ISOLATION AND CHEMICAL CHARACTERIZATION OF FLAVONOIDS FROM TEPHROSIA INTERRUPTA AND TEPHROSIA LINEARIS

BY

OBUYA WERE

A thesis submitted in partial fulfilment for the Degree of Master of Science in the University of Nairobi.

JULY, 1988

This thesis is my original work and has not been presented for a degree in any other University.

Signature ----------

OBUYA WERE
UNIVERSITY OF NAIROBI

This thesis has been submitted for examination with our approval as University Supervisors;

Signature ----------

PROF. RAPHAEL M. MUNAVU
DEPARTMENT OF CHEMISTRY
UNIVERSITY OF NAIROBI

Signature ----------

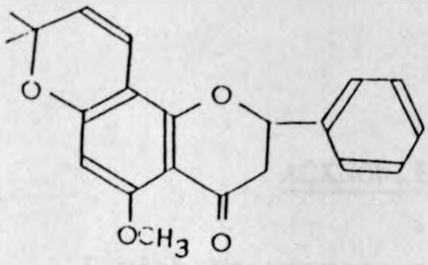
DR. W. LWANDE
CHEMISTRY AND BIOASSAY UNIT
ICIPE
P.O. Box 30772, NAIROBI

ABSTRACT

The thesis describes a chemical study of two tropical plant species, namely Tephrosia interrupta and Tephrosia linearis.

Chromatographic separation of the hexane, chloroform, ethyl acetate and methanol extracts of the leaves, roots, stem and pods of Tephrosia interrupta led to the isolation of the flavonoids 5-methoxyisolonchocarpin (26), Isopongaflavone (28), Pongachalcone (29), Rotenone (30a) and Deguelin (30b). The same technique was applied on Tephrosia linearis pods and roots to give Rotenone (30a), Deguelin (30b), Toxicarol (32), Tephrosin (33a) and 12a-hydroxy -rotenone (33b).

Structural elucidation of these compounds was performed on the basis of their spectroscopic data.



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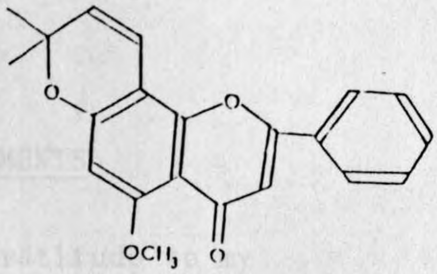

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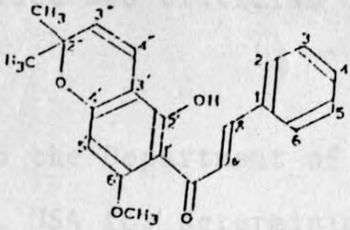

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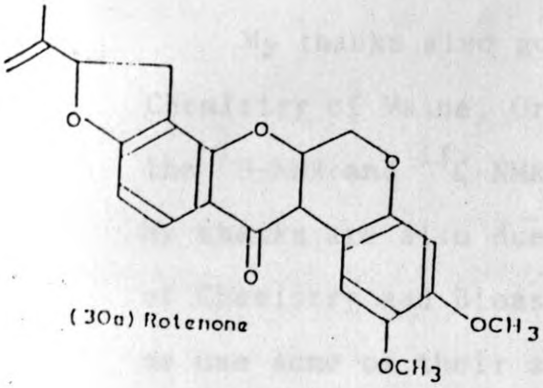
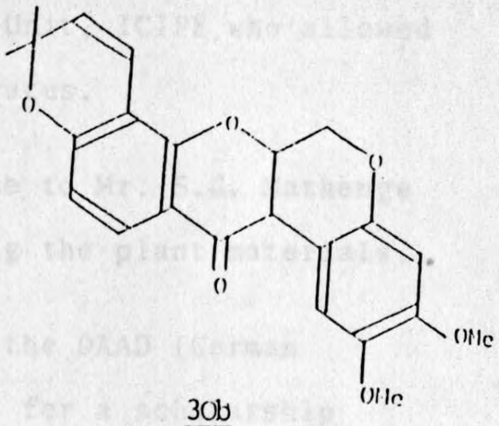
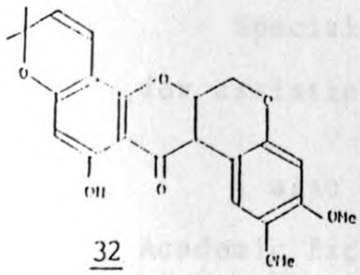


(30a) Rotenone



30b



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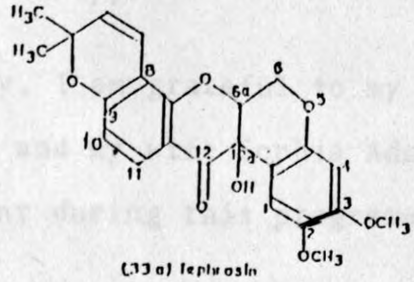
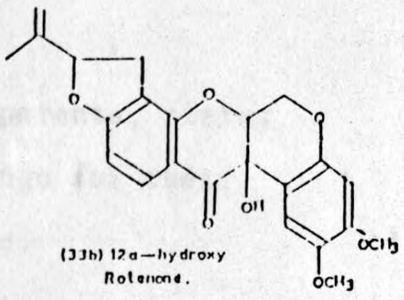


(33a) Tephrosin



(33b) 12a—hydroxy Rotenone.

ACKNOWLEDGEMENTS

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My thanks also go to the Department of Chemistry of Maine, Orono, USA for determining the ^{1}H-NMR and ^{13}C-NMR spectra for my samples. My thanks are also due to members of the Department of Chemistry and Bioassay Unit, ICIPE who allowed me use some of their apparatus.

Special thanks are due to Mr. S.G. Mathenge for assisting in collecting the plant materials.

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To my parents
and my children, Frank Bwire and Conceilia Nadidi

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CHAPTER 1

INTRODUCTION

1.1 General considerations

Throughout the world some 70 per cent of the people rely on traditional herbal remedies to cure a wide variety of ailments ranging from minor infections to asthma, dysentery and malaria[1].

In the Western world there is a growing demand for "alternative" herbal remedies and in the Third world it is now widely accepted that cheap, readily available herbal remedies should replace some of the expensive Western drugs in the market[1].

In Africa the use of traditional medicine is so well accepted that many countries support some kind of scientific research programme into traditional remedies. The Organization of African Unity's (OAU) scientific and Technical Committee and the World Health Organization (WHO) are two agencies spearheading a resurgence of interest in the Third World's traditional and medicinal heritage[1].

