppermost ring farthest to right and omitting atoms at ring junctions. To distinguish isomers, the peripheral sides of the parent compound are lettered a, b, c, etc., beginning with a for the side 1, 2, b for 2, 3, etc. To the letter as early in the alphabet as possible, denoting the side where fusion occurs, are prefixed, if necessary, the numbers indicating the positions of fusion of the other component; their order conforms to the direction of lettering of the base component. If should be noted that these numbers apply to the prefixed component (as a separate entity) and not to the combined system (which is numbered according to the usual rules).



Isoquinoline

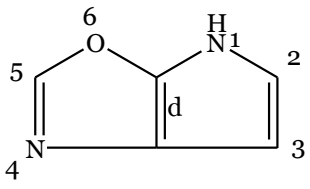


Benzo[h]isoquionline

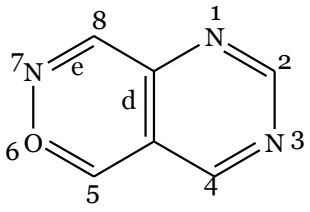


Thieno[2,3-b]furan

In addition to the fore going rules, there are the rules that the component chosen is the one containing the largest possible individual ring, or containing the greatest number or variety of heteroatoms, etc.



1H-Pyrazole[4,3-d]oxazole

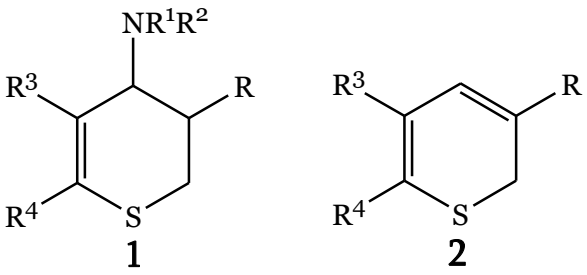


5H-Pyrido[2,3-d]-o-oxazine

1.5 REVIEW

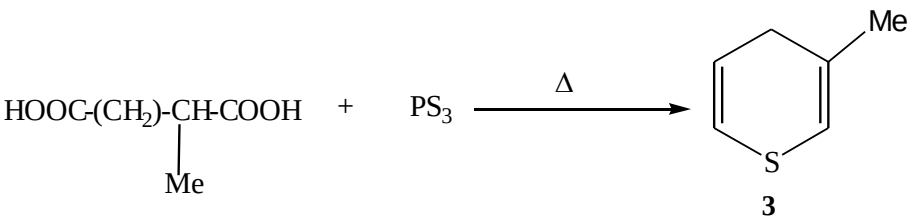
A variety of such heterocyclic compounds of different ring sizes are known (discussed earlier) but we shall restrict our study in this thesis only to the most important ones which are made of six-membered rings having one sulphur atom known as "**Thiopyran Compounds**" and some of its derivatives.

Thiopyrans of the type (**1**) were prepared by 1,4- cycloaddition of CH_2 =CHR with R^4CSCR^3=$CHNR^2R^1$. Cycloaddition- elimination gave the derivative of α-thiopyran (**2**), which were quantamised with $Ph_3C^+ClO_4^-$.[6]

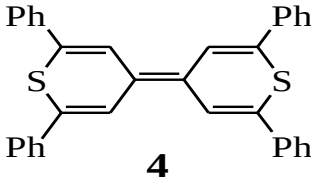


(1): $RCONH_2$, CN, Ac; $NR^1R^2NMe_2$, NEt_2, piperidino, 1-pyrrolidizyl, morpholino, NHC_6H_4Me-*p*; R^3 = H, Ph; R^4 = C_6H_4OMe-p, C_6H_4Br-*p*, C_6H_4Cl-p, 2-thienyl, SMe. (2): R = $CONH_2$, CO_2Me, CN, CHO; R^3 = H, Ph; R^4 = C_6H_4OMe-*p*, C_6H_4Br-*p*, SMe.

The 3- methyl-γ-thiopyran (**3**) has been prepared by heating α- methylglutaric acid with phosphorus trisulphide .[7]

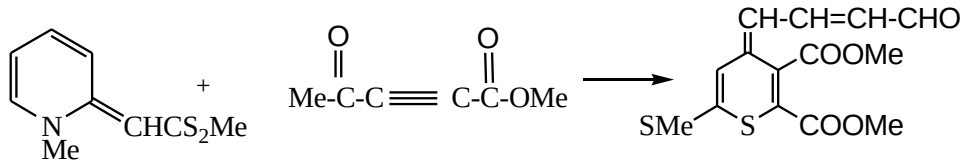


Derivative of γ- thiopyran (**4**) was prepared by the photolysis of 2,6- diphenyl-4H-thiopyran-4-thione thereby produces 2,2',6,6'-tetraphenyl-4-,4'-bithiopyranylidene (**4**) [8].



4

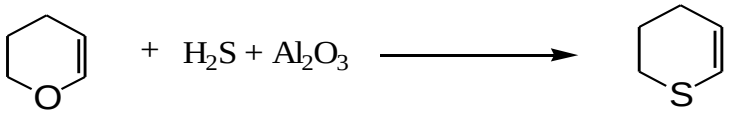
The reaction of 1-methyl-2-(methylthio)thiocarbenyl methylene-1,2-dihydropyridine with dimethyl acetylenedicarboxylate gave 15% thiopyran compound (**5**) [9].



5

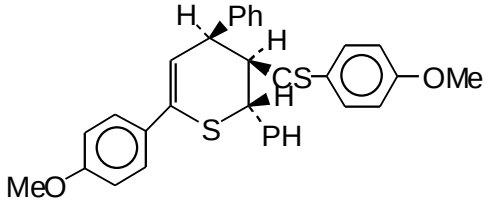
while 1-(dimethylaminophenyl)–2,4,6-triphenylthiabenzene (**6**) is more stable than the analogue without the *p*-dimethylamino group, it is decomposed by heat, light or acid to give a mixture of 4-(dimethylaminophenyl)–2,4,6-triphenyl-4H-thiopyran (**7**) and 2- (dimethylaminophenyl) – 2,4,6-triphenyl-2H-thiopyran (**8**). Both (**7**) and (**8**) are also given directly by the reaction of 2,4,6-triphenylthiopyrylium ion with *p*-dimethylaminophenylmagnesium bromide [10] .

Δ^2-Dihydrothiopyrans are (**9**) obtained when Δ^2-dihydropyran is heated with hydrogen sulphide and alumina at 425^oC (**8**) [11].



6

A derivative of Δ^2-dihydrothiopyrans were prepared by treating *p*-$MeOC_6H_4CSCH= CHNEt_2$ with phenylmagnesium bromide. On heating, (**10**) was isomerised irreversibly to its epimer at the 4-position. Similarly, when *p*- methylphenylmagnesium bromide was used as the Grignard reagent , (**11**) was obtained directly [12].



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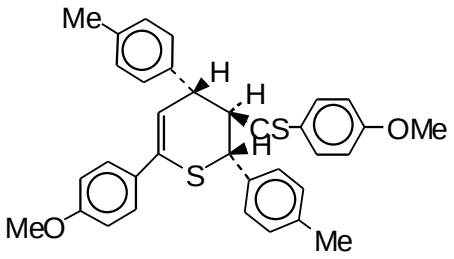
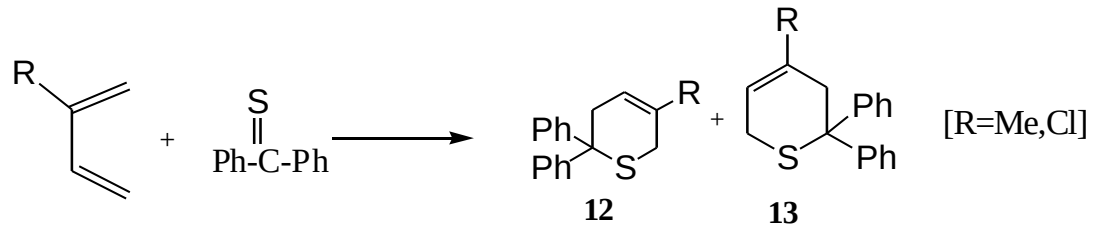


11

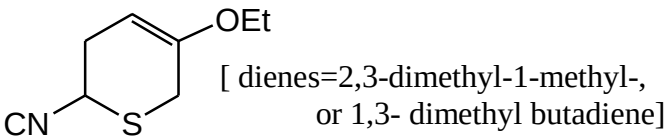
Δ^3-Dihydrothiopyran derivatives can be prepared by the "Diels-Alder Reaction". As for example, thiocarbonyl compounds react with dienes. The reactivity of these compounds is far greater than that of the corresponding oxygen analogues. Although most reactions proceed thermally, they appear to be catalysed by light. No detailed study on the mechanism has been made. However, the fact that unsymmetrically substituted dienes afford nearly equimolar amount of two possible adduct isomers (**12** & **13**) suggests a one-step radical-like mechanism [13].



12 **13**

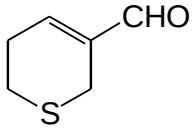
Methylation of 2- acyl-1,3-dithiolenes with $MeOSO_2F$ afford sulphonium salts which undergo cycloreversion to α- oxo dithioesters $RCOCS_2Me$ (R= Me, Ph). In the presence of dienes, good yields of 2- acyl-2-(methylthio)-3,6-dihydrothiopyrans are formed. Treatment of the adducts with thiophiles affords 2- acyl-3,6-dihydro-2H-thiopyrans.

Diels-Alder trapping of a reactive thioaldehyde (NCCHS) by 2-ethoxybutadiene gave 15% (**14**) [14].



14

Cyclisation of acrolein with hydrogen sulphide in dichloromethane containing Cu- turnings and triethylamine at -10^0 C gave 86% aldehyde (**15**) which was oximated with hydroxylamine to give 53% oxime (**16**). Similarly prepared was also (**17**) from the aldehyde [15] .



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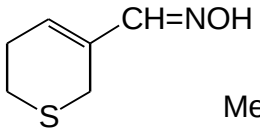

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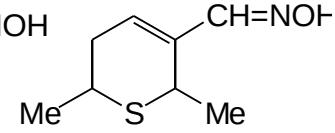
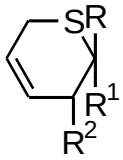


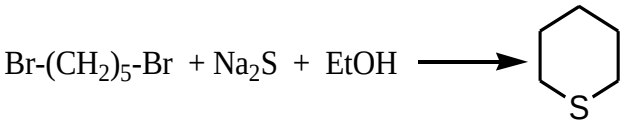
17

Thiopyranosides derivatives (**18**) and (**19**) i.e., novel carbohydrate derivatives with sulphur in the ring can be prepared by the "Diels-Alder reaction" of SC(CN)SMe with CH_2=CHCH=CHR (R= H, trans OMe). The structure of (**18**) and (**19**) were determined by NMR of their oxidation and reduction products [16].



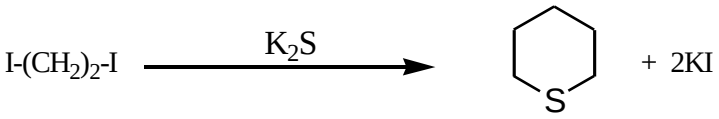
(**18**), R=CN,R^1=SMe, R^2=H
(**19**), R= SMe,R^1=CN, R^2=OME

Tetrahydrothiopyrans (**20**) were prepared from pentamethylene dibromide and sodium sulphide in ethanol[17].



20

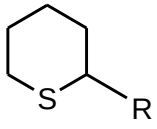
Tetrahydrothiopyran (**20**) can also be prepared from 1,5- diiodopentane and potassium sulphide[18] .



20

Thiocyclohexane (**20**) was also prepared by the reaction of 1,5-diketone such as $RCOCHR^1CHR^2CHR^1COR$ with H_2S-CF_3COOH. Sixteen different compounds were also prepared[19].

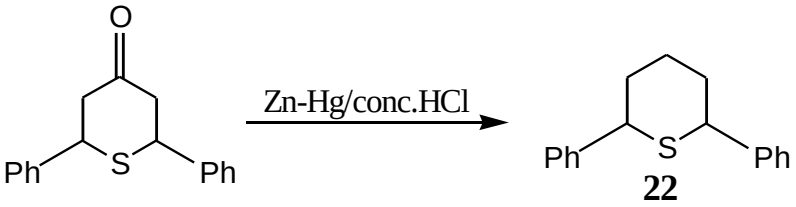
2-Substituted derivatives of tetrahydrothiopyrans (**21**) were prepared by the treatment of dihydropyran with RH[20].



R= MeO,BuO,PrS,BuS,$EtMe_2CS$,
PhS,$PHCH_2S$,$(MeO)_2P(S)S$,$(EtO)_2P(S)S$

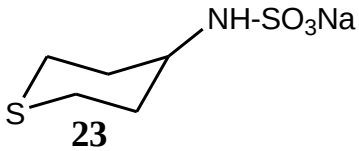
21

2,6-Diphenyltetrahydrothiopyran (**22**) can be obtained by the "Clemmensen reduction" of *cis*- and *trans*- 2,6-diphenyltetrahydro-4-thiopyrene to the corresponding 2,6-diphenyltetrahydrothiopyrans [21].



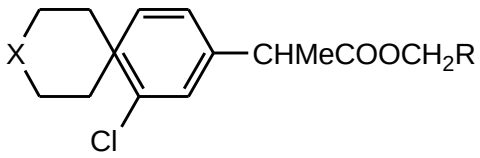
22

Sodium-4-thiocyclohexyl sulphamate (**23**) was prepared in 31% yield by oximation of ketones and reduction of the product (Na-EtOH), treatment with $ClSO_3H$ and converting into the salt (**23**) [22].



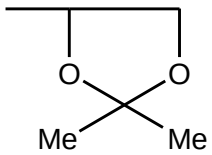
23

Phenylacetic acid ester derivatives of tetrahydrothiopyrans (**24** and **25**) were prepared by heating the corresponding pyranylphenylpropionic acids with thionyl chloride, cooling to 5^0 C and stirring with 2,2-dimethyl-1,3-dioxolane-4-methanol in pyridine, the dioxolanylmethyl propionates (**24**) formed treated with boric acid to give (**25**)[23].



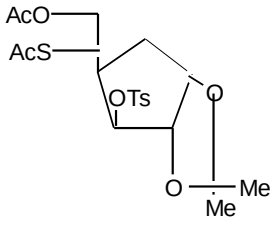
24, 25

(**24**), R=



(**25**), R=CH(OH)CH_2OH

5-Thio-**D**-allose and 5-thio-**D**-altrose can be prepared by the deacylation of the diacetate (**26**) (To S = 4-$MeC_6H_4SO_2$-) followed by isopropylidenation, desulfomylation, C-3 epimerisation by oxidation / reduction and acid hydrolysis gave 5-thio-**D**-allose (**27**) with methanol / hydrochloric acid gave the glycoside (**28**) which on sequential isopropylidenation, expoxidation. epoxide ring opening with sodium hydroxide (4:1 altrose:glucose), and hydrolysis gave 5-thio-**D**-altrose (**28**) [24].



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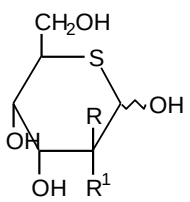

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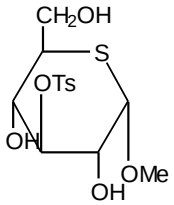
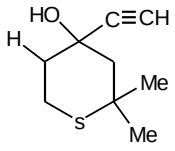


28

Heating (**29**) with $N_2H_4.H_2O$ 8 -10 hrs at 140^0-150 ^{0}C gave (**30**) (80-85%), also prepared by independent synthesis *via* ketones (**31**), which with $N_2H_4.H_2SO_4$ gave (**30**). Hydrogenation of (**30**) on *Ni-V* alloy gave saturated heterocyclic alcohols. Dehydration of (**29**) gave (**32**), which with $N_2H_4.H_2O$ gave (**33**)[25].



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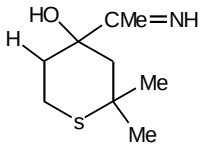

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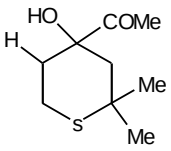

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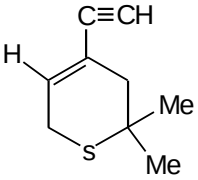

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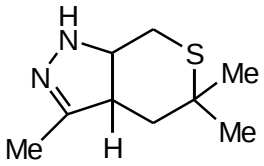
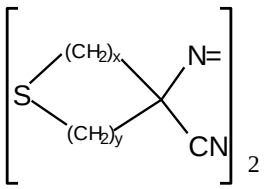


33

Azobis (1-cyanothiocyclohexane) compounds (**34**) were prepared by oxidising the corresponding hydrazines (**35**) [x,y = 0-4; x + y = 4], (**35**) [x = y = 2] in ethanol saturated with hydrochloric acid was treated with ethanolic solution of bromine with ice-cooling to give (77%) (**34**) [x = y = 2]. Similarly prepared was (**34**) [x = 1, y = 3][26].



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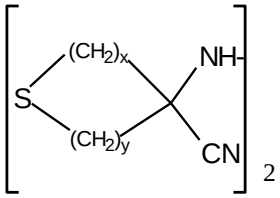
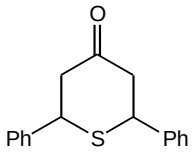


35

2,6-Diphenyl-4-ethynyl (phenylethynyl) tetrahydrothiopyran-4-ol (**37**) were prepared by the treatment of (**36**) with $RC{=}CH_2$(R= H, Ph) gave 77.3% (**37**) [R= H] and (**37**) 69.25% [R= Ph]. The compound (**37**) were hydrogenated to the saturated alcohols over Ni-Mo[27].



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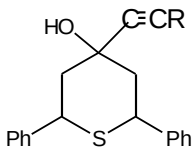
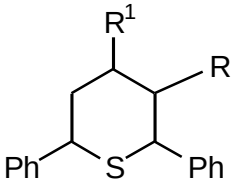


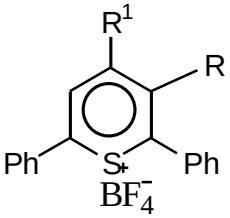
37

Cyclisation of $(BzCH_2)_2CH_2$ with BF_3-Et_2O and hydrogen sulphide at 20^0 C in ether for 100 hrs or in acetic acid for 20 hrs gave (**38**) 25-30% and (**39**)[28] 60%.



[R= R^1=H]

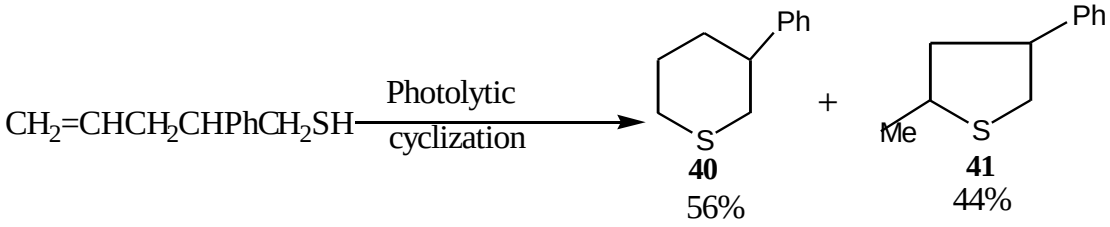
38



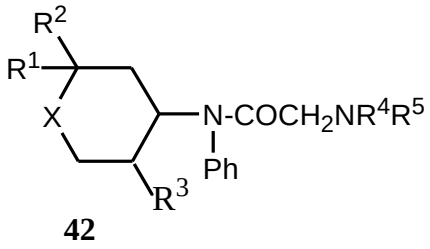
[R= R^1=H]

39

3-Phenyltetrahydrothiopyran (**40**) in 56% yield with minor amount of tetrahydrothiophene derivative (**41**) in 44% yield, were prepared by the photolytic cyclisation of CH_2=$CHCH_2CHPhCH_2SH$[29].



A series of tetrahydrothiopyran compounds (**42**) were prepared by the condensation of aniline with (**43**), reduction of formed Schiff's base[30], N-acylation by chloroacetylchloride and amination by the appropriate R^4R^5NH.



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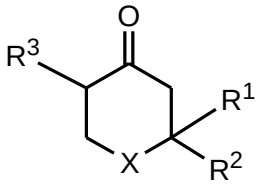
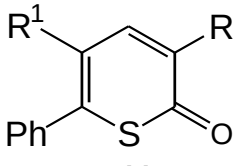


43

(**42**), R^1= Me; R^2, R^3= Me, H; N R^4R^5= NMe_2, NEt_2, pyrrolidino, piperidino, morpholino; X= S, O, NMe.

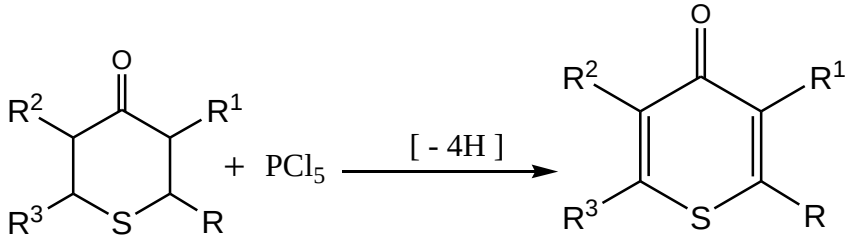
α-Thiopyrone compounds (**44**) formed by the Knoevenagel reaction of HSCPh=CPhCHO with $CH_2(CN)_2$ in 44% yield. This was also obtained in 62% yield from treating ClCPh=CPhCHO with $(CH_2)(CN)_2$ *via* 95% $ClCR^2=CR^1CH=CRCN$ (**45**) [R=CN, R^1= R^2= Ph] intermediate by reaction with sodium sulphide, hydrochloric acid and water. Similarly, seven other (**45**) were isolated in 70-95% yield but only in the case of (**45**) [R=CO_2Et, R^1=H, R^2= Ph], a further postulated intermediate MeSCPh=CHCH=C(CN)COOH was isolated. Similarly, prepared were (**44**) 42% [R= H, R^1= Ph] and (**44**) 46% [R= CN, R^1= H][31].



44

R=CN, R^1=Ph

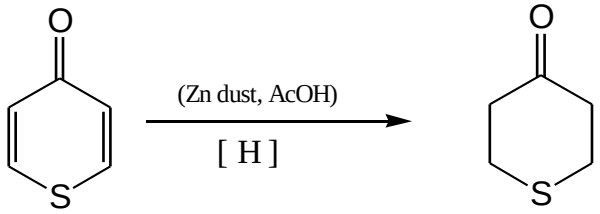
The γ– thiopyrones (**46**) and their derivatives i.,e., 2,6-dimethyl-1-thia-γ-pyrone (**47**), 2,6-diphenyl-1-thia-γ-pyrone (**48**), and 3,5-diphenyl-1-thia-γ-pyrone (**49**), can be prepared by the dehydrogenation of corresponding tetrahydrothiopyrones (**45**) with phosphorus(V) chloride[32].



45

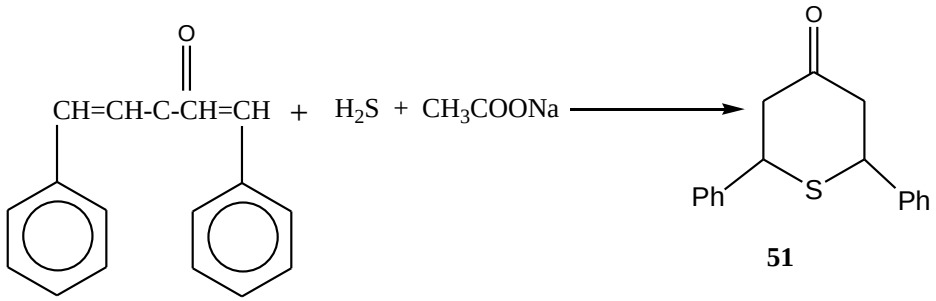
(**46**): R , R^1 , R^2 , R^3 = H
(**47**): R , R^3= Me, R^1, R^2 = H
(**48**): R , R^3 = Ph , R^1 , R^2 = H
(**49**): R^1 , R^2 = Ph , R , R^3 = H

Penthianone-4 i.e., tetrahydrothiopyrones (**50**) was prepared by the reduction of 1-thia-γ- pyrone with zinc dust and acetic acid[33].



50

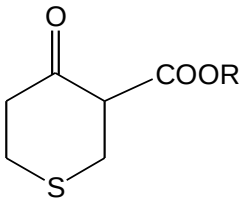
2,6-Diphenyltetrahydro-1-thiopyrone (**51**)[34] was prepared by the action of hydrogen sulphide on dibenzylideneacetone in the presence of sodium acetate



51

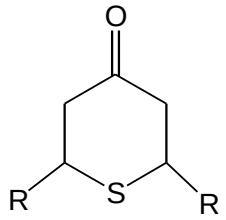
4-Oxotetrahydrothiopyran-3-carboxylic acid ester (**52**) [R= alkyl] were prepared by the reaction of 3,3'-thiodipropionic acid esters $S(CH_2CH_2COOR)_2$, (**53**) with alkali metals in the presence of hexaalkylphosphoramides $(R^1N)_3PO$ (54); [R^1= alkyl].

Thus a mixture of (**53**) [R= Me], (54) [R^1=Me] and sodium in ether was stirred for 19 hrs to give (**52**) 86% [R= Me][35].



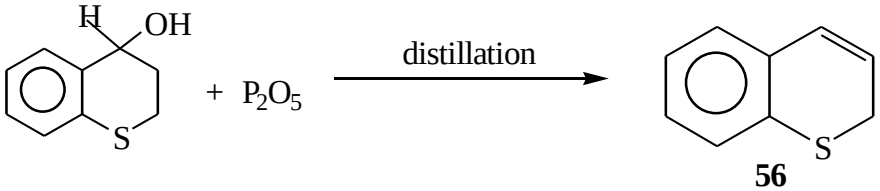
52

The *trans*-tetrahydrothiopyranone derivative (**55**) were prepared by the reaction of $(RCH=CH)_2CO$ with hydrogen sulphide in the presence of anhydrous sodium acetate in dioxane at reflux temperature[36].



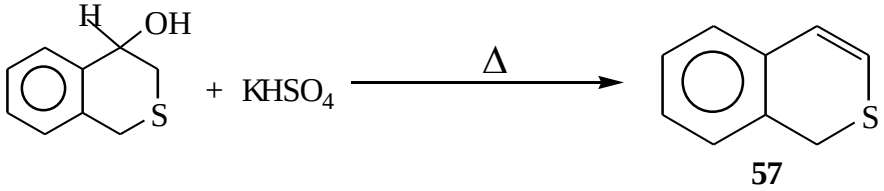
(**55**),R=Ph,$MeOC_6H_4(MEO)_2C_6H_3$

Benzo-α-thiopyran or thiachrom-3-ene (**56**) can be prepared from the thiachroman-4-ols by the distillation with phosphorus pentoxide[37].



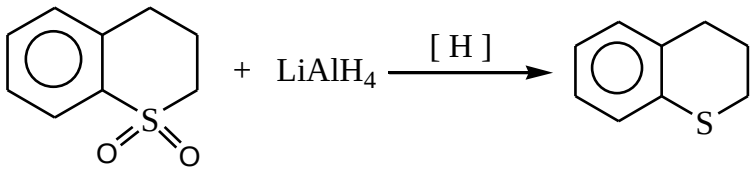
56

iso-Thiachromene (**57**) was obtained by heating isothiachroman-4-ol with potassium hydrogen sulphate [38].



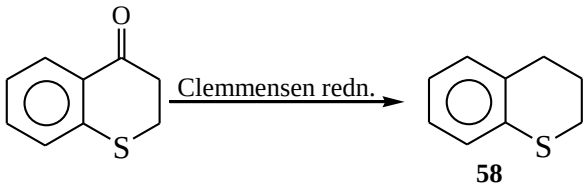
57

Thiachroman (**58**) is obtained in poor yield by reducing the sulphoxide with lithium aluminium hydride[39].



58

Thiachroman (**58**) also can be obtained by the Clemmensen reduction of 1-thiachroman-4-one[40].

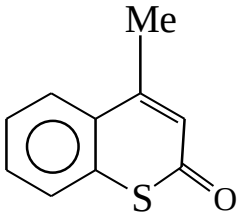


58

Also 2-methyl-, 3-methyl-, 4-methyl-, 6-methyl-, 6-ethyl- and 6,8-methyl-1-thiachroman (**59**) can be prepared by the same method[41].

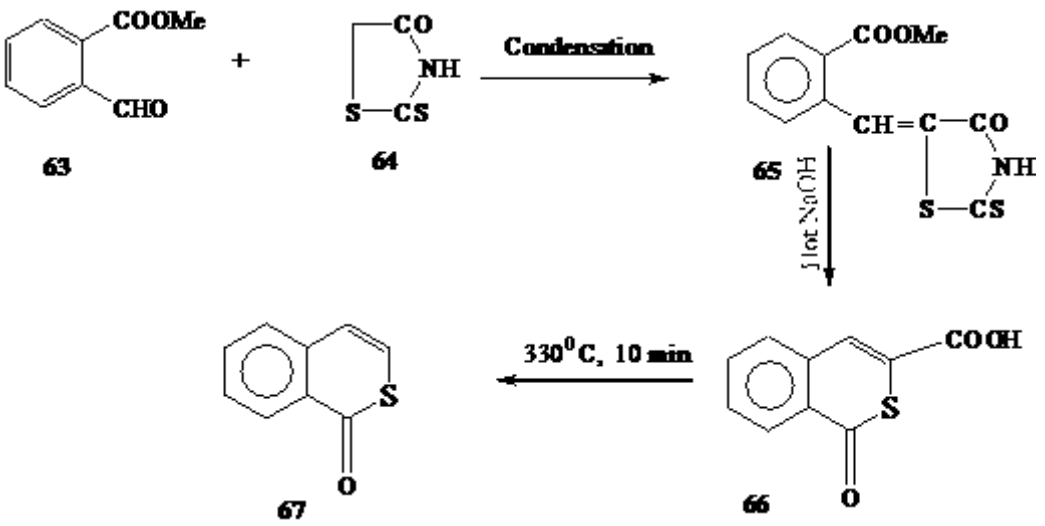
The product formed by the reaction of benzenethiols with diketone in the presence of sulphuric acid are (*E*)- and/or (*Z*)-β-(arylthio) crotonic acids and not, as has been prepared by Nakazumi and Kitao (1977), $RSCOCH_2COMe$ [(**60**), R= (un)substituted Ph]. (**60**) were prepared from benzenethiols and diketone in presence of triethylamine. The compound(**60**)reacted with various condensing agents to give thiaconmarins (**61**).

As for example, 4-methylthiaconmarin (**62**) were conventionally prepared by the reaction of (**60**) with anhydrous aluminium chloride in yields of 16-48%[42].



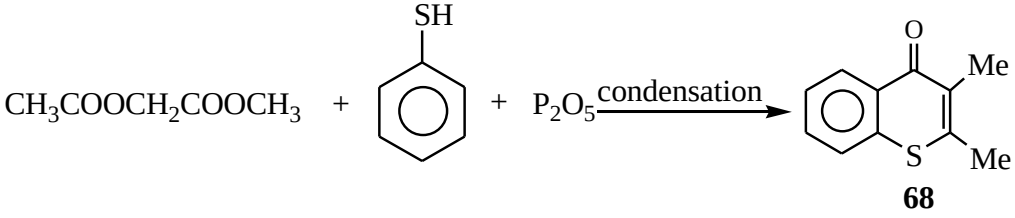
62

Methyl phthaldehyde (**63**) condenses with rhodamine (**64**) to give 5-O-methoxycarbonylbenzylidenerhodamine (**65**), yellow needdles,(Tm 215-216 ^{0}C), which with hot sodium hydroxide yields *iso*-thiaconmarin-3-carboxylic acid (**66**), $C_{10}H_6O_3S$, which when heated at 330^0 C for 10 minutes affords isothiaconmari (**67**)[43].



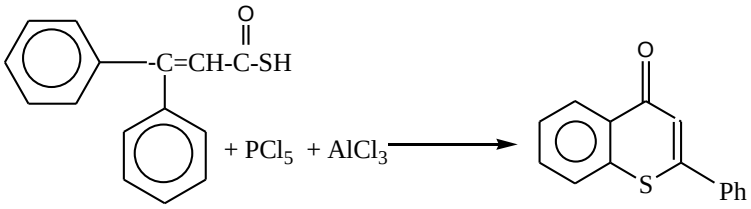
63 **64** **65** **66** **67**

2,3-Dimethyl-1-thiachoromone(**68**) was prepared by condensing methylaceto- acetate with thiophenol in the presence of P_2O_5[44].



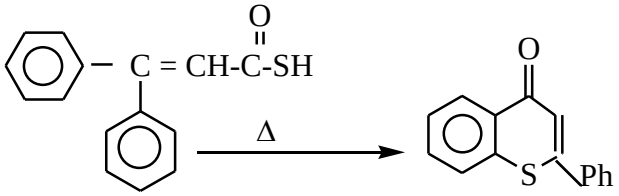
68

2-Phenyl thiaflavone (**69**) was prepared by the interaction of β-phenylthio- cinnamic acid, phosphorus pentachloride and aluminium chloride [45].



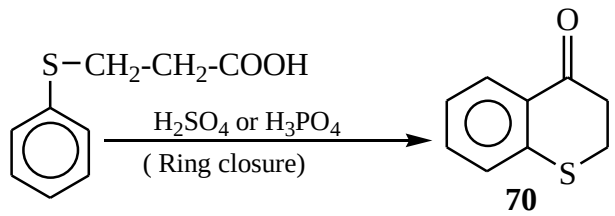
69

Compound(**69**)[46] was also prepared by heating β-phenylmercaptocinnamic acid.



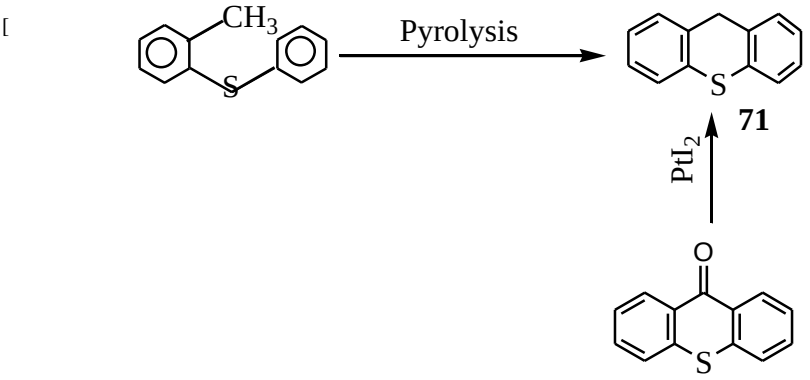
69

Thiachromanone(**70**) can be prepared by the ring closure of *β*-phenylmercapto-propionic acids by sulphuric acid[47] or phosphoric acid[48].



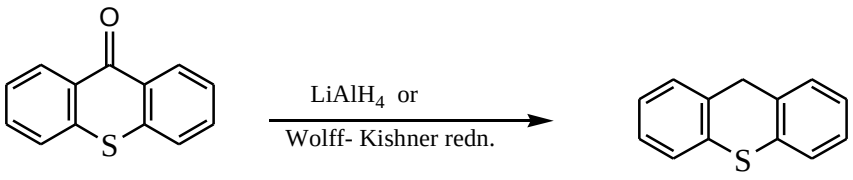
70

Thiaxanthene (**71**) was prepared by the pyrolysis of phenyl-**o**-tolyl sulphode or preferably by reducing thioxanthene by phosphorus and iodine[49].



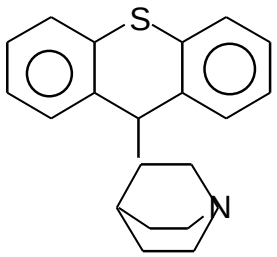
71

By reducing thioxanthene with lithium aluminium hydride[50] or the Wolff- Kishner method[51] one can get dibenzothiopyran (**72**) .



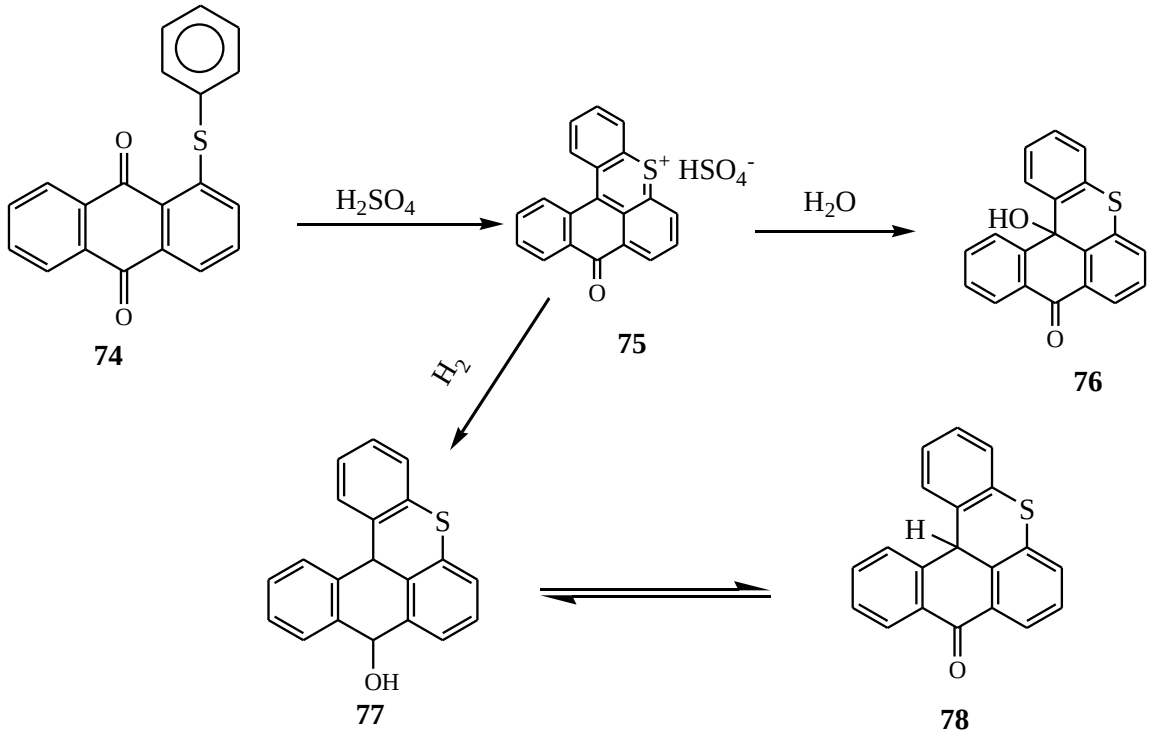
72

9-(3-Quinnclidinyl) thioxanthene (**73**) and their derivatives were prepared by a mixture of naphthalene, sodium and tetrahydrofuran, when treated with thioxanthene and 3-(phenylsulfonyloxy) quinuclidine [52].

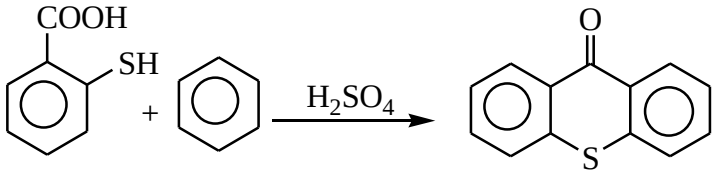


73

9-Hydroxycerothiene(**77**) on the keto form (**78**) were prepared when 1-phenylmercaptoanthraquinone(**74**) heated with sulphuric acid yields the sulphonium salts (**75**) which with water gives the carbinol bases (**76**) and reduction affords the above said compounds (**77** & **78**) [53].

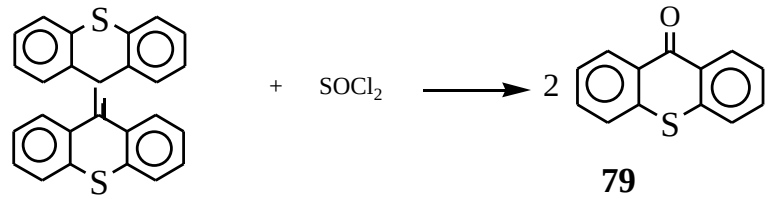


Thiaxanthone (**79**) was obtained by the condensation of *o*-mercaptobenzoic acid and benzene in presence of sulphuric acid [54].



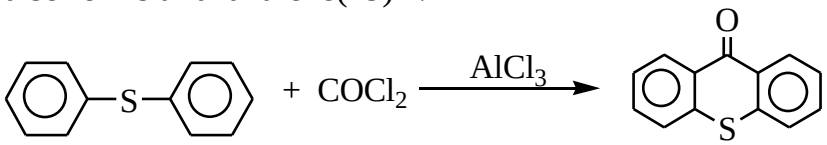
79

It(**79**) can also be prepared by the action of thionyl chloride on dithia- xanthylene [55].



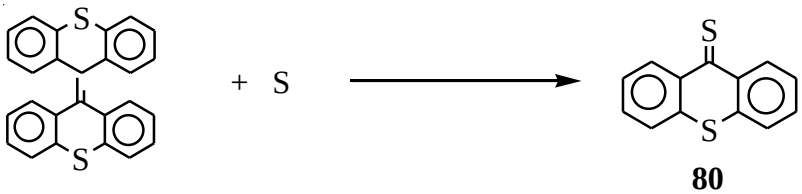
79

By the interaction of diphenylsulphide and carbonyl chloride with aluminium chloride as catalyst also forms thiaxanthene(**79**)[56].



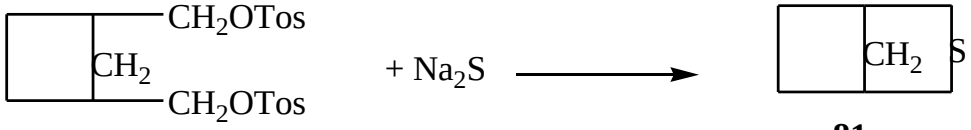
79

Thionothiaxanthone(**79**) can be prepared by the action of sulphur on dithiaxanthylene[57].



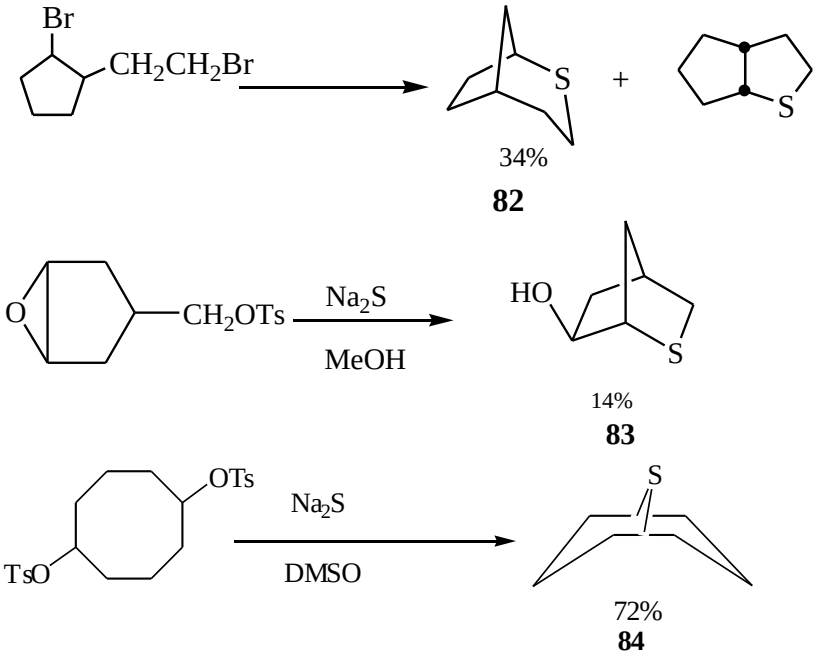
80

Bridged ring thiopyran compound i.e., *cis*-3-thiabicyclo[3,2,1] octane (**81**) can be prepared by the action of sodium sulphide on the ditosyl compound[58].

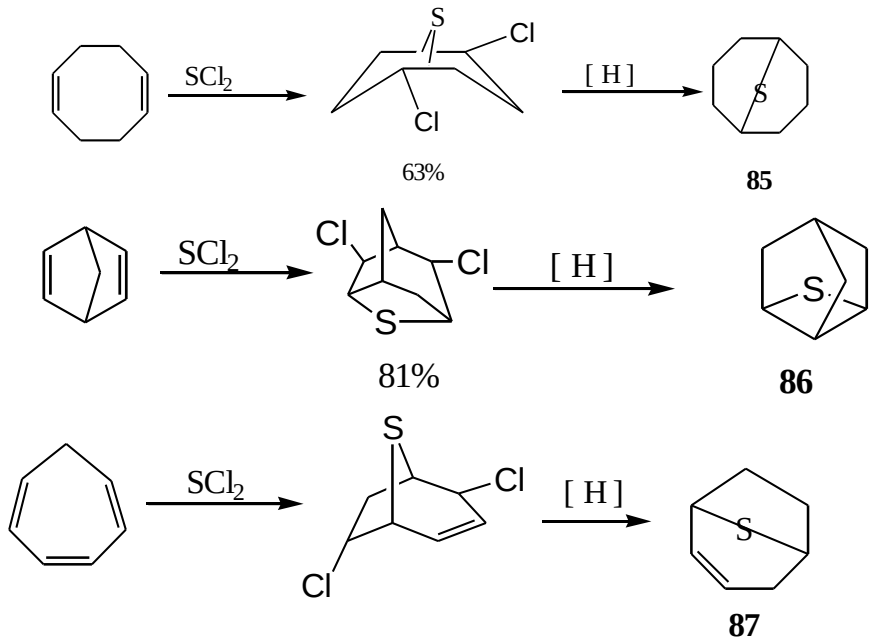


81

Six membered cyclic sulphides (**82, 83, 84**) may be prepared by refluxing an aqueous alcoholic solution of the corresponding dihalides and metallic sulphides [59].

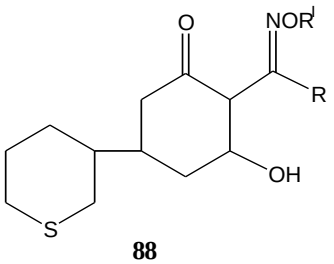


Electrophilic addition of sulphur dichlorides to olefins have opened new synthetic routes to a variety of cyclic sulphides (**85**, **86,** and **87**). Transannular addition takes place.For example, 1,5- cyclooctadienes and norbornadiene give the corresponding bicyclic dichoroslphides in good yields. Their reduction by metal hydride give the unsubstituted bicyclic sulphides(**85, 86,** and **87**)[60].



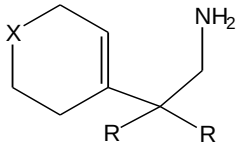
5-(Tetrahydrothiopyren-3-yl) cyclohexanonederivatives (**87**, R= acyl; R^1 = chloroalkyl) were prepared by treating 2-butyl-5-(tetrahydrothiopyren- -3-yl)

cyclohexan-1,3-dione in methanol was treated with trans-ClCH=CHCH$_2$ONH$_2$, HCl and NaHCO$_3$ to gave (**88**)[61].

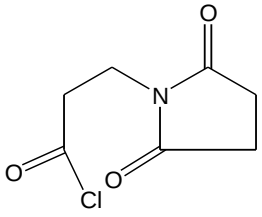


88

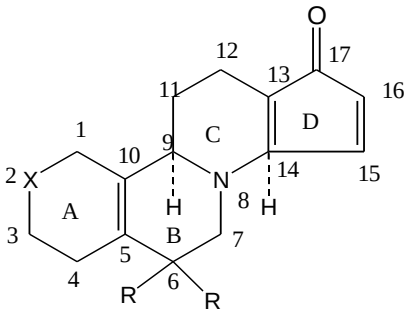
The total synthesis of heterocyclic steroids diszagonenoes (**91**) [x= CH$_2$, S; R= H, Me] was achieved starting from amines (**89**) as the A-ring precursors and 3-succinimidopropionyl chloride (**88**) as the D-ring moiety. (**89**) and (**90**) were condensed to yield the succinimides which subsequently by Bischler- Napieralski reaction and reductive cyclization produced (**91**)[62].



89

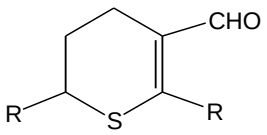


90



91

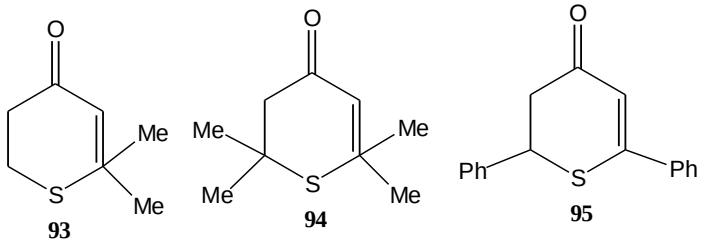
5,6-Dihydro-2H-thiopyran-3-carboxaldehyde (**92**) were prepared by treating acrolein or crotonaldehyde with hydrogen sulphide in a mineral oil, whose b.p. is higher than the b.p. of the starting material or end-product and recovering the (**92**) by distillation. Acrolein and hydrogen sulphide are charged into a vacuum gas-oil at 40-50^{o}C, 2 hrs, the mixture stirred 30 minutes, dodecylbenzenesulfonic acid added, and the mixture warmed ~1.5 hr to give 100-120^{o}C to give (**92**) [R= H][63].



92

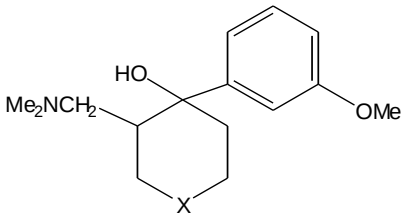
2,2-Dimethyl- (**93**), 2,2,6,6-tetramethyl- (**94**) and 2,6-diphenyltetrahydro-4H-thiopyran-4-ones (**95**) respectively were prepared by treating unsaturated ketones

RR^1C= $CHCOR_2$ (R= R^1= Me, R^2= $CH_2CH_2NEt_2$, $CH_2CMe_2NC_5H_{10}$; R= H, R^1= Ph, R^2= $CH_2CHPhNC_5H_{10}$) with hydrogen sulphide in ethanol 1 hr gave (**92**), (**93**) and (**94**) in 48, 78 and 75% respectively[64].



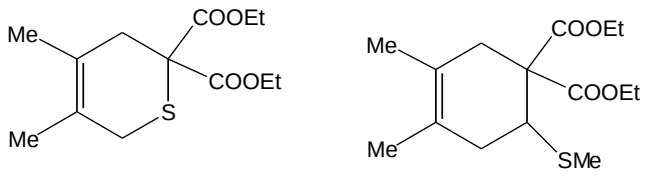
93 **94** **95**

3-(dimethylaminomethyl)-4-(3-methoxyphenyl) tetrahydro-4-pyranol (**96**) and -4-thiopyranol (**96**) i.e. the O- and S- analogues of tramadol were prepared by reacting 4-tetrahydropyranone and –thiopyranone with CH_2==$N^+Me_2Cl^-$ and 3-methoxyphenylmagnesium bromide[65].



96

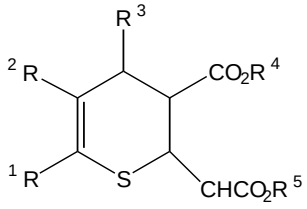
Cycloaddition of di-Et thioxomalonate to 1,3-diened proceeds regioselectively and in excellent yields to provide di-Et 3,6-dihydro-2H-thiopyran-2,2-dicarboxylates (**97**). Expose to the cycloadducts to either IDA or K bis (trimethylsilyl) amide at low temperature, followed by quenching with MeI, gives di-Et-2-(methylthio)-3-cyclopenten-1,1-dicarboxylates (e.g. **98**) with a high degree of diastereoselectivity (>85%d.e.).This novel 2-step transformation proceeds in overall yields of 55-84% [66].



96 **97**

2-Alkoxycarbonyl methylene-2H-thiopyran-3-carboxylates (**99**) [R^1,R^2 = H, alkyl, aryl, heteroaryl; R^4,R^5= H, alkyl] were prepared in one step by condensation of an enaminothioketone with a –2-chloropropenedicarboxylate. Thus, PhC = SCPh =

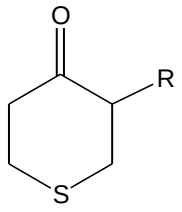
$CHNMe_2$ were stirred for 1 hr at room temperature and refluxed for 1 hr with MeO_2CCH_2Cl = $CHCO_2Me$ in benzene containing Et_3N to give 83% (**99**) [R^1 = R^2 = Ph, R^3 = H, R^4 = R^5 = Me][67].



99

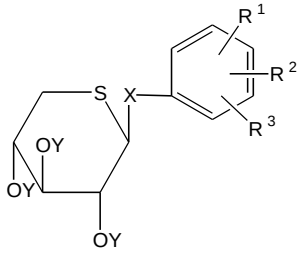
3-Alkyl-4-thianones (**100**) [R= Me, $PhCH_2$, allyl] can be conveniently prepared by the alkylation of 3-allyloxycarbonyl-4-thianone followed by deallyloxy- carboxylation mediated by tetrakis (triphenylphosphin) palladium in the presence of morpholine.

The corresponding sulfones, as well as 2,3-dialkyl-4-thianone derivatives can be prepared by analogues procedure [68].



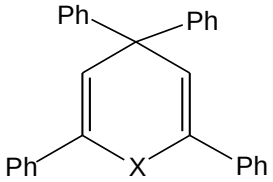
100

Phenylthio-β-xylosides (**101**) [X= S, O; R^1,R^3= H, cyano, COR; R= alkyl, CF_3, NH_2, NHAc etc.; Y= H, acyl] are prepared. Thus, 2,3,4-tri-O-acetyl-5-thio-α-D-xylopyranosyl bromide was condensed with 4-mercaptobenzonitrile-1,5- -thio-β-D-xylopyranoside[69].



101

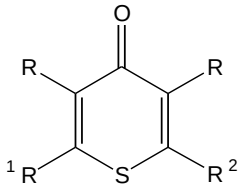
1,3,3,5-tetraarylpentane-1,5-diones react with tetraphosphodecasulphide in xylene at elevated temperature to give the corresponding 2,4,4,6-tetraaryl-4H-thiopyrans (**102**)[70].



(X = S, O)

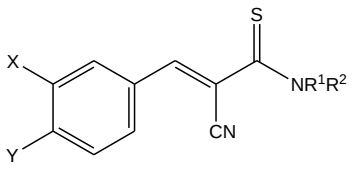
102

A novel synthesis of 2,6-diaryl-4H-thiopyran-4-ones [(**103)**; R= H, Me; R^1,R^2 = Ph, 2-pyridyl-, 2-thienyl] has been developed. The compound(**103**) were prepared by two sequential thio-Claisen condensation of a dialkylketone. RCH_2COCH_2R and two dithioesters, e.g. $R^1CS_2CH_3$. The intermediate β-thioxoketone corresponding β-(methylthio) enone for both protection and reactivity proposes. Facile addition-elimination of the methylthio moiety by a β-thioxoketone enolates generated in the second thio-Claisen condensation afforded the desired heterocycle (**103**) [71].

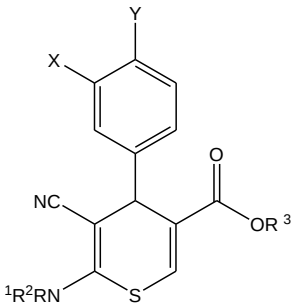


103

The 3-aryl-2-cyanothioacrylamides (**104**) [X= NO_2, OMe, Cl; Y= H, OMe, Cl; R^1,R^2= H, alkyl] reacted with methyl propionate and di-methylacetylene-dicarboxylate yielding 4-aryl-4H-thiopyrans (**105**) [same R^1,R^2,X,Y; Z=H,CO_2Me; R^3= Me, Et]. Reactions with less activated alkynes proceed sluggishly, if at all [72].

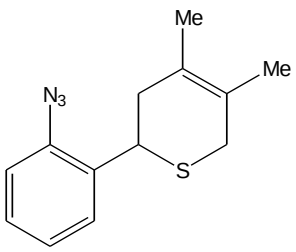


104



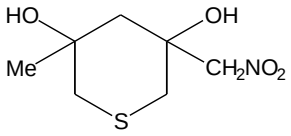
105

Treatment of aromatic and heteroaromatic O-azidoaldehydes with bis(trimethylsilyl) sulphide and a suitable catalyst affords an easy access to the corresponding O-azidothioaldehydes as their Diels-Alder cycloadducts (**104**) with dienes. e.g. ,reaction of $N_3C_6H_4CHO$ with bis(trimethylsilyl) sulphide in the presence of a 5-fold excess of 2,3-dimethylbutadiene,catalysed by $CaCl_2.6H_2O$, gave 76% thioaldehyde-diene cycloadduct (**106**) [73].



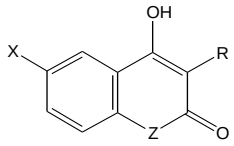
106

Perhydrothiopyran diols (**107**) were prepared by reaction of 2,2'-thiobis[cyclohexanone], 2-(phenylacylthio) cyclohexanone and diacetonyl sulphide with $MeNO_2$ in presence of MeONa[74].



107

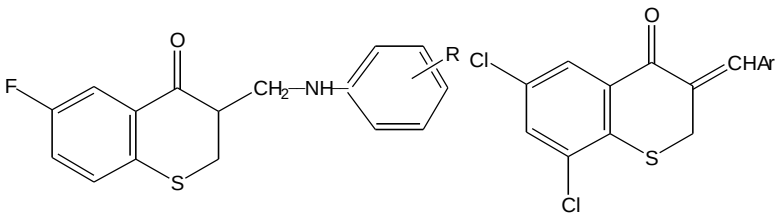
3-substituted 4-hydroxy-2H-1-benzothiopyran-2-one (**108**) and 4-hydroxy-2H-1-benzopyran-2-ones (**108**) [R= Me, Et, Pr, benzyl; X= H, Br, Cl; Z= S, O] were prepared by reaction of RCH_2CO_2Et with methylthiosalicylates or methylsalisylates followed by acid cyclisation[75].



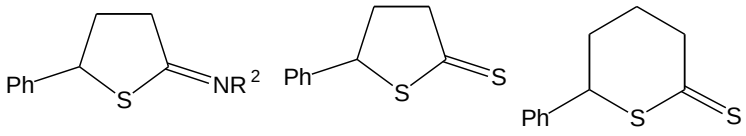
108

Nine new Mannich bases of 6-fluoro-thiochromanone (**109**) [R= 4-F, 4-Br, 2-NO_2 etc.] and eight new 3-substituted 6,8-dichlorothiochromanones, e.g., (**110**)[Ar= Ph, 2-ClC_6H_4, 2-$O_2NC_6H_4$, 4-$MeOC_6H_4$) were synthesized and their structures were confirmed by IR, ^{1}H- nmr and elementary analysis[76].

The treatment of 3-hydroxyamides $PhCH(OH)CH_2CONRR^1$ [R=Ph-,4-ClC_6H_4,4-MeC_6H_4,$PhCH_2$,Me_3C,Me_2CH;R^1=H,Me,Me_2CH,$PhCH_2$; RR^1= $(CH_2)_2O(CH_3)_2$] eith Lawesson's reagent exclusively gave thioenamides (E)-PhCH==CHCSNRR^1 in 58 – 98% yields. The treatment of 4-hydroxyamides $PhCH(OH)CH_2CHCONR^2R^3$ [R^2= Ph, 4-ClC_6H_4, 4-MeC_6H_4, $PhCH_2$; R^3= H, $PhCH_2$] with Lawesson's reagentyielded sulphur-containing heterocycles such as tetrahydrothiophene-2-imines (**111**) [R= Ph, 4-ClC_6H_4,4-MeC_6H_4, $PhCH_2$; R^3= H, $PhCH_2$] in 30 - 51% yields and tetrahydrothiophene-2-thione (**112**) in 15 –52% yield by cyclisation of the intermediate 4-mercaptamides$PhCH(SH)CH_2CH_2CONHR^2R^3$.The5-hydroxyamides $PhCH(OH)CH_2CH_2CH_2CONHR^4$ [R4= Ph, $PhCH_2$] also reacted with Lawesson's reagent to afford tetrahydrothiopyran-2-thione (**113**) as the sole product in 15 – 48% yield[77].

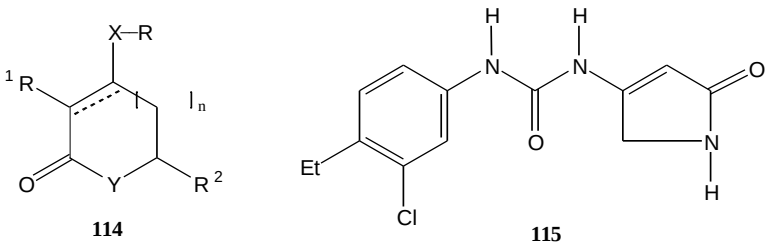


109 **110**



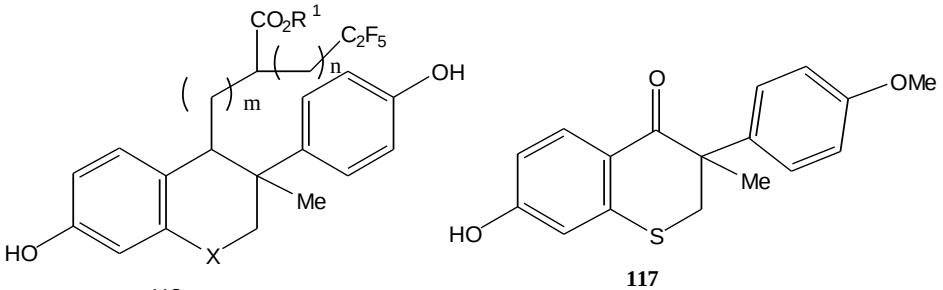
111 **112** **113**

The title compound (**114**) [Y= S,O,NH; n= 0-1; X= NHCONH, $NHCONHSO_2$ etc.; R^1= H, alkyl, $(CH_2)_pA$ (wherein p= 0-4; A= OH, NR^aR^b, CO_2R^c etc; R^aR^b= H,alkyl; NR^aR^b= a 4-7 membered heterocycle; R^c= H, alkyl, alkali or alkaline earth metal); R-alkyl, cycloalkyl etc; R^2= H, alkyl] were prepared. Thus. Reacting 3-chloro-4-ethylphenylurea with teramic acid in EtOH / PhMe afforded (**115**) [78].

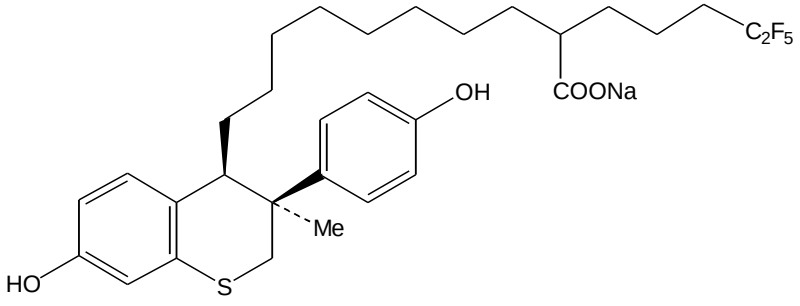


114 **115**

The preparation of *meta*; salts of 3-methylchromanone or thiochromanone derivatives, specifically (**116**) and their pharmaceutically acceptable salts; stereoisomers or hydrates [wherein X= S, O; R1= metal; m= 2-14; n= 2-7]. These specific examples (all sodium salts) were prepared and claimed. For instance, thiochromanone precursor (**117**) was converted to inversion compound (**118**) in 10 steps. (**117**) was markedly more soluble than either the free acid or the compression compound in artificial intestinal juice. (**118**) was also water-soluble nearly the same extent, whereas the other two compounds were essentially insoluble [79].

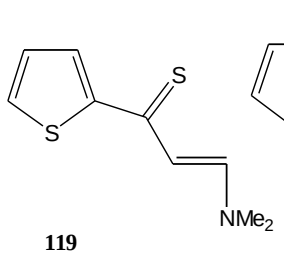


116 **117**

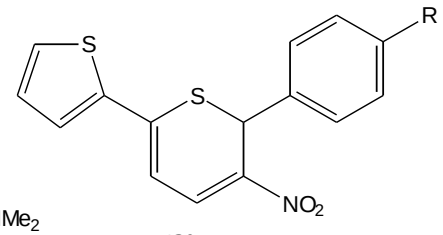


118

The hetero Diels-Alder reaction of 3-(dimethylamino)-1-(2-thienyl)-2-propene- -1-thione (**119**) (the diene) with β-nitrostyrenes, maleic acid and fumaric acid (the dienophiles) yielded 3,4-dihydro-4-(dimethylamino)-2H-thiopyrans. Treatment of some of the cycloadducts with acetic acid caused elimination of dimethylamine to yield stable 2H-thiopyrans, e.g., thienyl (nitro) thiopyrans (**120**) [R= H, Me, MeO]. Reaction of (**119**) with maleic anhydride gave a cycloadduct which underwent spontaneous rearrangement to give the thiopyrancarboxamide (**121**). Cycloaddition of (**119**) to maleimide, N-phenylmaleimide, maleic acid monoanilide, di-Et maleate, di-ethyl fumarate and 5H-furan-2-one in the presence of acetic anhydride were followed by elimination of dimethylamineto give stable 2H-thiopyrans[80].



119



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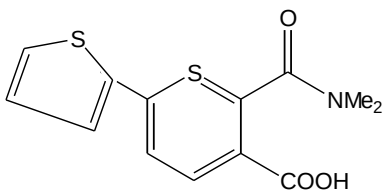
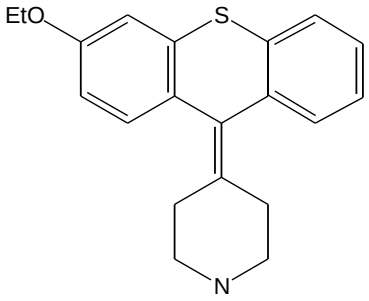


121

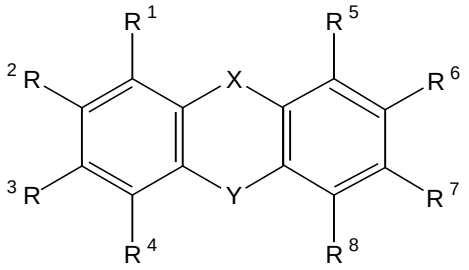
Preparation of 1-methyl-4-(3-ethoxy-9H-thioxanthene-9-ylidene)piperidine (**122**) was reported by reacting 3-ethoxythioxanthone with the Grignard reagent, prepared from 1-methyl-4-halopiperidine, preferably 1-methyl-4- -chloropiperidine followed by dehydration of the resulting alcohol[81].



122

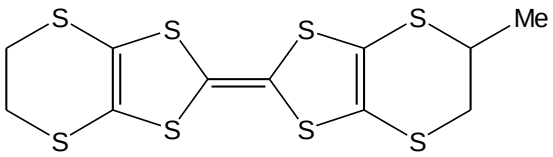
Tricyclic compounds (**123**) [X= (un) substituted CH_2, CH_2CH_2, CH=CH, NH, CH_2NH, CHN; Y= (un) substituted CH_2, CH_2CH_2, CH=CH, NH, CH_2NH, O, S, S(O), SO_2, CH_2O, CH_2S, CHN; R^1-R^8= H, halogen, haloalkoxy, alkylthio, alkylsulfinyl, alkylsulfonyl, haloalkylthio, haloalkylsulfinyl, haloalkylsulfonyl, dialkylaminosulfonyl, NO_2, CN, NH_2, CHO, acyl] were prepared. Thus, thioxanthen-

9-one was treated with 1-methyl-4-piperidinone to give 10-(1-methyl-9-piperidinylidene) dibenzo [b,e] thiane[82].



123

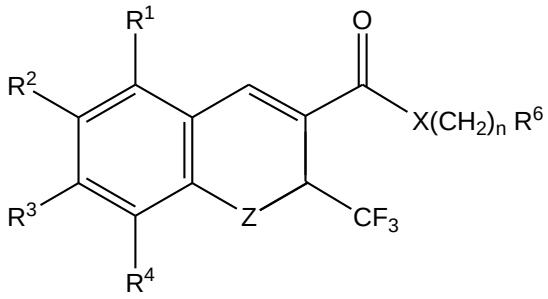
Bis (ethylenedithio) tetrafulvalene (ET) molecules with one alkyl chain (**123**), C_nET (n= 1-6) have been synthesised. The solubility in usual organic solvents is remarkably improved without changing the electron donating ability and good thin films are formed by the solution, east method. C_1ET (**124**) has the same dimer structure as ET, while the structures of the n= 2-4 molecules are composed of zig-zag columns[83].



124

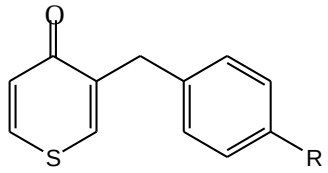
Nitrosated benzopyran compounds [(**125**); Z = S, O, NH; X = O, NH; n= 1-5; R^1-R^4 = H, alkanoyl, alkenyl, alkenylalkynyl, (substituted) alkyl, alkylamino, alkylheteroarylalkynyl, arylcarbonyl, aralkylthio, aralkynyl, arylaminoalkyl, alkoxycarbonylalkyl, alkoxyheteroaryl, OH etc.; R^1R^2, R^3R^4= atoms to form a cycloalkyl, heterocyclo, heteroaryl ring; R^6 = H, OH, ONO_2, halo, nitroxyalkylcarbonyl, SH, haloalkyl, alkoxy, aryl etc.] were prepared. Thus,4-hydroxybutyl(2S)-6,8-dichloro-2-trifluoromethyl-2H-chromene-3-carboxylate (preparation given) in CH_2Cl_2 at -5^oC was treated with a mixture of prepared from Ac_2O and HNO_3 to give 55% 4-nitroxybutyl(2S)-6,8-dichloro-2-trifluoromethyl-2H-

chromene-3-carboxylate (**125a**)[84].



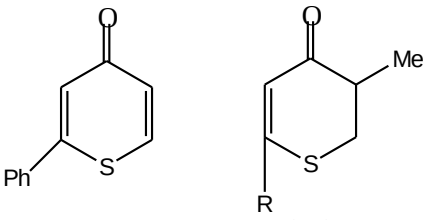
125

Efficient synthesis of several novel 3-substituted thiopyran-4-ones, e.g., **126** (R= H, Me, MeO, Cl or Br), was reported for the first time via Aldol condensation of various aromatic aldehydes with dihydrothiopyran 4-one derivatives under very mild conditions using magnesium bromide di-etherate and triethylamine . The product were characterized based on spectroscopic and X-ray diffraction experiments[85].



(126)

The synthesis of 5- to 8-membered cyclic thioethers, e.g., **127**, has been achieved through a simple two step sequence. The present methodology utilises the facile Friedel-crafts acylation of terminal alkynes with $NaSH.xH_2O$. The scope and utility of the tanden C(Sp3)-S and C(Sp2)-S bond formation was further extended with the synthesis of 1,2-dithiin-4-(3H)-ones,**128** (R=pentyl,e-hexyl, phenyl) and benzo[b] thiophene -3-(2H)-ones[86]**.**

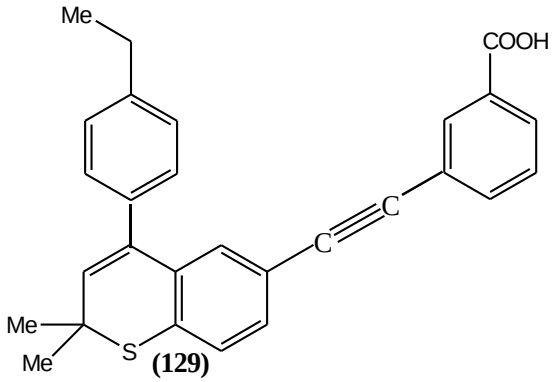


(127) (128)

The invention discloses a method for treating chemotherapy or radiation therapy side effects in a mammal undergoing chemotherapy and /or radiation therap, the method comprising administering a therapeutically effective amount of a RAR(retinoic acid receptor)antagonist or inverse agonist which binds to receptors of the RARα, RARβ and RARγ subtypes. Such side effects include chemoradiotherapy-induced alopecia, chemoradiotherapy-induced thrombo-

cytopenia, chemoradiotherapy-induced leucopenia and chemoradiotherapy-induced neutropenia.

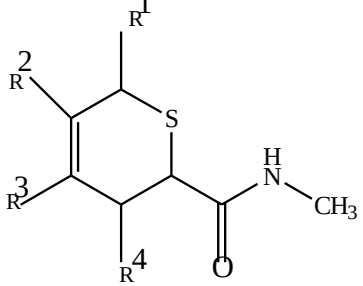
Preparation of VTP 194310 (**129**) is described[87]**.**



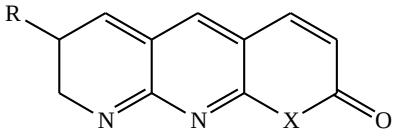
(129)

Oxazolidinones possessing a C-5carboxamide functionality(reverse amides) represent a new series of compounds that block bacterial protein synthesis. These reverse amides also exhibited less potency against monoamine oxidase (MAO) enzymes and thus possess less potential for the side effects associated with MAO, and excellent pharmacokinetic properties[88].

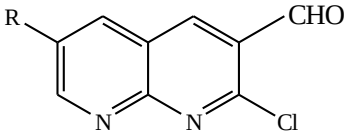
The title compound shows reduced *in- vivo* myclotoxicity compared to linezolid in a 14-day safely study in rats, potent *in –vivo* efficacyin murine systemic infectionmodels describes methods of treating obesity using DGAT-1(acyl CoA diacylglycerol acyltransferase –1) inhibitors according to formula , **130** (Z=aryl and heteroaryl, wherein each aryl and heteroaryl may be optionally substituted with 1 to 3 R^6; R^5 = alkyl, thioalkyl and halo; and R^6= alkyl and alkoxy) including all prodrug, solvates, pharmaceutically acceptable salts and stereoisomers[89]**.**



The title compounds, (**131**) [R=H,Me,MeO; X= O, S, Se] were prepared from naphthyridinecarboxaldehyde (**132**, same R) by Cl displacement with AcOH, Na_2S and NaHSe, followed by heterocyclisation with Ac_2 O over solid AcONa. Both steps were carried out in one pot under solvent free microwave irradiation assisted technique[90].

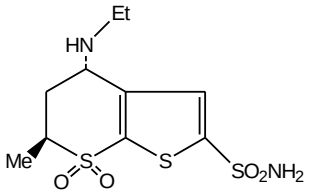


(131)



(132)

The present invention relates to an improved process for the preparation of 5,6-dihydro-4-(s)-(ethylamino)-6-(s)-methyl -4H-thieno-[2,3-b] thiopyran- 2-sulphonamide-7,7-dioxide–hydrochloride **133**. HCl, commonly known as Dorzolamide Hydrochloride, useful as an agent to reduce intraocular pressure by inhibiting carbonic anhydrase enzyme (no biol. data given) . Thus reacting the freshly prepared 2-thienylmagnesium bromide with S,Et_3N.HCl, crotonic acid and a base followed by treating the acid **134** with a chlorinating agent, cyclisation of the acid chloride in the presence of a Lewis acid , reacting **135** with chlorosulphonic acid and thionylchloride , and subsequently with ammonia, reducing **136**, oxidizing **137**, subjecting **138** to a Ritter reaction , reducing **139** , converting **140** to acid addition salt and recrystallisingenriched salt from the solvent and then converting salt to trans **133**, and resolving trans-**133** into (4S, 6S)- **133**[91].



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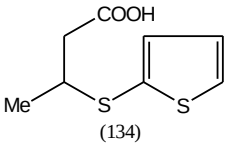

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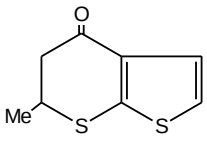

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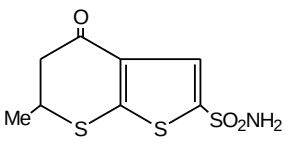

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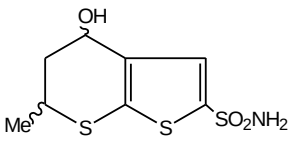
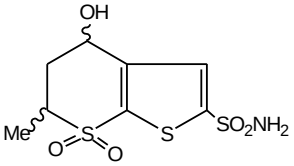


(137)



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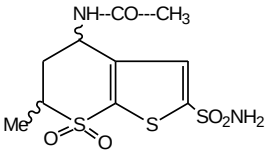

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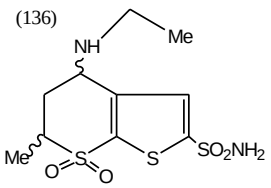
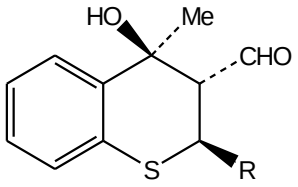


(140)

One pot reaction of aromatic aldehydes, cyanothioacetamide and malononitrile under microwave irradiation technique proved to be an efficient way for the synthesis of 2,6–diamino-4-aryl-4H-thiopyran-3,5-dicarbonitrles without any added catalyst[92].

The above mentioned title compound, [i.e.,3-(formyl)tetrahydrothiopyran] is an intermediate of cycloxydim [i.e., 2-{1-(ethoxyimino)butyl}-3-hydroxy- -5-(tetrahydro-2*H*-thiopyran-3-yl)-2-cyclohexen-1-one]. The cyclisation of acrolein (2-propenal) with hydrogen sulphide provided 5,6-dihydro-2H-thiopyran-3-carboxaldehyde. Hydrogenation of the later in the presence of a catalyst provided the title compound . Under optimized conditions the yield and purity of 3-formyl-5,6-dihydro-2H-thiopyran was more than 93% and 95% respectively. A new reaction mechanism was proposed based on optimised catalyst conditions. The target compound was obtained in a yield above 94% and product content above 92% by using Raney- Ni as catalyst[93].

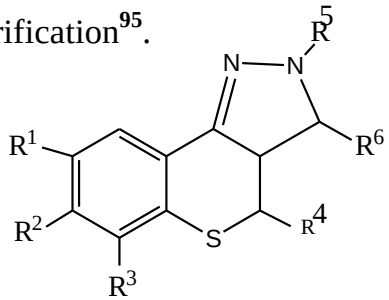
The authors described highly enantioselective organocatalytic dominothia-Michael/Aldol reactions between 2′-mercaptoacetophenoneand α,β-unsaturated aldehydes RCH:CHCHO (R = 4-XC_6H_4, X = H, cyano, Cl, Br, NO_2-). The reactions proceed with excellent chemo-, diastereo-, and enantioselectivity to give the corresponding benzothiopyran derivatives. (**141** same R) in 63-98% yields with upto >15:1dr and 96 to > 99% ee[94].



(141)

The title compounds **142** [wherein R^1 to R^3 = independently H, OH,CN, halo, or (un)substituted alkyl, etc; R^4 = H or alkyl; R^5=substituted alkyl; R^6 = substituted alkyl], including stereoisomers or pharmaceutically acceptable salts thereof, were prepared as fungicides. Thus, the invention compound **143** was prepared by condensation of 6-fluoro-2,3-dihydro-4*H*-1-benzothiopyran-4-one

with benzaldehyde followed by heterocyclisation with hydrazine in the presence of acetic acid and purification[95].



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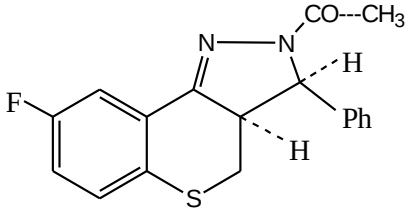
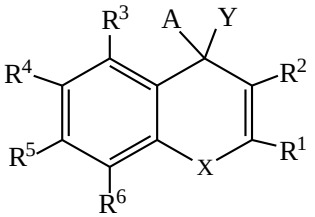


(143)

Title compounds, **144** [A = (un)substituted aryl,heteroaryl carbocyclyl, heterocyclyl when R^1= NHC(O)$R^{8;}$]; or A= (un)substituted quinolinyl, cinnolinyl, quinazolinyl, etc. When R^1= NR^7R^8, NHC(O)R^8,N=CHOR^8,etc, where R^7 and R^8 independently = H , (un)substituted alkyl, aryl , or R^7 and R^8 are also taken together within the N atom to form a(n) (un)substituted ring; X=O,S, or NR^1, where R^1= H, (un)substituted acyl, alkenyl, etc.,Y = H or alkyl; R^2 = CN, C(O)OR^9 , C(O)R^9, etc., where R^9= H, (un)substituted alkynyl, heteroaryl, etc.; R^3– R^6 independently = H, halo, NO_2, etc., or any two adjacent R^3 – R^6 are taken together with the C atoms to which they are attached to form a(n) (un)substituted ring], and their pharmaceutically acceptable salts, are prepared and disclosed as insulin- regulated amino peptides(IRAP) inhibitors.

For example, compound **145** was prepared via reaction of resorcinol with ethyl-2-cyano-3–quinoline–4-yl acrylate which was obtained by condensation of 4-quinolinecarboxaldehyde with ethylcyanoacetate. Select **144** were assayed for IRAP enzyme inhibition and found to possess IC50 values of 0.6 µM to 40 nM.

The invention is also directed to using **144** in therapeutic applications including memory enhancement and learning functions[96].



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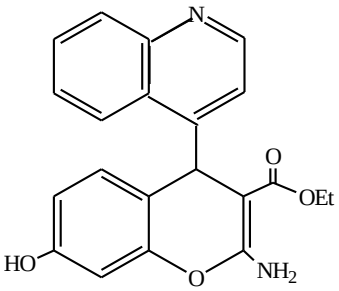
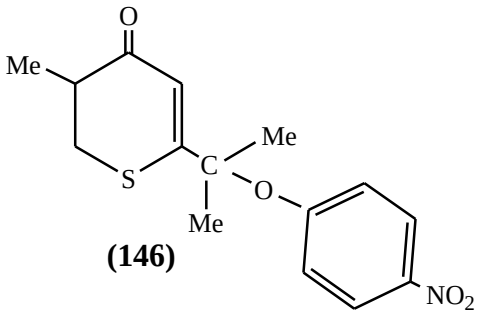


(145)

2,3-Dihydrothiopyran-4-one derivatives were readily prepared by Pd/Cu-catalysed reactions between α,β -unsaturated thioesters and propargyl alcohols in the presence of bases. E.g., in presence of $PdCl_2$, Cu_2I_2 and K_2CO_3, cyclisation of CH_2=CMeCOS$C_6H_4NO_2$-4 and CH≡$CCMe_2$-OH gave 60% 2,3-dihydrothiopyran-

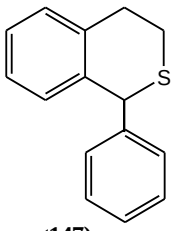
4-one, **146**. Of note, both carbon–sulphur bonds were cleaved as a result of the single procedure[97].



(146)

Organic transistors use organic semiconductor films which contain ≥1unsubstituted and X^1-X^2 substituted indeno[1,2,3-cd]pyrene derivatives, where X^1-X^2 = H, or halogen, direct- chain, branched or ring-shaped alkyl or alkoxy group, or substituted or non-substituted aryl group. The organic transistors have high mobility, large On/OFF current ratio, and excellent storage stability[98].

A new route to functionalized *iso* (thio)-chromans, i.e., **147**, is described. The compounds are accessible easily in a one-pot reaction by using different benzaldehydes and phenyl ethanethiol or phenyl ethanol in presence of bismuth triflate[99].

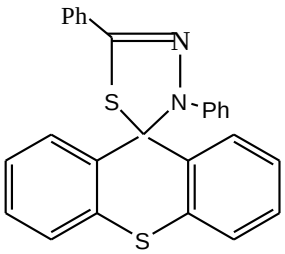


(147)

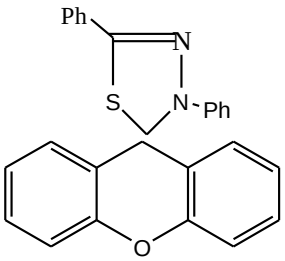
A convenient method is offered for double aldol condensation of tetrahydrothiopyran-4-one with aromatic aldehydes under lithium bromide catalysis. An X-ray analysis of (3z,5z)–3,5-dibenzylidene–tetrahydropyran– 4-one [monoclinic, P2, / n, a 10.035 (1), b 9.540(1), c 15.595(1) Å, β 95.32(1) Å, V 1486(55) A^3, Z4]verified the proposed structure[100].

The 1,3-dipolar cycloaddition of nitrile imines to 9H-thioxanthene-9-thione and 9H-xanthene-9-thione afforded novel spiro-thioxanthene-9′, 2-[1,3,4] thiadiazole, e.g.,

148, and spiro-xanthene-9′, 2-[1,3,4] thiadiazole, e.g.,**149** in good yields. Some of the newly synthesised compounds were tested for antiinflamatory and analgesic activities comparable toibuprofen. Some compound shown significant activity compared to the standard drug.The toxicity study revealed that neither death nor the behavioral or toxicological changes were observed on rats up to a dose as high as 200 mg/kg[101].

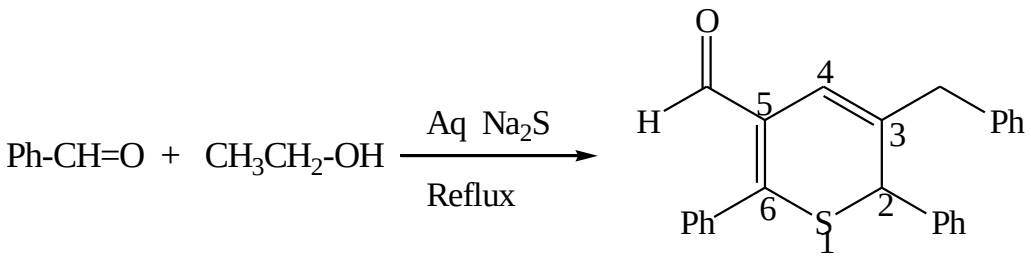


(148)



(149)

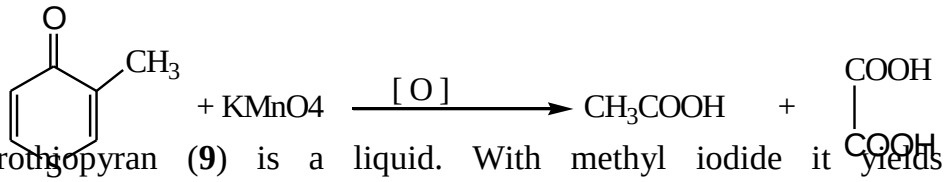
Latif and co-workers of Rajshahi University in 1957 have reported[102, 103] the one-pot preparation of 3-benzyl-2,6-diphenyl-2H-thiopyran-5-carboxaldehyde (**150**) by reacting benzaldehyde with ethanol in aqueous sodium sulphide at elevated temperature. Its structure was later confirmed by spectroscopic[104] and X-ray crystallographic studies[105, 106].



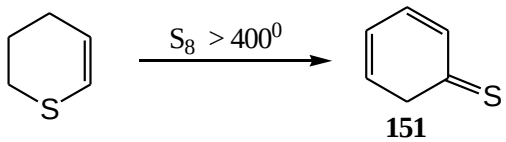
(150)

1.6 PHYSICAL AND CHEMICAL PROPERTIES OF THIOPYRAN TYPE COMPOUNDS

The 3-methyl-γ-thiopyran (**3**) is a volatile oil, having a smell similar to that of xylene. It resembles thiophene, giving a colour with isatin and H_2SO_4 and with phenanthraquinone and with acetyl chloride and aluminium chloride forming an acetyl compound. It is oxidised by potassium permanganate to acetic and oxalic acid[7].

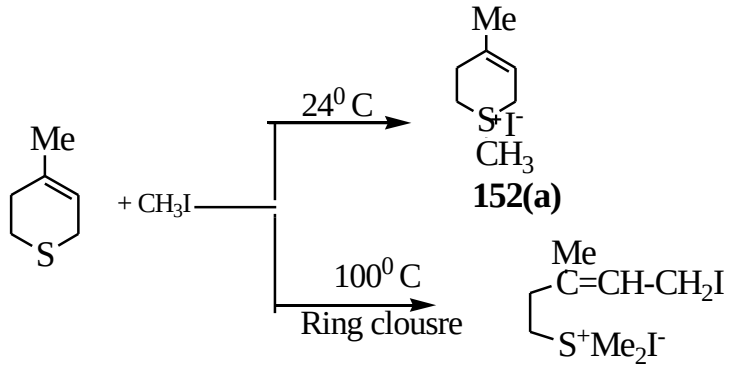


Δ^2-Dihydrothiopyran (**9**) is a liquid. With methyl iodide it yields mainly trimethylsulphonium iodide [11]. When treated with sulphur at greater than 400° C, by dehydrogenation forms thiopyran-2-thione. This is the first preparative method for α-dithiopyrones which formed as the end products [107].



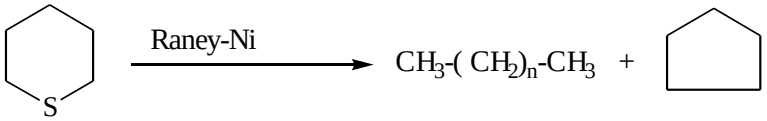
151

Δ^3-Dihydrothiopyran is a liquid. 4-Methyl- Δ^3-Dihydrothiopyran with methyl iodide at 24°C gives the methylsulphonium iodide **152(a)** (m.p.142.5° C) but at 100°C ring cleavage occurs giving dimethyl-5-iodo-3-methylpent-3-enylsulphonium iodide (**152**), which is a red brown needles[108].



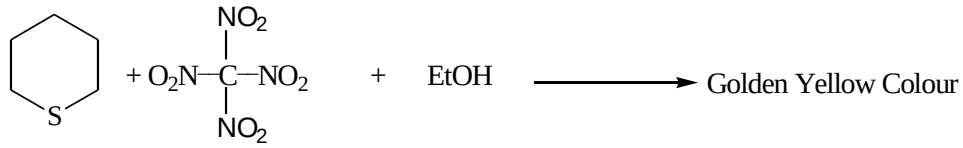
152

Tetrahydrothiopyran(**20**) is a liquid.With Raney-nickel it undergoes hydrogenolysis yielding n-pentane and cyclopentabe [109].



20

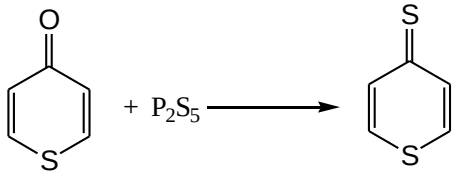
It gives golden yellow colour with tetranitromethane in ethanol[110].



20

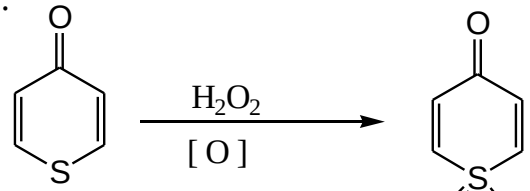
It yields the methylsulphonium iodide, subliming at 192°C, a sulphoxide, yellow liquid and the sulphone[111].

The γ-thiapyrone (**46**) with phosphorus pentasulphide yields 4-thiono-1-thia-γ- -pyrans (**153**) [112].



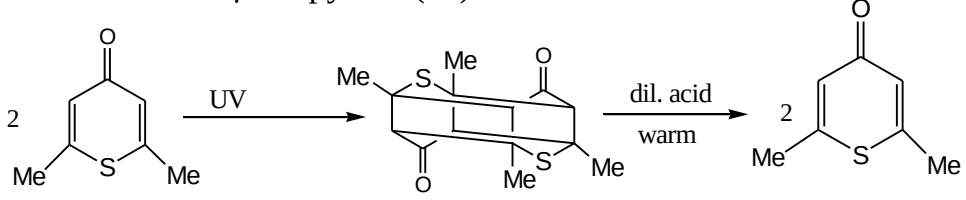
46 **153**

Oxidation of γ-thiapyrone (**46**) with hydrogen peroxide yields the S-dioxide (**154**), yellow crystals[113].



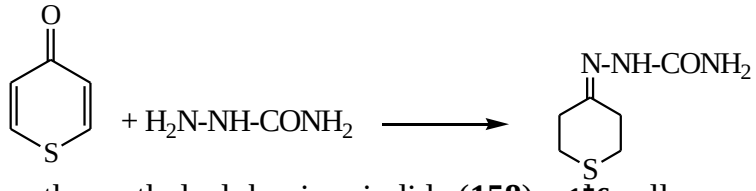
46 **154**

2,6-Dimethyl-γ-thiapyrone (**47**) is a solid compound. When irradiated with UV-light, gives a dimer (**155**) and the dimer has been shown to have a "cage" structure which reverts to the γ-thiapyrone (**47**) when heated with dilute acid [114].



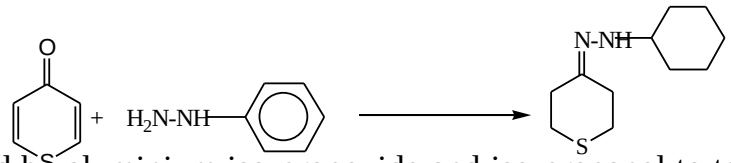
47 **155** **47**

Tetrahydrothiopyrones(**50**) is a solid. It forms semicarbazone (**156**) and 2,4-dinitrophenylhydrazones(**157**)with semicarbazide and 2,4-DNP respectively[115].



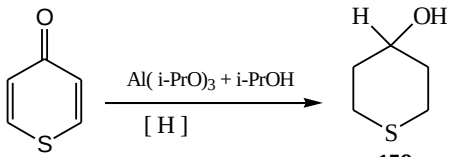
50 156

It forms the methylsulphonium iodide (**158**), pale yellow crystals with methyl iodide.



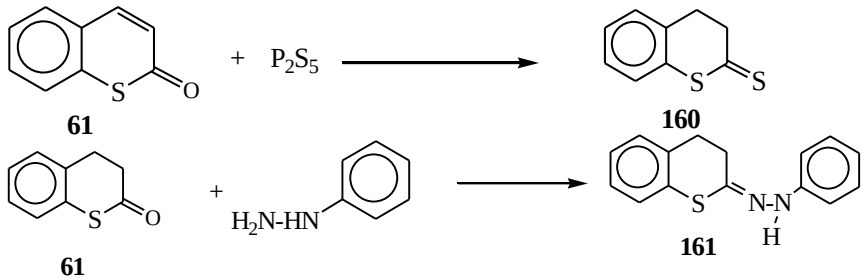
50 157

It is reduced by aluminium iso-propoxide and iso-propanol to tetrahydro- 1-thiopyran-4-ol (**159**)[33].



50 159

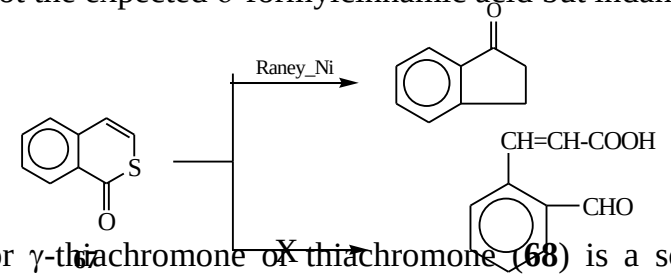
Phosphorus pentasulphide converts thiacoumarin (**61**) into thionothiacoumarin (**160**), which with phenylhydrazine affords a product, claimed to be thiacoumarin phenylhydrazone (**161**)[108].



61 160

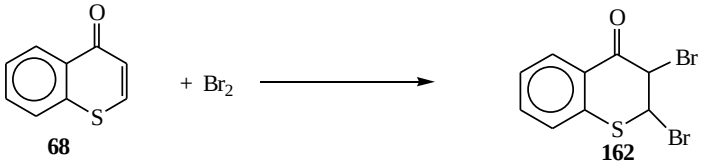
61 161

iso-Thiaconmarin (**67**) is a solid. With mthanolic ammonia it yields 1,2-dihydro-1- -oxo-isoquinoline which is a solid but does not reduce Fehling's solution. With Renay-nickel yields not the expected δ-formylcinnamic acid but indan-1- -one[115].



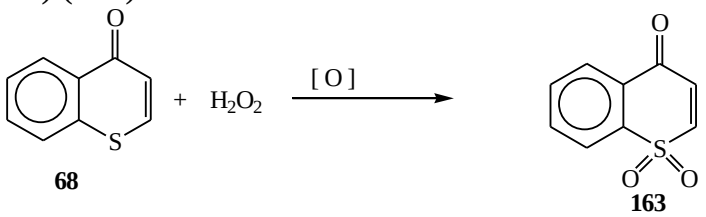
67 X

Benzo-γ-thiapyrone or γ-thiachromone or thiachromone (**68**) is a solid; has a blue fluorescence in sulphuric acid [116].



68 162

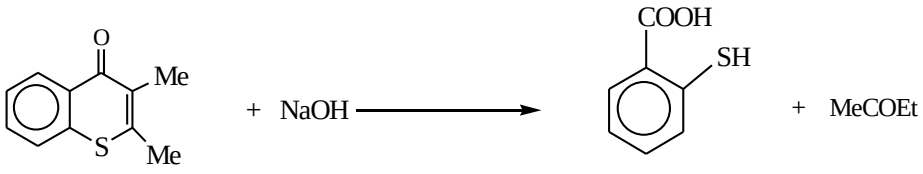
It gives a dibromide (**162**) with bromine and can be oxidised by hydrogen peroxide to S-dioxide (sulphones) (**163**) [116].



68 **163**

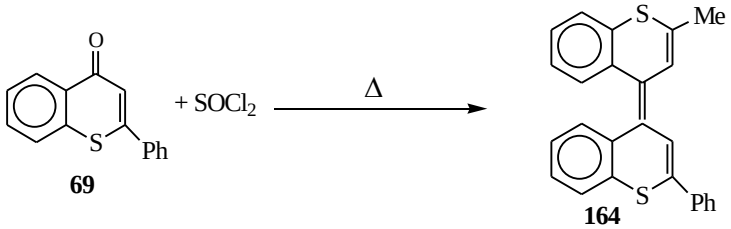
It is more resistant to alkali than chromones; for instance, long heating with sodium ethoxide being required to breakdown 1-thiaflavone to 2-mercaptoacetophenone, 2-mercaptobenzoic acid, acetophenone and other products[108].

2,3-Dimethyl-1-thiachromone (**68**) is a solid. With sodium hydroxide it yields O-mercaptobenzoic acid and methyl ethyl ketone[44].



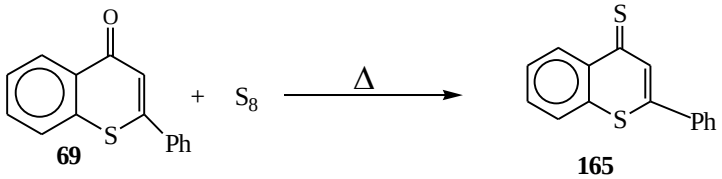
68

1-Thiaflavone (**69**) gives yellow needle shaped crystals and yellow colour in sulphonic acid but no fluorescence [45]. When thiaflavone (**69**) is heated with thionyl chloride, it yields dithiaflavylene (**164**), $C_{10}H_{20}S_2$, giving yellow crystals[119].



69 **164**

When 1-thiaflavanones (**69**) is heated with elemental sulphur, it yields 4-thionothiaflavone (**165**)[108].

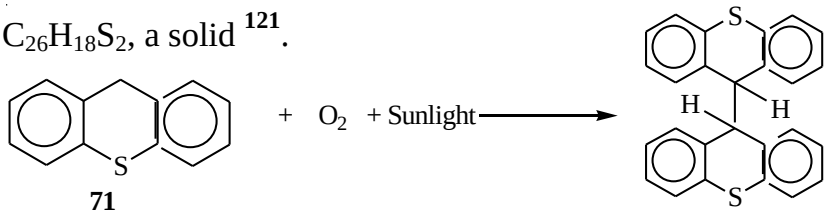


69 **165**

Thiachromanone is a solid. It gives intensely coloured solutions or solids with sulphuric acid and perchloric acid, 6-methyl-1-thiachroman-4-one, for instance,

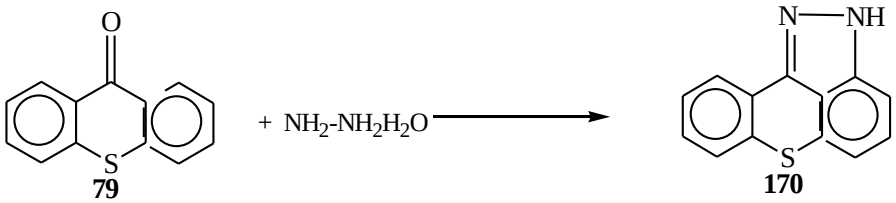
yielding an orange-red perchlorate[109]. The 1-thiachroman-4-one (**70**) reacts with Grignard reagents to give 1-thiachroman-4-ols (**166**) and in this way 4-methyl-, 4,6-dimethyl-, 4,6,8-trimethyl-, 4-ethyl-6-methyl- and 6-methyl-4-phenyl-1-thia-chroman-4-ol have been obtained[120].

Thiaxanthene (**71**) is a solid and gives a yellow solution in sulphuric acid with a faint fluorescence [54]. In the presence of oxygen and sunlight it gives dithiaxanthyl (**167**), $C_{26}H_{18}S_2$, a solid [121].



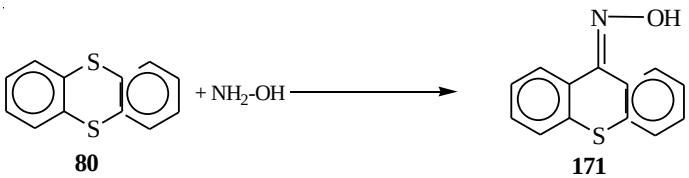
71 **167**

Thiaxanthone (**79**) are yellow crystals, gives yellow solution in sulphuric acid with green fluorescence[54]. It does not react with hydroxylamine or phenyl hydrazone perhaps because of the dipolar structure indicated by its high d.m., μ=5.4D[121]. It was oxidised by hydrogen peroxide or potassium persulphate to the corresponding S-dioxide. It was broken-down by fusion with alkali to S-phenyl-mercaptobenzoic acid[122]. 1-Chloro-4-methylthiaxanthone (**168**) reacting with aniline to give 1-aniline-4-methylthiaxanthone (**169**), orange needles. With hydrazinehydrate it (**79**) gives the product (**170**) below[123].



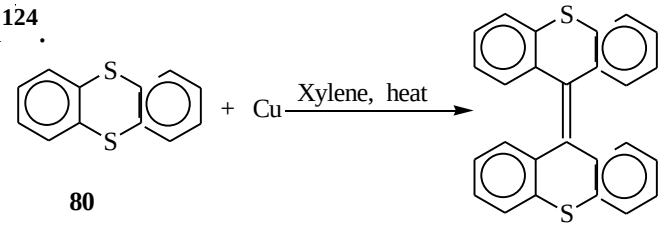
79 **170**

Thionothiaxanthone (**80**) is a solid.With hydroxylamine, it yields thiaxanthone oxime (**171**)[124].



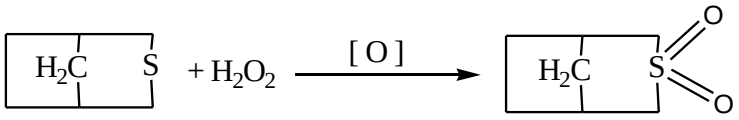
80 **171**

But when heated with copper in xylene, it yields dithiaxanthylene (**172**), $C_{26}H_{16}S_2$, which is a solid compound[124].



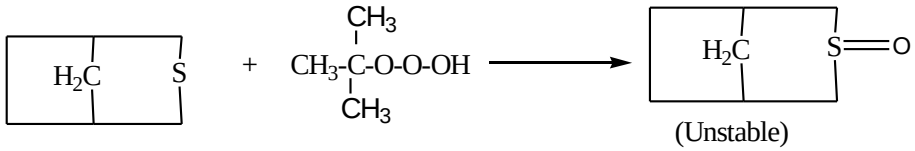
80 **172**

cis-3-thiabicyclo [3.2.1] octane (**81**) is a solid having the molecular formula, $C_7H_{12}S$; readily sublimes, has a camphor-like odour and forms S-dioxide (**173**) with hydrogen peroxide[58].



81 **173**

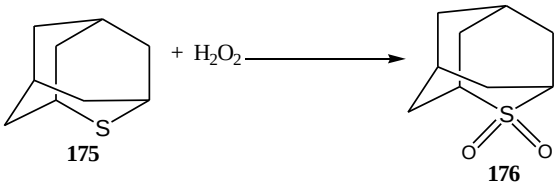
It gives an unstable sulphoxide (**174**) with tert-butylhydroperoxide[109].



81 (Unstable) **174**

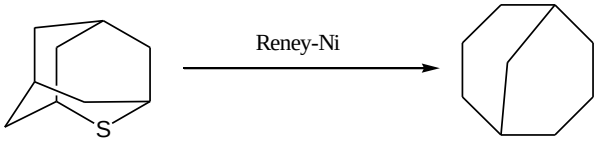
With Raney-nickel undergoes hydrogenolysis to yield cis-1,3-dimethyl- -cyclopentane which is a liquid and bicyclo [2.2.1] heptane[109].

Thiadamantane (**175**) is a liquid having the molecular formula, $C_9H_{14}S$; sublimes at 340^oC (decompose)[125]. It forms S-oxide (**176**) when oxidised by hydrogen peroxide[125].



175 **176**

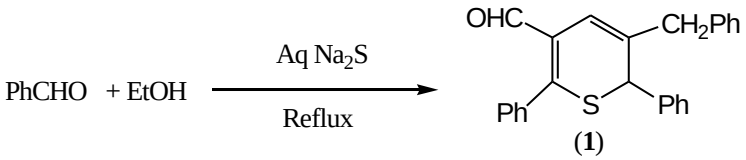
With Raney-nickel it yields bicyclo [3.3.1]- nonane (**177**)[125].



175 **177**

1.7 AIM OF THE PRESENT INVESTIGATION

Long back in 1922 Bruni and co-workers[152] reported a method for the preparation of dithiobenzoic acid (PhCSSH) by the action of ammonium polysulphide on benzaldehyde. In 1959, Latif *et al* [102,103] reported a reaction with sodium sulphide instead of ammonium polysulphide with a view to obtain dithiobenzoic acid but failed to obtain the same. Instead, they obtained the following yellow crystalline compound (1):



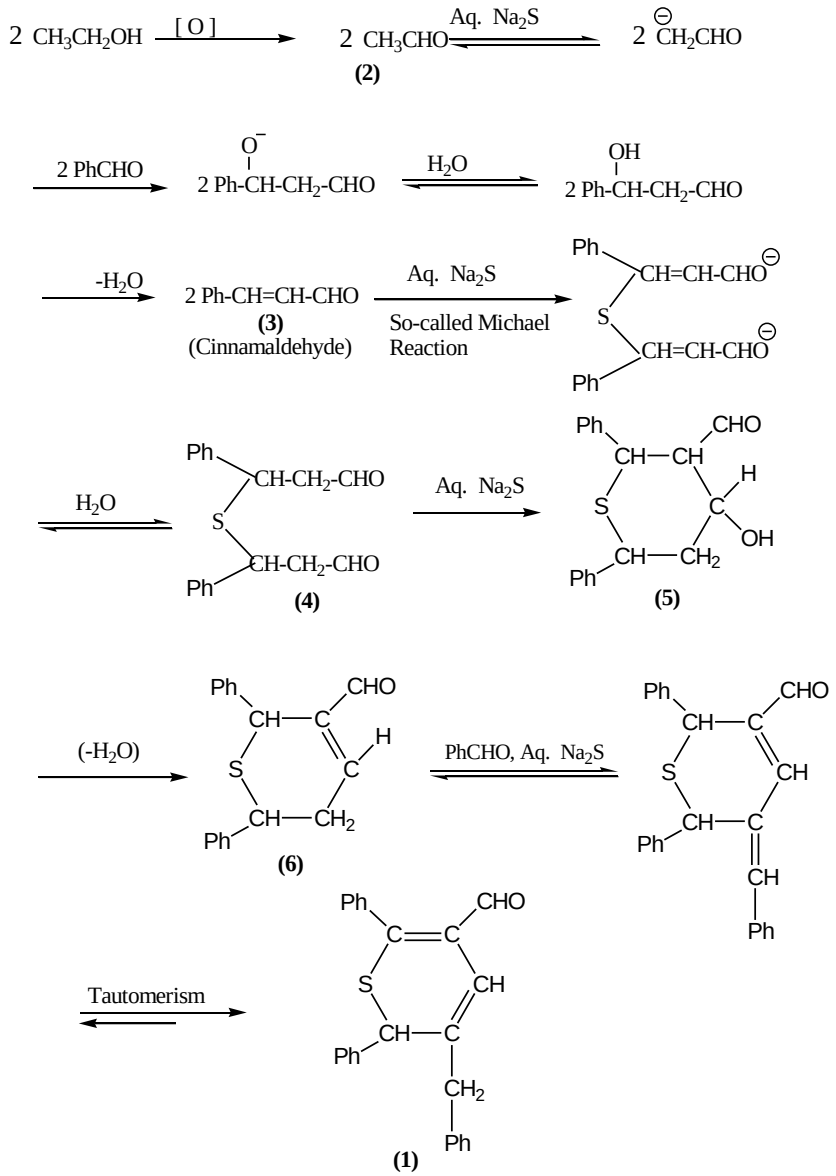
(1)

It was shown to contain a carbonyl[153] group which was reduced[153] to the corresponding alcohol. It was also shown[154] that the sulphur atom of this compound is a part of a ring, that is , the compound is a sulphur–heterocycle . The structure (1) of the compound was proposed by Cremer and Subbaratnam[104] with the help of spectroscopic studies and later was confirmed by x-ray crystallographic studies independently by Haque and Caughlan[105] as well as by Chowdhury *et al*[106]. Cremer and Subbaratnam[104] explained the formation of (1), by proposing a mechanism which when explained looked as below.(Scheme-1)

Cremer and Subbaratnam[104] did not give any experimental evidence in support of their mechanism.In order to support the mechanism proposed by Subbaratnam *et al*[104], it was necessary to repeat the reaction with some of the intermediates as proposed like ethanal and other compounds having α-hydrogen, cinnamaldehyde to see whether the compound (1) or something else are formed in presence or in the absence of ethanol.

Another important necessary investigation is to see the reaction of benzaldehyde with propanone in aqueous sodium sulphide followed by GLC for the mechanistic study to see whether the compound similar to (1) or other intermediate(s) compounds of the above scheme are formed or not.

It is imperative to see whether the compound (1) or similar to (1) are formed when substituted benzaldehydes are allowed to react with intermediates like ethanal, cinnamaldehyde, substituted cinnamaldehydes, some active α-hydrogen containing compounds like propanone, phenylethanone, etc.



Scheme -1

To investigate the generality of the above mechanism (Scheme-1) where ethanol was shown to oxidise to ethanal in the reaction condition, alcohols like 2-propanol could be used instead of ethanol so that one could end-up with compounds similar to (1). To substantiate the above proposition, propanone, the oxidised compound of 2-propanol, phenylethanone (an oxidised product of $PhCHOHCH_3$) could be used along with benzaldehyde containing sulphides in various forms.

The isolation of other relevant products will increase our understanding of how these thiopyran derivatives are formed and may supply further information on the acceptability of the mechanism.

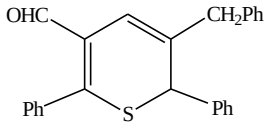
Latif reaction is interesting on two accounts. Firstly, it is a simple one-pot synthesis having a polyfunctional sulphur-heterocycle, thiopyran derivatives having high yield. Secondly, thiopyran derivatives has wide applications both in medicine such as antibacterial substances[104,126], analgesic and anti-inflamatory activities[23], analgesics and local anesthetics[30], anti-inflamatory and diuretic agents[128], anti-histaminic, anti-cholinergic, neuroleptic, tranquilising, and anti-depressive activities[52], bacteriostatic and carcinostatic activity[129] and used clinically in the treatment of human schistosomiasis[130],anti-malarial drugs[131],analgesic and anti-hypertensive activity[132], anti-anxiety, anti-depressants and tranqullisers[134], CNS-activity[135], glaucoma inhibitors[140], hypoglycemic activity[141], anti-diabetic activities[142] , anti-thrombotics[69], anti-tumor agents[78], estrogen-related diseases, particularly of the breast cancer[80] etc.

Besides, in the agricultural field such as insecticides [26, 82, 144, 150], herbicides[61], insecticides and herbicides[26], antifungal activity[76], antifungal and antibacterial *in vitro*[149]. Other importance in biological field such as juvenile hormone intermediate[151], plant protective agents and pharmaceuticals[63] etc as described in review.

It was also important to study the bioactivity of the synthesised compounds. So, the preparation of thiopyran derivatives and study of their biological activities has immense interest, thereby Chemists, Bio-chemists, Pharmacists, Microbiologists, and Genetic Engineers simultaneously may be engaged over it.

1.8 PRESENT INVESTIGATION

As has been mentioned earlier, that the Latif reaction[102,103] provides a simple one-step synthesis of polyfunctional sulphur-heterocycles such as (**1**), it was necessary to investigate the reaction thoroughly.



(**1**)

The present investigation was directed to

(a) examine how general the Latif reaction is for the synthesis of thiopyran derivatives by employing α-hydrogen containing compounds, any compounds which on oxidation converted to α-hydrogen containing compounds *in situ*, α,β-unsaturated carbonyl compounds and substituted benzaldehydes,

(b) establish the effects of substituents in the aromatic ring of benzaldehyde on the yield of thiopyrans

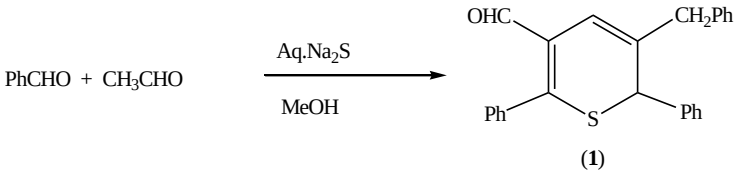
(c) test further the validity of the Cremer-Subbaratnam mechanism[104]

(d) compare the yield of product at different conditions

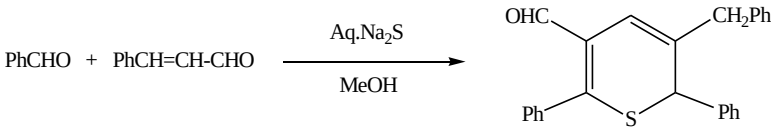
(e) test the biological activity of the synthesised compounds.

With this view in mind, we have succeeded in obtaining (1) by using ethanal with benzaldehyde and cinnamaldehyde with benzaldehyde separately in aqueous sodium sulphide. Compound (**1**) was not isolated when the Latif reaction was repeated in presence of methanol (instead of ethanol) indicating that ethanol in the Latif reaction was

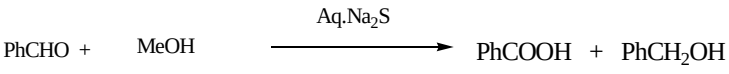
oxidised to ethanal which in turn converted to α,β-unsaturated carbonyl compound, cinnamaldehyde as mentioned by Latif and co-workers[102, 103].



(**1**)

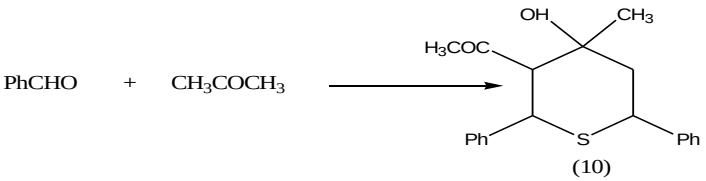


(**1**)

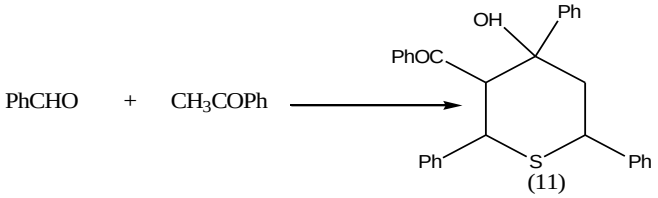


(12a) (12)

We obtained compounds (**10**) and (**11**) analogous to the intermediate product (**5**) (Scheme -1) by carrying out the reaction with propanone and phenyl ethanone ($PhCOCH_3$) instead of ethanol in the original Latif reaction that is with benzaldehyde in aqueous sodium sulphide solution.

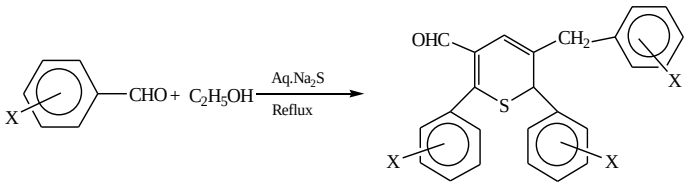


(10)

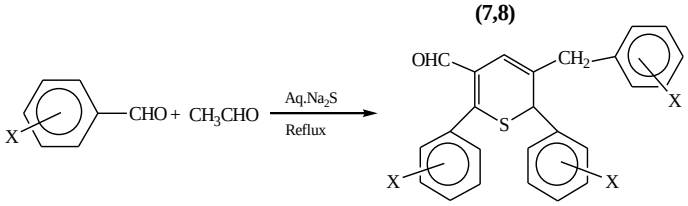


(11)

In our endeavour, we have also succeeded in obtaining (**7**) & (**8**) analogous to (**1**) by using 4-methoxy-, 2-methylbenzaldehyde respectively with ethanol and ethanal containing aqueous sodium sulphide in each case separately in reasonably good yield/s.



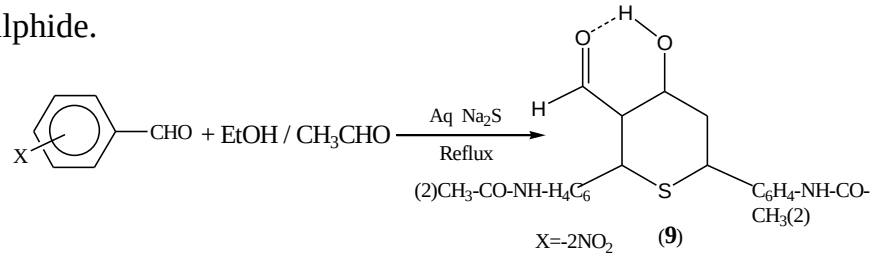
(7,8)



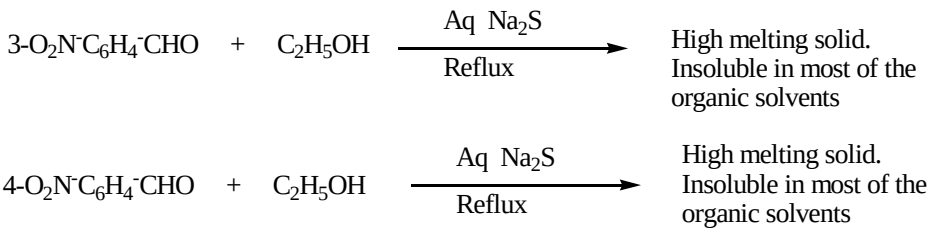
7: X = 4-OMe
8 : X =2-Me

(7,8)

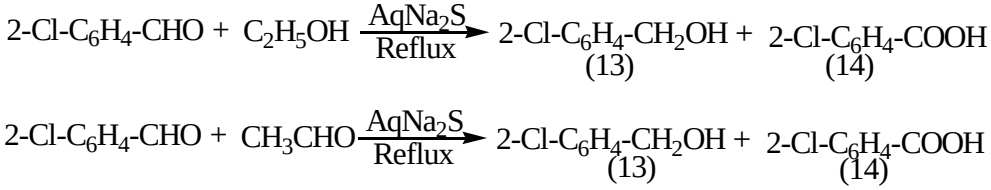
We have also succeeded in obtaining a new type of compound (**9**), not similar to (**1**), by using 2-nitrobenzaldehyde with each of ethanol and ethanal in presence of aqueous sodium sulphide.



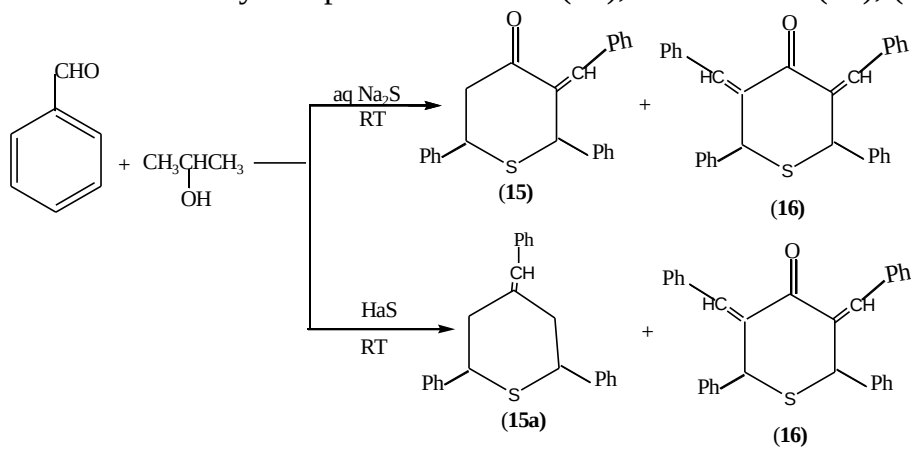
We obtained high melting (d >300^0 C) dark brown an$^{X=-2NO_2X=-2NO_2}$d reddish orange solids which form (in good quantity) very readily when 3- and 4-nitrobenzaldehydes were used with ethanol under identical conditions. Any structures to these solids could not be assigned due to their very low solubilities in most of the organic solvents.



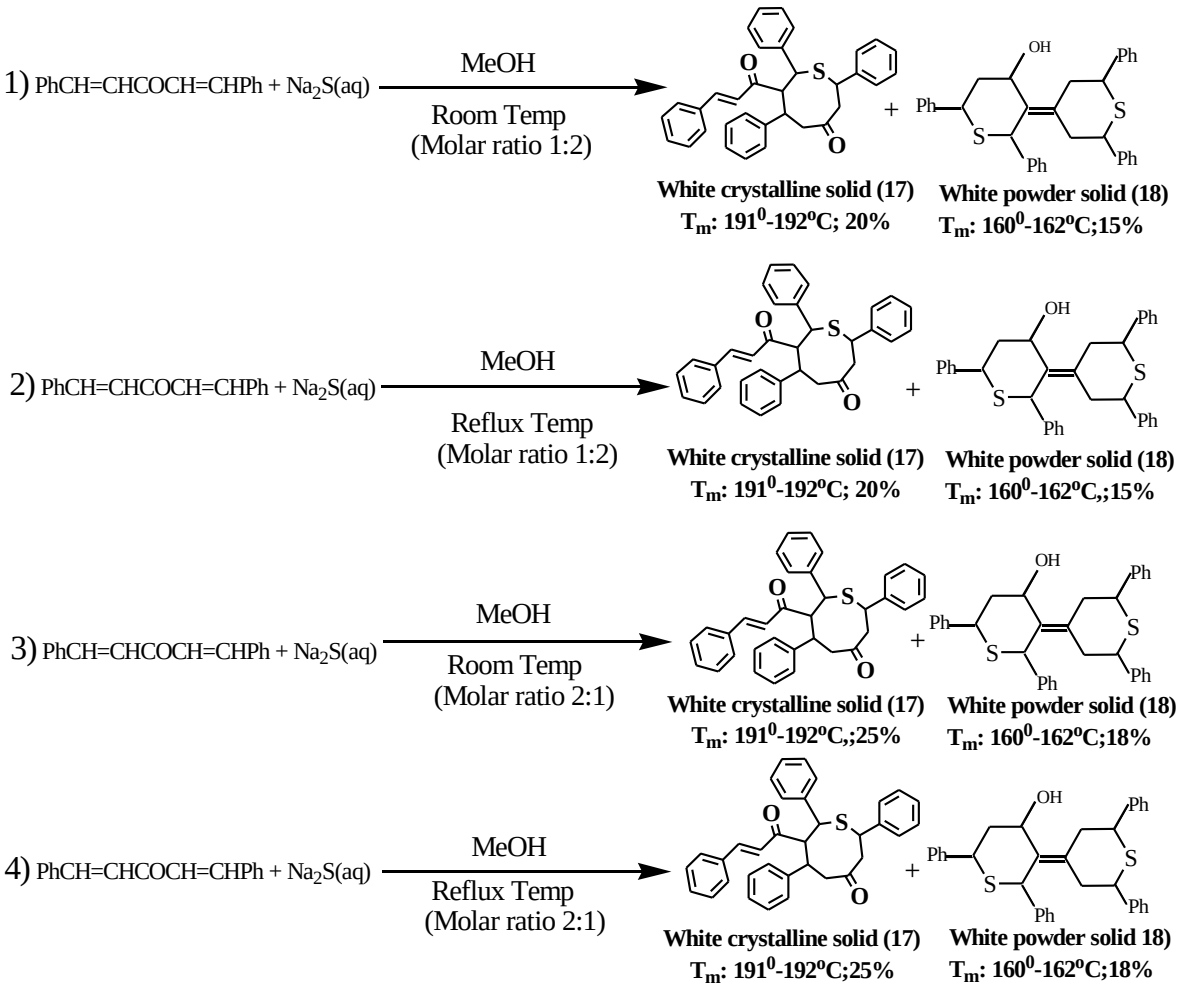
By employing 2-chlorobenzaldehyde in the Latif reaction, to our utter surprise, could not obtain any compound similar to (**1**), instead, obtained 2-chlorobenzyl alcohol (13) and 2-chlorobenzoic acid (14) – a redox product of 2-chlorobenzaldehyde.



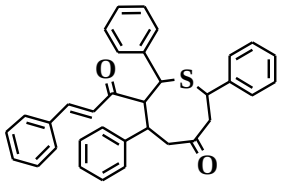
Use of 2-propanol in place of ethanol and aqueous sodium sulphide in the Latif reaction, we could not obtain any compound similar to (10), but obtained (15), (15a), and (16).



We have also succeded in obtaining a noble compound, white crystalline solid, T_m: 191^0–192^0 C (**17**) and a white powdered solid, T_m=160^0–162^0 C (**18**) not analogous to (10,15,15a or16) by using dibenzylideneacetone (PhCH=CHCOCH=CHPh) with sodium sulphide and ammonium sulphide separately in presence of methanol and/or ethanol under various reaction conditions.

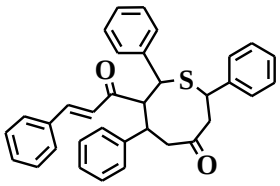


5) $PhCH=CHCOCH=CHPh + Na_2S(aq)$ —EtOH, Room Temp (Molar ratio 2:1)→



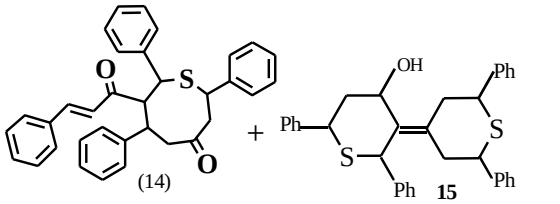
White crystalline solid (17)
T_m: 191^0-192^oC, 20%

6) $PhCH=CHCOCH=CHPh + Na_2S(aq)$ —EtOH, Reflux Temp (Molar ratio1:2)→



White crystalline solid (17)
T_m: 191^0-192^oC;20%

7) $PhCH=CHCOCH=CHPh + (NH_4)_2S(aq)$ —MeOH, Room Temp (Molar ratio1:1)→

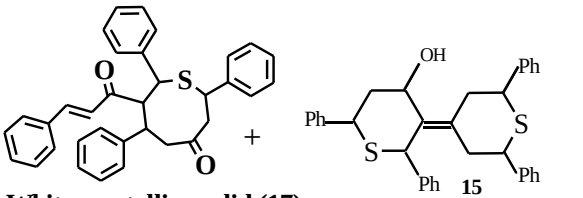


(14) + 15

White crystalline solid (17)
T_m: 191^0-192^oC; 15%

White powder solid (18)
T_m: 160^0-162^oC; 8%

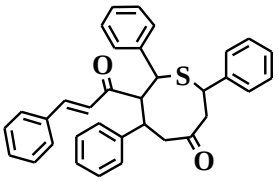
8) $PhCH=CHCOCH=CHPh + (NH_4)_2S(aq)$ —MeOH, Room Temp (Molar ratio2:1)→



White crystalline solid (17)
T_m: 191^0-192^oC;20%

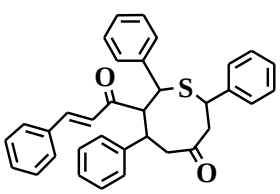
White powder solid (18)
T_m: 160^0-162^oC;12%

9) $PhCH=CHCOCH=CHPh + (NH_4)_2S(aq)$ —EtOH, Room Temp (Molar ratio1:1)→



White crystalline solid(17)
T_m: 191^0-192^oC;15%

10) $PhCH=CHCOCH=CHPh + (NH_4)_2S(aq)$ —EtOH, Room Temp (Molar ratio2:1)→



White crystalline solid(17)
T_m: 191^0-192^oC;20%

1.9 SCOPE OF THE PRESENT INVESTIGATION

It was observed from our present investigation that benzaldehyde reacts with ethanal and other active α-hydrogen containing compounds in aqueous sodium sulphide to yield Michael-type of adduct and/or cyclic sulphur–heterocycles such as thiopyran derivatives. It was also observed by GLC-study that benzaldehyde reacts with propanone in aqueous sodium sulphide formed benzylideneacetone in the reaction mixture and as the reaction progresses concentration of benzalacetone increases but at the cost of time it gradually decreases. It was also observed that benzaldehyde having electron releasing groups in the benzene ring such as (CH_3, OCH_3, etc) produces compounds similar to Latif product but benzaldehyde having electron withdrawing groups in the benzene ring such as (Cl, NO_2, etc) produces other than Latif product. In case of the reaction of chlorobenzene and ethanol in aqueous sodium sulphide, no compound analogous to Latif product forms , instead a Cannizzaro reaction product forms and for the case of nitrobenzene, polymeric type of compound forms except 2-nitrobenzaldehyde which formed substituted phenylethanamide.

So, it would be rather interesting to study the reaction of benzaldehyde and substituted benzaldehydes having electron-releasing groups such as $–NH_2$, -OH, -OR, -R, etc and electron-withdrawing groups such as $-NO_2$, -C≡N, -X, etc with α- hydrogen containing compounds like ethanal, propanone, phenylethanone, and α,β -unsaturated carbonyl compounds, 2-propanol, etc with different kinds of sulphides such as aqueous sodium sulphide, ammonoium sulphide, hydrogen sulphide, etc to isolate the compound(s) of the Scheme-1.

The Latif reaction may also be investigated to incorporate sulphur in the product by allowing the reaction to be carried out using elemental sulphur, polysulphides in place of sulphides and also all the above mentioned reactions may be carried out in the presence of UV- and microwave–assisted reactions.

The above reaction may also be investigated to replace the sulphur atom of the thiopyran ring moiety by suitable reagents.

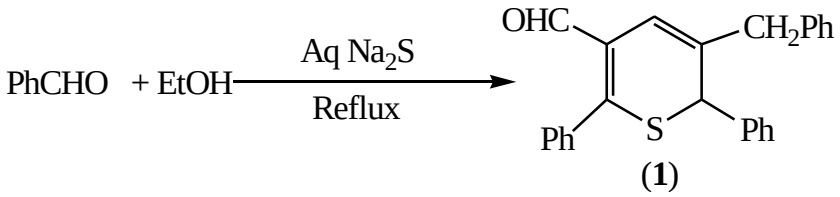
Besides, the direct replacement of the oxygen atom of pyranose ring of monosaccharide by suitable reagents like Na_2S,$(NH_4)_2S$, H_2S etc or the incorporation of nitrogen atom in the thiopyran moiety by allowing NH_3 gas, N_2-bubble in presence of a suitable solvent and/or UV/microwave-assisted reaction which will be echo-friendly and Green Chemistry also.

Chapter 2

RESULTS AND DISCUSSION

This chapter is a very important part which includes the results of the present work carried out during the preparation of this thesis.

In the aim of the present investigation it was mentioned earlier that this work was directed towards the investigation of the mechanism of formation of 3-benzyl-2,6-diphenyl-2H-thiopyran-5-carboxaldehyde (**1**) from te reaction of benzaldehyde with ethanol in aqueous sodium sulphide. The compound was originally prepared by Latif and co-workers[102, 103] and may be called the Latif product.



(**1**)

The compound (**1**) is elegant on two accounts -- (i) it is a multifunctional and complicated sulphur heterocycle which has been prepared[102, 103] in a straight forward simple one-pot preparative method, (ii) the reported yield is as high as 60% which appears to be exceptionally good considering the complexity of the method and the simplicity of its preparation. Moreover, the compound (**1**) is a sulphur heterocycle and it is a well-known fact that such compounds are medicinally and industrially exceedingly important.

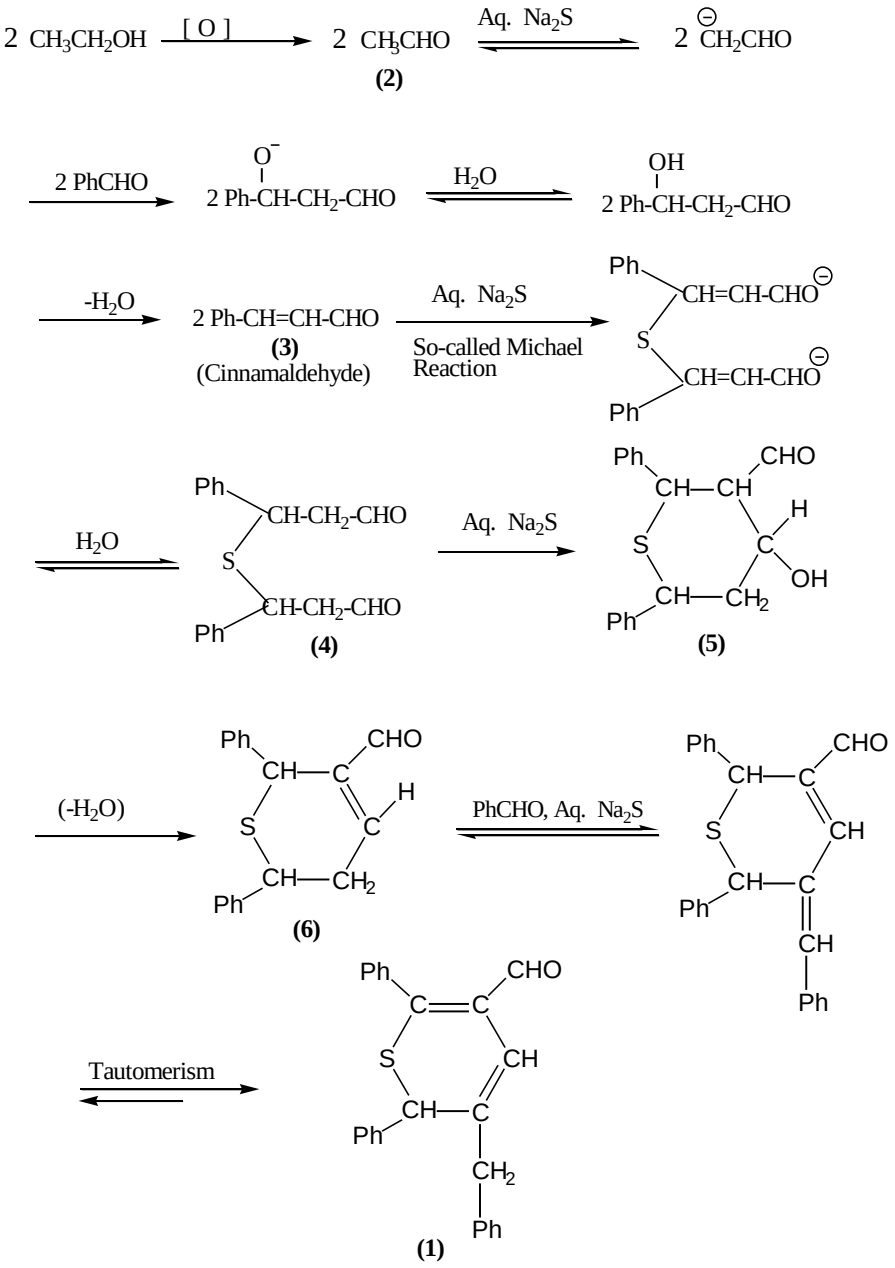
Latif and others[**102**-104] showed that presence of a carbonyl group in (**1**) which has been converted to corresponding alcoholic group to form a nice crystalline acetate derivative. They have also showed that sulphur atom of the molecule (**1**)

is a part of a ring. Cremer & Subbaratnam[104] were the first to give a complete structural formation of (**1**) from spectroscopic studies. Cremer-Subbaratnam's structure (1) was later confirmed by X-ray crystallographic studies independently by Haque and Caughlan[105] as well as Chowdhury *et al* [106]. Cremer and Subbaratnam[104] proposed a mechanism in which ethanol was oxidised to ethanal (**2**), an active α-hydrogen-containing carbonyl compound which later undergoes a cross-Aldol condensation with benzaldehyde (another starting material) in a highly alkaline reaction medium (pH $\simeq$ 13), generated by aqueous sodium sulphide to cinnamaldehyde(**3**)(**Scheme1**). None of these authors however could isolate ethanal or cinnamaldehyde–the two proposed intermediates. Cinnamaldehyde then undergoes a Michael addition-type reaction with sulphide ions and followed by a ring closure to give a thiopyran moiety– a sulphur heterocycle. This saturated thiopyran compound later condensed with another benzaldehyde molecule followed by tautomerism to yield the desired product (**1**). None of the earlier authors did provide any evidence in favour of the proposed mechanism by isolating the intermediates- ethanal and cinnamaldehyde.

It seems evident from the earlier discussion that ethanal (**2**), is an essential ingredient involved in the mechanism as proposed by Cremer and Subbaratnam[104] for the formation of (1). Similarly, cinnamaldehyde (**3**) is also an equally important intermediate compound for the formation of (**1**). Cremer and Subbaratnam[104] did not provide any experimental evidence in favour of this proposed mechanism.

It is therefore imperative and thought interesting to investigate the Cremer--Subbaratanam mechanism(Scheme-1) as thorough as possible for a fresh and strong evidence of the mechanism of and also for the generalisation of the Latif reaction as well as the biological activity of the synthesised compounds.

In this context, we carried out the reaction of (i) benzaldehyde with ethanal and (ii) benzaldehyde with cinnamaldehyde (in methanol) in aqueous sodium



Scheme 1

Sulphide at reflux condition separately. We also carried out the reaction of benzaldehyde with some active α-hydrogen containing compounds like propanone (acetone), phenylethanone (acetophenone) separately with ethanol in aqueous sodium sulphide at reflux condition. Consequently, we carried out the reaction of α,β-unsaturated carbonyl compounds like benzylidene acetone ($PhCH=CHCOCH_3$) and benzylidene acetophenone, PhCH=CHCOPh separately with benzaldehyde in presence of aqueous sodium sulphide in methanol at room temperature and at reflux condition.

We also carried out the reaction of some substituted benzaldehydes like 2-methyl, 4-methyl, 4-methoxy, 4-chloro, 4-N,N-dimethylamino, 2-nitro, 3-nitro and 4-nitrobenzaldehydes with ethanol in aqueous sodium sulphide.

In our endeavour, we succeeded in synthesing (**1**) by reacting benzaldehyde with ethanal (2) and benzaldehyde with cinnamaldehyde (3) in methanol containing aqueous sodium sulphide separately under reflux condition in the absence of ethanol. However, we failed to obtain (**1**) during the reaction of benzaldehyde and sodium sulphide in aqueous medium in presence of methanol (no ethanol was used as it was thought to be oxidised to ethanal) rightly suggests that ethanal is a part and parcel for the formation of (1).

In Cremer-Subbratnam mechanism[104], it was claimed that ethanal has been derived from the aerial oxidation of ethanol. However, we failed to trace the presence of ethanal when alone ethanol was refluxed with water for hours together in presence of aqueous sodium sulphide. The question then arises – how ethanal, an essential ingredients for (**1**) appears in the reaction mixture?

Now it is a matter of argument that if the Cremer–Subbaratnam mechanism[104] is correct, then compound (**1**) can be prepared in the absence of ethanol by employing (i) the reaction of benzaldehyde with ethanal and (ii) the reaction of benzaldehyde with cinnamaldehyde in presence of aqueous sodium sulphide or ammonium sulphide or hydrogen sulphide separately since these two compounds were proposed to be intermediates in the reaction and have been envisaged to have derived from ethanol. It may be noted that a GLC-study was monitored for the reaction of benzaldehyde with ethanol in presence of aqueous sodium sulphide at elevated temperature of each at certain time interval. It was observed that as the reaction progressed, at the starting no cinnamaldehyde peak (from the retention time) in glc was detected, gradually a peak of cinnamaldehyde appeared and finally another new peak appeared by diminishing the previous cinnamaldehyde peak also rightly suggests that cinnamaldehyde is also an intermediate for the formation of (1).

Our present studies is also directed for the further generalisation of Latif reaction. In this aspect, we took the project of the reaction of benzaldehyde with active α-hydrogen containing compounds like propanone, phenylethanone (acetophenone) and also α,β-unsaturated carbonyl compound namely benzylideneacetone (benzalacetone),and benzylidene acetophenone in methanol with aqueous sodium sulphide both at room temperature and at reflux temperature. Later, we carried out the reaction of benzaldehyde and 2-propanol (*iso*-propanol) in presence of aqueous sodium sulphide, ammonium sulphide, and hydrogen sulphide, etc.

Finally in a separate study, the reaction of benzaldehyde with dibenzylidene acetone (DBA) in methanol containing aqueous sodium sulphide or ammonium sulphide or hydrogen sulphide gas was carried out in different molar ratio.

Preparation of (1) from benzaldehyde and ethanal (2), and /or cinnamaldehyde (3)

In order to verify the hypothesis that ethanal is formed by the *in situ* oxidation of ethanol, benzaldehyde was chosen to react with ethanal containing aqueous sodium sulphide in methanol (no ethanol was employed) to obtain compound (1). In a similar manner, cinnamaldehyde (belived to be formed from benzaldehyde and ethanal) was allowed to react with benzaldehyde under identical condition as above to obtain (1) along with two other side products which were later identified as cinnamic acid and benzyl alcohol, might have formed by the Cannizzaro reaction in highly alkaline condition (aq Na_2S, pH ≈ 13).

Latif type reaction was also carried out with ethanal and benzaldehyde in aqueous methanolic sodium sulphide at refluxing condition for a period of two hours. In this case, we also obtained Latif product (1) in somewhat lesser yield (10%) along with cinnamaldehyde in a substantial amount (50%).

However, no cinnamaldehyde was obtained when the same reaction was carried out for a longer period (six hours).

Formation of Latif product (**1**) from benzaldehyde and ethanal, and from benzaldehyde and cinnamaldehyde in presence of aqueous sodium sulphide in methanol (without ethanol) indicates that both ethanal (probably the oxidised product of ethanol) and cinnamaldehyde are the two real intermediates for the formation of Latif product as shown in the scheme-1.

Identification of compound (1) obtained from the reaction of benzaldehyde and ethanal in aq sodium sulphide

i) The uv spectrum (**Fig** , page) of the yellow compound (1) obtained from the reaction of benzaldehyde with ethanal (**2**) in aqueous sodium sulphide showed the following peaks:

λ_{max}^{EtOH} (in nm) 363.0: $n \rightarrow \pi^*$ Transition of C=O

264.0: $\pi \rightarrow \pi^*$ Transition of C=C in conjugation with C=O

ii) The ir spectrum (**Fig** , page) exhibited the following assignable bands at ν_{max} /cm^{-1}:

ν_{max}^{KBr} 3350, 3050: C–H stretching of aromatic and olefinic system

2900: C–H stretching of aliphatic systems

1635: C=O stretching which in conjugation with phenyl group

1590, 1530: C=C stretching of aromatic ring

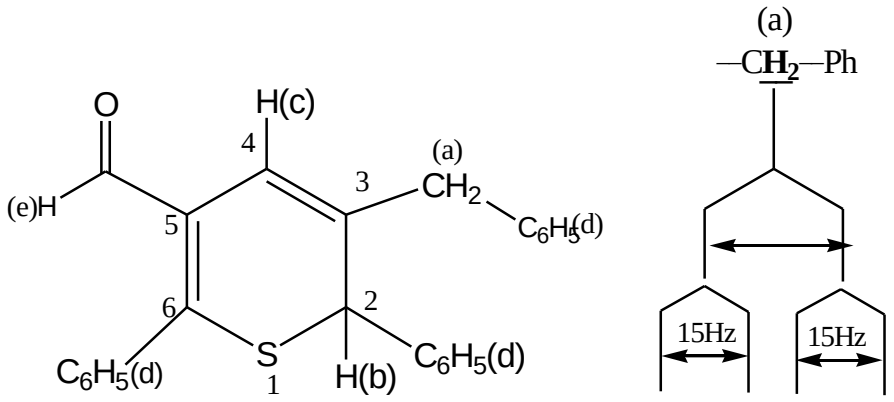
147: C–C stretching.

1125: C–O stretching

1475, 1350, 1210, 1160, 1085: C–H bending of alkyl group

910, 880, 835, 780, 760, 690: C–H bending of aromatic ring.

iii) The ^{1}H-nmr spectrum (**Fig** , page) of the compound exhibited the following signals: (chemical shift in δ/ppm) and were assigned as following:



(a) 3.3523 – 3.7300 (2H, dd, J = 15.12 Hz) : Two methylene proton of benzyl group, –C<u>H</u>$_2$–Ph at C-3

The expected methylene signal perhaps coupled with the methine proton (b) at C-2 and the olefinic proton (c) at C-4 to give a doublet which subsequently splitted to a doublet of a doublet (*dd*) by either of the above proton.

(b) 4.5266 (1H, *s*): Methine proton at C-2; adjacent to Ph group and ring S(-S-C<u>H</u>-Ph)

(c) 7.0000 (1H, s): Olefinic proton at C-4 (-C<u>H</u>=C-CH$_2$Ph)

(d) 7.2498 -7.4340
(15H, *m*; *J* = not resolved): Fifteen aromatic protons of two phenyl group at C-2,C-6 and one phenyl of benzyl group at C-3

(e) 9.2511(1H, s): One proton of –C<u>H</u>O group at C-5.

iv) The ^{13}C-nmr spectrum (**Fig** , page) of the compound exhibited following signals (chemical shift in δ/ ppm) which were assigned as follows:

a) 42.567 and 45.989: Aliphatic carbon of benzyl group, –<u>C</u>H$_2$Ph at C-3

b) 119.940 : Tertiary carbon (-<u>C</u>H-Ph) of heterocyclic ring at C-2

c) 126.810, 127.317, 128.167, 128.273, 128.680, 128.973, 129.140, 130.455, 131.177, 131.792, 133.803, 138.289, 140.861, and 154.856 : Four olefinic carbon (C-3, C-4, C-5 & C-6) of heterocyclic ring and six aromatic carbons of two Ph groups at C-2 , C-6 and one Ph of benzyl group at C-3

d) 186.755: Carbonyl carbon of –**C**HO at C-5.

The above spectral data is completely in agreement with that reported[104] earlier for the formulated structure (1) advocating that ethanal (**2**) as an intermediate in the formation of 3-benzyl-2,6-diphenyl-2H-thiopyran-5-carboxaldehyde (**1**) as proposed by Cremer & Subbaratnam**[104]**.

Identification of compound (1) obtained from the reaction of benzaldehyde and cinnamaldehyde in aq sodium sulphide

i) The uv- spectrum (**Fig** , page) of the needle- shaped yellow crystalline solid obtained from the reaction of benzaldehyde with cinnamaldehyde (**3**) in aqueous sodium sulphide had the following peaks:

λ_{max}^{EtOH}(in nm) 368.0 : $n \rightarrow \pi^*$ Transition of C=O

264.0 : $\pi \rightarrow \pi^*$ Transition of C=C in conjugation With C=O

ii) The ir- spectrum (**Fig** , page) exhibited the following assignable bands at ν_{max}/cm^{-1}:

ν_{max}^{KBr} 3350, 3050: C–H stretching of aromatic and olefinic system

2900: C–H stretching of aliphatic systems

1635: C=O stretching which in conjugation with phenyl group

1590, 1530: C=C stretching of aromatic ring

1475: C–C stretching

1125: C–O stretching

1475, 1350, 1210: C–H bending of alkyl group

1160, 1085, 910, 880,
835, 780, 760, 690: C–H bending of aromatic ring.

iii) The ^{1}H-nmr spectrum (**Fig** , page) exhibited the following proton signals (chemical shift in δ/ppm) assigned as follows:

:

(a) 3.3513 – 3.7280
(2H, *dd*, J = 15 Hz): The doublet of doublet (*dd*) of methylene of C-3 benzyl group, -CH_2Ph might have splitted due to C-2 & C-4 protons as described earlier

The expected methylene singlet was perhaps coupled any one of them with methane hydrogen atom (b) at C-2 and olefinic proton (c) at C-4 to give a doublet which subsequently splitted to a doublet of a doublet (*dd*) by either of the above proton.

(b) 4.5269 (1H, *s*): Methine proton at C-2; adjacent to Ph group and ring S (-S-C<u>H</u>-Ph)

(c) 7.0031(1H, *s*): Olefinic proton at C-4 (-C<u>H</u>=C-CH_2Ph)

(d) 7.2495 – 7.42 93
(15H, *m*; *J* = not resolved): Fifteen aromatic protons of two phenyl group at C- 2 & C-6, one phenyl of benzyl group at C-3.

(e) 9.4142(1H, *s*) : One proton of –C<u>H</u>O at C-5

Individual melting temperature of this compound and earlier compound prepared by ethanal were found unchanged. Identity of this compound with the previous compound again justifies cinnamaldehyde as another one intermediate

as described by Cremer and Subbaratanam[104] in the formation of 3-benzyl-2,6--diphenyl-2H-thiopyran-5- carboxaldehyde (**1**).

As we have obtained compound (**1**), by reacting ethanal and cinnamaldehyde in methanol separately with benzaldehyde and aqueous sodium sulphide medium, it may be said that Latif reaction is a generalised one as proposed by Cremer and Subbaratnam[**104**].

Reactions of substituted benzaldehydes with ethanol and/or ethanal in aqueous sodium sulphide

In support of our above hypothesis we carried out the reactions of substituted benzaldehydes such as 4-methoxy, 2-methyl, 2-nitro, and 2-chlorobenzaldehydes both with ethanol and ethanal but in case of 3-nitro- and 4-nitrobenzaldehyde only with ethanol under identical conditions.

The reaction of 4-methoxybenzaldehyde and sodium sulphide with each of aqueous ethanol and aqueous ethanal (no ethanol was employed) resulted the same compound (**9**) (T_m: 170^0 -172^0 C) in both cases in 3 % and 2 % yield respectively. Identity of the two products was evident from their mixed melting temperature and superimposable ir spectra.

i) The uv spectrum (**Fig** , page) of the compound gave the following peaks:

λ_{max}^{EtOH}(in nm) 331.0 : $n \rightarrow \pi^*$ Transition of C=O

274.0 and 225 : $\pi \rightarrow \pi^*$ Transition of C=C in conjugation with C=O

ii) The ir spectrum (**Fig** , page) showed the following assignable bands at ν_{max} /cm^{-1}:

3050: C–H stretching of aromatic and olefinic systems.

2900: C–H stretching of aliphatic systems.

1760, 1680: C=O stretching which in conjugation with phenyl group

1640, 1620, 1560: C=C stretching of aromatic ring.

1500, 1440: C–C stretching.

1110: C–O stretching.

1425,1380,1320,1290,
1250, 1180, 1030, 980: C–H bending of alkyl group.

900, 820, 810, 760 : C–H bending of aromatic ring.

iii) The ^{1}H-nmr spectrum (Fig , page) exhibited the following signals (chemical shift in δ/ppm):

(a) 3.5690 –3.7225
(2H, *J* = not resolved): Two methylene protons of $^{-}\underline{C}H_2{}^{-}C_6H_4{}^{-}$

(b) 3.7410 and 3.7510 (9H, *s*): Methoxy protons of three –OC$\underline{H}_3$

(c) 4.5145 – 4.5220 (1H, *s*) : C-2 one methine proton

(d) 6.7476 – 6.8264 (1H, *m*) : Olefinic proton of C-4

(e) 6.8857 – 7.7255 (12H, *m*) : Aromatic protons of $^{-}C_6\underline{H}_4{}^{-}$ at C_2, C_3 and C_6

(f) 9.7453 – 9.7556 (1H, *s*) : One proton of –C$\underline{H}$O at C-5

iv) The ^{13}C-nmr spectrum (**Fig** , page) of the compound exhibited the following few lines as assigned (chemical shift in δ/ppm):

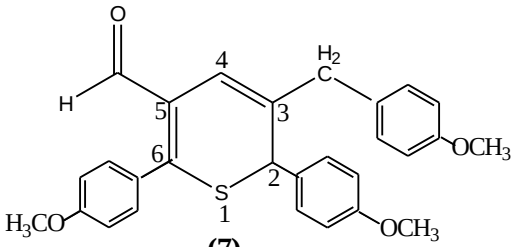
(a) 55.257, 55.423 : Methoxy carbon (–O**C**H_3)

(b) 114.049, 114.441, 123.500,
130.075, 142.676, 161.551 : Aromatic and heterocyclic ring carbon

(c) 188.860 : Carbonyl carbon of –CHO

However, the ^{13}C-nmr of the compound did not exhibit any signal for the secondary carbon of –$\underline{C}H_2$– Ar may be due to the low solubility of the compound in $CDCl_3$. We also could not obtain a fine ^{1}H-nmr spectrum for the compound may be due to the same reason.

On the basis of the above analysis, the following structure (**7**) was assigned for the 4- methoxybenzaldehyde product.



(7)

The reaction of 2-methylbenzaldehyde with each of ethanol and ethanal (in methanol) containing sodium sulphide in water produced the same yellow solid (T_m: 134^0 -138^0 C) in both cases.

i) The uv spectrum (**Fig** , page) of the compound gave the following . peaks

λ_{max}^{EtOH} (in nm) 345.0 : $n \rightarrow \pi^*$ Transition of C=O

284.0 and 233.0 : $\pi \rightarrow \pi^*$ Transition of C=C in conjugation with C=O

ii) The ir spectrum (**Fig** , page) showed the following assignable bands at ν_{max} /cm^{-1}:

3010: C–H stretching of aromatic and olefinic systems.

2900, 2850: C–H stretching of aliphatic systems.

1790, 1680: C=O stretching which in conjugation with phenyl group.

1640, 1590, 1580: C=C stretching of aromatic ring.

1475, 145: C–C stretching.

1110: C–O stretching.

1375, 1275, 1210,
1155, 1030, 1000: C–H bending of alkyl group.

940, 860, 830, 740: C–H bending of aromatic ring.

iii) The ^{1}H-nmr spectrum (**Fig** , page) of the compound exhibited the following signals (chemical shift in δ/ppm) some of them were assigned as follows:

(a) 10.2785 and 9.1620 (1H , *s*) : One proton of the C-5 aldehydic proton.

(b) 7.1473-7.2842 (12H, *m*): Aromatic protons, two each of C-2 and C-6 aryl group, Me-$C_6\underline{H}_4$- and C-3 Me-$C_6\underline{H}_4$-CH_2-

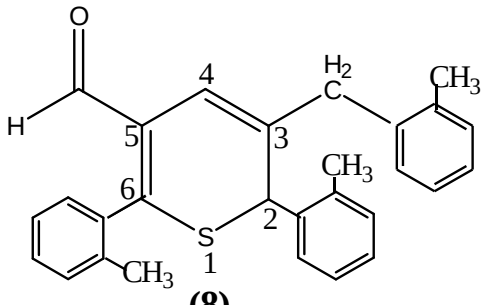
(c) 6.9180 (1H, *s*): C-4 olefinic proton.

(d) 4.7030-4.7358
(1H, *d*; *J* = 13.0 Hz): C-2 methine proton, -S-C<u>H</u>-Ar

(e) 3.594-3.665 and 3.405-3.441 } (2H, *t*, *J* = 13 -16 Hz) : C-3 benzylic protons, - $C\underline{H}_2$-C_6H_4-Me

(f) 2.2859, 2.2909, 2.3053 (9H, *s*) : Three singlets each integrating for there methyl protons of C-2 and C-6, -C_6H_4-$C\underline{H}_3$ and C-3 –CH_2-C_6H_4-$C\underline{H}_3$

The above spectrum contained some signals in addition to the above described. The additional signals which could not be assigned might have appeared from impurities such as unreacted 2-methyl benzyldehyde. It amounts that the compound was not pure but contaminated with some unreacted reactants. However, from the assigned signals, one of the compounds in the above mixture may be an analogue of Latif product (**1**).



(8)

The reaction of 2-nitrobenzaldehyde with ethanol in aqueous sodium sulphide resulted a pale yellow solid [T_m: > 300 ^oC (d)].

i) The uv spectrum (**Fig** , page) of the compound gave the following peaks (in nm):

368.0 : $n \rightarrow \pi^*$ Transition of C=O

286.0 and 236.0: $\pi \rightarrow \pi^*$ Transition of C=C in conjugation

ii) The ir spectrum (**Fig** , page) of the compound showed the following bands at ν_{max} /cm^{-1}:

3250 (*br*)	: O–H stretching and the N-H stretching might have merged with O-H bond.
3050	: C–H stretching of aromatic and olefinic Syste
2905	: C–H stretching of aliphatic systems
1720, 1660	: C=O stretching
1600	: C=C stretching of aromatic ring.
1470	: C–C stretching.
1110	: C–O bending
1440, 1360, 1300, 1240, 1220, 1150, 1080, 1050:	C–H bending of alkyl group
990, 970,880, 860, 840, 790, 780, 745:	C–H bending of aromatic ring

iii) The ^{1}H-nmr spectrum (**Fig** , page) of the compound exhibited the following proton signals (chemical shift in δ/ppm) which were assigned a :

(a) 4.555 (6H, *s*) : 6 protons of two N- acyl group of –NHCOC<u>H$_3$</u>

(b) 5.330 (1H, *s*)

(c) 5.522 – 5.567 (2H, *d*)

(d) 6.667 – 6.687 (1H, *d*)

(e) 6.731 – 6.768 (1H, *t*)

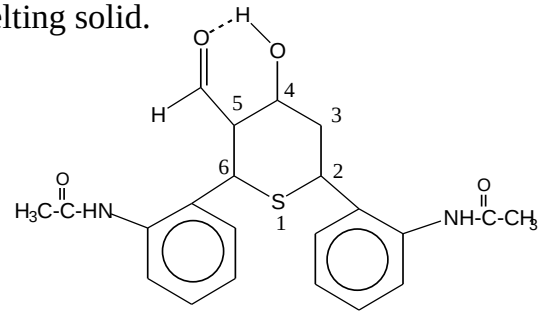
(f) 6.929 – 6.947 (1H, *t*)

(g) 7.104 – 7.122 (1H, *d*)

All these signals are for six heterocyclic ring protons and one $^{-}$OH proton at C- 4

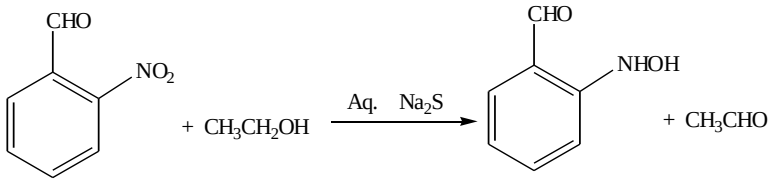
The C- 4 hydroxy proton might have resonated so downfield (in aromatic region) due to its hydrogen bonding with the C- 5 aldehydic oxygen. Signal (s) for –NH proton might have merged with the broad hydroxy signal of solvent methanol at δ ppm 4.836. However, we did not get any signal for the aldehydic hydrogen.

On the basis of the above analysis, the following structure (**9**) was proposed for the above high melting solid.

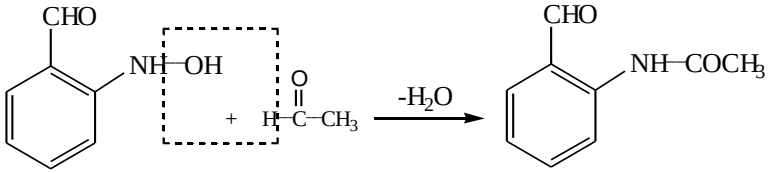


(9)

We have already proposed earlier that ethanol, may not be all, has been oxidised by hydride shift to ethanal and benzaldehyde has been reduced to the corresponding benzyl alcohol by Cannizzaro type reaction. In this case, ethanol and 2-nitrobenzaldehyde have been disproportionated to ethanal and 2-hydroxyaminobenzaldehyde.



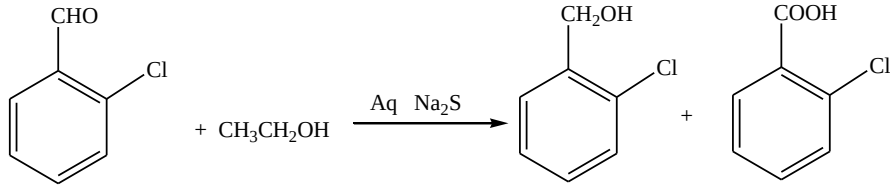
Then the 2-hydroxyaminobenzaldehyde might have condensed with acetaldehyde as follows to produce the N-acetylatedaminobenzaldehyde.



Now, the corresponding 2-N-acetylaminobenzaldehyde reacts with ethanol or acetaldehyde in aqueous sodium sulphide in a manner to that of benzaldehyde and ethanol to produce the above compound (**9**) similar to (**5**) proposed in the mechanism given by Cremer and Subbaratnam[104].

We have also carried out the reaction of 3- and 4-nitrobenzaldehyde with ethanol in aqueous sodium sulphide under identical conditions to that of Latif reaction and obtained a blackish brown and a reddish orange high melting solids respectively. Any structure could not be proposed to these solids due to their very low solubilities in most of the organic solvents such as ethanol, methanol, TFA, DMSO, etc. It may be mentioned here that these solids resulted readily and in a very high yield.

We have also carried out the reaction of 2-chlorobenzaldehyde with each of ethanol and ethanal in presence of aqueous sodium sulphide isolated neither Latif-type of product nor any of the product(s) analogues to the intermediate(s) in the proposed mechanism. Rather, we isolated the 2-chlorobenzyl alcohol,(13) T_m: 68^0-70^0 C (lit[152], 68^0-71^0C) and 2-chlorobenzoic acid,(14)T_m:138^0-140^0 C (lit[152], 139^0-142^0 C) oxidation-reduction products of 2-chlorobenzaldehyde probably by simple Cannizzaro reaction.



(13) (14)

i) The ir spectrum (**Fig , page**) of the compound, showed the following bands at ν^{KBr}_{max} /cm^{-1}:

3300-3200	: O–H stretching KBr
2900	: C–H stretching of aliphatic systems
1640, 1560	: C=C stretching of aromatic ring.
1460, 1430	: C–C stretching.
1115	: C–O stretching.
1360, 1050, 1025	: C–H bending of alkyl group.
980,940, 860,800, 740	: C–H bending of aromatic ring.

ii) The ^{1}H-nmr spectrum (**Fig** , page) of the alcohol exhibited the following proton signals (chemical shift in δ/ppm) :

(a) 1.707 (1H, *bs*) : One proton of the alcoholic –O<u>H</u>

(b) 3.786 (2H, *s*) : Methylene protons of –C<u>H</u>$_2$OH

(c) 7.222 – 7.367 (4H, *m*) : Aromatic protons.

Above spectral analysis advocates the solid to be 2-chlorobenzyl alcohol.

The another solid obtained from the aqueous part of the above reaction after acid treatment to be 2-chlorobenzoic acid from its melting temperature, 138^0-140^0 C (lit[152], 139^0-142^0 C).

i) The ir spectrum (**Fig , page**) of the compound showed the following bands at ν_{max} /cm^{-1}:

3600, 3400 (*br*)	: O–H stretching of –(CO)O-H group
3050	: C–H stretching of aromatic systems
2950	: C–H stretching of aliphatic systems
1680	: C=O stretching of –(C=O)-OH
1580	: C=C stretching of aromatic ring
1430	: C–C stretching
1040	: C–O stretching
1400, 1300, 1250, 1040	: C–H bending of alkyl group
950, 910, 810, 790,740	: C–H bending of aromatic ring.

Formation of compound (**1**) starting with benzaldehyde, ethanol (**2**) and cinnamaldehyde (**3**) does not necessarily justify that ethanal and cinnamaldehyde are the two real intermediates in the Latif reaction. Our next approach was to find out if ethanal and cinnamaldehyde are formed in the reaction medium during the preparation of (**1**) by Latif method[102, 103]. We repeated the Latif reaction under the usual conditions and followed it by glc. We found that at the start of the reaction, no cinnamaldehyde was present in the medium, but as the reaction progressed, cinnamaldehyde was formed; its concentration increased gradually to a steady-state and then dropped. These results, when considering along with the fact that (**1**) can be prepared either from ethanal or cinnamaldehyde even in the absence of ethanol, may be taken to indicate that ethanal and cinnamaldehyde are real intermediates in the Latif reaction and ethanol is converted to ethanal during the reaction as was proposed by Cremer and Subbaratnam[104].

We could, to some extent, conclude above by glc studies that ethanal (**2**) and cinnamaldehyde (**3**) are intermediates in the formation of (**1**). Our second strategy if compounds such as (**4**) and (**5**), the other two proposed intermediates are also formed in the Latif reaction. For that, we thought to follow at somewhat milder conditions for the preparation of (**1**) ; accordingly reacted benzaldehyde with sodium sulphide in aqueous ethanol at 60^0–70^0 C, instead of reflux (not at the optimum yield temperature) for two hours, and the mixture was worked out to isolate (**1**) in somewhat decreased yield (38%), elemental sulphur (6%), benzoic acid (14%), benzyl alcohol (24%) and a brown semi-solid mass (1% yield) separated by preparative tlc. Based on its ir and ^{1}H nmr data, structure (**5**) as assigned to the last product. Any product corresponding to (**4**) could not be isolated. The proposed open-chain intermediate (**4**), a Michael addition-type product of sulphur with cinnamaldehyde (**3**), could be obtained perhaps at milder condition than 60^0–70^0C. Intermediate cyclic product (**5**) did not eliminate water (could have eliminated water at refluxing condition) on its way to form (**1**). Moreover, intermediate (**5**) is extra-stabilised by the formation of a six membered ring through H-bonding.

Our current understanding does not permit us to fully explain how ethanol is oxidised in the reaction medium. One possibility is that ethanol is oxidised by aerial oxygen during the reaction which is conducted in vessels open to the atmosphere. However, we have failed to detect the presence of ethanal in the Latif reaction. Neither could we detect its presence in the reaction mixture obtained when ethanol and benzaldehyde were refluxed in water in open atmosphere for two hours. On the other hand, we were able to prepare (**1**), though in a reduced yield (20%), by refluxing degassed solutions of ethanolic benzaldehyde and aqueous sodium sulphide under oxygen-free nitrogen atmosphere. These experiments indicate that ethanal obtained in the Latif reaction is probably not all formed by the aerial oxidation of ethanol and when it is formed, is immediately consumed in the strong alkaline[104] reaction condition (pH $\simeq$ 14) and thus escapes detection. We feel that a good part of ethanol is oxidised in the strongly basic reaction medium to ethanal by a reaction in which a hydride ion, is transferred from ethanol to benzaldehyde reducing the latter to benzyl alcohol. When benzaldehyde is refluxed with sodium sulphide in water, it undergoes a Cannizzaro reaction, among others to produce benzoic acid and benzyl alcohol. During Latif reaction, at least, a substantial percentage of benzyl alcohol and benzoic acid is always formed. It is possible that this excess benzyl alcohol is formed by the hydride transfer mechanism.

In conclusion, we can say that this work has provided strong experimental support for the mechanism proposed by Cremer and Subbaratnam[104]. It also indicates that not all of the ethanol is oxidised by air alone to ethanal, but some of it is probably oxidised in the system by a hydride-ion transfer mechanism reaction from ethanol to benzaldehyde under the basic conditions. This work also shows that the intermediates such as (**5**), a sulphur heterocyclic in the Latif reaction,can be isolated by using milder reaction conditions, such as at a temperature of 60^0–70^0 C. It was also observed that nitro- and chlorobenzaldehydes behave differently with ethanol and aqueous sodium sulphide under identical conditions which at the moment, could not be explained.

Reaction of benzaldehyde with propanone in presence of aqueous sodium sulphide at room temperature

In order to test the hypothesis given by Cremer and Subbaratnam[104] that ethanol is converted to active α-hydrogen atom containing compound, ethanal, we carried out the reaction of benzaldehyde (0.1 mol) with propanone (1.36 mol) in presence of sodium sulphide (0.3 mol) in water at room temperature. The mixture was stirred for six hours when a white solid (**10**) precipitated out. The solid was recrystalised from propanone–pet ether mixture had the melting temperature 183^0-185^0 C (yield 30%).

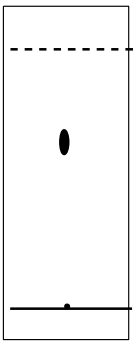
Characterisation of the product obtained from the reaction of benzaldehyde and propanone in presence of aqueous sodium sulphide

In the above reaction when a mixture of benzaldehyde and propanone was stirred in water containing sodium sulphide at room temperature, the clear solution started becoming turbid and white precipitates separated out immediately. The stirring was continued for four-hours when no further precipitate formed. The precipitated product was separated by filtration, the remaining filtrate was extracted with ether, dried and evaporated to result the same white compound. The combined white compound,(**10**) (yield 28 %) was then recrystallised from a mixture of propanone-pet ether which had the melting temperature, T_m : 183^0-185^0 C.

1) Tlc behaviour:

The compound showed a single spot in each case on tlc when run in a number of different solvents.

Solvent: $CHCl_3$–pet ether (1:1)
R_f = 0.55



2) Chemical characteristics:

The comoupnd produced yellow precipitate when added to a DNP reagent showing the presence of carbonyl group in it. The nature of the carbonyl function was also confirmed to be ketonic from its negative reactions both with Fehling's and Tollen's reagents.

3) Spectral behaviour:

i) The uv- spectrum (Fig , page) of the compound showed the following peak(s):

λ_{max}^{EtOH} (in nm) 289.0: $n \rightarrow \pi^*$ Transition of C=O
251.0: $\pi \rightarrow \pi^*$ Transition of C=C of phenyl ring

ii) The ir-spectrum (Fig , page) exhibited the following assignable bands at ν_{max} /cm^{-1}:

3410 : O-H stretching of bonded (with C-3 acetyl oxygen) C-4 O-H

3000, 2900, 2830: C–H stretching of aromatic system

1690: C=O stretching of bonded (with C-4 OH) C=O of C-3 acetyl group

1600(w), 1510(w): C=C stretching of aromatic ring skeletal

700(s), 695(s) : C–H bending of aromatic skeletal

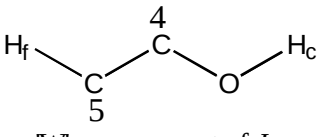
iii) The ^{1}H-nmr spectrum (**Fig , page**) of the compound exhibited signals (chemical shifts in δ / ppm) which were assigned as follows:

(a) 1.272 (3H, s) : Three C-4 methyl protons

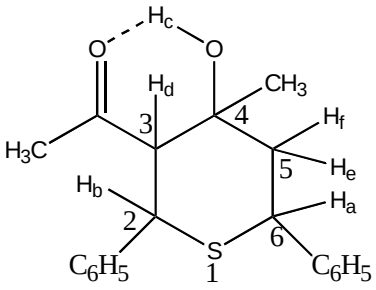
(b) 1.717 (3-H, s) : Three C-3 acetyl protons ($C\underline{H}_3CO$-)

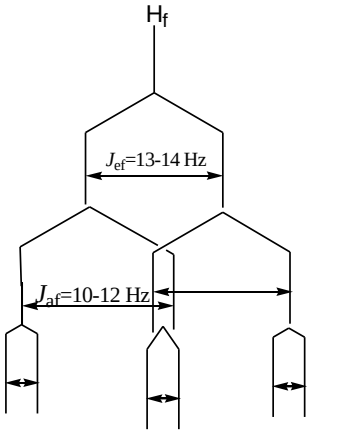
(c) 1.972-2.037 (1H, *dt*)
(J_{ef} =14Hz, J_{af} =12Hz, J_{cf} =3Hz) :One of the protons (*axial*, H_f) of C-5 methylene group

Of the two C-5 methylene protons (H_e and H_f), one will be equatorial and another will be axial. H_f will interact with the H_e through germinal coupling (J_{ef} =14 Hz) to give a doublet which subsequently will be splitted (J_{af} =12Hz) by C-6 H_a to give a doublet of a doublet resulting to a triplet. This triplet will again couple (J_{cf}=3Hz) with C-4 O-H_c proton probably thorough a "W"--arrangement (through a long range coupling, J = 3-4 Hz) to result doublet of a triplet (dt). The C-4 O-H_c is bonded to C-3 acetyl carbonyl oxygen forming a 6-membered ring and hence locks the proton forming a "W"-shaped arrangements with H_f proton.



'W'- arrangement of J_{cf}

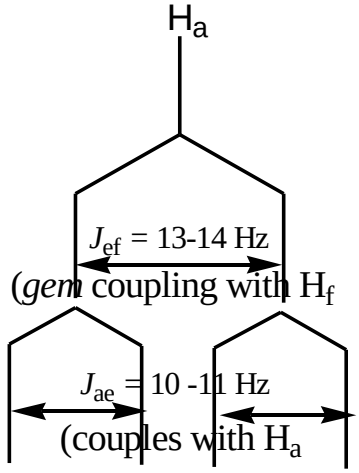




J_{cf}=3-4 Hz('W'-arrangement)= 3Hz

d) 2.206-2.40 (1H,dd)
(J_{ef} = 14Hz, J_{ae} = 3Hz): One of the protons of C-5 methylene protons (-C$\underline{H}_eH_f$-)

This proton splits into a doublet of doublet (dd) by a gem coupling with H_f which further splits into another doublet by C-6 methine proton (H_a,-S-C$\underline{H}_a$Ph-) resulting a doublet of a doublet(dd).



e) 3.209-3.237(1H, *d*, J_{bd} = 11.0 Hz) : One C-3 methine proton (-C$\underline{H}_d$COCH$_3$)

This signal splits into a doublet due to the coupling with C-2 methine proton (-CH$_b$Ph-)

f) 4.051(1H,d, J_{cf} = 3.0 Hz) : One C-4 O-$\underline{H}$c proton.

C-4 O-$\underline{H}_c$ proton signal is splited into a doublet by the long-range "W"-type arrangement coupling with one of the C-5 methylene protons (-CH$_c$H$_f$ -), the H_f proton as described earlier for the H_f proton.

g) 4.554-4.582(1H, d, J_{bd} = 11.0 Hz) : One C-2 methine proton (-S-C$\underline{H}_b$Ph-)

This signal splits into a doublet by the adjacent C-3 methine proton (H_d).

h) 4.617-4.647 (1H, d, J_{ae} = 12.0 Hz) : One C-6 methine proton (-S-C$\underline{H}_b$Ph-)

This signal splits into a doublet by the adjacent C-5 methylene proton (H_e).

i) 7.260-7.276 (2H, m, *J* = not resolved): One para proton each of two phenyl groups

j) 7.295-7.338 (4H, m, *J* = not resolved): Two meta proton each of phenyl groups

k) 7.372-7.403 (4H, m, *J* = not resolved): Two ortho proton each of phenyl groups

iv) The ^{13}C-nmr spectrum (**Fig , page**) of the compound (**1**) shows several signals (chemical shifts in δ /ppm) which were assigned as follows

a) 216.36 : Aliphatic carbonyl carbon of C-3 acetyl group (-$\underline{C}OCH_3$)

b) 140.78,138.38: Quaternary aromatic carbon of C-2 & C-6 phenyl group

c) 127.51-128.86: Aromatic tertiary carbon

d) 71.24 : C-4 Quaternary carbon ()

e) 63.79 : C-3 Tertiary carbon of 6-membered ring contain S-atom

f) 47.34 : C-2 Tertiary carbon of the same ring

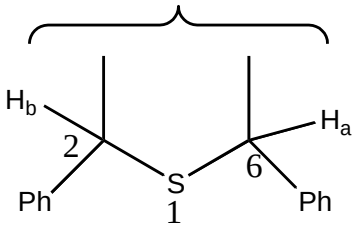
g) 46.33 : C-5 Primary carbon of 6-membered sulphur heterocycle

From ^{13}C-nmr (DEPT-135) (**Fig , page**) spectrum, the negative signal (chemical shift in δ/ppm) 46.33 is for carbon attached to even number of hydrogen atoms (i.e. -C-5–CH_2- group)

h) 42.88 : C-6 Tertiary carbon of the ring.

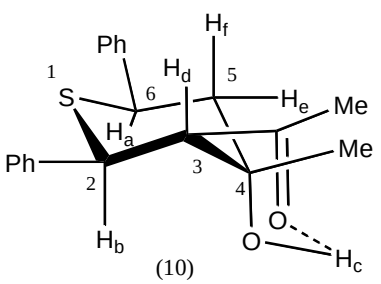
i) 34.78 & 30.01 : C-4 Methyl & C-3 acetyl methyl carbon.

From the chemical shift of C-2 & C-6 methine protons (H_b and H_a respectively, δ = 4.554-4.582 and δ = 4.617-4.647) indicates that these protons are connected to electronegative elements and/or have aromatic rings. One possibility is that these two protons may be connected to sulphur through carbons which are in linked to aromatic rings suggesting the presence of the following fragments in the compound (**I**).

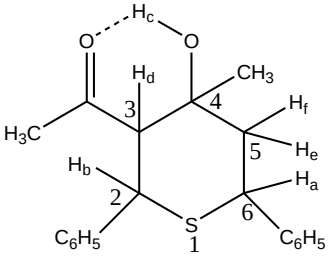


The mass spectrum (**Fig , page**) of the compound (**10**) exhibited the following important peaks at *m/z*: 326 [M^+], 308 [M-H_2O], 265 [M- H_2O,-CH_3CO], 250 [M-2H_2O, - CH_3CO, - CH_3], 91[$C_7H_7^+$, tropylium ion], 77[Ph], 65 ($C_5H_5^+$, cyclopentadienium ion). From the expanded mass spectrum of compound (10) at *m/z*: 340 to 320 (**Fig , page**) shows that the peak at 327 is about 20% of that at 326 indicating that there is about 20 carbons in the molecule . The peaks at 328 is about 4% of that at 326 indicating that the parent compound contains sulphur (the natural isotopic abundance of sulphur is ^{32}S: 95.0%; ^{33}S: 0.76%, and ^{34}S: 4.22%).The isotopic abundance of ^{12}C: 98.89% and ^{13}C: 1.11%).

On the basis of the above spectral analyses, the structure for the white solid obtained by the reaction of benzaldehyde and propanone (acetone) in presence of aqueous sodium sulphide at room temperature having melting temperature 183^0-185^0C was assigned as 3-acetyl-2,6-diphenyl-4-methyltetrahydrothio- -pyran-4-ol (**10**).



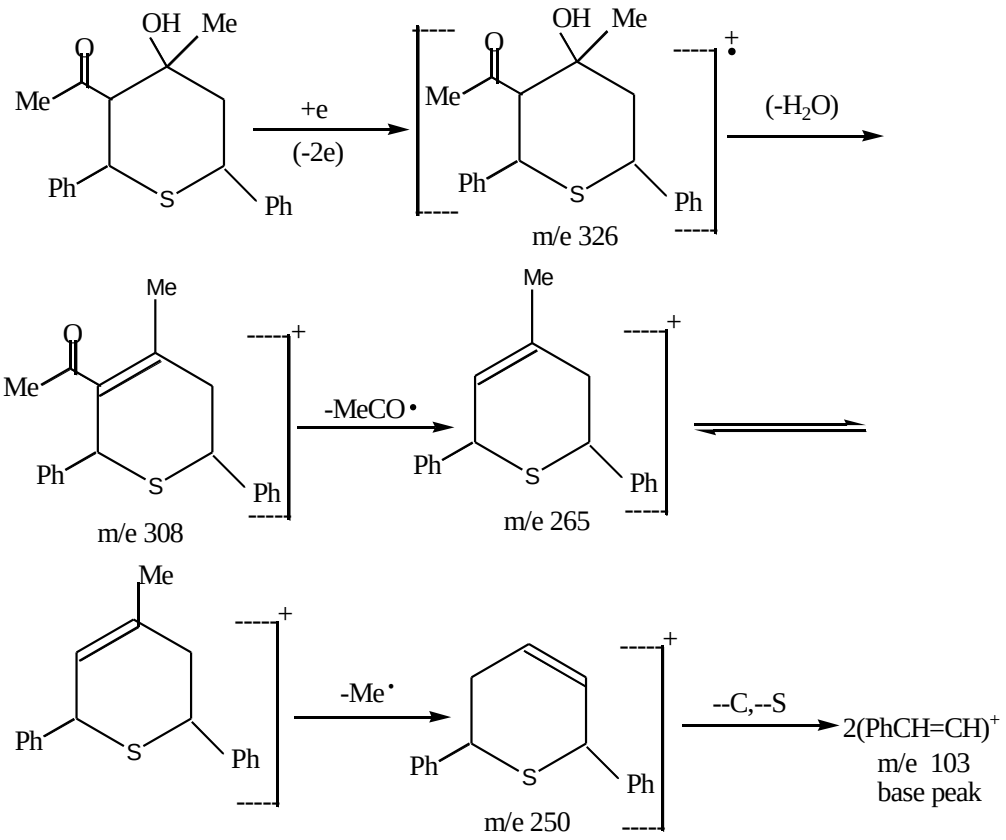
(10)



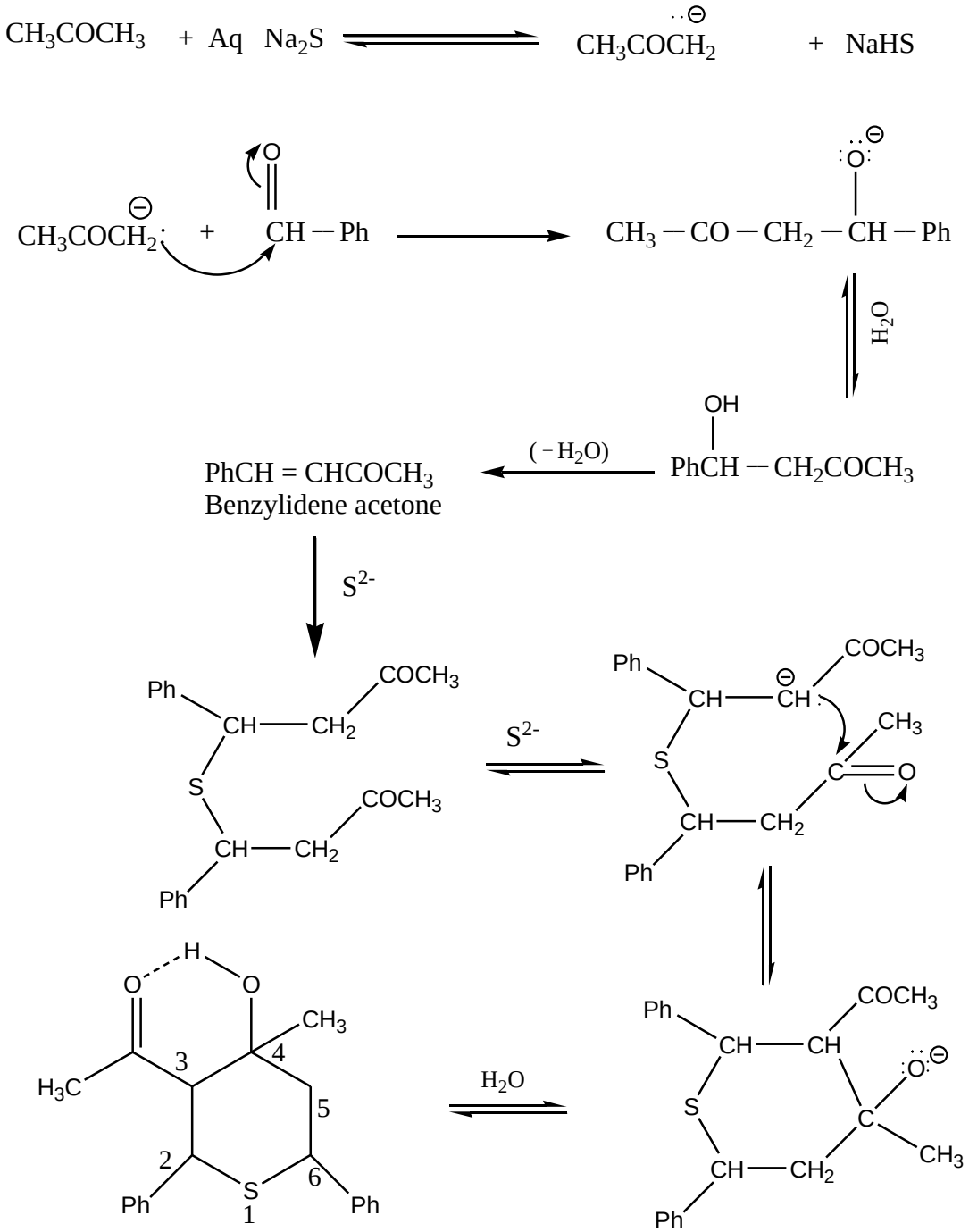
3-acetyl-2,6-diphenyl-4-methyl-tetrahydrothiopyran-4-ol(10)

From the distinct difference in coupling constants of axial and equatorial protons (H_e and H_f), it is likely that the ring system present in the compound (**10**) is six member. All the above spectral properties strongly indicate that the structure of the compound is as above.

On the basis of this structure the main reactions taking place in the ionisation chamber of the mass spectrometry may be explained as formulated below:



A possible mechanism to that proposed by Cremer and Subbaratnam[104] may be put forward for the formation of 3-acetyl-2,6-diphenyl-4-methyltetra--hydrothiopyran-4-ol (10).



(10) Scheme-**2**

Reaction of benzaldehyde with propanone in presence of aqueous sodium sulphide at reflux temperature

The reaction of benzaldehyde with propanone containing sodium sulphide was also carried out at reflux temperature for six hour with a view whether compound (10) and / or any other compounds is obtained. In the endeavour we obtained the same compound (10) in somewhat lesser yield (20%) with compared to that at room temperature described earlier. In this process, we also obtained another light yellow solid which was characterised as benzylidene acetone (30%, mixed melting temperature and superimosable ir-spectra (Fig , page) and a white crystalline solid which was characterised in the last reaction as 7-cinnamoyl-2,6,8-triphenyl-thiocane-4-one (5%).

Characterisation of the product obtained from the reaction of benzaldehyde and propanone in presence of aqueous sodium sulphide at reflux temperature

The compound obtained (yield 20 %) during the reaction of banzaldehyde with propanone in presence of sodium suphide at reflux temperature was found to be the one isolated (30 %) in the reaction of same reactants at room temperature in all respect such as melting tempature, tlc, uv-visible, ir, ^{1}H-nmr spectra etc.

Reaction of benzaldehyde and benzylideneacetone in aqueous methanolic solution at reflux temperature

Benzaldehyde was allowed to react with benzylidene acetone (T_m 38^0- 42^0 C) containing sodium sulphide in methanol (without ethanol) at reflux temperature. This reaction was carried out with a view to obtain compound (10) formed perhaps through the formation of benzylidene acetone (scheme-1, page). In this reaction we obtained the product (10) as evident from mixed melting temperature and super imposable ir spectra (Fig , page). It may be noted that here from the aqueous layer after usual treatment obtained benzyl alcohol and benzoic acid.

Characterisation of the product obtained from the reaction of benzaldehyde and benzylidene acetone in presence of aqueous sodium sulphide

The compound obtained during the reaction of benzaldehyde and benzylidene acetone in presence of aqueous methanolic sodium sulphide at reflux temperature was found to be the one isolated from the reaction of benzaldehyde with propanone in presence of sodium sulphide at room temperature.in all respect such as melting temperature, tlc, uv, ir, ^{1}H-nmr, etc.