The idea that pharmaceutical preparations have adequately served the human race is questionable[1]. Among their drawbacks were the residues in the system that become a burden to the sick body. No chemical drugs are ever free from side effects[1].

Modern medicines have also concentrated on healing symptoms rather than the living system. The modern drugs have been unecessarily expensive for Third world countries whose resources are meagre. Herbs have nutritive complementality in the form of proteins, vitamins, minerals and hormones which the plant in question may contain "thus simultenously nourishing as well as healing the living body[1]".

Some of the chemicals used in conventional medicine such as quinine and penicillin have been isolated from plants but wholesome original herbs containing all the known nutritive and curative principles are more effective than isolated chemicals[1].

Scientific analysis of medicinal plants has led to the discovery of important modern drugs and some experts believe that plants may

well hold the secrets to combat Diabetes, Cancer and AIDS[1].

In Kenya, the University of Nairobi "Miti-Shamba" Drug Research Centre has for many years been carrying out some clinical tests on the efficacy of some preparations administered by these herbalists. The results have indicated that herbal medicine is a generally powerful source of biological activity[1].

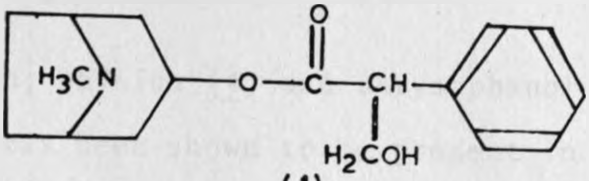
In Ghana, the centre for scientific research into plant medicine is clinically assessing Desmodium adscendues for the treatment of asthma[1].

It is estimated that only about 10 per cent of the earth's flowering plant species have been used in traditional medicine and only about one per cent of those have been acknowledged by scientists to have real therapeutic value. This draws attention to the urgent need of conservation of natural habitats containing untapped resources of potentially useful plant life[1].

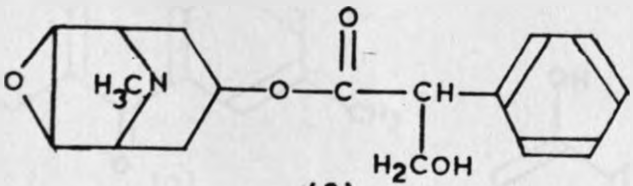
1.2 Types of active chemical components from plants

The chemical plant constituents that are generally responsible for the curative powers of various plants are:- alkaloids, anthraquinones, terpenoids and flavonoids.

A number of Datura spp. contain the alkaloids hyoscyamine (1) and hyposcine (scopolamine) (2).



(1)



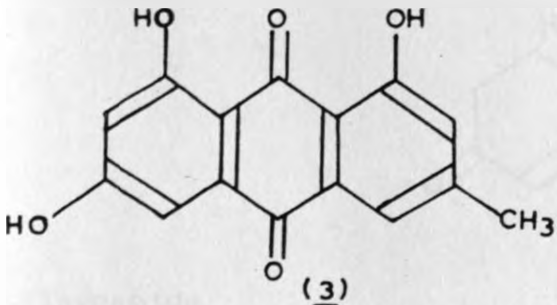
(2)

When isolated from the plant, the natural isomer (-) hyoscyamine racemises to form atropine which is a mixture of (-) hyoscyamine and (+) hyoscyamine.

Atropine is used in eye surgery as it dilates the pupil of the eye. It is also used in minute amounts in preparations to combat diarrhoea as it is antispasmodic and calms the muscles of the intestines[2].

A number of Cassia spp. contain compounds which have laxative properties. The Anthraquinone emodin (3) is obtained from Indian senna (Cassia angustifolia) which is grown in Somalia. Emodin occurs as a glycoside in the plant.

Emodin, physion (4) and chrysophanol (5) have recently been shown to be present in Kenyan Rumex spp[3,4]. These plants are used extensively in



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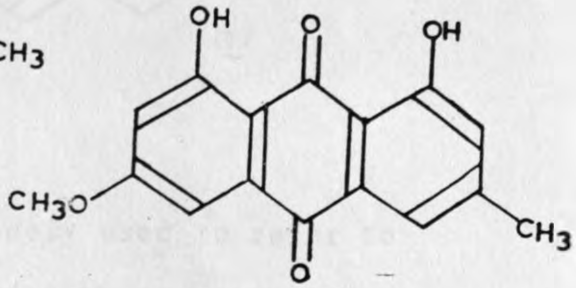

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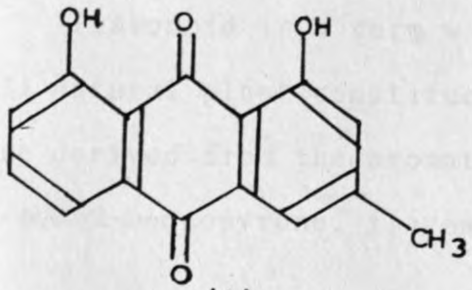
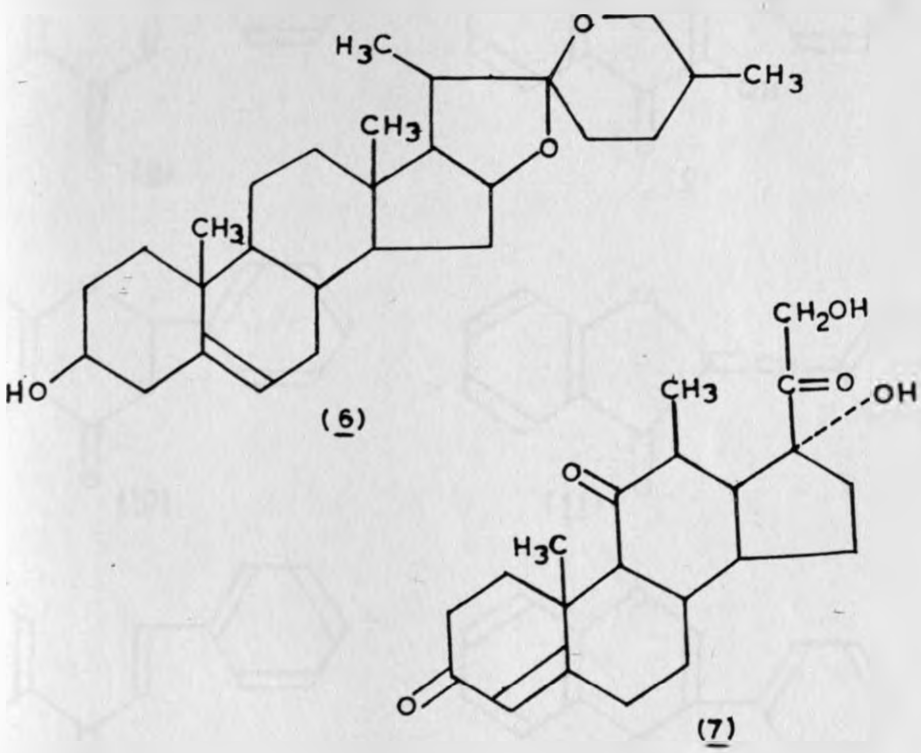


(5)

this country as enthno-pharmocological anthelmintic agents.