Reaction of benzaldehyde with phenylethanone in presence of aqueous sodium sulphide at reflux temperature

Latif *et al*[102,103] reported the reaction of benzaldehyde and ethanol in presence of aqueous sodium sulphide and obtained a six membered sulphur heterocycle,3-benzyl-2,6-diphenyl-2H-thiopyran-5-carboxaldehyde(1) where it was suggested that ethanol was oxidised to ethanal. Keeping Latif and his co-workers view in mind we already described the reaction of benzaldehyde and propanone and obtained the copmpound 3-acetyl-2,6-diphenyl-4-methyltetrahydrothiopyran-4-ol(10) which is similar to (5) of Scheme-1, page).Keeping analogy to propanone, we decided to study the reaction of benzaldehyde and phenylethanone under identical condition and ended-up with a solid, 3-benzoyl-2,4,6-triphenyltetrahydrothiopyran-4-ol (11) ; melting temperature ,T_m:178^0-180^{0}C (20% yield).

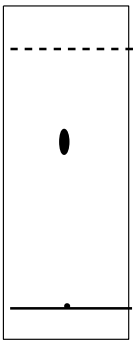
Characterisation of the product obtained from the reaction of benzaldehyde and phenylethanone in presence of aqueous sodium sulphide at reflux temperature

The white needled shaped crystals (**11, 20% yield)**, had melting temperature, T_m: 178^0-180^0 C (propanone-pet ether,2:3).

1) Tlc behaviour:

The compound showed a single spot in each case on tlc when run in a number of different solvents.

Solvent: $CHCl_3$–pet ether (1:1)
R_f = 0.50



2) Chemical characteristics:

When the solution of the conpound were treated with lead acetate solution,a black precipitate formed indicating the presence of sulphur in it.

$$S^{2-} + (AcO)_2Pb \longrightarrow \underset{\text{black ppt}}{PbS\downarrow}$$

The comoupnd produced yellow precipitate when added to a DNP reagent showing the presence of carbonyl group. The nature of the carbonyl group was found to be ketonic from its negative reactions both with Fehling's and Tollen's reagents.

The sulphur containing ions shows the characteristic isotopic pattern in mass spectrometry.

3) Spectral behaviour:

i) The uv- spectrum (Fig , page) of the compound showed the following peak(s):

λ_{max}^{EtOH} (in nm) 346.0: $n \rightarrow \pi^*$ Transition of C=O
265.0: $\pi \rightarrow \pi^*$ Transition of C=C of phenyl ring

ii) The ir-spectrum (Fig , page) exhibited the following assignable bands at ν_{max}^{KBr}/cm^{-1}:

3390 : O-H bonded stretching with C-5 acetyl oxygen and C-4 O-H
3080-3020 : Aromatic C–H stretching
1647 : >C=O stretching of extended conjugation
1600, 1582 : C=C stretching of aromatic ring skeletal system
760-700 : C–H bending of aromatic skeletal

The pattern of the ir-spectra of compound (11) looks similar as that of 3-acetyl-2,6-diphenyl-4-methyltetrahydrothiopyran-4-ol (10) except that the resonances occur at different positions.

iii) The ^{1}H-nmr spectrum (**Fig , page**) of the compound exhibited signals (chemical shifts in δ / ppm) which were assigned as follows:

(a) 2.36 (1H, dd, H_e; J_{ae}= 3.0 Hz, J_{ef} = 11.0 Hz) (3H, s) :

(b) 2.57 (1H, ddd, H_f; J_{ef} = 11.0 Hz, J_{af} = 12.0 Hz, J_{cf} = 3.0 Hz)

(c) 4.56 (1H, d, H_d; J_{bd} = 11.0 Hz)

(d) 4.92 (1H, dd, H_a; J_{af} = 12.0 Hz, J_{ae} = 3.0 Hz)

(e) 4.97 (1H, d, H_b; J_{bd} = 11.0 Hz)

(f) 5.27 (1H, d, H_c; J_{cf} = 3.0 Hz)

(g) 6.91-7.52 (20H,m) : Aromatic protons of four phenyl groups at C-2,C-4, C-5,and C-6.

iv) The ^{13}C-nmr spectrum (**Fig , page**) of the compound showed several signals (chemical shifts in δ /ppm) which were assigned as follows

a) 43.4 : (C-7 tertiary carbon)

b) 46.9, 48.4 : (C-6 and /or C-8)

c) 58.1 : (C-5 tertiary carbon,)

d) 76.0 : C-4 quaternary carbon > C (OH) Ph

e) 124.4, 126.8, 127.3-127.9, 128.0-128.4, 132.6 : Aromatic tertiary carbon

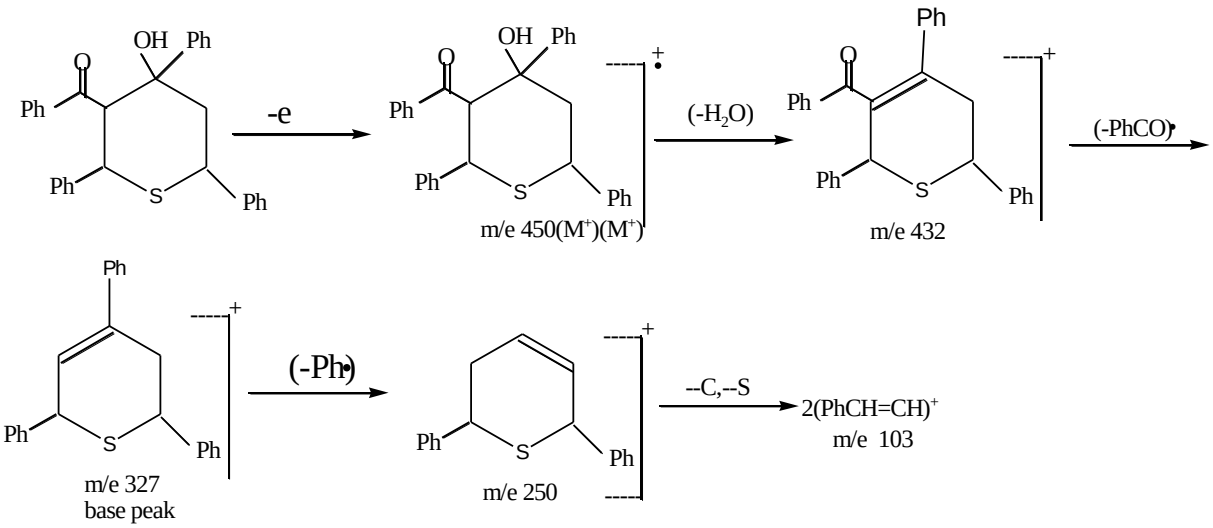
f) 137.4, 137.6, 140.4, 145.4 : Four quaternary aromatic carbons at C-2, C-4, C-5, and C-6)

g) 206.6 : Carbonyl carbon.

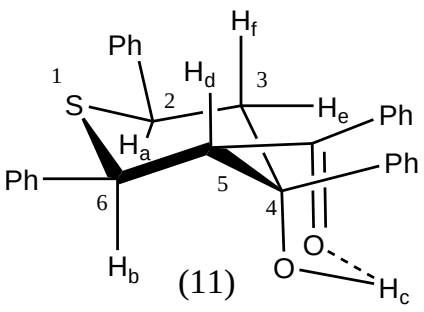
v) The mass spectrum (**Fig , page**) of the compound (**11**) also showed the following assignable peaks at ms (*m/z*)

51(Cyclobutadienum ion), 77$(Ph)^+$, 91$(PhCH_2^+)$, 105$(PhCO^{+)}$,327(M-(H_2O+PhCO)), 432(M-H_2O), 450 (M^+)

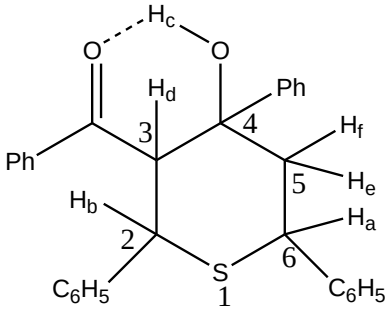
A possible fragmantation taking place in the ionisation chamber of the mass spectrometry may be explained as formulated below:



On the basis of the above spectral analyses, the structure for the white solid (11) was assigned as 3-benzoyl-2,4,6-triphenyltetrahydrothiopyran-4-ol (**11**).

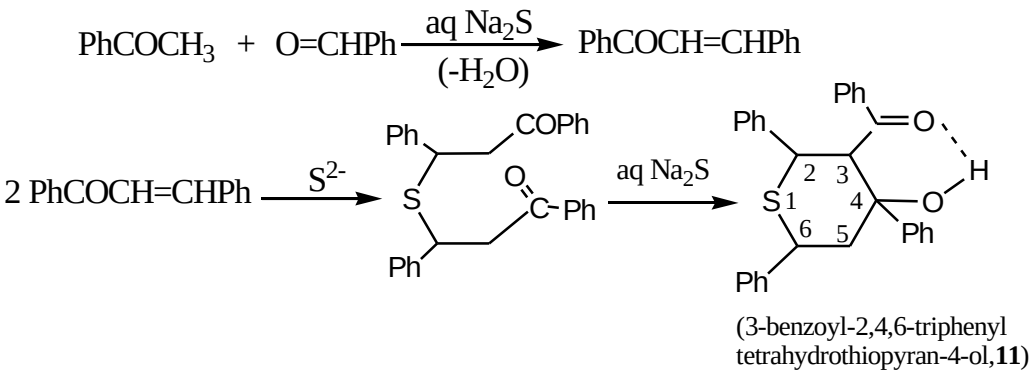


(11)



3-benzoylyl-2,4,6-triphenyl tetrahydrothiopyran-4-ol(11)

A possible mechanism may be put forward for the formation of 3-benzoyl-2,4,6-triphenytetrahydrothiopyran-4-ol (**11**) , (Schcme-) .



(3-benzoyl-2,4,6-triphenyl tetrahydrothiopyran-4-ol,**11**)

In the Cremmer-Subbaratnam's mechanism (Scheme-1) it was proposed that ethanol is oxidised *in situ* to ethanal, so instead of ethanol , we employed propanone and phenylethanone in two earlier reactions; the oxidised products of 2-propanol and 1-phenylethanol respectively and obtained two analogous derivatives of tetrahydrothiopyranol (**10**) and (**11**) equivalent to the product (**5**) of scheme-1.With this view in mind, we carried out the reaction of 2-propanol with benzaldehyde i) in aqueous sodium sulphide and ii) hydrogen sulphide gas separately at room temperature to see whether they produce the same product(s) (**10**) or not as found in the case of the reaction of benzaldehyde with propanone in aqueous sodium sulphide at room temperature in an earlier reaction. However, to our utter surprise, instead of the intermediate (10), we obtained three other compounds i.e, in case of sodium sulphide reaction (**15**), (**16**) and in case of hydrogen sulphide reaction (**15a**),(16) .

The reaction of benzaldehyde with 2-propanol in the presence of aqueous sodium sulphide at room temperature

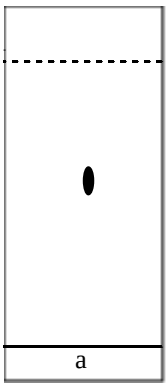
In order to investigate the hypothesis given by Cremer and Subbaratnam [104] that ethanol is oxidised to active hydrogen containing compound, ethanal, we carried out the reaction of benzaldehyde with 2-propanol in presence of aqueous sodium sulphide at room temperature for about 6 hours. The reaction mixture was extracted with Et_2O, dried over anhydrous sodium sulphate and resulted a pale yellow liquid . The pale yellow liquid on tlc examination showed the presence of three compounds, which was separated by column chromatography (silica gel, 60-120 mesh; eluent: pet ether-PhH,1:1) as a white crystalline solid (15) T_m: 137^0-139^0 C (20% yield) and another pale yellow solid (16) T_m: 105^0-107^0 C (10% yield) and a very trace gummy mass which was discarded.

Characterisation of the white crystalline solid (15) product obtained from the reaction of benzaldehyde with 2-propanol in presence of sodium sulphide

(1) Physical appearance: White crystalline solid, T_m: 137^0-139^0 C

(2) Solubility: The compound is readily soluble in propanone. chloroform, ethoxyethane etc.

(3) Tlc behaviour: The compound showed a single spot in each on tlc when run in a number of different solvents**.**



Solvent: $CHCl_3$ –pet ether (2:1) ; R_f = 0.60

(4) Chemical characteristics: The compound produced yellow precipitate when added to a DNP reagent showing the presence of carbonyl group in it. The nature of the carbonyl function was also confirmed to be ketonic from its negative reactions both with Fehling's and Tollenn's reagents. The usual qualitative elemental analysis indicated the presence of sulphur in the compounds.

(5) Spectral behaviour:

i) The uv-visible spectrum (Fig , page) of the compound showed the following peak(s):

$\lambda_{max}^{CHCl_3}$ 301 nm : $n \rightarrow \pi^*$ Transition of C=O conjugated with C=Cdouble bond
. 205 nm : $\pi \rightarrow \pi^*$ Transition of C=C double bond

ii) The ir spectrum (Fig , page) of the compound exhibited a number of bands, some of which were assigned as the follows at ν_{max}^{KBr} cm^{-1}:

3020	: Aromatic C-H stretching
2870	: Aliphatic C-H stretching
1640	: >C=O stretching conjugated with >C=C<
1590	: >C=C< skeletal vibration for aromatic ring.
1480, 1440 1210, 1170, 1060	: -CH_2- bending.
1020, 800, 745, 690	: Aromatic out of plane C-H bending

iii) The ^{1}H-nmr spectrum (Fig , page) of the compound exhibited signals (chemical shift in δ /ppm) which were assigned as follows:

a) 1.53 (1 H, s) : One C-2 methine proton (-S-C$\underline{H_a}$Ph-)

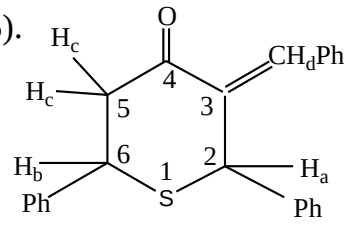
b) 1.70-1.76 (1 H, *t*, J_{bc} = 7 Hz) : One C-6 methine proton (-S-C$\underline{H_b}$Ph-)
This signal splits into a triplet by the two adjacent C-5 methylene protons (H_c).

c) 5.46 – 5.49 (2 H, *d*, J_{cb}= 8Hz) : Two C-5 methylene protons (-CO-C$\underline{H_2}$-)
This signal splits into a doublet by the adjacent C-6 methine proton (H_b)

d) 5.63 (1 H, s) : One benzylidene proton at C-3(=C$\underline{H_d}$Ph-)

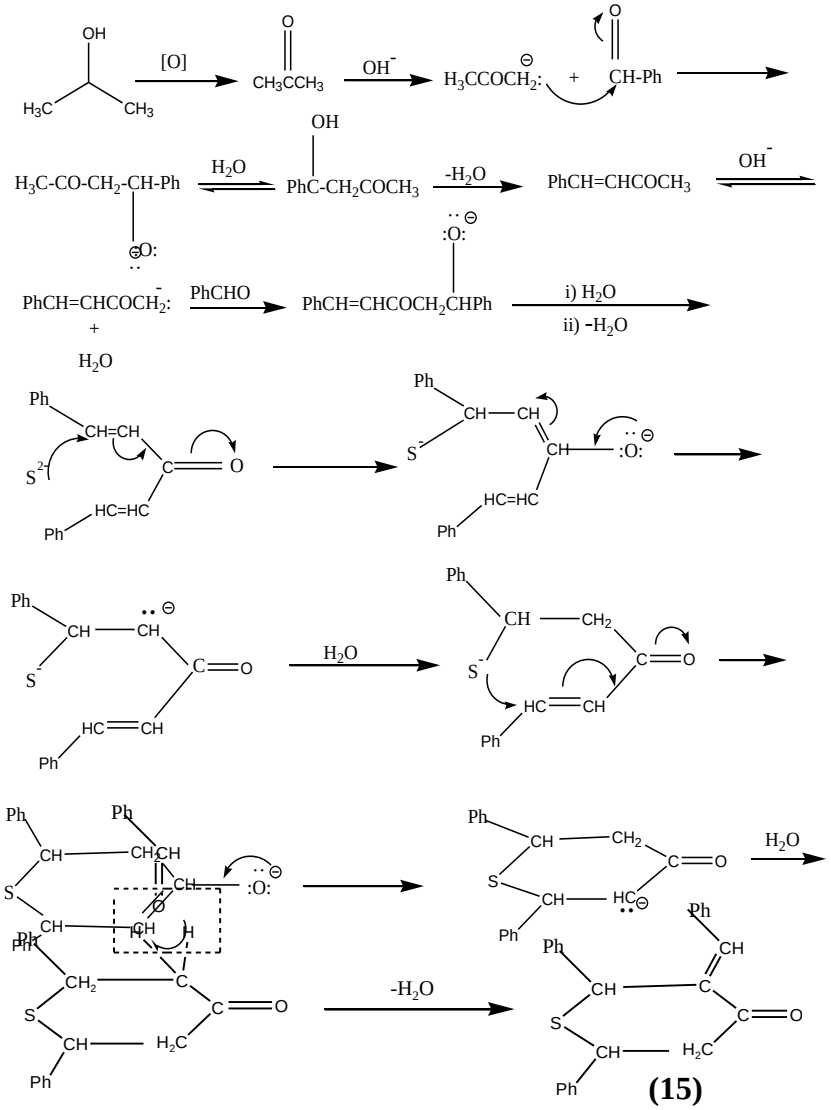
e) 7.05-7.89 (15 H, *m*, *J* = unresolved): Phenyl protons at C-2 and C-6 and benzylidene protons at C-5 ($C_6\underline{H_5}$-C$\underline{H}$=)

On the basis of the above analyses, the structure of the white crystalline solid (T_m:137^0-139^0 C) obtained from the reaction of benzaldehyde and 2-propanol in presence of aqueous sodium sulphide was assigned as 3-benzylidene-2,6-diphenyltetrahydro-4-thiopyrone(15).



3-benzylidene-2, 6-diphenyltetrahydro-4-thiopyrone(**15**)

A possible mechanism to that proposed by Cremer & Subbaratnam may be put forward for the formation of 3-benzylidene-2, 6-diphenyltetrahydro-4-thiopyrone.



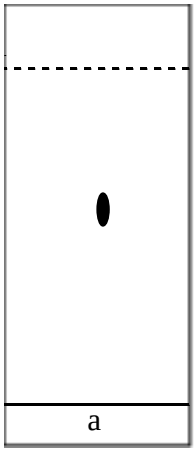
(15)

Characterisation of the pale yellow solid (16) obtained from the reaction of benzaldehyde with 2-propanol in presence of aqueous sodium sulphide

(1) Physical appearance: Light yellow solid, T_m: 105^0-107^0C.

(2) Solubility: The compound is readily soluble in propanone, chloroform, ethoxyethane, etc.

(3) Tlc behaviour: The compound showed a single spot in each on tlc when run in a number of different solvents**.**



Solvent: $CHCl_3$ –pet ether (2:1); R_f : 0.50

(4) Chemical characteristics: The compound produced yellow precipitate when added to a DNP reagent showing the presence of carbonyl group in it. The nature of the carbonyl function was also confirmed to be ketonic from its negative reactions both with Fehling's and Tollenn's reagents. The usual qualitative elemental analysis indicated the presence of sulphur in the compounds.

(5) Spectral behavior:

i) The uv-visible spectrum (Fig , page) of the compound showed the following peak(s):

$\lambda_{max}^{CHCl_3}$ 220, 301 nm : $n \rightarrow \pi^*$ Transition of C=O conjugated with . C=C double bond

ii) The ir-spectrum (Fig , page) of the compound exhibited a number of bands, some of which were assigned as the follows at ν_{max}^{KBr} cm^{-1}:

3350, 3120	: C-H stretching of aromatic and olefinic system
2340	: C-H stretching of aliphatic system
1615	: Conjugation of C=O with>C=C<
1440, 1390	: Medium to strong absorptions of aromatic ring
880, 770, 690	: Aromatic out of plane C-H bonding.

iii) The ^{1}H-nmr spectrum (Fig , page) of the compound exhibited signals (chemical shift in δ/ ppm) which were assigned as follows:

a) 1.000-1.273(2H, *dd, J* = unresolved) : Two C-2 and C-6 methine protons (S-C**H**a-Ph)

b) 7.261-7.452 (22H, m, J = unresolved): Twenty aromatic protons of two
& 7.515-7.902 phenyl groups at C-2 and C-6, and two benzylidene phenyl groups at C-3and C-5, and two methine protons of benzylidene groups (=CH-Ph) at C-3 and C-5.

iv) The ^{13}C-nmr spectrum (Fig , page) of the compound exhibited signals (chemical shift in δ /ppm) which were assigned as follows:

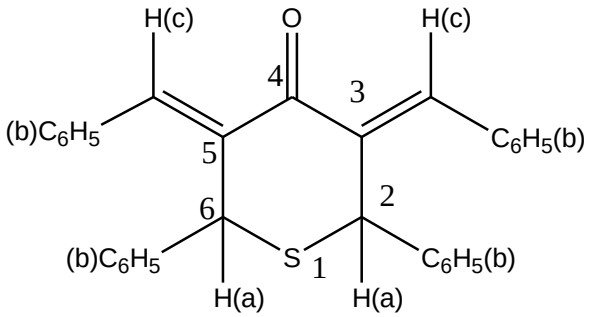
a) 29.608 : Two aliphatic tertiary carbons of heterocyclic ring (=<u>C</u>H-Ph)

b) 126.801, 128.417, 131.932 : C-3 and C-6 phenyl and C-3 and C-5 benzylidene aromatic ring carbons

c) 202.813 : Carbonyl carbon of (>**<u>C</u>**=O) at C-4.

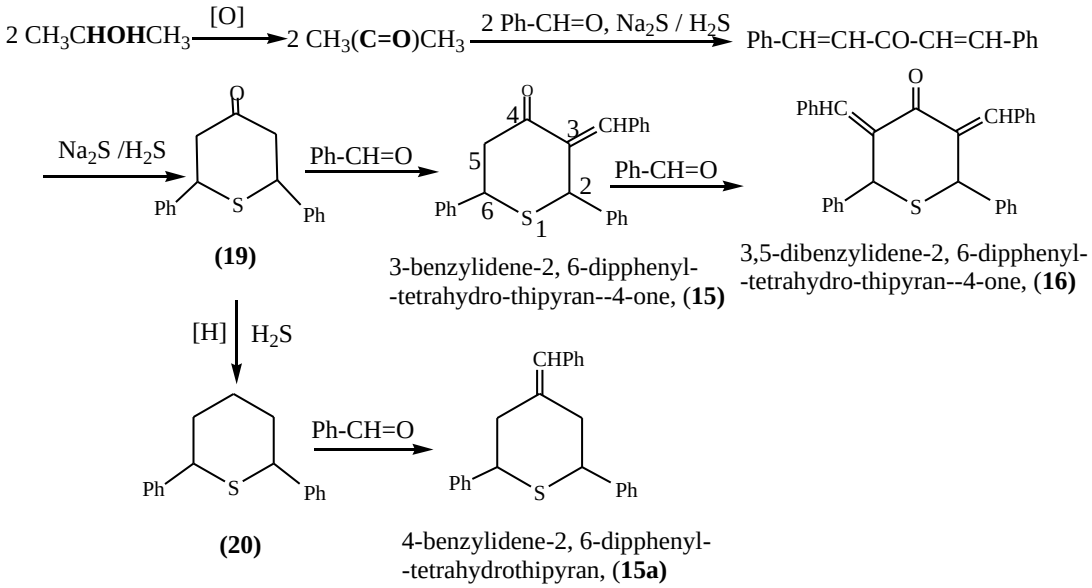
v) The ^{13}C-DEPT-135 nmr spectrum (chemical shift in δ/ppm, Fig , page) of the compound exhibited no inverse signal(s) indicating the absence of any -<u>C</u>H_2- carbon.

On the basis of the above analyses, the structure of the pale yellow solid (T_m:105^0-107^0 C) obtained from the reaction of benzaldehyde and 2-propanol in presence of aqueous sodium sulphide was assigned as 3,5-dibenzylidene-2,6-diphenyltetrahydrothiopyran-4-one (**16**).



3,5-Dibenzylidene-2,6-diphenyl-tetrahydrothiopyran-4-one(**16**)

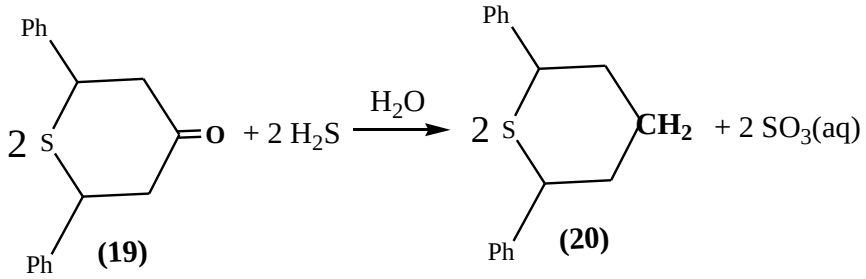
A possible pathway for the formation of (**15**) ; (**15a**) and (**16**) in presence of aqueous sodium sulphide or hydrogen sulphide respectively are shown as follows:



Scheme-

In the reactions of benzaldehyde with 2-propanol in presence of sodium sulphide and hydrogen sulphide, 2-propanol might have oxidised to propanone which then under the reaction condition, condensed with benzaldehyde to give dibenzylideneacetone(DBA). The dibenzylidene acetone then undergoes a double Michael addition reaction with sulphides to give a 6-membered sulphur heterocycle 2,6-diphenyl-tetrahydro-4- thiopyranone, (**19**) Later copmpound (**19**) condensed with benzaldehyde to give 1:1 and 1:2 products—(**15**) and (**16**) respectively. Besides that compound (**19**) in presence of hydrogen sulphide, a good reducing agent, produces the corresponding reduced product of (**19**)--2,6-diphenyltetrahydrothiopyran (20). However, compounds (**19**) and (**20**) could not be isolated. Compound (20) under the reaction condition gives (**15a**), a 1:1 product of (20) and benzaldehyde.

Thiopyranone (**19**) might have reduced by hydrogen sulphide under the reaction condition to give (20) and sulphur (VI) oxide (SO_3) a highly water soluble gas as follows:



The reaction of benzaldehyde with 2-propanol in the presence of hydrogen sulphide at room temperature

The reaction of benzaldehyde with 2-propanol in presence of hydrogen sulphide was carried out at room temperature for about 6 hours, extracted with Et_2O, dried(anhyd Na_2SO_4) resulted a pale yellow liquid . The pale yellow liquid on tlc examination showed the presence of three compounds, which was separated by column chromatography (silica gel, 60-120 mesh; eluent: pet ether-PhH,1:1) as an off-white solid (15a) T_m: 62^0-65^0 C (15% yield) and another pale yellow solid (16) T_m: 105^0-107^0 C (10% yield) and a very trace gummy mass which was discarded.

Characterisation of the off-white crystalline solid (15a) product obtained from the reaction of benzaldehyde with 2-propanol in presence of hydrogen sulphide

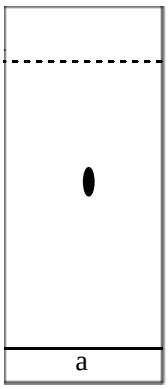
(1) Physical appearance: An off-white crystalline solid, T_m: 62^0-65^0 C

(2) Solubility: The compound is readily soluble in propanone. chloroform, ethoxyethane,ethanol etc.

(3) Tlc behaviour: The compound showed a single spot in each on tlc when run in a number of different solvent systems**.**

Solvent: $CHCl_3$ –pet ether (2:1)

$R_f = 0.65$



(4) Chemical characteristics: The compound did not produced any yellow precipitate when added to a DNP reagent indicating the absence of carbonyl group in it. The usual qualitative elemental analysis indicated the presence of sulphur in the compounds.

(5) Spectral behaviour:

i) The uv-visible spectrum (Fig , page) of the compound showed the following peak(s):

$\lambda_{max}^{CHCl_3}$ 288.0 nm : $\pi \rightarrow \pi^*$ Transition of C=C double bond

ii) The ir spectrum (Fig , page) of the compound exhibited a number of bands, some of which were assigned as the follows at ν_{max}^{KBr} cm^{-1}:

3340,3000 : C-H stretching of aromatic and olefinic system.

2850 : Aliphatic C-H stretching.

1570,1530 : >C=C< skeletal vibration for aromatic ring.

1490, 1215 : -CH_2- bending.

890, 760, 690 : Aromatic out of plane C-H bonding.

iii) The ^{1}H-nmr spectrum (Fig , page) of the compound exhibited signals (chemical shift in δ/ ppm) which were assigned as follows:

a) 3.640 (5H, s) : five proton of C-2,C-3,C-5 and methane arbon of the phenyl methyl group.

b) 4.066 (1H, s) : One proton of –O-H group attached to phenyl methyl group.

c) 4.192 – 4.237 (1H, t) : one C-6 methine proton.

d) 7.268–7.394 (15H, m) : protons of two C-2, C-6 Phenyl groups (2x-H_5C_6), and one phenyl of C-4 methyl phenyl group.

iv) The ^{13}C-nmr spectrum (Fig , page) of the compound showed several signals which were assigned as follows:

a) 43.087 :one saturated hetarocyclic ring carbon of C-6.

b) 43.208 : one saturated hetarocyclic ring carbon of C-2.

c) 43.572 : one saturated hetarocyclic ring carbon of C-3

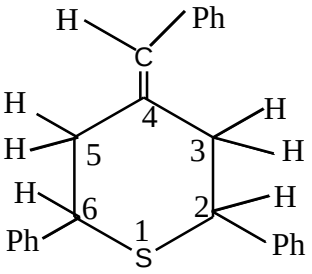
d) 44.200 : one saturated hetarocyclic ring carbon of C-4

e) 77.388 : methine carbon of the phenyl methyl group.

f) 137.297 : one quatenary carbon of the aromatic ring.

g) 127.361 -129.352 : all aromatic carbons of the phenyl rings.

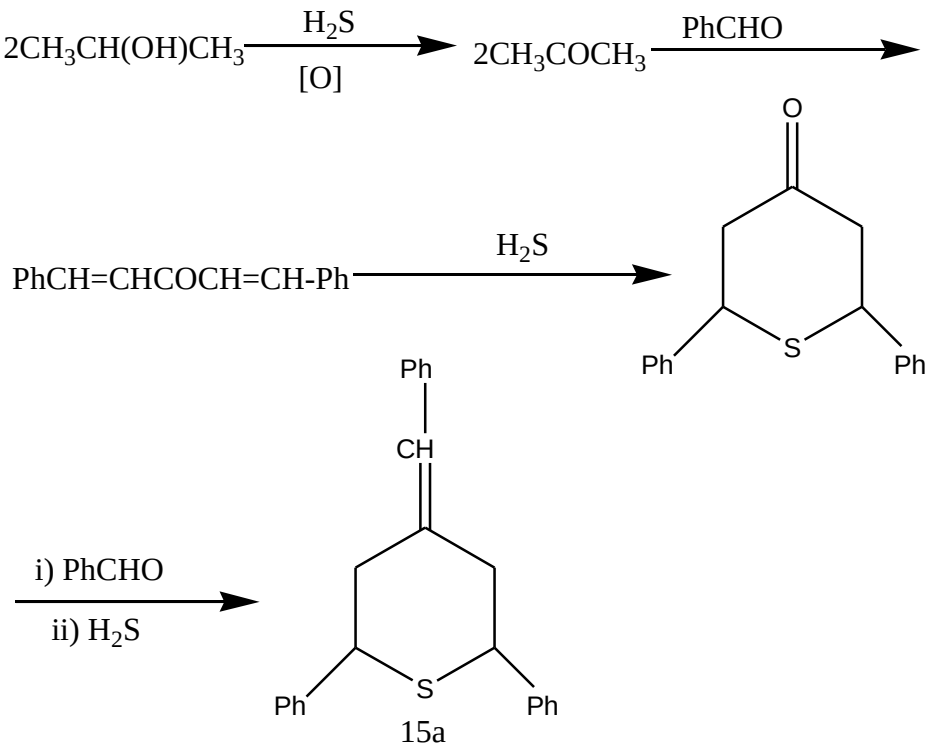
On the basis of the above analyses, the structure of the off-white solid (15a), melting temperature,T_m: 62^0-65^0C obtained from the reaction of benzaldehyde and 2-propanol in presence of hydrogen sulphide at room temperature was assigned as 4-benzylidene-2,6- diphenyltetrahydrothiopyran (15a).



(15a)

4- benzylidene -2,6- diphenyl-tetrahydrothiopyran(15a)

A possible pathway for the formation of the compound is given below:



15a

Characterisation of the pale yellow solid (16) obtained from the reaction of benzaldehyde with 2-propanol in presence of hydrogen sulphide

In the reaction of benzaldehyde with 2-propanol in presence of hydrogen sulphide produced the same pale yellow solid as that in presence of sodium sulphide as evident from melting temperatures, superimposable ir-spectra, etc.

Finally,in order to justify the hypothesis that 2-propanol is oxidised to propanone which later condensed with benzaldehyde to give benzylideneacetone and dibenzylideneacetone (DBA) in presence of sodium sulphide, we carried out our investigation starting with one of the intermediates (not isolated by them)–dibenzylideneacetone (DBA) with sodium sulphide or ammonium sulphide separately in molar ratios 1:1, 1:2, 2:1 at room temperature and at reflux temperature in aqueous or alcoholic medium under various reaction conditions to obtain a white crystalline solid (14,T_m: 191^0–192^0 C) and another white powdered solid (15,T_m: 160^0 – 162^0 C). Structure of the white crystalline solid having melting temperature (191^0–192^0 C) was analysed (as described earlier) as 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one (**17**)and that of white powdered solid having melting temperature(191^0–192^0C) as 2,6,2',6'-tetraphenyl-tetrahydro-[3,4']-bithiopyranyliden-4-ol(18).

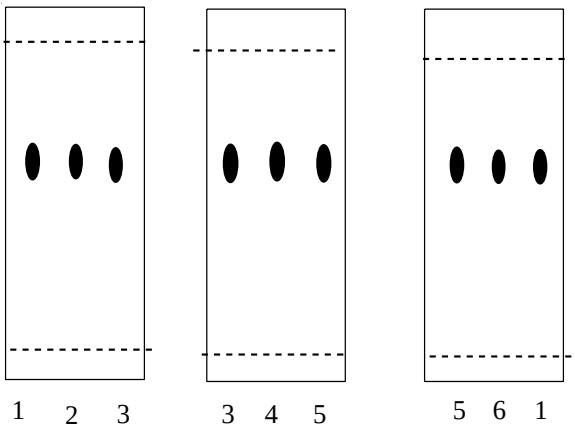
Reaction of dibenzylideneacetone (DBA) and aq sodium sulphide in methanol (molar ratio 1:2)

At room temperature:

In order to investigate the hypothesis put forward by us that 2-propanol is oxidised to propanone which subsequently reacts with benzaldehyde to give benzylidene acetone and dibenzylideneacetone, we carried out the reaction of dibenzylideneacetone (2.34 g, 0.01 mol) with sodium sulphide (1.56g, 0.02 mol) in methanol (60 cm^3). The mixture was stirrered for 20 hours when a white solid precipitate out. The solid (17) was recrystallised from acetone and had the melting temperature of 191^0-192^0 C (yield: 20%). After the separation of the above solid, the mother liquor was extracted with ethoxy ethane, Which on evaporation results another white powered solid(18), melting temperature 160^0-162^0 C (yield: 15%).

The reaction of dibenzylideneacetone (DBA) with sodium sulphide at room temperature and at reflux temperature in molar ratios 1:2 and 2:1 gave the two products, melting temperatures 191^0-192^0 C and 160^0-160^0 C respectively. The reaction of dibenzylideneacetone was repeated under identical condition and molar ratios with ammonium sulphide to obtain also two products of melting temperature 191^0-192^0 C and 160^0-160^0 C. These twelve products were found to be actually two products as evident from their thin layer chromatography and ir spectral behaviours.

(i) All the white crystalline solid compounds gave single spot in co-tlc when run in different solvent systems.

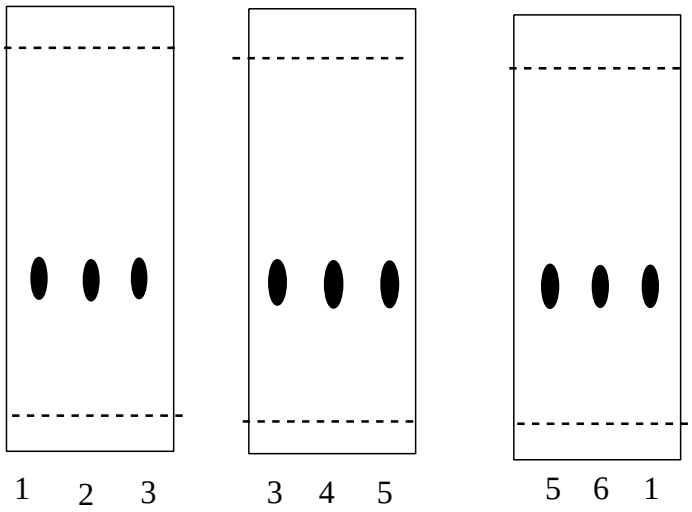


$CHCl_3$: Pet Ether = 1:1 ; R_f = 0.60

Tlc of white crystalline solid products (14) obtained at

1) room temperature for the reaction of dibenzylideneacetone and Na_2S in meathanol(molar ratio 1:2)
2) reflux temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 1:2)
3) room temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 2:1)
4) reflux temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 2:1)
5) room temperature for the reaction of dibenzylideneacetone and $(NH_4)_2S$ in methanol (molar ratio 1:1)
6) room temperature for the reaction of dibenzylideneacetone and $(NH_4)_2S$ in methanol (molar ratio 2:1)

(ii) All the white powdered solid compounds (15) gave single spot in co-tlc when run in different solvent systems.



$CHCl_3$: Pet Ether = 1:1; R_f value = 0.30

Tlc of white powered solid products obtained at

1) room temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 1:2)
2) reflux temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 1:2)
3) room temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 2:1)
4) reflux temperature for the reaction of dibenzylideneacetone and Na_2S in methanol (molar ratio 2:1)
5) room temperature for the reaction of dibenzylideneacetone and $(NH_4)_2S$ in methanol (molar ratio 1:1)
6) room temperature for the reaction of dibenzylideneacetone and $(NH_4)_2S$ in methanol (molar ratio 2:1)

From the above tlc study we can find that all the six white crystalline products had the same R_f value and the six white powered products had also the same R_f value though obtained from different reaction conditions, reactant and molar ratio.

(iii) The mixed melting temperature of all the six white crystalline products obtained at different conditions, reactant and molar ratio had the same melting temperature and that was 191^0-192^0 C. Similarly for white powered compounds the mixed melting temperatures found to be 160^0-162^0 C.

(iv) The ir-spectra of white crystalline products obtained from six reactions at different conditions with the different sulphides and different molar ratios gave superimposible peaks. Similarly also for the six white powered compounds gave superimoposible ir spectra.

From the above studies it can be concluded that all six white crystalline solids and white powered solids compounds were two different single compounds for six different reaction conditions, with the different sulphides and different molar ratio.

Characterisation of the white crystalline solid obtained from the reaction of dibenzylideneacetone and sodium sulphide (or ammonium sulphide) in presence of methanol at different reaction condition

Reaction of dibenzylideneacetone (DBA) with sodium sulphide (or ammonium sulphide) in presence of methanol at different reaction condition gave a white crystalline solid melting temperature; 191^0-192^0 C.

The white crystalline solid, exhibited the following characteristics:

a) Melting temperature:

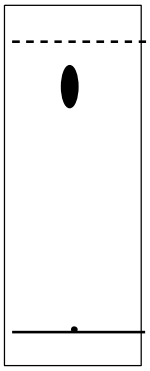
The white solid melted at 191^0-192^0 C

(b) Solubility:

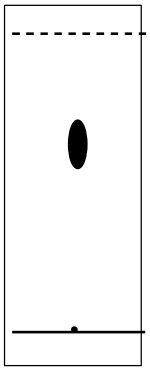
The above white crystalline solid was readily soluble in chloroform, acetone etc.

c) Tlc behavior:

The compound showed a single spot on tlc in each case when run in a number of different solvent systems.



(EtOAc-Pet.ether = 1:4) ; R_f =0.90



($CHCl_3$- Pet.ether = 1:1) ; R_f =0.60

d)Chemical behaviour:

The yellowish solid when added to 2, 4-DNPH reagent gave yellow precipitate indicating the presence of carbonyl group in it. The nature of the carbonyl function was also confirmed to be ketonic from its negative reactions to both with Fehling's and Tollen's reagents.

The usual qualitative elemental analysis indicated the presence of sulphur in the compound.

(e) Spectral behaviour:

i) The uv- visible spectrum (Fig , page) of the compound showed the following peak(s)

$\lambda_{max}^{CHCl_3}$ (in nm) 301 : $n \rightarrow \pi^*$ Transition of C = O

212 : $\pi \rightarrow \pi^*$ Transition of C = C

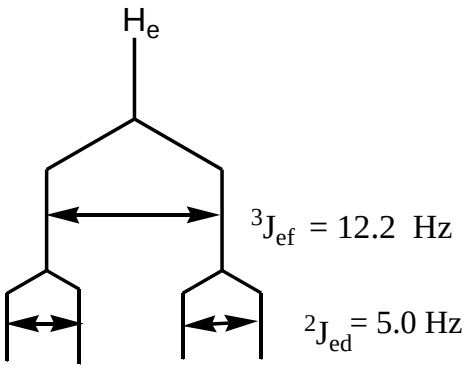
ii) The ir spectrum (Fig: , page:) of the compound exhibited a number of bands /cm^{-1}. Some of which were assigned as the follows at ν_{max}^{KBr}:

3450, 3005	: Aromatic C-H stretching
1700	: >C=O ring carbonyl
1640	: >C=O stretching conjugated with >C=C<
1580	: >C=C< skeletal vibration for aromatic ring
1485, 1440	: -CH_2- bending
1325, 1190, 1120 1090, 1060, 1020, 990, 880, 750, 695	: Aromatic out of plane C-H bending.

iii) The ^{1}H-nmr spectrum (Fig: , page:) of the compound exhibited signals(chemical shift in δ/ppm) which were assigned as follows:

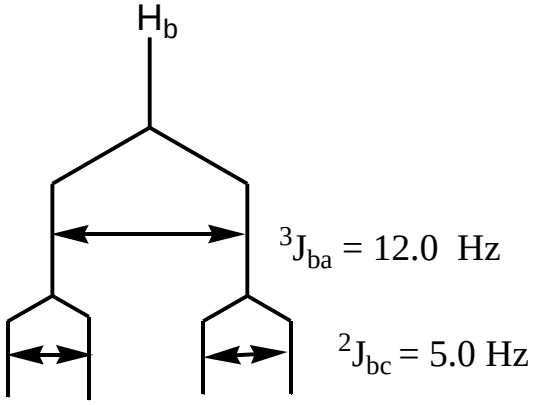
a) 2.82123 – 2.85715 (1H, *dd*) : One of the C-5 ketomethylene (*axial*) protons (H_e) (*J* = 12.2 and 5.0Hz)

This axial proton (H_e) coupled with the axial C-6 proton (H_f), *trans* coupling; ($^3J_{ef}$ = 12.2 Hz) to give a doublet which further coupled (*gem*-coupling) with another C-5 ketomethylene equitorial proton (H_d) to give the doublet of a doublet ($^2J_{ed}$ = 5.0 Hz).



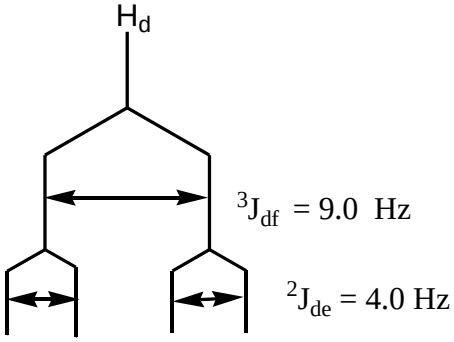
b)2.96582 – 3.02136 (1H, *dd*,
$^{2}J_{bc}$ = 5.0 Hz, $^{3}J_{ba}$ = 12.0 Hz): One of the C-3 ketomethylene (*axial*) protons (H_b)

This proton (H_b) might have coupled with the adjacent C-2 axial methine proton (H_a) to give a doublet (*d*, $^{3}J_{ba}$ = 12.0 Hz) which then further coupled with the rest C-3 of ketomethylene equitorial proton (H_c, *gem*- coupling,$^{2}J_{bc}$ = 5.0 Hz) to give a doublet of a doublet (*dd*).

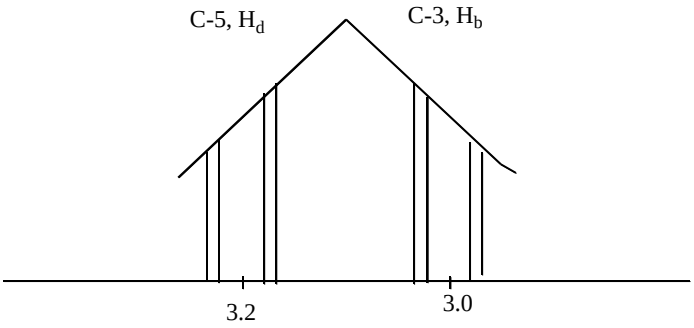


c) 3.13953 – 3.20245 (1H, *dd*,
J_{df} = 9.0Hz, J_{de} = 4.0 Hz)**:** One of the C-5 ketomethylene (*equitorial*) protons (H_d)

This proton (H_d) might have coupled with the adjacent C-6 axial methine proton (H_f) to give a doublet (*d*, $^{3}J_{df}$ = 9.0 Hz) which then further coupled with the rest C-5 of ketomethylene proton (H_e, *gem*-coupling, $^{2}J_{de}$ = 5.0 Hz) to give a doublet of a doublet (*dd*).



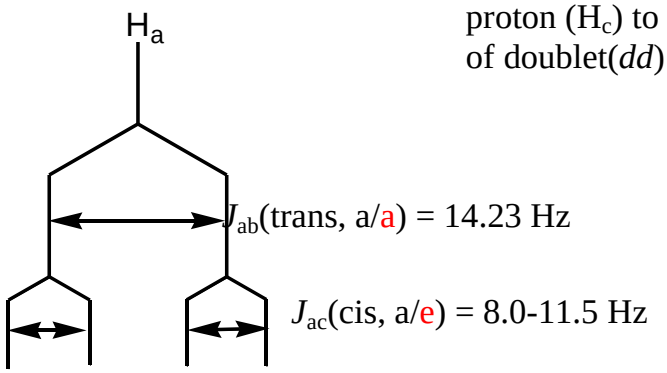
The above two doublet of doublets (dd) centred at 2.96582 – 3.02136 and at 3.13953-3.20245 are the counter parts of the each other for a proton, H_b of C-3 and another proton H_d of C-5 show a roof-effect as evident from the spectrum.



d) 3.34582 – 3.41319 (2H, *p*, J_{cb} = 5.0 Hz, J_{ca} = 8.0 Hz): One of the C-3 ketomethylene (*equitorial*) protons (H_c) and one C-7 methine protons (H_g, axial)

e) 3.92984 (1H, *s*),
3.98991– 4.01551(1H, d, J = 10.25): two C-6 and C-8 methine protons (H_a/ H_f and H_h respectively)

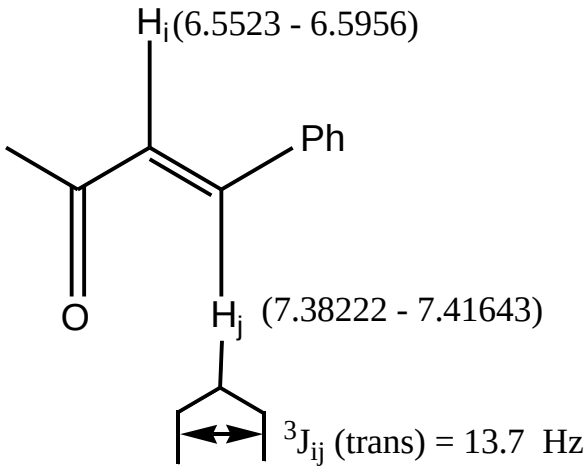
f) 4.45393 – 4.51404(1H, m/dd, $^2J_{ac}$ = 8-11.5 Hz, $^3J_{ab}$=14.23Hz): One C-2 methine proton (*axial* H_a) which was splitted to a doublet by the adjacent C-3 axial proton (H_b) which further underwent coupling with C-2 equitorial proton (H_c) to give rise finally a doublet of doublet(*dd*)



g) 6.5523-6.5956 (1H, *d*, $^{3}J_{ji}$ = 16.17 Hz)**:**One of the C-7 cinnamoyl (H_j) proton of -COCH=C<u>H</u>Ph

h) 7.38222-7.41643(1H, *d*, $3J_{ij}$ = 13.7 Hz)**:**Another C-7 cinnamoyl (H_i) proton of -(COC<u>H</u>=CHPh)

These two cinnamoyl protons exhibited two separate doublets far away from each other (at $/ppm$: 6.5739 and 7.3939) due to their different environment as evident from the structure. Most of the aromatic proton-signals (7.2075 – 7.4561) have resonated in between these two ethenyl signals and some of the signals (both ethenyl and aromatic) might have merged with one another. As a result, the total number of splitted lines for the aromatic protons do not always match with the expected lines. Moreover, the two ethenyl proton-signals of the cinnamoyl moiety (at C-7 of the thiocane ring) also clearly exhibited the roof-effect (Fig , page) as described earlier (for H_b at C-3 and H_d at C-5) advocate further for their identity (trans-coupling of ethenyl protons, $^{3}J_{ij}$ = 13.7 Hz).



i) 7.20750 – 7.45641 (20 H, *m*): Twenty aromatic protons (4 × $C_6\underline{H}_5$)

The above twenty aromatic protons for the three phenyl groups at C-2, C-6, and C-8, and one phenyl group of the cinnamoyl moiety at C-7 were alalysed ( ) as below:

7.2075 (two *ortho*-H of C-6 Ph), 7.2235 (one *para*-H of C-8 Ph), 7.3472 (one *para*-H of C-6 Ph), 7.2718 (one *para*-H of C-2 Ph), 7.2956 (two *ortho*-H of C-2 Ph), 7.3163 (two *ortho*-H of C-8 Ph), 7.3287 (two *meta*-H of C-2 Ph), 7.3447 (two *meta*-H of C-8 Ph), 7.3602 (two *meta*-H of C-6 Ph), 7.3822 (two *ortho*-H of C-7 Ph of cinnamoyl), 7.4164 3822 (two *meta*-H of C-7 Ph of cinnamoyl), and 7.4564 (one *para*-H of C-7 Ph of cinnamoyl).

iv) The ^{13}C-nmr spectrum (Fig: , page:) of the compound showed several signals (chemical shift in δ /ppm) which were assigned ( ) as follows:

a) 41.623 (C-7 aliphatic ring C), 43.840 (C-6 aliphatic ring C), **45.278** (C-5 aliphatic ring C, inversed in DEPT-135 spectrum), 45.555 (C-2 aliphatic ring C), **48.671** (C-3 aliphatic ring C, inversed in DEPT-135 spectrum), 58.965 (C-8 aliphatic ring C), and 211.945 (C-4 carbonyl ring C)

The two ^{13}C- signals at δ/ppm 45.278 and 48.671 were inversed in the DEPT-135 ^{13}C-nmr sepctru (Fig , page) indicating that these two are methylene carbons such as C-3 and C-5.

b) 126.093 (C-7 ethenyl C of cinnamoyl moiety (-(C=O)-**C**H=CH-Ph), 142.877 (C-7 ethenyl C of cinnamoyl moiety (-(C=O)-CH=**C**H-Ph), and 197.504 (C-7 carbonyl C of cinnamoyl moiety (-(**C**=O)-CH=CH-Ph)

c) 127.044 (two *ortho* C of C-2 Ph), 127.400 (two *ortho* C of cinnamoyl Ph at C-7), 127.733 (two *ortho* C of C-8 Ph), and 127.985 (two *ortho* C of C-6 Ph).

d) 128.194 (two *meta* C of C-2 Ph), 128.296 (two *meta* C of C-8 Ph), 128.469 (two *meta* C of C-6 Ph), and 128.793 (two *meta* C of cinnamoyl Ph at C-7)

e) 128.887 (one *para* C of C-6 Ph), 129.136 (two *para* C of C-2 and C-8 Ph), and 130.550 (one *para* C cinnamoyl Ph at C-7)

f) 130.505 (C-8 quaternary C of Ph), 134.350 (quaternary C of Ph of cinnamoyl moiety at C-7), 141.429 (C-2 quaternary C of Ph), and 141.518 (C-6 quaternary C of Ph)

v) The mass spectrum (Fig: , page:) of the compound (**17**) showed the following major peaks at m/z:

77(23%), 91(33), 104(21), **131**(100%), 132(53), 154(73), 193(35), 239(28), 267(56), 307(11), 371(05), 381(03), 411(03), 425(03), **502** (44%, M^+), and 504 (10%, M^++2).

Appearance of some of the above peaks in the mass spectrum of compound (**17**) may be explained as below (Scheme: , page:):

a) $C_{34}H_{30}O_2S$ (M^+, m/z: **502**) – Ph(**77**) = m/z: **425** ($C_{28}H_{25}O_2S$)

b) ($C_{28}H_{25}O_2S$, m/z: **425**) – CH_2(14) = m/z: **411** ($C_{27}H_{23}O_2S$)

c) $C_{34}H_{30}O_2S$ (M^+, m/z: **502**) – (cinnamoyl, C_9H_7O, m/z: 131) = ($C_{25}H_{23}OS$, m/z: **371**)

d) ($C_{25}H_{23}OS$, m/z: **371**) – $PhCH{=}CH_2$ (m/z: **104**) = m/z: **267** ($C_{17}H_{15}OS$)

e) ($C_{17}H_{15}OS$, m/z: **267**) – CO (m/z: 28) = m/z: **239** ($C_{16}H_{15}S$)

f) ($C_{16}H_{15}S$, m/z: **239**) – $S{=}CH_2$ (m/z: 46) = m/z: **193** ($C_{15}H_{13}$)

g) ($C_{15}H_{13}$, m/z: **193**) – C_3H_3 (m/z: 39) = 2 Ph (m/z: 154)

h) Ph• + •Ph = Ph-Ph (m/z: **154**)

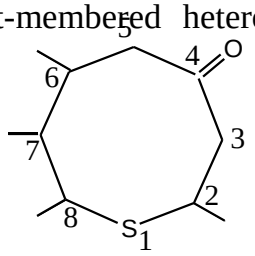
i) Ph• + :CH_2 ⟶ Ph-CH_2^+ (tropylium ion, ⊕ m/z: 91)

Dibenzylidene acetone (DBA,Ph-CH=CH-CO-CH=CH-Ph) containing sodium sulphide (or ammonium sulphide) were allowed to react both at room temperature and at reflux temperature in molar ratios 1:1, 1:2, and 2:1 in methanol (and ethanol) as solvent to obtain a crystalline solid, melting temperature: 191^0 - 192^0 C in various percentage of yields (Table: , page:).

The compound in its classical tests exhibited the presence of carbonyl group and sulphur atom in it. The presence of two different carbonyl (at δ/ppm: 211.945 and 197.504) groups was evident from its ^{13}C-nmr spectrum.

Ring carbonyl carbons generally resonate at lower filed than those of open-chain carbonyl carbons. One of the above two carbonyl groups, the low field one (at δ/ppm: 211.945) may be a part of a ring and the rest one is in a side chain.

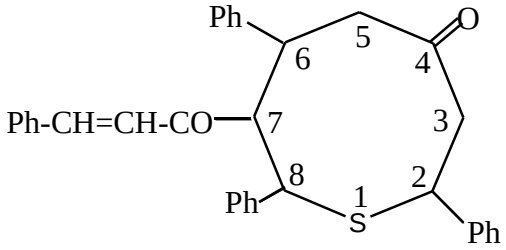
The ^{13}C-nmr spectrum of the compound also indicates the presence of six aliphatic carbons (at δ/ppm: 41.623, 43.840, 45.278, 45.555, 48.671, and 58.965) of which two are methylene carbon (at δ/ppm: 45.278 and 48.671) as evident from their reversed signals in DEPT-135 spectrum. One carbonyl carbon (the lower field one at δ/ppm: 211.945), one sulphur and the above six aliphatic carbons might have comprised an eight-membered heterocyclic ring known as thiocane as below:



Analysis of the ^{1}H- and ^{13}C-nmr spectral data indicates the presence of four phenyl groups (4 $C_6\underline{\mathbf{H}}_5$). Besides, ^{1}H-nmr also advocates for the presence of two ethenyl protons (trans to each other). Of these four phenyl groups, one may be a part of a cinnamoyl moiety, **Ph**-CH=CH-(C=O)- . The ^{1}H-nmr spectrum also indicates that the compound contains a total of thirty protons. Of these, twenty (4 × $C_6\underline{\mathbf{H}}_5$) belong to four phenyl

groups, two protons belong to two ethenyl protons of the cinnamoyl moiety. The rest of the eight protons (2 × -C**H**$_2$- and 4 × >C**H**-) belongs to thiocane ring.

Of the above eight protons, four belongs to two methylene function (2× -C**H**$_2$-) as evident from the DEPT-135 and the left over four protons belong to four different methine carbons (4 × >C**H**-) of the thiocane ring . Four methine carbons, two methylene carbons, one carbonyl carbon, and one sulphur atom can be formulated to an eight-membered ring having sulphur atom as a part of it. Now of the four phenyl groups, three can be attached to three different carbon of thiocane ring and the left over phenyl group may be a part of the cinnamoyl moiety joined to the fourth carbon of the ring. The rest four atoms of the eight-membered ring, one goes to sulphur, one to carbonyl carbon, and two to two ketomethylene each for the formation of 7-cinnamoyl-2,6,8--triphenylthiocane-4-one (**17**).



(**17**)

7-cinnamoyl-2,6,8-triphenyl –thiocane-4-one (**17**)

The formulated structure of 7-cinnamoyl-2, 6, 8-triphenyl –thiocane-4-one (**17**) fits to the molecular formula $C_{34}H_{30}O_2S$ (molecular mass = 502g mol^{-1}). The mass spectrum of the compound exhibited several peaks at m/z: 77(23%), 91(33), 104(21), 131(100%), 132(53), 154(73), 193(35), 239(28), 267(56), 307(11), 371(05), 381(03), 411(03), 425(03), 502 (44%, M^+), and 504 (10%, M^++2). The peak at m/z: 504 has relative intensity of about 20% of that of at m/z: 502. So the peak at m/z: 504 may be due to the contribution of sulphur-34 isotope. Appearance of some of the above peaks in the mass spectrum of compound (**17**) may be explained as below (Scheme: , page:):

i) $C_{34}H_{30}O_2S$ (M^+, m/z: **502**) – Ph (**77**) = m/z: **425** ($C_{28}H_{25}O_2S$)

ii) ($C_{28}H_{25}O_2S$, m/z: **425**) – CH_2 (14) = m/z: **411** ($C_{27}H_{23}O_2S$)

iii) $C_{34}H_{30}O_2S$ (M^+, m/z: **502**) – (cinnamoyl, C_9H_7O, m/z: 131) = ($C_{25}H_{23}OS$, m/z: **371**)

iv) ($C_{25}H_{23}OS$, m/z: **371**) – $PhCH=CH_2$ (m/z: **104**) = m/z: **267** ($C_{17}H_{15}OS$)

v) ($C_{17}H_{15}OS$, m/z: **267**) – CO (m/z: 28) = m/z: **239** ($C_{16}H_{15}S$)

vi) ($C_{16}H_{15}S$, m/z: **239**) – $S=CH_2$ (m/z: 46) = m/z: **193** ($C_{15}H_{13}$)

vii) ($C_{15}H_{13}$, m/z: **193**) – C_3H_3 (m/z: 39) = 2 Ph (m/z: 154)

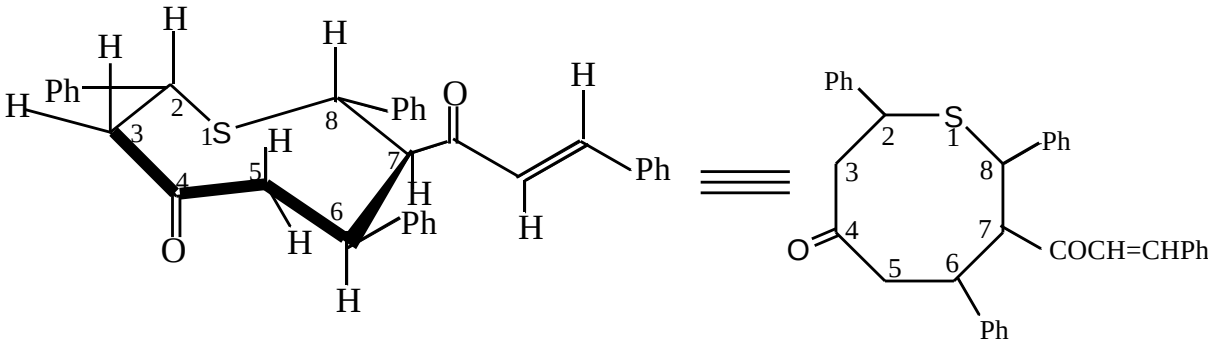
viii) $Ph\bullet + \bullet Ph$ = Ph-Ph (m/z: **154**)

ix) $Ph\bullet + :CH_2 \longrightarrow Ph\text{-}CH_2^+$ (tropylium ion,  , m/z: 91)

Finally, from all the above analyses, it may be concluded that the above mentioned white crystalline solid (**17**) has the above structure.

Moreover, the ^1H- and ^{13}C-nmr data of the compound (14) match in all respect to those obtained from a website [117, 118] (Fig: , page: , Fig: , page: ,Table: , page: , Table: , page:)..

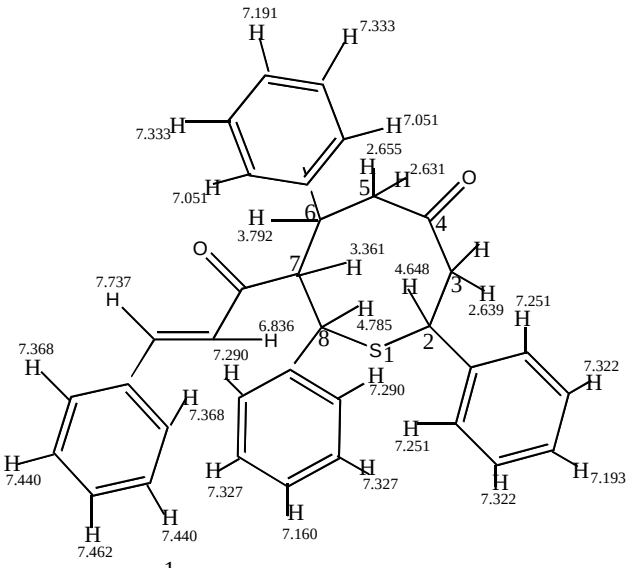
One of the stable conformations of eight-membered ring compounds such as the thiocane is a eight-membered chair conformation as below (**17**):



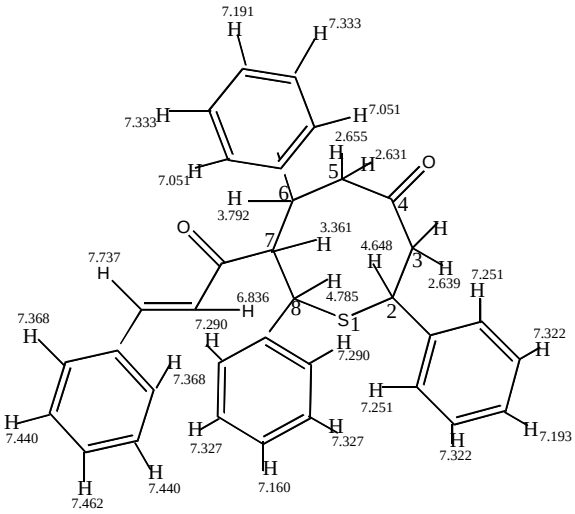
A comperative study of ^{1}H- and ^{13}C-nmr data of the compound 7-cinnamoyl--2,6,8-triphenyl-thiocan-4-one (**17**) was made with those obtained from a website [117] and Chem Draw Ultra 7.0 [118] (Table: , page: ; Fig: , page:), and was found to be almost identical in all respect.

^{1}H-nmr sprctrum obtained from a website [ ] exhibit signals for different type of protons with expected splitting patterns but do not show any proton signals for hydrogen attached to oxygen. The above mentioned website method of generating ^{1}H-nmr signals are theoretical and have their chemical shift values (in δ-scale) may not always match with those of experimental values. However, we generated ^{1}H- and ^{13}C-nmr signals for our compound(**17**) and was found that both the ^{1}H- and ^{13}C-nmr signals for this compound obtained by nmr machine (BRUKER 400 MHz) are quite similar to those generated by www.nmrdb.org in terms of both chemical shifts and splitting patterns.

Our ^{1}H- and ^{13}C-nmr data of the synthesised compound matched with those generated values as obtained from the website www.nmrdb.org.These tables and data are given below :



Values of ^{1}H-nmr obtained from the synthesised compound 7-cinnamoyl-2,6,8-triphenyl--thiocan-4-one(**17**)



Values of H-nmr obtained from the software www.nmrdb.org for the compound 7-cinnamoyl-2,6,8-triphenyl-thiocan-4one(**17**)

Fig 4.1: Comparison of the ^{1}H–nmr values (chemical shift in δ/ ppm) of synthesised compound and the software www.nmrdb.org.

Table: Comparison of ^{1}H-nmr data (in δ/ppm) of compound 7-cinnamoyl -2, 6, 8-triphenylthiocan-4-one (**17**) with those obtained from a web site [117]

Nature of proton	Chemical shift for the synthesised compound (**17**, in δ /ppm)	Chemical shif (in δ /ppm) obtained from the web site www.nmrdb.org
C-2 phenyl protons	*o*-7.295, *m*-7.328, *p*-7.271	*o*-7.251, *m*-7.322, *p*-7.193
C-6 phenyl protons	*o*-7.207, *m*-7.360, *p*-7.347	*o*-7.051, *m*-7.373, *p*-7.191
C-8 phenyl protons	*o*-7.316, *m*-7.344, *p*-7.223	*o*-7.290, *m*-7.327, *p*-7.160
C-7 cinnamoyl phenyl protons	*o*-7.382, *m*-7.416, *p*-7.456	*o*-7.368, *m*-7.440, *p*-7.462
ethenyl protons of C-7 cinnamoyl	6.595 - 6.652, 7.382 - 7.416	6.836, 7.737
C-2 methine proton	4.453 – 4.514	4.648
C-3 methylene protons	2.965 – 3.021, 3.345 – 3.413	2.639, 2.904
C-5 methylene protons	2.821 – 2.851, 3.139 – 3.202	2.631, 2.655
C-6 methine proton	3.929	3.792
C-7 methine proton	3.413	3.361
C-8 methine proton	3.989-4.0156	4.785

Table: Comparison of ^{13}C-nmr data (in δ/ppm) of compound 7-cinnamoyl -2, 6, 8-triphenylthiocan-4-one (**17**) with those obtained from a web site[117]

Nature of carbon	Chemical shift for the synthesised compound (**14**, in δ /ppm)	Chemical shif (in δ /ppm) obtained from the web site www.nmrdb.org
C-2 phenyl carbons	*o*-127.044, *m*-128.194, *p*-129.136 , *q*-141.429	*o*-126.720, *m*-128.574, *p*- 128.924, *q*-137.633
C-6 phenyl carbons	*o*-127.985, *m*-128.469, *p*-128.887, *q*-141.518	*o*-127.990, *m*-128.636, *p*- 128.924, *q*-140.520
C-8 phenyl carbons	*o*-127.733, *m*-128.296, *p*-129.131, *q*-130.505	*o*-127.800, *m*-128.574, *p*- 128.924, *q*-133.750
C-7 cinnamoyl phenyl carbons	*o*-127.400, *m*-128.793, *p*-130.550, *q*-134.350	*o*-127.331, *m*-128.748, *p*- 129.924, *q*-134.718
C-7 cinnamoyl carbonyl carbon	197.504	199.852
ethenyl carbons of C-7 cinnamoyl	126.093, 142.877	125.950, 143.800
C-2 methine carbon	045.555	047.159
C-3 methylene carbon	048.671	043.858
C-4 carbonyl carbon	211.945	208.104
C-5 methylene carbon	045.278	044.537
C-6 methine carbon	043.840	043.909
C-7 methine carbon	041.623	040.950
C-8 methine carbon	058.965	052.933

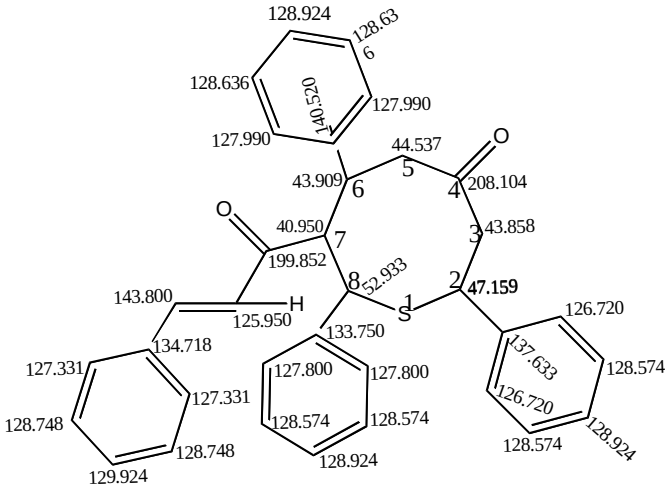
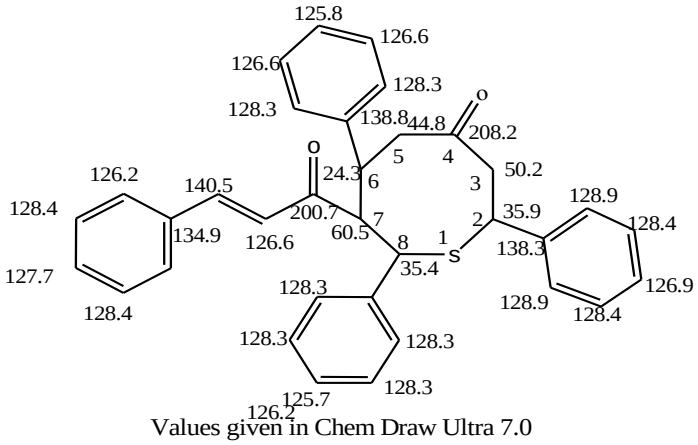
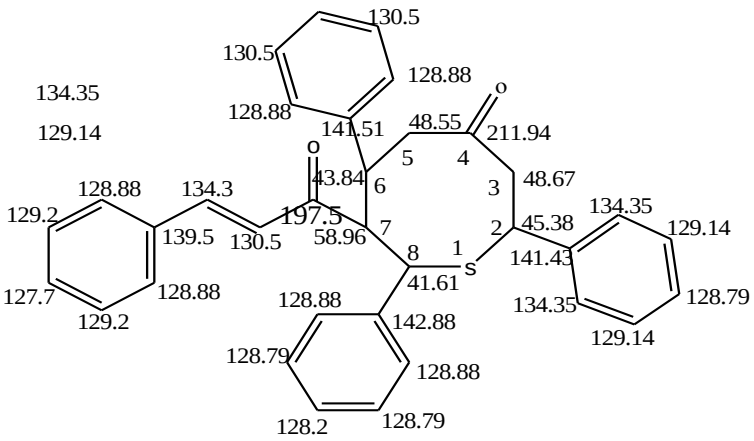


Fig 4.2 : Comparison of the ^{13}C- nmr values (chemical shift in δ /ppm) of synthesised compound and the software www.nmrdb.org.

Table 2: Data for the comparison of the values of ^{13}C- nmr obtained from the synthesised compound and the software www.nmrdb.org

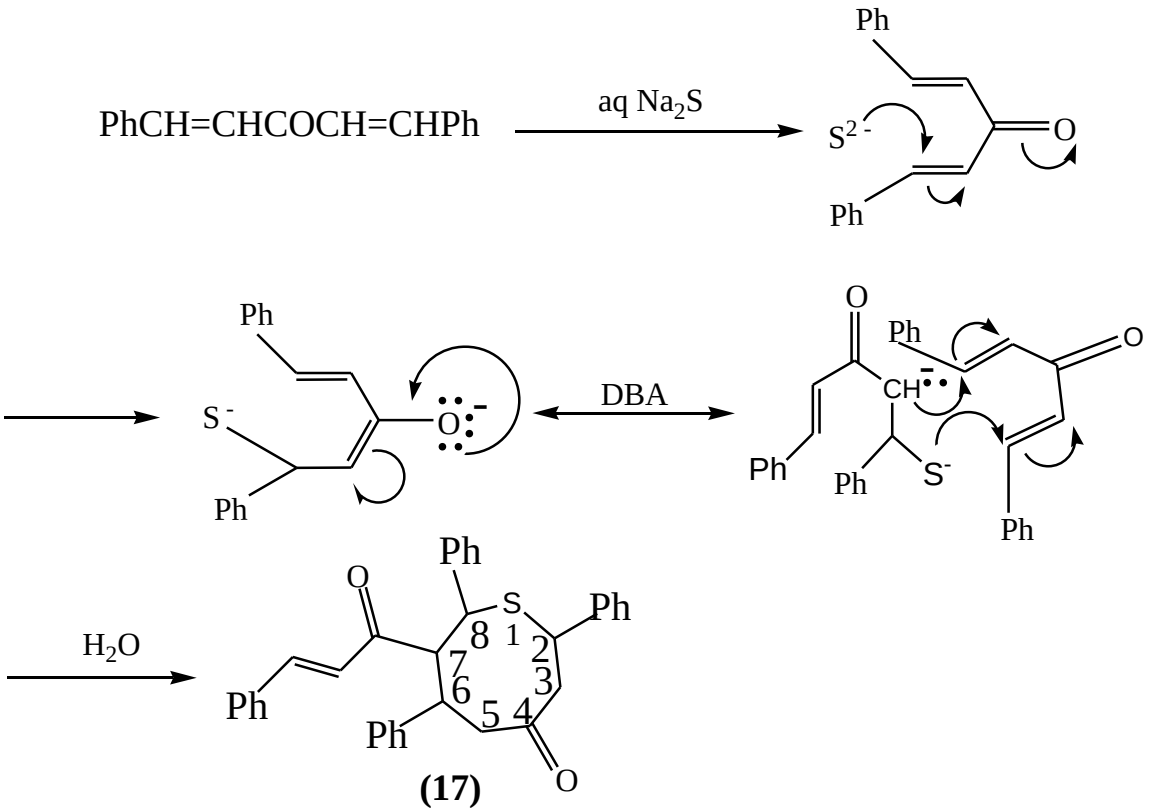


Values given in Chem Draw Ultra 7.0



Values of the prepareded compound

A possible mechanism for the formation of (**17**) is given below:



(17)

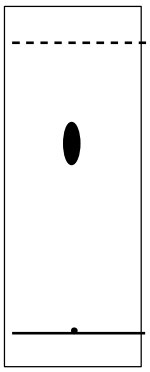
Characterisation of the white powdered solid obtained from the reaction of dibenzylideneacetone (DBA) and sodium sulphide or ammonium sulphide in presence of methanol at different reaction condition.

In the above reaction, when a clear solution of dibenzylideneacetone in methanol was stirred containing sodium sulphide (or ammonium sulphide) at different reaction condition room temperature, the clear solution started becoming turbid and white precipitates separated out immediately. The stirring was then continued for several hours when no further precipitates were formed. The precipitated product was separated by filtration, the remaining filtrate was extracted with diethyl ether, dried and evaporated to result a white powdered solid (T_m: 160^0-162^0 C).

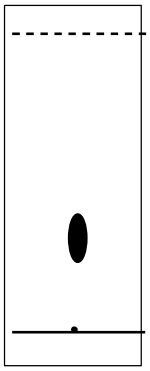
(1) Melting temperature : The white solid melted at 160^0 - 162^0 C.

(2) Solubility: The above white crystalline solid was readily soluble in chloroform, acetone etc.

(3) Tlc behaviour: The compound showed a single spot on tlc in each case when run in a number of different solvent systems.



(EtOAc : Pet ether = 1: 4)
R_f = 0.70



($CHCl_3$: Pet ether = 1:1),
R_f = 0.30

(4) Chemical behaviour: The white powdered solid was when added to a 2,4-DNPH reagent gave no yellow precipitate indicating the absence of carbonyl group in it. The usual qualitative elemental analysis indicated the presence of sulphur in the compound.

(5) Spectral behaviour:

i) The uv-visible spectrum (Fig: , page:) of the compound showed the following peak(s):

$\lambda_{max}^{CHCl_3}$ (in nm) 368,314 nm : $n \rightarrow \pi^*$ Transition of C = O
264 nm $\pi \rightarrow \pi^*$ Transition of C = C

ii) The ir spectrum (Fig: , page:) of the compound exhibited a number of bands / cm^{-1}, some of which were assigned as the follows :

ν_{max}^{KBr} 3400	: O-H stretching of alcohol
3000	: C-H stretching of aromatic and olephanic system
2900	: C-H stretching of aliphatic system
1600	: C=C stretching of the aromatic ring
1490, 1440	: C-C stretching frequency
1350, 1250, 1100, 1060, 1020	: C-H bending of alkyl group
970, 750, 720, 690, 500, 550, 610	: C-H bending of aromatic ring.

iii) The ^{1}H-nmr spectrum (Fig: , page:) of the white powdered compound exhibited signals (in chemical shift) (at δ / ppm) which were assigned as follows:

a) 1.28639 (*b.s* , 1H) : C-4, -O<u>H</u> proton

b) 2.06886 (*d*, 1H),
2.37083 - 2.49212 (*d*, 3H) : four methylene protons of C-3' and C-5'

c) 2.48506 – 2.96192 (*t*, 2H) : Two protons of each C-2', C-6' methine

d) 4.12673 (*s*, 1H) : One C-2 methine proton

e) 4.66034 (*s*, 2H) : Two C-5 methylene proton

This signal would have appeared as a triplet or as a doublet of a doublet (*dd*) instead of a singlet which at the moment could not be explained.

f) 6.89125 – 7.09725 (*m*, 2H) : One C-4 and one C-6 methine protons

g) 7.05775 - 7.51217 (*m*, 20H) : Twenty(4 X $C_6\underline{H}_5$-) aromatic protons of each C_6H_5- group each at C-2', C-6', C-2' and C-6'

(iv) The ^{13}C-nmr spectrum (Fig: , page:) of the compound showed several signals (chemical shift in δ / ppm) which were assigned as follows:

a) 31.646, 40.929, 47.746 : Three aliphatic C-5, C-3', and C-5' methylene carbons of heterocyclic ring

These three peaks appeared as inverse signals in DEPT-135 spectrum (Fig: , page:) indicating that they are for three $-CH_2-$ moity.

b) 43.708, 44.150,
48.470, 73.865 : Four C-2, C-6, C-2'and C-6' carbons

c) 41.623, 43.840, 45.277,
58.965, 48.669 : Six saturated heterocyclic ring carbons

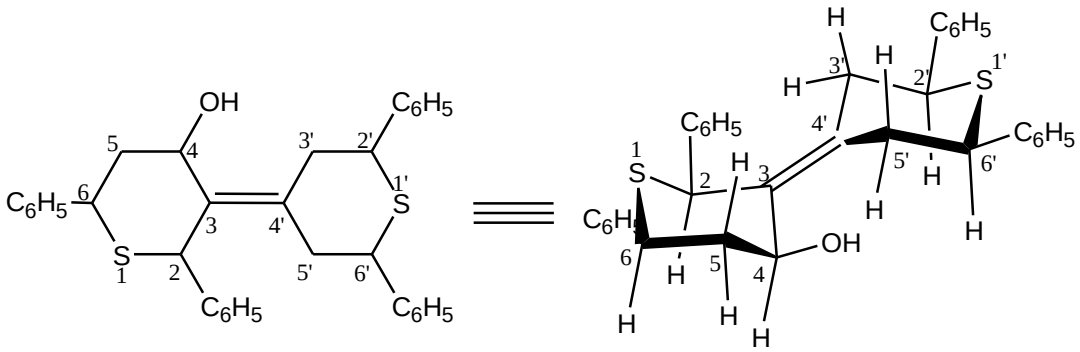
d) 126.334, 127.186, 127.440,
127.706, 127.820, 128.575,
128.820, 135.873, 141.077, 141.268 : Aromatic ring carbons of C-2, C-6, C-2', C-6' phenyl group

e) 131.042, 142.813 : Heterocyclic ring quaternary carbon of C-3, C-4'

f) 162.840 : C- 4, heterocyclic ring carbon.

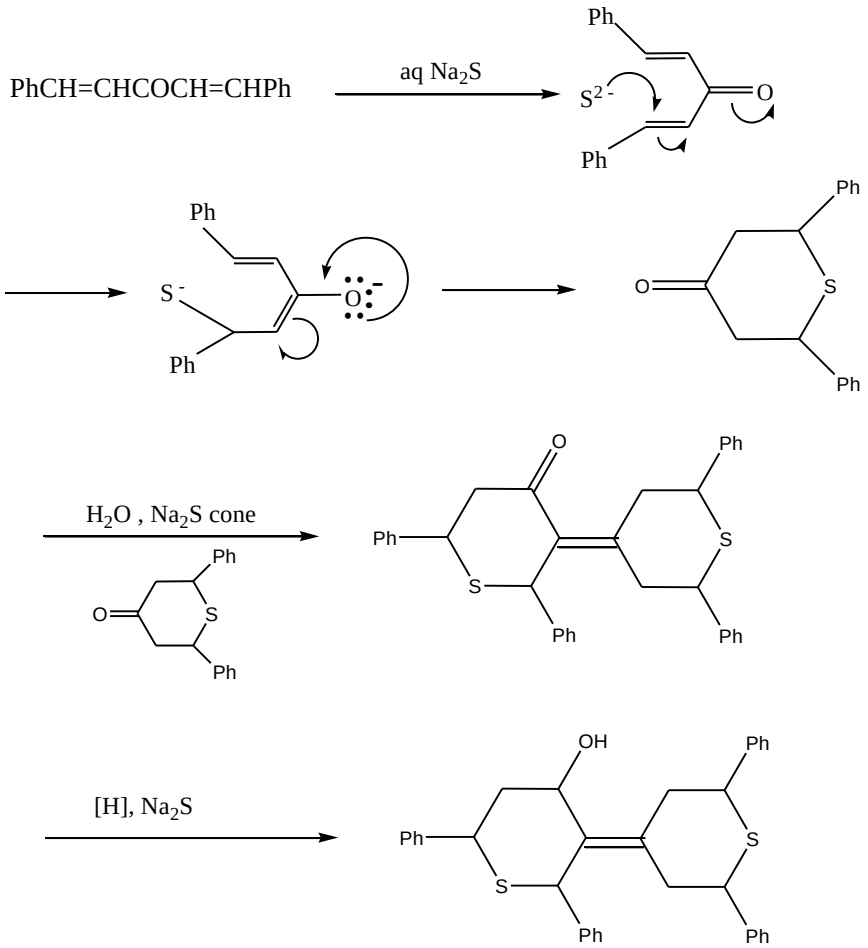
Total number of protons (32 H) in the above structure match with those signalsfouned in its ^{1}H-nmr spectrum. Three reverse DEPT-135 (**Fig**: , page:) signals indicate the presence of three –CH_2–.

On the basis of the above analyses, the structure of the white powdered compound may be as follows:



2,6,2',6'-Tetraphenyl-tetrahydro-[3,4']bithiopyranyliden-4-ol (**18**)

A possible mechanism for the formaltion of the compound(18) is given below:



2,6,2',6'-Tetraphenyl-tetrahydro-[3,4']bithiopyranyliden-4-ol (**18**)

Table : Percentage yield of the products obtained from different reaction

Reaction No.	Dibenzylideneacetone/ (mole)	Na_2S/(mole)	$(NH_4)_2S$/(mole)	Condition	Solvent	% Yield of Compound	
						Thiocane T_m :191^0-192^{0}C	T_m: 160^0-162^{0}C
1.	1	2		Room Temp.	MeOH	25	18
2.	1	2		Reflux Temp.	MeOH	25	18
3.	2	1		Room Temp.	MeOH	30	20
4.	2	1		Reflux Temp.	MeOH	30	20
5.	1	2		Room Temp.	EtOH	25	
6.	1	2		Reflux Temp.	EtOH	25	
7.	1		1	Room Temp.	MeOH	15	8
8.	2		1	Room Temp.	MeOH	20	12
9.	1		1	Room Temp.	EtOH	15	
10.	2		1	Room Temp.	EtOH	20	

Chapter 3

EXPERIMENTAL

This chapter contains details of experimental techniques followed during the Present study.

3.1 GENERAL TECHNIQUES AND EQUIPMENT

Various general techniques and equipments that have been employed during this work are briefly described below

3.1.1 General Techniques

i) Purification of solvents using distillation[155] apparatus

Most of the solvents used in this research were distilled prior to use .

Fig 58 : Distillation set used in Organic Research Laboratory

ii) Concentration of solution using vacuum rotary evaporator

The rotary evaporating flask containing the solution of samples was heated in a water bath. Vacuum was created in the system by a gallenkamp filter water pump. Evacuation with simultaneous heating caused rapid evaporation of the solvent (s) and a solution could be concentrated with a short time without heating to a high

Fig 59 : Vacuum rotary evaporator used in Organic Research Laboratory

temperature because excessive heating may decompose thermally and oxidatively unstable sulphur compounds. In this system the solvent was collected in a receiving flask.

iii) Washing the solid products

Most of the organic products are formed in presence of one or more solvents. So it is necessary to wash the solid products to remove such solvents completely. The ideal washing solvent should comply as far as possible with the following conditions:

i) It should have no solvent action upon the product, but dissolve foreign substances easily.

ii) It should have no dispersive action on the product.

iii) It should be easily volatile at the temperature of drying the product.

iv) Drying and storing of products

Products after separation and purification of solids were dried and also stored under vacuum in a desiccator containing blue silica gel.

v) Determination of physical constants

The most widely used physical constants in the characterisation of the organic compounds are melting temperatures. The techniques of the determination of these are given below:

a) Determination of melting temperature

In general a sharp melting temperature (within 1.0^0 C) is one of the most characteristic properties of a pure organic compound.

The melting temperature and mixed melting temperature were determined either by Gallenkamp melting point apparatus or by using a capillary tube in a paraffin oil bath.

Fig 60 : Melting point apparatus used in Organic Research Laboratory

vi) Separation of the reaction mixtures

The reaction mixture was separated by the following methods:

a) Solvent extraction[156]

The process of extraction with solvent was employed either for the isolation of dissolved substances from solution or from solid mixtures or for the removal of undesired soluble impurities from mixture. The solvents generally used were diethyl ether, chloroform and petroleum ether. The selection of solvent depended upon the solubility of the substance to be extracted in that solvent and upon the case with which the solvent could be removed from the solute. Diethyl ether, owing to its powerful solvent properties and its low boiling point (35^{o} C) was the most used one during this work.

b) Fractional crystallisation [157]

Solid organic compounds when isolated from organic reactions were seldom pure; they were usually contaminated with small amount of other compounds, some of which may be unreacted substances and some of which may be produced along with the desired product. The purification of impure crystalline compounds is usually affected by crystallisation from a suitable solvent or mixture of solvents. Solid substances were purified by recrystallisation. The solvents generally used for recrystallisation were acetone, rectified spirit, and ethanol, pet. ether (60^{o}-80^{o} C).

vii) Chromatographic analysis

Chromatography is the most widely used separation technique of chemical mixtures, exploiting differences in their physical/chemical properties. All chromatography relies on the differential distribution of compounds between two phases; one of which is a stationary phase and the other is a mobile phase.

(a)Thin layer chromatography (tlc) [158]

Fig 61 : Tlc-plate prepared in the laboratory

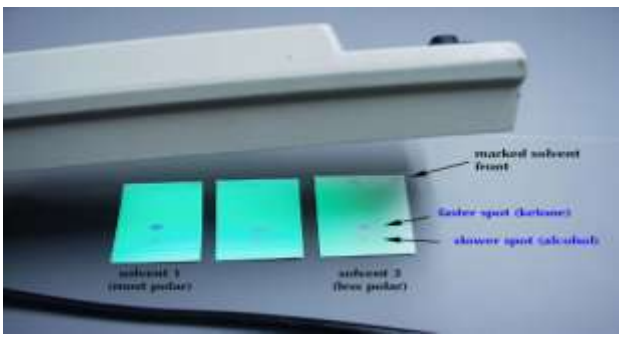


Fig 62 : Visualisation/detection of compounds under UV lamp

The separation and identification of reaction mixture were carried out by thin layer chromatography(tlc). Tlc-grade silica gel G (E Merck) was used for making the plates (stationary phase). Slurry was made with silica- gel and chloroform (1:2) in a previously cleaned and dried reagent bottle. All the air bubbles were removed from the slurry by stirring with a glass rod. Then a pair of clean and dried slides (7.6 x 2.6 cm) were dipped into the slurry and removed immediately.

A uniform silica-gel layer was formed on the plate. The thickness of the layer was 0.2 mm. The plates were dried in the air.

To spot a plate, at first a mark was made about 1cm above the bottom of the plate and the sample solution was spotted by means of a fine narrow capillary glass tube. A suitable solvent or a mixture of solvents were used as the mobile phase in the chromatographic tank of suitable size and fitted with a lid. The spotted TLC plate was then dipped into the solvent so that the spotted marks of the samples remained above the solvent. The plates were removed when the solvent front had reached near the top of the plate, allowed to dry and then the chromatograms were developed in an iodine chamber or visualised in UV light.

The R_f value

The retardation factor (R_f) is the ratio of the distance the compound travel to the distance the solvent front moves.

$$R_f = \frac{\text{Distance from sample front}}{\text{Distance from solvent front}}$$

Usually, the R_f value is constant for any given compound and it corresponds to a physical property of that compound.

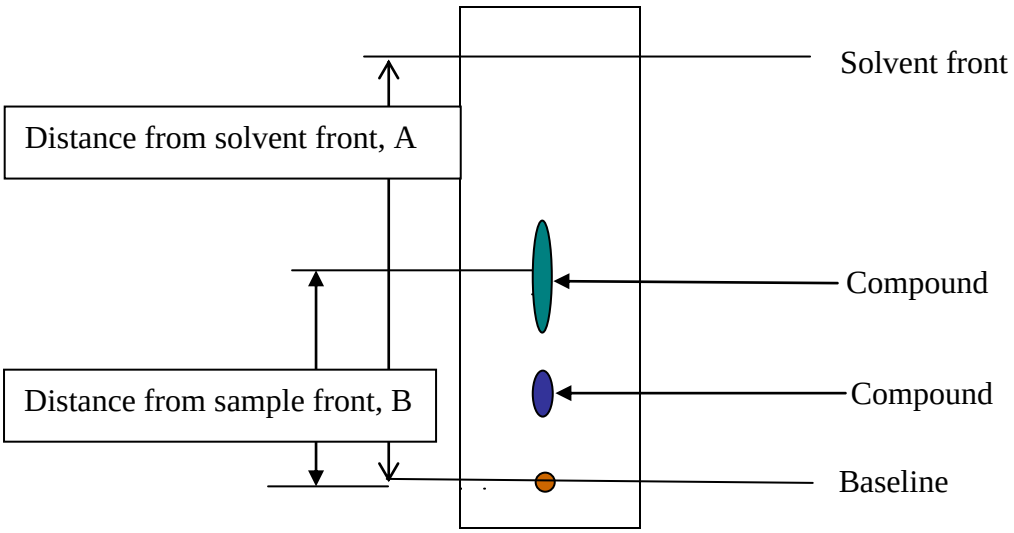


Fig 63 : A Plate for the calculation of R_f value

(b) Column chromatography(cc)[159, 160, 161]

The column (glass, 100cm long, 3cm in diameter) was prepared by the slurry method, silica gel of particle size 60-120 mesh (E. Merck), being the stationary phase. Petroleum ether(60-80^0 c), chloroform,dicholoromethane(DCM), ethyl acetate etc. were used as solvents for making the column. The adsorbent was supported on a plug of glass wool placed at the bottom of the column. Exclusion of air bubble was effected by making the column as quickly as possible. The surface of the column was covered with glass wool also. The mixture of products was applied as concentrated a solution as possible or in a powdered form at the top of the column. The mixture was allowed to be adsorbed on the surface of the column and eluted with the appropriate solvent. The fractions coming out of the column were collected in small portions (20cm^3) in conical flask, concentrated and were monitored by thin layer chromatography to check the progress of separation and where possible identification. The column was gradually eluted with solvents of increasing polarity.

Fig 64 : A column chromatographic separation of a reaction mixture

(c) Gas liquid chromatography(glc) [160]

The GLC was recorded in the department of Chemistryof the University of Dhaka using a “PYE-UNICAM PU 4500 GCD CHROMATOGRAPH”.

3.1.2 Equipments

Spectroscopic analyses

a) Ultraviolet (uv) Spectroscopy

UV-spectra were recorded using a UV-160A, UV-VISIBLE RECORD SPECTROPHOTOMETER, SIMADZU, JAPAN.

b) Infra-red (ir) spectroscopy

The IR spectra of solids were recorded as KBr-pellets or as thin liquid film between sodium chloride plates using an IR-470, SIMADZU, JAPAN, INFRA-RED SPECTROPHOTOMETER.

b) ^{1}H-Nuclear Magnetic Resonance (^{1}H- nmr) Spectroscopy

The ^{1}H-nmr spectra were recorded on a VARIAN XL-400 MHz and BRUKER AVANCE 400 MHz ULTRASHIELD high resolution nmr machine using TMS as an internal standard at BCSIR Laboratories, Dhaka.

c) ^{13}C-Nuclear Magnetic Resonance (^{13}C - nmr) Spectroscopy

The ^{13}C-nmr spectra were recorded on a VARIAN XL-400 MHz and BRUKER AVANCE 400 MHz ULTRASHIELD high resolution nmr machine using TMS as an internal standard at BCSIR Laboratories, Dhaka

3.2 PURIFICATION AND DRYING OF REACTANTS AND SOLVENTS

All solvents and analytical or laboratory grade reactants used during the investigation were manufactured by E Merck (Germany) and BDH (England).

3.2.1 Purification of Reactants

All reactants were used directly after procurement from scientific stores.

3.2.2 Purification and Drying of Solvents

i) Diethyl ether: Diethyl ether was dried over sodium wire and decanted prior to use.

ii) Petroleum ether: (40^0 – 60^0 C and 60^0 - 80^0 C): It was dried over sodium wire and distilled prior to use.

iii) Chloroform: It was dried over sodium wire and distilled prior to use.

iv) Acetone: It was purified by drying over sodium sulphate and collecting the liquid distilling at 54^0 – 56^0 C.

v) Methanol: Commercial grade methanol was purified by distillation and collecting the distillate at 65^0 – 66^0 C.

vi) Ethanol: Commercial grade ethanol was purified by distillation and collecting the distillate at 78^0 – 79^0 C.

vii) Ethyl acetate: Commercial grade ethyl acetate was purified by distillation and collecting the distillate at 76^0 – 78^0 C.

viii) Dichloromethane: It was dried over sodium wire and distilled prior to use

ix) UV-grade ethanol: The commercial grade ethanol was distilled using a calcium chloride guard tube. Fraction distilling at 78^0 – 79^0 C was collected. the distilled ethanol was refluxed with magnesium turnings for about four hours using a calcium chloride guard tube. Ethanol was then distilled at 78^0- 79^0 C. the distilled ethanol was then again refluxed with sodium hydroxide pellets for about three hours. Absolute alcohol was then collected by distillation (T_b: 78^0–79^0 C). This distilled ethanol was further refluxed with silver nitrate for about three hours and finally the liquid distilling at 78^0 – 79^0 C was collected and preserved for using it as UV-grade solvent

The reaction of benzaldehyde with ethanal (in methanol) containing aqueous sodium sulphide:

A mixture of BzH (5.30 g, 0.050 mol) and AcH (1.45 g, 0.033 mol) were dissolved in MeOH (10.0 cm^3) and was transferred to a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel, and a magnetic stirrer. A soln of Na_2S (36.0 g, 0.150 mol) in H_2O (100.0 cm^3) was added dropwise from a dropping funnel to the above mixture while it was stirred continuously at room temperature over a period of ½ hr when the whole mixture became turbid and yellowish. The mixture was then heated to reflux for 2 hr with constant stirring. After this period, the whole content of the flask was left overnight at room temperature. The mixture was then extracted with Et_2O (3×50.0 cm^3), washed (H_2O, 2×25.0 cm^3), dried (anhy Na_2SO_4), and concentrated to a gummy mass which on tlc ($CHCl_3$-pet ether; 2:1) showed the presence of two components. These were separated by column chromatography (silica gel) using $CHCl_3$-pet ether (60^0-80^0 C, 1:1), as an eluent to obtain (**1**, 0.85g, 16%), as the 1st fraction;T_m:153^0-154^0 C (AcMe), lit[102, 103] 153^0 C; R_f: 0.57(silica gel, PhH); uv (λ_{max}^{EtOH}, Fig: 1, page:213): 346.0 and 265.0 nm; ir (ν_{max}^{KBr} ; Fig:2 , page: 214): 3350, 3050, 2900, 2800, 1635, 1590, 1530, 1475, 1350, 1210, 1160, 1125, 1085, 910, 880, 835, 780, 760, 740, 690 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 3 , page: 215): 3.5432-3.7292, 4.5291, 7.0061, 7.2367-7.4128, 9.4161; ^{13}C-nmr (δ/ppm; Fig:4, page:218): 42.567, 45.989, 119.940, 126.810, 127.317, 128.167, 128.273, 128.680, 128.973, 129.140, 130.445, 131.177, 131.792, 133.803, 138.289, 140.861, 154.856, 186.755; and cinnamaldehyde (**2**, 2.71g,(51%), T_b: 248^0- 250^0 C, lit[152] 252^0 C as 2nd fraction. ir(ν_{max}^{KBr} ;Fig: , page:): 3400, 3025, 2875, 2695, 1950, 1870, 1800, 1662, 1600, 1480, 1440, 1360, 1315, 1210, 1170, 1140, 1070, 1030, 910, 750, 690, 590 cm^{-1}.

The reaction of benzaldehyde, cinnamaldehyde and sodium sulphide in aqueous methanol:

BzH (2.65 g, 0.025 mol) and PhCHCHCHO (6.60 g, 0.050 mol) in MeOH (20.0 cm^3) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the methanolic solution over a period of 10 min. During the

addition of Na_2S soln, the whole reaction mixture became brownish in colour. The mixture was then heated to reflux for 2 hrs.. A tlc study showed that it contained 2 components, one of which was the unreacted PhCHCHCHO and another one the Latif product (1). The same reaction with $C_6H_5CHCHCHO$, and Na_2S in MeOH was refluxed for 6 hr and tlc monitoring showed no unreacted $C_6H_5CHCHCHO$. The mixture was then cooled and kept at room temperature overnight. A brown coloured solution having a few precipitates resulted. The whole reaction mixture was extracted with Et_2O (3 × 25.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhy Na_2SO_4), and concentrated in *vacuu* and kept in a refrigerator overnight. A brownish-yellow needle-shaped crystals of compound(**1**) separated out; 0.792 g (12%), T_m:153^0-154^0 C (AcMe), lit[**102, 103**] 153^0 C; R_f : 0.57 (silica gel, PhH); uv (λ_{max}^{EtOH} Fig: 1 , page: 213): 368 and 264 nm; ir (ν_{max}^{KBr} ; Fig: 2 , page: 214) : 3350, 3050, 2900, 2800, 2310, 1635, 1590, 1530, 1478, 1440, 1350, 1260, 12120, 1180, 1150, 1125, 1085, 1025, 1000, 908, 880, 830, 780, 760, 740, 690, 660 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig:3 , page:215): 3.3513-3.7280, 4.5269, 7.0031, 7.2495, 7.4293, 9.4142. ^{13}C-nmr (δ/ppm; Fig: 4 ,page: **218**):42.567, 45.989, 119.940, 126.810, 127.317, 128.167, 128.273, 128.680, 128.973, 129.140, 130.445, 131.177, 131.792, 133.803, 138.289, 140.861, 154.856, 186.755.

The aqueous layer from the above was acidified (aq HCl, 6 M), a heavy precipitate formed which was separated by filtration, then the filtrate was neutralised (aq $NaHCO_3$, 5%). A yellow solid formed, separated by filtration and dried, both weighed; 1.08g, 6%),T_m: 117^0 C, lit[**162**] 118^0 C. The T_m and mixed T_m of the solid with an authentic sample of elemental sulphur, was undepressed.

The filtrate after separation of S, was again acidified (aq HCl, 6 M), thereby formed a white needle-shaped crystals; 3.23g, 50 %), T_m:133^0 C (H_2O), lit[162] 132^0-134^0 C. The T_m and mixed T_m of this compound with an authentic sample of $PhCHCHCO_2H$ was found to be undepressed. The identification of this compound as cinnamic acid was also confirmed by superimposable ir spectrum of authentic

sample. ir (ν_{max}^{KBr} Fig: , page:): 3700, 3025, 2850, 2650, 2550, 2250, 1950, 1880, 1840, 1790, 1680, 1655, 1620, 1480, 1440, 1410, 1300, 1270, 1215, 1170, 1090, 1070, 1020, 975, 940, 910, 870, 850, 760, 710, 680, 620, 580, 540 cm^{-1}.

The reaction of benzaldehyde with sodium sulphide in aqueous methanol:

BzH (2.65 g, 0.025mol) was taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the BzH kept in the three-necked flask over a period of about 15 min. The mixture was then heated to reflux for 2.0 hr with constant stirring. The mixture was cooled and kept at room temperature overnight. The solution was then extracted with Et_2O (3×25.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhyNa_2SO_4), and concentrated. A tlc study ($CHCl_3$-pet ether;1:3) of the concentrate showed that it contained $PhCH_2OH$, and $PhCO_2H$ (comparing the R_f values with those of authentic samples). The concentrated ethereal extract was distilled to obtain $PhCH_2OH$ (T_b: 205^0 C, 25%, lit[162] 205^0 C). The identification of this compound as $PhCH_2OH$ was further confirmed by suerimposable ir spectrum of authentic benzyl alcohol. ir (ν_{max}^{KBr} ; Fig: 55 , page:): 3500-3200, 3052, 2900, 2850, 1585, 1482, 1440, 1400, 1200, 1175, 1050, 730, 690 cm^{-1}.

The aqueous layer left after ethereal extract was acidified (aq HCl, 6M) when heavy precipitate was formed. The filtrate was further acidified, a white needle-shaped crystals formed (T_m: 122^0 C (H_2O), 15 %), lit[162] 121^0- 123^0 C. The T_m and mixed T_m of this compound with an authentic sample of $PhCO_2H$ was undepressed. The identification of this compound as benzoic acid was also confirmed by superimposable ir spectrum of an authentic benzoic acid. Ir (ν_{max}^{KBr} ; Fig 56 , page) : 3700, 3650, 3050, 2825, 2775, 2650, 2550, 2275, 1905, 1790, 1700, 1660, 1590, 1570, 1440, 1414, 1320, 1280, 1180, 1120, 1090, 1060, 1020, 990, 930, 805, 700, 680, 540 cm^{-1}.

The reaction of benzaldehyde with propanone in aqueous sodium sulphide at room temperature:

BzH (5.306 g, 0.05mol) and AcMe(50.0cm^3,0.68 mol) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (11.7 g, 0.015 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the soln of BzH over a period of 15 min. The mixture was then stirred for 6 hr with constant stirring when no further precipitate formed. The precipitates were then separated by filtration, washed(H_2O).The remaining filtrate was extracted with Et_2O (3× 25.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhyNa_2SO_4), and concentrated(in *vacuu*) resulted the same white solid. The combined precipitates were recrystallised (AcMe:pet ether,1:1) formed white needle shaped crystal (**10**, 1.6242 g, 10%); T_m:183^0-185^0 C. A tlc study of the crystals showed the presence of a single compound ($R_{f:}$ 0.55; $CHCl_3$-pet ether, 1:4).uv (λ_{max}^{EtOH}; Fig: 18 , page: 239):289.0 and 251 nm; ir (ν_{max}^{KBr} ; Fig:19 , page: 240): 3410, 3000, 2900, 2300, 1690, 1600, 1510, 1490, 1430, 1415, 1330, 1320, 1300, 1250, 1210, 1195, 1130, 1115, 1030, 1010, 890, 625, 800, 700, 695 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig:20, page:241): 1.272, 1.717, 1.972-2.037,2.206-2.40, 3.209-3.237, 4.501, 4.554-4.582, 7.260-7.276, 7.295-7.338, 7.372-7.403; ^{13}C-nmr (δ/ppm; Fig:21 , page: 246): 30.01, 34.78, 42.88, 46.33, 47.34, 63.79, 71.24, 127.51-128.86, 138.8, 140.78, 216.36. ms (*m/z* ; Fig: 23, page: 250): 65, 77, 91, 250, 265, 308, 326 (M^+).

From the mother liquor on tlc study showed the presence of three components which corresponded to benzylidene acetone, benzoic acid and benzyl alcohol.

The reaction of benzaldehyde with propanone in aqueous sodium sulphide at reflux temperature:

BzH (5.306 g, 0.05mol) and AcMe (50.0 cm^3, 0.68 mol) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (11.7 g, 0.015 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the soln of BzH over a period of about 15 min. The mixture was then refluxed for 4 hr with constant stirring .cooled and kept standing overnight. The reaction mixture formed two layers in which the

upper layer was organic. The upper layer was extracted with Et_2O (3×25.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhyNa_2SO_4), concentrated(in *vacuu*), few drops of benzene added and finally kept in refrigerator for few days resulted white solid which were recrystallised (AcMe:pet ether, 1:1) formed white needle shaped crystal (**10**, 3.1584 g, 18%); T_m:183^0-185^0 C. A comperative tlc study, T_m , mixed T_m of the crystals showed the presence of a same single compound (**10**, $R_{f:}$ 0.55; $CHCl_3$-pet ether, 1:4).

The mother liquor on tlc study, showed the absence of benzylidene acetone but the presence of benzoic acid and benzyl alcohol.

The reaction of benzaldehyde with benzylidene acetone in aqueous sodium sulphide at reflux temperature:

BzH (1.325 g, 0.0125 mol) and PhCHCHAc (3.65 g , 0.025 mol in 10.0 cm^3 MeOH) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (11.7 g, 0.15 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the above soln over a period of about 15 min. The mixture was then refluxed for 4 hr with constant stirring, cooled and kept standing overnight. The reaction mixture formed two layers in which the upper layer was organic. The upper layer was extracted with Et_2O (3×25.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhyNa_2SO_4), concentrated(in *vacuu*) , few drops of benzene added and finally kept in refrigerator for few days resulted white solid which were recrystallised (AcMe-pet ether, 1:1), formed white needle shaped crystals (**10**, 3.1602 g, 20%); T_m:183^0-185^0 C. A tlc study of the crystals showed the presence of a single compound ($R_{f:}$ 0.55; $CHCl_3$-pet ether, 1:4).uv (λ_{max}^{EtOH} ; Fig: 18 , page: 239): 289.0 & 251.0nm; ir (ν_{max}^{KBr}; Fig: 24, page: 252): 3450, 3000, 2900, 1700, 1600, 1490, 725, 695 cm^{-1}. From the aqueous layer after acidification (aq HCl,6N) and ether extraction was concentrated. A tlc study showed a mixture of two compounds.The crystals formed were separated.(**12a,** T_m:122^0 C(H_2O),18 %), lit[162] 121^0- 123^0 C. The T_m and mixed T_m of this compound with an authentic sample of $PhCO_2H$ was undepressed. The rest liquid mass was distilled to obtain $PhCH_2OH$ (**12,** T_b: 203^0-204^0 C,20%,lit[162] 205^0 C).

The reaction of benzaldehyde with phenylethanone and sodium sulphide in aqueous methanol at reflux temperature:

A mixture of BzH (1.06 g, 0.01 mol), AcPh (2.4 g, 0.02 mol) and MeOH (25.0 cm^3) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (2.34 g, mol) in H_2O (25.0 cm^3) was added dropwise from the dropping funnel to the above mixture over a period of about 10 min. The mixture was then refluxed for 4 hr with constant stirring .cooled and kept standing overnight. The mixture was extracted with Et_2O (3×25.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhy Na_2SO_4), concentrated (in vacuu) resulted a yellowish liquid. A tlc study of the yellowish liquid showed the presence of three compounds (R_f: 0.20, 0.40, 0.50; $CHCl_3$-pet ether, 1:4) which could not be separated by crystallization. The components of the mixture were separated by column chromatography using a mixture of dichloromethane-pet ether (60^0-80^0 C) as eluent of mobile phase and silica-gel (60-120 mesh) stationary phase respectively. Several weeks were necessary to complete the column efficiently and successfully. From the three fractions collected, first and last fraction were discarded due to very little amount. The middle fraction i.e the second fraction produced a white needled-shaped crystals (**11**, 1.7106 g, 20 %); T_m:178^0-180^0 C. uv (λ_{max}^{EtOH}; Fig: 25 , page: 253) :346 & 265 nm; ir (ν_{max}^{KBr}; Fig: 26 , page:254): 3390, 3080-3020, 1647, 1600, 1582, 1495, 1335, 1305, 1275, 1235, 1205, 1170, 1072, 1040, 1020, 1000, 760, 742, 705, 687 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 27 , page:255):2.37, 2.57,4.56, 4.92, 4.97,5.27, 6.91-7.52; ^{13}C-nmr (δ/ppm; Fig: 28 , page:257): 43.4, 46.9, 48.4, 58.1, 76.0,124.4, 126.8, 127.3-127.9, 128.0-128.4, 132.6, 137.4, 137.6, 140.4, 145.4, 206.6; ms (*m/z* ; Fig: 29 , page: 260): 51, 77, 91, 105, 327, 432, 450 (M^+).

The reaction of benzaldehyde with acetone in the presence of sodium sulphide in water --- A glc study:

To a mixture of BzH (5.3 g, 0.05 mol) and AcMe (4 cm^3, 0.05 mol) in a three necked round bottomed flask equipped with a reflux condenser and a magnetic stirrer was added from a pressure equalising dropping funnel containing an aq soln of Na_2S (11.7 g, 0.15 mol) in H_2O (75 cm^3) dropwise to the reaction vessel over

a period of 30 min. The mixture was heated with constant stirring at 90^0-95^0 C for 2hr on an oil-bath. The reaction was then followed by glc , (Fig: 57 , page: **301**) (5% 1m OV-1 at 120^0 C, N_2-detector temp 250^0 C). An aliquots were withdrawn every 30 min and examined by glc for the presence of benzylidene acetone. The following table shows the variation of the concentration of benzylidene acetone with the progress of the reaction.

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Table 5: Summarised glc-record of the reaction of benzaldehyde with acetone in the presence of sodium sulphide in water

Time in hr	Benzylidene acetone
0	Absent
½	Present
1	Present (highest $conc^n$ of BA))
1½	Present
2	Trace

The reaction of benzylideneacetone with sodium sulphide in water:

To a three-necked round bottomed flask equipped with a reflux condenser and a magnetic stirrer, PhCHCHAc (3.65 g, 0.025 mol) was placed inside the vessel. A pressure-equalising dropping funnel containing an aqueous soln of Na_2S (5.85 g, 0.075 mol in 37.5 cm^3 H_2O) was added dropwise to the vessel over a period of 15 min and then refluxed at for 2 hr on an oil-bath with constant stirring and kept standing overnight. The mixture was then extracted with Et_2O (3× 25.0 cm^3), washed (H_2O), dried (anhy Na_2SO_4), concentrated (by *vacuu*) and finally kept in a refrigerator after the addition of a little amount of benzene in order to remove the gummy condition of the yellowish liquid mass for a week thereby formed white crystals (**10**, 3.1704 g, 20 %); T_m : 183^0-185^0 C. A tlc study of this crystal showed that it was a single compound (R_f: 0.55; $CHCl_3$-pet ether, 1:4).

The reaction of 4-methoxybenzaldehyde with ethanol in aqueous sodium sulphide:

(4)-MeO-C_6H_4CHO (3.40 g, 0.025 mol) and EtOH (50.0 cm^3 in excess) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure--equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the above mixture at 50^0 C over a period of 15 min while the whole mixture became yellow in colour. The mixture was the heated to reflux for 3 hr with constant stirring, cooled, kept at room temperature overnight. A dark yellow coloured soln formed with some brownish needle-shaped crystals which were separated, washed (aq EtOH, 5%) gave 2,6-bis(4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran-5-carboxaldehyde (**7**; 0.102 g, 3%),T_m :170^0-172^0 C ($CHCl_3$-pet ether,1:9); R_f : 0.54 (silica gel , pet. ether-$CHCl_3$ 1:9). . uv(λ_{max}^{EtOH}; Fig: 5 , page: 229): 331.0, 274.0, 225.0 nm; ir (ν_{max}^{KBr}; Fig: 6 , page: 221): 3400, 3050, 2982, 1940, 1890, 1850, 1800, 1750, 1690, 1640, 1620, 1590, 1580, 1500, 1450, 1410, 1380, 1340, 1320, 1280, 1250, 1180, 1110, 1030, 980, 900, 830, 750, 530 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 7, page: 222): 3.5690-3.7225, 3.7410, 3.7510, 4.5145-4.5220, 6.7476-6.8264, 6.8857-7.7255, 9.7453-9.7556; ^{13}C-nmr (δ/ppm; Fig 8:, page: 225): 55.257 , 55.423, 114.049, 114.441, 123.500, 127.637, 130.075, 142.676, 161.551, 188.860.

***The reaction of 4-methoxybenzaldehyde with ethanal in aqueous sodium sulphide*:**

(4)-MeO-C_6H_4CHO (1.70 g, 0.0125 mol) and AcH (0.3625 g, 0.0082 mol) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping-funnel and a magnetic stirrer. A soln of Na_2S (9.0 g, 0.0375 mol) in H_2O (25.0 cm^3) was added dropwise from the dropping funnel to the above mixture over a period of 5 min. The mixture was stirred for ½ hr at room temperature in an ice-water jacket and then refluxed for 2 hr. During heating, the mixture became yellow gradually in colour. After that, the mixture was cooled, kept at room temperature overnight. A dark yellow coloured solution formed

with some brownish needle-shaped crystals which were separated, washed (aq EtOH, 5%), dried (anhy Na_2SO_4)gave 2,6-bis(4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran-5-carboxaldehyde (7, 0.0306g,2%),T_m:171^0-173^0 C ($CHCl_3$-pet ether,1:9).A tlc study showed(silica gel ,$CHCl_3$-pet ether, 1:9) that the above was a single compound, R_f : 0.54; The T_m , mixed T_m ,uv and ir spectra of this compound was identical to compound **7.** uv (λ_{max}^{EtOH} **;** Fig:5 , page: 220): 331.0, 274.0, 225.0 nm; ir (ν_{max}^{KBr} ; Fig:6 , page: 221): 3400, 3050, 2982, 1940, 1890, 1850, 1800, 1750, 1690, 1640, 1620, 1590, 1580, 1500, 1450, 1410, 1380, 1340, 1320, 1280, 1250, 1180, 1110, 1030, 980, 900, 830, 750, 530 cm^{-1}.

The reaction of 2-methylbenzaldehyde with ethanol in aqueous sodium sulphide:

(2)-Me-C_6H_4CHO (1.20 g, 0.01 mol) and EtOH (20.0 cm^3 in excess) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure- -equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (7.2 g, 0.03 mol) in H_2O(20.0 cm^3)was added dropwise from the dropping funnel to the ethanolic soln of (2)Me-C_6H_4CHO contained in the three-necked round bottomed flask at 50^0 C over a period of about 5 min. During the addition of Na_2S soln, the mixture became pale yellow as the temperature raised to reflux and continued for 3 hr with constant stirring, cooled, kept at room temperature overnight. A yellow coloured soln formed with some solid at the bottom of the flask adhering with the magnet. The mixture was extracted with Et_2O (3×25.0 cm^3), washed with H_2O (25.0 cm^3), dried (anhy Na_2S), concentrated. A tlc study showed ($CHCl_3$- pet ether, 2:1) that the mixture contained two compounds. The above viscous liquid was kept in a refrigerator for a few days. The precipitate was separated, filtered and dried under *vacuu* thereby formed the compound2,6-bis(4-methylphenyl)-3-(4-methylbenzyl)-2H-thiopyran-5-carboxal- -dehyde,(8, 0.1272g,11%),T_m:138^0-140^0 C (PhH-pet ether,1:3); R_f : 0.58 (silica gel ,PhH-pet ether, 1:2); uv(λ_{max}^{EtOH} ; Fig: 9, page: 226): 345.0, 284.0, 233.0 nm; ir (ν_{max}^{KBr}; Fig: 10, page: 227): 3010, 2900, 2850, 2725, 2325, 1940, 1910, 1790, 1680, 1640, 1590, 1580, 1475, 1450, 1375, 1275, 1210, 1155, 1110, 1030, 1000, 940, 860, 830, 740, 660, 635, 610 cm^{-1};

^{1}H-nmr (δ/ppm; Fig: 11 , page: 228): 10.2785, 9.1620, 7.1473-7.2842, 6.9180, 4.7030-4.7358, 3.594-3.669, 3.405-3.441 δ (ppm)

The reaction of 2-methylbenzaldehyde with ethanal in methanol containing aqueous sodium sulphide:

(2)-Me-C_6H_4CHO (1.20 g, 0.01mol) and AcH (0.2634 g, 0.006 mol) in MeOH (10.0 cm^3) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S(7.2 g, 0.03 mol) in H_2O (20.0 cm^3) was added dropwise from the dropping-funnel to the methanolic mixture of (2)-Me-C_6H_4CHO and AcH contained in the three-necked round bottomed flask at 50^0 C over a period of about 5 min. The mixture was stirred for half an hour in an ice-cold jacket and then refluxed for 2 hr with constant stirring. During the heating, mixture became white turbid to pale yellow, cooled, kept at room temperature overnight. A yellow coloured solution formed. The mixture was extracted with Et_2O (3×25.0 cm^3), washed with H_2O (25.0 cm^3), dried (anhy Na_2SO_4), concentrated. A tlc study showed ($CHCl_3$-pet ether, 2:1) that the mixture contained two compounds. The above viscous liquid was kept in a refrigerator for a few days. The precipitate separated was filtered, washed (aq EtOH, 2%), dried under *vacuu*. thereby formed the compound (**8**, 0.125 g, 10%); T_m: 138^0-140^0 C (PhH-pet ether, 1:3); R_f : 0.58 (silica gel ,PhH-pet ether, 1:2). uv (λ_{max}^{EtOH}; Fig:9, page: 226): 345.0, 284.0, 233.0 nm; ir (ν_{max}^{KBr}; Fig:10 , page: 227): 3010, 2900, 2850, 2725, 2325, 1940, 1910, 1790, 1680, 1640, 1590, 1580, 1475, 1450, 1375, 1275, 1210, 1155, 1110, 1030, 1000, 940, 860, 830, 740, 660, 635, 610 cm^{-1} .

The reaction of 2-nitrobenzaldehyde with ethanol in aqueous sodium sulphide:

(2)-O_2N-C_6H_4CHO (3.778 g, 0.025 mol) and EtOH (50.0 cm^3, in excess) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping-funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping-funnel to the above mixture over a period of 15 min at 50^0 C. The mixture was kept for a

further period of 2 hr with constant stirring at the same temperature, cooled, kept at room temperature overnight. The content in the flask was extracted with Et_2O (3×50.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhy Na_2SO_4). The ethereal soln was concentrated in *vacuu*. A tlc study ($CHCl_3$) showed that the mixture contained two compounds.

The above gummy mass was dissolved in AcMe and kept in a refrigerator for a few days. A yellow solid separated out (**9**, 0.7 g, 18%); T_m: 270^0- 275^0 C (d). uv ($\lambda_{max}^{CHCl_3}$; Fig: 12, page: 231): 368.0, 286.0, 236.0 nm; ir ν_{max}^{KBr} ; Fig: 13 , page: 232): 3250, 3050, 2905, 2850, 2700, 2305, 1935, 1900, 1790, 1720, 1660, 1600, 1470, 1440, 1360, 1300, 1240, 1220, 1150, 1110, 1080, 1050, 990, 970, 880, 860, 840, 790, 780, 745 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 14 , page: 233): 4.555, 5.330, 5.522 - 5.567, 6.667 - 6.687, 6.731 - 6.768, 6.929-6.947, 7.104 - 7.122.

***The reaction of 2-nitrobenzaldehyde with ethanal in methanol containing aqueous sodium sulphide*:**

(2)-NO_2-C_6H_4CHO (3.778 g,0.025 mol) and AcH(0.88 g, 0.02 mol) in MeOH (10.0 cm^3) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping-funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping-funnel to the methanolic mixture of (2)-NO_2-C_6H_4CHO and AcH over a period of 15 min. The mixture was stirred for half an hour in an ice cold jacket and then refluxed for 2 hr with constant stirring. During the addition of Na_2S solution, the mixture became light brown to dark brown gradually. The mixture was then cooled, kept at room temperature overnight. The mixture was extracted with Et_2O (3 × 50.0 cm^3), washed (H_2O, 50.0 cm^3), dried (anhy Na_2SO_4) and concentrated in v*acuu*. A tlc study ($CHCl_3$) showed that the mixture contained two compounds.

The above concentrated gummy mass was dissolved in AcMe and kept in a refrigerator for a few days when a yellowish solid separated out (**9**, 0.40 g, 30 %); T_m : 270^0-275^0 C (d). This compound was insoluble in most of the organic solvents. uv($\lambda_{max}^{CHCl_3}$; Fig:12, page: 231): 368.0, 286.0, 236.0 nm; ir (ν_{max}^{KBr}; Fig: 13 , page:232): 3250, 3050, 2905, 2850, 2700, 2305, 1935, 1900, 1790, 1720, 1660, 1600, 1470,

1440, 1360, 1300, 1240, 1220, 1150, 1110, 1080, 1050, 990, 970, 880, 860, 840, 790, 780, 745 cm^{-1}.

***The reaction of 3-nitrobenzaldehyde with ethanol in aqueous sodium sulphide*:**

(3)-O_2N-C_6H_4CHO (3.778 g, 0.025 mol) and EtOH (50.0 cm^3 in excess) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel to the ethanolic solution of (3)-O_2N-C_6H_4CHO over a period of about 15 min at 50^0C.. The mixture was then refluxed for 2 hr with constant stirring, cooled, kept at room temperature overnight. The dark brown solution having solids at the bottom was filtered, washed, (aq AcMe),(1.5g,), T_m:>300^0C(d)]. The compound was insoluble in most of the organic solvents.

***The reaction of 4-nitrobenzaldehyde, ethanol and sodium sulphide in aqueous methanol*:**

(4)-O_2N-C_6H_4CHO (3.778 g, 0.025 mol) and EtOH (50.0 cm^3 in excess) were taken in a three-necked round bottomed flask fitted with a reflux condenser,a pressure-equalising dropping funnel and a magnetic stirrer.A soln of Na_2S(18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel over a period of about 15 min. The mixture was heated to reflux for 2 hr with constant stirring. The mixture was then cooled, kept at room temperature overnight. The solid formed was filtered, washed with aq AcMe(7.0 g , T_m:>290^0C (d)). The compound was insoluble in most of the organic solvents.

***The reaction of 2-chlorobenzaldehyde with ethanol in aqueous sodium sulphide*:**

(2)-Cl-C_6H_4CHO (3.5125 g, 0.025 mol) and EtOH (50.0 cm^3 in excess) were taken in a three-necked round bottomed flask fitted with a reflux condenser,

a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping funnel over a period of 15 min. The mixture was heated to reflux for 3 hr, cooled and kept at room temperature overnight. A solid formed, filtered, washed (aq EtOH, 5%), dried under *vacuu* (0.9132 g, 25%),T_m: 66^0-70^0 C , Lit[162] 68^0-71^0 C; and was identified as 2-chlorobenzyl alcohol (13) by T_m and ir spectrum. ir ($\nu_{max}^{CHCl_3}$; Fig: 15 , page: 235): 3300, 3200, 2900, 2350, 1640, 1560, 1460, 1430, 1360, 1115, 1050, 1025, 980, 940, 860, 800, 740 cm^{-1}.

The aqueous layer was acidified (aq HCl, 6M), heavy precipitate formed, filtered. The filtrate was again acidified (aq HCl, 6N), a white needle-shaped crystals formed (2.0 g, 55%),T_m: 138^0-140^0 C, lit[162]139^0-142^0C; and was identified as 2-chlorobenzoic acid(14) by T_m and ir spectrum. Ir (ν_{max}^{KBr}; Fig:17, page: 238): 3400, 3050, 2950, 2325, 1680, 1580, 1460, 1430, 1400, 1300, 1250, 1040, 950, 910, 810, 790, 740 cm^{-1}.

The reaction of 2-chlorobenzaldehyde with ethanal in methanol containing aqueous sodium sulphide:

(2)-Cl-C_6H_4CHO (3.5125g, 0.025 mol) and AcH (0.88 g, 0.02 mol) in MeOH (10.0 cm^3) were taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (18.0 g, 0.075 mol) in H_2O (50.0 cm^3) was added dropwise from the dropping-funnel to the above mixture over a period of 14 min and then heated to reflux for 2 hr. The mixture was cooled, kept at room temperature overnight. A greenish-brown coloured soln with some brown precipitate formed, filtered, washed (aq EtOH, 5%), dried under *vacuu*. (1.0 g, 20%), T_m: 66^0-70^0 C (aq EtOH, 50%), lit[162] 68^0-71^0 C; and was identified as 2-chlorobenzyl alcohol by T_m and ir spectrum. Ir (ν_{max}^{CHCl3}; Fig:15 , page: 235): 3300, 3200, 2900, 2350, 1640, 1560, 1460, 1430, 1360, 1115, 1050, 1025, 980, 940, 860, 800, 740 cm^{-1}.

The aqueous layer was acidified (aq HCl, 6M), needle-shaped crystals appeared, (2.0 g, 48%), T_m: 138^0-140^0 C (H_2O), lit[152] 139^0-142^0 C; and was identified as 2-chlorobenzoic acid(14) by T_m , mixed T_m and ir spectrum. Ir (ν_{max}^{KBr}; Fig:17 , page: 238): 3400, 3050, 2950, 2325, 1680, 1580, 1460, 1430, 1400, 1300, 1250, 1040, 950, 910, 810, 790, 740 cm^{-1}.

The reaction of benzaldehyde and 2-propanol with aqueous sodium sulphide:

***At room temperature*:** BzH (2.15 g, 0.02 mol) in 2-PrOH (50.0 cm^3) was taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure--equalising dropping funnel and a magnetic stirrer. A soln of Na_2S (4.68g,0.06mol) was added dropwise from the dropping funnel to the above mixture over a period of 15 min at room temperature. During the addition of Na_2S soln, the system was stirred constantly with a magnetic stirrer and the stirring was continued for 6 hours. The reaction mixture was extracted with ethoxyethane, dried (anhyd Na_2SO_4) and resulted a pale yellow liquid . The liquid on tlc examination showed the presence of three compounds, which was separated by column chromatography using (silica gel, 60-120 mesh) as stationary phase and (pet ether-PhH,1:1) as mobile phase resulted a white crystalline solid (15) T_m: 137^0-139^0 C (20% yield). A tlc study of the crystals showed the presence of a single compound (15) (R_f: 0.60; $CHCl_3$-pet ether, 2:1). uv (λ_{max}^{EtOH}; Fig: 30 , page: 261a): 301.0 &205.0 nm; ir (ν_{max}^{KBr}; Fig: 31 , page: 262): 3020, 2870, 1640,1590,1480,1440,1210, 1170, 1060, 1020, 800, 745, 690 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig:32, page: 263): 1.53, 1.70 -1.76, 5.46-5.49, 5.63, 7.05-7.89 ; Another compound was a pale yellow solid (16, 1.332g, 10%), T_m: 105^0-107^0 C. A tlc study of the solid showed the presence of a single compound (R_f: 0.65; $CHCl_3$-pet ether, 1:1). uv (λ_{max}^{EtOH} ; Fig: 33 , page: 267): 301.0 & 220.0 and 221.0 nm; ir (ν_{max}^{KBr}; Fig:34 , page: 268): 3350, 3120, 2340, 1615, 1440, 1390, 880, 770, 690 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 35, page: 269): 1.000, 1.273, 7.261-7.452, 7.515 -7.902.; ^{13}C-nmr (δ/ppm; Fig: 36, page: 270): 29.608, 126.801, 128.417, 131.932, 202.813. A very trace amount of gummy mass was discarded.

The reactions of benzaldehyde and 2-propanol were also repeated under various conditions such as (i) elevated temperature, (ii) reflux temperature in presence and in the absence of alkali as above. Each of these reaction was also worked out by following the same procedure as earlier and obtained the same compounds (**15** & **16**).

The following table (Table: 6, page: 159) summarises reaction condition, percent yield, melting temperature, etc :

Table 6: Summarised results of the reactions of benzaldehyde and 2-propanol at different conditions

Reaction Condition			Product (%)	
Temp	with alkali	without alkali	Comp (15)	Comp (16)
Room Temp	_	✓	25	15
Elevated Temp	_	✓	18	10
Reflux Temp	_	✓	10	5
Room Temp	✓	_	30	20
Elevated Temp	✓	_	20	15
Reflux Temp	✓	_	15	8

The reaction of benzaldehyde and 2-propanol with hydrogen sulphide:

***At room temperature*:** BzH (2.15 g, 0.02 mol) in 2-PrOH (50.0 cm^3) was taken in a three-necked round bottomed flask fitted with a reflux condenser. Hydrogen sulphide gas was passed to the above mixture over a period of one hour at 0^0 C in an ice-bath. The mixture was shaked occasionally and passing of H_2S was continued for eight hours . Then the reaction mixture was extracted with ethoxyethane, dried (anhyd Na_2SO_4) and resulted a pale yellow liquid. The liquid on tlc examination showed the presence of three compounds, which was separated by column chromatography using (silica gel, 60-120 mesh) as stationary phase and (pet ether-PhH,1:1) as mobile phase, resulted an off-white solid (15a) T_m: 62^0-65^0 C (15% yield). A tlc study of the solid showed the presence of a single compound (R_f: 0.70; $CHCl_3$-pet ether, 2:1). uv (λ_{max}^{EtOH}; Fig: 38 , page: 272): 288.0 nm; ir (ν_{max}^{KBr}; Fig:39 , page: 273): 3340, 3000, 2850,1570,1530,1490,1215, 890, 760, 690 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 40, page:274): 3.640, 4.066, 4.192, 7.268 -7.394; ^{13}C-nmr (δ/ppm; Fig:41, page: 275):43.087, 43.208, 43.572, 44.200, 127.361-129.352, 137.297.

Another compound was a pale yellow solid (16, 0.6592g, 5%), T_m: 105^0-107^0 C. This pale yellow solid was same as that pale yellow solid produced in presence of sodium sulphide as evident from the melting temperature, mixed melting temperature, superimposable ir-spectra, etc.

The reaction of dibenzylideneacetone(DBA) and sodium sulphide in methanol at room temperature (molar ratio1:2):

Dibenzylideneacetone,$(PhCHCH)_2CO$ (DBA) (2.34 g, 0.01 mol) in MeOH (60.0 cm^3) was taken in a three-necked round bottomed flask fitted with a reflux condenser, a pressure-equalising dropping funnel and a magnetic stirrer were placed in an oil-bath and was heated (≈ 50^0 C) for about 8-10 min with stirring when the DBA dissolved in MeOH. The three-necked flask was then taken out of the oil bath and was continuously stirred at room temperature. A soln of Na_2S (1.56g, 0.02 mol) in H_2O (100 cm^3) was added dropwise from the dropping funnel to the above mixture over a period of 15 min at room temperature and the system was stirred continuously for 20 hr when no further precipitates formed. The precipitates were then separated by filtration, washed (aq AcMe), recrystallised (AcMe) to a fine white crystalline solid (**17**, 1.0072 g, 20 %); T_m: 190^0-192^0 C. A tlc study of the solid showed the presence of a single compound (R_f: 0.55; $CHCl_3$-pet ether, 1:4). uv (λ_{max}^{EtOH} ; Fig: 42 , page: 276) : 368.0,314.0 and 264 nm; ir (ν_{max}^{KBr} ; Fig: 43, page: 277): 3450, 3005, 2325, 1690, 1640, 1580, 1485, 1440, 1325, 1190, 1090, 1060, 1060, 1020, 980, 750, 695 $.cm^{-1}$; ^{1}H-nmr (δ/ppm; Fig: 44 , page: 278): 7.4564-7.2075, 6.5957-6.5552, 4.4849, 4.0155-3.9298, 3.4132-3.3458, 3.1596-3.1395, 3.0214-3.0087, 2.8572-2.8212; ^{13}C-nmr (δ/ppm; Fig:45 , page: 281): 211.945, 197.504, 142.877, 141.518, 141.429, 139.457, 134.350, 130.505, 129.136, 128.887, 128.793, 128.469, 128,296, 128.194, 127.984, 127.733, 127.400, 127.044, 126.093, 58.965, 48.669, 45.277, 43.840, 41.623; ms (*m/z* ; Fig:47, page: 287):502, 371, 268, 191, 131, 115.

The remaining filtrate was extracted with Et_2O (3×50 cm^3), separated, washed (H_2O), dried (anhy Na_2SO_4) which on evaporation resulted a white powdered solid (**18**, 0.7684g, 15 %); T_m: 160^0-162^0 C. A tlc study of the solid showed the presence of a single compound (R_f: 0.60; $CHCl_3$-pet ether, 1:4). uv ($\lambda_{max}^{CHCl_3}$; Fig:50 , page:288): 291.0 nm; ir ν_{max}^{KBr} ; Fig:51 , page: 289): 3400, 3000, 2900, 2350, 1600, 1480, 1440, 1350, 1250, 1100, 1060, 1020, 970, 750, 720, 690, 610, 500, 550 cm^{-1}; ^{1}H-nmr (δ/ppm; Fig: 52 , page: 290): 1.28693, 2.06886, 2.37083, 2.49212, 2.96192-

2.84506, 4.12673, 4.66034, 7.51217-6.89125; ^{13}C-nmr (δ/ppm; Fig: 53 , page:293): 31.686, 40.929, 43.708, 44.150, 47.746, 48.470, 73.865, 126.344, 127.186, 127.443, 127.706, 128.575, 128.820, 131.042, 135.072, 135.873, 141.077, 141.268, 142.813, 162.840.

The reactions of dibenzylideneacetone (DBA) and Na_2S were repeated at different reaction conditions using various DBA and Na_2S molar ratios following the same procedure as above and was also worked out similarly to obtain the same compounds **17** and **18.**

The following table summarises the reactants, their molar ratio, reaction conditions, solvent, percentage yields, T_m, etc.

Table : Summarised results of the reactions of DBA with sulphides at different reaction

Dibenzylideneacetone (DBA) /(mole)	**Na_2S (mole)**	**$(NH_4)_2S$ (mole)**	**Condition**	**Solvent**	**% Yield of Compound**	
					Thiocanone	**Bithiopyra- lidenes**
1	2	-	Room Temp	MeOH	25	18
1	2	-	Reflux Temp	MeOH	25	18
2	1	-	Room Temp	MeOH	30	20
2	1	-	Reflux Temp	MeOH	30	20
1	2	-	Room Temp	EtOH	25	_
1	2	-	Reflux Temp	EtOH	25	_
1	-	1	Room Temp	MeOH	15	8
2	-	1	Room Temp	MeOH	20	12

conditions

1	-	1	Room Temp	EtOH	15	_
2	-	1	Room Temp	EtOH	20	_

Chapter 4

BIOACTIVITY

PART II

This section mainly contains

- A general discussion on the biological activities
- A concise review on the biological activities of six membered sulphur heterocycle thiopyrans and its derivatives
- Aim of the Present Investigation
- Present Investigation
- Results & Discussion
- Scope of the Present Investigation.

1.1 GENERAL

The first use of synthetic organic chemicals for interference with life- processes was probably started when ether and chloroform were introduced for anesthesia during the first half of the nineteenth century. In consequence, early efforts to find synthetic drugs were concentrated on anesthetics and hypnotics and eventually analgesics. Chloralhydrate appeared in 1869 and paraldehyde in 1862, and the sulphone hypnotics were discovered by accident in 1888. The local anesthetic properties of ethyl *p*-aminobenzoate were known in 1890 and led to the development of procaine hydrochloride (Novocain), the structure of which is based on some features of the cocaine molecule which was introduced as a local anesthetic in 1884. Phenacetin also appeared during this period and its discovery resulted from the observation of hydroxylation and conjugation of aniline in the animal body. This was probably the first drug to be designed as a result of knowledge of a biochemical transformation. Aspirin was introduced in 1899 and it resulted from an attempt to reduce the nausea caused by the salicylates, which had been used as antipyretics. Antipyrine was discovered from investigations of the chemistry of quinine, at this time, and the urethane hypnotics also resulted from the study of compounds produced by the chemical industry.

The next period in the development of medicinal agents was dominated by Paul Ehrlich. He was appointed Director of the Institute for Experimental Therapy in Frankfurt in 1899 at the age of fourty five. By this time, synthetic analgesics, anesthetics and antipyretics were being manufactured by German Chemical Industries. It occurred to him that since these molecules differentiate between cells in man, other molecules might differentiate between cells of man and his parasites.This belief was strengthened by his previous experience in studying the selective staining of various tissues of the mammalian body by

dyes, as well as by his studies of the selectivity of an antibody for the corresponding antigen. Ehrlich was responsible for the discovery of a number of biologically active compounds, although few of them were put to use. Perhaps his greatest contribution to the advancement of medicinal chemistry was the original ideas he had on the modes of drug action. He proposed that receptors exist in mammalian cells and that both antigens and chemotherapeutic agents (a term he coined) possess haptophoric (anchorer) and toxophilic (poisoner) groups. Chemotherapeutic agents, he considered, combine with the receptor areas of the cells by ordinary chemical reactions, a concept still valid, although modified to include more types of bond formation. He also stated that, "the union between the alkaloid and the chemoreceptor is labile and reversible and not firmly bound"- a view still held. His early work on tissue staining techniques led to the discovery of the antimalarial activity of methylene blue in 1891. Further work with dyes resulted in the discovery of the trypanocidal action of trypan red and later of Trypaflavin. A direct result of the later discovery was the finding of the antibacterial acriflavine. His work with arsenicals led to the introduction in 1910 of Salvarsan for treatment of syphilis, only five years after the causative organism of syphilis had been identified. He established the structure of Atoxyl, previously found by Breinl and Thomas to have a favorable effect on human trypanosomiasis.

Drug resistance was also discovered in Ehrlich's laboratories and supported his hypothesis of drug action though chemical combination with cellular receptors. Ehrlich concluded that resistance developed when the drug was no longer absorbed by the parasite (trypanosomes), and in the cause of the arsenicals, the amino group present was the haptophore and the arsenoxide the toxophile. His ideas were thus supported by experimental facts, but they were slow to gain acceptance. Several discoveries were made in the ensuing period by modification of compounds found to be active by Ehrlich and his school. The synthetic antimalarials, Plasmaquine (1926) and Atabrine (1932) were based on the structure of methylene blue, which was found to have antimalarial activity by Ehrlich in 1891. Two of Ehrlich's arsenicals were found to have clinical application for

disease other than trypanosomiasis, carbarsone for amebiasis and oxophenarsine for syphilis. A method for growing Entamoebae *in vitro* was not developed until 1928, which handicapped the discovery of amoebicides before that time. This period following Ehrlich's discoveries and his postulation of a theory of drug action and prior to the discovery of the sulphonamides and antibiotic was characterized by increasing knowledge of the chemistry of natural substances, particularly enzymes. This was greatly assisted by new physical, chemical and biological methods, perhaps the most important being microchemical techniques for determination of structure. Optical techniques, such as X-ray analysis and ultraviolet spectroscopy were also introduced during this period and became of great value.

The first direct evidence of enzymatic involvement by a drug was found by Loewi and Navratil, who showed in 1926 that physostigmine inhibits the enzymatic hydrolysis of acetylcholine. Other drug-enzyme interactions were found in the following years, but it was not until the sulphonamides had been introduced and their mode of action studied that it became evident that drugs might, in general, interfere with normal chemical processes in the cells. By 1940, when Fildes put forward his thesis that chemotherapeutic substances might be designed to compete with essential metabolites or growth factors, thereby destroying an invading organism by competitive antagonism of the function of the essential metabolite, the stage was prepared for the development of present-day medicinal chemistry.

The concept of the drug "Receptor" the portion of a macromolecule that undergoes reaction or "coupling" with a drug molecule to produce a physiological change or pharmacological response, has undergone much modification in the 1960s, 70s and 80s. In contrast to the static "lock and key" view of Ehrlich, receptors are now considered flexible and capable of some variation in different locations (analogous to enzymes) as well as of lateral movement within the framework in which they are embedded (often in the plasma membrane or cell

organelle membrane).Recognized receptors are lipoproteins, proteins (as in enzymes), occasionally lipids and nucleic acids, many antibiotics and anticancer agents interfere with DNA replication or transcription and steroid hormones react with DNA.

The approach to the practice of medicinal chemistry has developed from an empirical one involving organic synthesis of new compounds, based largely on modification of structures of known activity, to a more logical and less intuitive approach. This is mostly because of the findings in molecular biology, pharmacology and enzymology and the mechanics of drug-receptor interactions. Manfred Wolff refers to the present development of medicinal chemistry as a renaissance, stating that: Underlying this new age is a foundation that includes the explosive development of molecular biology since 1960, the advances in physical chemistry and physical organic chemistry made possible by high-speed computers and new powerful analytic methods.

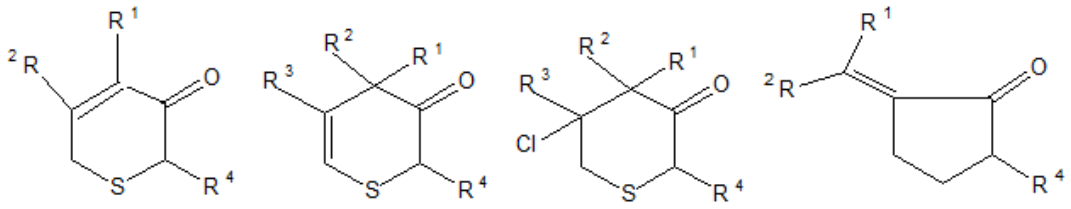
1.2 CONCISE REVIEW ON THE BIOLOGICAL ACTIVITIES OF SOME THIOPYRANS AND ITS DERIVATIVES

Thiopyran compounds and their derivatives have wide applications. In practical field some of the thiopyran compounds and their derivatives are used in medicine, whereas others may be cited to demonstrate outstanding biological and industrial importance. Some of these uses are described below:

1.2.1 Use in Medicine

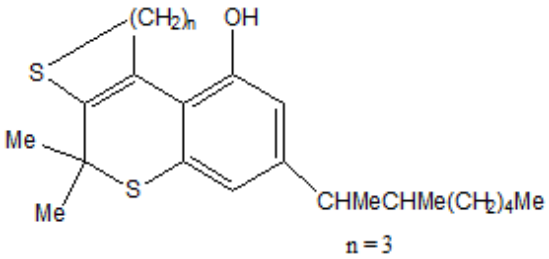
N-(5,6–dihydro-2H-thiopyran-4-yl)tetracycline are antibacterial substances[126] and N-(tetrahydrothiopuran-4-yl) tetracycline were effective against tetracycline-resistant bacteria[127]. Some tetrahydrothiopyran derivatives of phenyl acetic acid ester have the analgesic and anti-inflamatory activities[25]. Carboxymethylpenicillins of the type and/or their Na/Ca–salts are useful as bactericides, food additives, and drugs for the treatment of malitis[24]. A derivative of tetrahydrothiopyran (**42**) is useful as analgesics and local anesthetics [30].

Derivatives of dihydrothiopyran-3-one (**178**) and a tetrahydrothiopyran-3-one derivative are antiinflamatory and diuretic (**179**) agents[128].



9-(3-Quinuclidinyl)thioxanthene and various derivatives has antihistaminic, anticholinergic, neuroleptic, tranquilizing, and antidepressive activities[52]. Miracil-D;LucanthoneB.P.thatis1-dialkylamino-alkylamino-4methylthio xanthones (180) is associated with a decrease in bacteristatic and carcinostatic activity[129] and used clinically in the treatment of human schistosomiasis[130]. The 2,4-diamino-5,7-dihydro-6H-thiopyrano[4',3':4,5]–thieno[2,3-d]pyrimidine can be used as antimalarial drugs[131].Thiopyranobenzopyran derivatives(**1180**) exhibit analgesic and antihypertensive activity[132]. Thiopyrano[3,4-b]indole derivatives can be used as antidepressant[133]. Thiopyranobenzopyran compound can be used as an

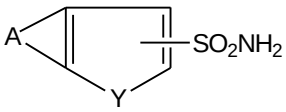
antianxiety,antidepressants and tranqullisers[134]. Another kind of thiopyranobenzopyrans (**181**) had the test for CNS activity[135].



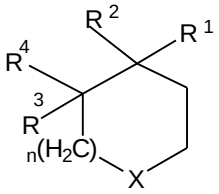
(181)

Aminoalkylthiopyranopyrrols (**182**) were used as anti-inflammatory and antiarrhythmics[136]. 6-Thiatetracyclines[137] are antibacterial agent, superior to all known tetracyclline[138]. Thiopyranopenicillins (**183**) showed bactericidal activity superior to that of carbenicillin[139].

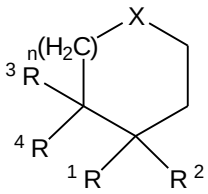
The following compounds (**184, 185, 186**) are cited to be effective glaucoma inhibitors[140].



184



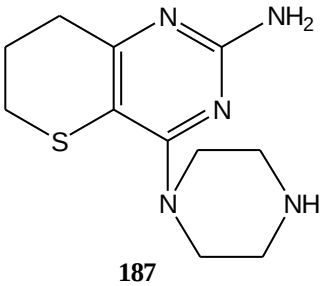
185



186

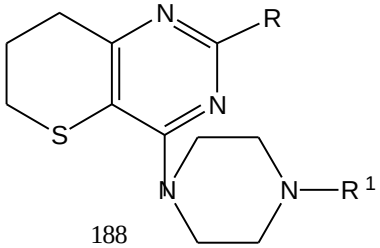
Y = S; NR^3 A= **185**, **186** where R^{1-4} = H, OH, amino etc.
X = S
n = 1,2

In a report it has been revealed that the compound (**187**) has hypoglycemic activity[141].



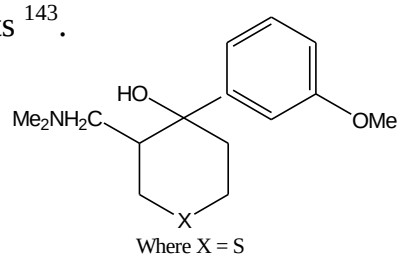
187

Other compounds with similar structure moiety (**188**) have been described as having antidiabetic activities[142] .



188

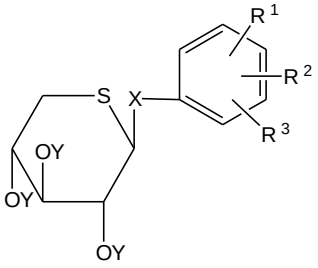
The O- and S- analogue of tramadol exhibited significant analgesic activity in animal experiments [143].



Where X = S

189

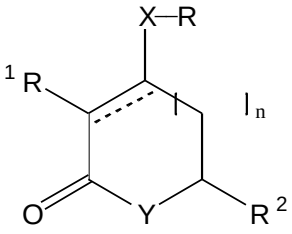
Phenylthio-β-xylosides and derivatives (**101**) are useful as antithrombotics (no data)[71].



X= S, O
R1,R2,R3= H, cyano, COR,
R= alkyl, CF3, NH2, NHAc etc;
Y= H, acyl

101

The compound (**114**) of the type below are useful as medicaments, in particular as antitumor agents (no data)[78].

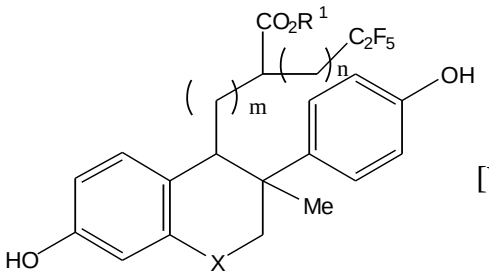


114

Where, Y = S, O, NH; n= 0-1 ;
X = NHCONH, NHCO, $NHSO_2$ etc.
R^1 = H, alkyl, $(CH_2)_pA$ (where in p =0-4;
A = OH, NR^aR^b, CO_2R^c etc. R^aR^b= H, alkyl; NR^aR^b = a 4-7 membered heterocycle; R^c = H, alkyl, alkali or alkaline earth metal; R = alkyl, cycloalkyl, arylalkyl etc.; R^2= H, alkyl

The metal salts of 3-methyl-thiochromanone and chromanone derivative (**116**) exhibit good antiestrogenic activity without substantial agonistic effects, even

when administered orally. Moreover, they are useful for the treatment of estrogen-related diseases, particularly breast cancer[80].

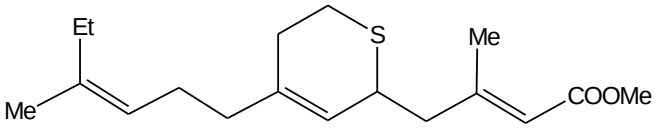


[Where X= S, O; R1= metal; m= 2-14; n= 2-7]

116

1.2.2 Use in insecticides, fungicides and herbicides

The compound (**190**) used against *Dysdercus intermedius* larvas, hence acts as insecticides[144].



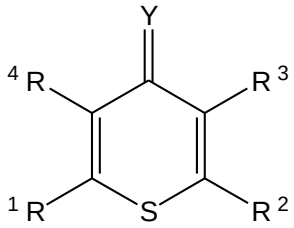
190

Azobis (1-cyanothiocyclohexane) type compound (**34**) can be used as herbicides and insecticides[26]. Thiopyrones of the type (**191**) and their dihydro- and tetrahydro derivatives used as fungicides[145].

Where Y= S, O;

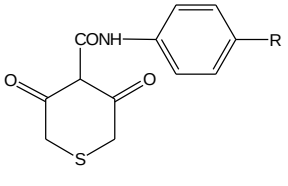
R^1,R^2= H, alkyl, pyridyl, furyl;

R^3,R^4= H, Halo etc.

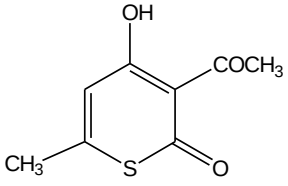


191

Bromoderivatives of 6-methyl-4-methoxy-2H-thiopyran-2-one (**192**) possessed bactericidal and fungicidal antimycotic activities[146]. Various mono- and polybromo derivatives of 3-acetyl-4-hydroxy-6-methyl-2H-thiopyran-2-one (**193**) had the fungicidal and bactericidal activity[147]. A kind of γ-thiopyrone derivatives, apalcillin, piperacillin (**194**) has antibacterial action against *Pseudomonas aeruginosa*[148].3,5-Dioxo-2,3,5,6-tetrahydro-4H-thiopyran-4-carboxanilide derivatives (**171**) were antifungal and antibacterial at 1–100 mg/ml in vitro[149].

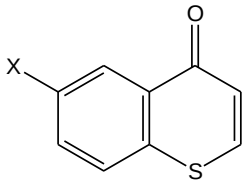


195



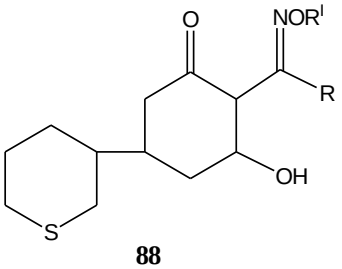
193

The compound (**196**), a benzo-γ-thiopyrone halo derivatives are useful as an insecticides[150].



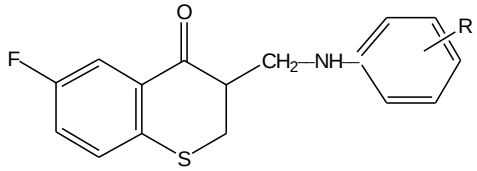
196

5-(Tetrahydrothiopyran-3-yl) cyclohexanone derivatives (**88**) are useful as herbicides (no data)[61].

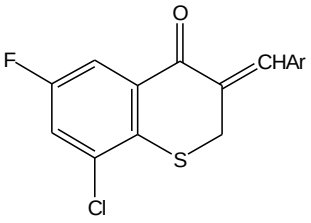


88

Most of the 6-fluoro-thiochromanone (**109**) derivatives and 3- substituted 6,8-dichloro thiochromanones (**110**) showed excellent antifungal activity[76].



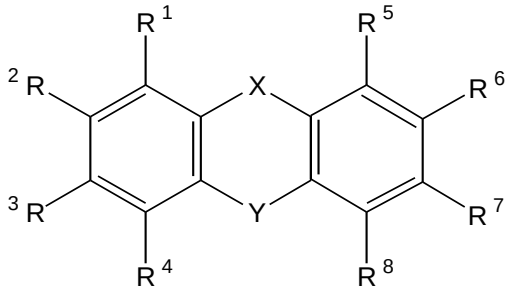
109



110

Where R= 4-F, 4-Br, 2-NO_2 Ar=Ph,2-ClC_6H_4-,2-$NO_2C_6H_4$-, 4-$MeOC_6H_4$-

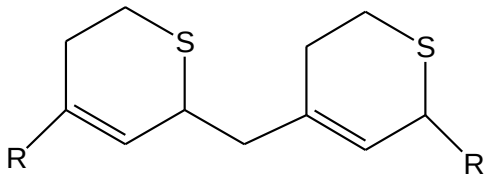
Tricyclic compounds of the type (**123**) can be used as insecticides[82].



123

1.2.3 Use as Biological Importance

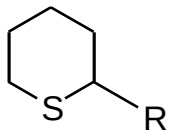
A derivative of Δ^3-dihydrothiopyran that is 5,6-dihydro-2H-thiopyran (**197**) compounds in the following form can be used as recemic cecropia juvenile hormone intermediate[151].



Where R = $CH_2CHMeOH$, $CH_2CMe = CHCH_2OH$

197

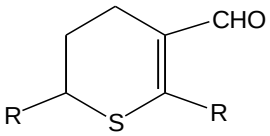
2-Substituted tetrahydrothiopuran of the type (**21**) were useful in the control of mosquitoes[20].



R= MeO,BuO,PrS,BuS,$EtMe_2CS$, PhS,$PHCH_2S$,$(MeO)_2P(S)S$,$(EtO)_2P(S)S$

21

5,6-dihydro-2H-thiopyran-3-carboxaldehyde (**92**) are useful as intermediates for plant protective agents and pharmaceuticals[63].



92

1.3 AIM OF THE PRESENT INVESTIGATION

Thiopyran compounds and their derivatives have wide applications in medicine such as antibacterial substances[104,126], analgesic and anti-inflamatory activities[23], analgesics and local anesthetics [30], anti-inflamatory and diuretic agents[128], anti-histaminic, anti-cholinergic, neuroleptic, tranquilising, and anti-depressive activities[52], bacteriostatic and carcinostatic activity[129] and used clinically in the treatment of human schistosomiasis[130],anti-malarial drugs[131],analgesic and anti--hypertensive activity[132], anti-anxiety, anti-depressants and tranqullisers[134],CNS--activity[135],glaucoma inhibitors[140], hypoglycemic activity[141], anti-diabetic activities[142] , anti-thrombotics [69], anti-tumor agents [78], estrogen-related diseases, particularly of the breast cancer[80].

Besides that, in the agricultural field such as insecticides[26,82,144,150], herbicides [61], insecticides and herbicides [26], antifungal activity[76], antifungal and antibacterial *in vitro*[149]. Other importance in biological field such as juvenile hormone intermediate[151], plant protective agents and pharmaceuticals[63] etc as described in review.

The aim of the present study includes the evaluation of the synthesised sulfur heterocycles for their potentialities as antioxidants, antimicrobial agents against some selective gram-positive and gram-negative bacteria, and fungi as well as effect of these compounds on chromosomal DNA level and at protein level.

1.4 PRESENT INVESTIGATION

The current study includes evaluation of the synthesised sulfur heterocycles for their potentialities as antioxidants, free radical scavengers, and also as antimicrobial agents against some selective bacteria and fungii. The selective gram-positive bacteria were *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megatricum* and *Staphylococcus aureus* and gram-negative bacteria were *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri*, *Shigella boydii* and *Vibrio cholera*. Two fungal strains *Candia albicans* and *Saccharromyces cerevisiae* were studied.

1.4.1 Materials and Methods

A large part of diseases are mainly linked to oxidative stress due to free radicals[163]. Antioxidants can interact with the oxidation process by reacting with free radicals, chelation, catalyzing metals, and also by acting as oxygen scavengers[164].

Literature reviews have shown that there was much effort to invent medicine to overcoming the death. But until recently the actual cause of aging was not known. There is considerable recent evidence that free radical induce oxidative damage to biomolecules. This damage causes aging, diabetes, cancer, malaria, neurodegenerative diseases and other pathological events in living organisms[165]. Antioxidants which scavenge free radicals are known to posses an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidant effects of compounds derived from plants, which could be relevant in relations to their nutritional incidence and their role in health and diseases[166-170].

A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids[171]. Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidant such as *tert*-butyl-1-hydroxitoluene (TBHT), *tert*-butylhydroquinone (TBHQ),butylated

hydroxianisole (BHA) and propyl gallate (PG) are widely used as food additives to increase shelf- life, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, TBHT and BHA are known to have not only toxic and carcinogenic effects on humans[172] but also abnormal effects on enzyme systems[173].

Thus, the interest for synthesised sulphur heterocycles,i.e, thiopyrano derivatives were employed for antioxidant, antibacterial, antifungal bioassay as well as effect of the synthesised compounds on chromosal DNA and at protein level activity study also.

1.4.2 Quantitative Analysis of Total Antioxidant Capacity

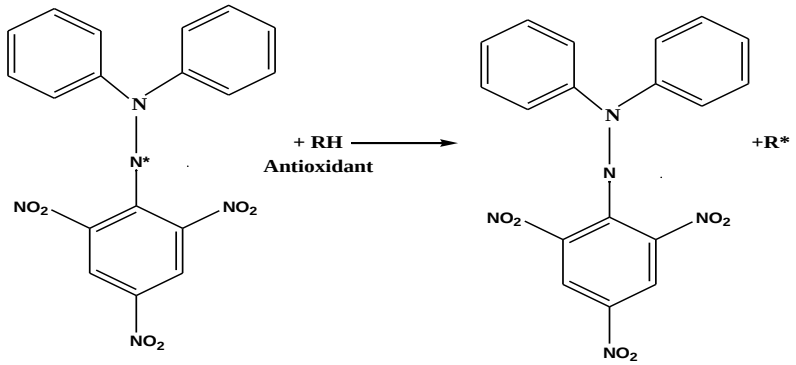
Oxidation processes are intrinsic in the energy management of all living organisms[174]. However, the excessive production of free radicals, which results from biochemical reactions or from external factors is involved in a range of physiological and pathological process[175]. Excess free radicals generated in the body beyond its antioxidant capacity can even cause DNA strand breakage, carbonylation of cellular proteins and lipids peroxidation leading to chronic health problems such as cancer. Dietary intake of antioxidants may be an important strategy for inhibiting or slowing down the oxidation of susceptible cellular substrates and is thus relevant in disease prevention.

The assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. In this assay Phosphomolybdenum reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) was used. To perform the, 100 μg of each compound was mixed with 700 μL methanol in each test tube and was mixed well. After that 6 mLof Phosphomolybdate reagent solution was added in each tube and mixed well. Then all the tubes were incubated at 95°C for 90 minutes. After incubation absorbance was measured at 695 nm and recorded for further analysis. 100 μg ascorbic acid was used as a positive control. The antioxidant capacity is expressed as the number of gram equivalents of ascorbic acid. These experiments were done in triplicate.

1.4.3 DPPH Free Radical Scavenging Activity[176]

The free radical scavenging activity (antioxidant capacity) of the synthesised compounds on the persistent radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) were estimated by the method of Blois (1958). The hydrogen atom or electron donation abilities of some pure compounds were measured by the bleaching of a purple coloured methanol solution of DPPH. The free radical scavenging activities of the synthesised compounds were measured by 1, 1-diphenyl-2-picryl-hydrazil (DPPH·) using the method of Blois . Wherein the bleaching rate of a stable free radical, DPPH· is monitored at a characteristic wavelength in the presence of the sample. In its radical form, DPPH absorbs at 517nm, but upon reduction by an antioxidant or a radical species its absorption decreases.

A possible anti-oxidant reaction mechanism of DPPH is shown below:



***DPPH (oxidized form)** **DPPH (reduced form)**

DPPH = 2,2-diphenyl-1-picrylhydrazyl

When a hydrogen atom or electron was transferred to the odd electron in DPPH·, the absorbance at 517 nm decreased proportionally to the increases of non-radical forms of DPPH.

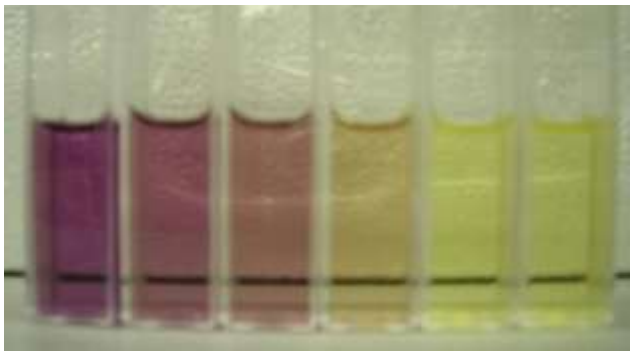


Fig : Colour variation of DPPH solution after samples treatment

Briefly, 0.1 mM solution of DPPH in methanol was prepared and 2 mL of this solution was added to 2 mL of sample containing 100 μg of each compound in methanol. The mixture was shaken vigorously and allowed to stand at room temperature for up to 120 minutes. Then the absorbance was measured at 517 nm in a spectrophotometer in different time interval, at 0 minutes, 30 minutes, 60 minutes and 120 minutes. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity[177]. The capability to scavenge the DPPH· radical was calculated using the following equation[178]:

$$\text{DPPH radical- Scavenging activity (\%)} = [(Ac-As)/ Ac] \times 100$$

where Ac =Absorbance of the control
and As =Absorbance in the presence of samples or standards

Ascorbic acid was used as a positive control in this experiment and the DPPH free radical scavenging assay estimated as IC_{50} for each compound in μg/mL. These experiments were done in triplicate

1.4.4 Antibacterial Activity Assay

Microbial infection is one of main causes of human diseases in the world and it is accounting for approximately one-half of all deaths in tropical countries. Perhaps it is not surprising to see these statistics in developing nations, but what may be remarkable is that mortality rates due to infectious disease are actually increasing in developed countries, such as the United States. Deaths from infectious diseases, was ranked 5th in 1981, and has become the 3rd leading cause of death in 1992, with an increase of 58%. It is estimated that infectious disease is the underlying cause of death in 8% of the deaths occurring in the United States. This is really alarming as it was once believed that we would estimate infectious disease by the end of the millennium. The increases are attributed to increase in respiratory tract infections and HIV/AIDS. Other contributing factors are an increase in antibiotic resistance in nosicomial and community acquired infections. Again, the most

dramatic increases are occurring in the 25-44 year old age group[179]. Microbes are responsible for many infectious diseases. The increasing clinical importance of drug resistant microbial pathogens has lent additional urgency to antimicrobial research.

These negative health trends call for a renewed interest in infectious diseases in the medical and public health communities and renewed strategies on treatment and prevention. It is this last solution that would encompass the development of new antimicrobial agents[180]. The antimicrobial screening, which is the first stage of antimicrobial drug research, is performed to ascertain the susceptibility of various microbes (bacteria and fungi) to any agent. This measures the ability of each test sample to inhibit the *in vitro* microbial growth and can be estimated by any of the following three methods.

(1) Disc diffusion method

(2) Serial dilution method

(3) Bioautographic method

Actually there is no standardised method for expressing the results of antimicrobial screening[181]. Some investigators use the diameter of zone of inhibition and/or the minimum weight of extractive to inhibit (MIC) the growth of microorganisms. However, a great number of factors viz., the extraction methods[182], inoculums volume, culture medium composition[183]. pH [184]and incubation temperature[185,186]can influence the results.

Among the mentioned techniques, the disc diffusion technique[187]. is a widely accepted *in vitro* investigation for preliminary screening of test agents, which may possess any antimicrobial activity. It is essentially a quantitative or qualitative test indicating the sensitivity or resistance of the microorganisms to the test materials. However, no distinction between bacteriostatic and bactericidal activity can be made by this method[188].

1.4.5 Principle of Disc Diffusion Method

In diffusion method, antibiotics diffuse from a confined source through the nutrient agar gel and create a concentration gradient. Solutions of known concentration (μg/mL) of the test samples are made by dissolving measured amount of the samples in definite volume of solvents. Dried and sterilized filter paper discs (6 mm diameter) are then impregnated with known amounts of the test substances using micropipette. Discs containing the test material are placed on nutrient agar medium uniformly seeded with the test microorganisms. Standard antibiotic discs and blank discs (impregnated with solvents) are used as positive and negative control. These plates are then kept at low temperature (4^oC) for 24 hours to allow maximum diffusion. During this time dried discs absorb water from the surrounding media and then the test materials are dissolved and diffused out of the media. The diffusion occurs according to the physical law that controls the diffusion of molecules through agar gel[189]. As a result there is a gradual change of test materials concentration in the media surrounding the discs. The plates are then inverted and incubated at 37^oC for 24 hours to allow maximum growth of the organisms. If the test materials possess antimicrobial property, it inhibited microbial growth in the media surrounding the discs and thereby yielded a clear, distinct area as 'Zone of Inhibition'. The antimicrobial activity of the test agent is then determined by measuring the diameter of zone of inhibition expressed in millimeter[190].

New sources of antimicrobial agents need to be discovered due to the existence and continuous evolution of resistant microorganisms, the emergence of new infectious diseases and the toxicity concerns of some of the currently used antimicrobial drugs. The indiscriminate use of conventional antibiotics and synthetic antimicrobial drugs has resulted in the emergence of resistance microbes such as methicillin resistance *Staphylococcus aureus* (MRSA). In addition, the high costs of the effective western medicines have also pushed people in the developing countries to turn to alternative therapy as source of drug for the management and treatment of diseases[191].

1.4.6 Test Bacterial Strains

In order to determine the antibacterial activity of the synthesised sulfur heterocycles, four different gram-positive bacteria such as *Bacillus cereus*, *Bacillus subtilis*, *Bacillus megatricum* and *Staphylococcus aureus* and five different gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, *Shigella flexneri*, *Shigella boydii* and *Vibrio cholera* were used. All these bacteria were collected from the Department of Genetic Engineering and Biotechnology, University of Dhaka repository. These bacterial strains were multidrug resistant.

1.4.7 Preparation and Standardisation of Inoculums

The above mentioned bacterial strains were grown in nutrient agar at 37°C and reactivated them for further use in nutrient broth. The different compounds were tested for antimicrobial activity against the test organism using the disc diffusion method[192]. Four to five colonies from pure growth of each test organism were transferred to 5 mL of Muller Hinton Broth (MHB). The broth was incubated at 35-37°C for 4 to 5 hrs.

1.4.8 Antibiogram by Disc Diffusion Method

To determine the anti-bacterial activity of the compounds, disk diffusion method was used. This is the most frequently used method and it is also known as the NCCLS (National Committee for Clinical Laboratory Standards) or *Kirby-Bauer* method. In this method, a number of small, sterile filter paper disks of uniform size (6 mm) that have each been impregnated with a defined concentration of an antimicrobial agent are placed on the surface of an agar plate previously inoculated with a standard amount of the organism by a cotton swab to be tested. After incubation, the plates are examined for the presence of zones of inhibition of bacterial growth (clear rings) around the antimicrobial disks. If a zone of inhibition present surrounds the disk, the organism could be considered susceptible to the drug being tested depending on the size.

The antimicrobial activity of the test materials was determined by measuring the diameter of the zone of inhibition in millimeter with transparent scale (Figure)

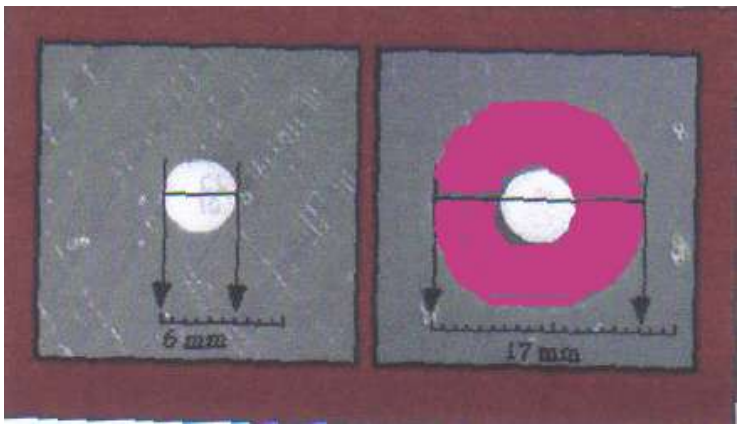


Fig :Determination of clear zone of inhibition

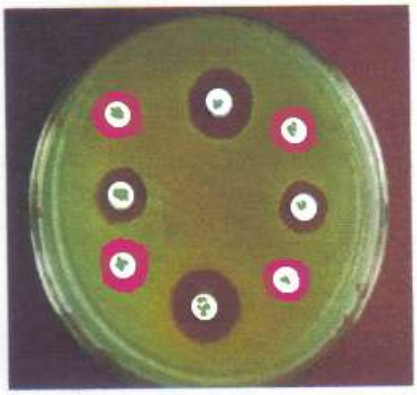


Fig :Clear zone of inhibition

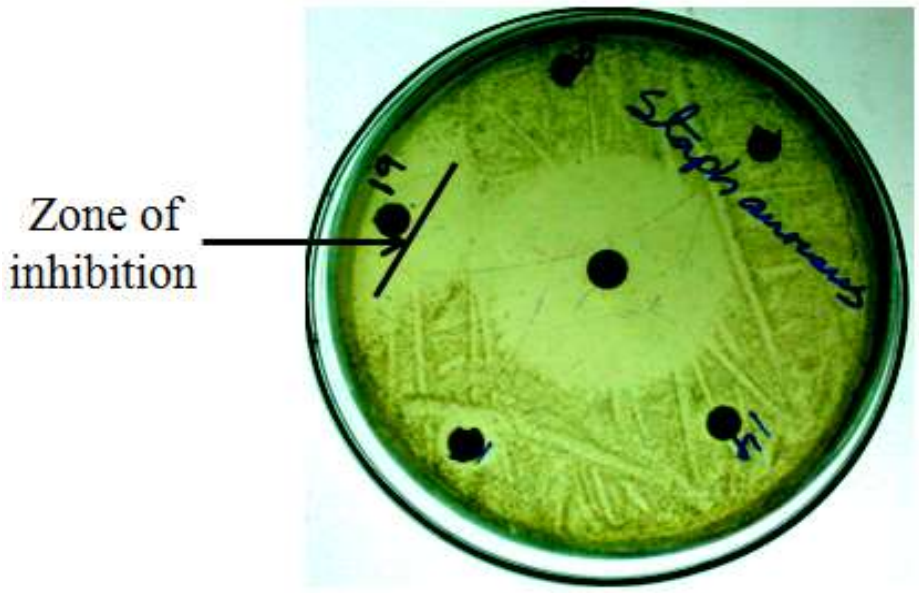


Fig : Representative figure of zone of inhibition of bacterial growth during antibiotic treatment by disc diffusion method

The antibacterial activities of the compounds were done on Muller-Hinton agar (Difco, USA) plate. For this, 0.5 McFurland standard culture of each bacteria was spread over the plate. Sterile paper discs containing 100 μg of each compound were applied on the plate. A disc soaked in chloroform, was also applied on the plate and considered as negative control. A sterile disc was applied too and was considered as a blank to justify if the paper material show any activity. Then the plate was incubated overnight at 4 °C to allow the diffusion of the compounds from the disc and then the plate was incubated at 37°C until the bacteria grow. Then the clear zone was measured and recorded. These experiments were done in triplicate and average was considered.

1.4.9 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC was determined by broth microdilution method. For this, 10^8 CFU/mL of the bactrerial strains in fresh nutrient broth were treated with each compound with positive antibacterial activity. The concentrations of each compound ranged from 100-600 μg/ml. After overnight incubation at 37°C, absorbance was measured at 600 nm against a blank nutrient broth. Untreated similar culture was used as control. The concentration at which no bacterial growth obtained was considered as MIC. All the experiments were done in triplicate.

For MBC, 5 μL of the above samples were spotted in nutrient agar plate and was allowed to soak. Then the plates were incubated overnight at 37°C. The next day, the plates were observed for visible bacterial growth. The concentration where no bacterial growth was observed or less than 10 CFU was observed was considered as MBC. All the experiments were done in triplicate.

1.5.0 Effect of the Compounds on Chromosomal DNA

For this, 10^8 CFU/mL of the bactrerial strains in fresh nutrient broth were treated with each compound at MBC. Untreated or 60 μL chloroform treated bacteria were used as a control. After overnight incubation at 37°C, the bacterial cultures were spun down at 10,000 rpm for 10 minutes at 4°C to harvest the cells. The cells were washed thrice with normal saline (0.9% NaCl). Then, the cells were treated with cell lysis buffer (500 μL solution consisting 50 mM Tris, 25 mM EDTA, 400 mM NaCl, 10% SDS bringing to pH 8 with HCl) followed by the addition of 2 μL of proteinase K (20 mg/ml) and 6 μL of Rnase (10 mg/mL). After that, 500 μL of phenol:chloroform:isoamylalcohol (25:24:1) was added to it and mixed well. Then, the samples were centrifuges at 10,000 rpm for 10 minutes at 4°C to separate the protein and to collect the aqueous layer. Then, equal volume of isopropanol was added to the collected aqueous fraction and mixed well. After centrifugation at 10,000 rpm for 10 minutes at 4°C, DNA was precipitated. The

precipitated DNA was washed once with 70% ethanol and then air dried. Then the DNA was solubilized with 25 mLTE buffer (10 mM Tris, 1mM EDTA at pH 8.0). Then 10 μL samples were mixed up with 3 μL of 0.25% (w/v) bromophenol blue in 40% (w/v) sucrose. Then the samples were run on 1.5% agarose gel with 0.1 μg/mL ethidium bromide. Electrophoresis was done using TE (Tris-EDTA) buffer (10 mM Tris and 1 mM EDTA in 1 L distilled water bringing to pH 7.5 with HCl) at 60 V. After the end of electrophoresis, the gel was photographed under UV transilluminator. The whole experiments were repeated three times.

1.5.1 Effect of the Compounds at Protein Level

For this, 10^8 CFU/mL of the bacterial strains fresh in nutrient broth were treated with each compound at MBC. Untreated or 60 μL chloroform treated bacteria were used as a control. After overnight incubation at 37°C, the bacterial cultures were spun down at 10,000 rpm for10 minutes at 4°C to harvest the cells. The cells were washed thrice with normal saline (0.9% NaCl). Proteins in the cells were analyzed by Sodium Dodecyl Sulphate PolyAcrylamide Gel Electrophoresis (SDS-PAGE) according to the protocol of Laemmli as described before[114]. For this, glass plates were properly cleaned with 70% ethanol and were assembled. 10% separating gel mixture was prepared as described in Table 2.1 and was poured very carefully, ensuring no bubble formed, into the assembly leaving 2 centimeters from top for stacking gel. Then the gel was overlaid with water very carefully and was allowed to polymerise for 30 minutes. 4% stacking gel was prepared as described in Table 2.1. Water was decanted from the separating gel and the stacking gel preparation was poured onto the top of separating gel. The comb was placed immediately and when the stacking gel was solidified, the glass plate assembly was transferred to the electrophoresis tank containing 1X SDS running buffer (19.6 mM glycine (Sigma, USA), 0.01% SDS, 5 mM Tris-HCl, pH 8.3). Then tank was filled up with the 1X SDS running buffer and the comb was removed. Protein samples were prepared by mixing the bacterial cells with 10 μL of the purified phage preparation or the negative control with 10 μL of 2X SDS sample buffer (0.125 M Tris-HCl,

pH 6.8, 4% (w/v) SDS, 20%(w/v) glycerol,10% (v/v) 2-mercaptoethanol (Sigma, USA), 0.001% (w/v) bromophenol blue (Merck, India) and was boiled at 100 ºC for 5 minutes. Then the samples were spun down and loaded on to the respective well. Seablue pre-stained protein standards (Life Technologies, USA) were used as protein marker. Then the apparatus was connected up with power supply and electrophoresis was started at 60 volts. When the samples passed the stacking gel voltage was increased to 90 volts. Electrophoresis was performed until the bromophenol dye reached at the bottom. After electrophoresis, glass plates were disassembled and the gel was soaked with Coomassie brilliant blue solution (0.2% CBB in 45:45:10 % menthanol:water:acetic acid) at room temperature for 2 hours at 37 ^{o}C with gentle agitation. The gel was then destained with destaining solution (25% methanol, 65% water and 10% acetic acid) with agitation until the proteins were visualized. Then, the gel was dried on filter paper by gel dryer and photographs were taken thereafter. The whole experiments were repeated three times.

Table : Composition of separating and stacking gels

Chemicals (Company, Country)	**10% Separating Gel**	**4% Stacking Gel**
30% (w/v) acrylamide (Sigma, USA) with 0.8% bis-acrylamide (Promega, Canada)	10.0 mL	900 μL
1 M Tris-HCl, pH 8.8 (Sigma, USA)	11.1 mL	0 mL
1 M Tris-HCl, pH 6.8 (Sigma, USA)	0 mL	750 μL
Distilled deionized H_2O	8.52 mL	4.22 mL
10% SDS (Sigma, USA)	300 μL	60 μL
TEMED (Sigma, USA) (added immediately before pouring)	25 μL	10 μL
10% APS (Sigma, USA) (added immediately before pouring)	300 μL	60 μL

1.5.2 Antifungal Activity Assay

Antifungal activity of the synthesised compounds was determined by disc diffusion method as described above. Two fungal strains, *Candia albicans* and *Saccharromyces cerevisiae* were collected from the Department of Genetic Engineering and Biotechnology, University of Dhaka repository. These fungal strains were cultured in potato-dextrose agar medium. Simply, a colony of individual fungal strain was suspended in 100 μL sterile normal saline. Then the solution was spread over the agar medium. Sterile paper discs containing 100 μg of each compound were applied on the plate. A disc soaked in chloroform, was also applied on the plate and considered as negative control. A sterile disc was applied too and was considered as a blank to justify if the paper material show any activity. Then the plate was incubated overnight at 4 °C to allow the diffusion of the compounds from the disc and then the plate was incubated at 37°C until the fungal grow. Then the clear zone was measured and recorded. These experiments were done in triplicate and the average was considered.

2.0 RESULTS AND DISCUSSION

2.1 Results

The selected 11 sulphur heterocycles were tested for different biological activities. For the ease of representation, these compounds were designated with different symbol as mentioned in Table.

Table : List of sulphur heterocycles synthesised using sulphides

Sl No	Chemical name of the Compound	Symbol
1	7-cinnamoyl-2,6,8-triphenylthiocan-4-one	B 1
2	2,6,2',6'-tetraphenyl-tetrahydro-[3,4-b]bithiopyranyliden-4-ol	B 2
3	3-benzylidene-2,6-diphenyl-tetrahydrothiopyran-4-one	B 4
4	3,5-dibenzylidine-2,6-diphenyl-tetrahydrothiopyran-4-one	B 5
5	3-benzyl-2,6-diphenyl-2***H***-thiopyran-5-carboxaldehyde	B 8
6	3-acetyl-2,6-diphenyltetrahydrothiopyran-4-ol	B 9
7	3-benzoyl-2,4,6-triphenyltetrahydrothiopyran-4-ol	B 10
8	3-(p-methylbenzyl)-2,6-(p-methyldiphenyl)-2***H***-thiopyran-5-carboxaldehyde	B 11
9	3-(o-methylbenzyl)-2,6-(o-methyldiphenyl)-2***H***-thiopyran-5-carboxaldehyde	B 12
10	3-(4-methoxybenzyl)-2,6-(4-methoxydiphenyl)-2***H***-thiopyran-5-carboxaldehyde	B 14
11	3-formoyl-2,6-(2-acetamidodiphenyl)-tetrahydrothiopyran-4-ol	B 15

2.2 Total Antioxidant Capacity

The total antioxidant capacity of the chemical compounds were measured and compared to that of ascorbate. It was found that for the 100% activity of 100 µg ascorbate, the chemical compounds gave the antioxidant activity as given in the chart below. The compound B 10 (3-benzoyl-2,4,6-triphenyltetrahydrothiopyran-4-ol) was the most potent of the compounds having antioxidant activity about 51.55±1.37 % of that of ascorbate. On the other hand the least antioxidant activity was shown by B 2, B 12 and B 5. Their activity was about 14%. However, B 11 had 23%, and B 14 had 21% of the activity of ascorbate.

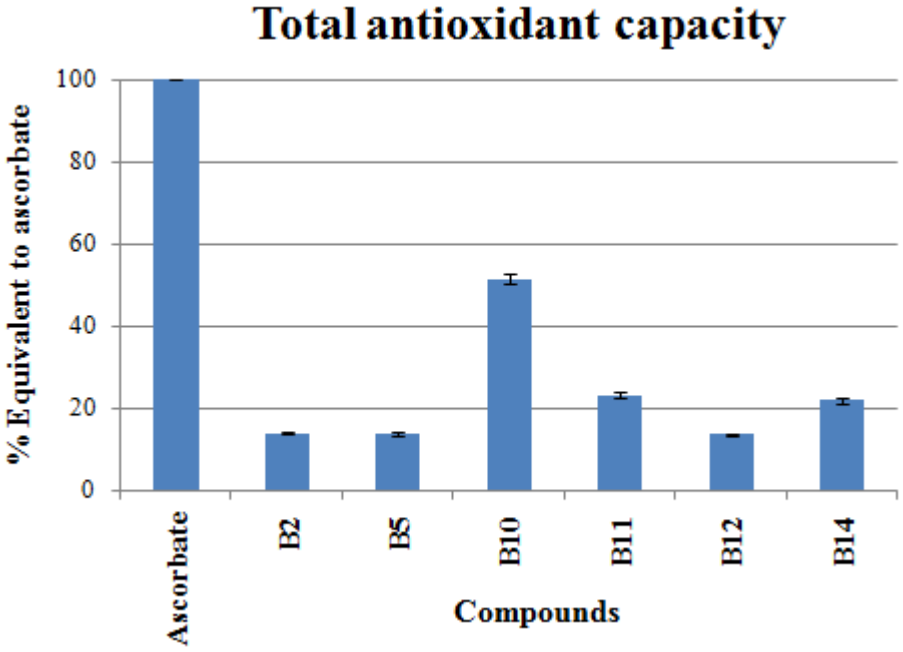


Fig : Total antioxidant activity of the synthesised compounds compared to ascorbate.

2.3 DPPH Free Radial Scavenging Assay

The DPPH free radical scavenging assay was also carried out for the chemical compounds and compared to that of Ascorbate. There were some promising results. B11 had almost similar IC_{50} concentration (17.37±1.05 µg/mL) compared to ascorbate after 120 minutes. B5 could not scavenged DPPH at 60 minutes that much but was promising at 120 minutes as the IC_{50} was close to the ascorbate (25.73±0.87 µg/mL). B4 with IC_{50} equal to 55.53±16.57 µg/mL at 120 minutes was very poor to scavenge DPPH free radicals. B10 had no DPPH free radical scavenging although it had antioxidant activity indicating it may function in some other reaction mechanism. Therefore, among the compounds, B11 [3-(p-methylbenzyl)-2,6-(p-methyldiphenyl)-2***H***-thiopyran-5-carboxaldehyde] scavenged DPPH free radicals the most.

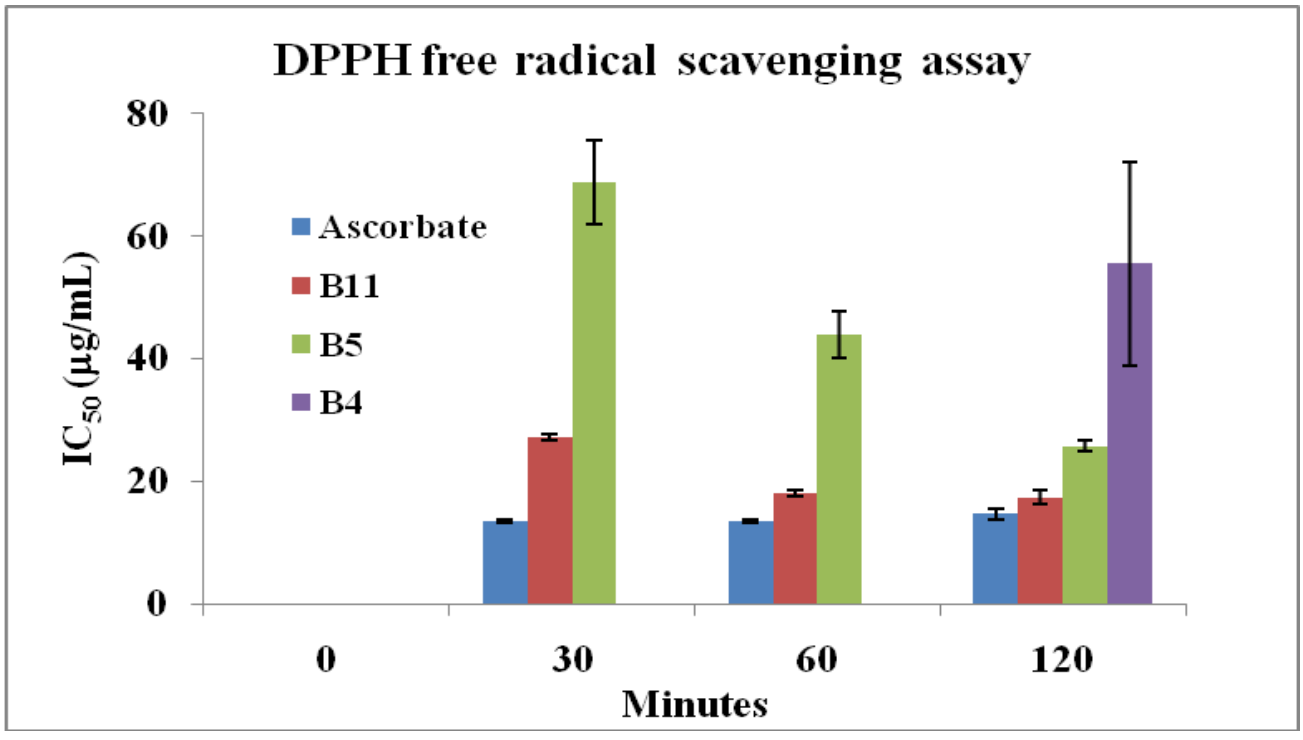


Fig : The DPPH free radical scavenging assay of the synthesised compounds compared to ascorbate

2.4 Antibacterial Activity Assay

The compunds were used to observe antibacterial activity on various bacterial strains: both Gram-positive and Gram-negative. This activity was quantified by using the disk diffusion method after preparation of the bacterial strains and development of inoculum. The zone of inhibition created by each compund is show in the tables.