The triterpenoid diosgenin (6) is extracted from yams (Dioscorea spp). It is important as a starting material for the commercial synthesis of cortisone (7) and its derivatives, and also for the synthesis of the hormones used in oral contraceptives[2].



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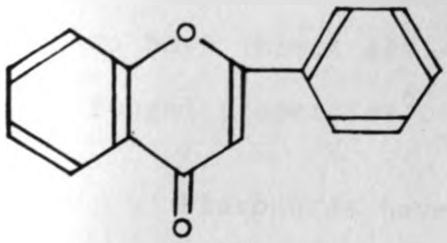
Flavonoids:

Flavonoid is a term widely used to refer to all natural plant constituents whose structures are derived from the aromatic heterocycle, 3-phenyl-benzopyrone, flavone (8). They are

usually divided into classes depending on the oxidation level of the central pyran ring. The most familiar are:- flavone (8), flavonol (9), flavanones (10), aurones (11), chalcones (12), isoflavones (13) and anthocyanidins (14).



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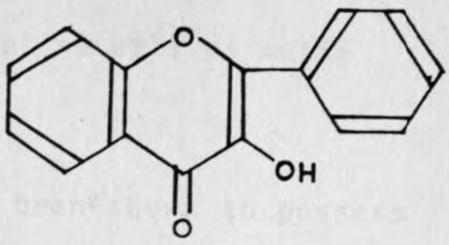

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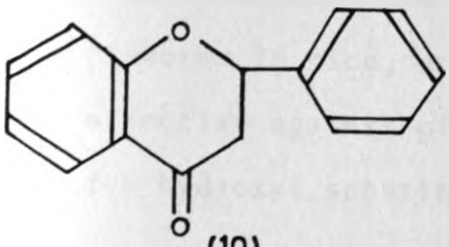

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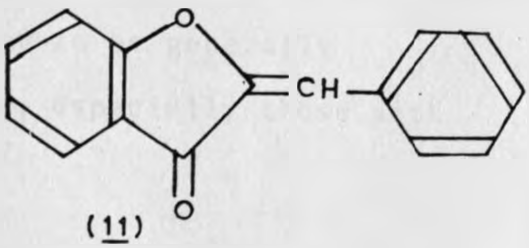

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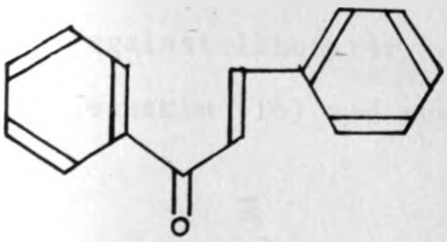

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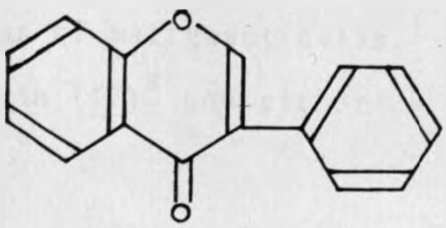

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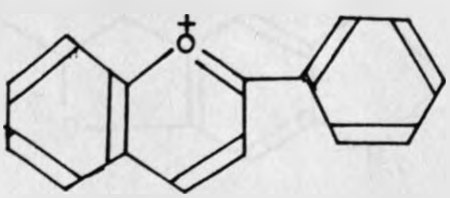

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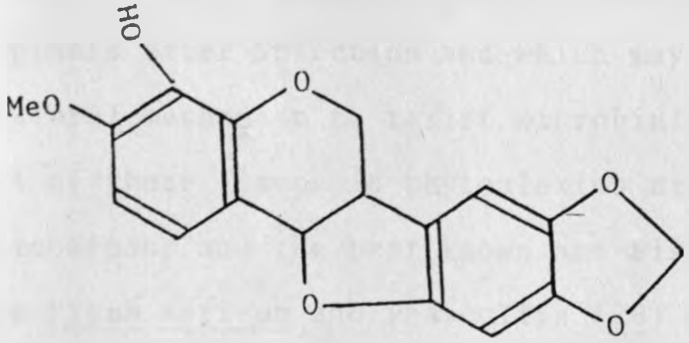
Flavonoids have many uses. In considering attractants and repellents in higher animals, it was reported that taste preference seems to be quite similar in man and in a number of other animals[5]. The flavonoid hildecarpin (15) from the roots of Tephrosia hildebrandtii Vatke has been found to have insect anti-feedant as well as anti-fungal properties[6].

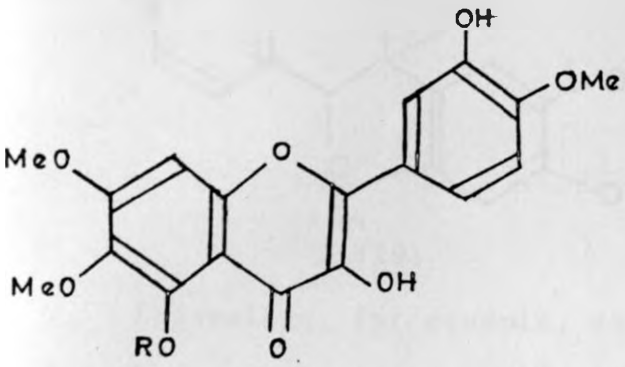
Flavonoids have also been shown to possess anthelminthic activities. One hundred and sixteen (116) chalcones and analogues were evaluated on pinworms in mice, and found to be generally effective against pinworms, especially those with few hydroxyl substituents[7].