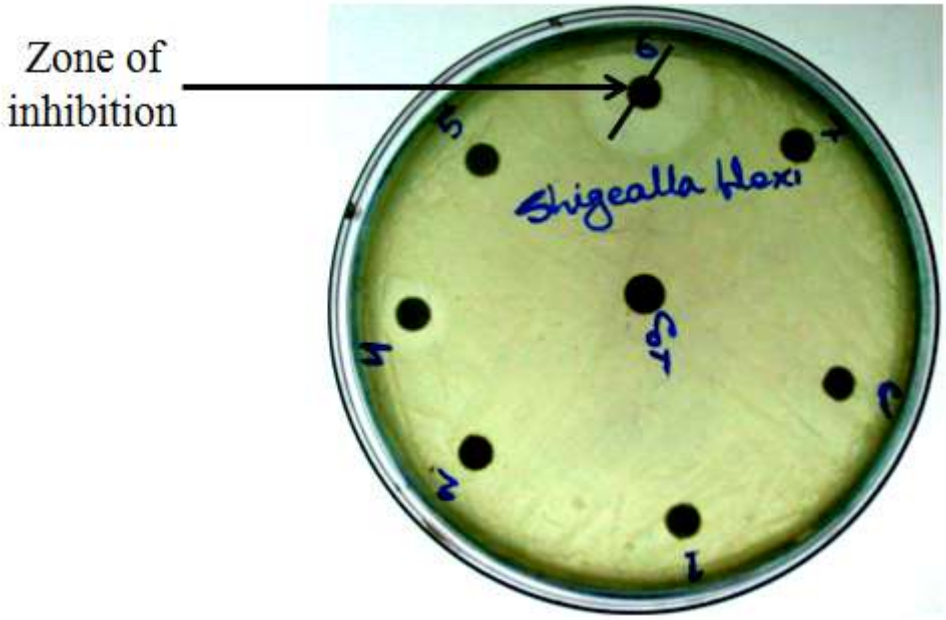


Fig : A representative figure showing activity of compound B4 (no. 6) against *Shigella flexneri*

B5 was the most potent antibacterial agent giving larger zone of inhibitions against all strains. B4 was most active against *B. megaterium*. B2 and B10 had moderate activity against all strains. B15 was most active against *S. aureus* and it also affected *B. cereus*.

Table : The antibacterial activity of the compounds against Gram-positive bacteria

Organism	$CHCl_3$	B2	B4	B5	B8	B10	B15
Bacillus megaterium	-	10.1 ± 0.1	13.9 ± 0.1	11.9 ± 0.1	10.0 ± 0.1	10.0 ± 0.0	-
Bacillus subtilis	-	9.9 ± 0.1	-	9.9 ± 0.1	14.9 ± 0.1	9.9 ± 0.1	-
Bacillus cereus	-	9.9 ± 0.1	-	9.9 ± 0.1	12.9 ± 0.1	9.9 ± 0.1	10.0 ± 0.0
Staphylococcus aureus	-	9.9 ± 0.1	-	11.9 ± 0.1	-	11.9 ± 0.1	14.0 ± 0.0

Against Gram-negative bacteria none of the compounds affected all bacterial strains. B1 had the weakest activity which worked against only *K. pnuemoniae* and *E. coli*. B4 and B5 worked best against *V. cholera*. B10 was the most effective against *S. boydii*.

Table : The antibacterial activity of the compounds against Gram-negative bacteria

Organism	$CHCl_3$	B1	B4	B5	B10
Shigella boydii	-	-	11.9 ± 0.1	13.9 ± 0.1	15.9 ± 0.1
Klebsiella pneumoniae	-	11.9 ± 0.1	-	10.2 ±0.6	11.9 ±0.1
Escherichia coli	-	11.9 ± 0.1	-	-	9.9 ± 0.1
Shigella flexneri	-	-	11.9 ± 0.1	-	-
Vibrio cholerae	-	-	13.9 ± 0.1	13.9 ± 0.1	-

2.5 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values of the compounds extracted are given in the charts and tables below. For *S. aureus*, B15 allowed growth till 100 µg/mL but the bacteria still survived till 400 µg/mL and finally died at 600 µg/mL. B4 was active against *S. flexnei*. Its growth rapidly dropped to 0 % at about 400 µg/mL and the bacteria did not die even at about 600 µg/mL. Both B4 and B5 were active against *V. cholerae*. For B4 the bacteria grew till 100 µg/mL, but above 200 µg/mL the bacteria could not survive. For B5, however the bacteria could not survive at even 100 µg/mL. B8 worked on *B. subtilis* and although it grew till 100 µg/mL, the bacteria was killed slowly at this concentration. B8 also had activity on *B. cereus*. The bacteria grew till 400 µg/mL and died at 600 µg/mL. *S. boydii* was affected by both B5 and B10. Both B5 and B10 did not allow the bacteria to grow much and

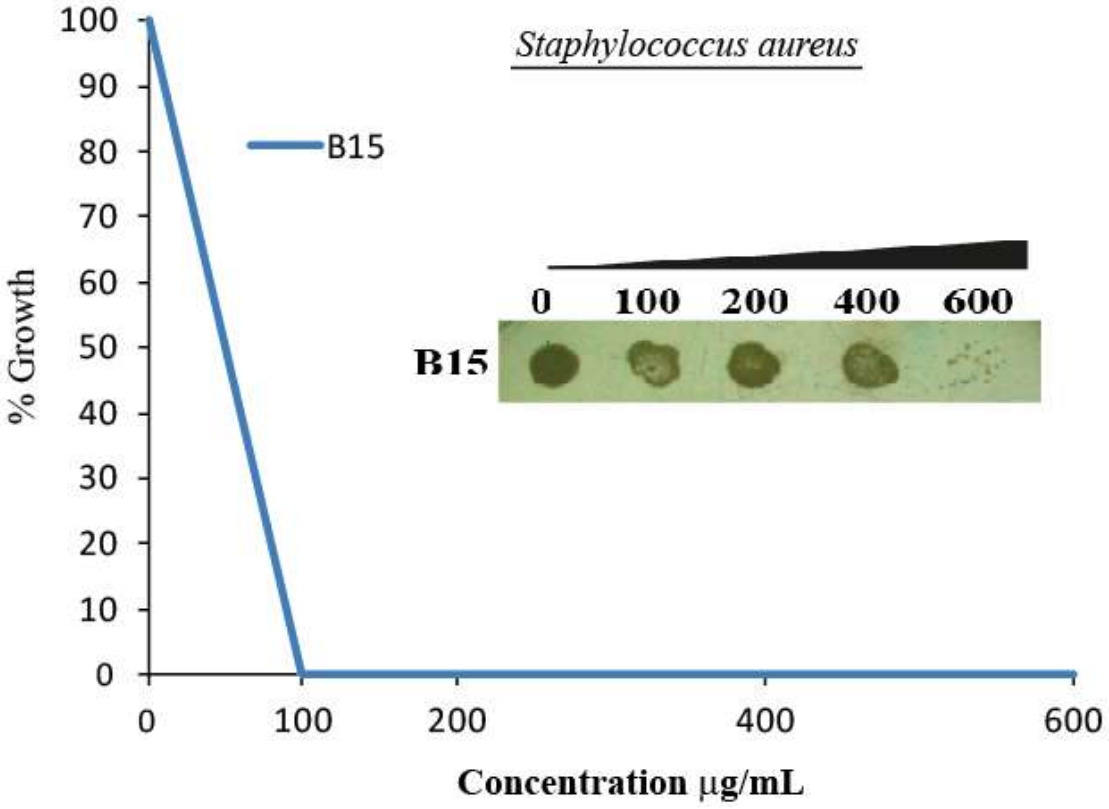


Fig : The MIC and MBC values of B15 against *S. aureus*

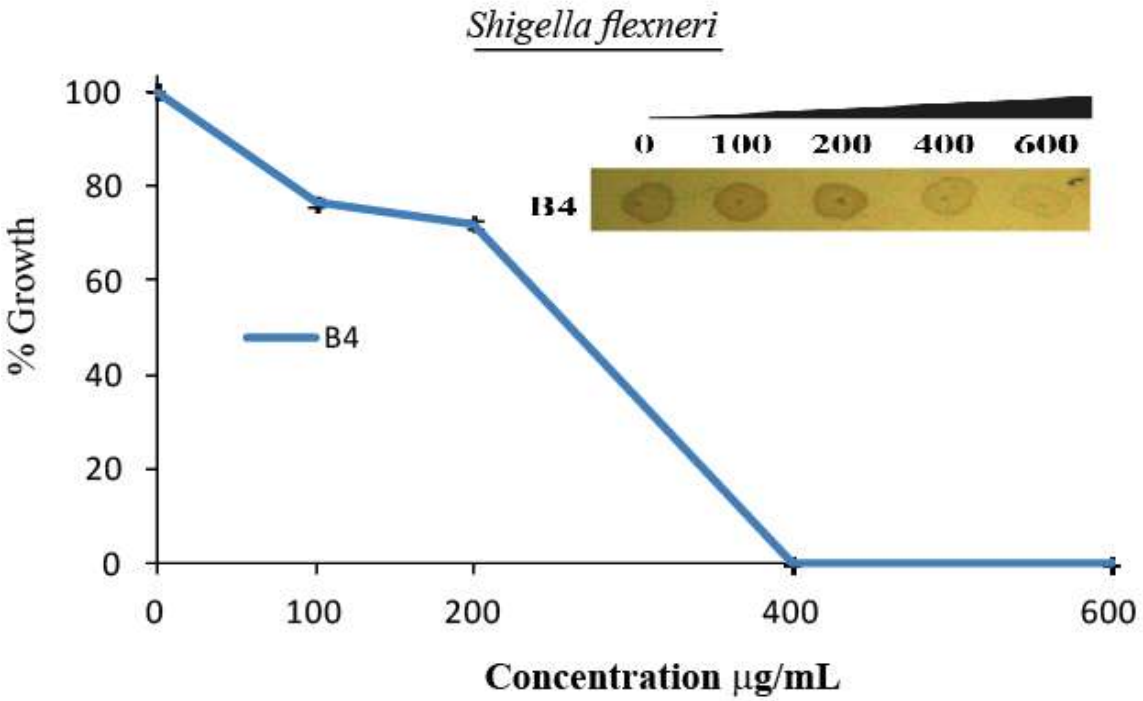


Fig : The MIC and MBC values of B4 against *S.flexneri*

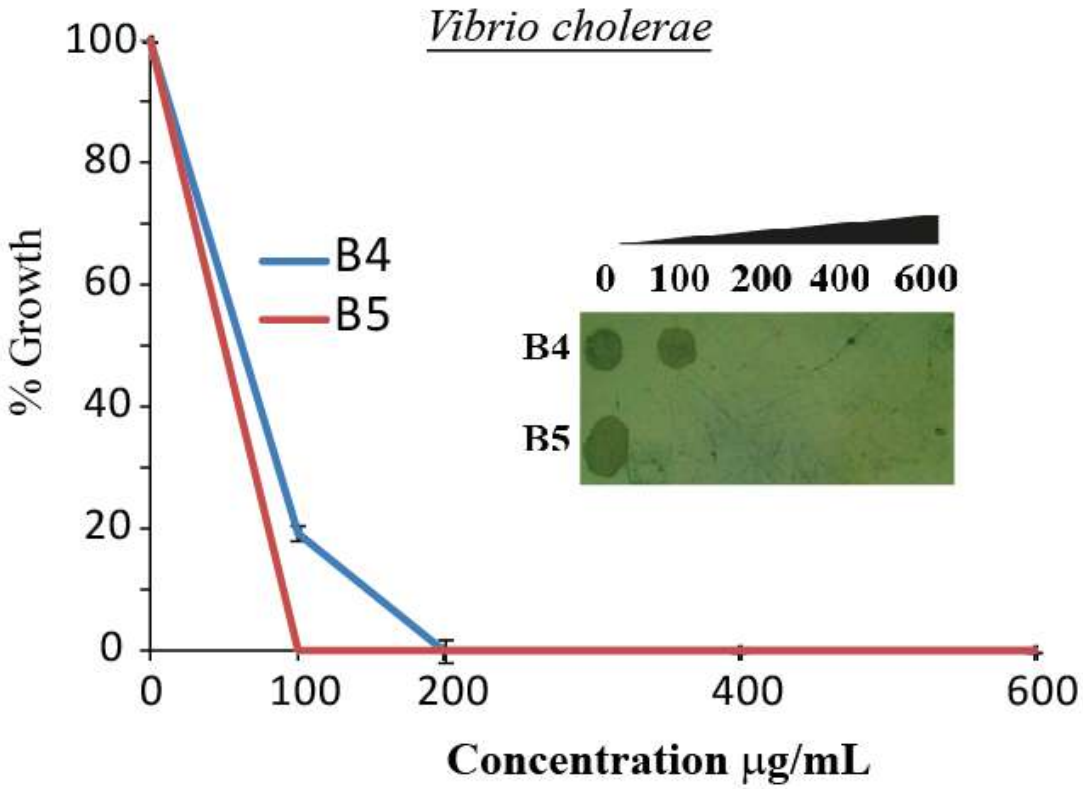


Fig : The MIC and MBC values of B4 against *V.cholerae*

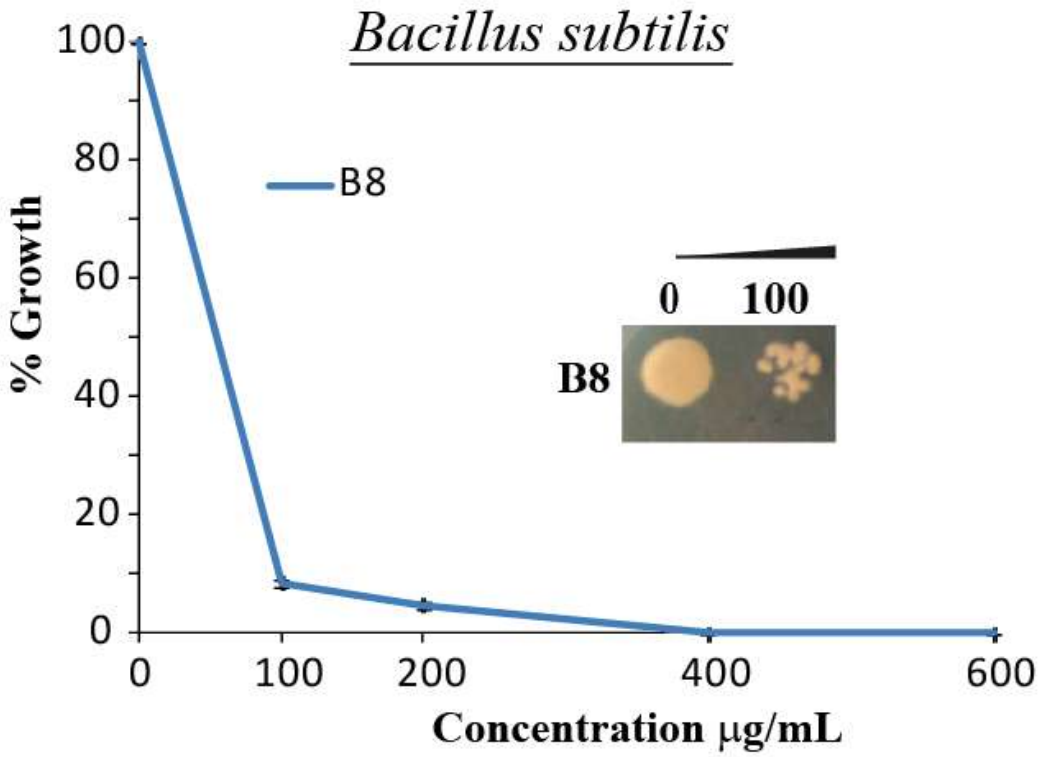


Fig :The MIC and MBC values of B 8 against *B. subtilis*

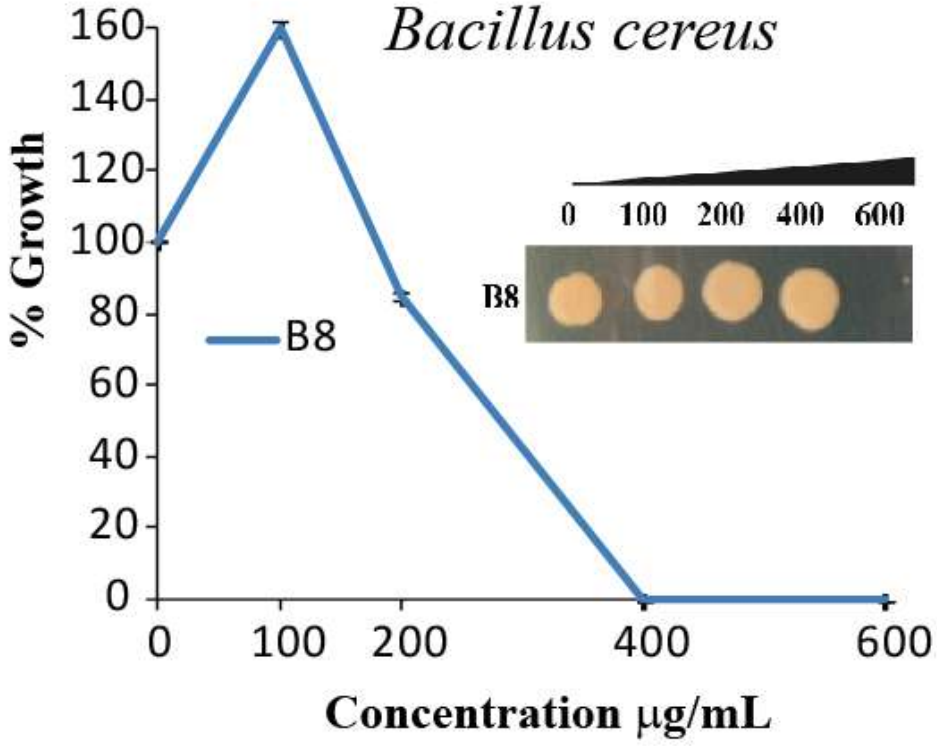


Fig :The MIC and MBC values of B8 against *B. cereus*.

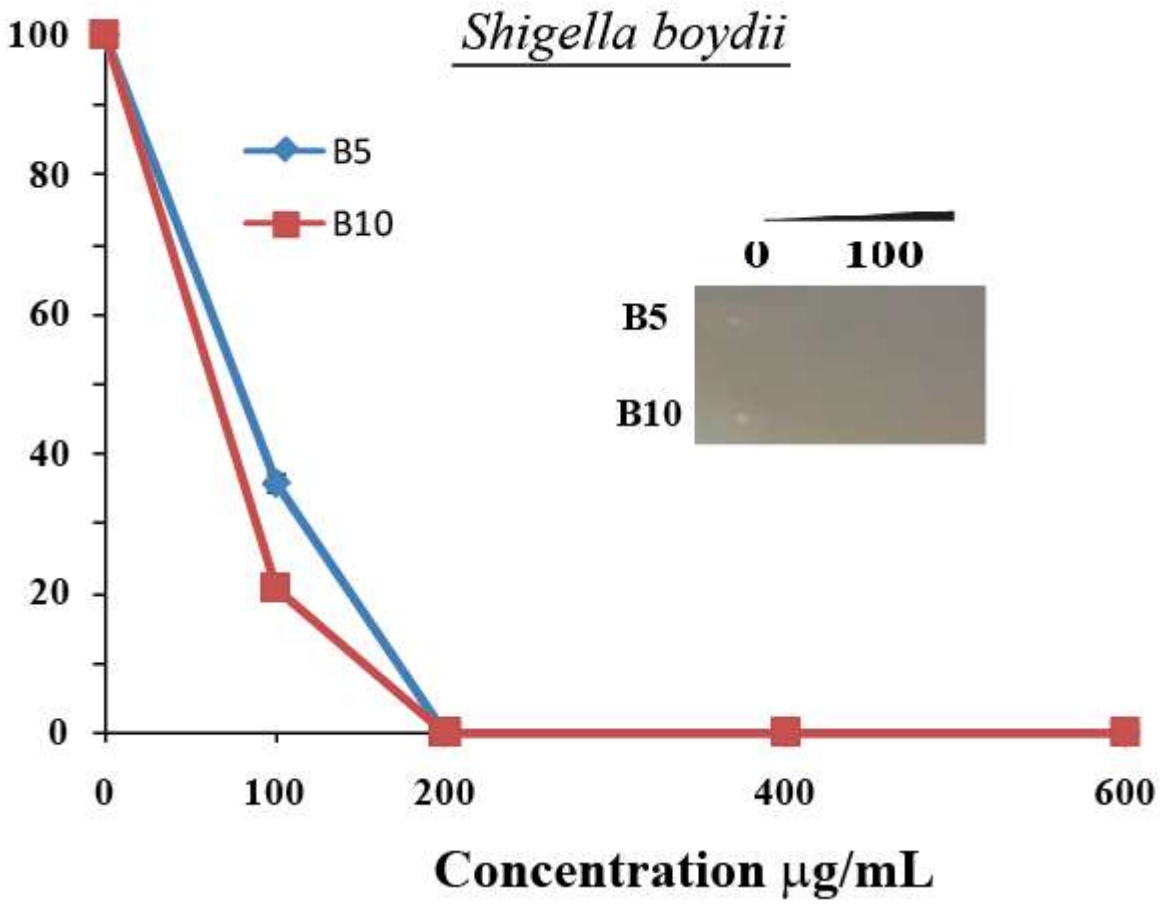


Fig :The MIC and MBC values of B5 and B10 against *S. boydii*.

Table : Summary of the MIC and MBC values of all the compounds against specific bacteria

Organism	B4		B5		B8		B10		B15	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
Vibrio cholerae	200	200	100	100	-	-	-	-	-	-
Shigella boydii	-	-	100	100	-	-	100	100	-	-
Shigella flexneri	400	-	-	-	-	-	-	-	-	-
Bacillus subtilis	-	-	-	-	400	400	-	-	-	-
Bacillus cereus	-	-	-	-	400	600	-	-	-	-
Staphylococcus aureus	-		-	-	-	-	-		100	600

2.6 Effect of the Compounds on Chromosomal DNA and at Protein Level

The effect of the compounds on the bacterial chromosomal DNA and at the protein level was also elucidated as can be seen in the following figures.

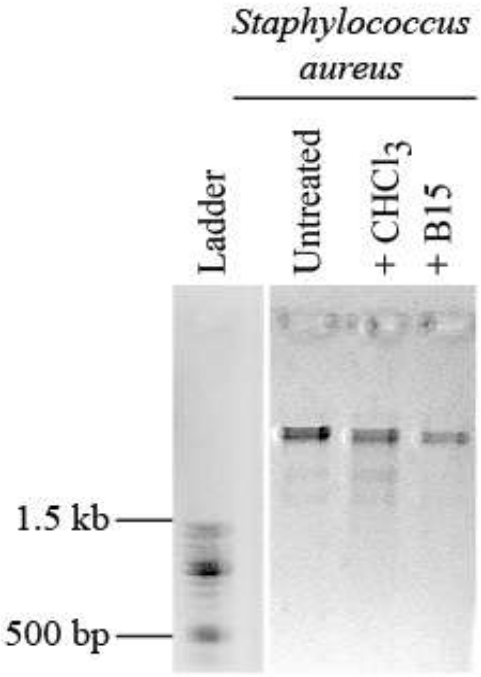


Fig : The agarose gel electrophoresis of the genomic DNA of *S. aureus* after the bacteria was untreated, treated with $CHCl_3$ and B15

B 15 did not cause any DNA damage to *S. aureus* similar to the untreated samples. The band of DNA remained undamaged and a clear band was observed. So B15 must act on the bacteria in some other way. At the protein level, however a slight change in protein expression profiles can be observed. B 15 caused a protein of about 120 kDa to be destroyed as evident in the SDS-PAGE. The band of the protein is missing. So it affects a specific protein of *S. aureus*. Both B5 and B10 affected *S. boydii*. It damaged the DNA of the bacteria as can be seen in the agarose gel electrophoretic movement of the damaged DNA.

The clear band of the DNA as in the untreated sample has been damaged and turned into a smear of DNA by both B5 and B 10.

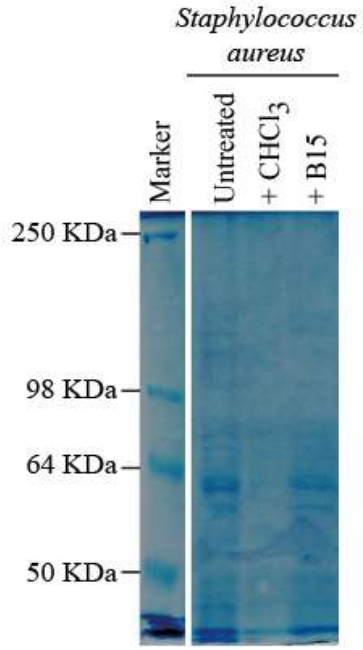


Fig :The SDS polyacrylamide gel electrophoresis of the entire protein content of *S. aureus* after the bacteria was untreated, treated with $CHCl_3$ and B 15

At hindsight, B5 and B10 may seem to have no effect on the bacteria at the protein level, but the expression of some proteins can be seen to have altered at closer examination.

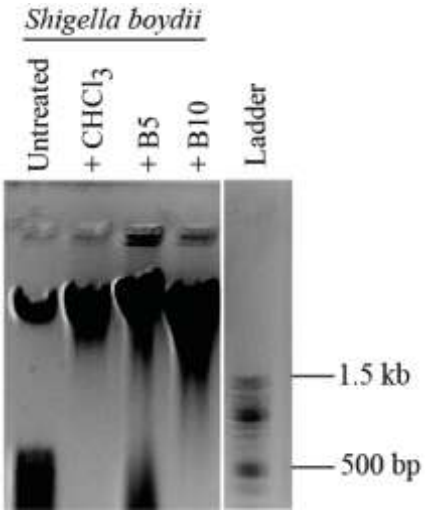


Fig :The agarose gel electrophoresis of the genomic DNA of *S. boydii* after the bacteria was untreated, treated with $CHCl_3$, B5 and B10

A few bands of protein (about 90 kDa) has grown faint owing to reduced expression while some bands of protein (about 50 kDa) have grown darker indicating increased expression.

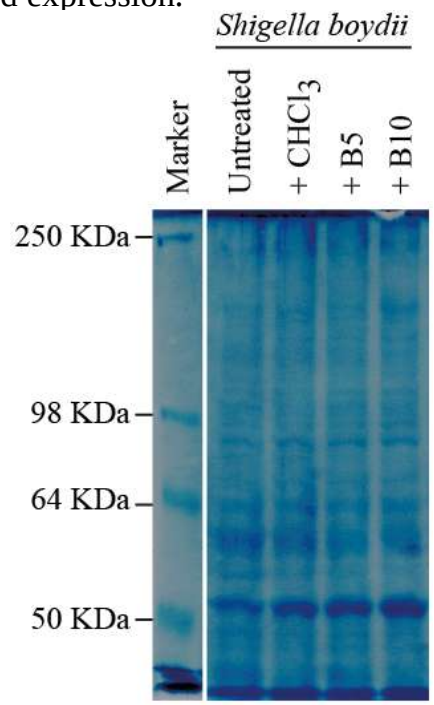


Fig : The SDS polyacrylamide gel electrophoresis of the entire protein content of *S. boydii* after the bacteria was untreated, treated with $CHCl_3$, B5 and B 10

2.7 Antifungal Activity Assay

The antifungal activity of the compounds were also measure as shown in the table below. The results are not very promising. Only B10 was active against both *C. albicans* and *S. cerevisiae*. B5 was poorly active against only *S. cerevisiae*.

Table 6.0:The antifungal activity of the compounds against specific fungi

Organism	$CHCl_3$	Control	B5	B10
Candida albicans	-	-	-	10.0±0.1
Saccharomyces cerevisiae	-	-	10.0±0.1	10.4±0.1

2.7 Discussion

The use of synthetic organic compounds has become a thing of necessity in the modern world. The abuse of antibiotics and other antibacterial and antifungal agents has led to the development of resistant strains of these harmful organisms. These pathogens seem to be proliferating at a drastically increased rate. This has led to the outbreak of many a disease especially in the developing countries of today. The outbreak of anthrax in this subcontinent is a prime example. So newer drugs other than antibiotics, are needed to be developed to prevent resistance and to battle this growing concern.

This is where thiopyran compounds and their derivatives come into play. In the modern day, they have been noted to have immense biological and industrial importance. They has been used in medicine to treat a number of diseases. They can also be used as insecticides, fungicides and herbicides. They can be used for agricultural as well as public health related actions.

The potency of thiopyran compounds and their derivatives was the main aim of this study.The primary investigation carried out was the total antioxidant activity. This was carried out by comparing the compounds to ascorbate. It was observed that B 10 was the most potent antioxidant having activity 51% of that of ascorbate. The least activity was shown by B2, B5 and B12 at just 14% of ascorbate.

The DPPH free radical scavenging assay was carried out next. B11 had the best DPPH free radical scavenging with IC_{50} just 17 μg/ml very close to that of ascorbate. On the other hand, B5 and B4 were not very good at DPPH free radical scavenging. It should be noted that although B10 had the most antioxidant activity it had no DPPH free radical scavenging, indicating it may work with some other mechanism.

Antibacterial assay was also carried out via the disk diffusion method. For gram--positive bacteria B2, B 5 and B 10 were all-round agents affecting all the bacterial strains. B4 was most active against *B. megaterium*, B8 was most effective against

B.subtilis and B 15 worked best on *S. aureus*. For gram-negative bacteria, no all--round agents were found. B4 and B5 were most effective against *V. cholera* while B10 was most effective against *S. boydii*.

The MIC and MBC values were also calculated based on the investigation. Only B 5 showed the lowest MIC and MBC value against *V. cholerae* and *S. boydii*. However, the compound B15 was the only compound active against *S. aureus*. However, both the compounds B5 and B10 showed similar MIC and MBC values against *S. boydii*. Such observations led to the idea that B5 and B10 may exert some molecular effect on *S. boydii*, and B15 may on *S. aureus*.

Next the effect of the compounds on the bacteria at the chromosomal and protein level was elucidated. B15 did not cause any DNA damage to *S. aureus* but it affected the protein profile. It caused a protein of about 120 kDa to disappear, meaning it must have inhibited the protein expression or it degraded the protein completely. B 5 and B10 caused great DNA damage to the *S. boydii* bacteria and as a result the bacteria were unable to survive. They also affected the protein expression profiles.They caused both the upregulation and downregulation of some proteins.

Antifungal assay of the compounds was carried out at the end. The results were not pleasing at all. Only B10 and B5 had antifungal activity. B10 affected both *C.albicans* and *S. cerevisiae* but B5 affected only *S. cerevisiae*.

Finally, it can be said that some of the thiopyran compounds and their derivatives are really effective in the field of antibacterial medicine. They seem to have a lot of antioxidant activity. They are also great at acting as inhibitory and bactericidal agents. The overall observation suggested that the compound B5 (3,5-dibenzylidine-2,6-diphenyl-tetrahydrothiopyranone-4)andB10(3-benzoyl-2,4,6-triphenyl-thiopyran- -4-ol) could be potential therapeutic agents

3 SCOPE OF THE PRESENT INVESTIGATION

It was observed from our present investigation that a few of our synthesised compounds has total antioxidant property (B10, B11) , DPPH free radical scavenging activity (B11), antibacterial activity(B1,B2,B4,B5,B10,B15), MIC and/or MBC (B5,B10), antifungal activity (B5,B10), effect on the chromosal DNA(B5,B10), and at protein level (B15) as evident in the SDS-PAGE on some of the selective stains such as Staphylococcus aureus, Bacillus megaterium, Bacillus subtilis and Bacillus cereus as gram-positive bacteria and Shigella boydii, Kllebsiella pneumonia, Escherichia coli, Shigella flexneri and Vibrio cholera as gram-negative bacteria. Two of the selective fungi such as Candida albicants and Saccharomyces cerevisiae were poorly active on B5, B10.

Since thiopyran derivatives shows diversified applications in medicine that is why our synthesised compounds can also be studied in so many other gram-positive and gram-negative bacteria which were not studied by us as well as fungi also. In addition, by applying the same experimental procedure for other electron donating and electron withdrawing groups present in aldehydes by heating or uv-irradiation technique. New thiopyran derivatives can be prepared and also other various medicinal activities as mentioned earlier on different bacterias, fungi as well as viruses can be studied.

Therefore, due to above mentioned applications, this research field is very diversified and simultaneously Chemists, Bio-Chemists, Pharmacists, Microbiologists, and Genetic engineers may be engaged over it.

S U M M A R Y

The thesis entitled, **"Synthesis, Characterisation and Study of Biological Activity of Some Sulphur Heterocycles"** contains two parts--- (i) **Part I** and **Part II**. The Part I contains the chemical investigation, synthesis, and characterisation, and Part II contains the biological activity of synthesised compounds .

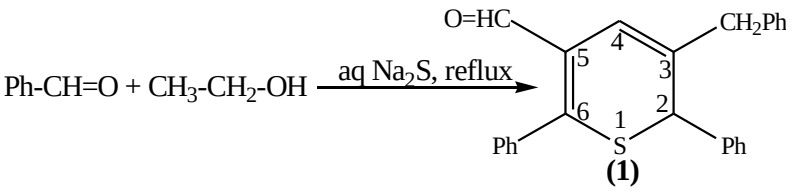
Part I

1. An extensive literature survey was carried out on the compounds containing thiopyran skeleton where they were classified as (a) different-membered heterocycles, (b) their different possible routes of synthesis, and (c) their physical and chemical properties.

2. The aim of the present investigation where we wanted to justify that why did we attempt to do this work.

3. The present investigation which includes our findings in short.

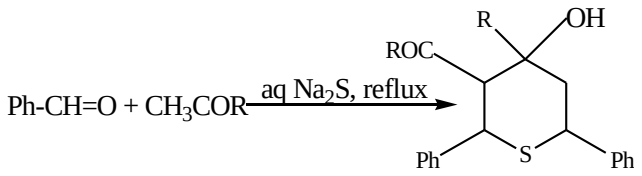
(a) The reaction of benzaldehyde with ethanol in presence of aqueous sodium sulphide at reflux temperature forms a thiopyran derivatives, 3-benzyl-2,6-diphenyl--2H-thiopyran-5-carboxaldehyde (1) is referred as the Latif recation and the product as the Latif product. The earlier workers proposed the structure (1) on the basis of modern instrumental tools and they also gave a possible mechanism for its formation through some intermediates which nor they could isolate and neither could give any concrete evidence in favour of the formation of the intermediate(s) proposed.



(1)

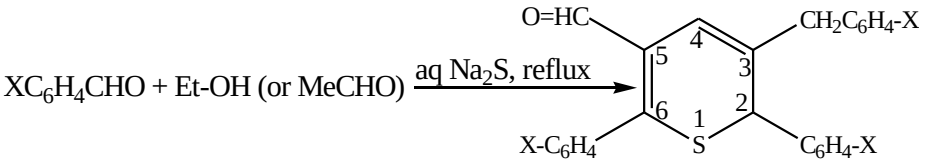
(b) In order to examine the validity of the Latif reaction, the reaction of benzaldehyde with ethanal and the reaction of benzaldehyde with cinnamaldehyde separately in aqueous methanolic sodium sulphide were carried out and the same original Latif product (1) was obtained which indicated the involvement of ethanal and cinnamaldehyde as the intermediates in the formation of (1). When benzaldehyde alone was reacted with methanolic sodium sulphide, no Latif product was found but benzyl alcohol and benzoic acid formed possibly followed by a Cannizzaro reaction indicated that in the formation of Latif product (1), ethanol or ethanal was also necessary in addition to benzaldehyde.

(c) Two analogous intermediates of the proposed scheme (Scheme 1) were obtained as multifunctional thiopyran derivatives (7) and (8) by following the Latif reaction using benzaldehyde and propanone in case of (7) and phenyl ethanone in case of (8). This was further supported by the fact that benzylidene acetone was detected by a GLC-study during the reaction of benzaldehyde with propanone in aqueous sodium sulphide.

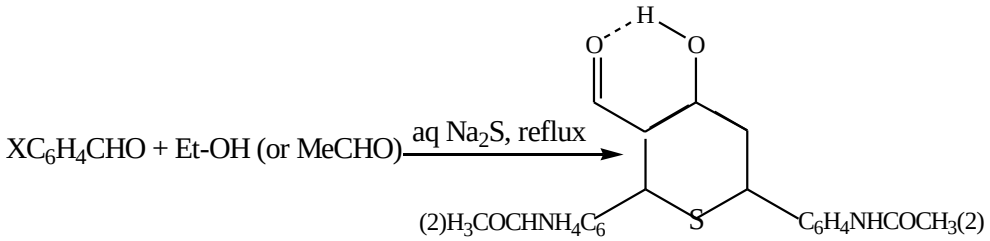


7, R = CH_3
8, R = Ph

(d) To examine the generality of the Latif reaction, the reaction of benzaldehyde with electron releasing groups such as 4-OMe, 2-Me-C_6H_4-CHO, both with each of ethanol and ethanal was carried out to obtain the products (9) and (10), analogous to (5) in good yields. In case of electron withdrawing groups such as 2-NO_2, 3-NO_2 and 4-NO_2-C_6H_4-CHO, only 2-NO_2-C_6H_4-CHO produced a new type of compound (11), not similar to (5) with each of ethanol and ethanal separately. But 3-NO_2 and 4-NO_2-C_6H_4-CHO with only ethanol under reflux produced instantly high melting solids which are insoluble in most of common organic solvents and seems to be polymeric in nature.



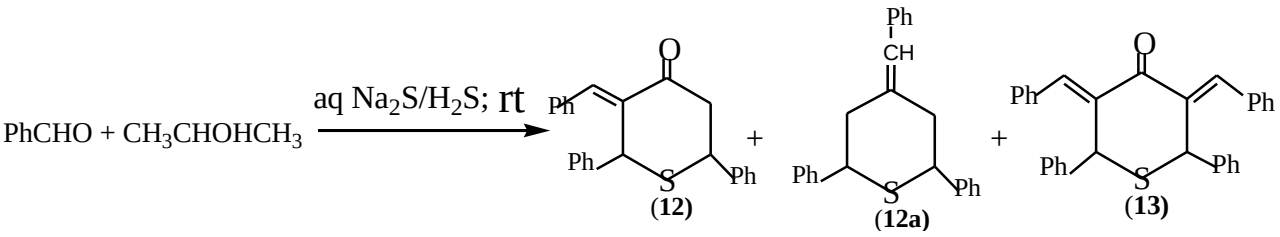
9, X = 4-OMe
10, X = 2-Me



(**11**)

(e) Our study were then extended to include a different type of compound like 2-Cl-C_6H_4-CHO with ethanol under identical conditions as above. To our utter surprise, we could not obtain any product(s) similar to (1) instead, obtained the corresponding redox products---2-chlorobenzyl alcohol and 2-chlorobenzoic acid respectively.

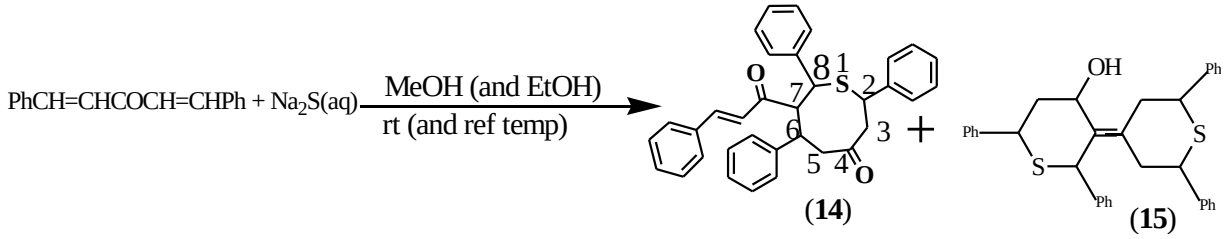
(f) In order to substantiate the mechanism of Latif reaction put forward by Cremmer and Subbaratnam (Scheme-1) where they mentioned that ethanol was oxidised *in situ* to ethanal, 2-propanol was employed instead of ethanol to see whether compound (7) forms or not but we ended up with compounds (12),(13) in case of sodium sulphide and (12a) ,(13) in case of hydrogen sulphide separately.



(**12**) (**12a**) (**13**)

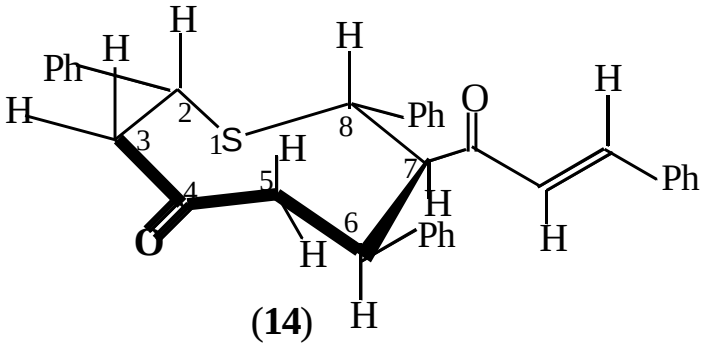
(g) In support of the generalisation of Bahar *et al* hypothesis which were also quite in agreement with the Cremmer-Subbaratnam mechanism, we carried out the reaction of dibenzylidene acetone (DBA) with sodium and ammonium sulphides separately at room and reflux temperatures using different stoichiometry in methanol and ethanol separately

and obtained compound (15) and a noble eight-membered sulphur heterocycle as a white crystalline solid, T_m: $191^0 – 192^0$ C, 7-cinnamoyl-2,6,8-triphenylthiocan-4-one (**14**) whose structure was proposed as follows:



(**14**) (**15**)

The compound (14) has a molecular mass 502 (M^+). The presence of (M+2) peak (≈ 20% of M^+) at 504 indicated the presence of sulphur in it and its formula was $C_{34}H_{30}O_2S$. The compound also has two carbonyl groups one in a ring and the other as a cinnamoyl side chain (^{13}C- and ^{1}H-nmr). The ^{13}C-study (DEPT-135) also indicated the presence of two methylene groups. The ^{1}H-nmr indicated the presence of four phenyl groups one of which is a part of cinnamoyl moiety. All the above study advocated the structure (14) as mentioned. Moreover, the ^{1}H- and ^{13}C-nmr data and spectra of the compound match in all respect with those generated from Chem Draw Ultra 7.0 and www.nmrdb.org.



(**14**)

4. In the scope of present investigation we made some recommendations which could be done in future.

Part II

1. This part of the thesis like that of Part I contains a general introduction , literature on biological activity of thiopyrans, aim of the present investigation, and scope of the present investigation.
2. The present investigation includes:
   a) The quantitative analysis of total antioxidant capacity (TAC) of eleven synthesised compounds. The compound B10 was found to be the most potent with respect to ascorbate (standard), B11 and B14 showed moderate and in significant for B2, B5, and B12.

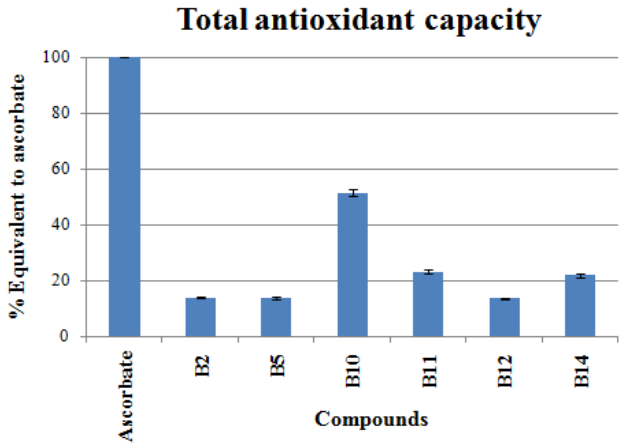


Figure : Total antioxidant activity of the synthesised compounds compared to ascorbate.

   b) The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was carried out on B4, B5, and B11 of which B11 was the most prominent

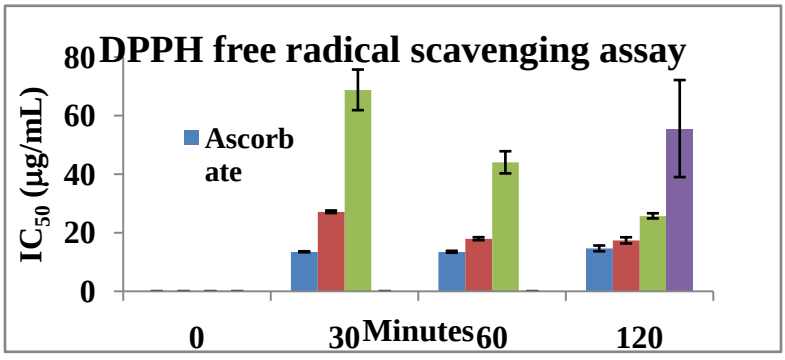


Figure : The DPPH free radical scavenging assay of the synthesised compounds compared to ascorbate

c) The antimicrobial activity were performed on four selective gram-positive bacteria such as *B. cereus, B. subtilis, B. megatorium* and *S. aureus* and five different gram-negative bacteria such as *E. coli, K. pneumoniae, S. flexneri , S. boydii, V. cholerae* and antifungal on two fungi such as *Candida albicans* and *Saccharomyces cerevisiae.* The compound B5 was the most potent antibacterial agent against all stains. The B4 was most active against *B. megatorium*, B2 and B10 had moderate activity against all strains The B15 was the most active against *S. aureus* and it also affected *B.cereus*. The compound B5 and B10 was active against all gram-positive bacteria tested. Against gram-negative bacteria none of the compounds affected all bacterial strains. The B4 and B5 worked best against *V. cholera*, B10 was the most effective against *S.boydii* but B1 had the weakest activity which worked against only *K. pneumonia* and *E. coli* . The compound B4 and B5 was active against most of the gram-negative bacteria tested.

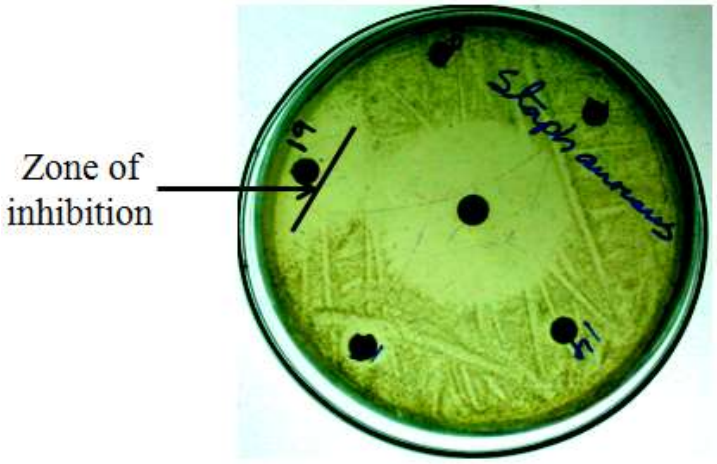


Fig : Representative figure of zone of inhibition of bacterial growth during antibiotic treatment by disc diffusion method

d) The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) study were also carried out. The B4 was active against *S. flexneri* and *Vibrio cholera* but B5 was only active against *Vibrio cholerae.* The compound B8 had activity on *Bacillus cereus*. *S.* boydii was affected by both B5 and B10 and both B5 and

B10 did not allow the bacteria to grow much and killed it at 100 µg/mL

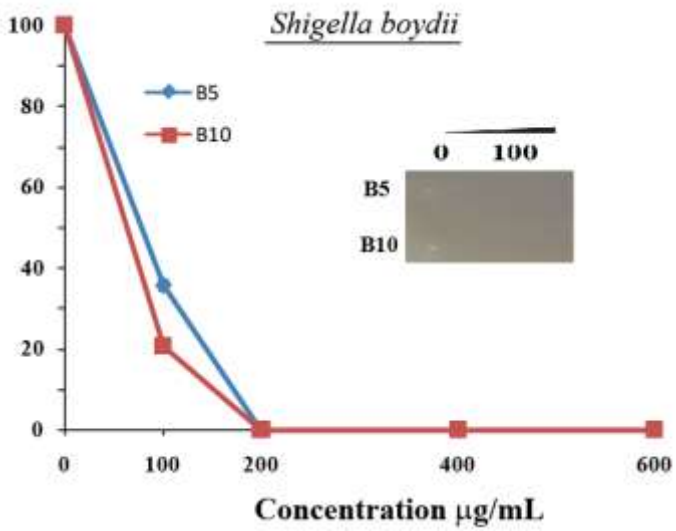


Figure : The MIC and MBC values of B5 and B10 against *Shigella boydii*

e) Effect of the compounds on chromosomal DNA and at protein level was also carried out on *S.aureus* and *S. boydii*. Both the compound B5 and B10 affected *S. boydii*. It damaged the DNA of the bacteria as can be seen in the agarose gel electrophoretic movementof the damaged DNA. The clear band of DNA as in the untreated sample has been damaged and turned into a smear of DNA by both B5 and B10.

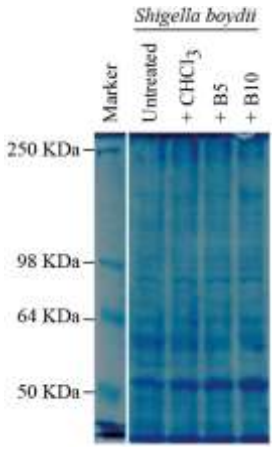


Fig : SDS polyacrylamide gel electrophoresis of entire protein content of *S.boydii* after the bacteria was untreated, treated with $CHCl_3$, B5 and B10

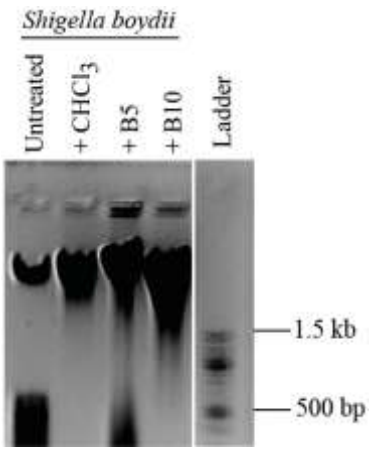


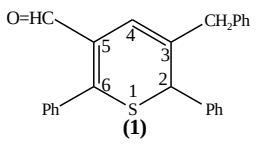
Fig : The agarose gel electrophoresis of the genomic DNA of *S.boydii* after the bacteria was untreated, treated with $CHCl_3$, B5 and B10

f) At the end, the antifungal activity was carried out on two fungi such as. *Candida albicans* and *Saccharomyces cerevisiae.* The compound B5 and B10 had the antifungal activity.The compound B5 affected only *S. cerevisiae* but B10 affected both by *Candida albicans* and *Saccharomyces cerevisiae*.

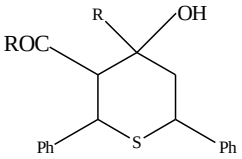
A B S T R A C T

Long back in 1959, Latif and co-workers, department of Chemistry , Rajshahi University , Bangladesh in an attempt to prepare dithiobenzoic acid [Ph-(C=S)-SH] from a mixture of benzaldehyde and ethanol in presence of aqueous sodium sulphide ended up with structure (1), 3-benzyl -2,6-diphenyl-2H-thiopyran-5-carboxaldehyde. .The structure of (1) was later confirmed by a number of workers who also proposed mechanism for its formation. But none of them could either isolate or gave any evidence in support of the intermediates proposed in the mechanism. Keeping this view in mind , we took this project to justify their mechanism by using some of the intermediates and some more compounds which could be transformed into those intermediates *in situ* and ended up with compound (1) , compounds analogous to (1) are (9), (10) , and some other products not analogous to (1) are (11), (12), (13), (14), and (15). The compound(7) and (8) are analogous to the intermediate (5).

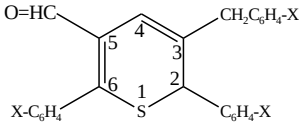
One of the products (14) was isolated for the first time is an interesting noble compound having sulphur in an eight membered ring (7-cinnamoyl-2,6,8-triphenyl-thiocane-4-one) .



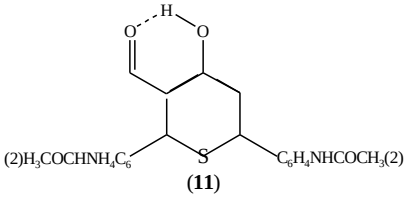
(1)



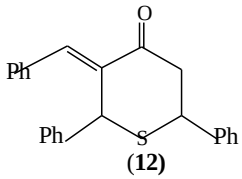
7, R = CH_3
8, R = Ph



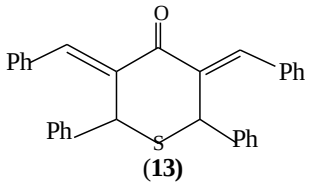
9, X = 4-OMe
10, X = 2-Me



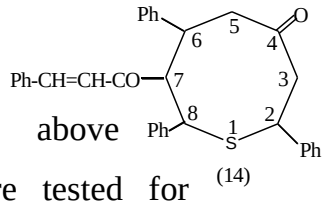
(**11**)



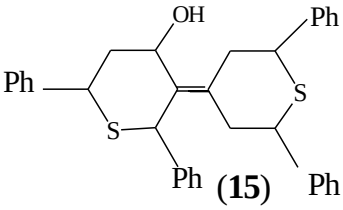
(**12**)



(13)



(14)



(15)

Some of the above synthesised compounds were tested for their biological activity against four selective gram-positive bacteria such as *Bacillus cereus, Bacillus subtilis, Bacillus megatorium* and *Straphylococcus aureus* and five gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Shigella flexneri , Shigella boydii ,Vibrio cholerae*. Some of which were very promising and fewer moderate. Two fungi such as *Candida albicans* and *Saccharomyces cerevisiae* also assayed whose results were not very promising.

Chapter 5

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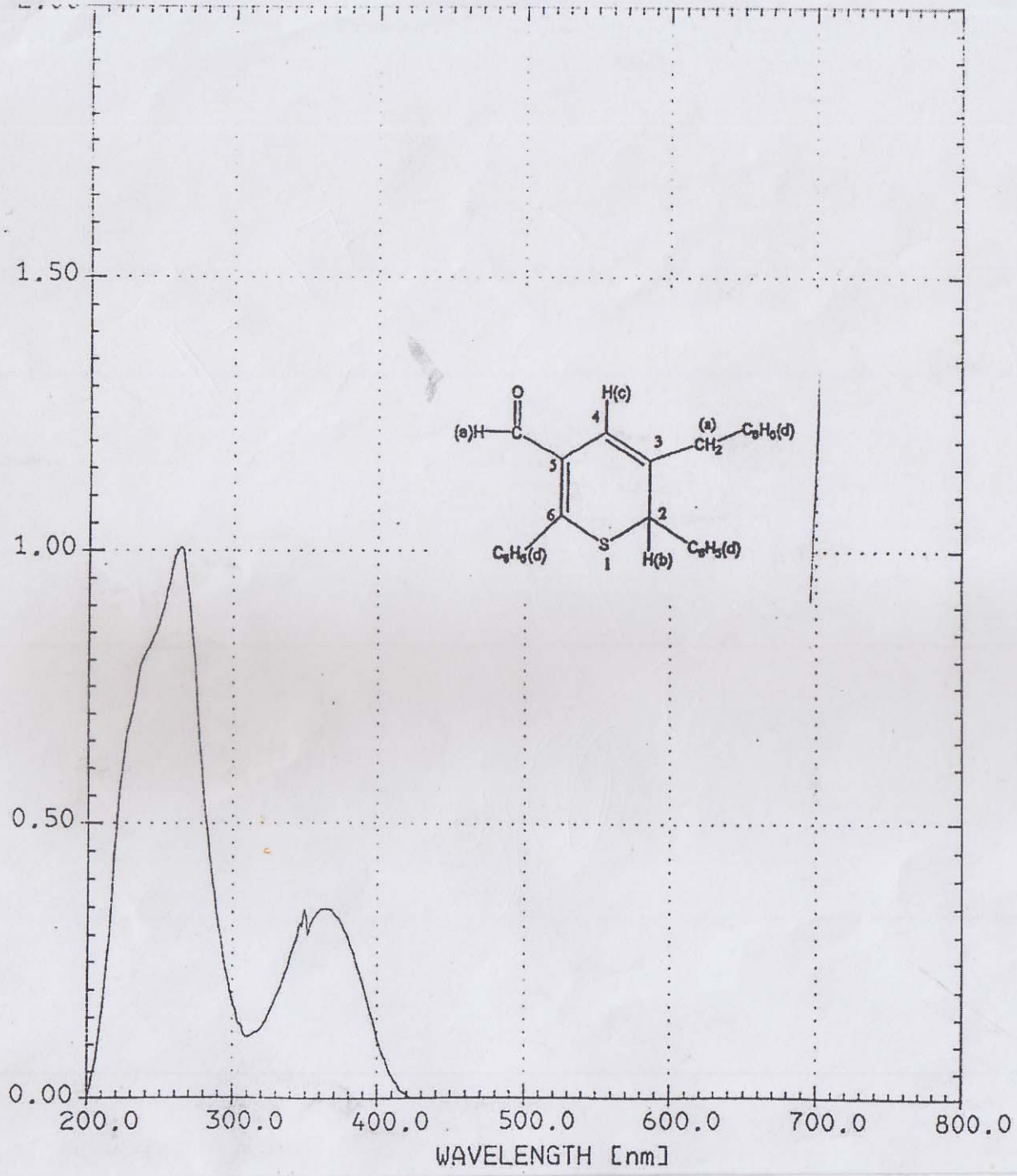


No.	PEAK λ	PEAK ABS	VALLEY λ	VALLEY ABS
1	363.0	0.349	539.0	-0.015
2	264.0	1.007	310.0	0.116

2/3

Fig 1: UV-Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde (**1**)

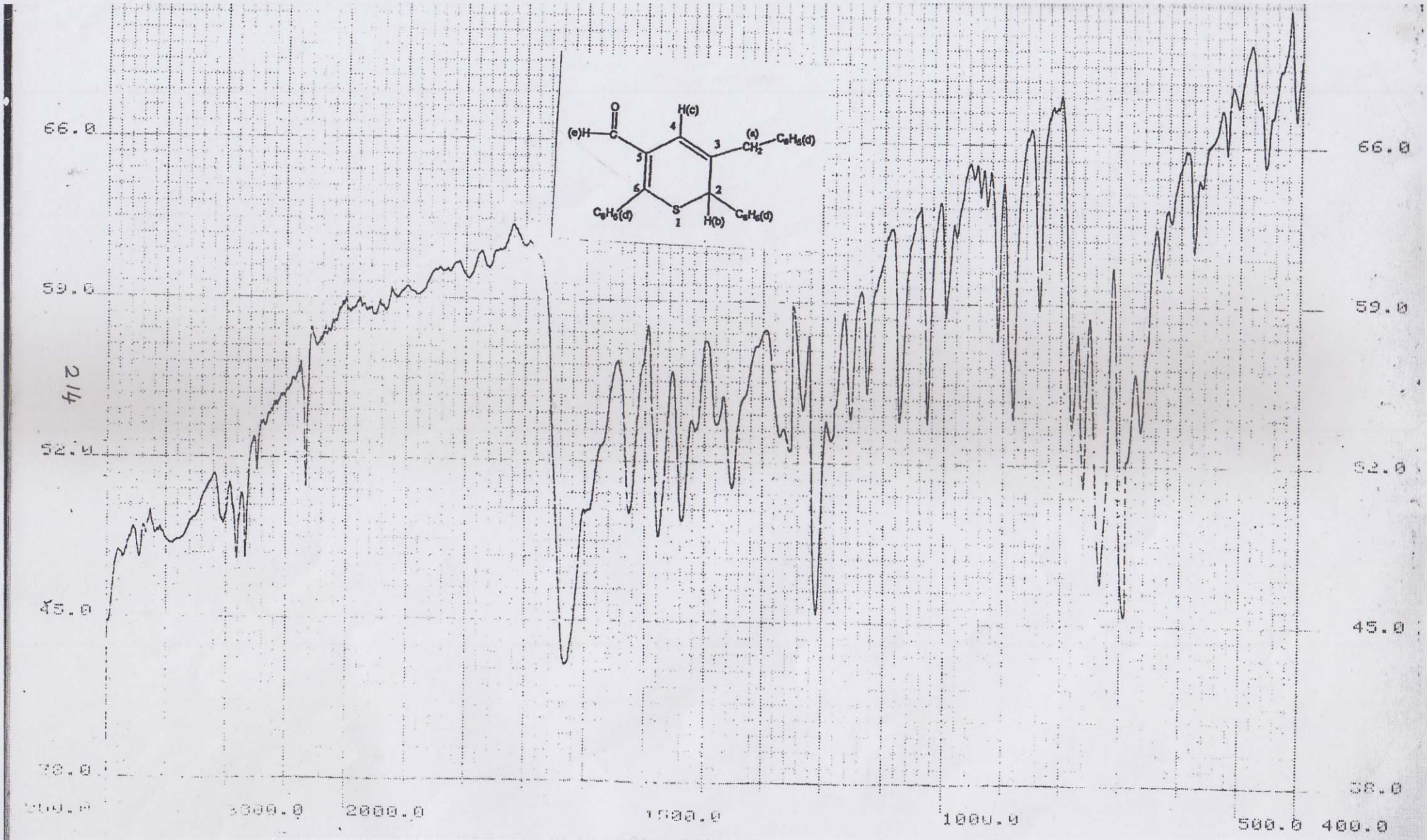


Fig 2: IR- Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde (**1**)

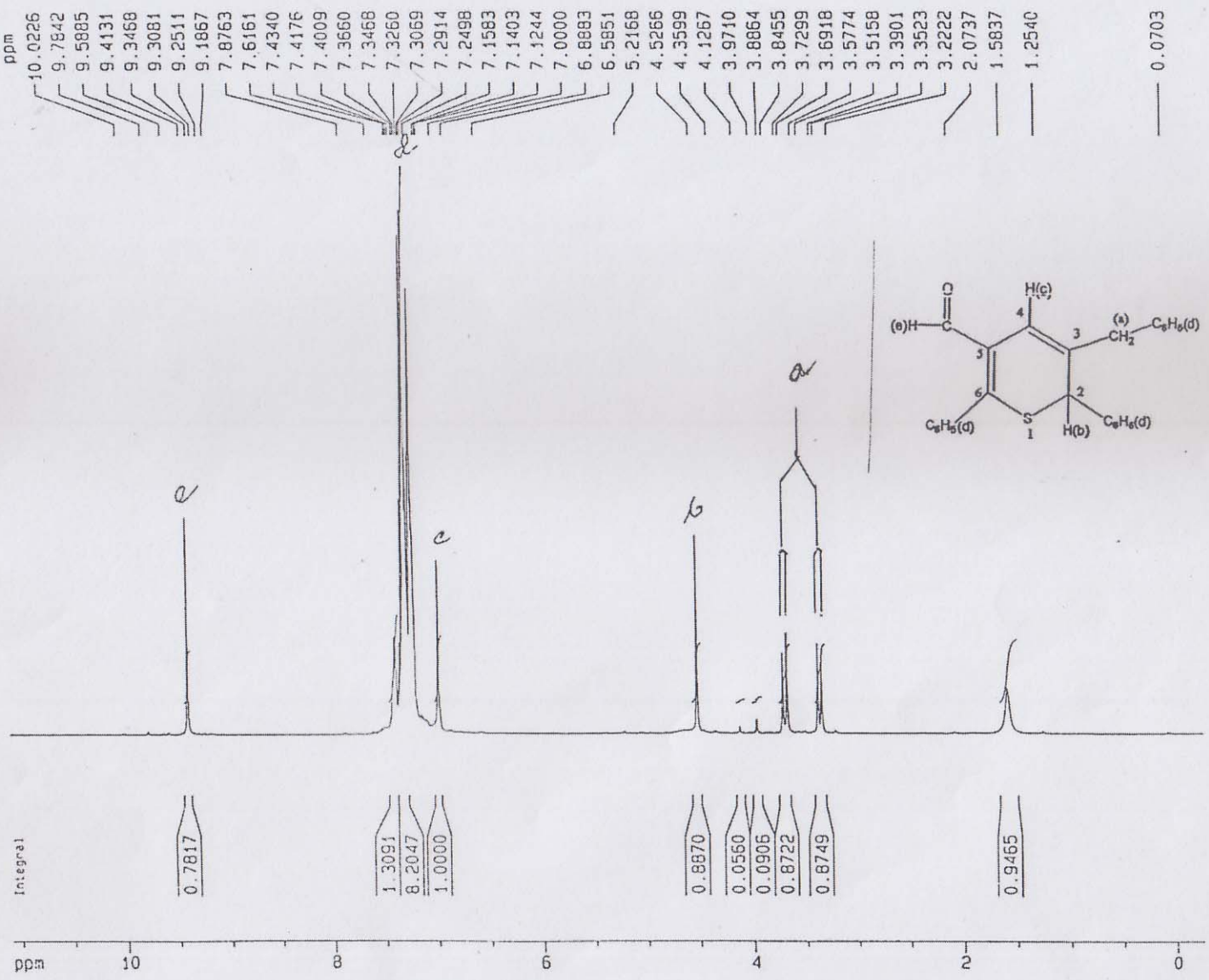


Fig 3 : ^{1}H-nmr Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde (**1**)

Fig 3a : ^{1}H-nmr Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde (**1**) [expanded part]

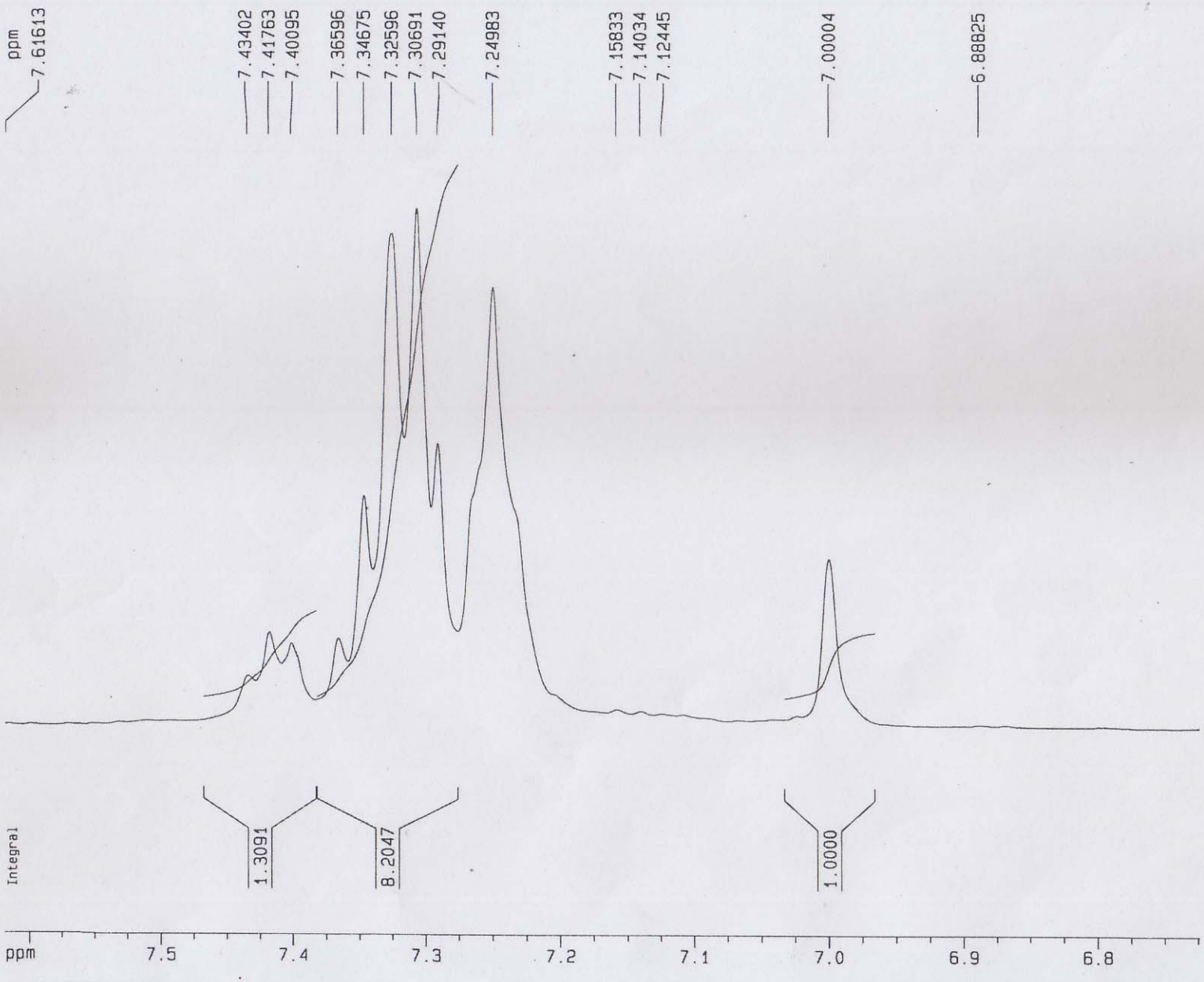
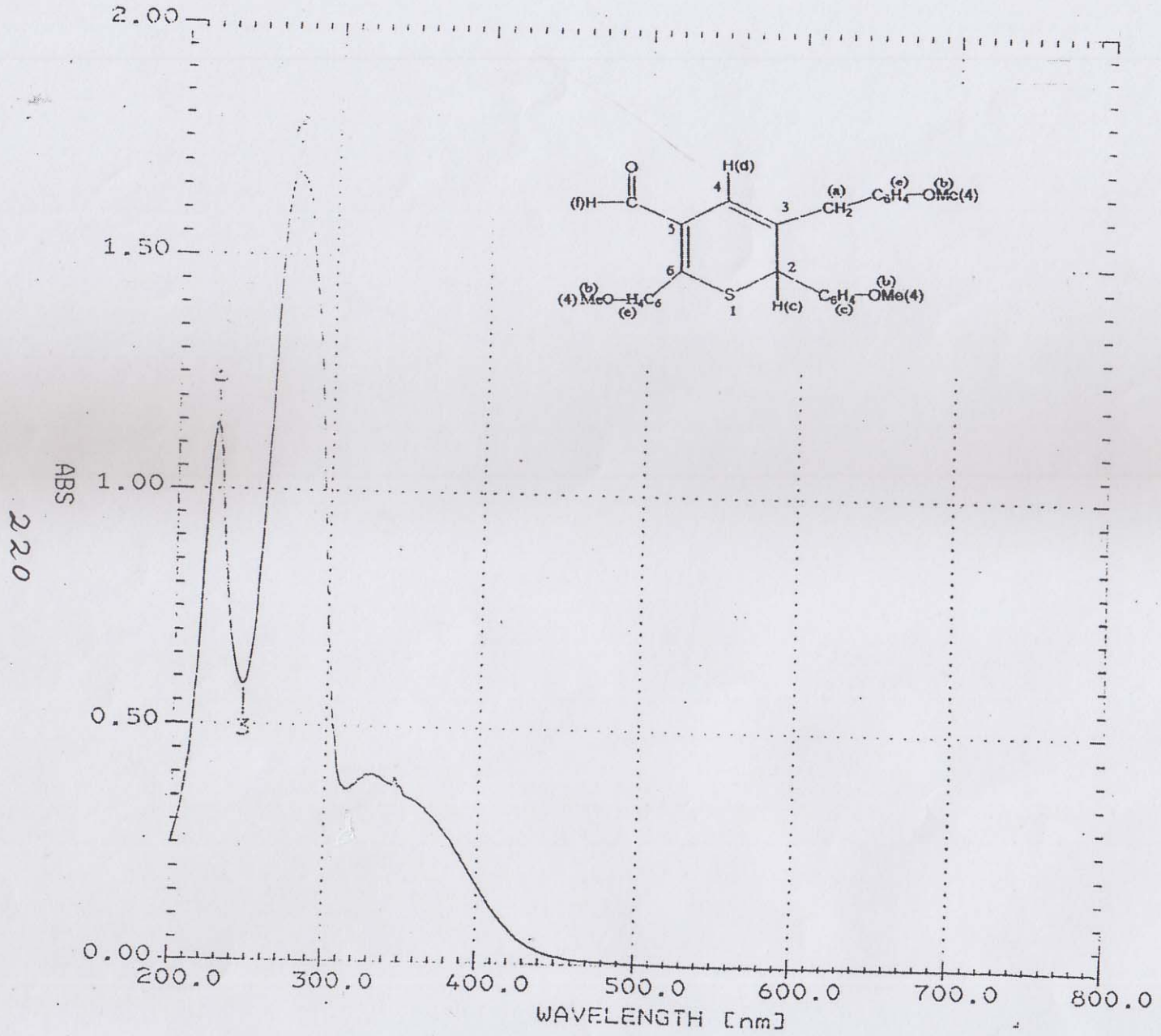


Fig3b : ^{1}H-nmr Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde **(1)** [expanded part]

Fig 4 : ^{13}C-nmr Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde (**1**)

Fig 4a : ^{13}C-nmr Spectrum of 3-benzyl-2,6-diphenyl-2H-thiopyran -6-carboxaldehyde (**1**) [expanded part]



*** PEAK-PICK ***

	--- PEAK ---		-- VALLEY --	
Nc.	λ	ABS	λ	ABS
1	331.0	0.401	748.0	0.001
2	274.0	1.676	314.0	0.369
3	225.0	1.145	244.0	0.585

Fig 5 : UV-Spectrum of 2,6-bis(4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran-5-carboxaldehyde ((7)

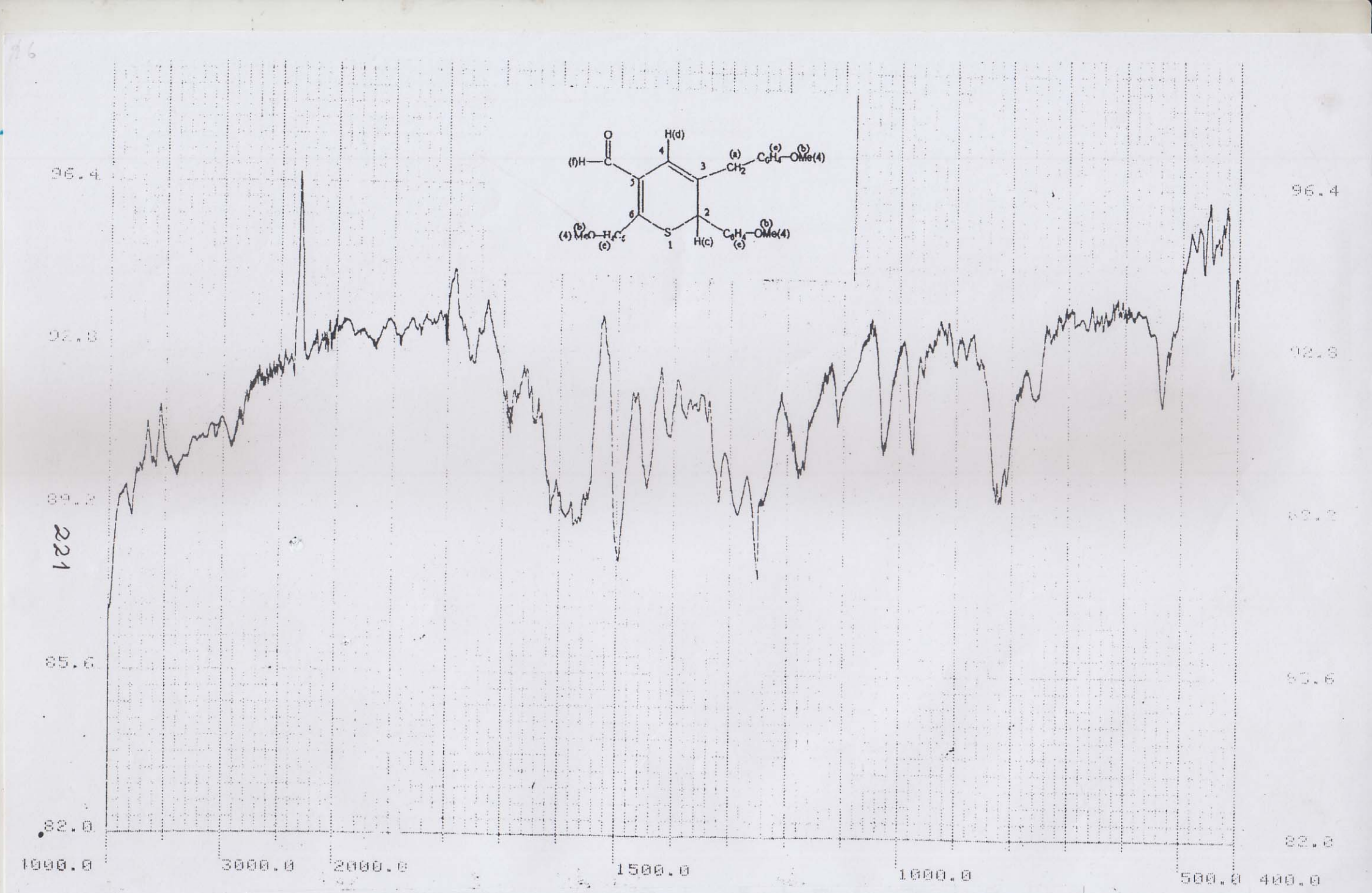


Fig 6 : IR-Spectrum of 2,6-bis(4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran-5-carboxaldehyde ((7)

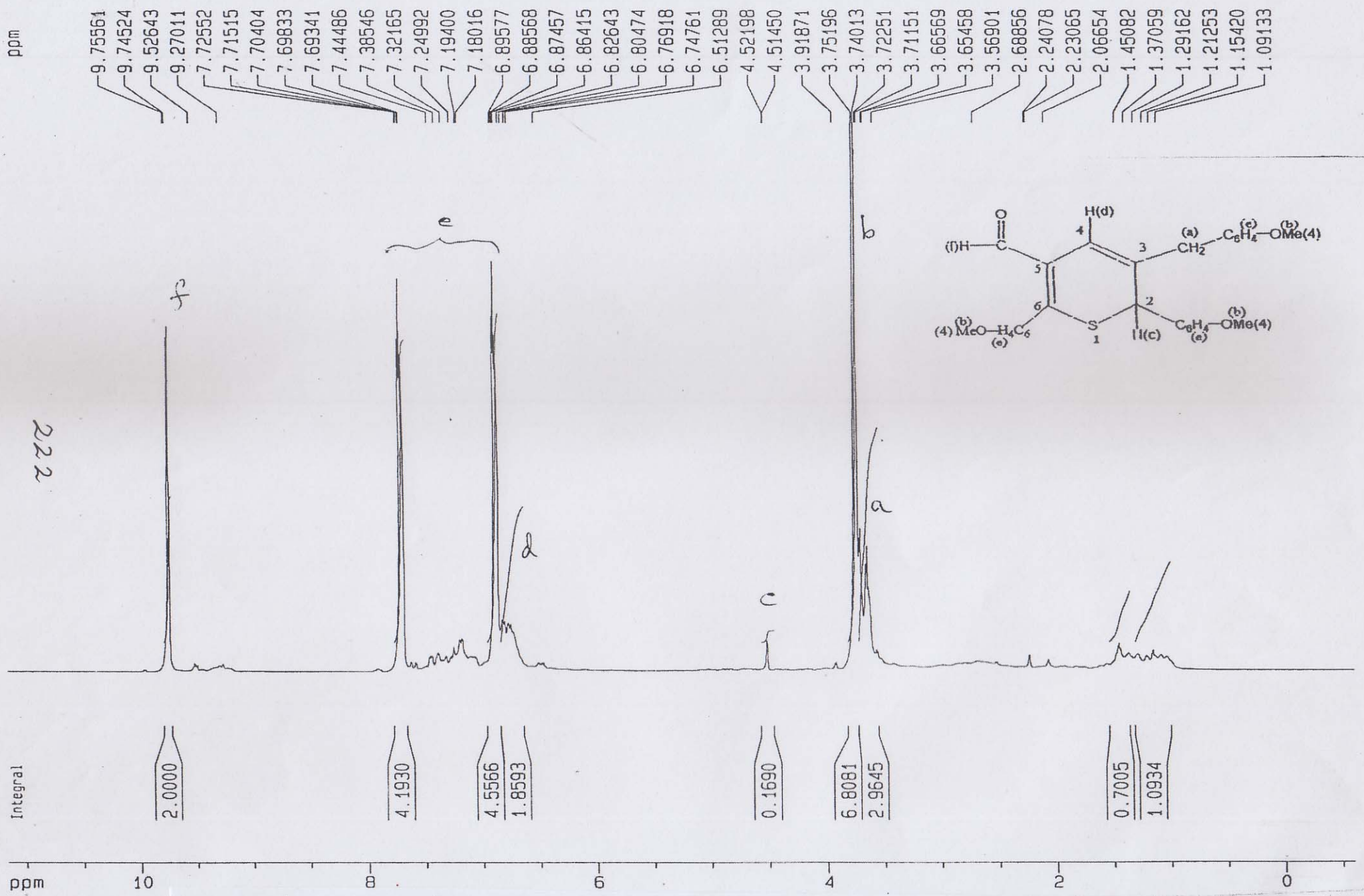


Fig7: ^{1}H-nmr spectrum of 2,6-bis(4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran--5-carboxaldehyde (7)

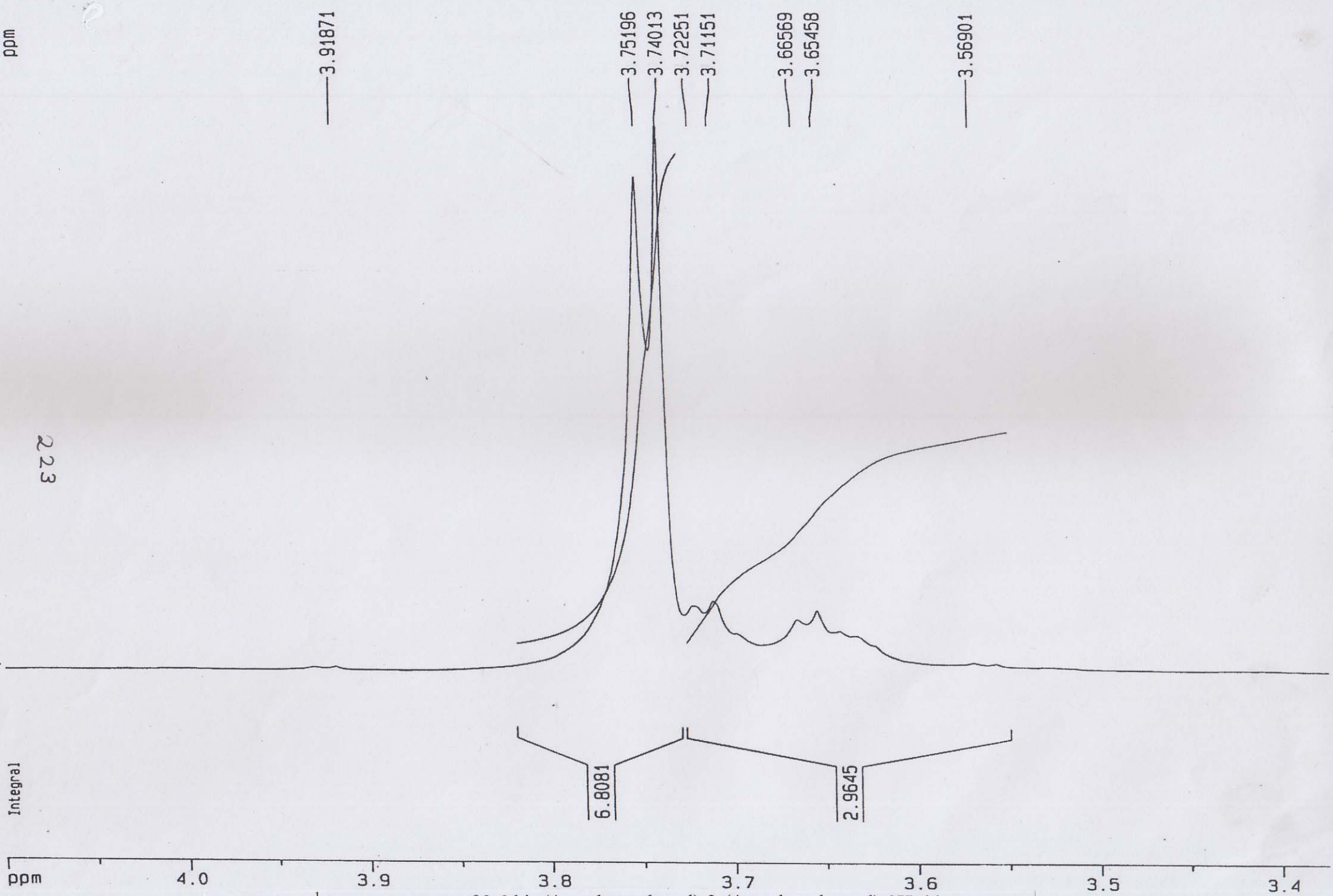


Fig 7a : ^{1}H-nmr spectrum of 2,6-bis (4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran--5-carboxaldehyde(7) [expanded part]

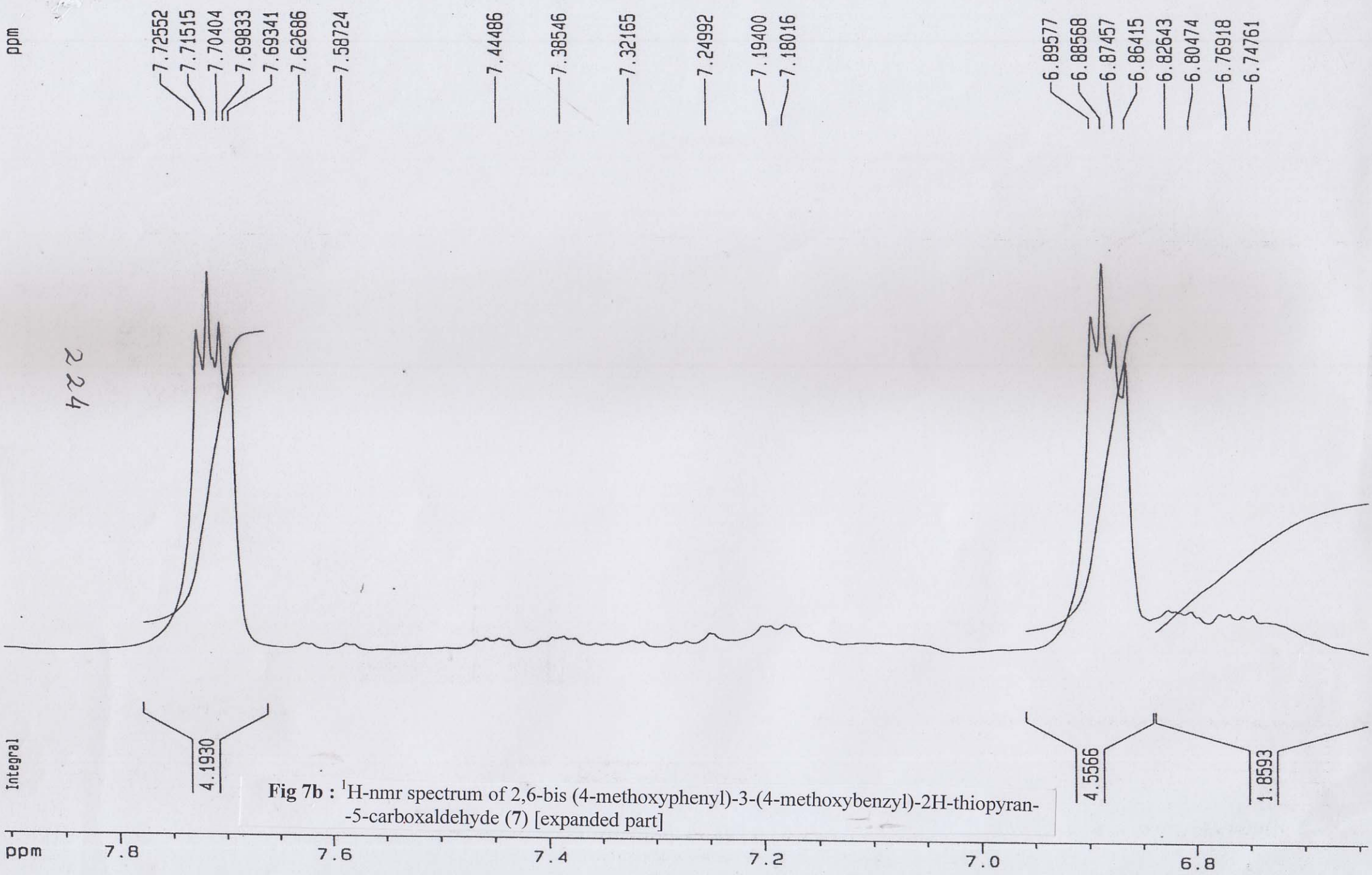


Fig 7b : ^{1}H-nmr spectrum of 2,6-bis (4-methoxyphenyl)-3-(4-methoxybenzyl)-2H-thiopyran--5-carboxaldehyde (7) [expanded part]

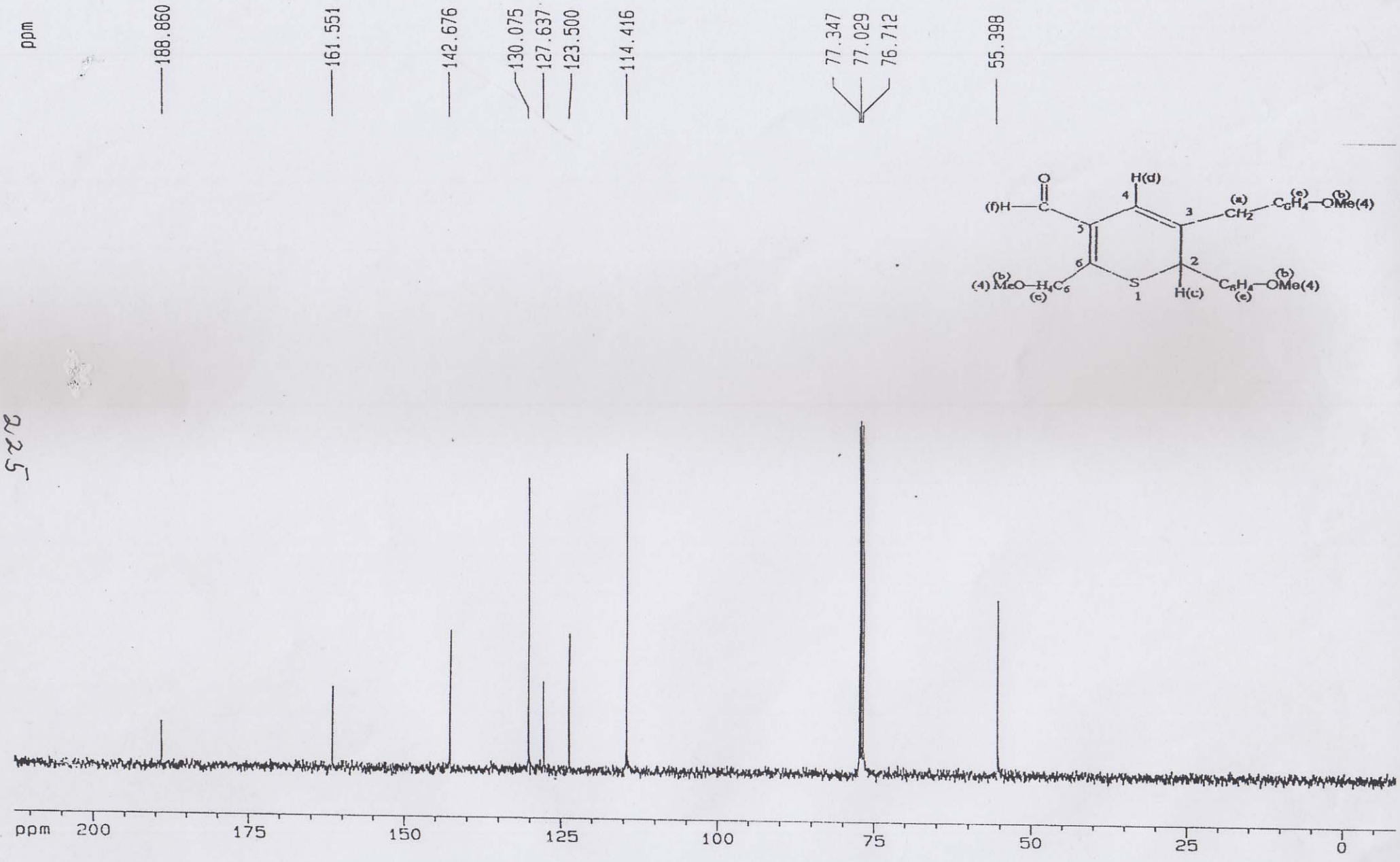
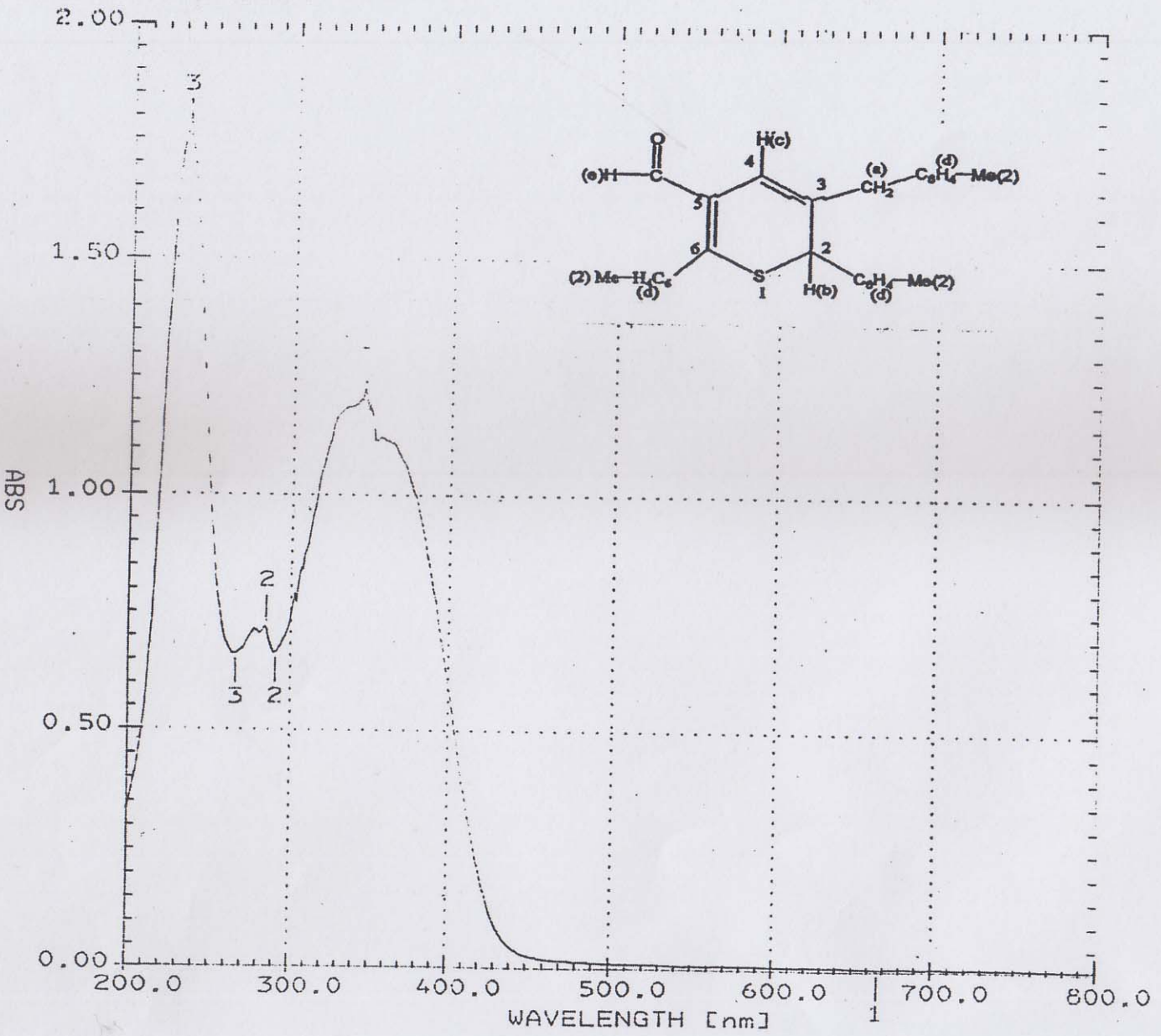