Several flavonoids are moderately effective against laboratory cultures of malignant cells. eupatin (16) and eupatoretin (17)[8] and either

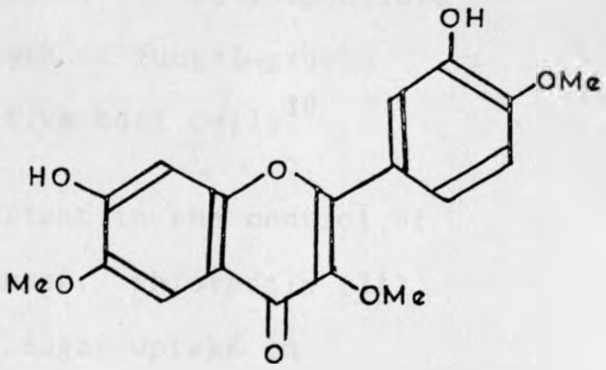


(15)

centaureidin (18) or 6-demethoxy-centaureidin are all moderately effective against a carcinoma from the nasopharynx[9].

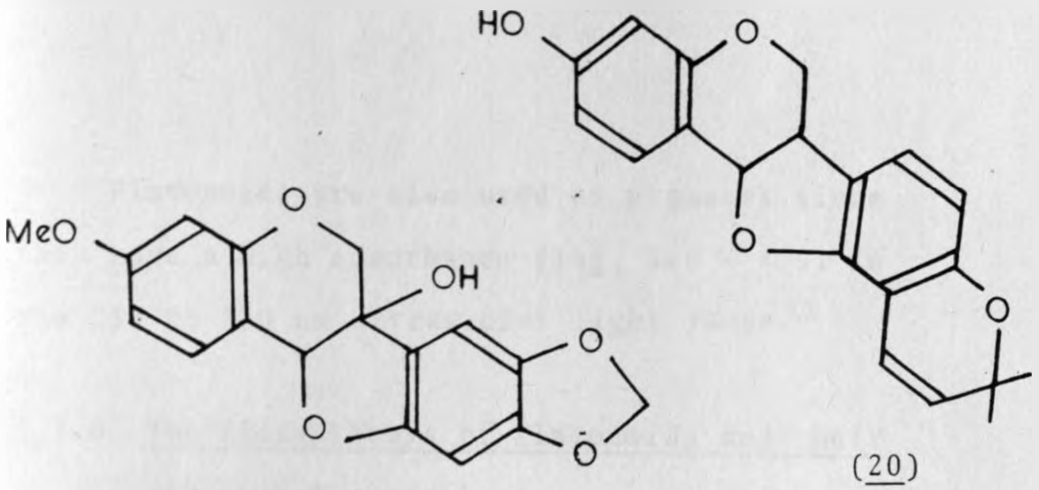


(16), Eupatin R = H ;
(17) Eupatoretin R = Me



(18) Centaureidin

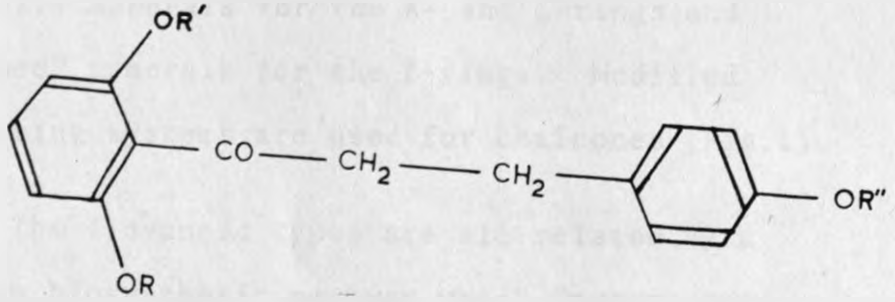
To date, over twenty flavonoid phytoalexins have been chemically characterized. These are a group of toxic compounds which accumulate in plants after infection and which may represent a natural mechanism to resist microbial attack. Most of these flavonoid phytoalexins are pterocarpans and the best known are Pisatin (19) from Pisum sativum and Phaseollin (20) from Phaseolus vulgaris.



(19) (20)

Phaseollin, for example, may be responsible for the cessation of growth of fungal-growtn germ tubes in hypersensitive host cells[10].

Flavonoids are important in the control of plant growth and development. Phloredzin (21) is a potent inhibitor of sugar uptake in animals and serves as cofactor for Indole Acetic Acid (IAA) oxidase in plants[11].



R = R' = R'' = H)

(21)

Flavonoids are also used as pigments since they have a high absorbance ($\log_{\varepsilon}$ 4.0 - 4.5) in the 250 to 270 nm Ultraviolet light range.[12]

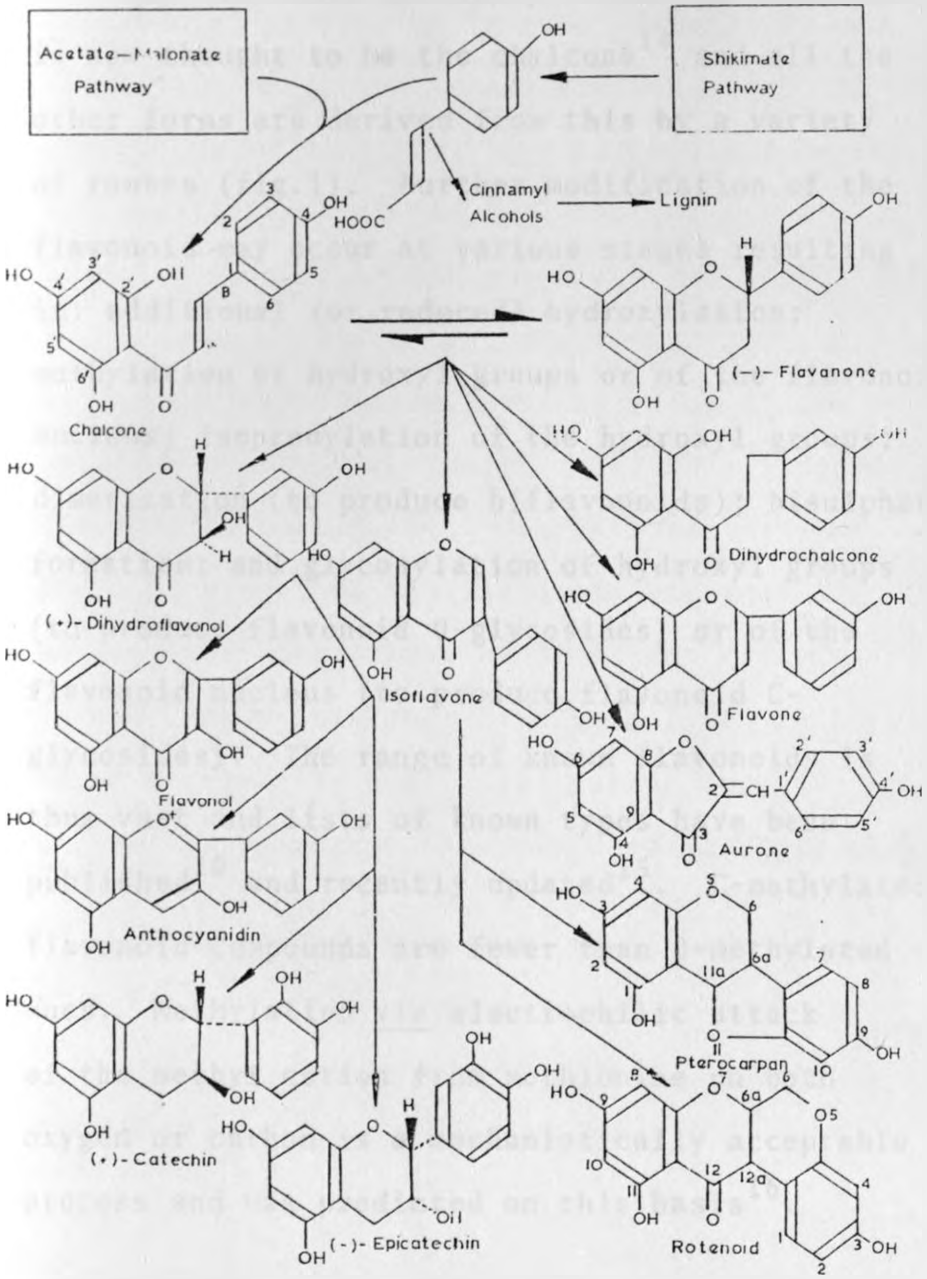
1.3.0 The Biosynthesis of flavonoids and their structural variations

In plants, flavonoids without attached sugars (aglycones) occur in a variety of structural forms.

All contain fifteen carbon atoms in their basic nucleus and these are arranged in a C_6-C_3-C_6 sequence; i.e. two aromatic rings linked by a three carbon unit which may or may not form a third ring. The rings are labelled A,B and C and the individual carbon atoms are referred to by a numbering system which utilizes ordinary numerals for the A- and C-rings and "primed" numerals for the B-rings. Modified numbering systems are used for chalcones (Fig.1).

The flavonoid types are all related by a common biosynthetic pathway which incorporates precursors from both the "Shikimate" and "Acetate Malonate"[12,13]. The first flavonoid is produced following confluence of the two pathways (Fig. 1).

Figure 1. Currently proposed interrelationships between flavonoid monomer types (supported by varying levels of experimental evidence[13]



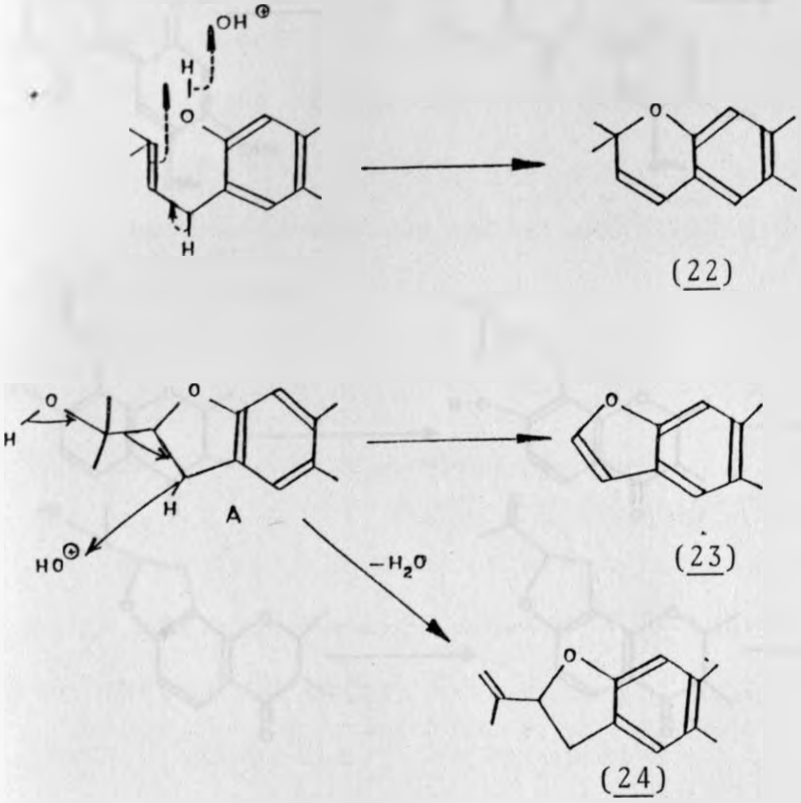
The flavonoid initially formed in biosynthesis is now thought to be the chalcone[14] and all the other forms are derived from this by a variety of routes (fig.1). Further modification of the flavonoid may occur at various stages resulting in: additional (or reduced) hydroxylation; methylation of hydroxyl groups or of the flavonoid nucleus; isoprenylation of the hydroxyl groups; dimerization (to produce biflavonoids); bisulphate formation; and glycosylation of hydroxyl groups (to produce flavonoid O-glycosides) or of the flavonoid nucleus (to produce flavonoid C-glycosides). The range of known flavonoids is thus vast and lists of known types have been published[10] and recently updated[15]. C-methylated flavonoid compounds are fewer than O-methylated ones. Methylation *via* electrophilic attack of the methyl cation from methionine on both oxygen or carbon is a mechanistically acceptable process and was predicted on this basis[16].

The introduction of isoprenoid units by alkylation on carbon, on the other hand, is much more common compared with O-alkylation.

Birch[16] has suggested that since the biochemically fundamental process of terpene elaboration requires alkylation on carbon, other processes such as alkylation of aromatic rings may reflect deviation via modification of the C-alkylating enzymes of normal terpenoid biosynthesis. Both C-methylation and C-isoprenylation are more commonly found in ring A of flavonoids presumably owing to the greater nucleophilic character of this ring in the species undergoing C-alkylation. At what stage or stages of the flavonoid biosynthetic pathway the alkylations occur is not clear. Notably, many chalcones have been found in nature to be highly alkylated[17] indicating that C-isoprenylation can take place at an early stage.