Fig 8 : ^{13}C-nmr spectrum of 2,6-bis (4-methoxyphenyl)-3-(4-methoxybenzyl)-2H- - thiopyran-5-carboxaldehyde (**7**)



*** PEAK-PICK ***

No.	PEAK λ	PEAK ABS	VALLEY λ	VALLEY ABS
1	345.0	1.226	666.0	0.006
2	284.0	0.720	290.0	0.664
3	233.0	1.768	265.0	0.662

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Fig 9 : UV-spectrum of 2,6-bis (2-methyl phenyl)-3-(2-methylbenzyl)-2H-thiopyran - 5-carboxaldehyde **(8)**

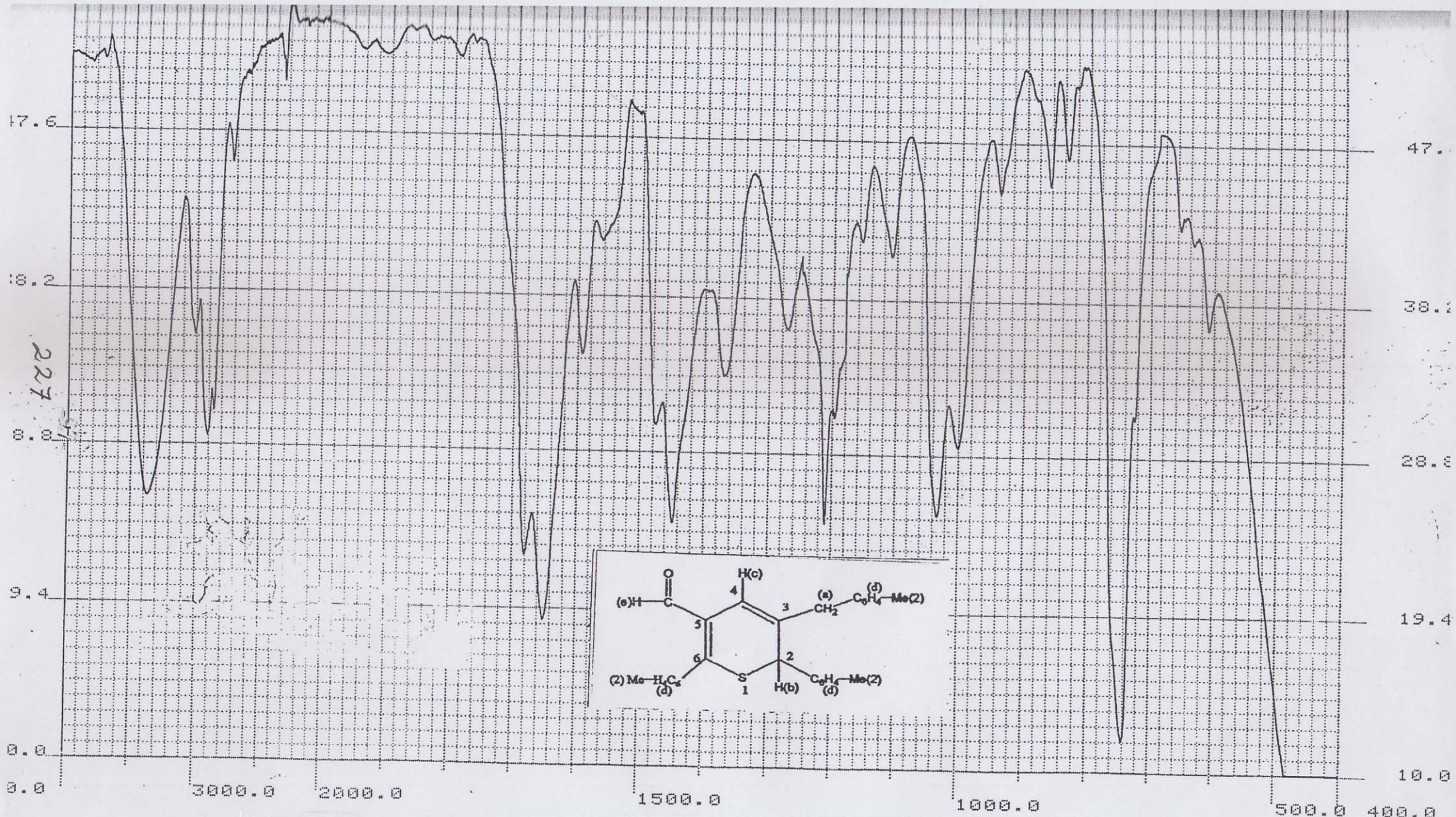


Fig 10 : IR-spectrum of 2,6-bis (2-methyl phenyl)-3-(2-methylbenzyl)-2H-thiopyran--5-carboxaldehyde (**8**)

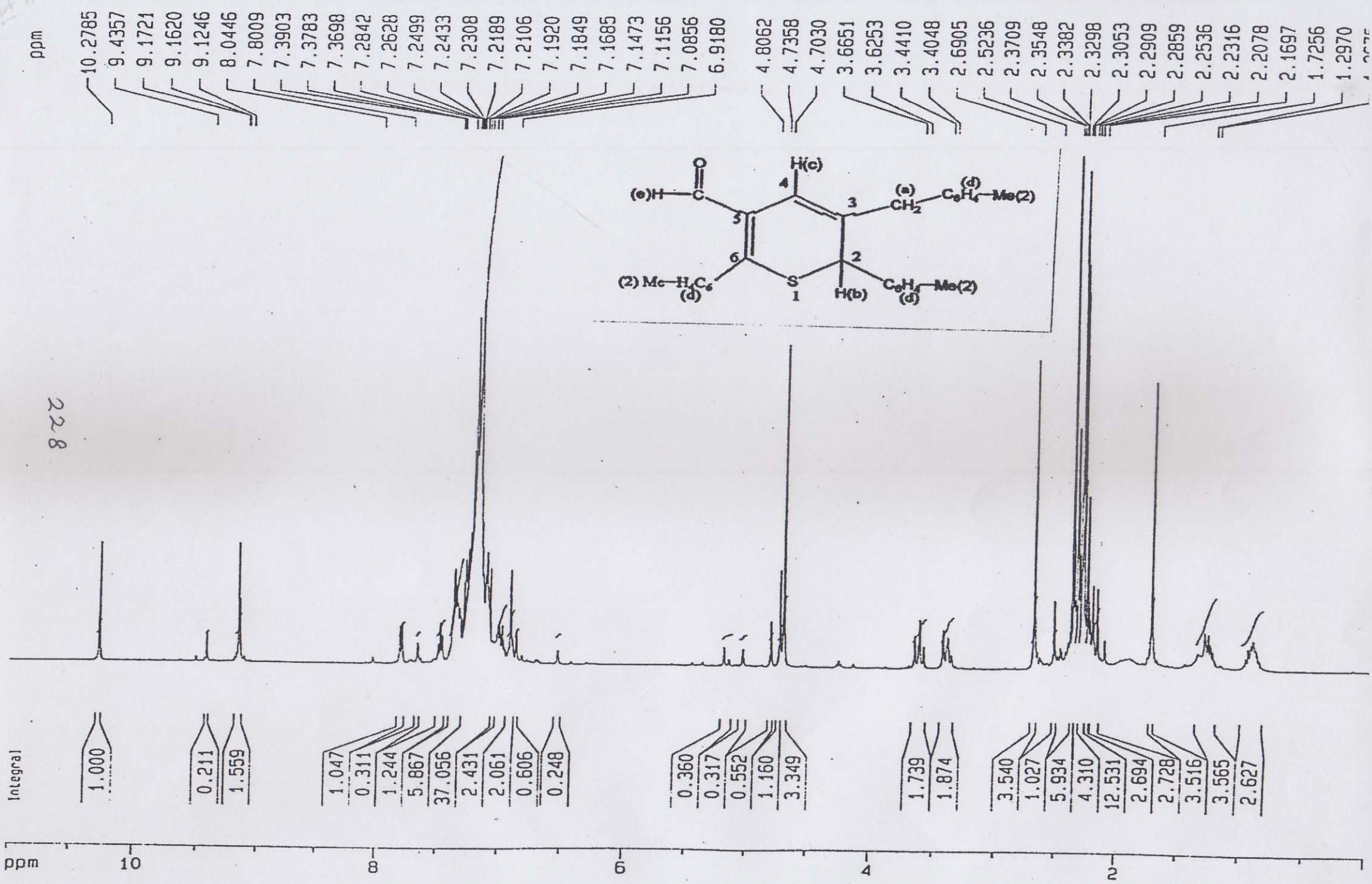


Fig 11 : ^{1}H-nmr spectrum of 2,6-bis (2-methyl phenyl)-3-(2-methylbenzyl)-2H-thiopyran--5-carboxaldehyde (**8**)

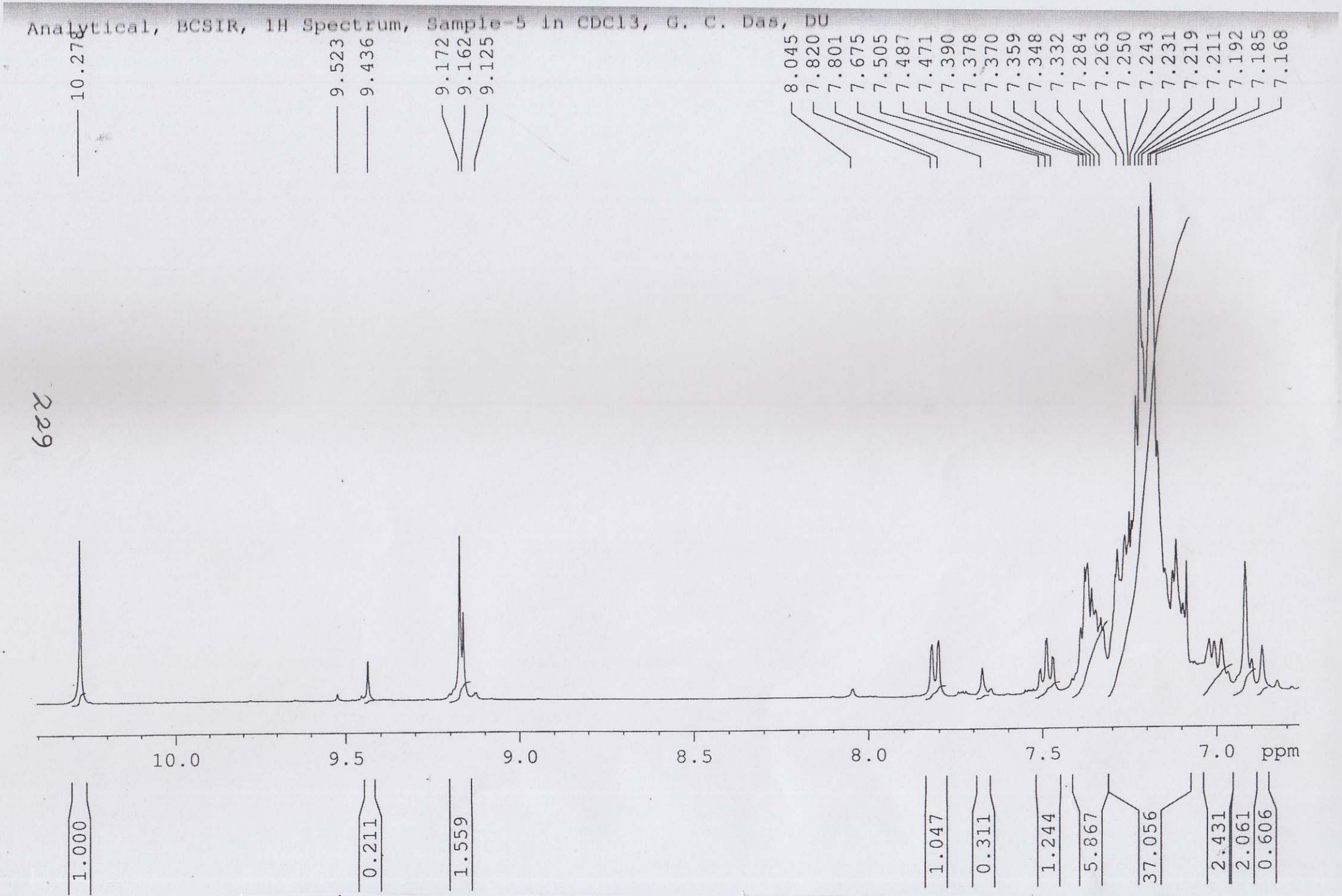


Fig 11a : ^{1}H-nmr spectrum of 2,6-bis (2-methyl phenyl)-3-(2-methylbenzyl)-2H-thiopyran--5-carboxaldehyde (**8**) [expanded part]

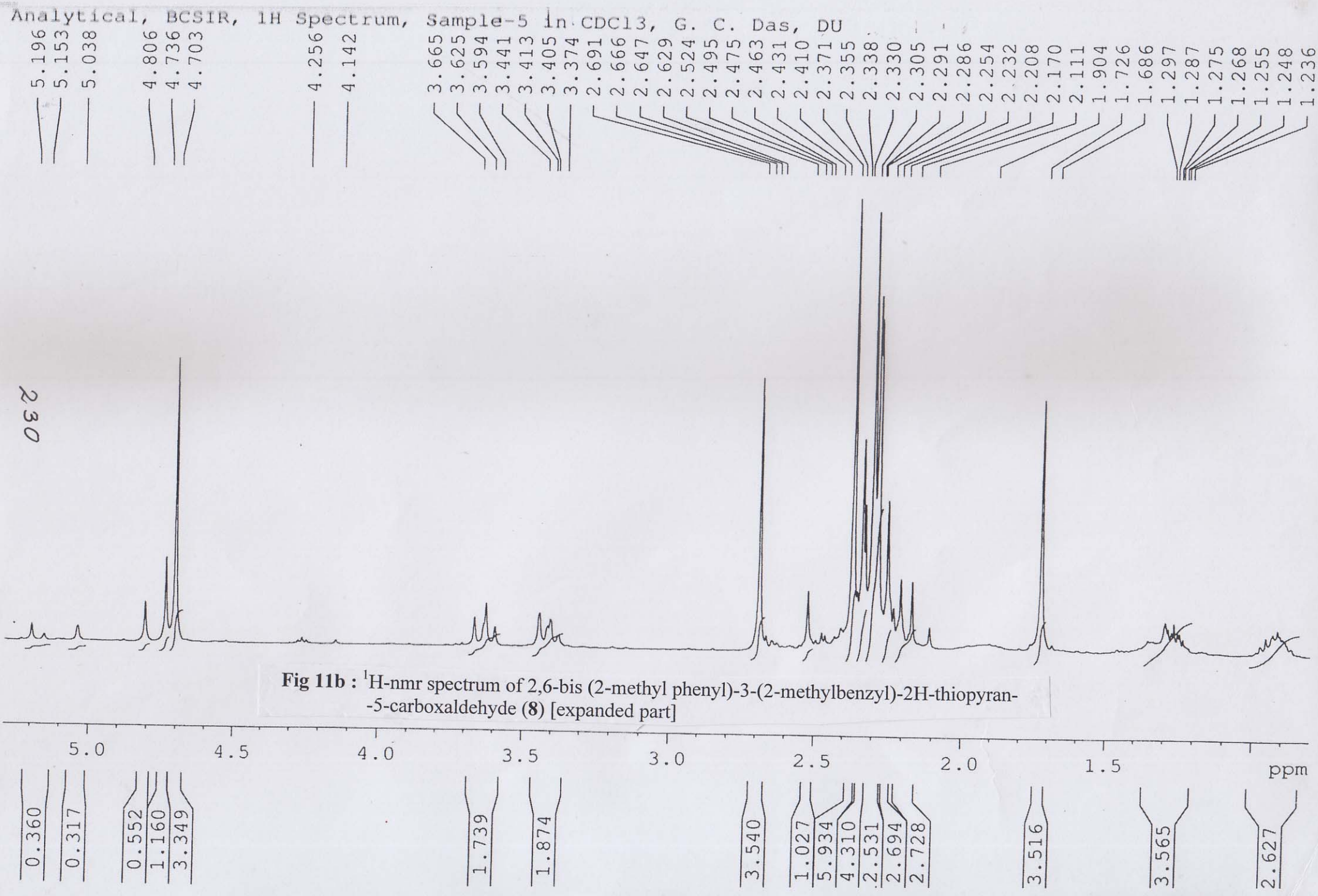
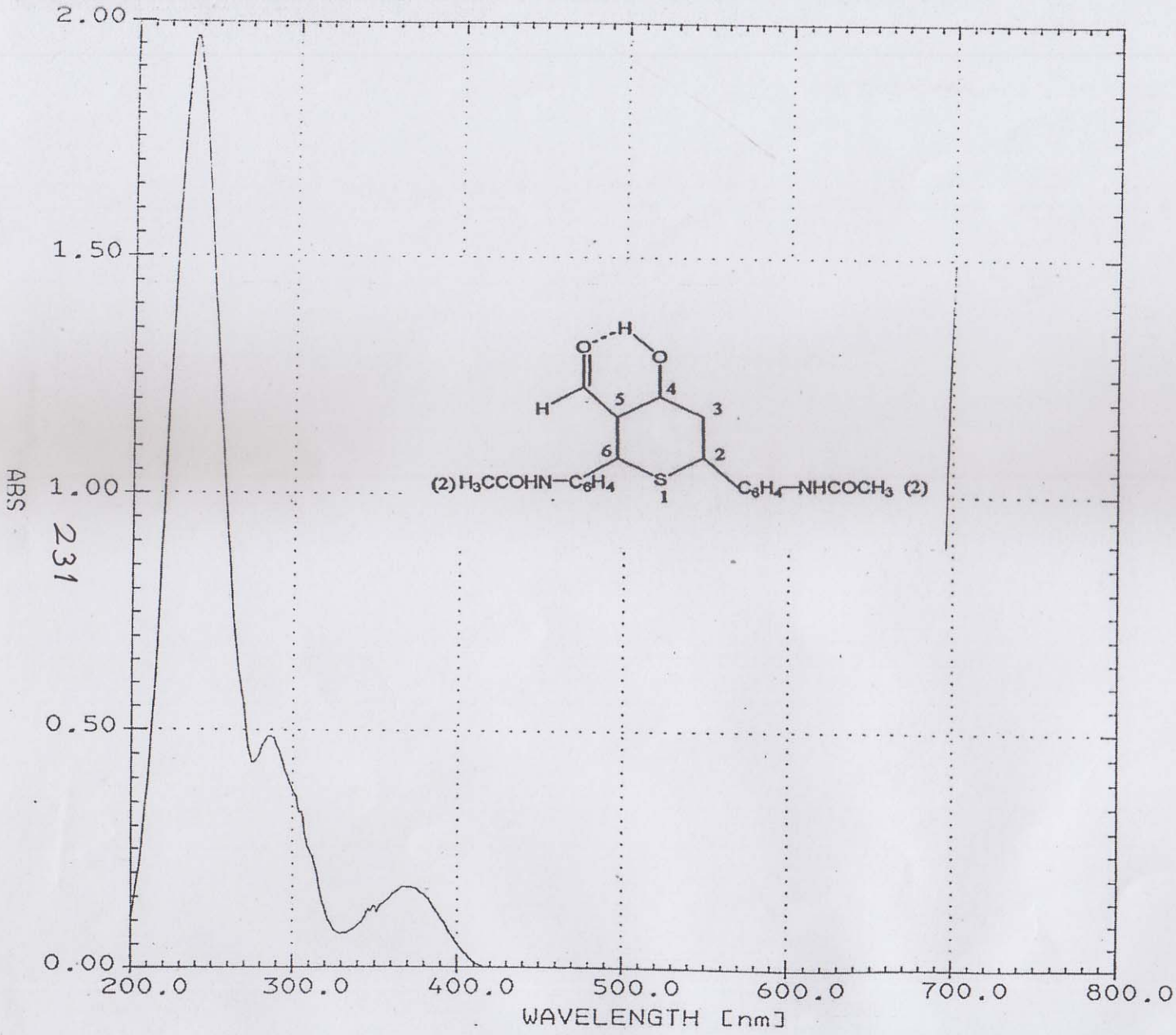


Fig 11b : ^{1}H-nmr spectrum of 2,6-bis (2-methyl phenyl)-3-(2-methylbenzyl)-2H-thiopyran--5-carboxaldehyde (**8**) [expanded part]



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	368.0	0.175	440.0	-0.016
2	286.0	0.489	329.0	0.072
3	236.0	1.968	275.0	0.434

Fig 12: UV-spectrum of 2,6-bis (2-N-acetylaminophenyl) tetrahydrothiopyran--4-hydroxy-5-carboxaldehyde (**9**)

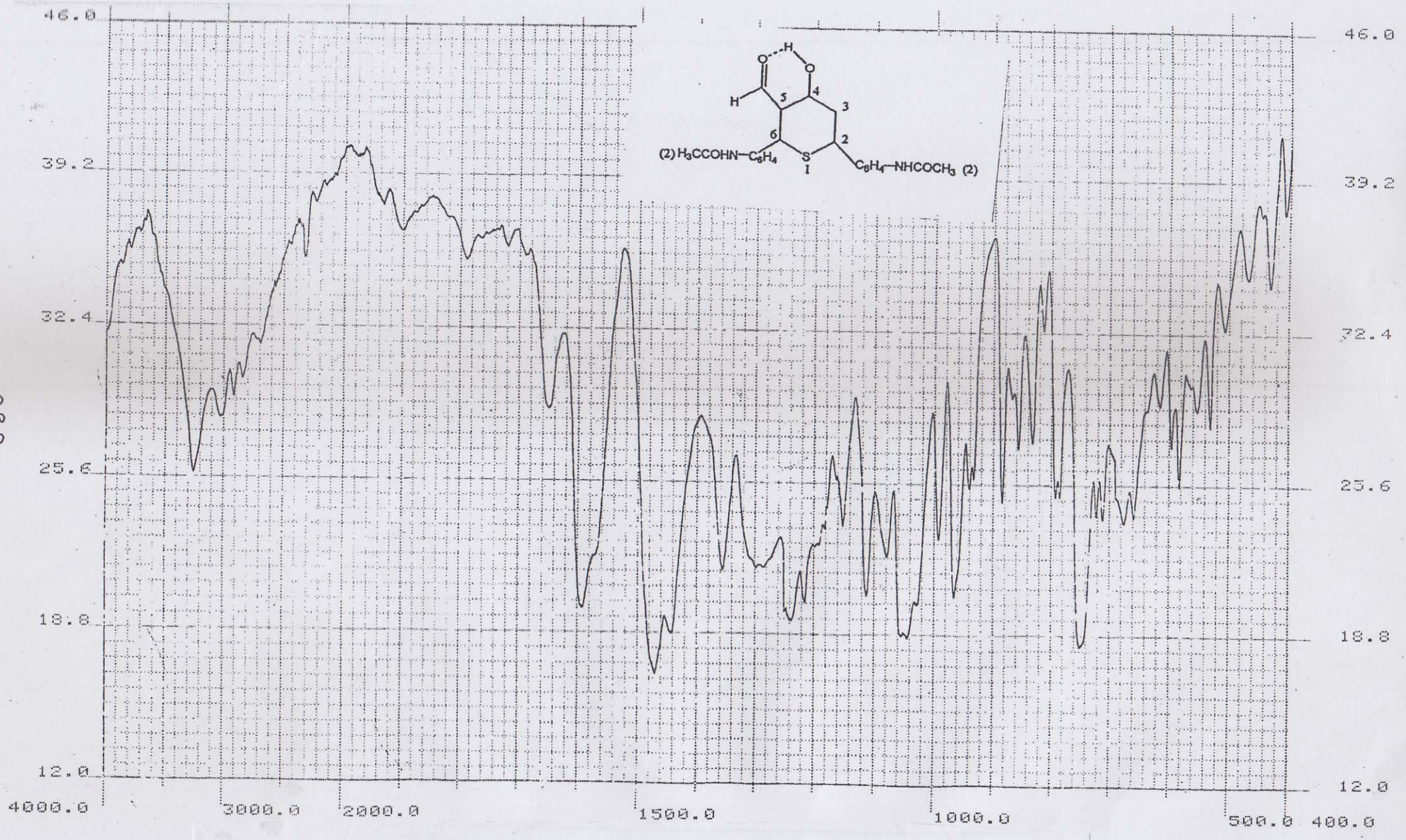
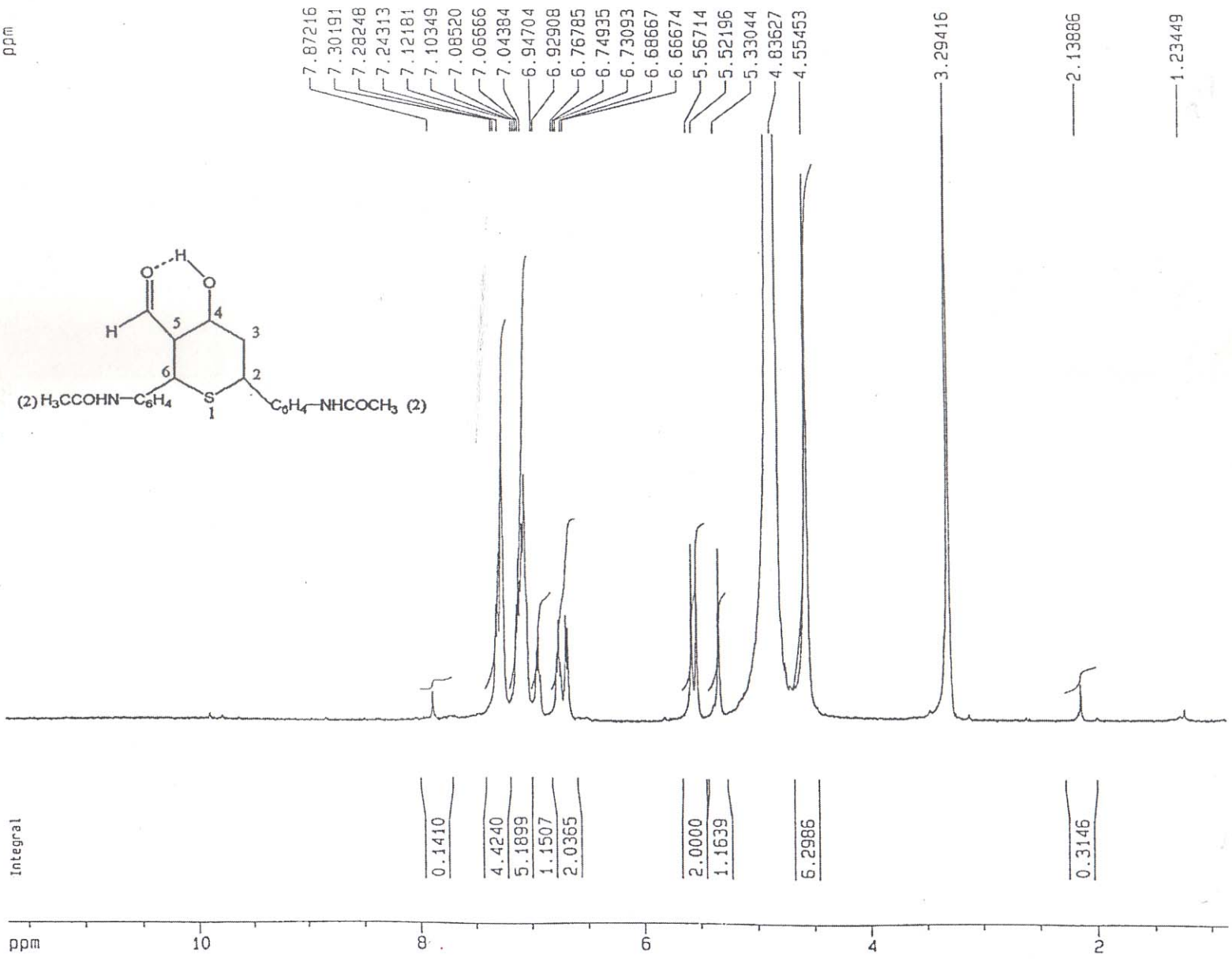


Fig 13: IR-spectrum of 2,6-bis (2-N-acetylaminophenyl) tetrahydrothiopyran-
-4-hydroxy-5-carboxaldehyde (**9**)



233

Fig14: ^{1}H-nmr spectrum of 2,6-bis (2-N-acetylaminophenyl) tetrahydrothiopyran--4-hydroxy-5-carboxaldehyde (**9**)

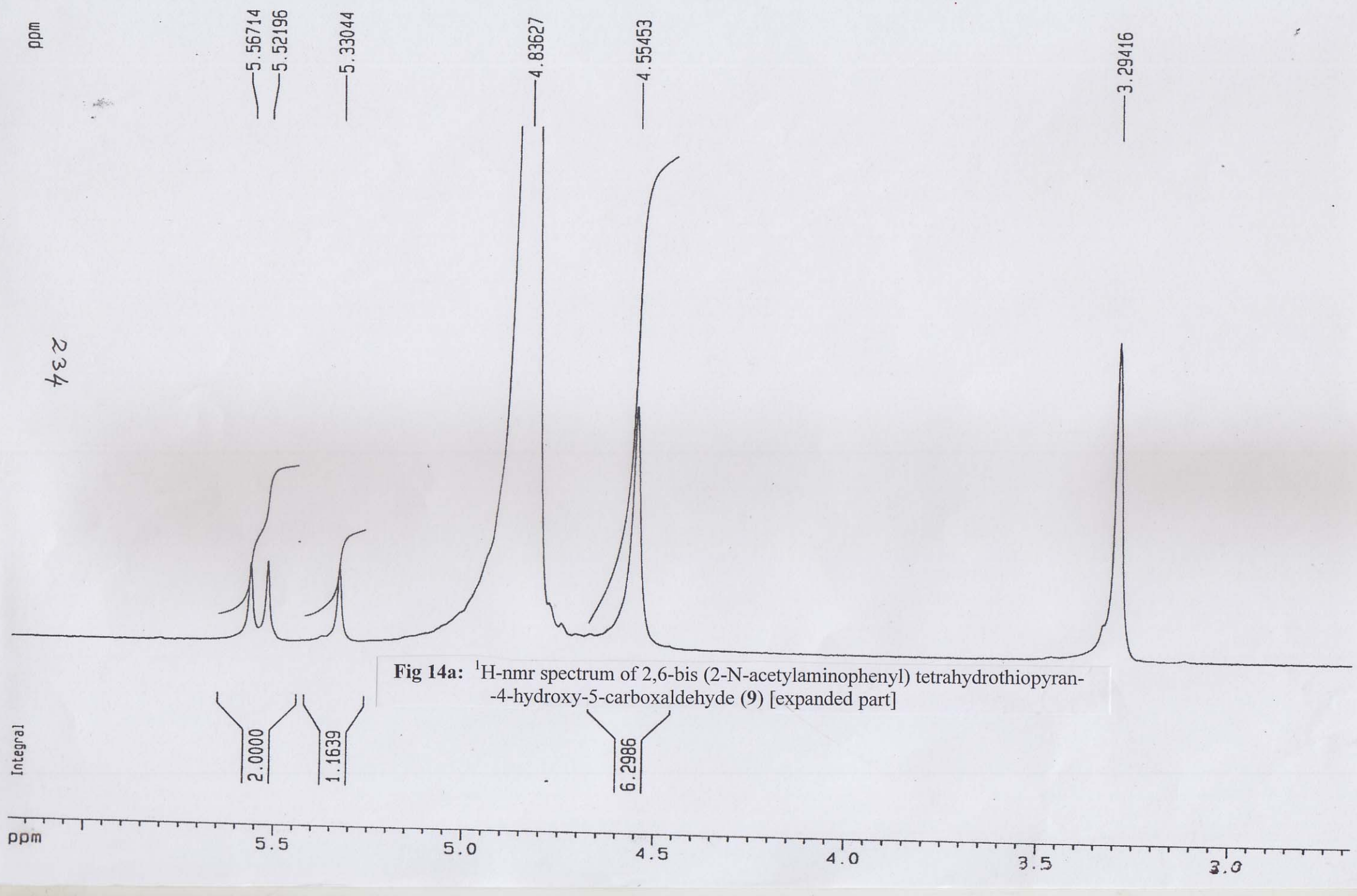


Fig 14a: ^{1}H-nmr spectrum of 2,6-bis (2-N-acetylaminophenyl) tetrahydrothiopyran--4-hydroxy-5-carboxaldehyde (**9**) [expanded part]

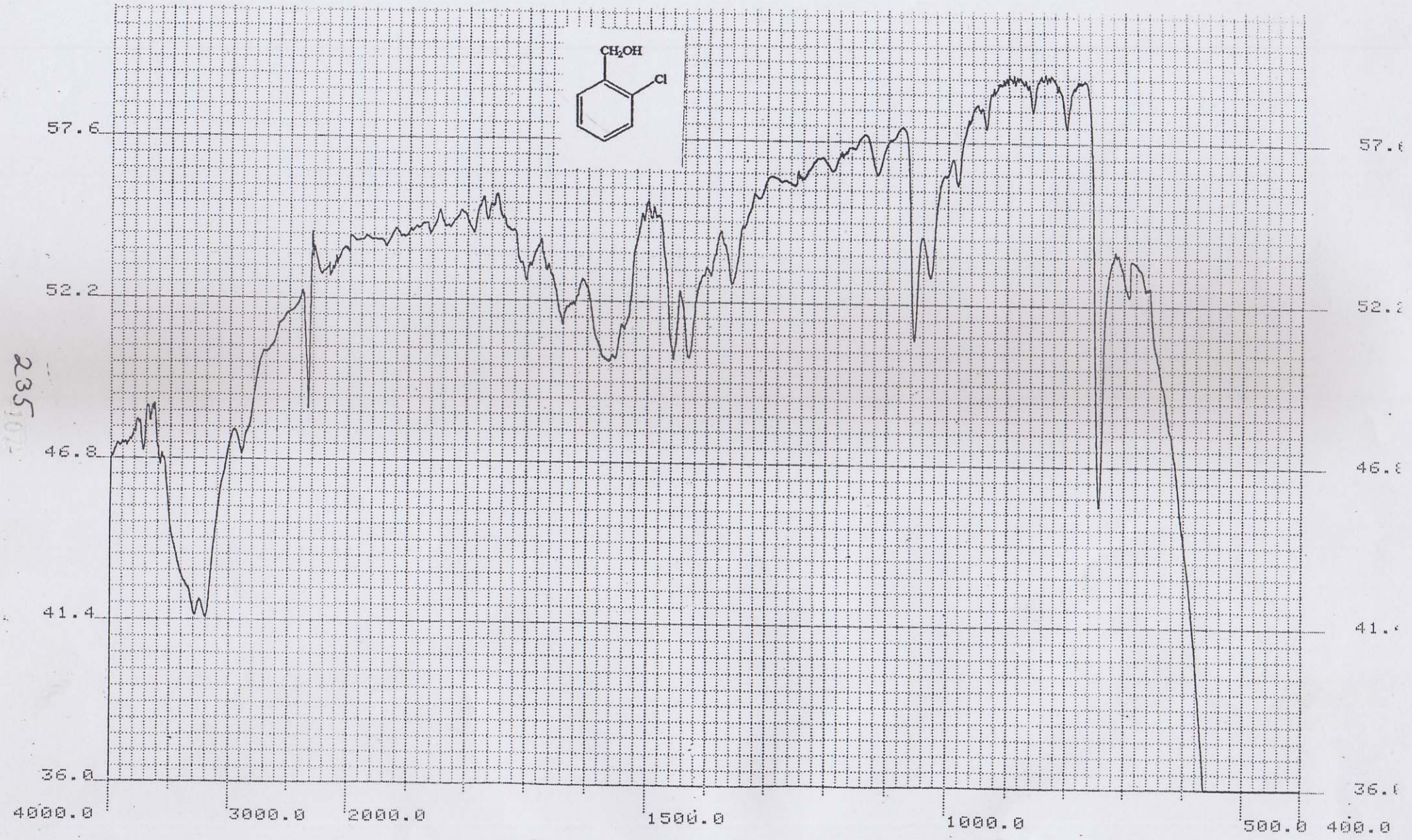


Fig 15 : IR-spectrum of 2-chlorobenzyl alcohol **(13)**

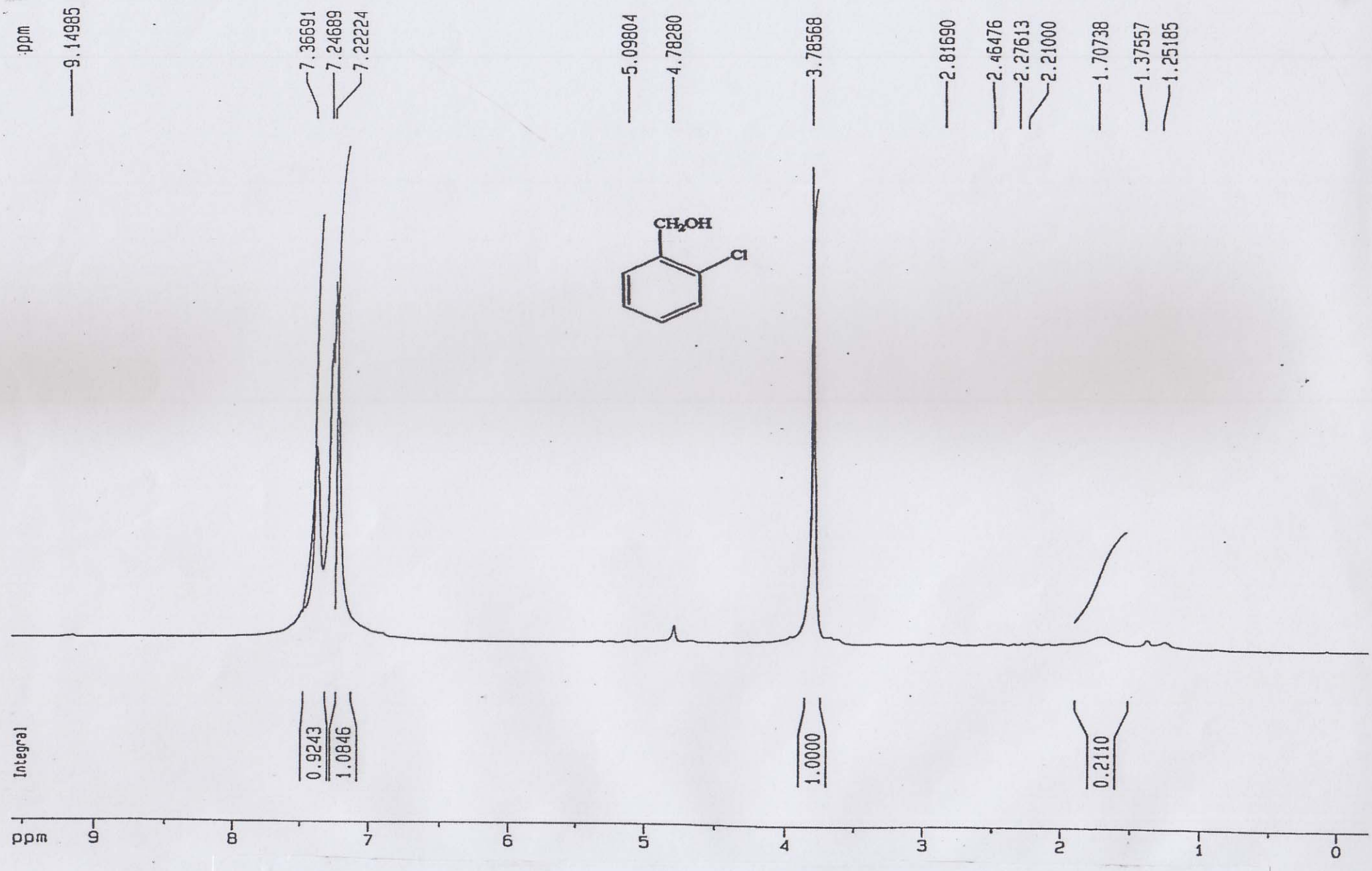
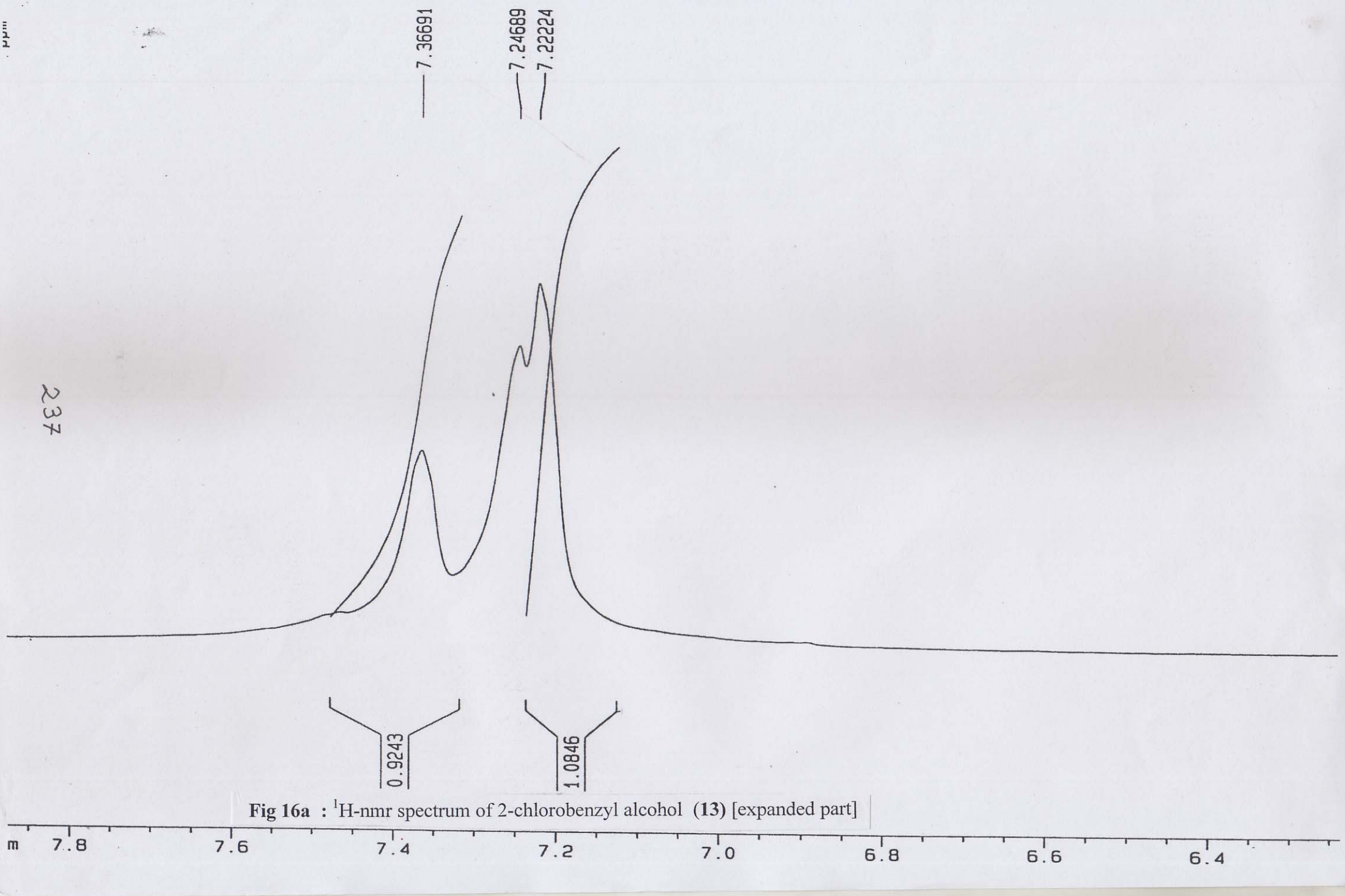


Fig 16 : ^{1}H-nmr spectrum of 2-chlorobenzyl alcohol **(13)**



237

Fig 16a : ^{1}H-nmr spectrum of 2-chlorobenzyl alcohol **(13)** [expanded part]

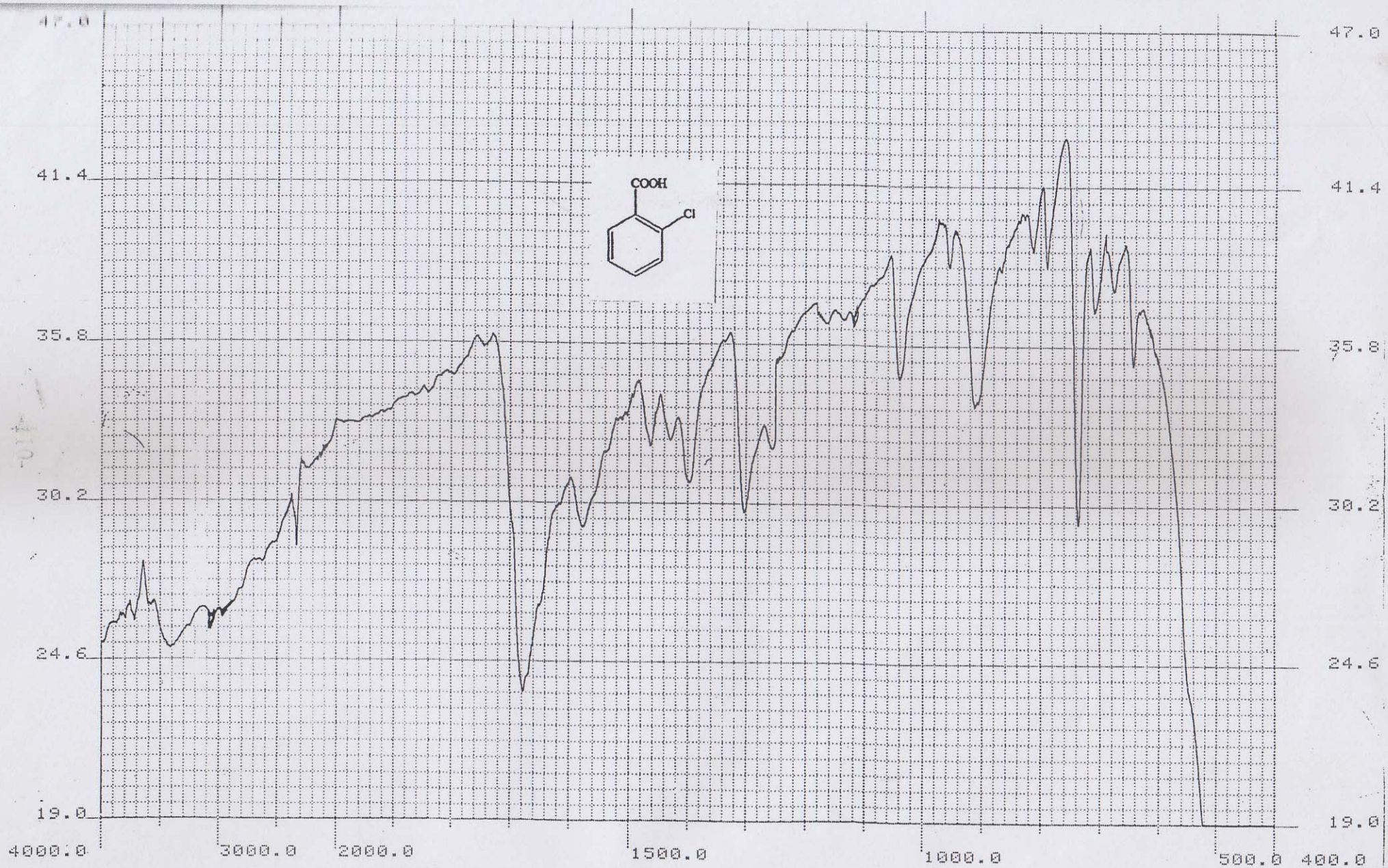
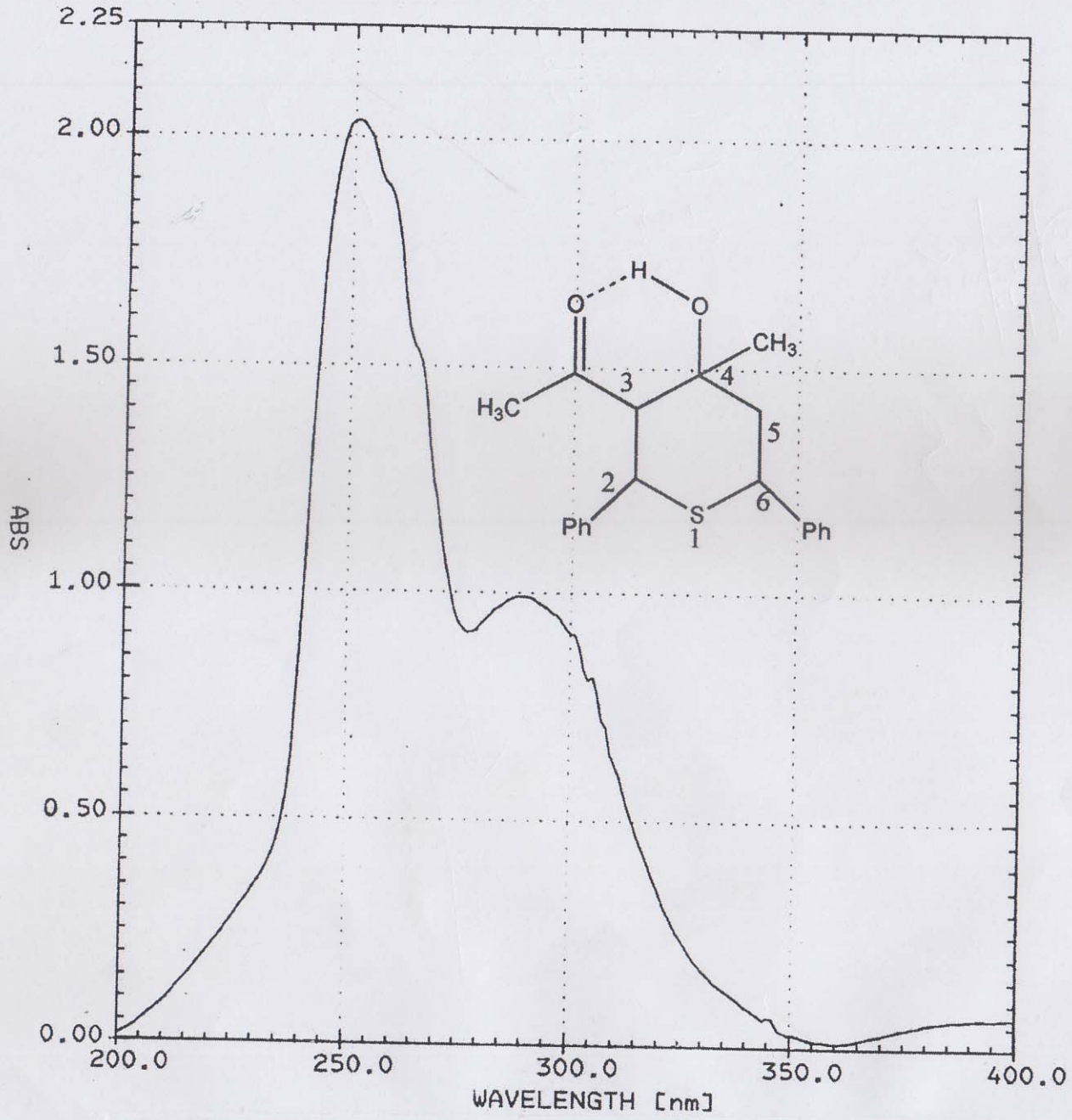


Fig 17 : IR-spectrum of 2-chlorobenzoic acid (14)



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	289.0	0.990	360.0	0.012
2	251.0	2.037	277.0	0.910

Fig 18 : UV-spectrum of 3-acetyl-2,6-diphenyl-4-methyl-tetrahydrothiopyran-4-ol **(10)**

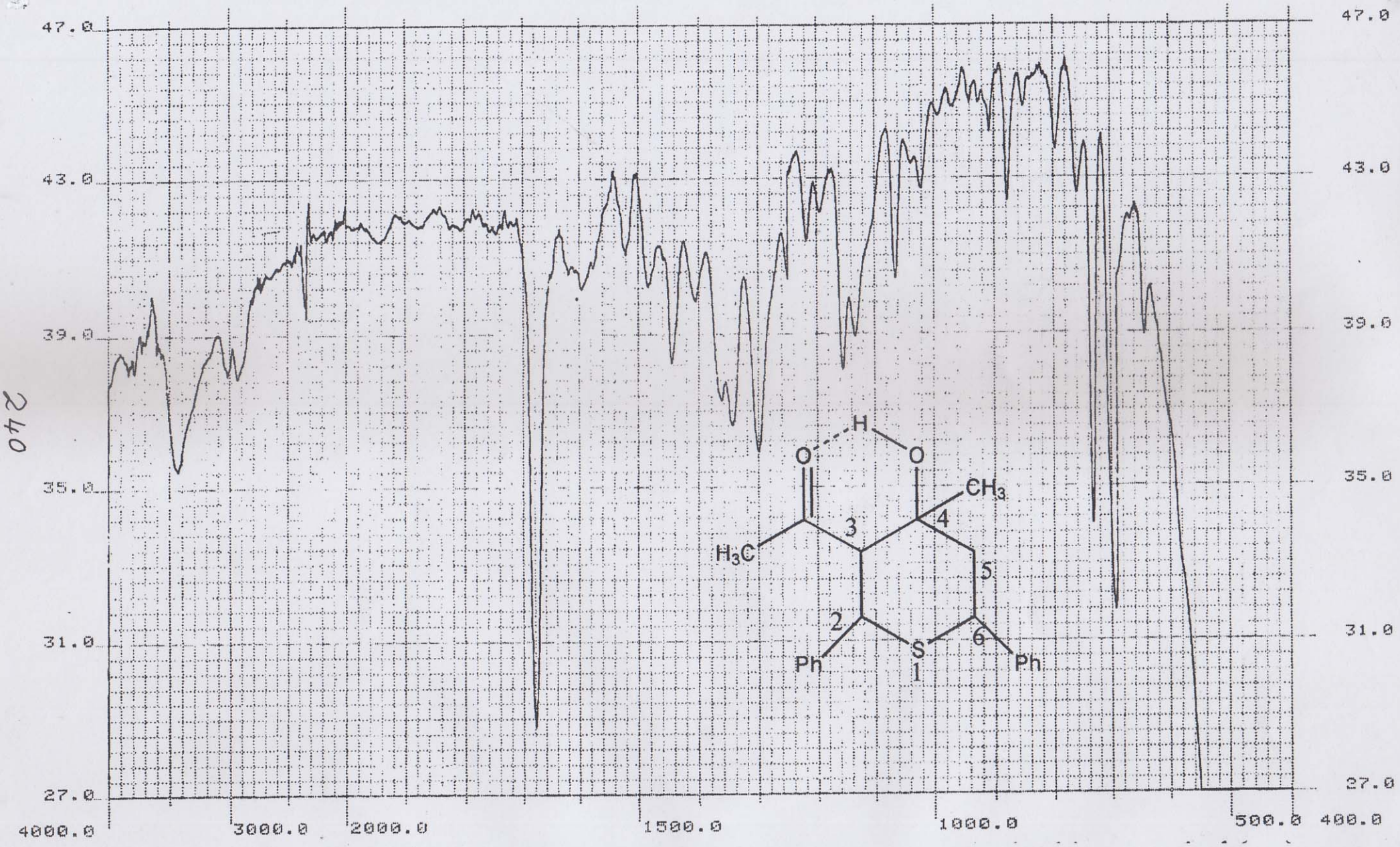


Fig 19 : IR-spectrum of 3-acetyl-2,6-diphenyl-4-methyl-tetrahydrothiopyran-4-ol **(10)**

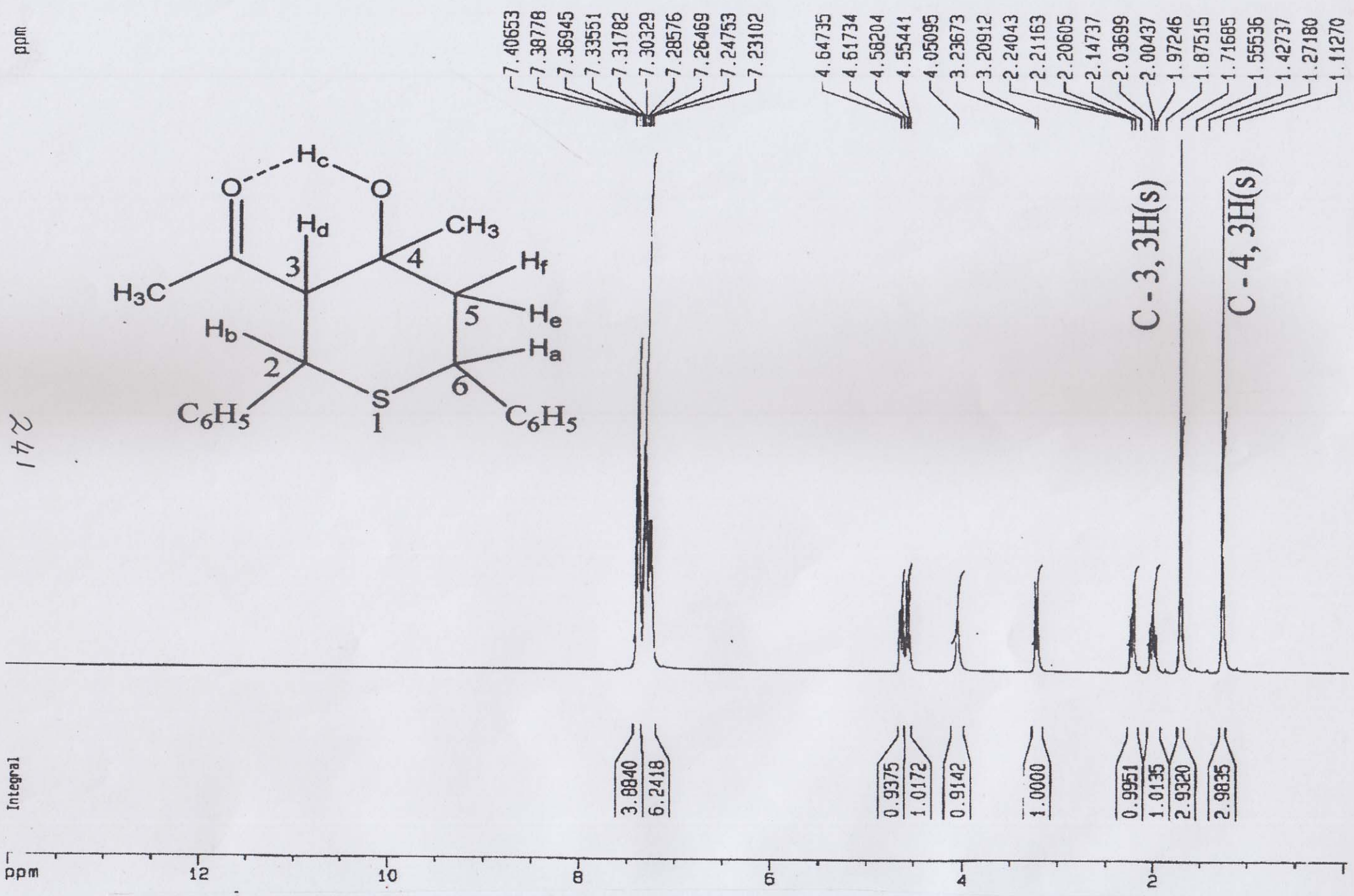


Fig 20 : ^{1}H-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl-tetrahydrothiopyran-4-ol **(10)**

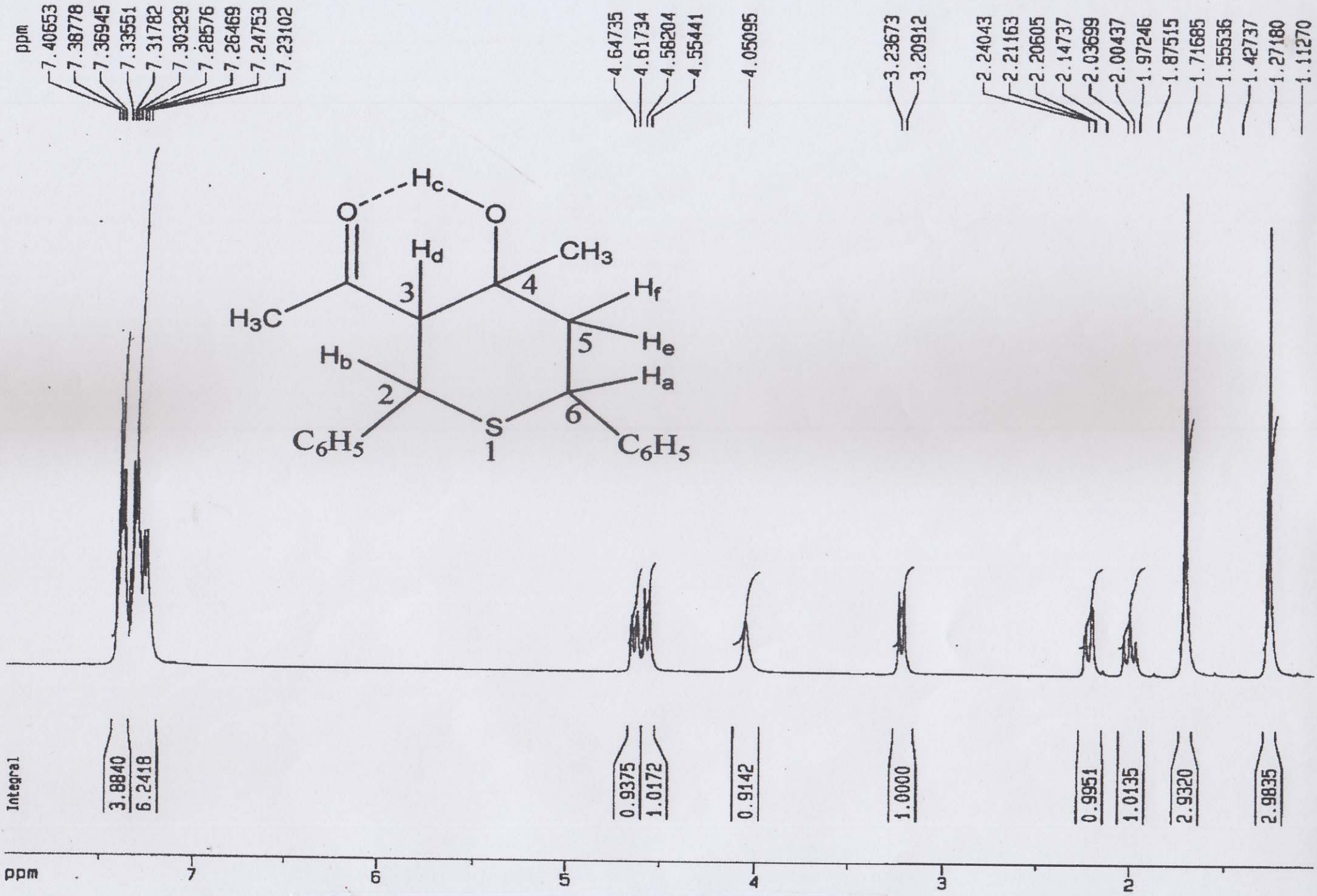


Fig 20a : ^{1}H-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)** [expanded part]

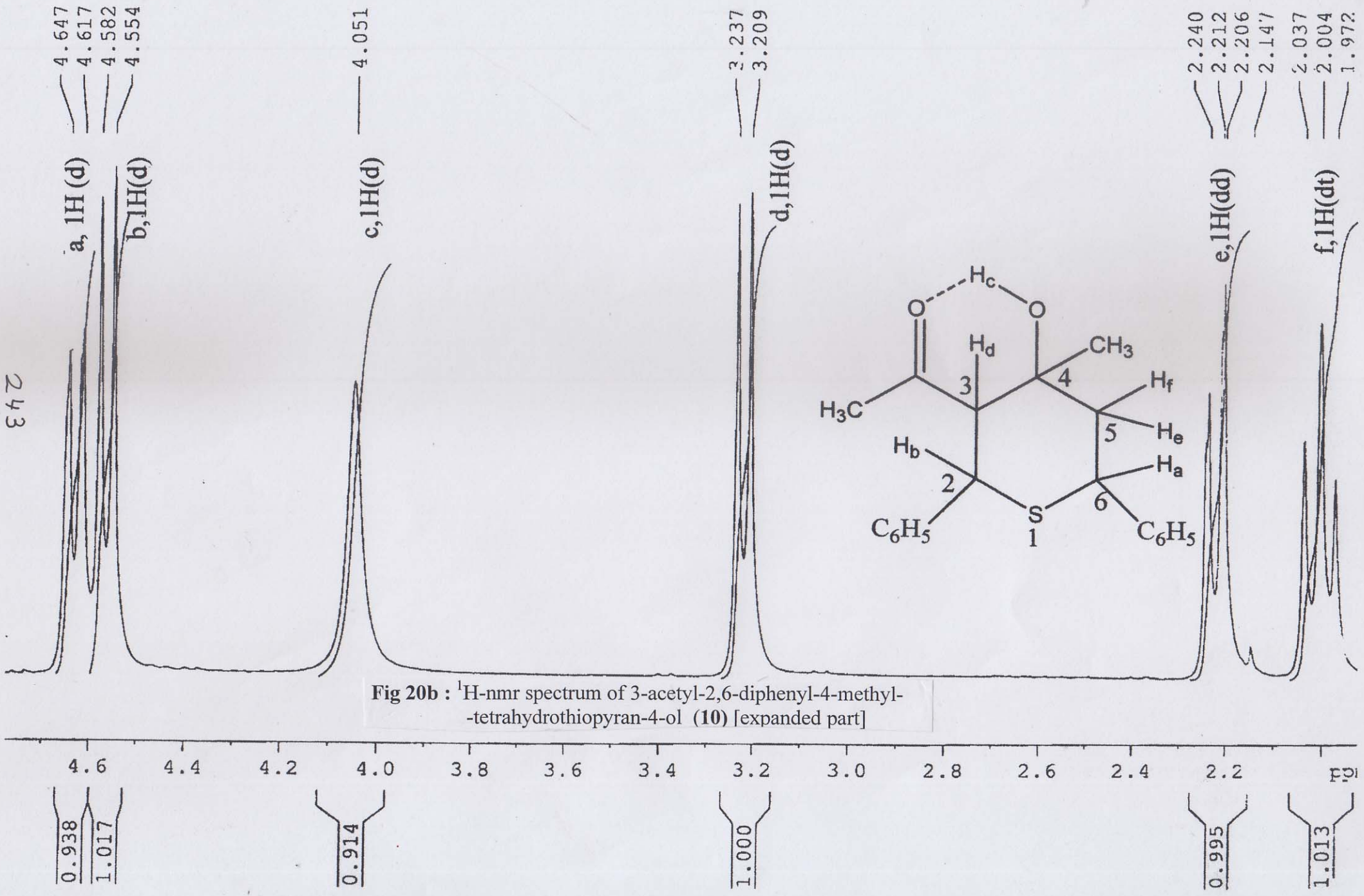


Fig 20b : ^{1}H-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)** [expanded part]

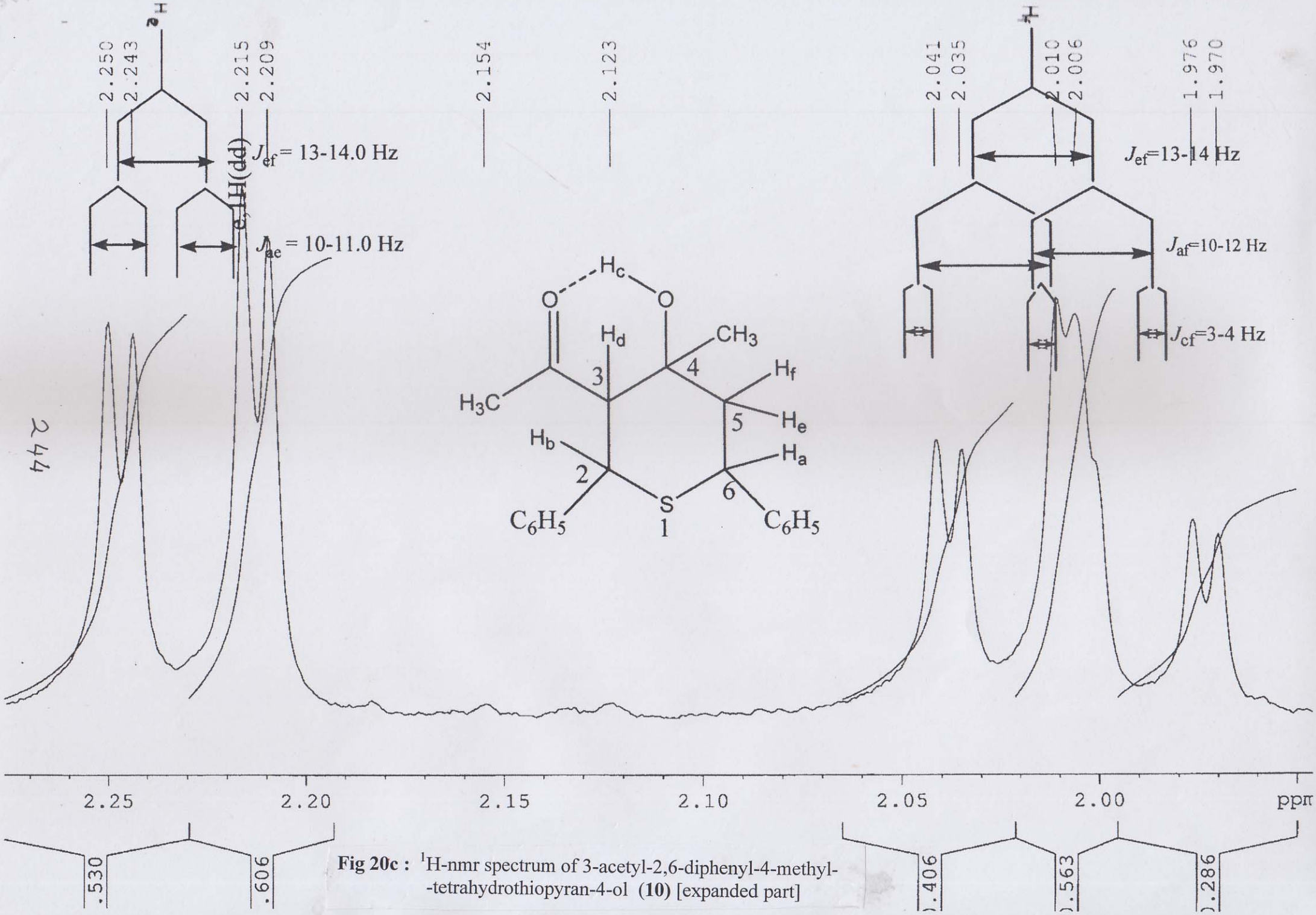


Fig 20c : ^{1}H-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)** [expanded part]

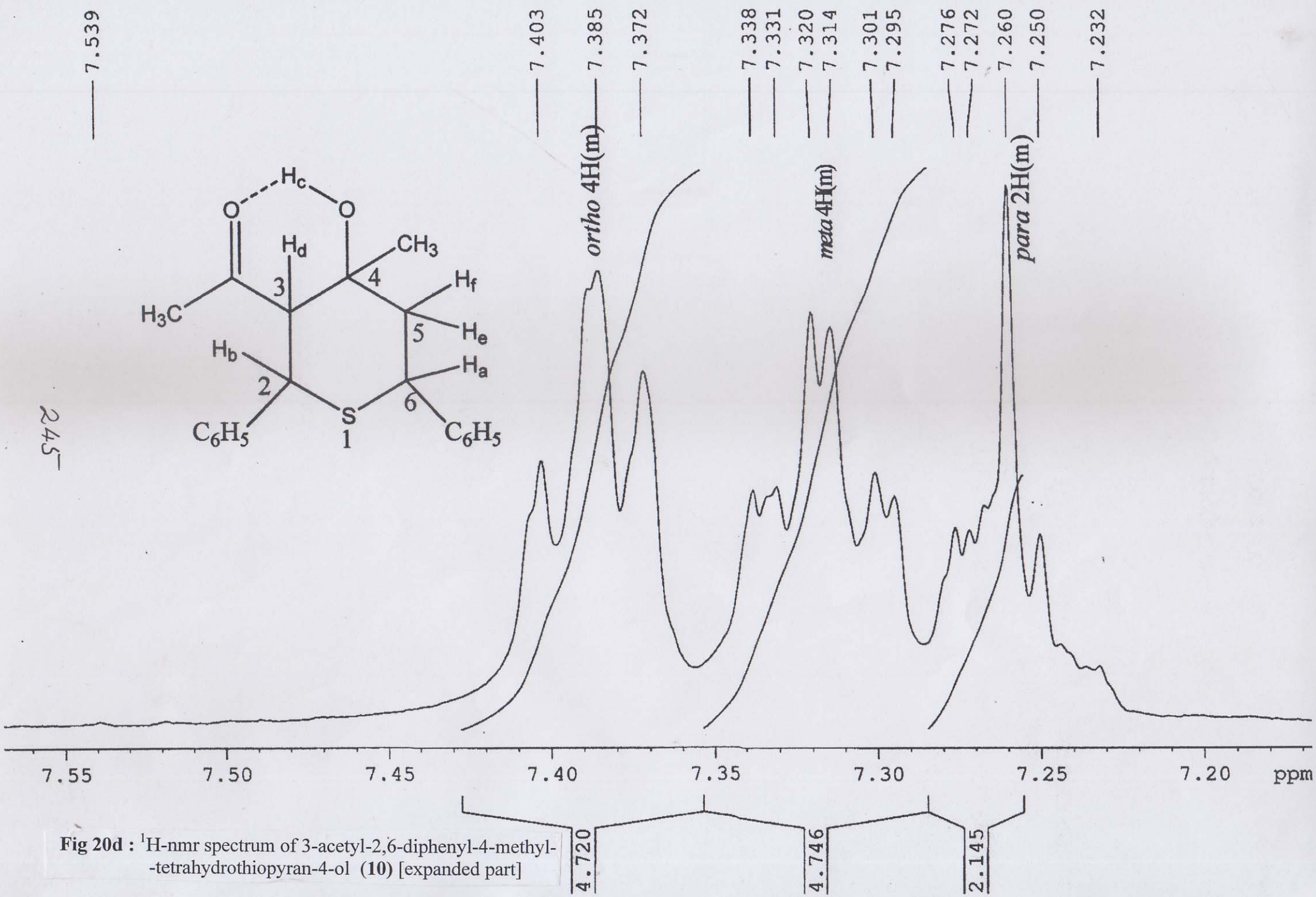


Fig 20d : ^{1}H-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)** [expanded part]

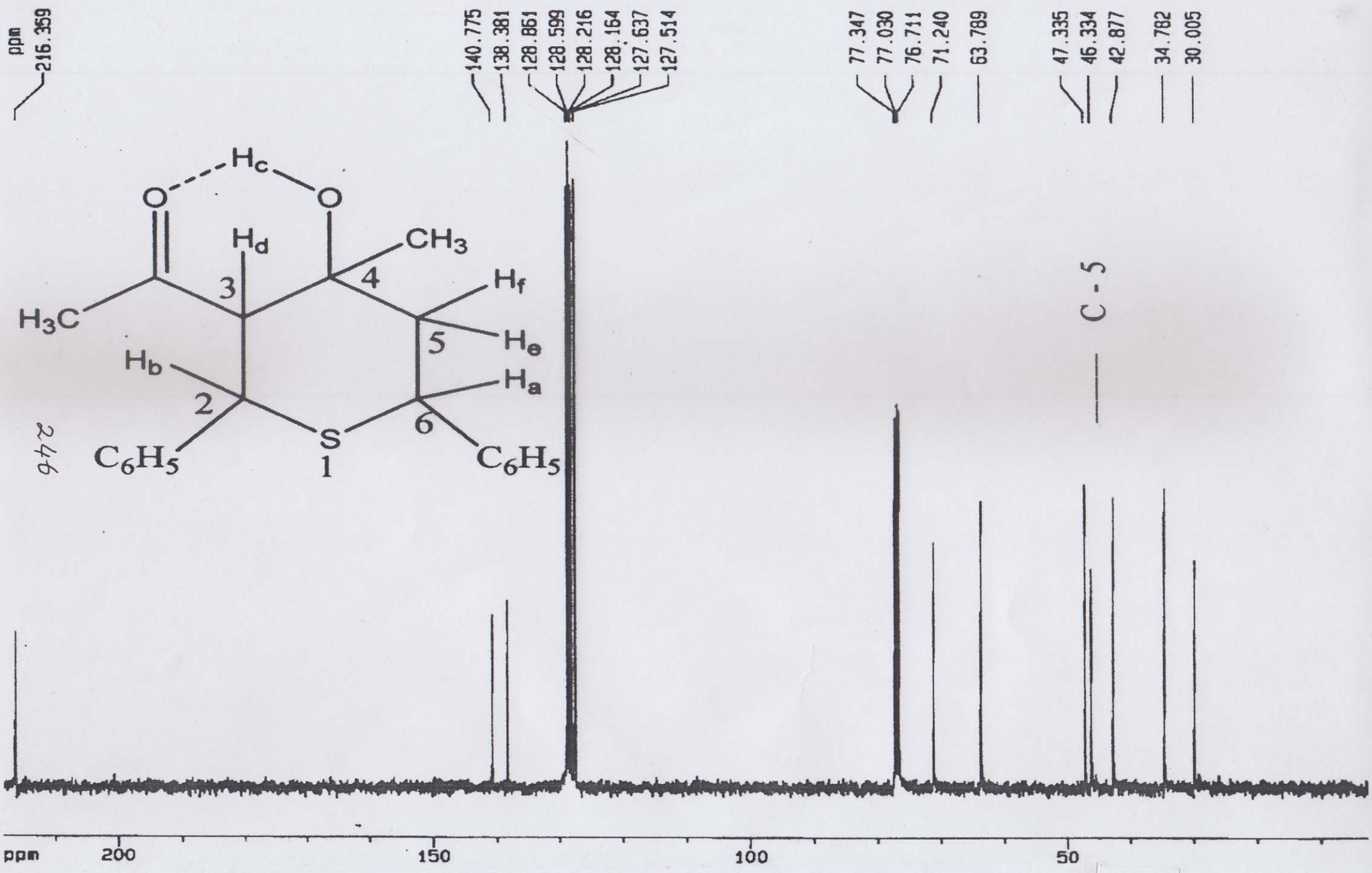


Fig21: ^{13}C-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)**

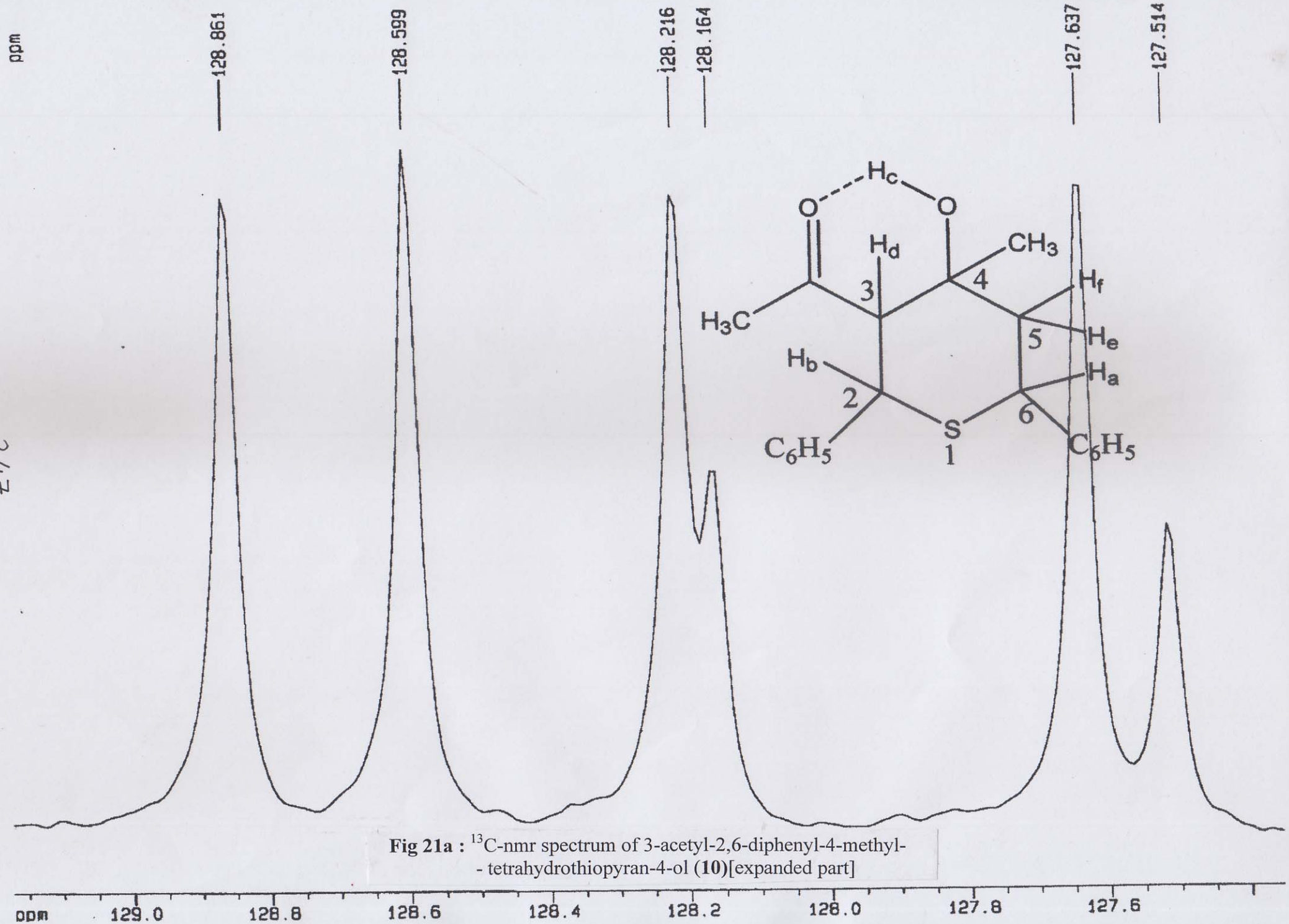


Fig 21a : ^{13}C-nmr spectrum of 3-acetyl-2,6-diphenyl-4-methyl- - tetrahydrothiopyran-4-ol **(10)**[expanded part]

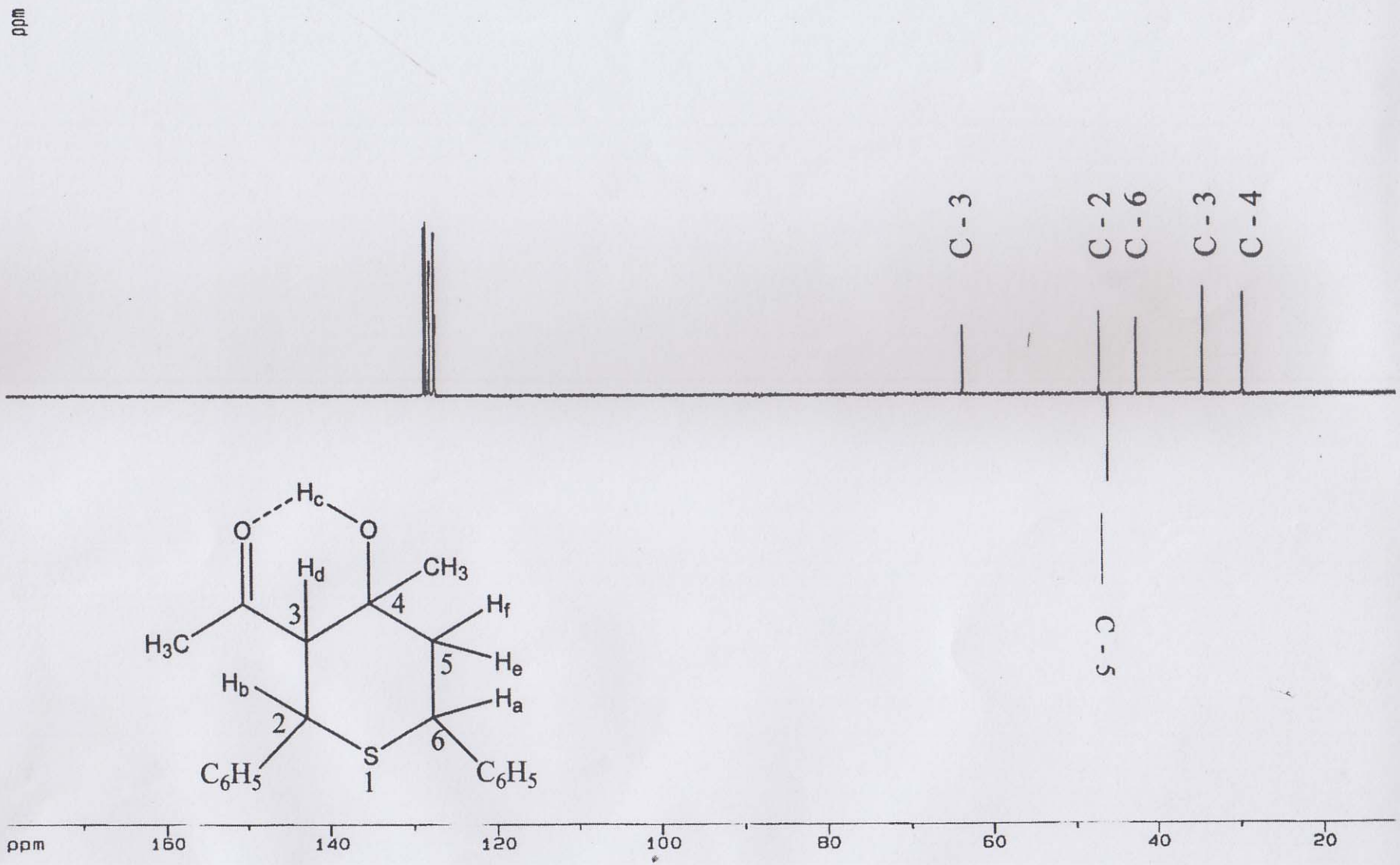


Fig 22 : ^{13}C-nmr (DEPT-135) spectrum of 3-acetyl-2,6-diphenyl-4-methyl- - tetrahydrothiopyran-4-ol **(10)**

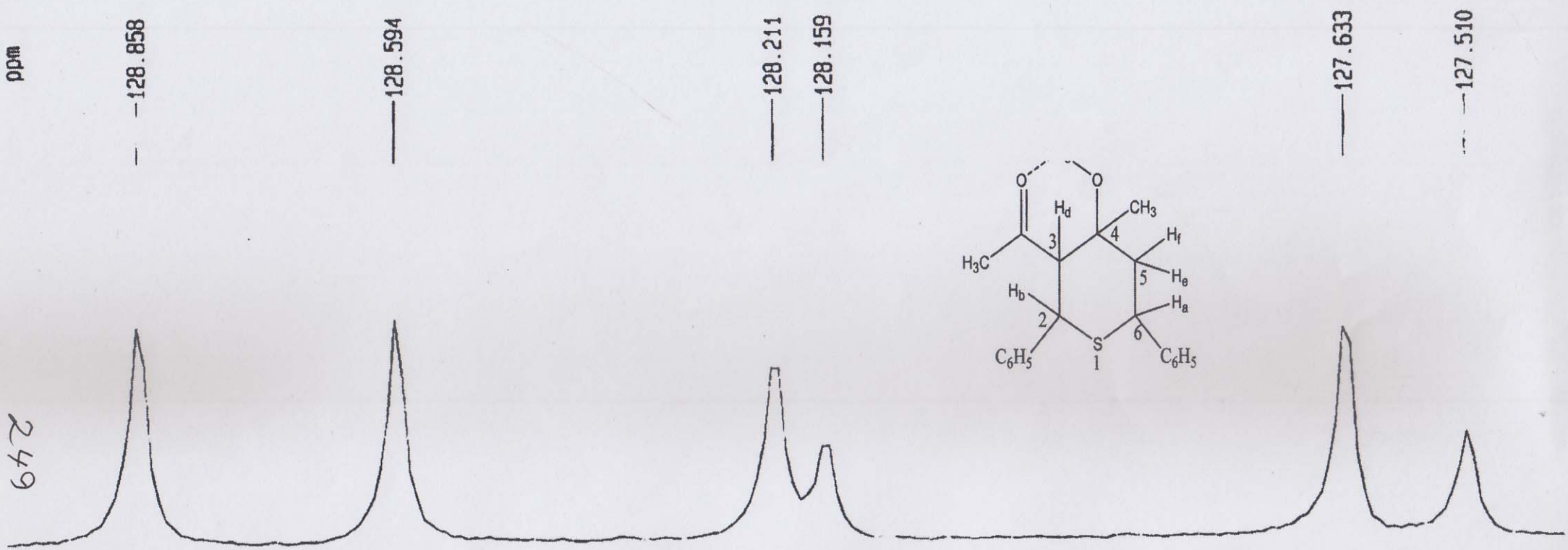
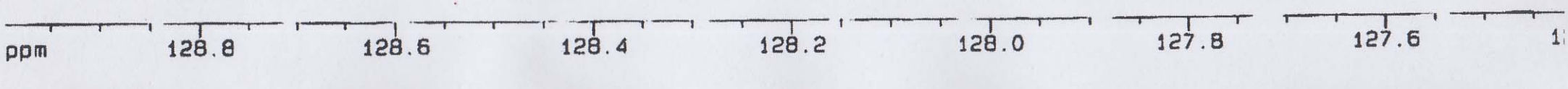


Fig 22a : ^{13}C-nmr (DEPT-135) spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)** [expanded part]



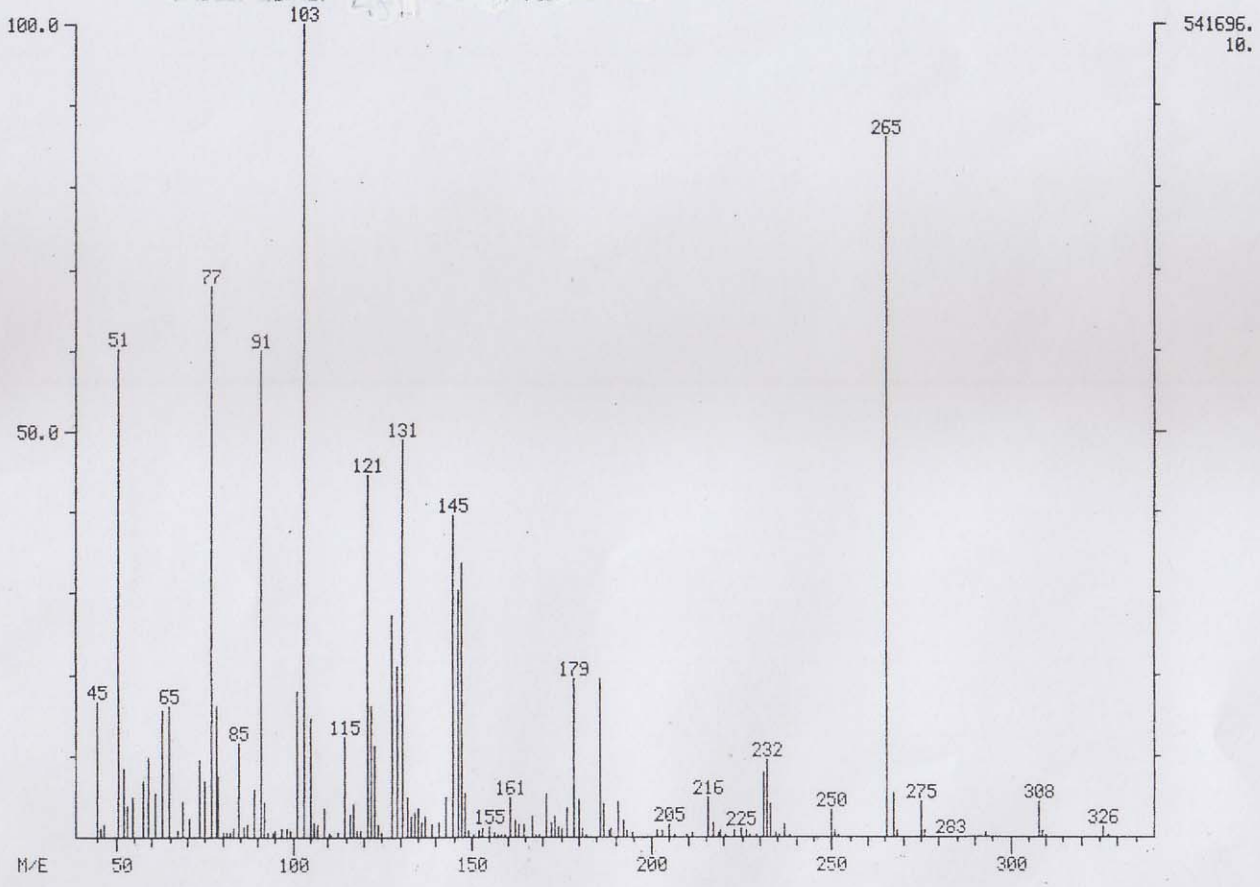


Fig 23 : Mass spectrum of 3-acetyl-2,6-diphenyl-4-methyl- - tetrahydrothiopyran-4-ol **(10)**