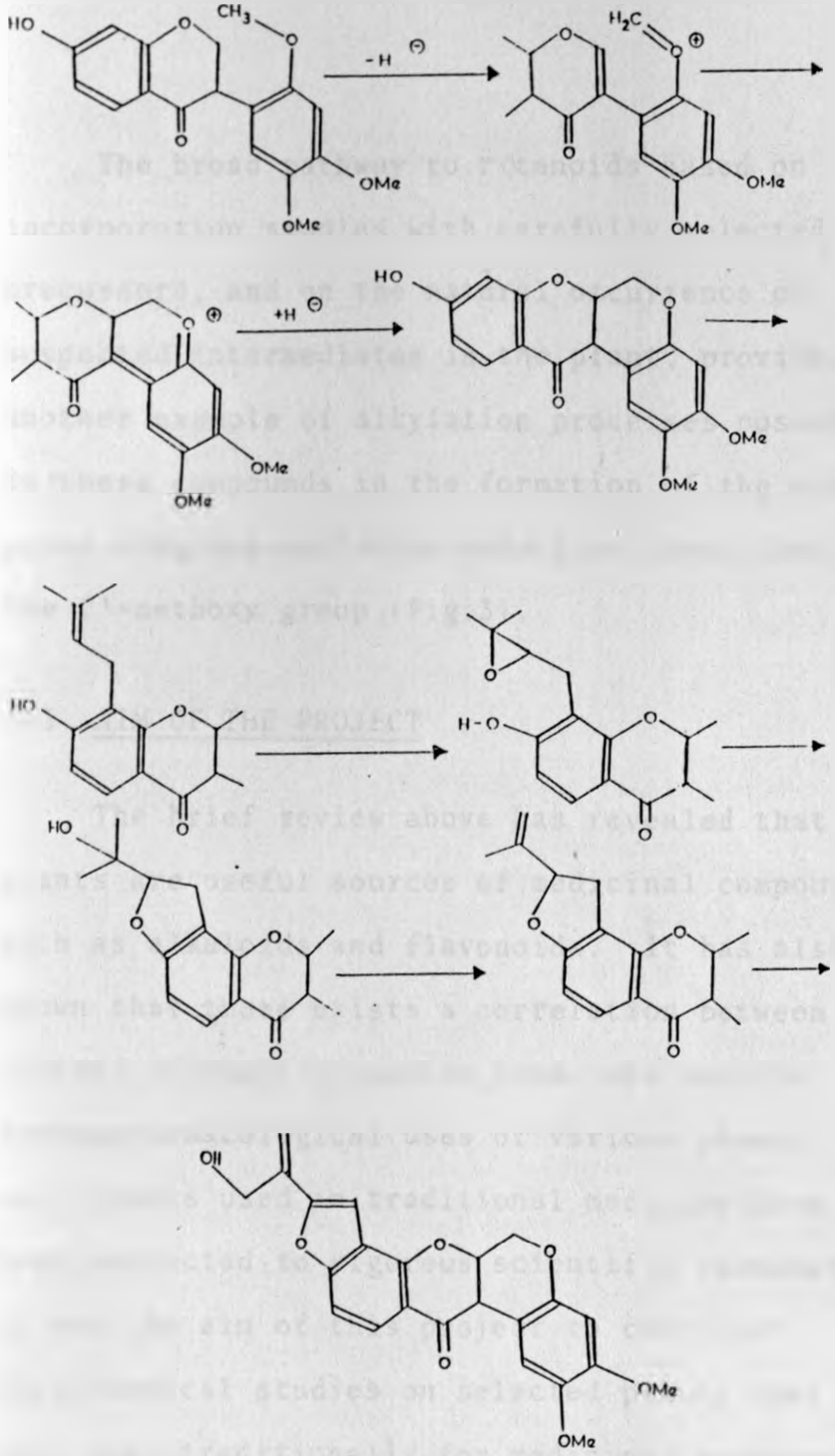
Isoprenoid substituents in flavonoids are very frequently manifested as the 2,2-dimethyl-chromene (22) or the 2-isopropenyl-coumaran (23) and benzofuran (24) ring systems.

Figure 2. Possible mechanism for the derivation of some common isoprenoid substituents



The formation of (22) and (23) via oxidative mechanisms can be variously formulated, as for example in Fig.2. Genesis of the 2-hydroxyisopropyl coumaran ring system is illustrated in the proposed scheme for the late steps of the pathway to the rotenoid amorphigenin (Fig. 3) (25).

Fig. 3. Proposed Scheme for the biosynthesis of rotenoid amorphigenin for a 2'-methoxysoflavone[18]



(25) Amorphigenin

The broad pathway to rotenoids based on incorporation studies with carefully selected precursors, and on the natural occurrence of suspected intermediates in the plant, provides another example of alkylation processes possible in these compounds in the formation of the extra pyran ring via oxidation-reduction steps involving the 2'-methoxy group (Fig.3).

1.3 AIM OF THE PROJECT

The brief review above has revealed that many plants are useful sources of medicinal compounds such as alkaloids and flavonoids. It has also shown that there exists a correlation between the content of these bioactive chemicals and the enthnopharmacological uses of various plants. Since many plants used in traditional medicine have not been subjected to vigorous scientific examination, it was the aim of this project to carry out phytochemical studies on selected plants that have been used traditionally for medicinal purposes in Kenya. The study was carried out by isolating, purifying and characterizing the flavonoids using physico-chemical methods. The two plants used in this study were Tephrosia interrupta and T. linearis.

1.4 The Genus Tephrosia Pers

Bentham[19] recognised three subfamilies of the family leguminosae namely Minosoideae, Caesalphnioideae and Lotoideae (Papilionoideae). The Lotoideae is much larger than either of the other two sub-families, having about 500 genera and 12,000 species. The Genera of the Lotoideae arranged according to Hutchinson[20] are 50. A few of these and their examples are listed in table 1.

In recent years taxonomic opinion[20] has favoured a circumscription of Tephrosieae to include, among other genera, Derris, Lonchocarpus, Milletia, Mundulea, Piscidia, Pongamia, Tephrosia and Wisteria. This led Ceres[21] to recommend that the traditional division given by Bentham[22] is unsatisfactory. In connection with the International Conference on Leguminosae held in London in 1978, a systematic investigation of flavonoids and rotenoids in the Leguminosae with a special emphasis on Lonchocarpus and Derris has revealed the two to be closely related genera[23]. Work is already in progress to try and use a chemotaxonomic approach to clarify the taxonomic situation of the tribe Tephrosieae[24]. Thus, the taxonomic status of the genera Tephrosieae is not yet clear.

Table 1. The Genera of the Lotoideae arranged according to Hutchinson[20].

Genera	Examples
Sophoreae spreng	Afromosia Harms, sophora L.
Genisteae	Genista L; Petleria C. Presl.
Crotalarieae Hutch	Crotalaria L.
Robinieae Hutch	Robina L.
Millettieae Miq.	Millettia wight. Arn. Wisteria Nutt.
Lonchocarpeae Hutch	Lonchocarpus H. Bak Pongamia vent. Derris Lour Piscidia L.
Pterocarpeae Hutch	Pterocarpus L., Tipuana Benth.
Tephrosieae Hutch	Tephrosia Pers. Paratephrosia Domin.

However, the genus Tephrosia pers is a large genus of perennial and woody herbs that are distributed in the tropical and subtropical regions of the world[25,26]. Between 300-400 species of Tephrosia are known[27]. A number of Tephrosia species have been used traditionally in various parts of the world for medicinal purposes. Table 2 lists the uses of some Tephrosia species.

A number of Tephrosia species occurring in Kenya have not been examined. Tephrosia linearis and Tephrosia interrupta were examined for the presence of flavonoids.

Tephrosia linearis is a plant common in grassland and rocky, bush slopes especially in higher rainfall areas[31]. The juice of boiled leaves is used as medicine for babies, but no particular disease was mentioned[32]..

Tephrosia interrupta roots are roasted and ground, mixed with a little salt and used as a cough cure. Roots may also be pounded, mixed with porridge and eaten by women after childbirth to give them strength[32].

Table 2. Uses of some Tephrosia species

Plant Species	Uses	Ref
T. aequilata Bak	Relief of abdominal pains	28
T. atroviolacea E.G. Bak	Administered to a woman after birth	28
T. candida DC	Fish poison	28
T. capensis Pers.	Emetic for biliousness palpitation and arrow poison	28
T. dasyphylla welw. Ex. Bak	Fish poison	28
T. densiflora Hook. f.	Arrow poison, piscicidal effect	28
T. diffusa Harv.	Parasiticide high insecti-dal value	28
T. elegans scham & Thonn	Arrow poison	28
T. grandiflora Pers.	Parasiticide, fish poison, High insecticidal value	28
T. knaussiana meisn	Cure cough	28
T. lucida sond	As an emetic	28
T. lupinifolia DC	For procuring abortion, For committing suicide	28

Table 2 (Contd..)

Plant Species	Uses	Ref
T. macropoda Harv.	For stupefying fish, as a vermin killer, as an anthelmintic in cattle	28
T. purpurea Pers.	As fish poison, as an anthelmintic, as a purgative, as deobstruent, as diuretic, useful in bilious fevers, as a flavouring	28,29
T. semiglabra sond.	For chest colds	28
T. toxicaria Pers.	As an insecticide, as a fish poison, as an arrow poison	28
T. vogelii Hook f.	Fish poison. Parasticide against the flea, the louse and tick	28
T. linearis	As a fodder for horses, sheep, cattle and goats	30

1.5 Tephrosia Flavonoids

Phytochemical screening of a number of species of Tephrosia have revealed the presence of rotenoids, isoflavones, flavanones, chalcones, flavonols and flavones[33]. No species have yet been reported as flavonoid-free. A wide range of flavonoids and rotenoids have previously been reported in the genus Tephrosia. A few of the species and the flavonoids and rotenoids that have been isolated from them are summarized in Table 3. Tephrosia flavonoids are prenylated resulting in various types of prenyl derived substituents as shown in Figure 4. Several species of Tephrosia contain rotenone and deguelin. None of the Tephrosia species that have been examined, contain as much rotenone, deguelin and other insecticidal constituents as are found in Derris or Cube. For example, the maximum rotenone and deguelin content of Tephrosia virginiana root is about 4 per cent, whereas samples of Derris and Cube often contain 10 per cent or more. So far as is known, the insecticidal constituents of Tephrosia are identical with those of Derris and Lonchocarpus[28].

Table 3. The distribution of flavonoids and rotenoids in Tephrosia pers.

Tephrosia species	Flavonoid + Rotenoid	Ref
T. candida (Roxb) DC	3,7-dihamnoside of 6-hydroxy-kaempferol-4'-methylether	34
T. elongata E. mey	elongetin	35
T. falciformis Ramasw	elliptone, deguelin, rotenone, tephrosin	36
	Falciformin, 7-hydroxy-8' (γ,γ-dimethyl-allyl flavanone	37
T. lupinifolia DC	Lupinifolin, lupinifolinol	38, 39
T. macropoda (E. mey.) Harv.	Rotenone	40
T. maxima (L.) Pers.	Maxima isoflavone A,B and C	41, 42
T. multijuga Young	multijugin, multijuginol	43
T. obovata merril	rotenone, tephrosin, α-toxicarol	44
T. praecana Brummitt	Praecansone A and B; 5-methoxyisolonchocarpin, 5-methoxy-6"; 6"-dimethyl-chromene (7,6:2",3" flavone)	45
T. purpurea (L.) Pers. (T. piscatoria Pers.), T. lanceolata Gra ex Wall.)	rutin;	46
	(-)-isolonchocarpin, pongamol, lanceolatin A and B; rotenone, dehydroisoderricin, deguelin	47,36 48-51