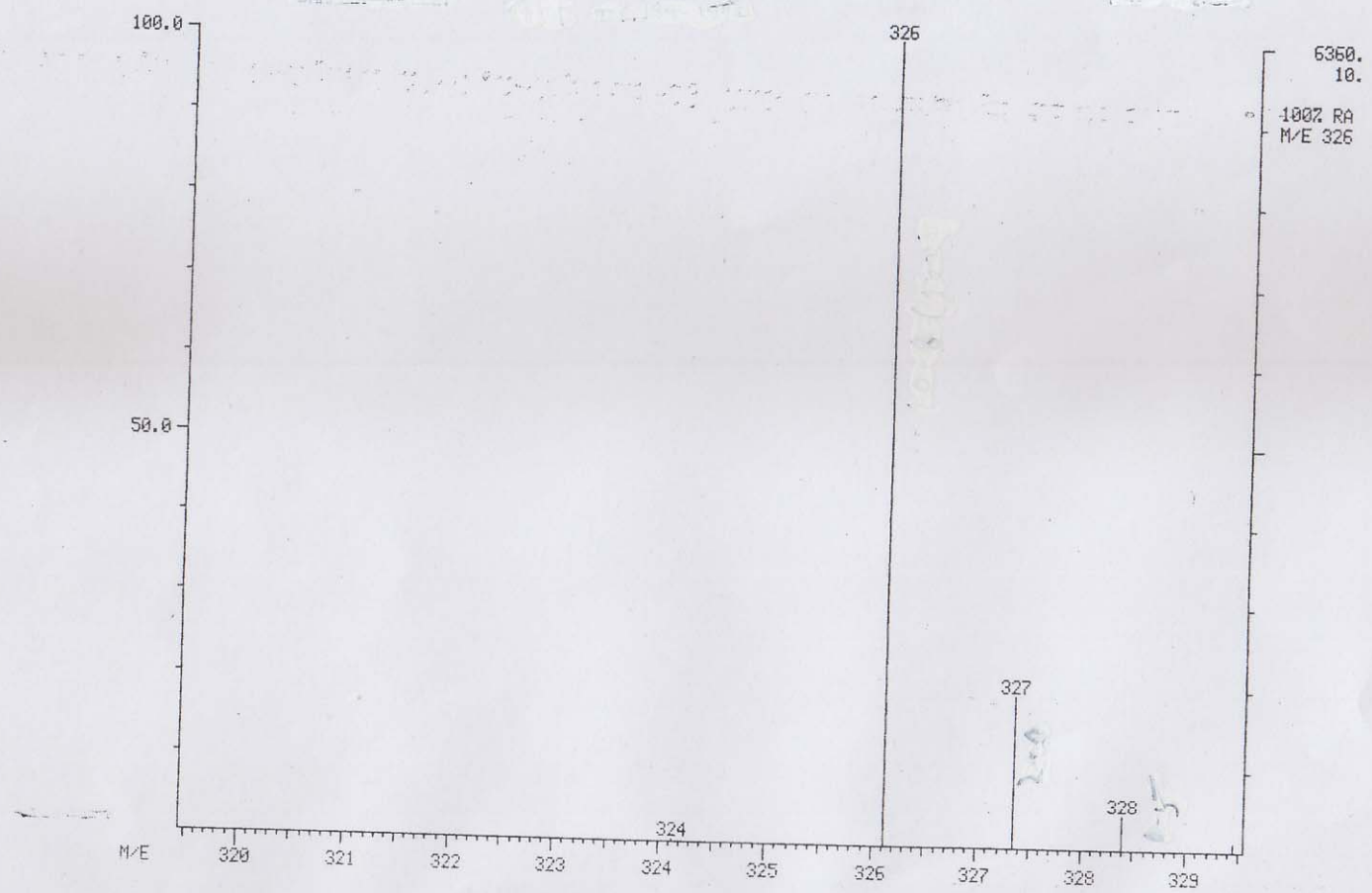


Fig 23a : Mass spectrum of 3-acetyl-2,6-diphenyl-4-methyl--tetrahydrothiopyran-4-ol **(10)** [expanded part]

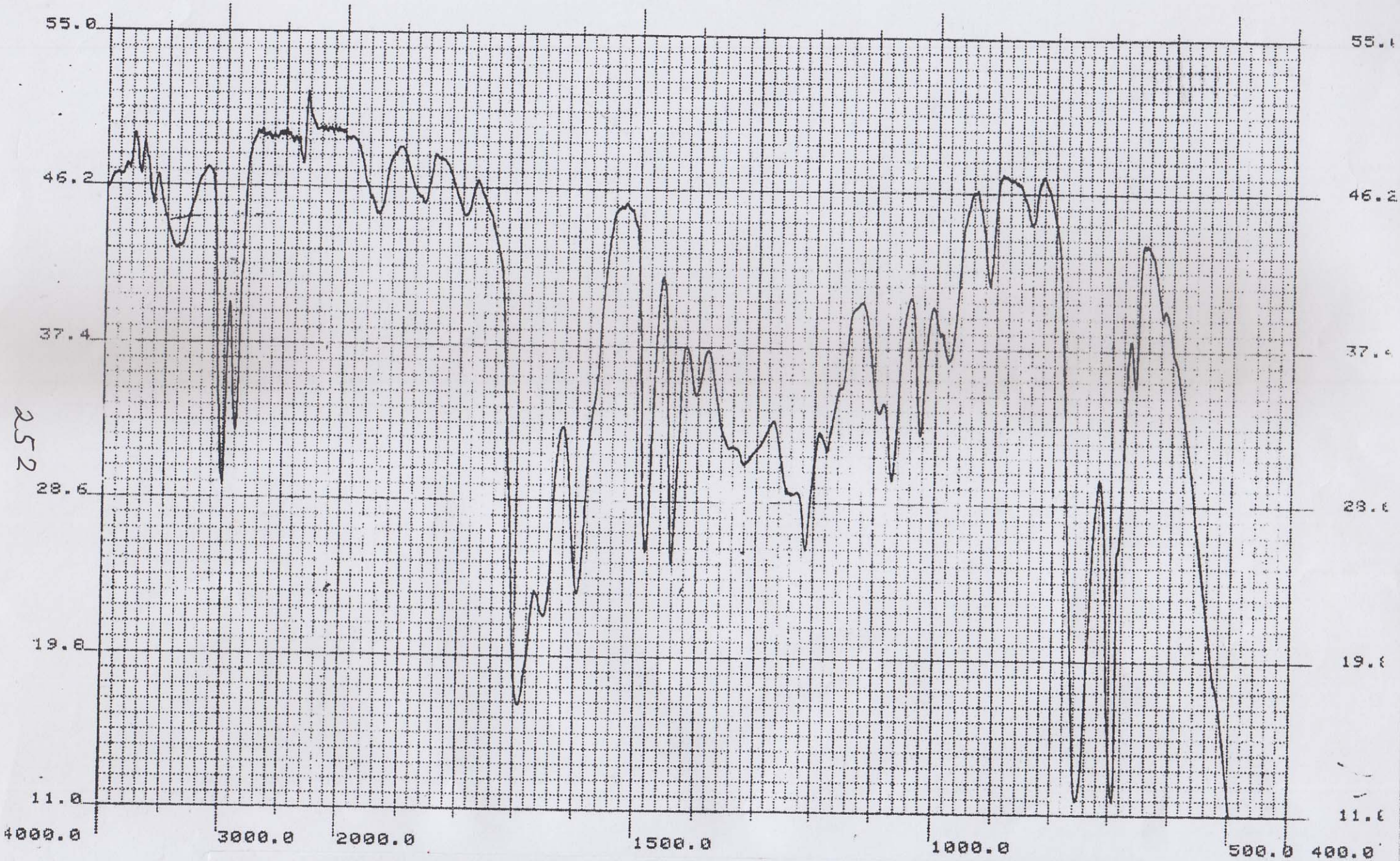
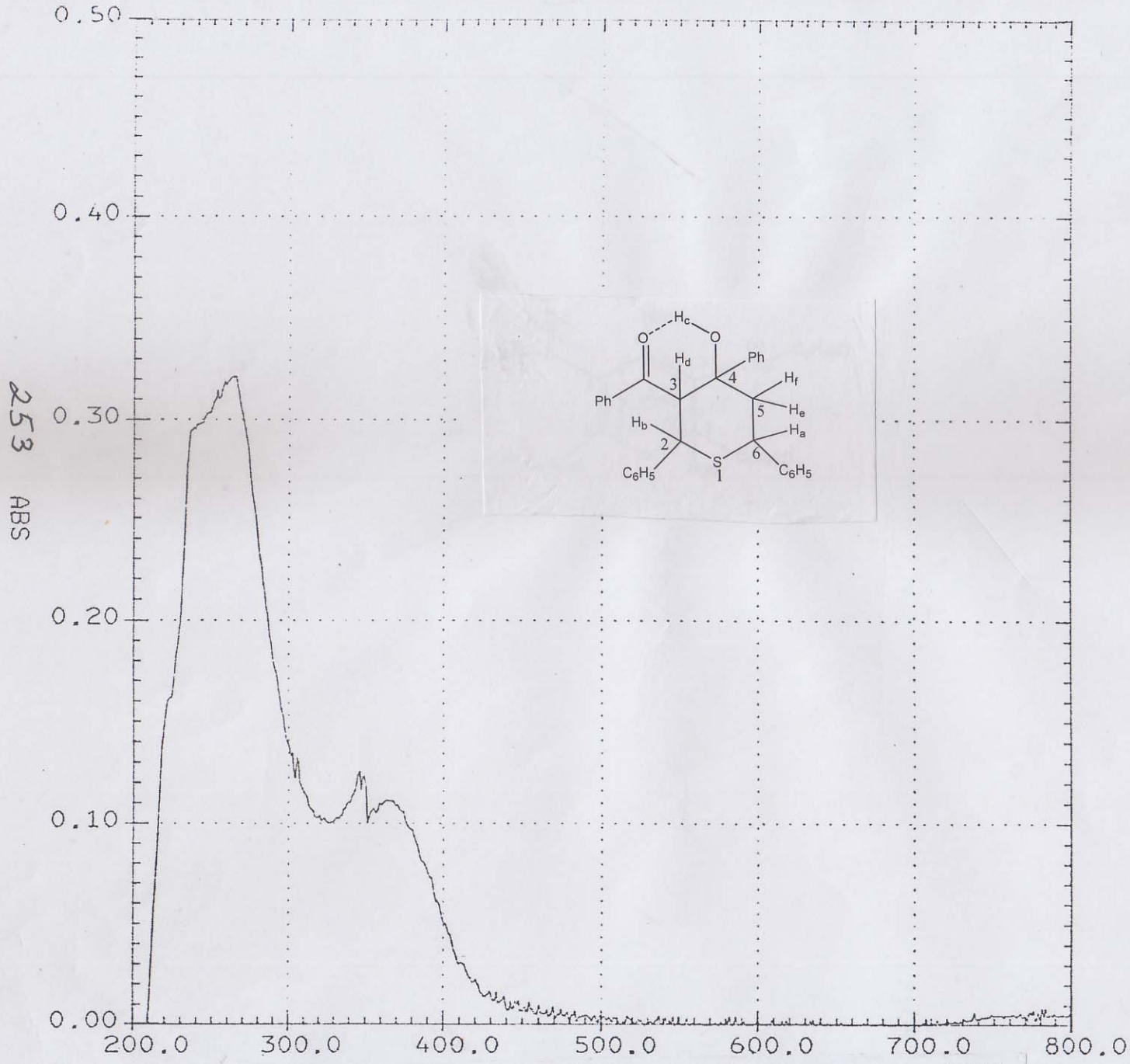


Fig 24 : IR-spectrum of compound (10) obtained from benzaldehyde and benzylideneacetone with aq sodium sulphide reaction



*** PEAK-PICK ***

	-- PEAK --		-- VALLEY --	
No.	λ	ABS	λ	ABS
1	346.0	0.126	327.0	0.10
2	265.0	0.322		

Fig 25 : UV-spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran-4-ol **(11)**

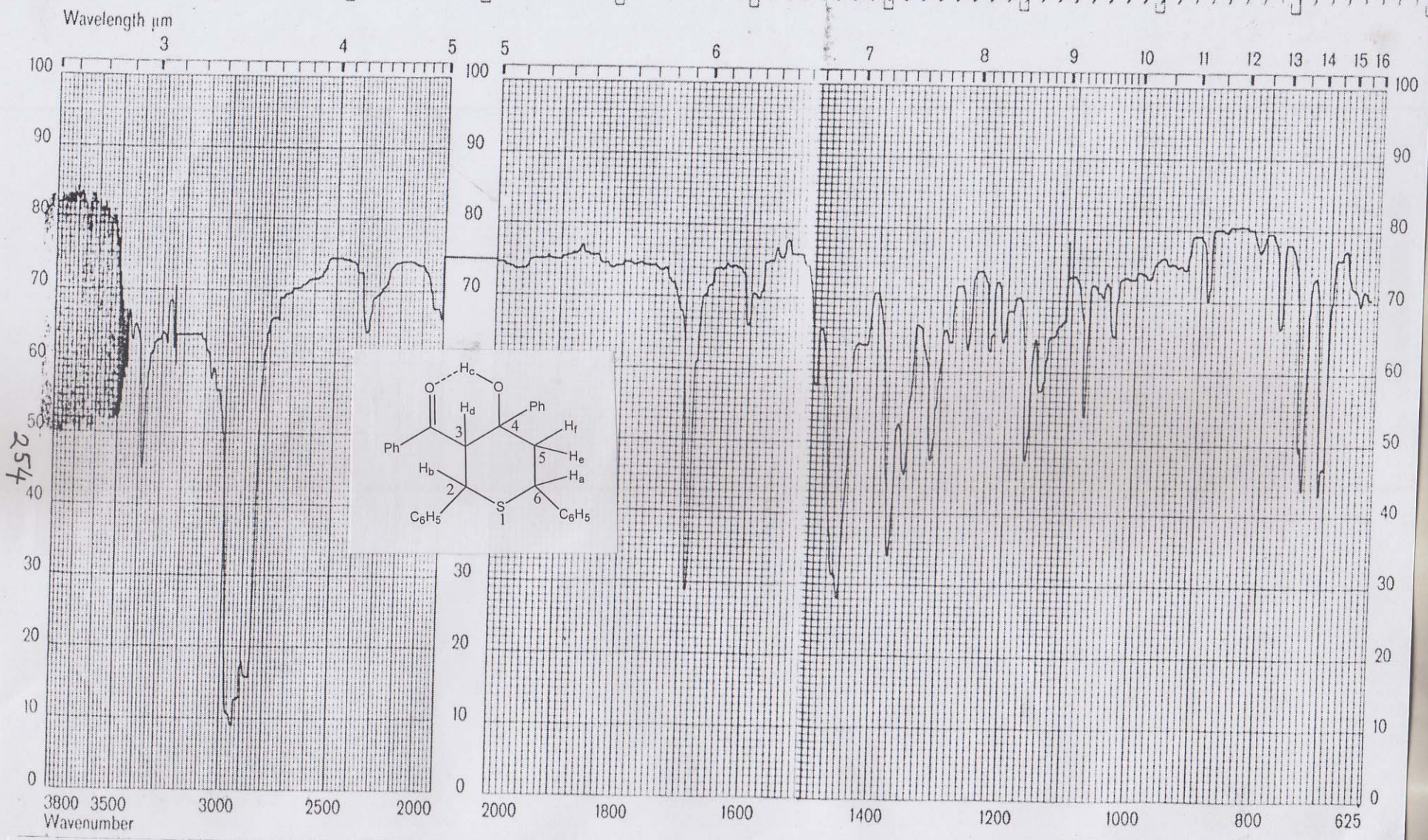


Fig 26 : IR-spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran-4-ol **(11)**

Fig 27 : ^{1}H-nmr spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran-4-ol **(11)**

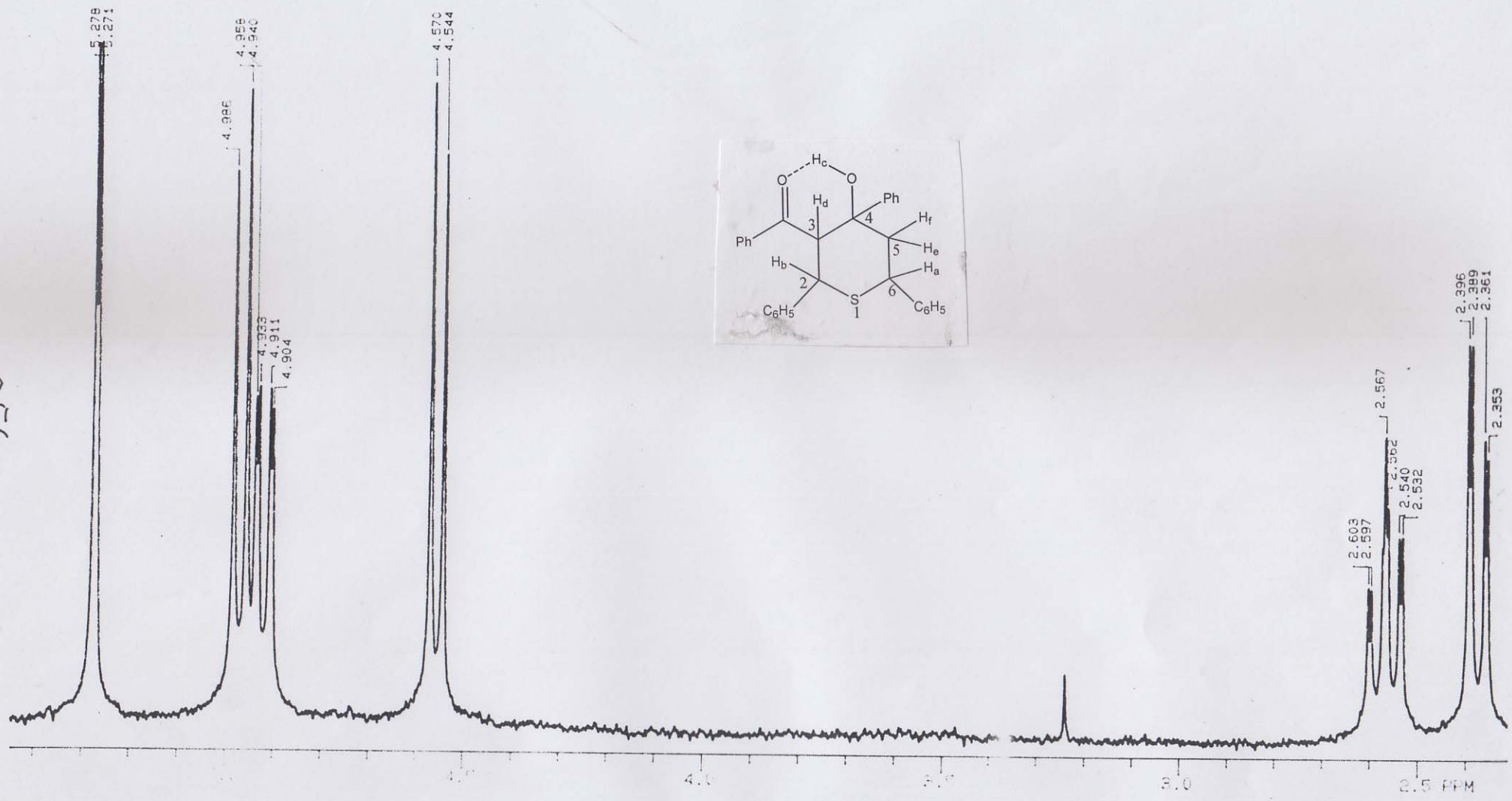


Fig 27a : ^{1}H-nmr spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran--4-ol **(11)** [expanded part]

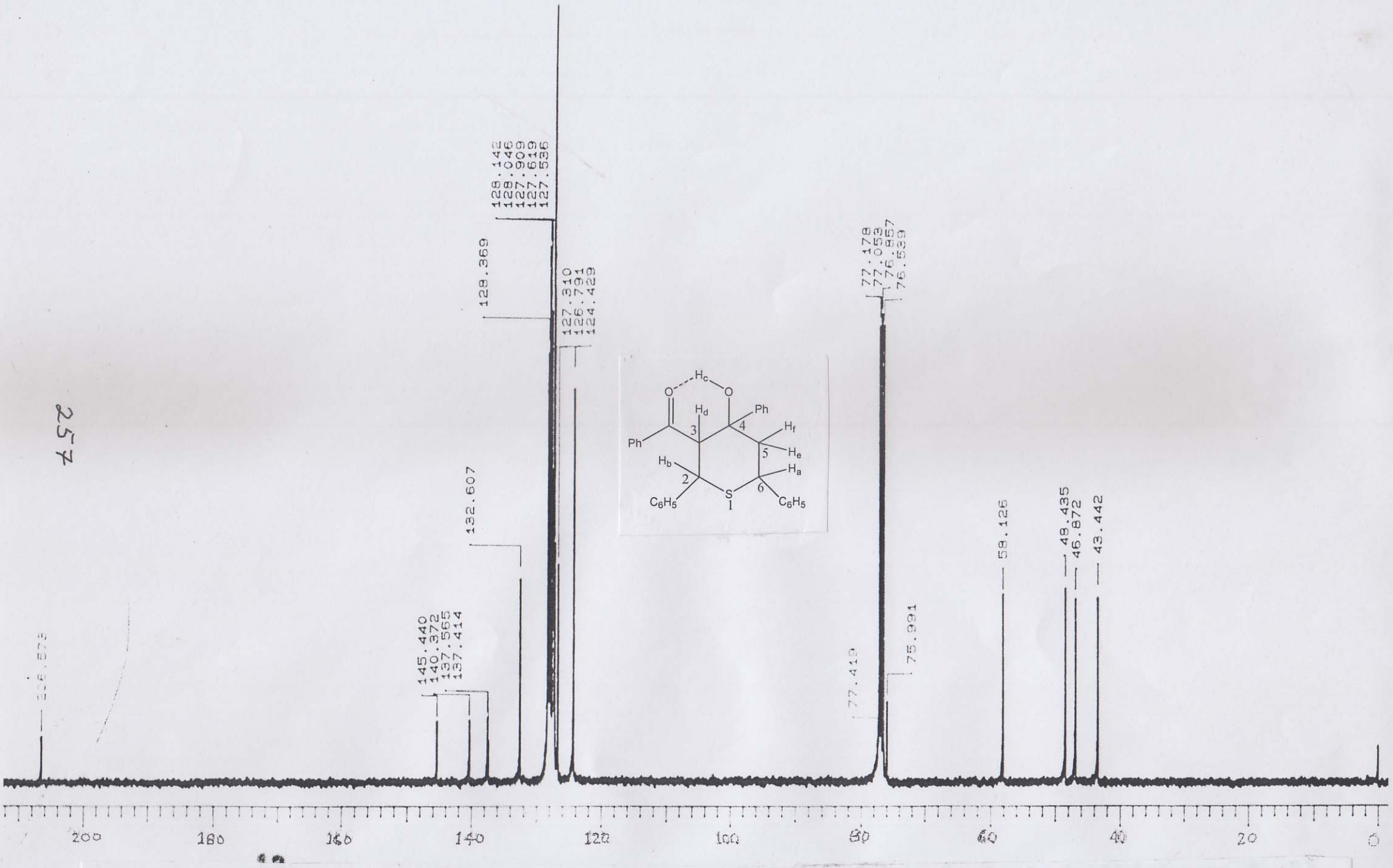


Fig 28 : ^{13}C-nmr spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran-4-ol **(11)**

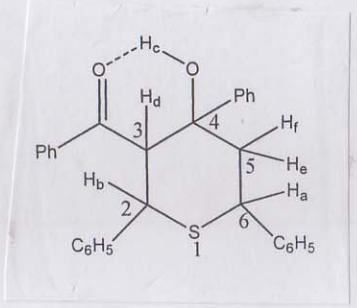


Fig 28a : ^{13}C-nmr spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran--4-ol **(11)** [expanded part]

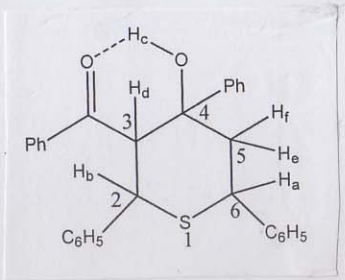


Fig 28b : ^{13}C-nmr spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran--4-ol **(11)** [expanded part]

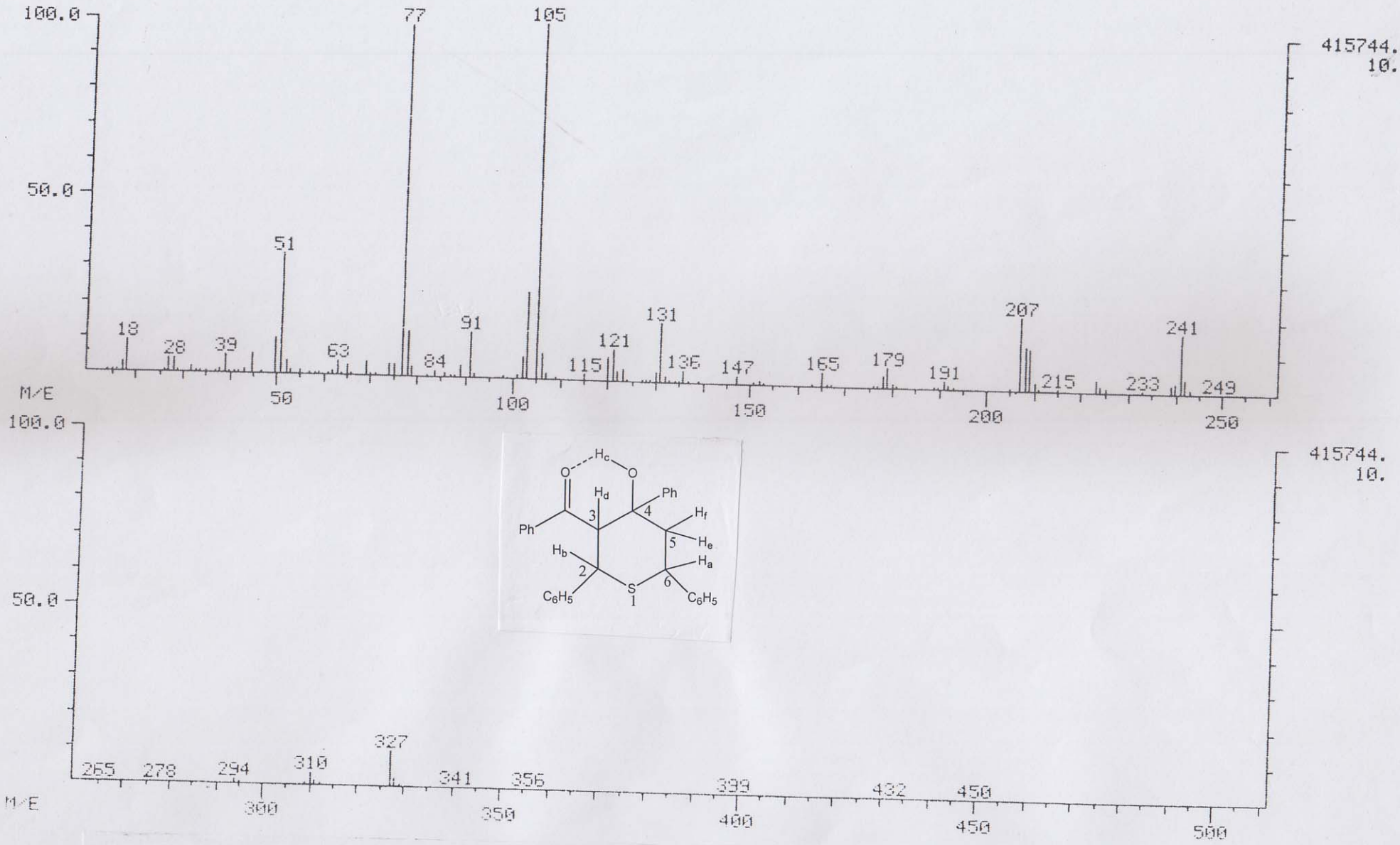


Fig 29 : Mass spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran-4-ol **(11)**

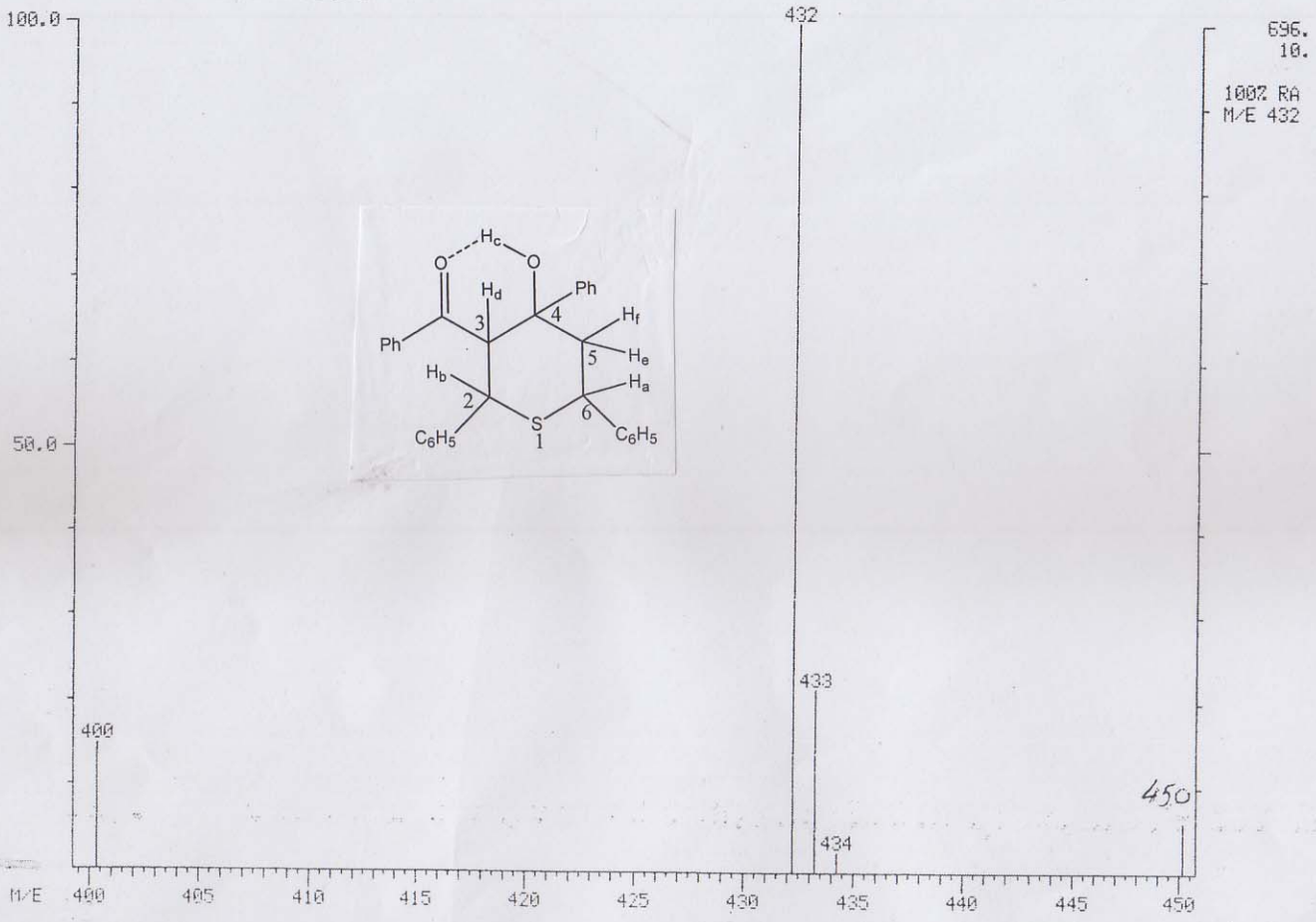
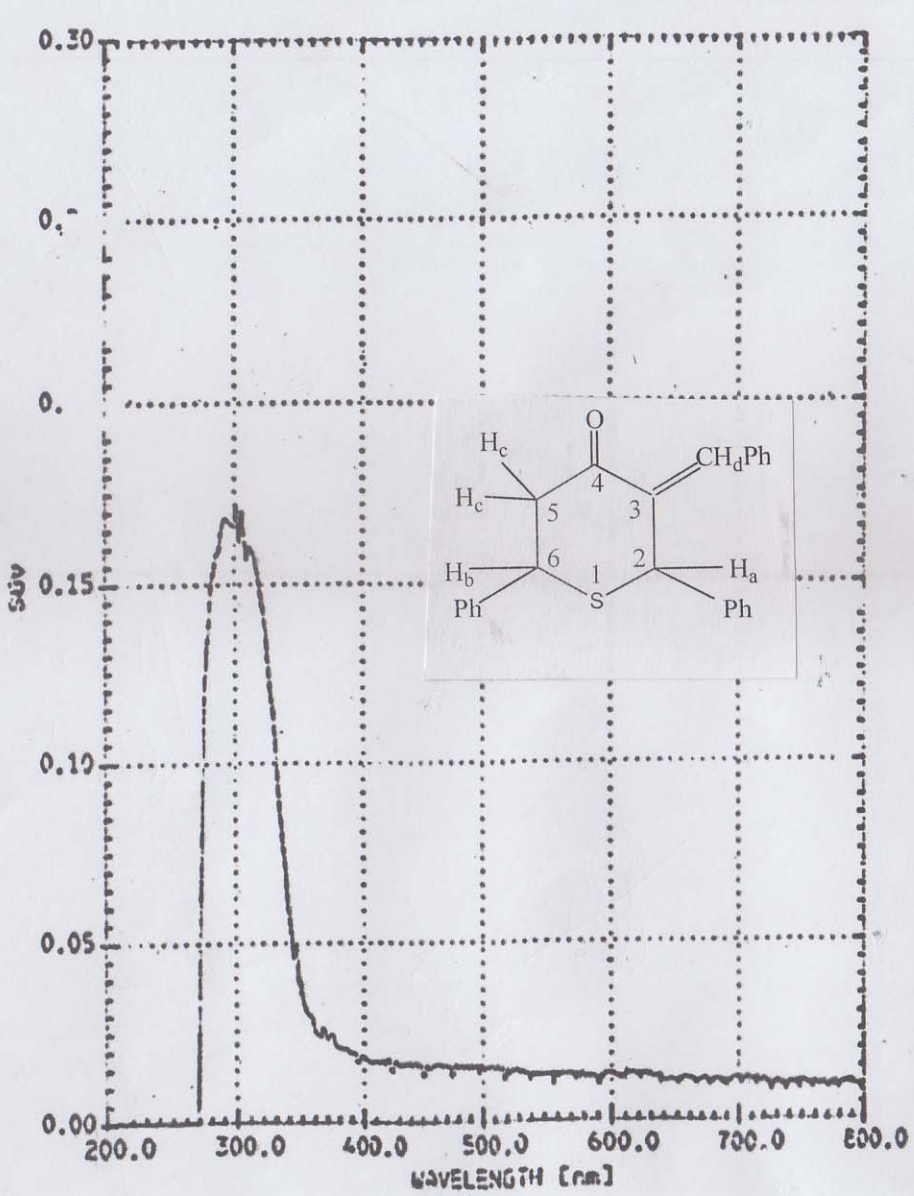


Fig 29a : Mass spectrum of 3-benzoyl-2,4,6-triphenyl-tetrahydrothiopyran--4-ol **(11)** [expanded part]



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	300.0	0.172	205.0	-0.280

Fig 30 : UV-spectrum of 3-benzylidene-2,6-diphenyltetrahydrothiopyran-4-one **(15)**

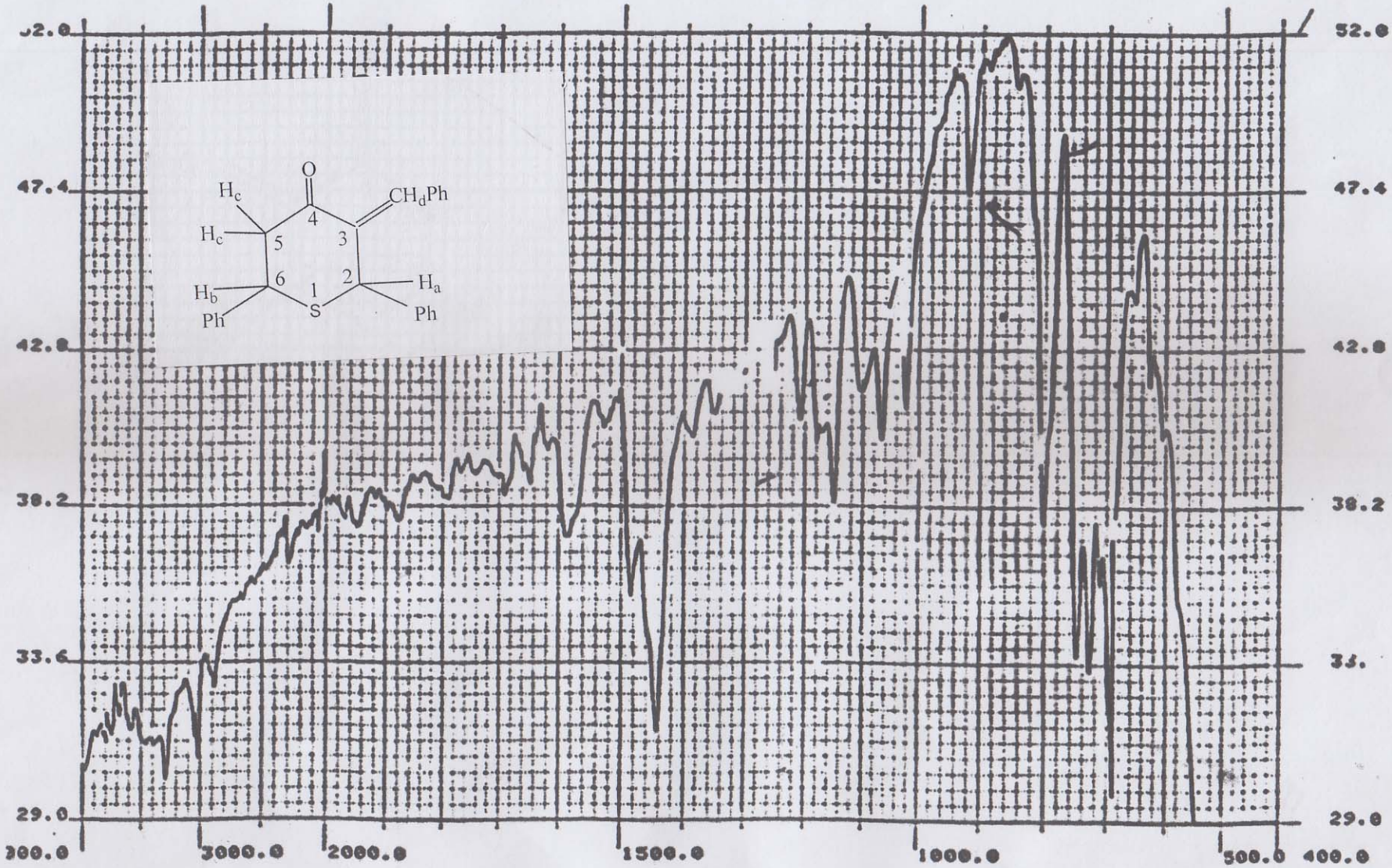


Fig 31 : IR-spectrum of 3-benzylidene-2,6-diphenyltetrahydrothiopyran-4-one **(15)**

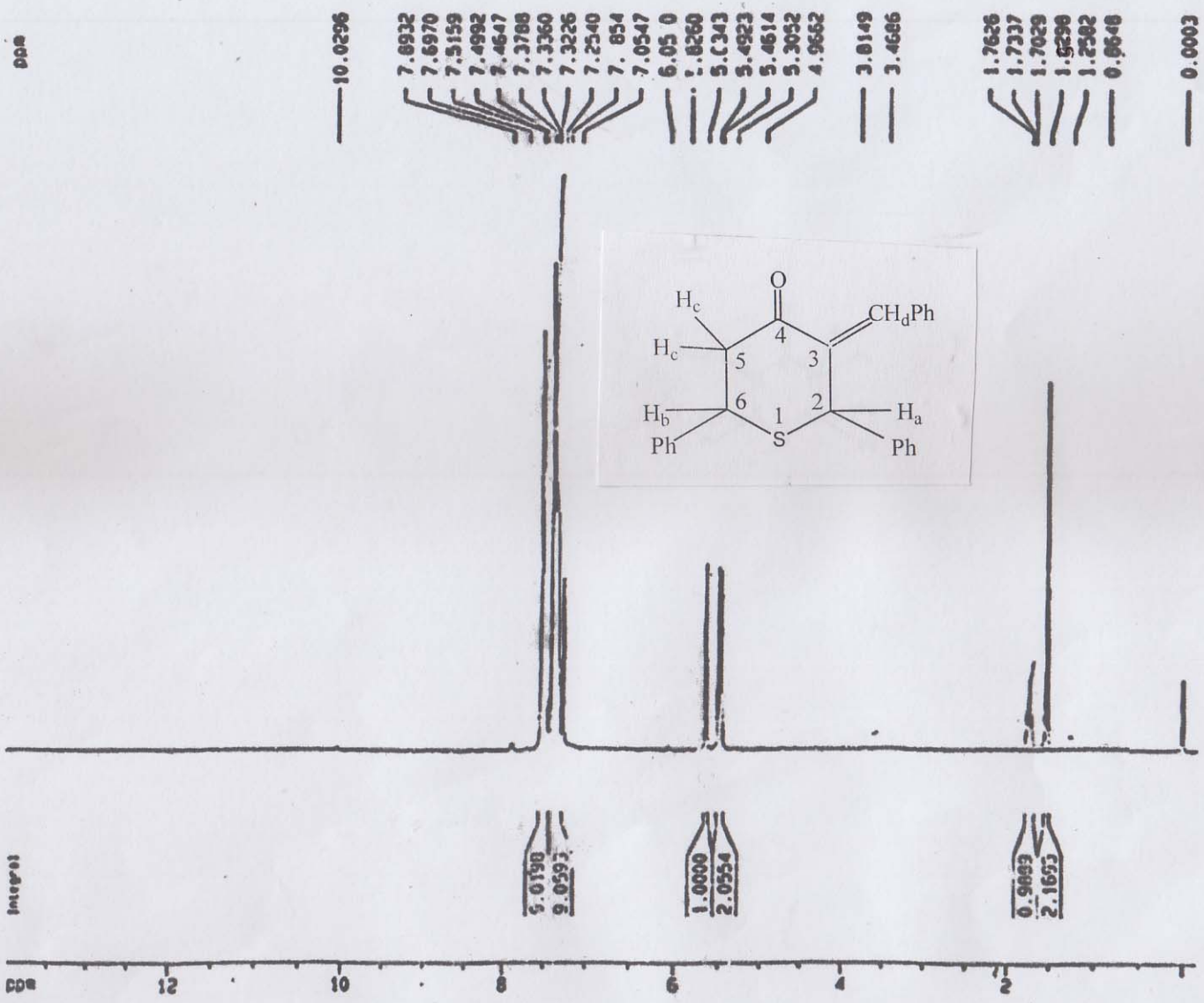


Fig 32 : ^{1}H-nmr spectrum of 3-benzylidene-2,6-diphenyltetrahydrothiopyran-4-one **(15)**

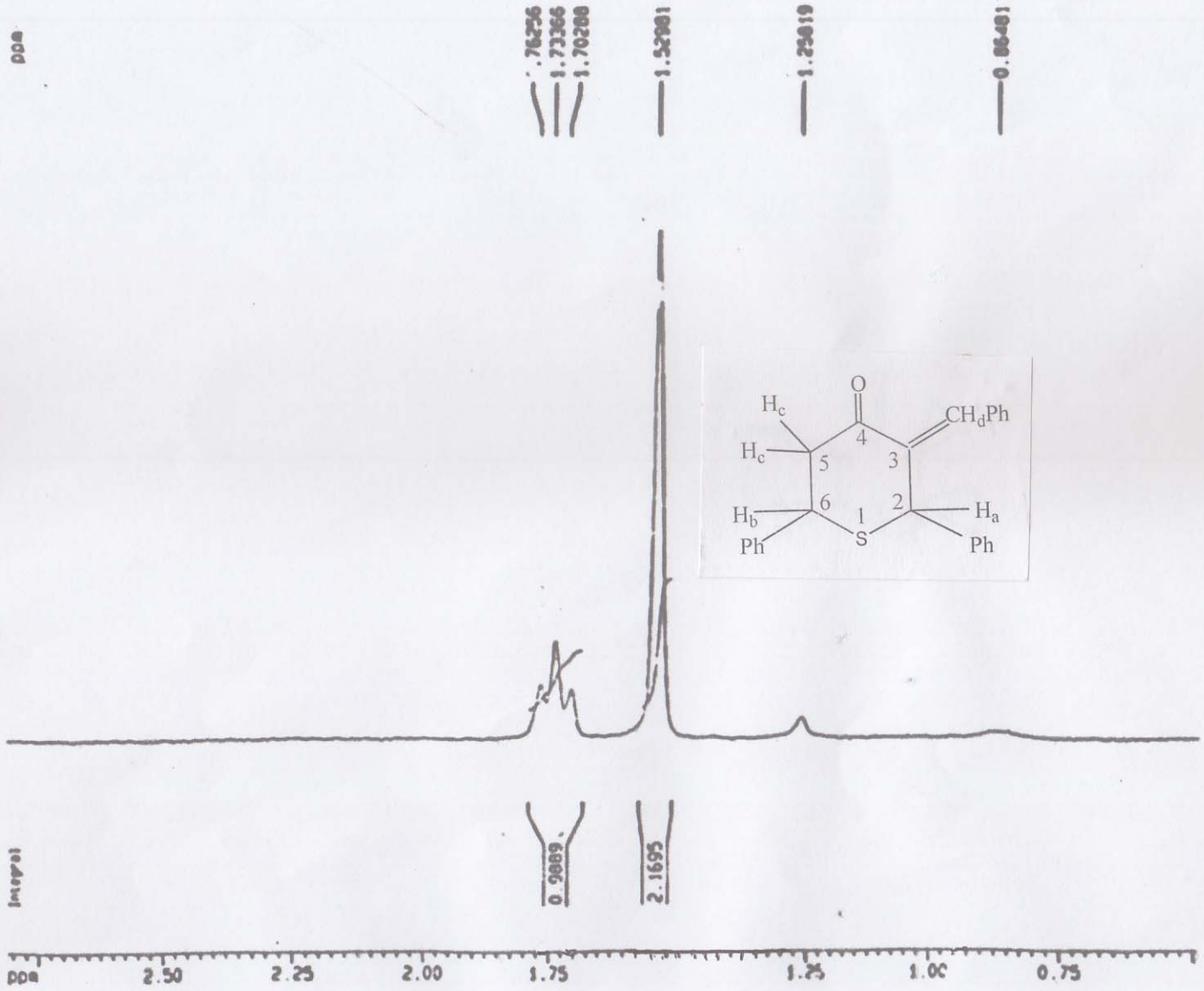


Fig 32a : ^{1}H-nmr spectrum of 3-benzylidene-2,6-diphenyltetrahydro--thiopyran-4-one **(15)** [expanded part]

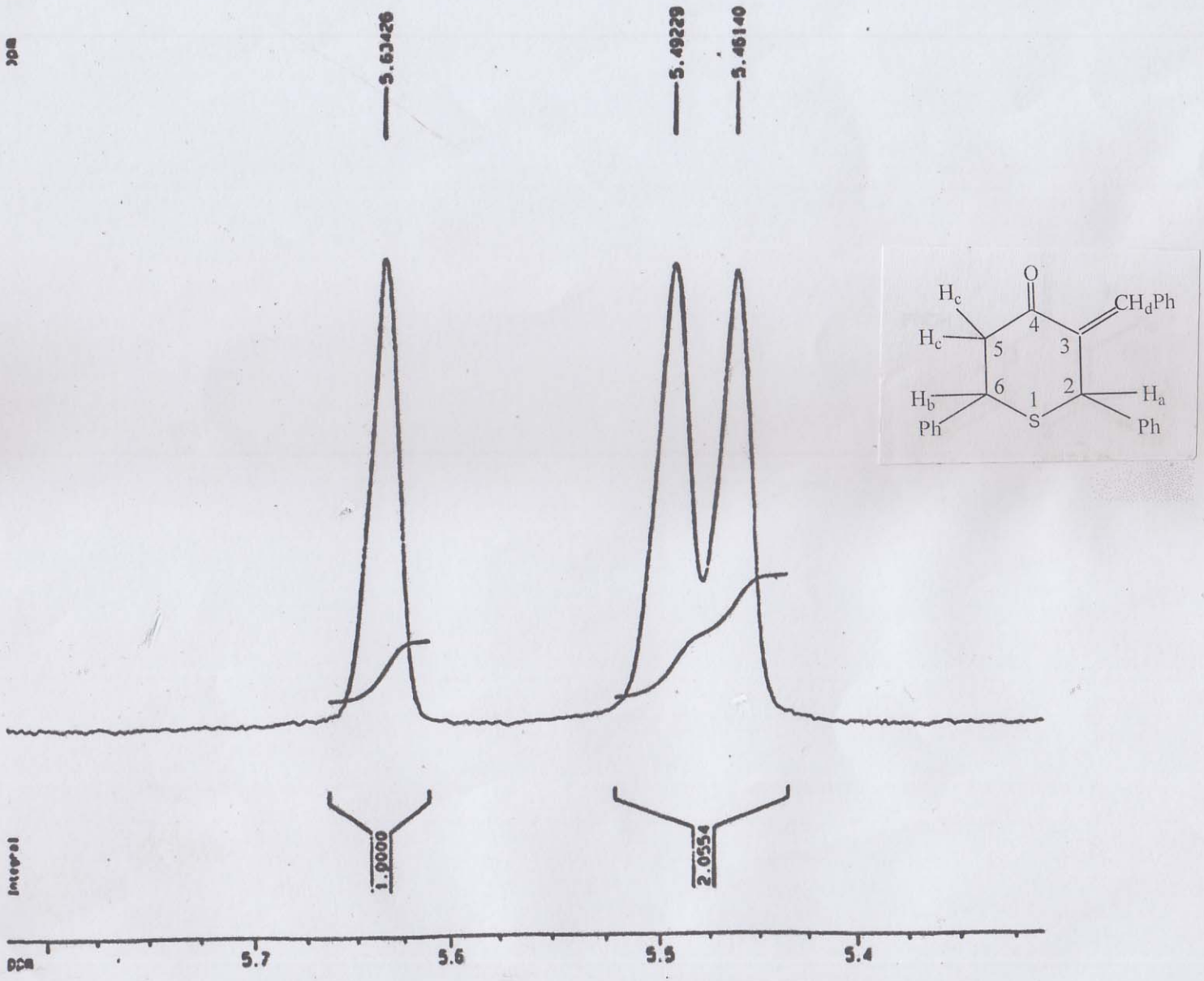


Fig 32b : ^{1}H-nmr spectrum of 3-benzylidene-2,6-diphenyltetrahydro--thiopyran-4-one **(15)** [expanded part]

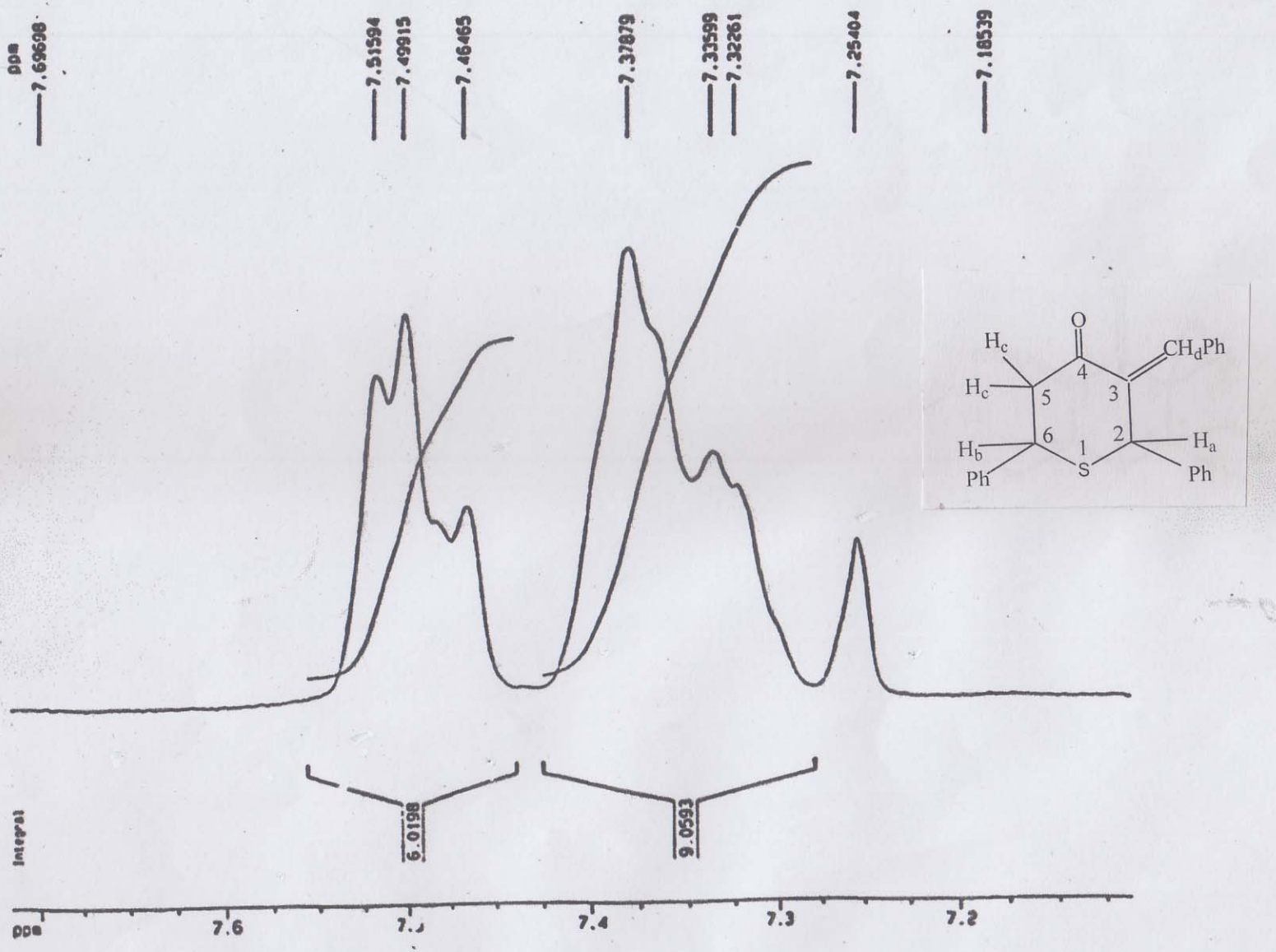
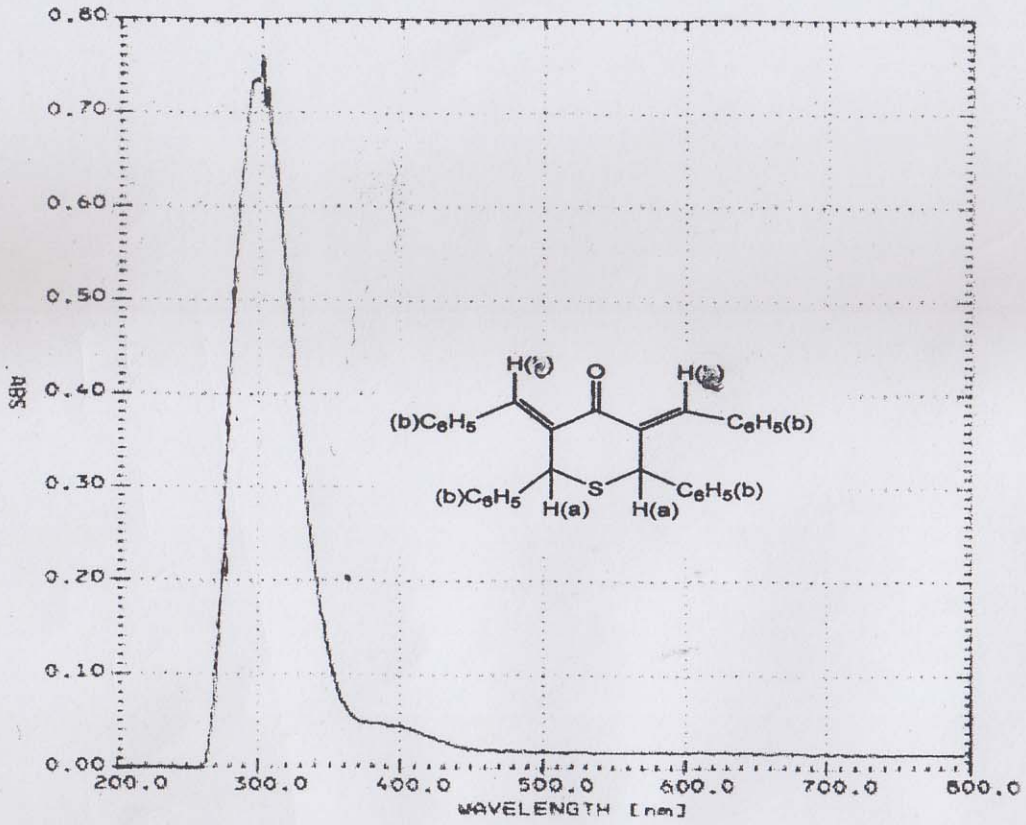


Fig 32c : ^{1}H-nmr spectrum of 3-benzylidene-2,6-diphenyltetrahydro--thiopyran-4-one **(15)** [expanded part]



*** PEAK-PICK ***

	-- PEAK --		--- VALLEY --	
No.	λ	ABS	λ	ABS
1	301.0	0.760	220.0	-0.126

Fig 33 : UV-spectrum of 3,5-dibenzylidene-2,6-diphenyltetrahydrothiopyran-4-one **(16)**

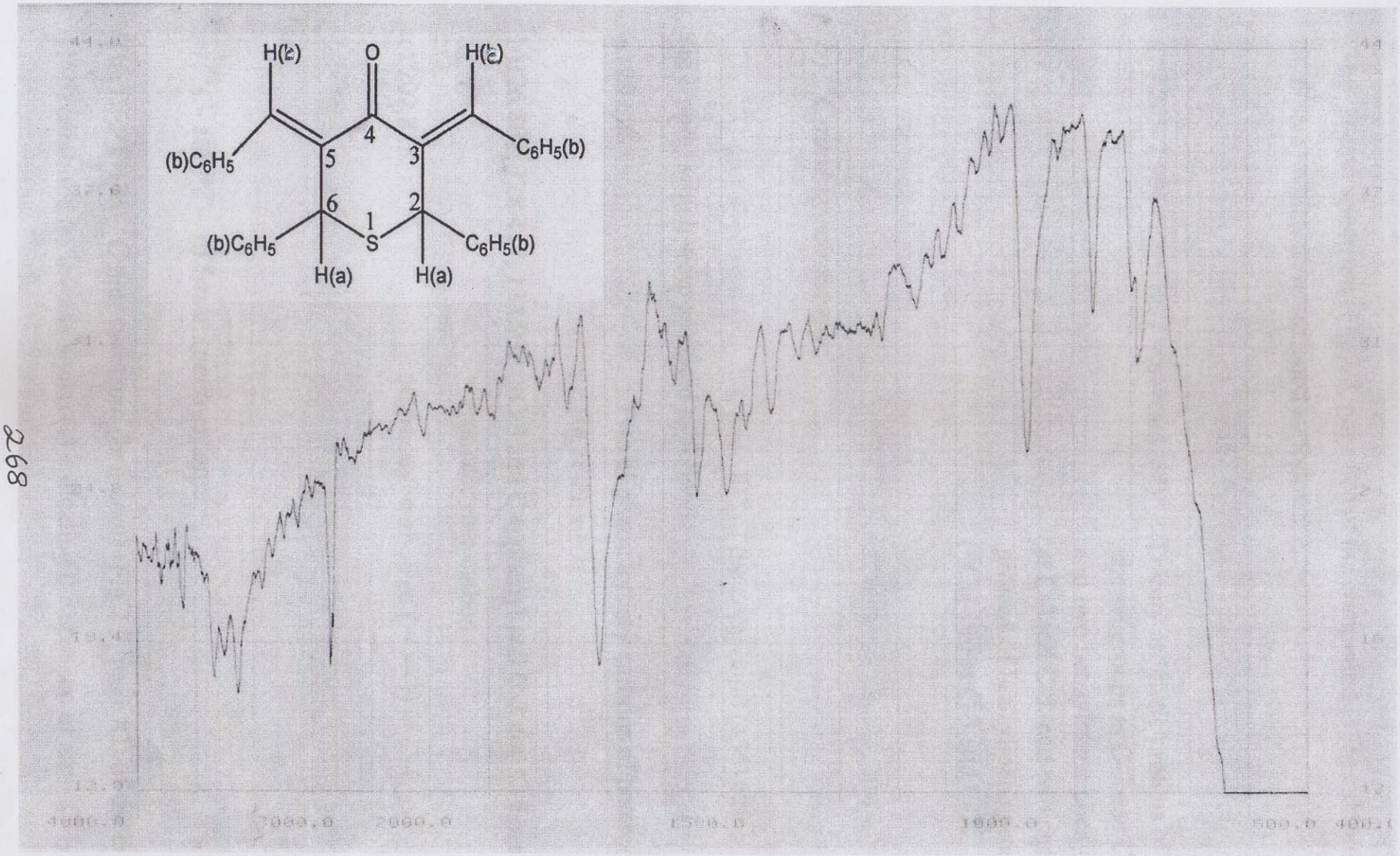


Fig 34 : IR-spectrum of 3,5-dibenzylidene-2,6-diphenyltetrahydrothiopyran-4-one (16)

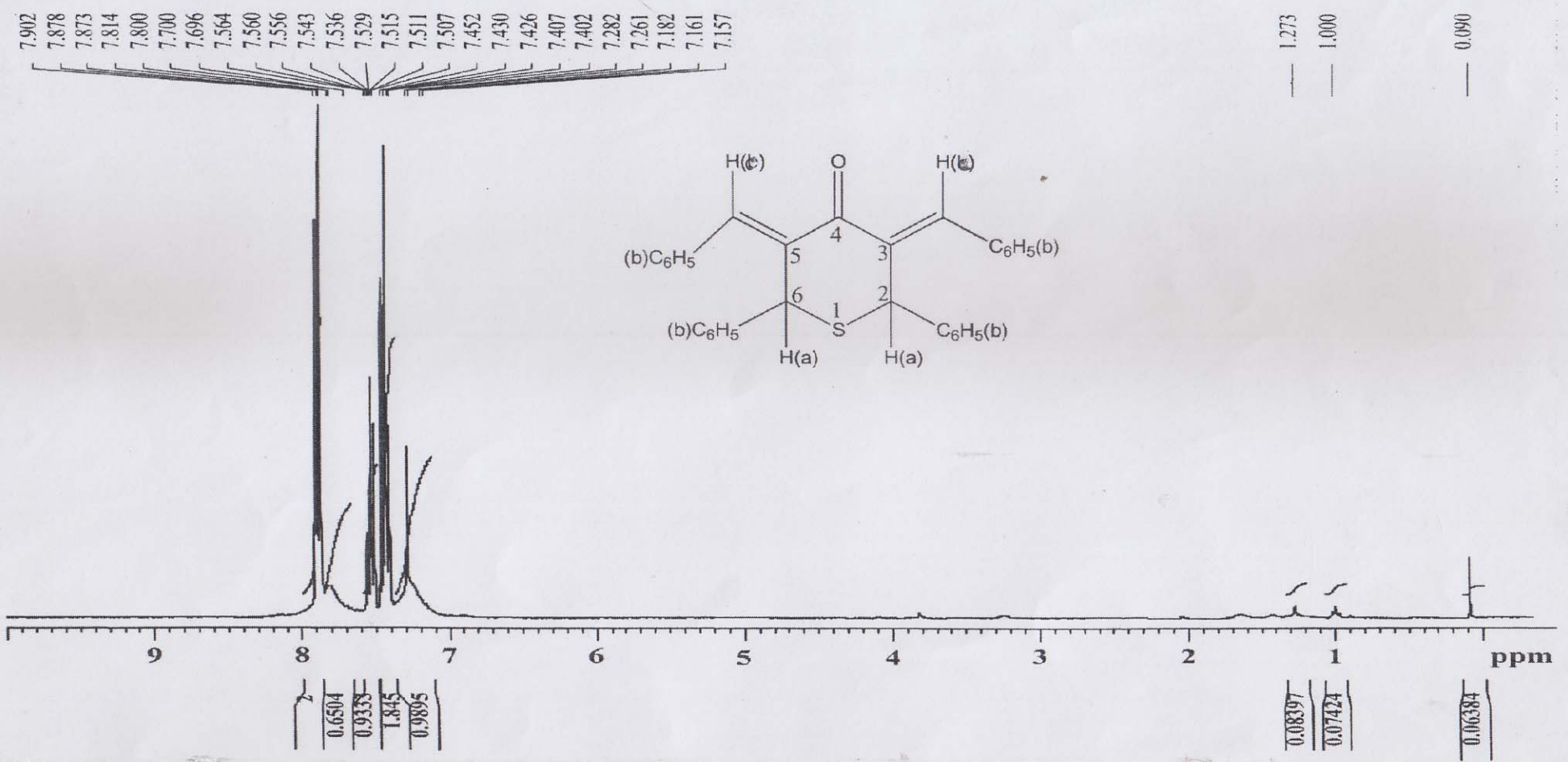


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Fig 35 : ^{1}H-nmr spectrum of 3,5-dibenzylidene-2,6-diphenyltetrahydrothiopyran-4-one **(16)**

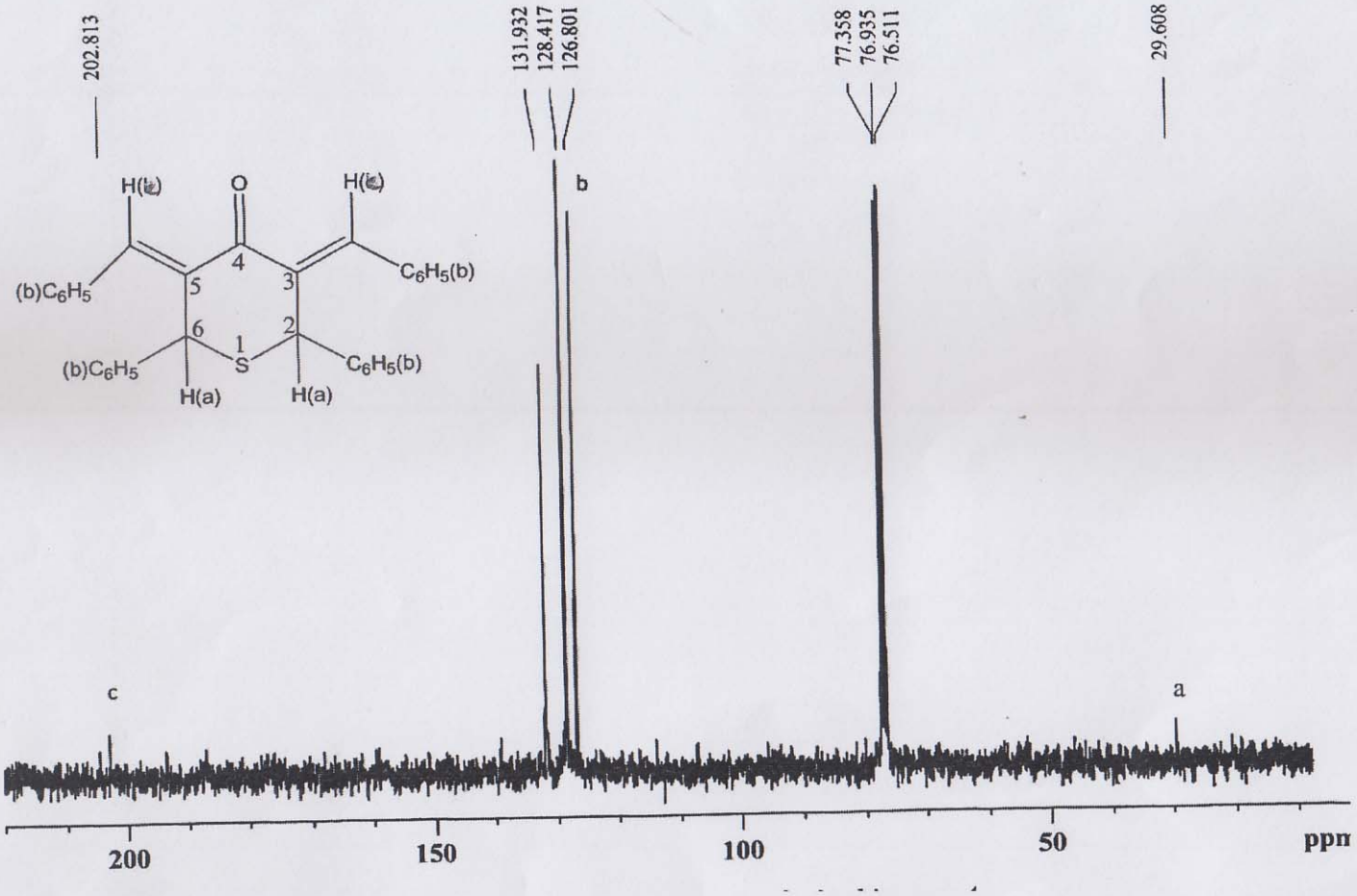


Fig 36 : ^{13}C-nmr spectrum of 3,5-dibenzylidene-2,6-diphenyltetrahydrothiopyran-4-one **(16)**

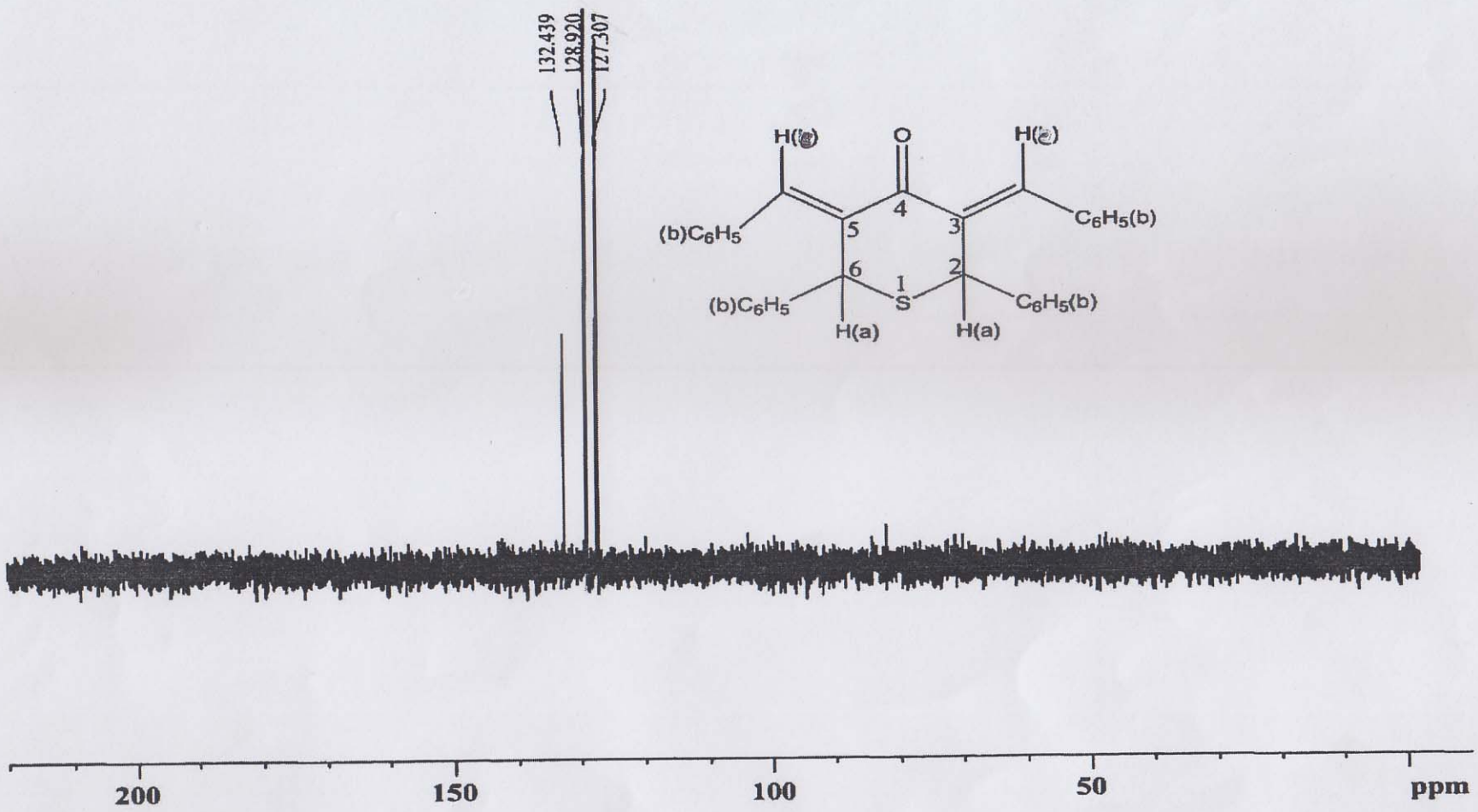


Fig 37 : ^{13}C-nmr (DEPT-135) spectrum of 3,5-dibenzylidene-2,6--diphenyltetrahydrothiopyran-4-one **(16)**

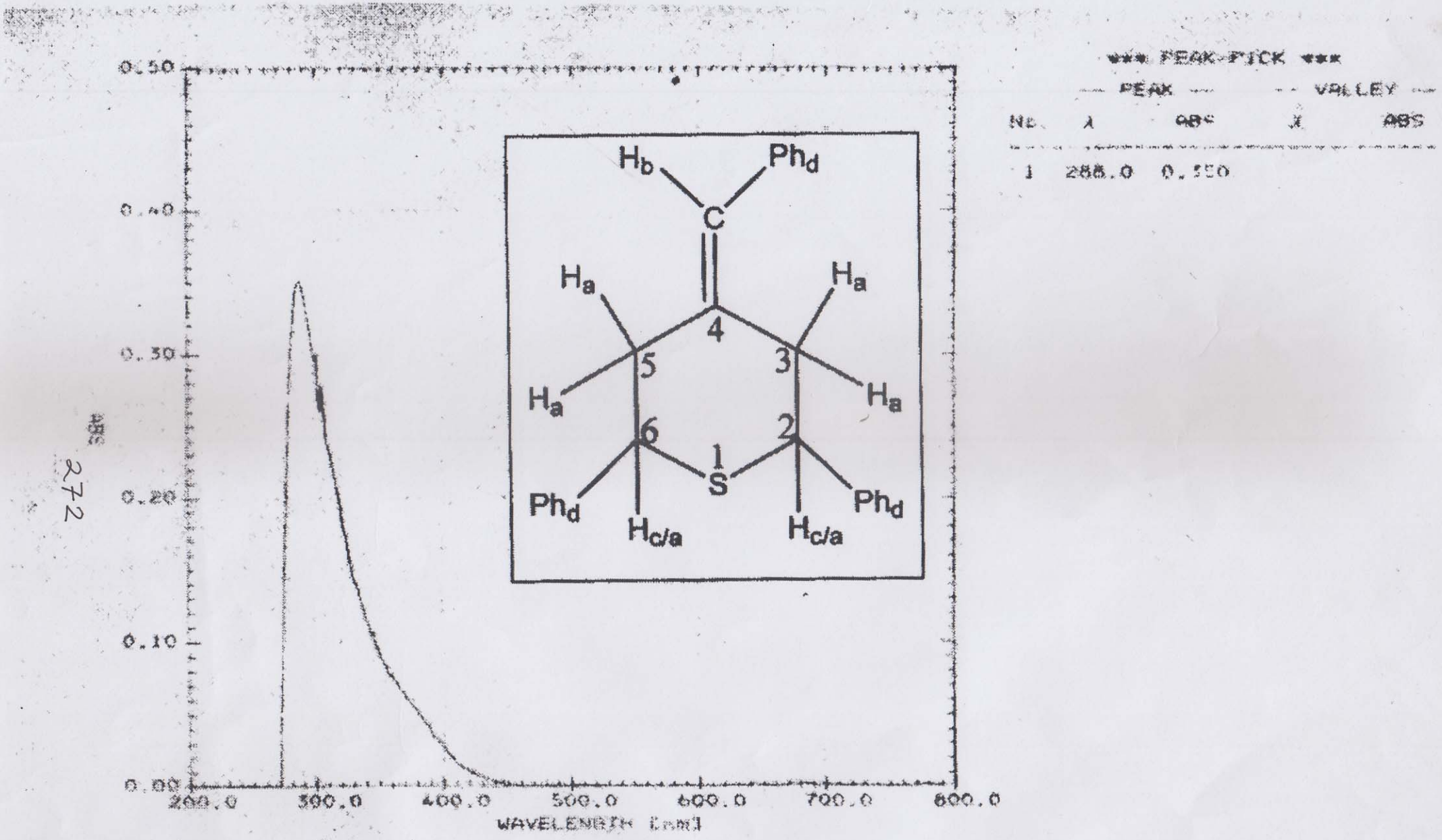


Fig 38 : UV-spectrum of 4-benzylidene-2,6-diphenyltetrahydrothiopyran **(15a)**

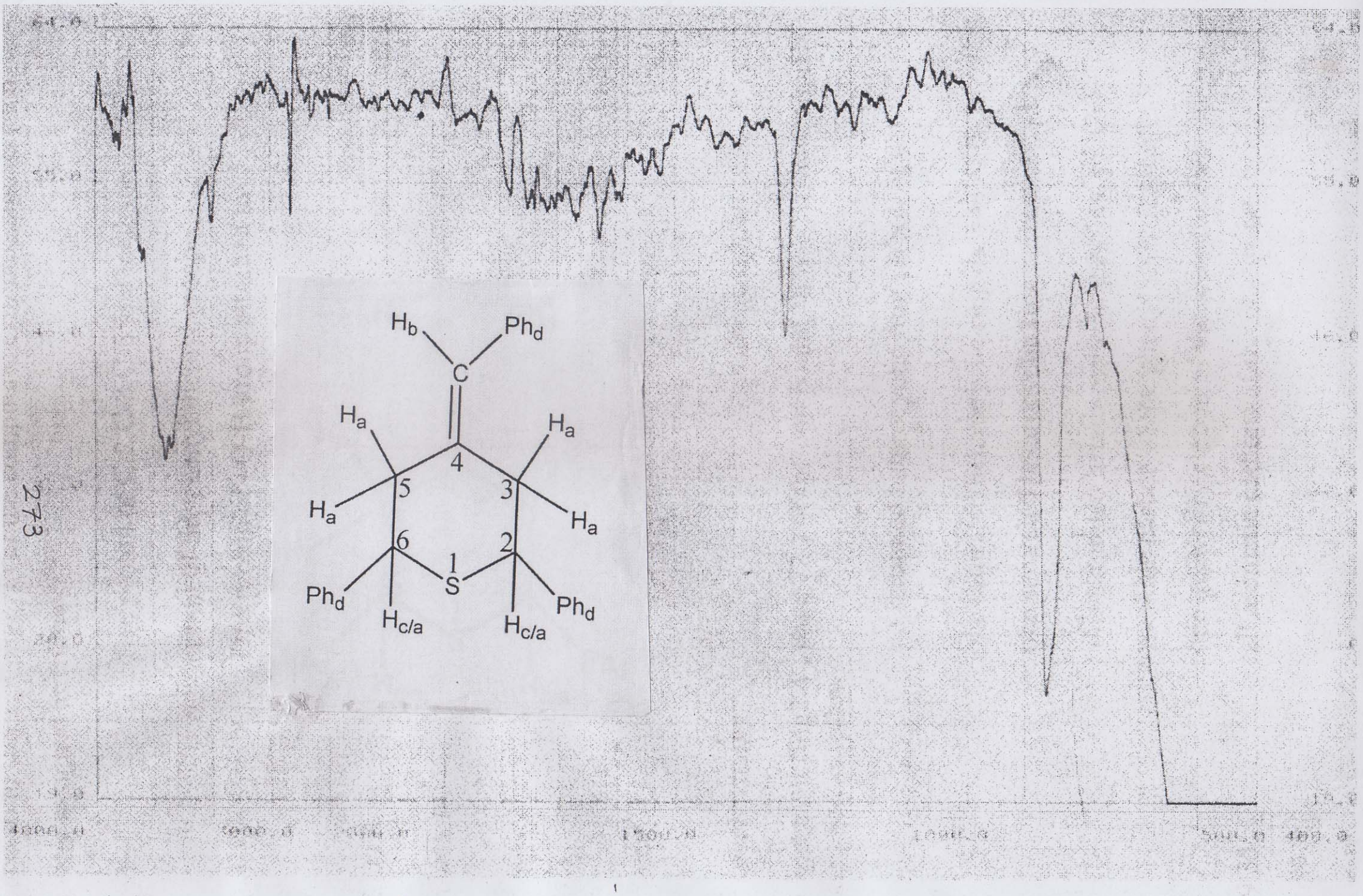


Fig 39 : IR-spectrum of 4-benzylidene-2,6-diphenyltetrahydrothiopyran **(15a)**

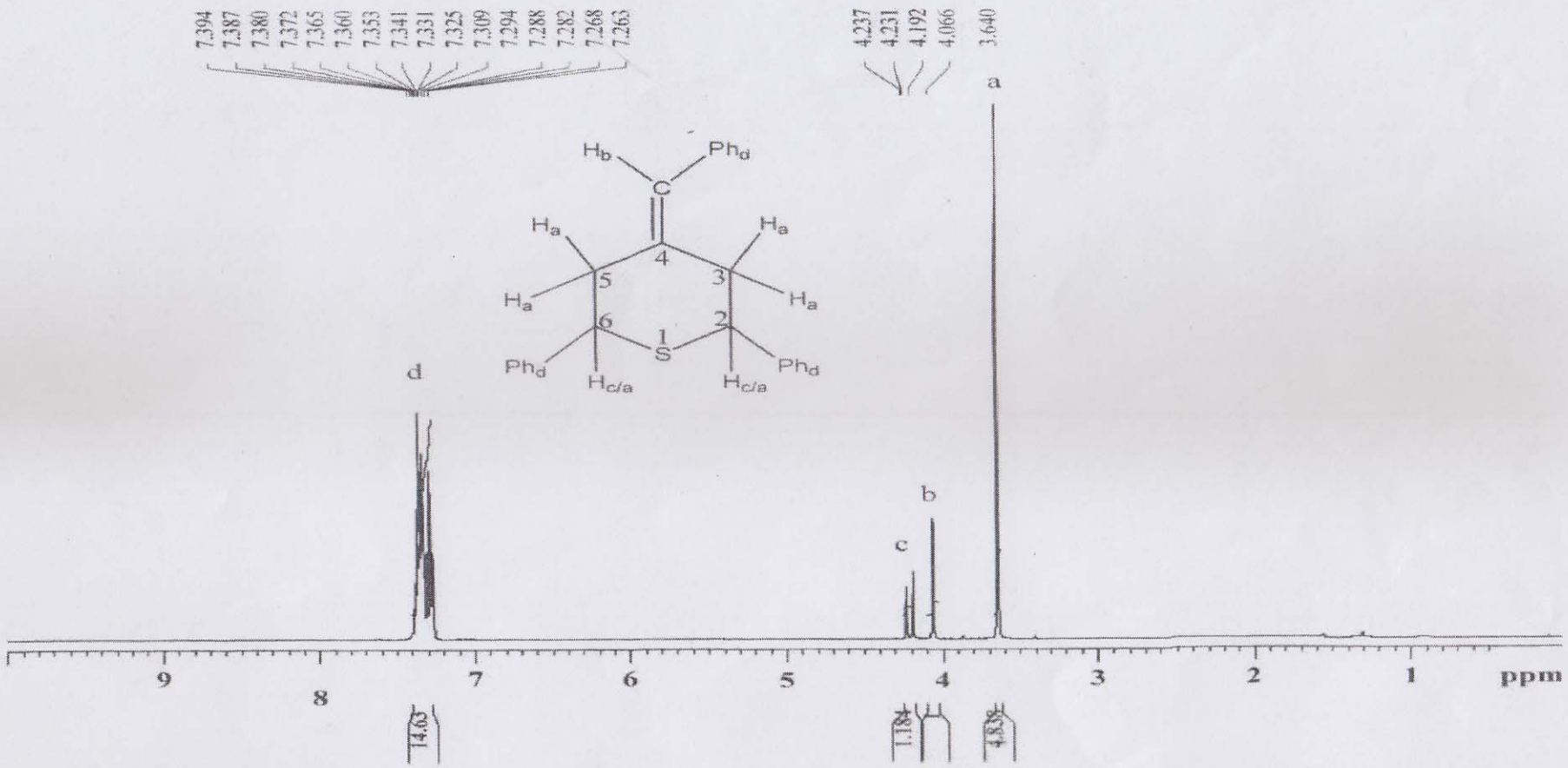


Fig 40 : ^{1}H-nmr spectrum of 4-benzylidene-2,6-diphenyltetrahydrothiopyran **(15a)**

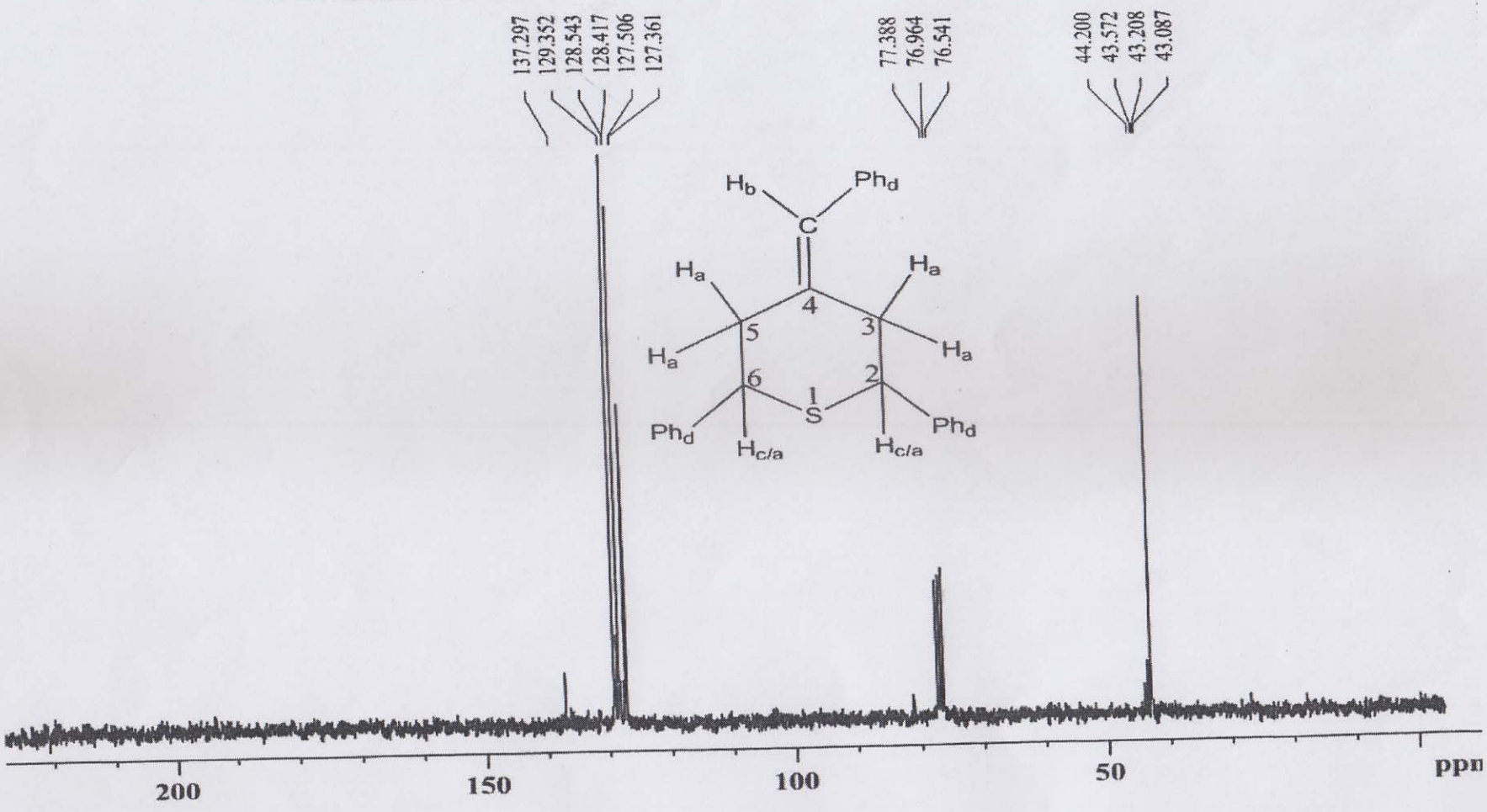
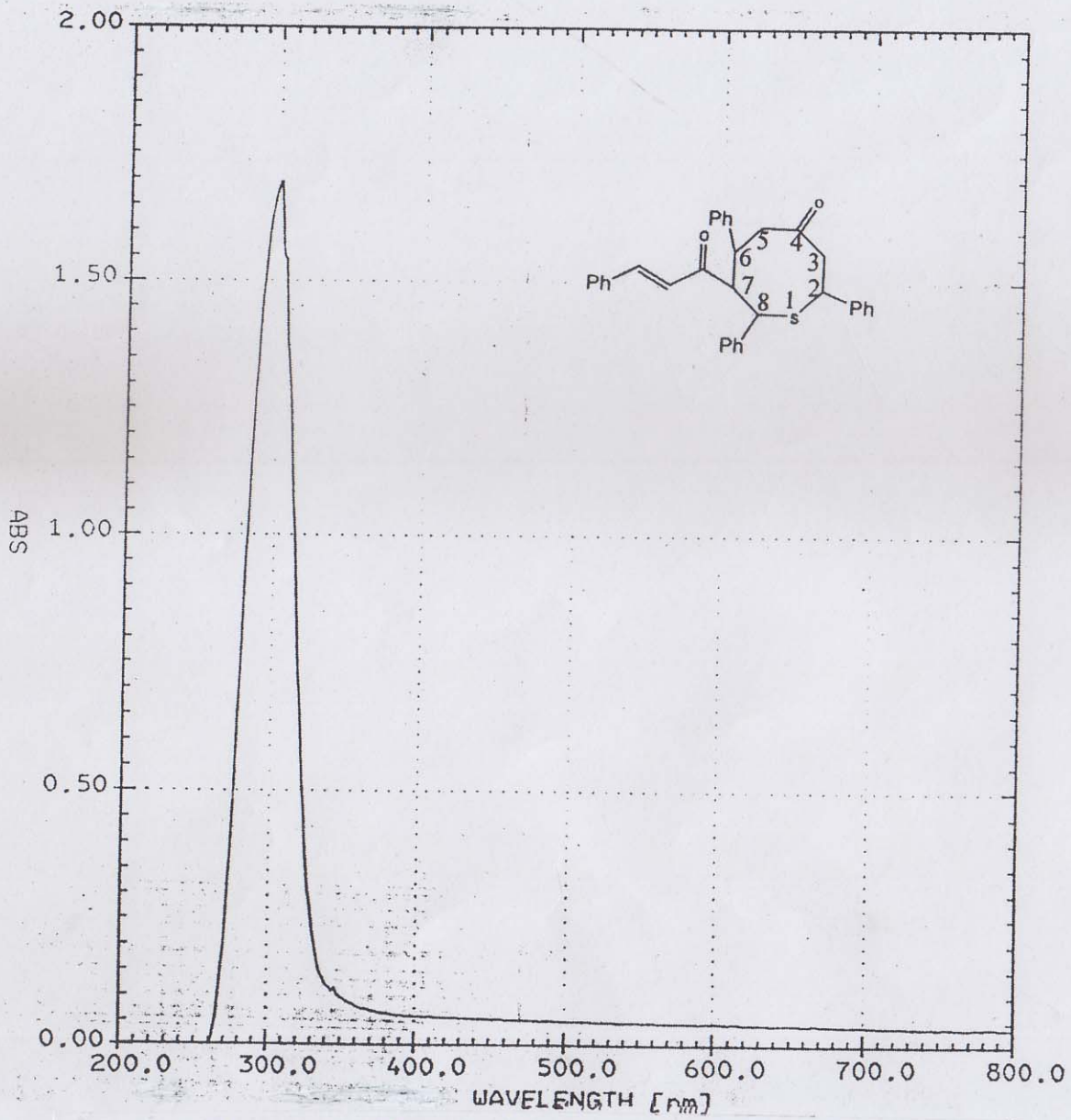


Fig 41 : ^{13}C-nmr spectrum of 4-benzylidene-2,6-diphenyltetrahydrothiopyran **(15a)**



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	301.0	1.694	212.0	-0.266

Fig 42 : UV-spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**

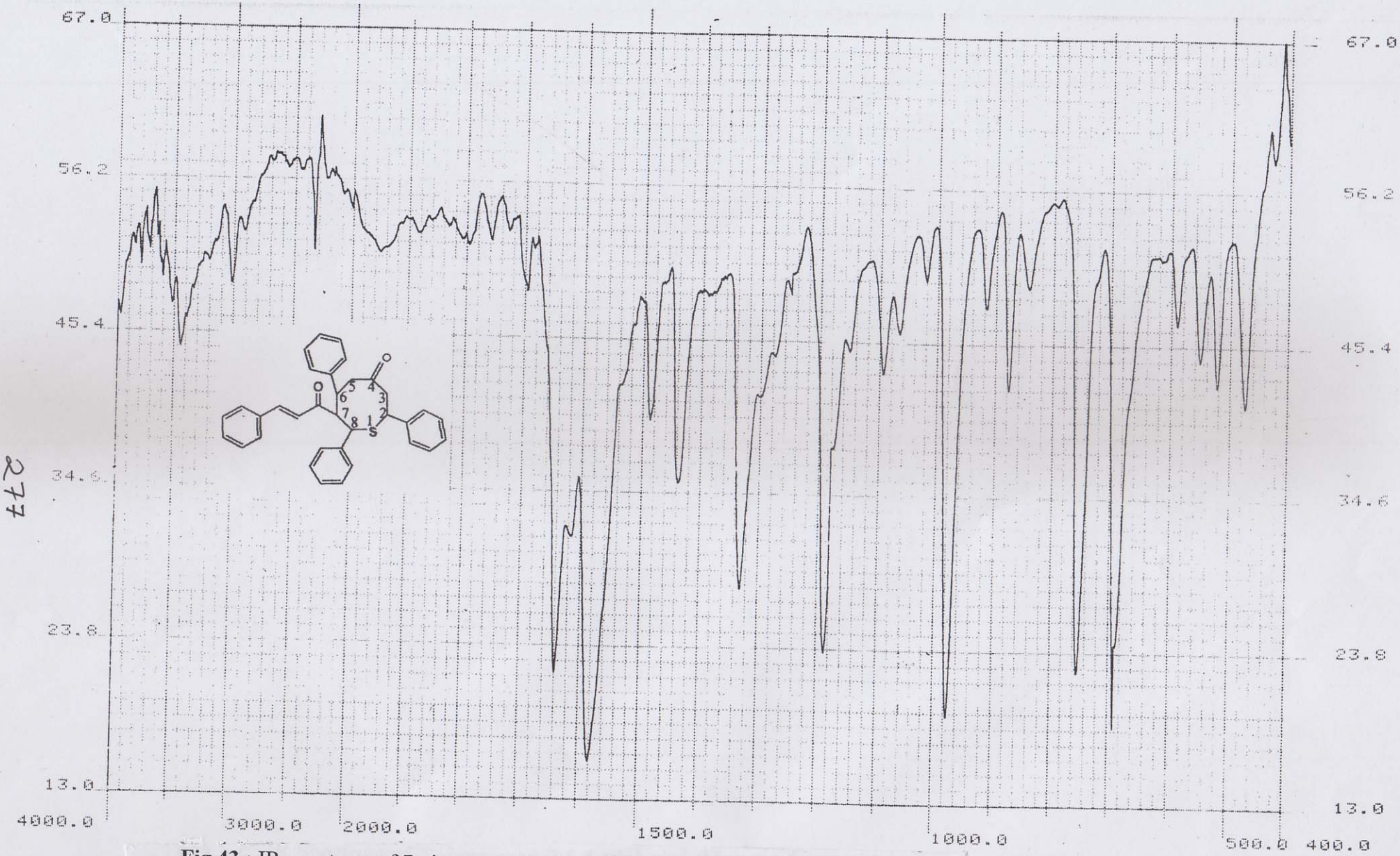


Fig 43 : IR-spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**

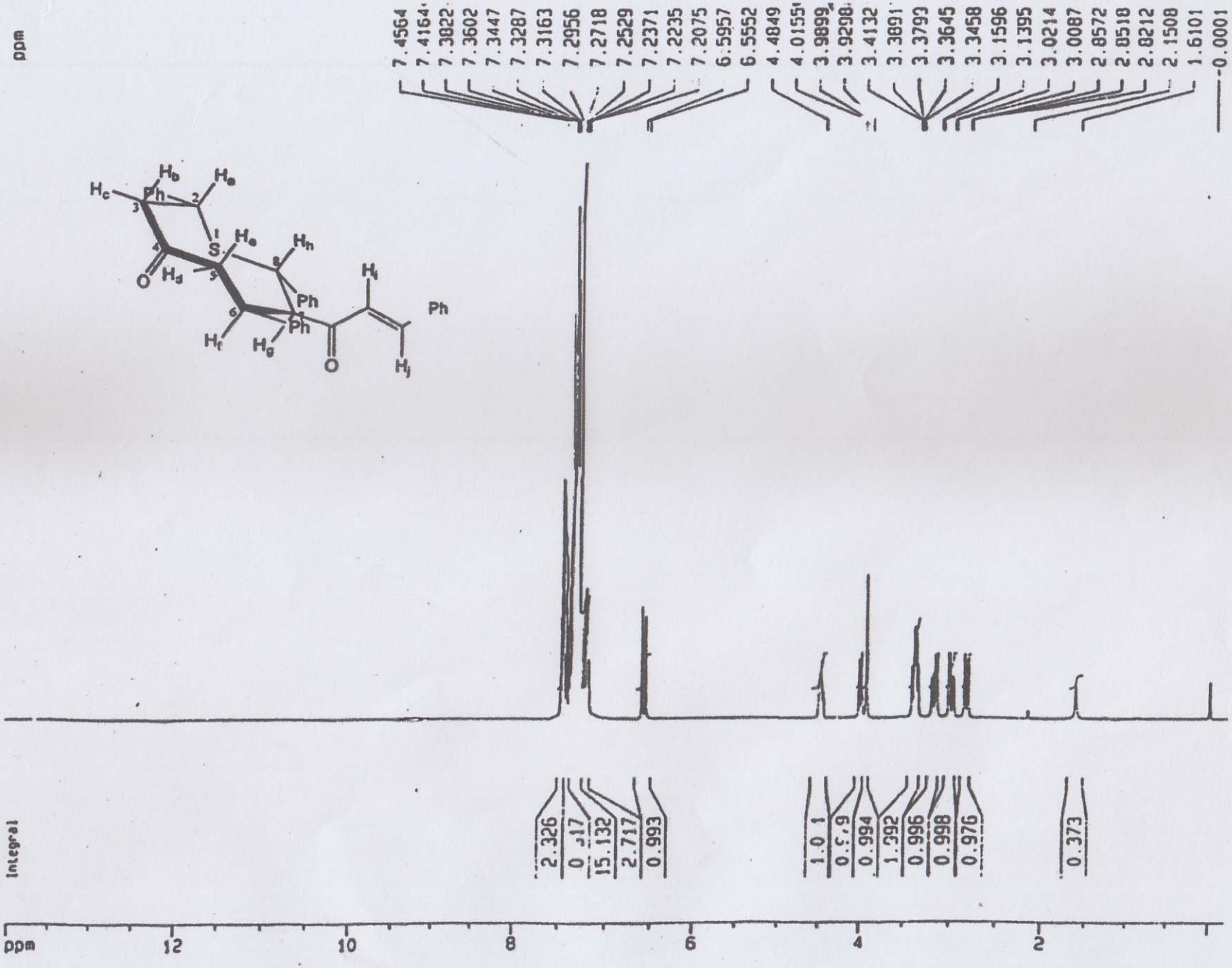


Fig 44 : ^{1}H-nmr spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**

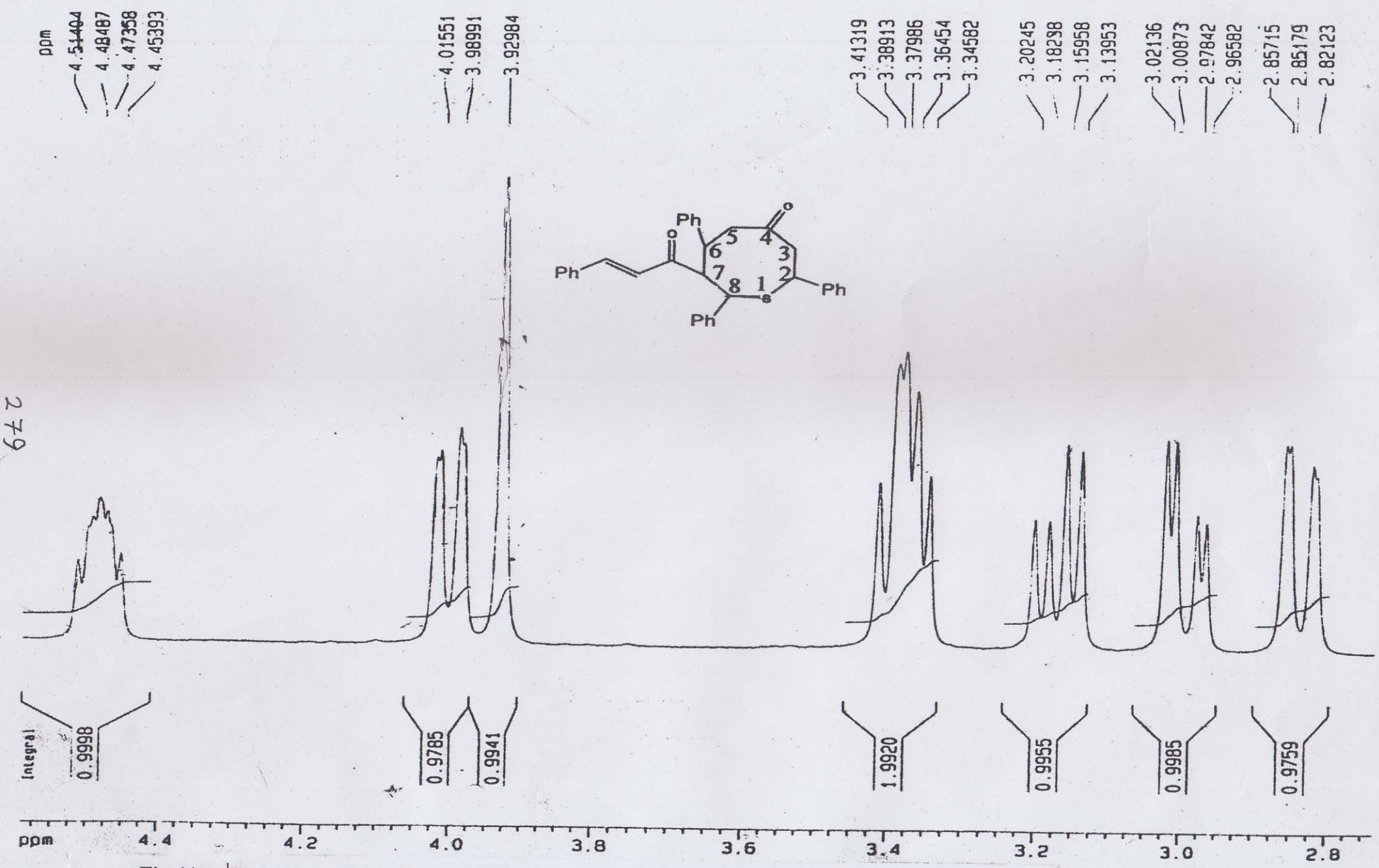


Fig 44a : ^{1}H-nmr spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**[expanded part]

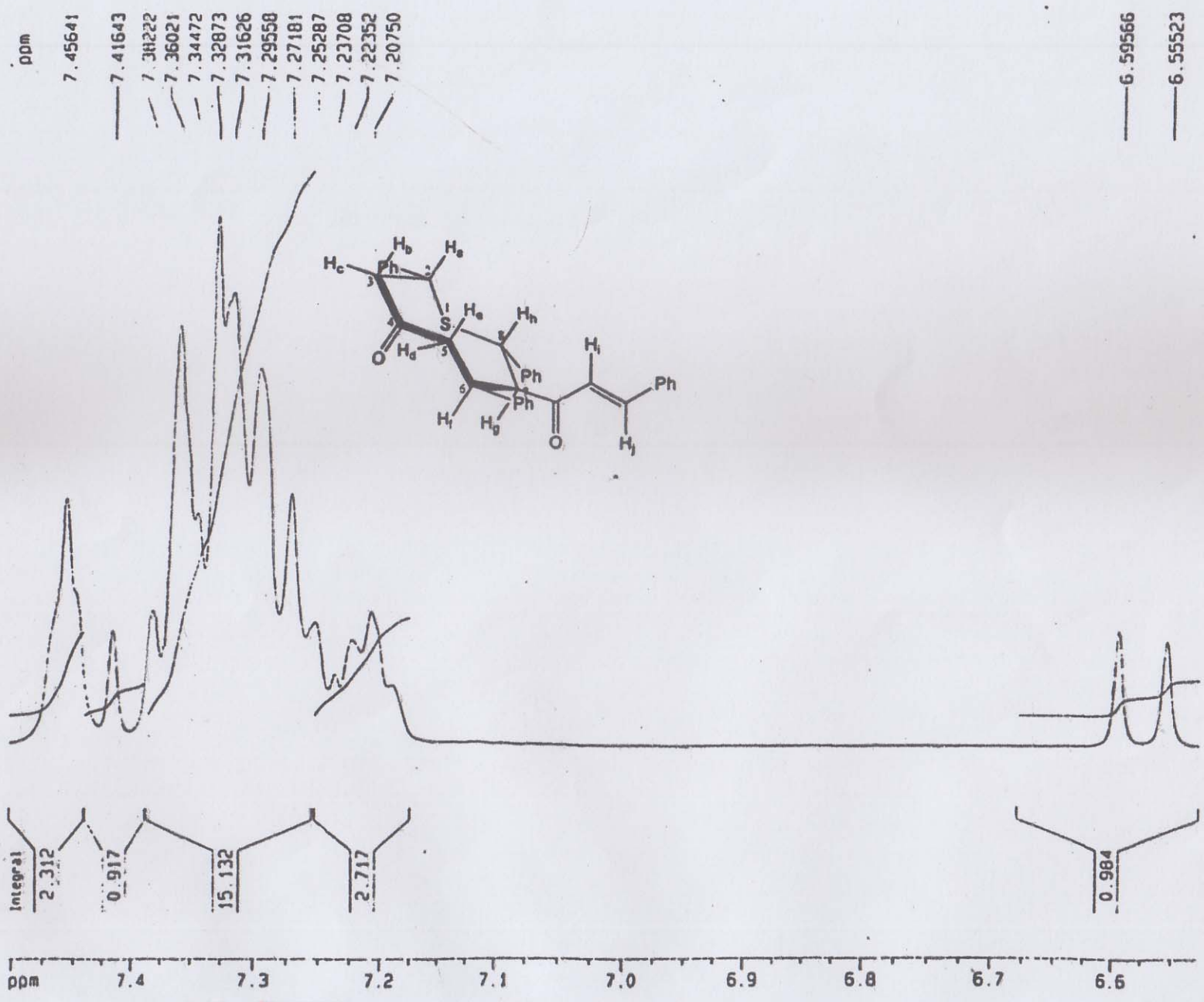


Fig 44b : ^{1}H-nmr spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**[expanded part]

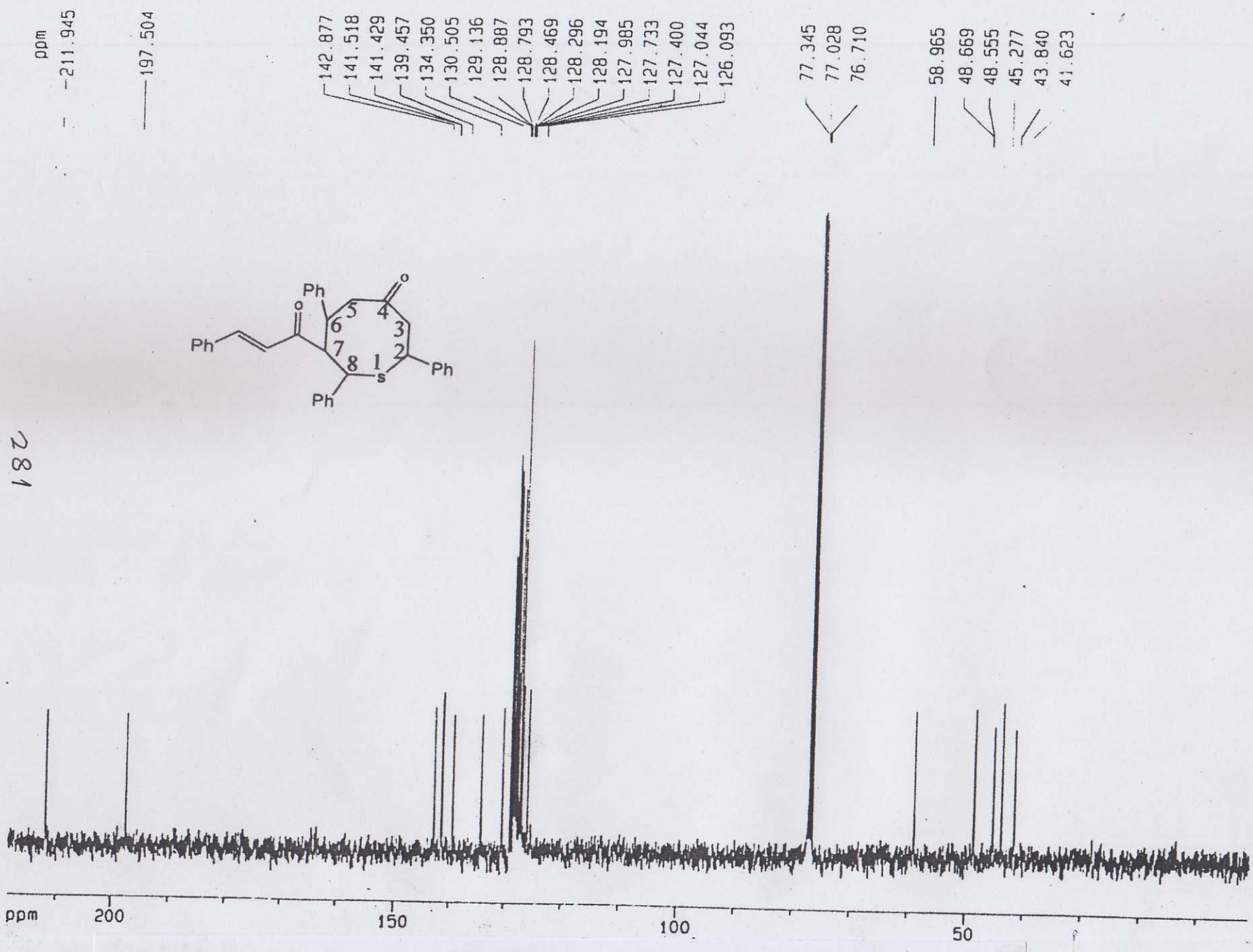


Fig 45 : ^{13}C-nmr spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**

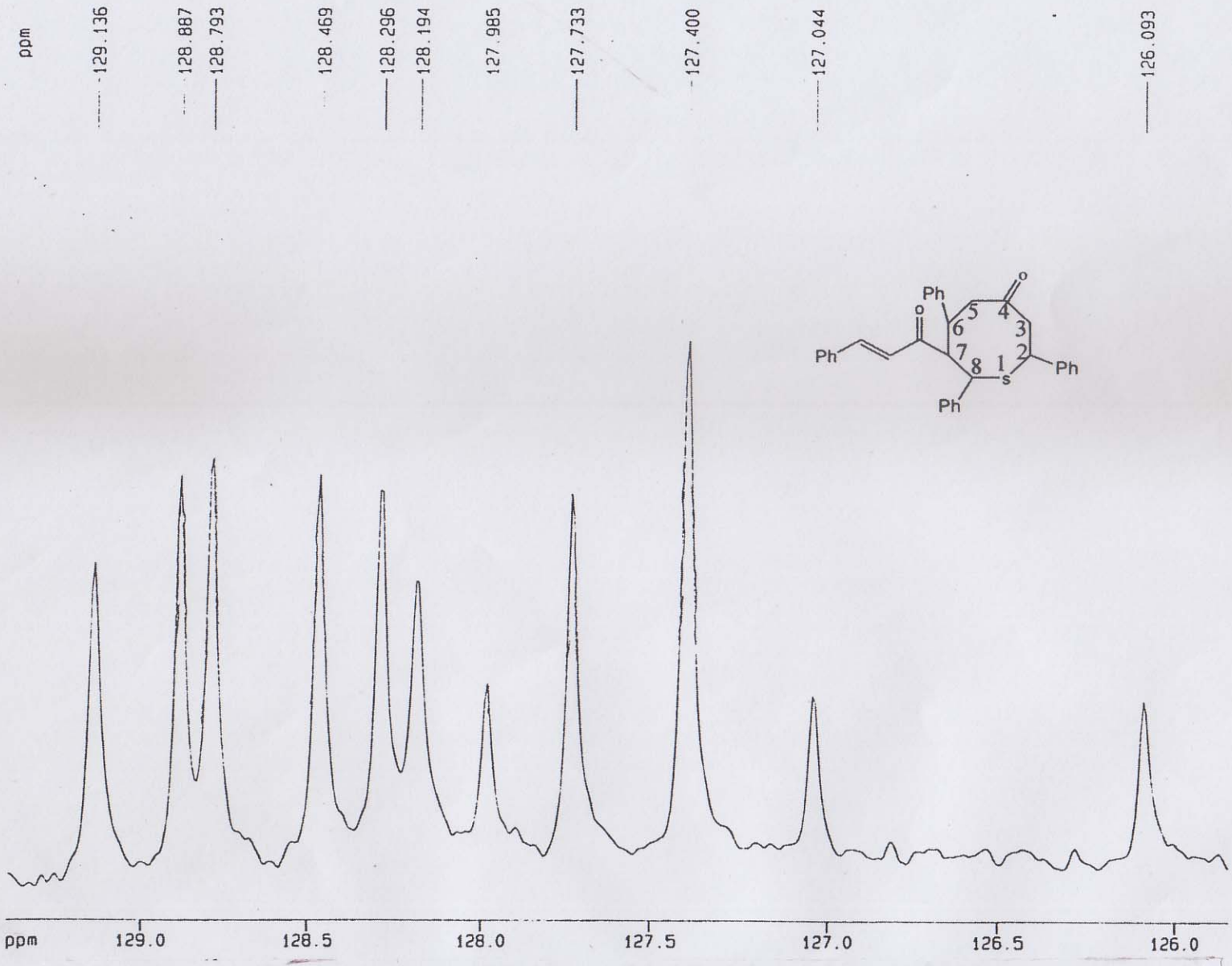


Fig 45a : ^{13}C-nmr spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**)[expanded part]

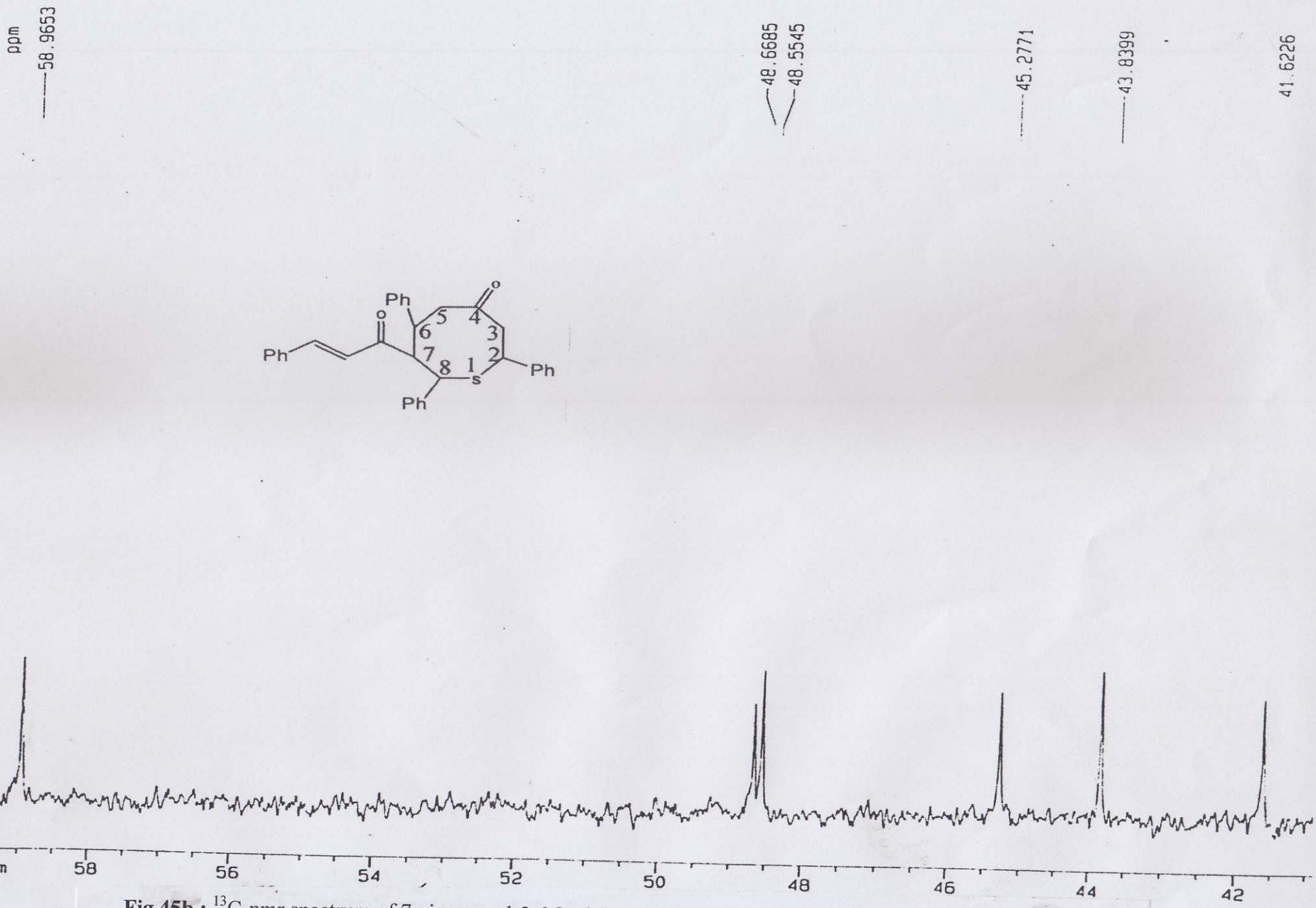


Fig 45b : ^{13}C-nmr spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one **(17)**)[expanded part]

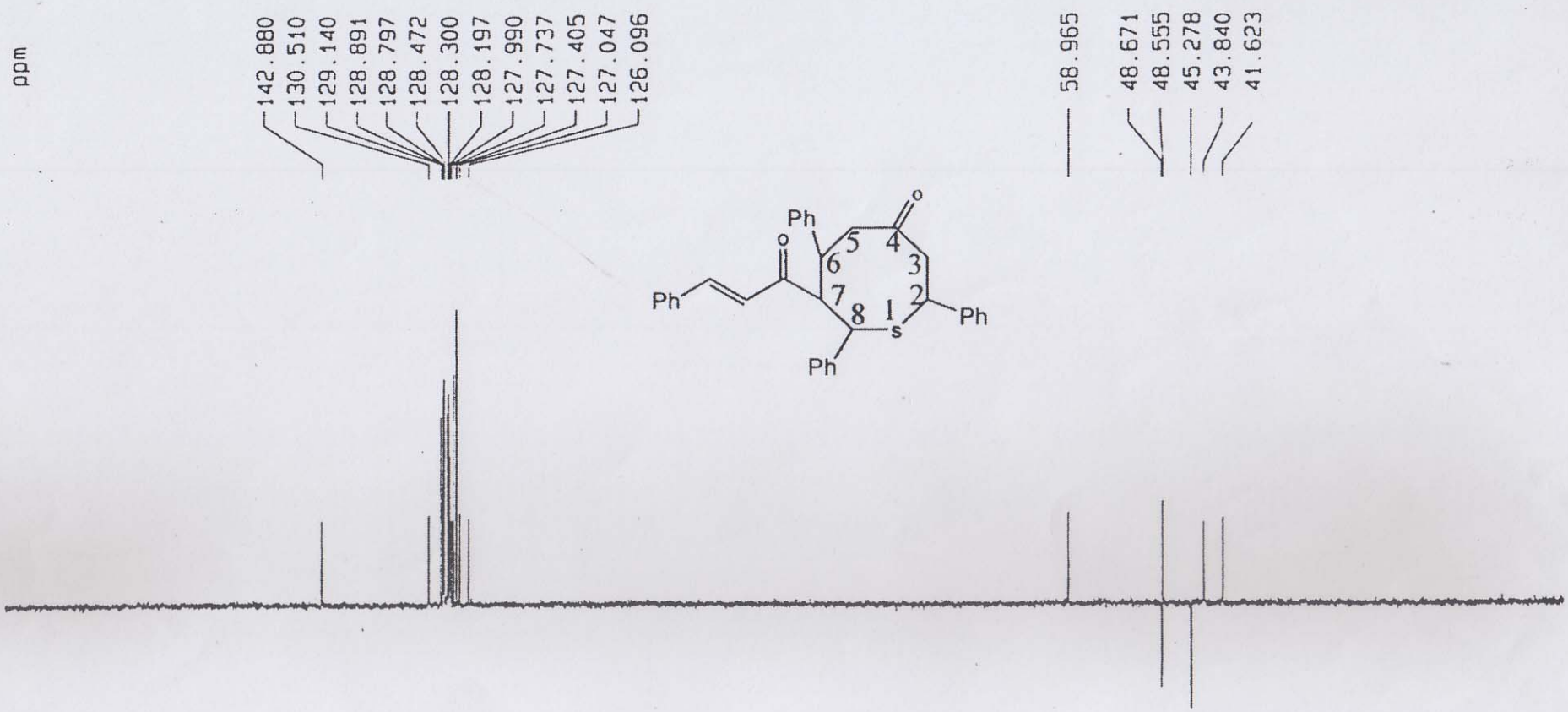


Fig 46 : ^{13}C-nmr(DEPT-135) spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one (**17**)

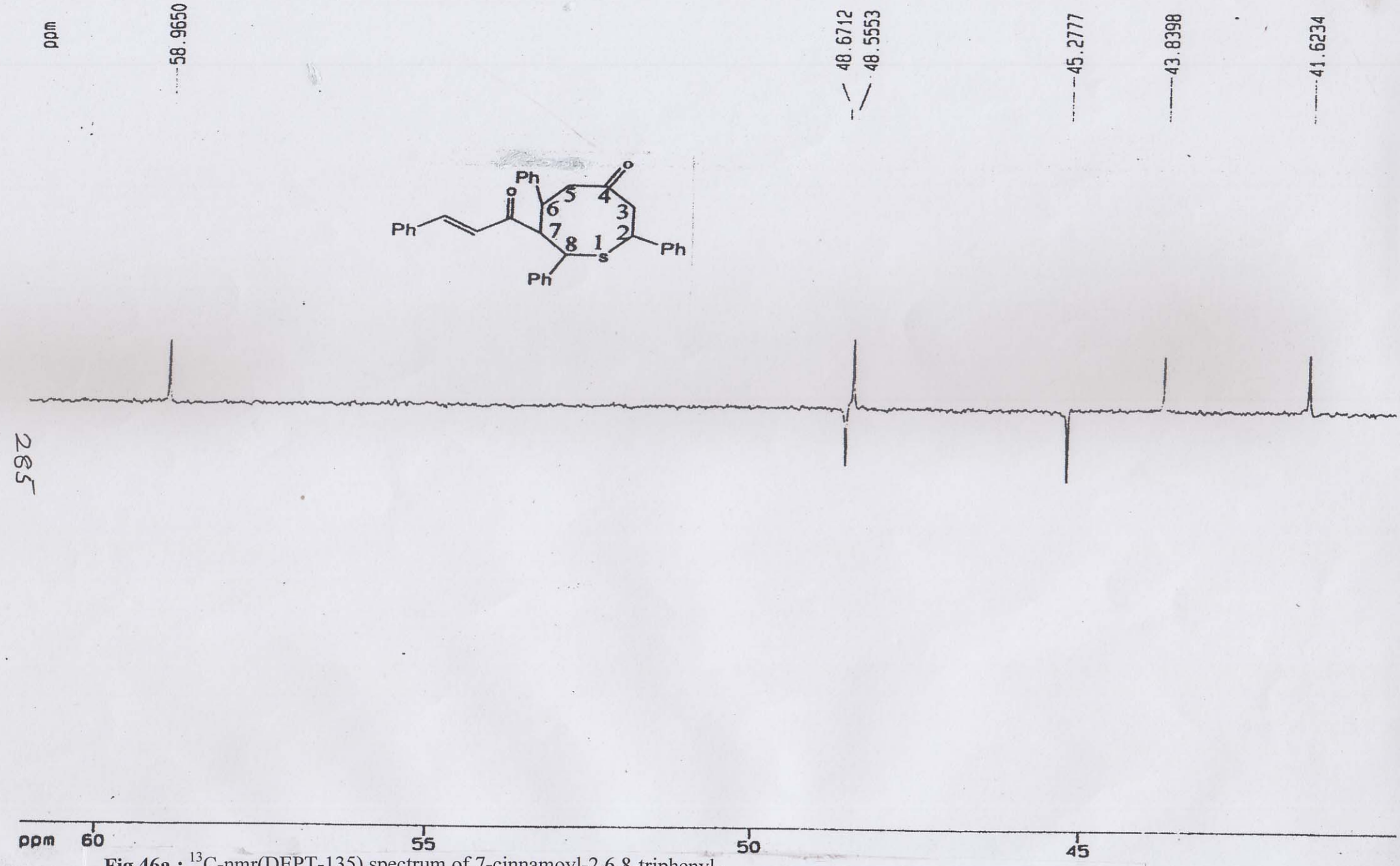


Fig 46a : ^{13}C-nmr(DEPT-135) spectrum of 7-cinnamoyl-2,6,8-triphenyl-

-thiocan-4-one (**17**) [expanded part]

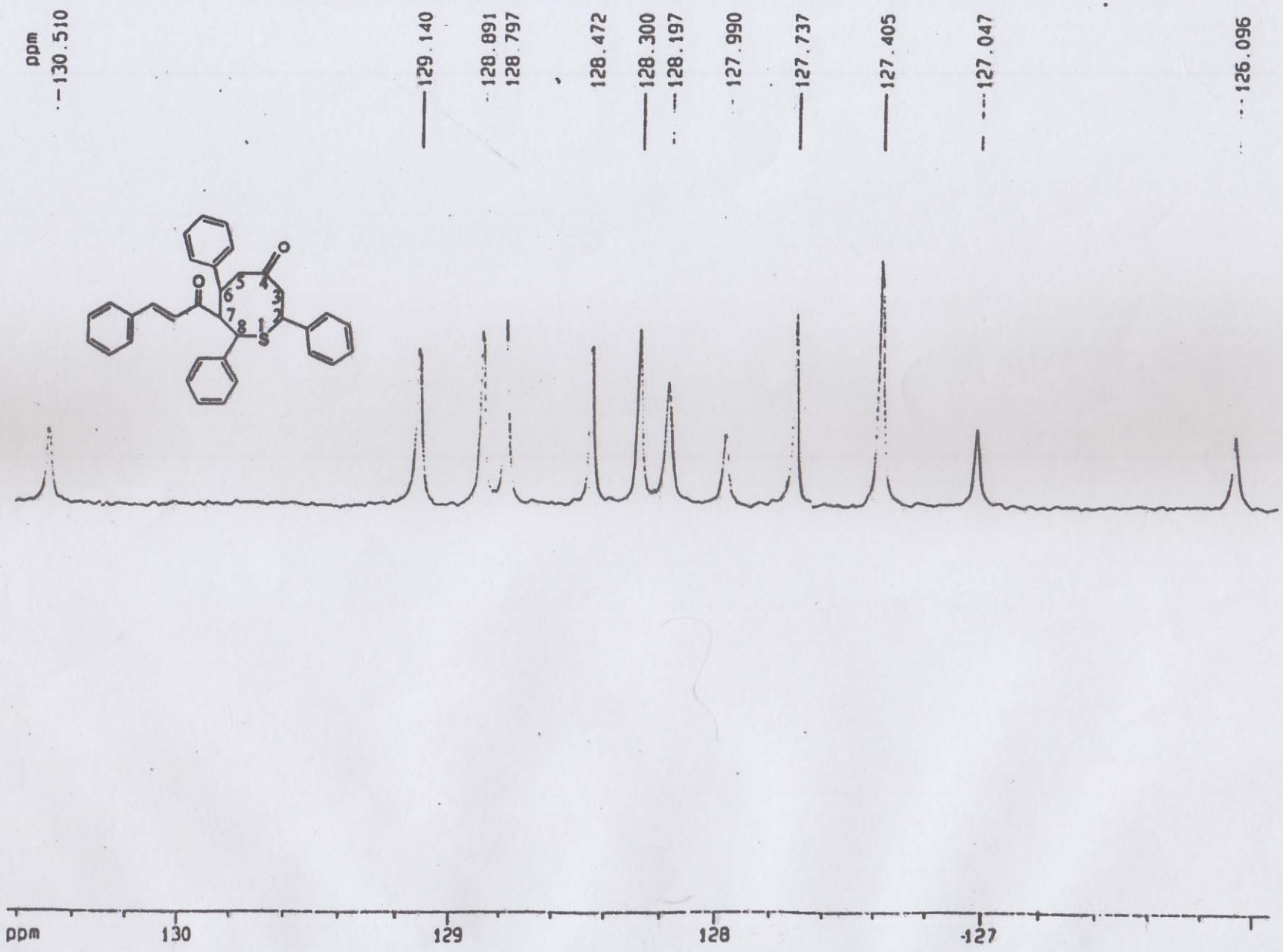


Fig 46b : ^{13}C-nmr(DEPT-135) spectrum of 7-cinnamoyl-2,6,8-triphenyl--thiocan-4-one (**17**) [expanded part]

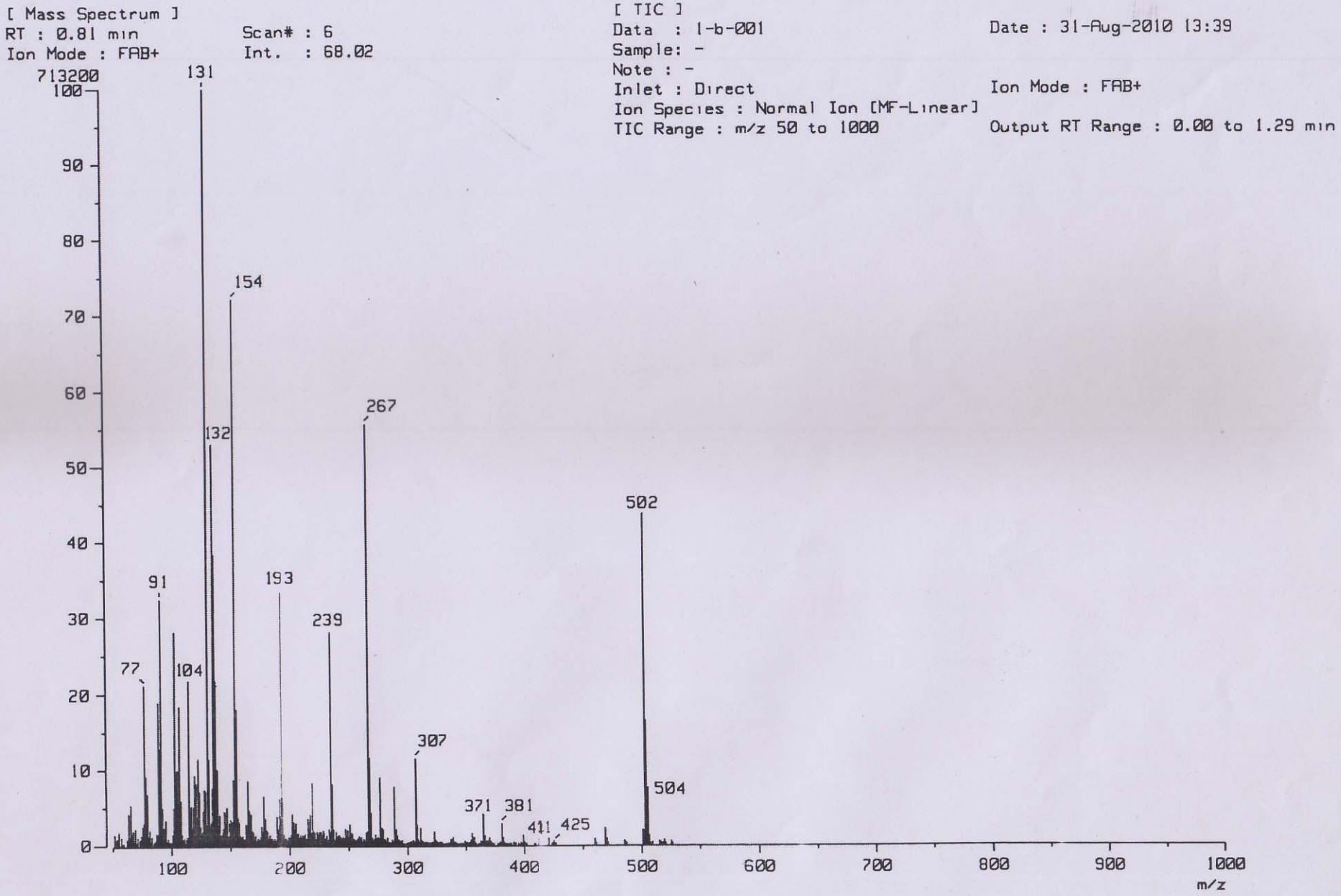
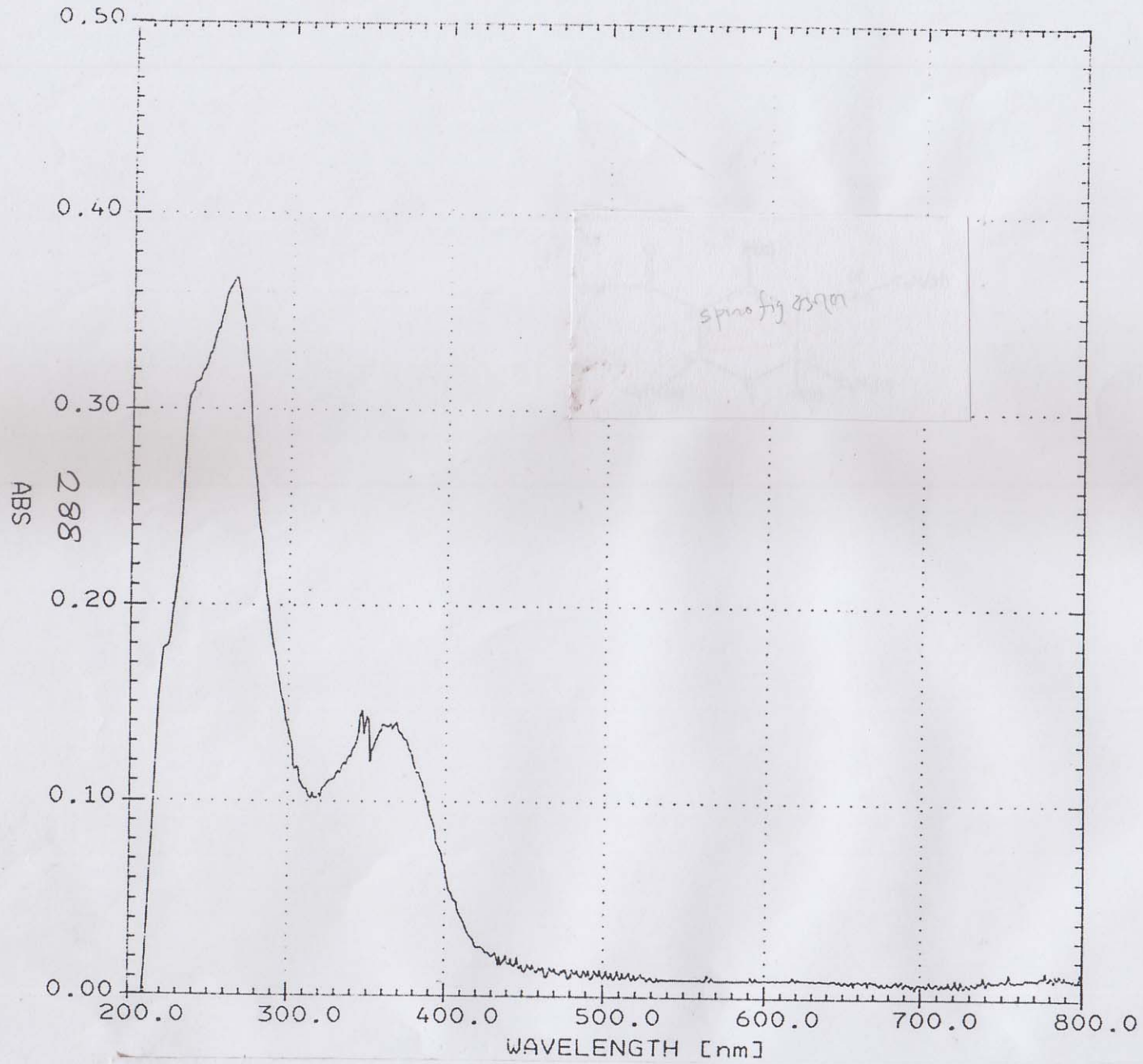


Fig 47 : Mass spectrum of 7-cinnamoyl-2,6,8-triphenyl-thiocan-4-one (**17**)



*** PEAK-PICK ***

No.	-- PEAK -- λ	ABS	-- VALLEY -- λ	ABS
1	368.0	0.140	314.0	0.101
2	264.0	0.368		

Fig 50 : UV-spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4'] bithiopyranyliden-4-ol **(18)**

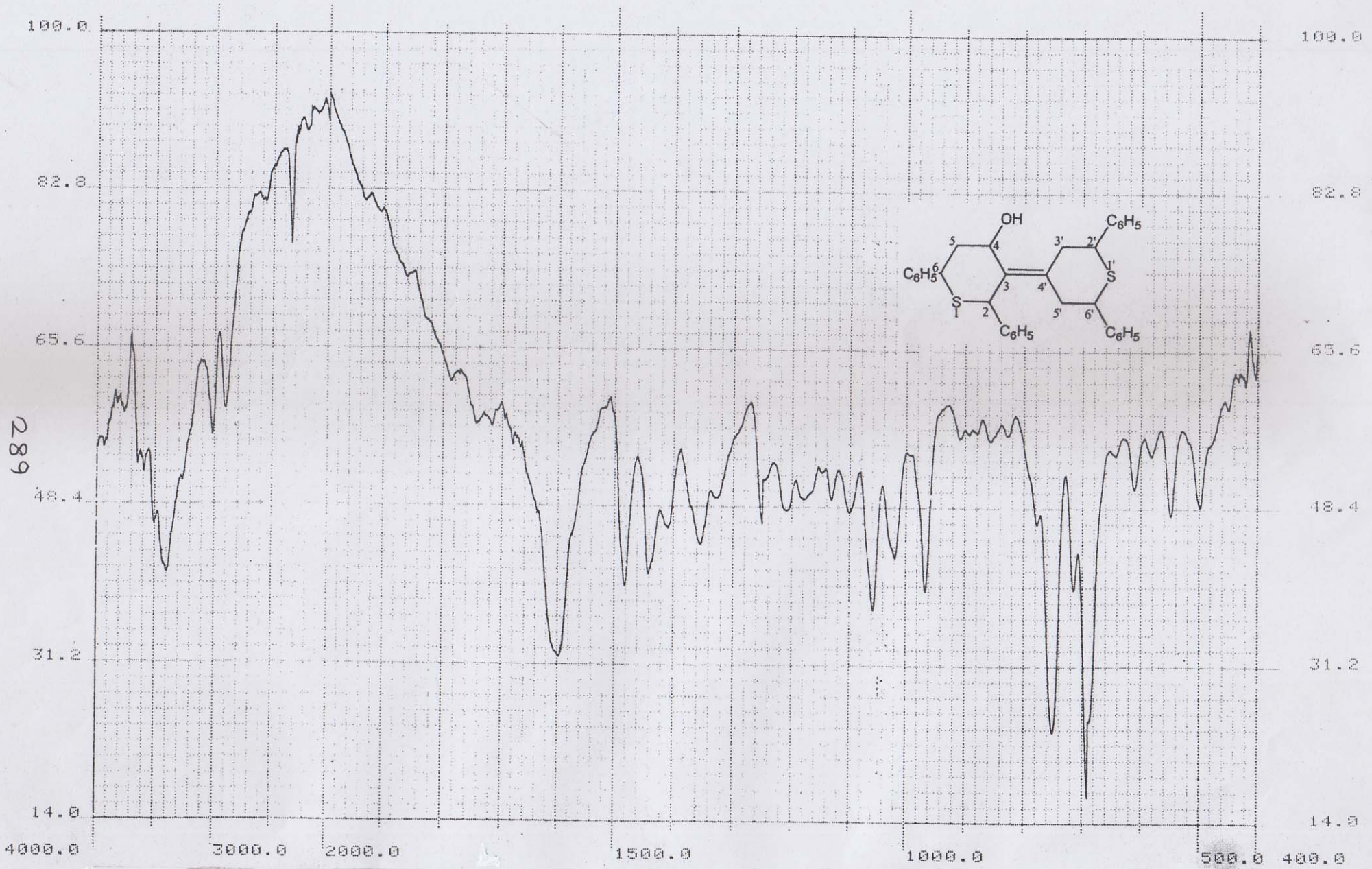


Fig 51 : IR-spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4'] bithiopyranyliden-4-ol **(18)**

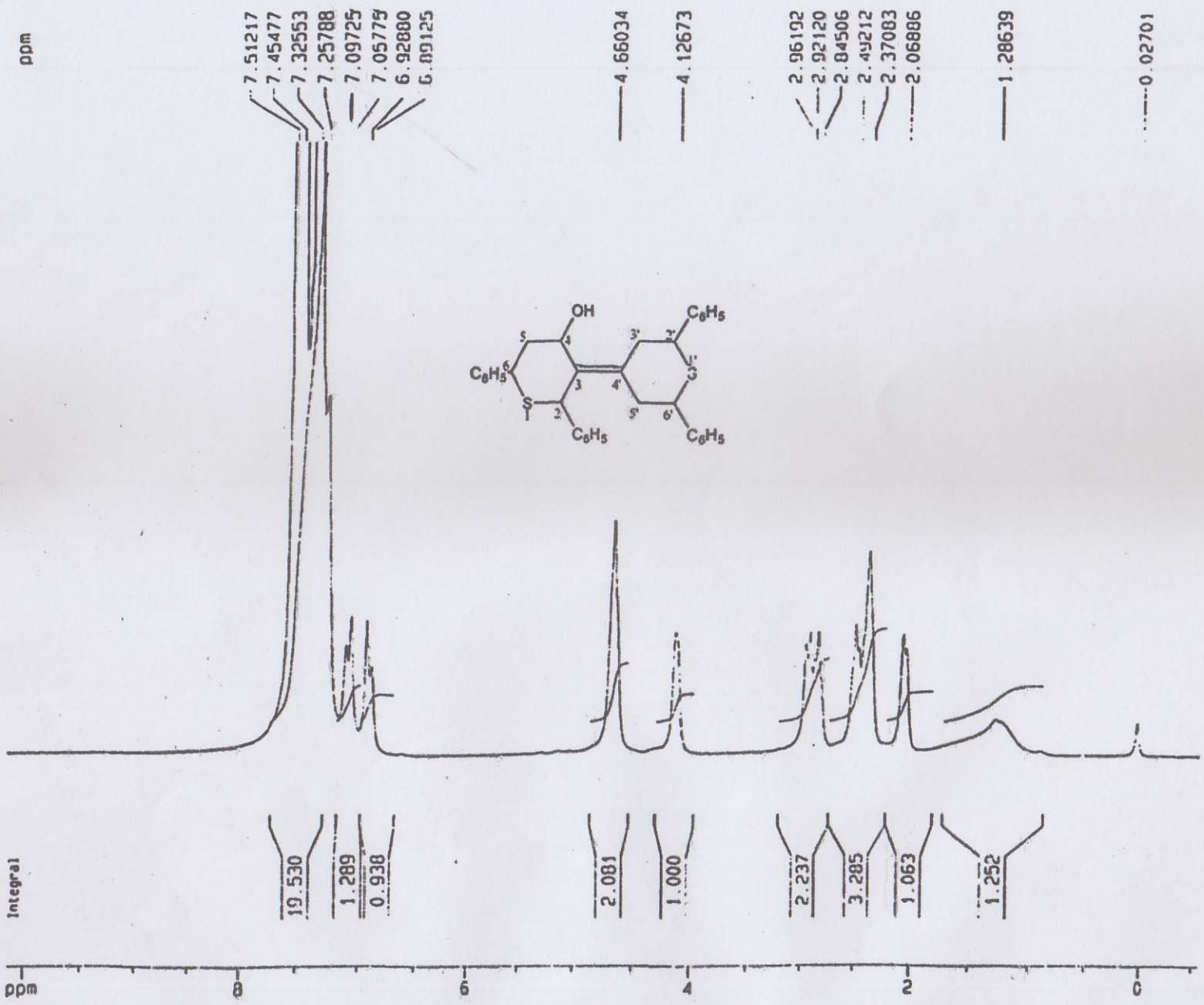


Fig 52 : ^{1}H-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4'] bithiopyranyliden-4-ol **(18)**

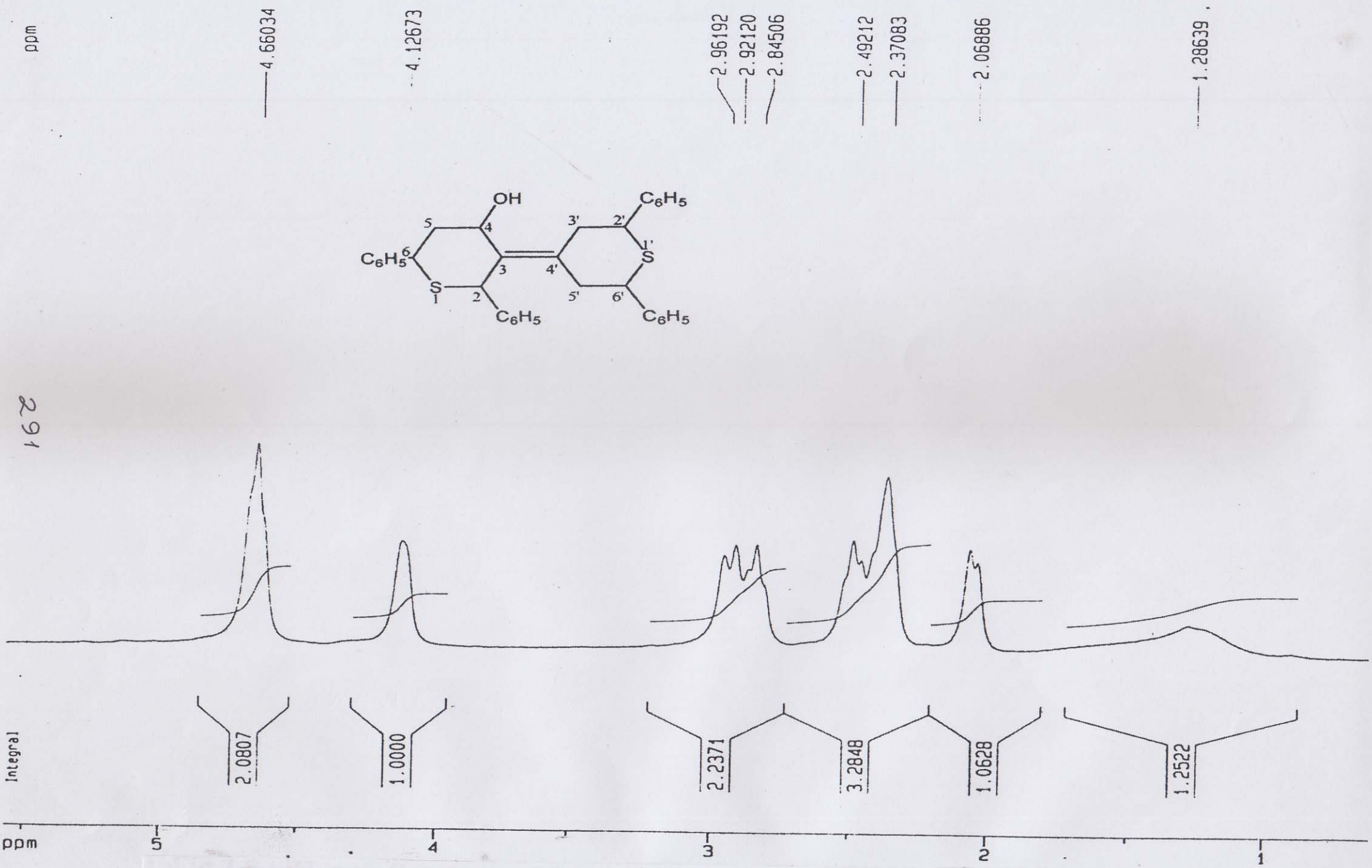


Fig 52a : ^{1}H-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4']-
-bithiopyranyliden-4-ol (**18**)[expanded part]

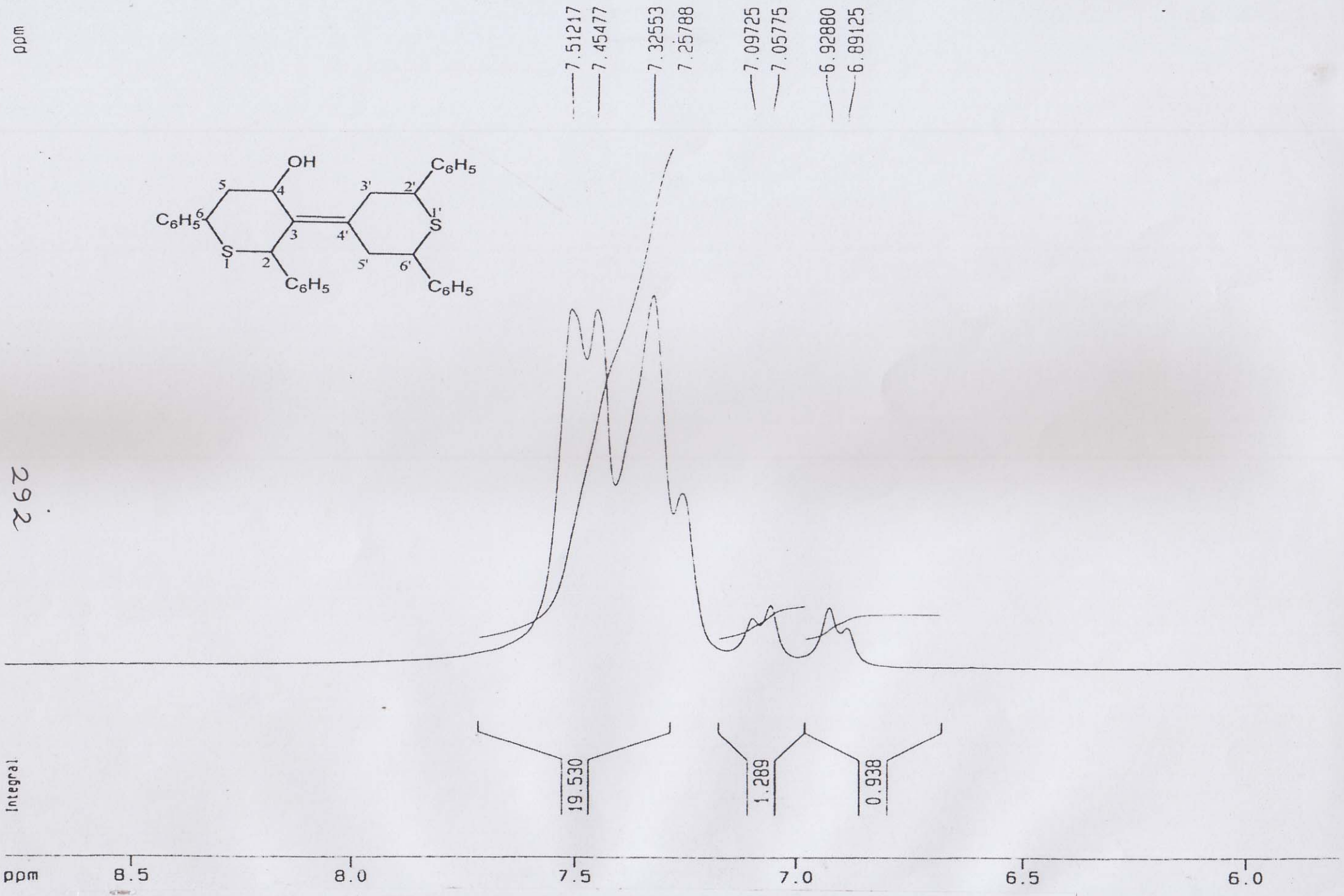


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Fig 52b : ^{1}H-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4']-
-bithiopyranyliden-4-ol (**18**)[expanded part]

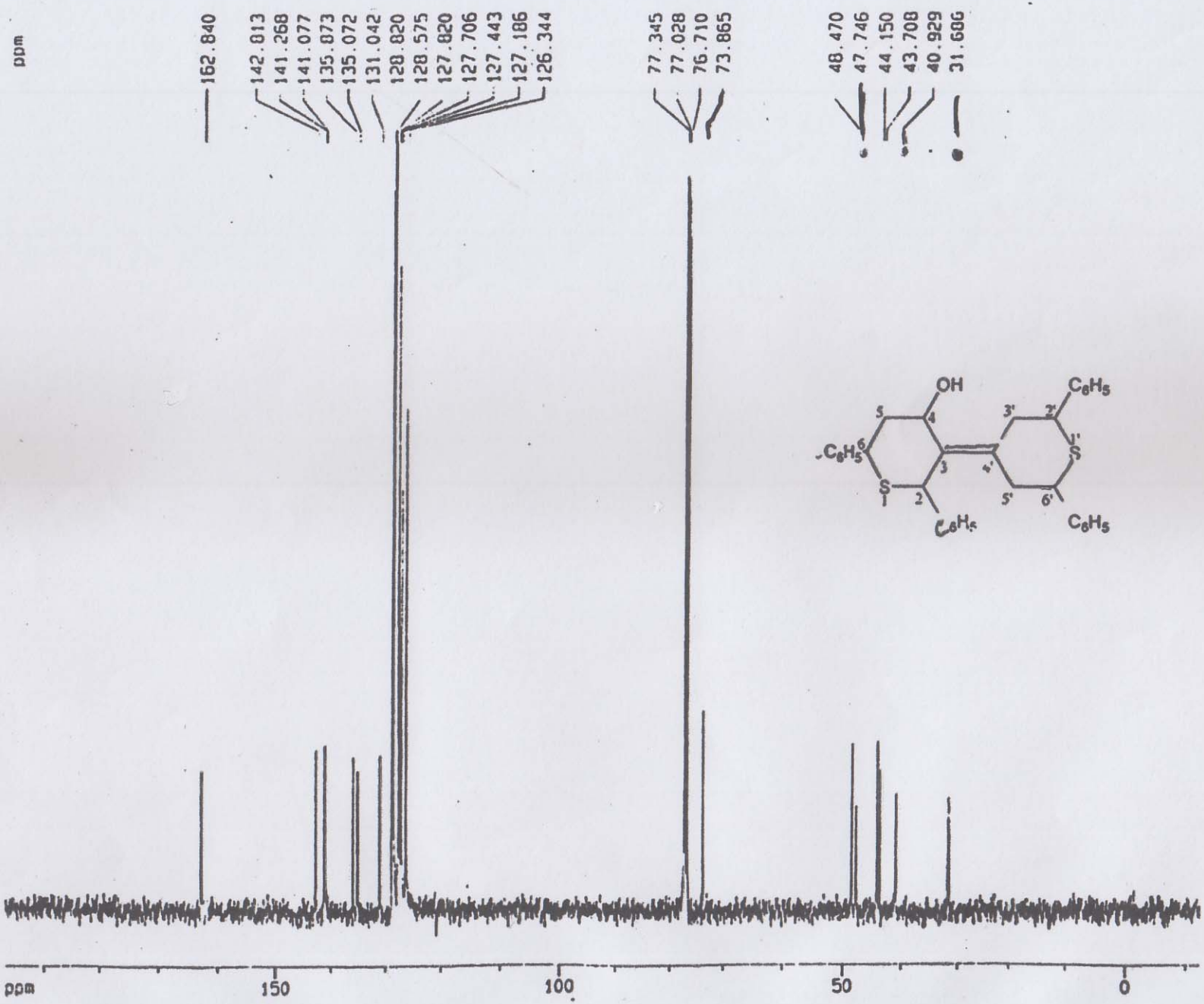

Fig 53: ^{13}C-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4'] bithiopyranyliden-4-ol **(18)**

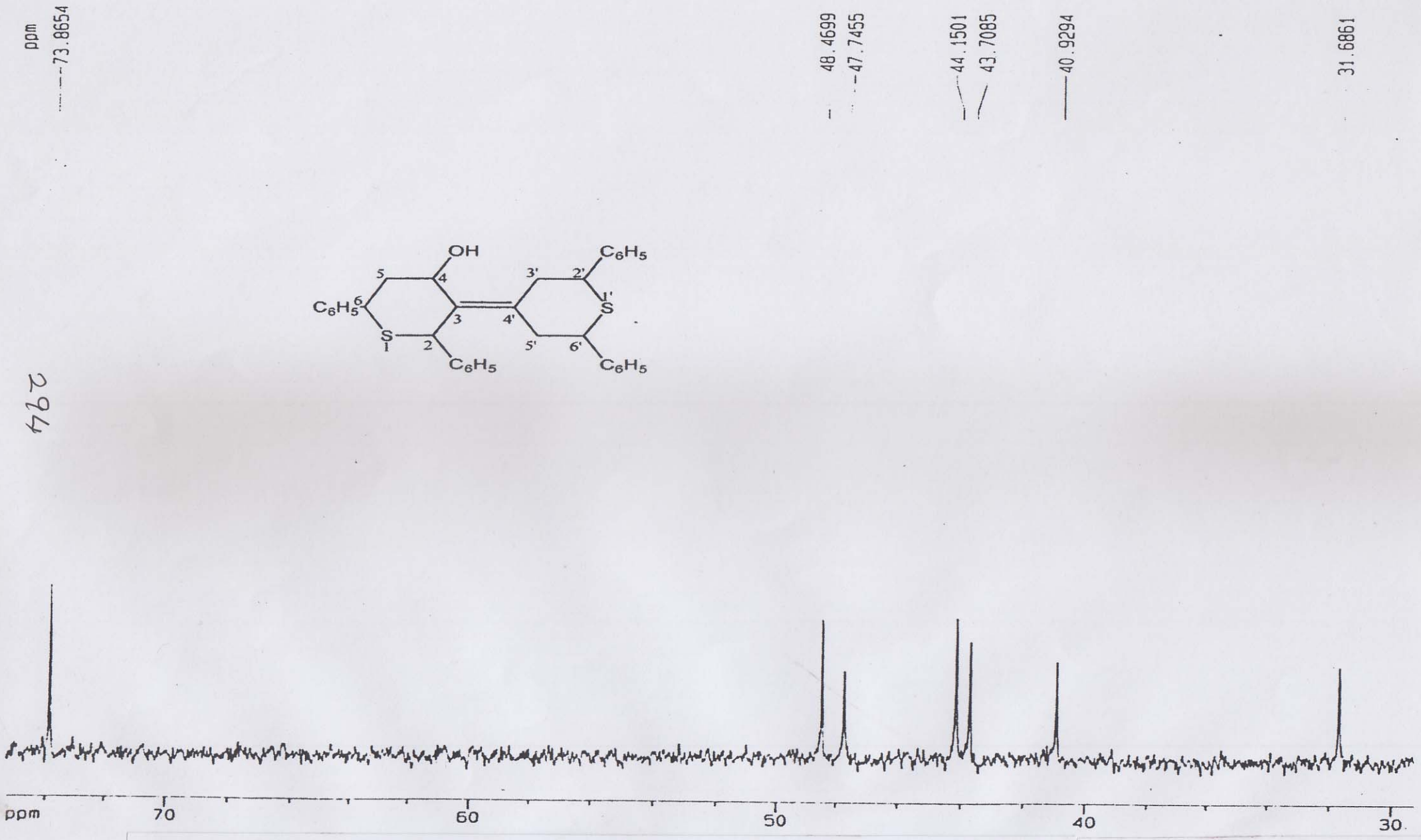

Fig 53a: ^{13}C-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4']-
- bithiopyranyliden-4-ol **(18)**[expanded part]

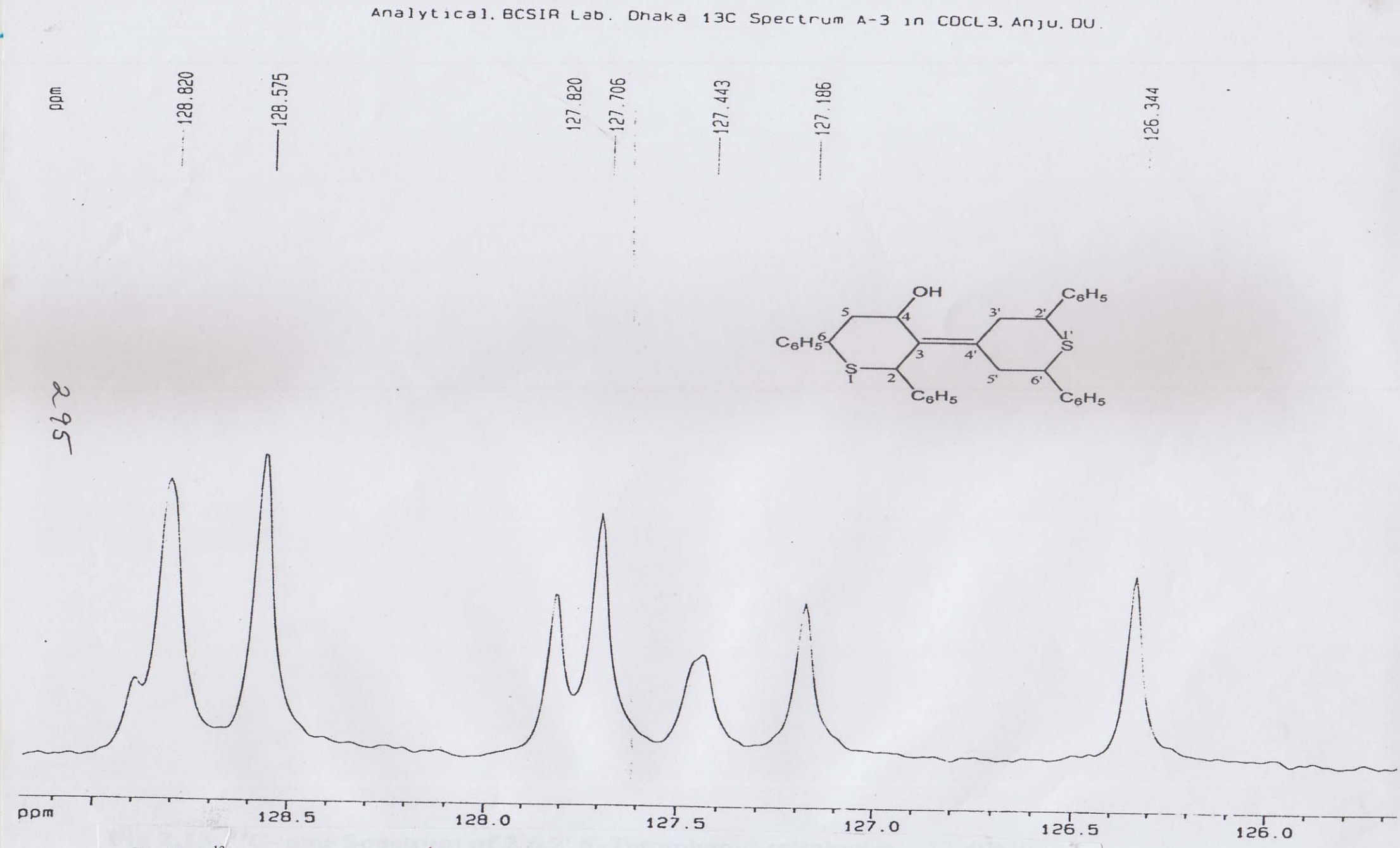

Fig 53b: ^{13}C-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4']-
- bithiopyranyliden-4-ol (**18**)[expanded part]

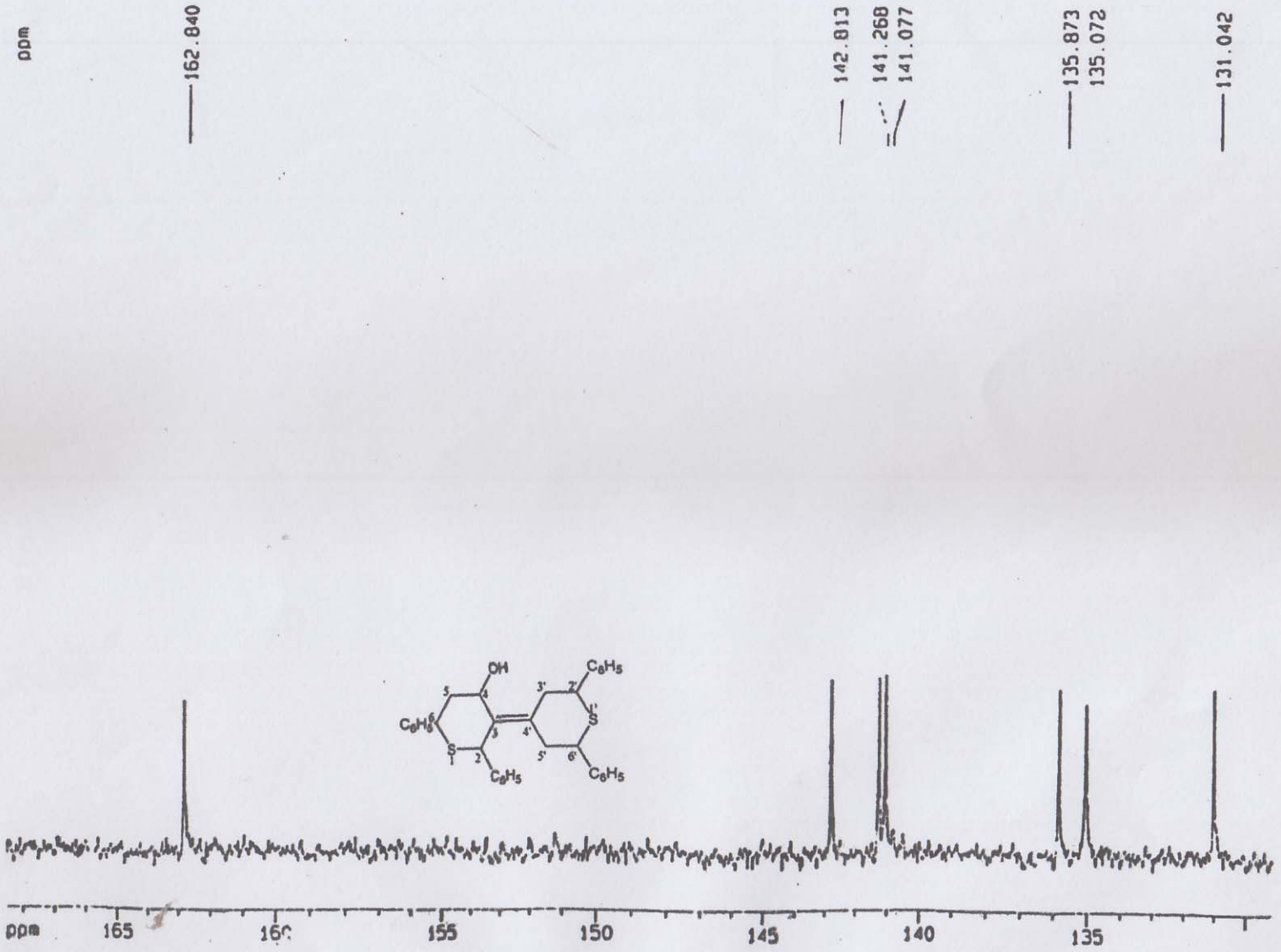

Fig 53c: ^{13}C-nmr spectrum of 2,6,2',6'-tetraphenyl-tetrahydro-[3,4']-
- bithiopyranyliden-4-ol **(18)**[expanded part]

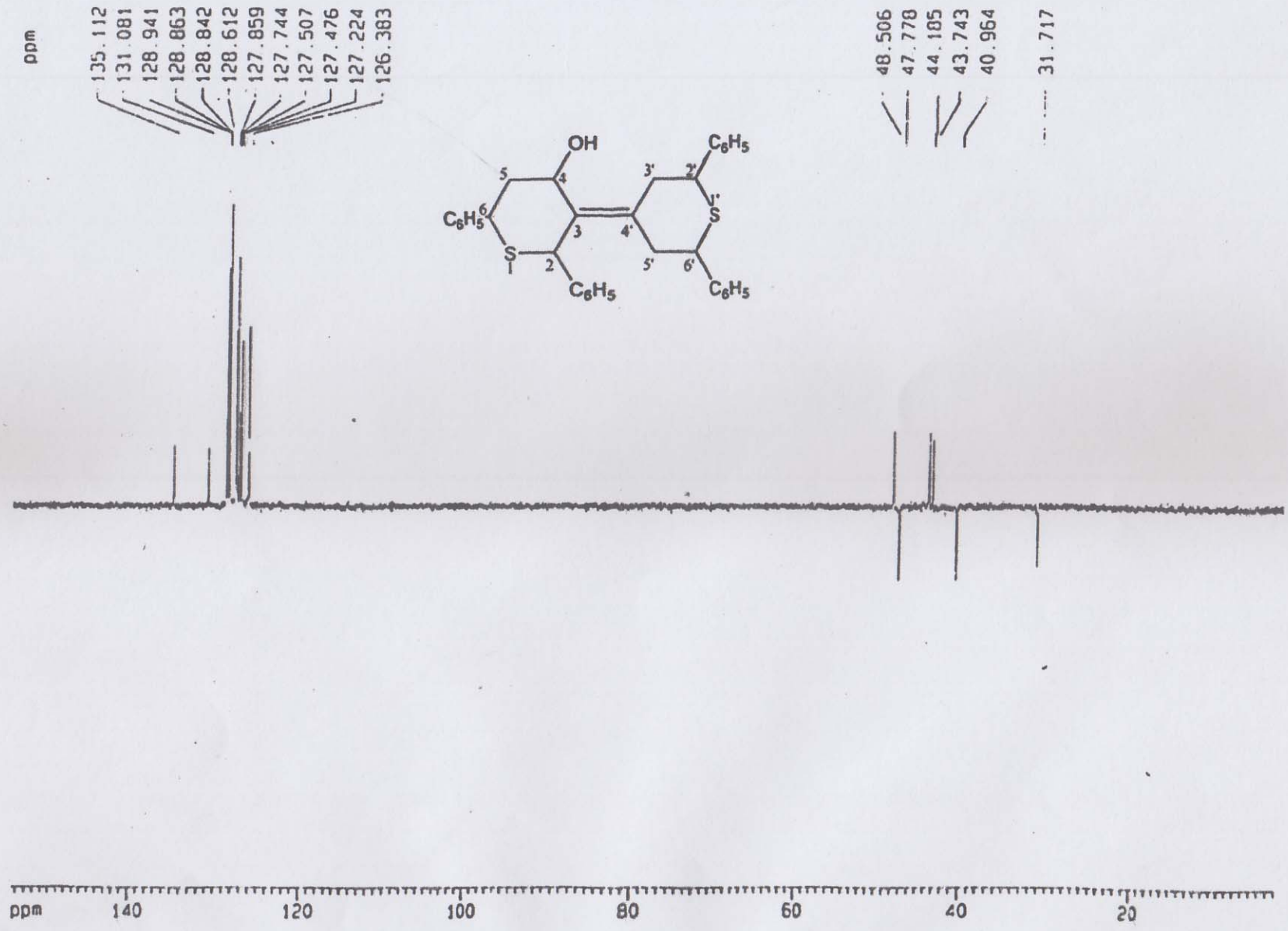

Fig 54: ^{13}C-nmr(DEPT-135) spectrum of 2,6,2',6'-tetraphenyl--tetrahydro-[3,4'] bithiopyranyliden-4-ol **(18)**

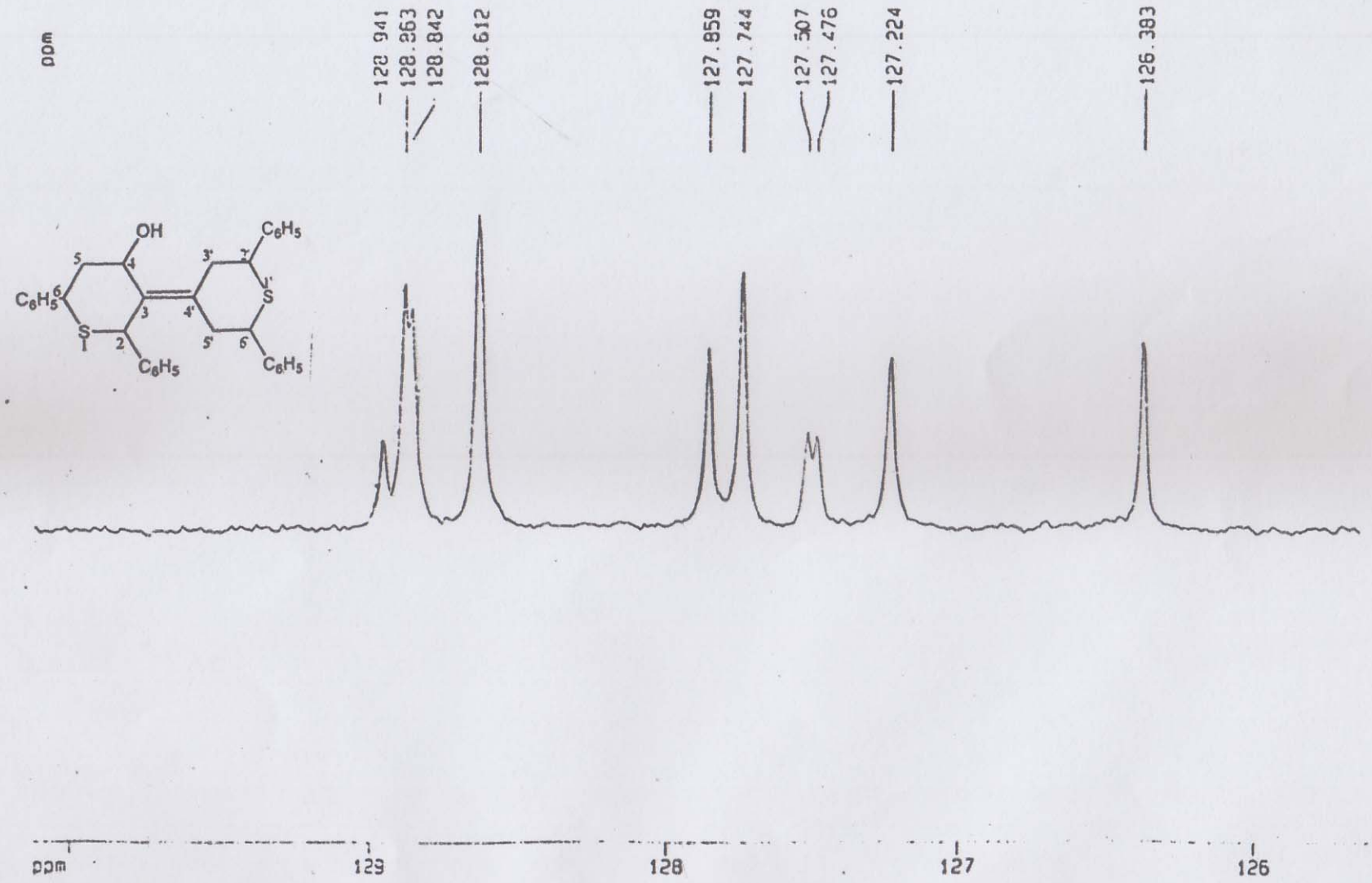

Fig 54a: ^{13}C-nmr(DEPT-135) spectrum of 2,6,2',6'-tetraphenyl--tetrahydro-[3,4'] bithiopyranyliden-4-ol **(18)** [expanded part]

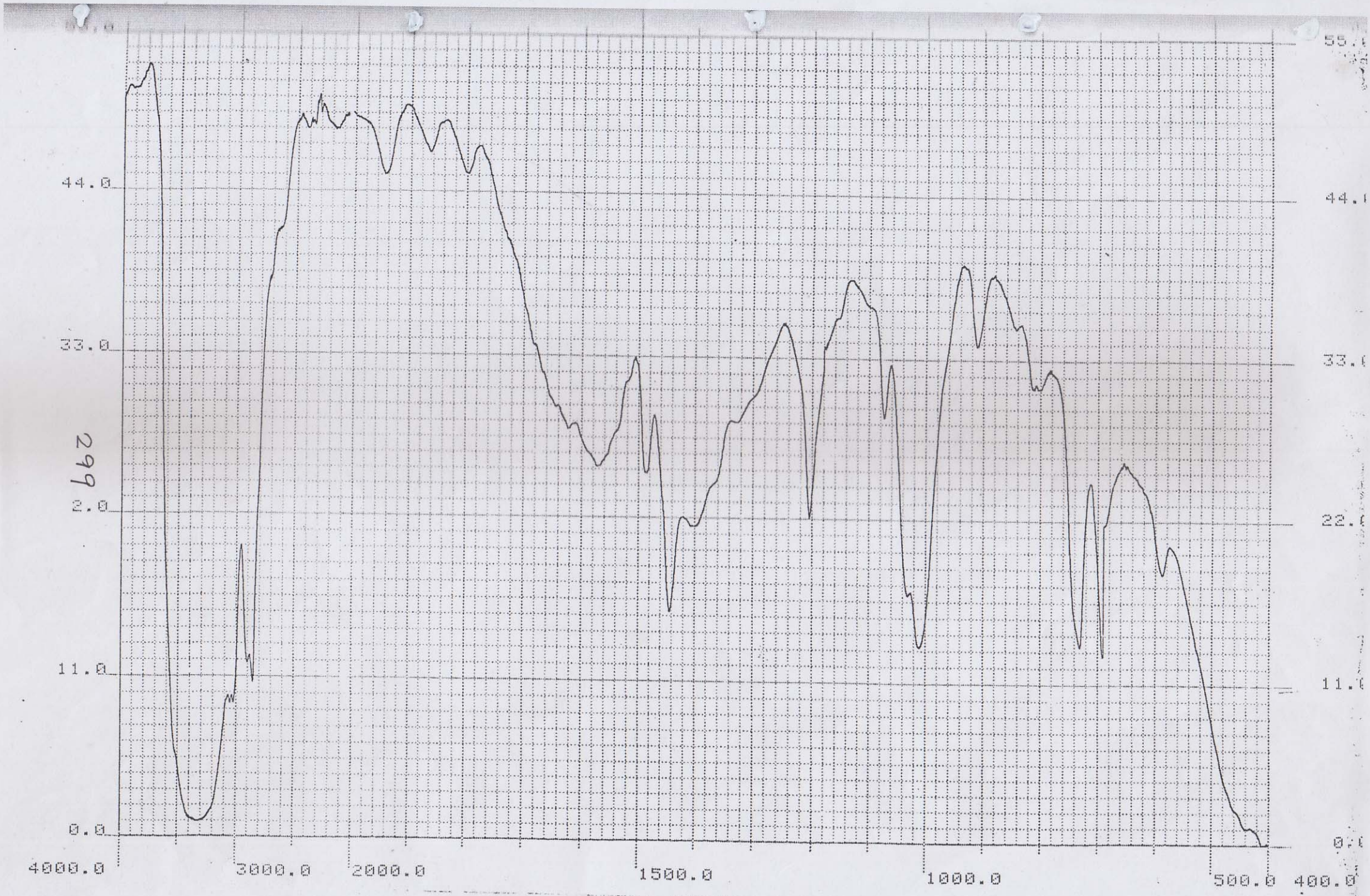

Fig 55: IR-spectrum of benzyl alcohol (12)

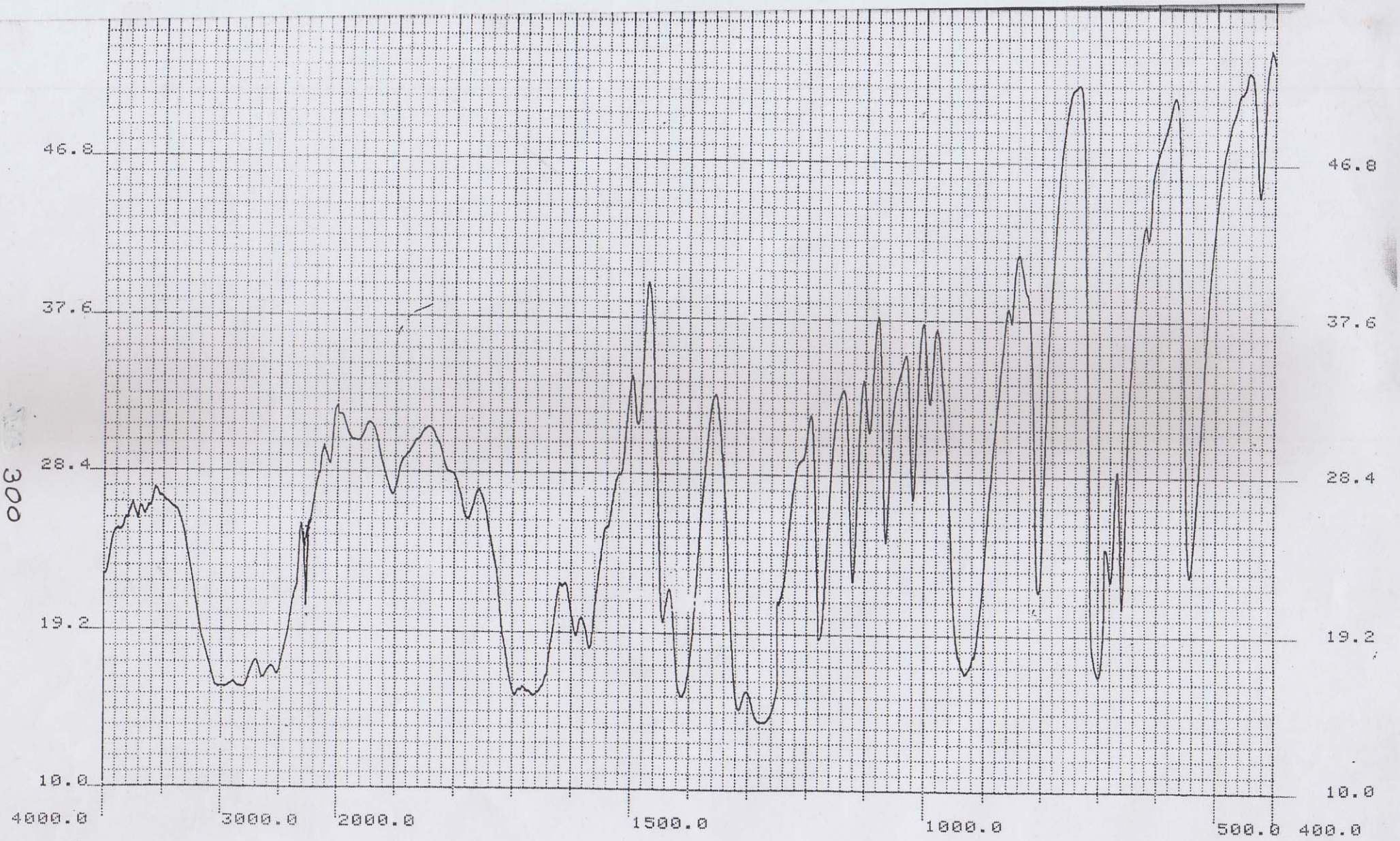

Fig 56: IR-spectrum of benzoic acid (12a)

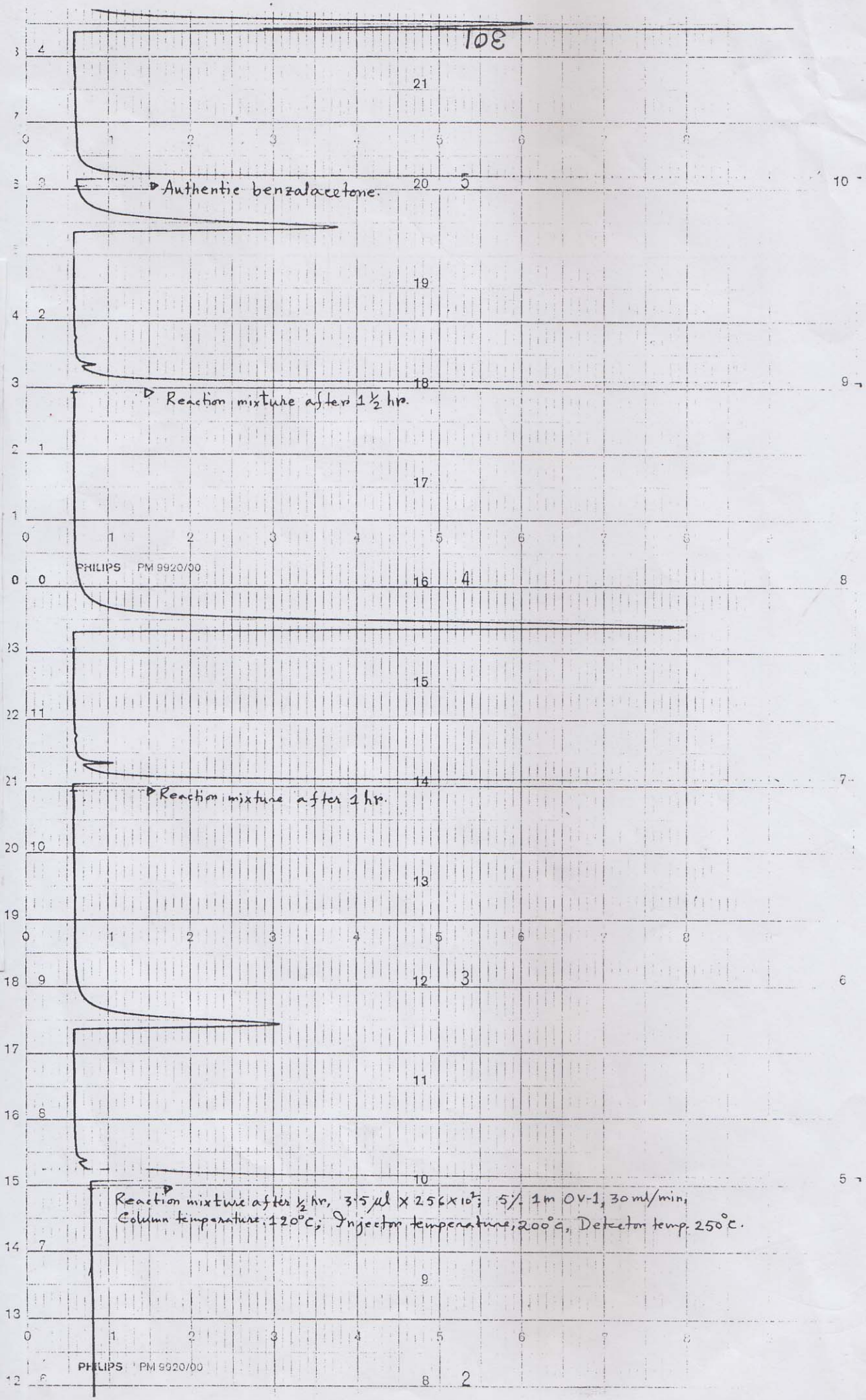

Fig 57 : Chromatogram (GLC)for the detection of benzylideneacetone