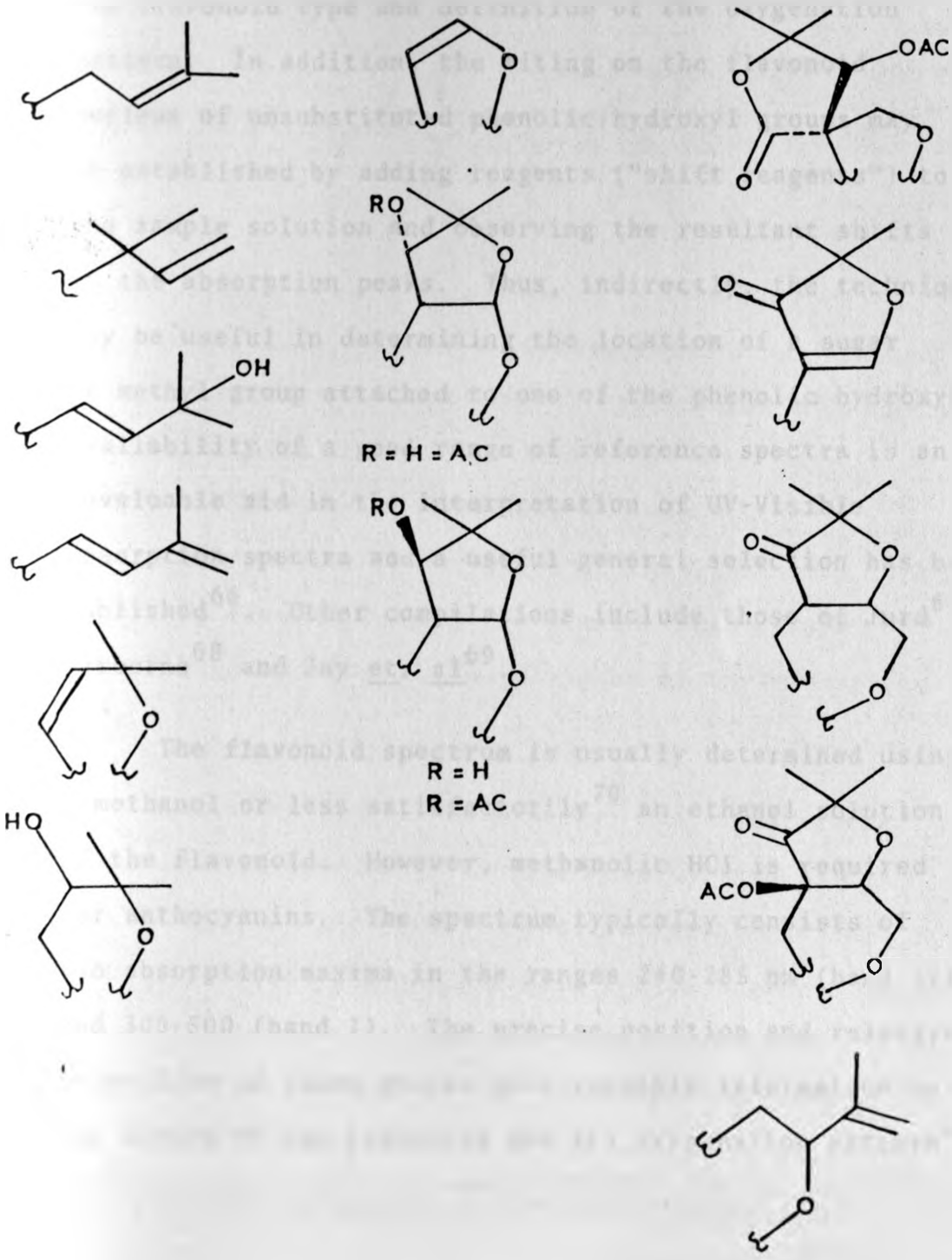
Table 3. (Contd..)

Tephrosia species	Flavonoid + Rotenoid	Ref.
T. rhodesica Bak. f. (T. polystachyoides Bak. f.)	tachrosin, stachyoidin tephrodin	52, 53
T. semiglabra sond	glabratephrin, semiglabrinol	54, 55
T. sinapou (Buc'hoz) A. chev. (T. toxicaria (SW.) Pers.)	rotenone, deguelin, α-toxicarol, sumatrol	56, 57
T. sp.(from Guatemala)	deguelin, tephrosin	58
T. villosa (L.) Pers.	Villosin, Villosone, Villol, Villinol, 6a, 12a-dehydro-Sumatrol, 12a-hydroxy-Sumatrol.	59 60
	rotenone, 12a-hydroxy-rotenone, deguelin, tephrosin, 5-hydroxy-isoderricin	50
T. virginiana (L.) Pers. (Cracca virginiana)	rotenone, deguelin, 6a, 12a-dehydro -rotenone α-toxicarol	61, 62
T. vogelii Hook f.	deguelin, 6a, 12a-dehydrodeguelin, tephrosin, vogeletin, vogeletin-3-arabino-sylrhamnoside, deguelin, tephrosin	47, 63, 57
	deguelin, tephrosin, 5-methoxyisolonchocarpin	50

Table 3 (Contd..)

Tephrosia species	Flavonoid + Rotenoid	Ref
T. hildebrandtii Vatke	6a-hydroxypterocarpin,	64,6
	8-C-Prenylated flavones	65
	4-B-substituted flavans	110,111
T. elata Deflers	Isopongaflavone, Tephrosin, obovatin methyl ether, Warangalone (scandenone), (+)-pisatin, (-)-maackiain, 8-(3,3-dimethylally 5,7-dimethoxyflavonone.	112, 101

Figure 4: Prenyl derived substituents in flavonoids from Tephrosia



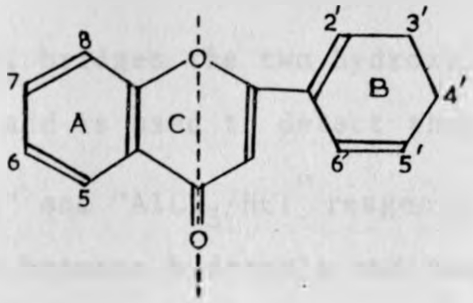
1.6.0 Structure determination of flavonoids

1.6.1 Ultraviolet-Visible Absorption Spectroscopy

The technique is used to aid both identification of the flavonoid type and definition of the oxygenation pattern. In addition, the siting on the flavonoid nucleus of unsubstituted phenolic hydroxyl groups may be established by adding reagents ("shift reagents") to the sample solution and observing the resultant shifts in the absorption peaks. Thus, indirectly, the technique may be useful in determining the location of a sugar or methyl group attached to one of the phenolic hydroxyls. Availability of a good range of reference spectra is an invaluable aid in the interpretation of UV-Visible absorption spectra and a useful general selection has been published[66]. Other compilations include those of Jurd[67], Harborne[68] and Jay et. al[69].

The flavonoid spectrum is usually determined using a methanol or less satisfactorily[70] an ethanol solution of the flavonoid. However, methanolic HCl is required for anthocyanins. The spectrum typically consists of two absorption maxima in the ranges 240-285 nm (band II) and 300-500 (band I). The precise position and relative intensities of these maxima give valuable information on the nature of the flavonoid and its oxygenation pattern[66].

In general terms the band II absorption may be considered as having originated from the A-ring benzoyl system and band I from the B-ring cinnamoyl system (See below)



(Benzoyl Cinnamoyl)

Full interpretation of the set of spectra can often only be achieved when information from other sources is also available. However, a good deal of information is obtainable from the spectra alone. The first step is to "type" the flavonoid using the general form of the methanol spectrum and the wavelengths of the absorption bands.

The second step is to consider the significance of the changes induced in the spectrum by the various shift reagents. The "$NaOCH_3$" spectrum represents that of the flavonoid with all phenolic hydroxyl groups ionized to some extent. It is therefore generally a good "fingerprint" indicator of the hydroxylation pattern as well as being useful for the detection of the more acidic hydroxyl groups in unsubstituted form.

Sodium acetate causes significant ionization of only the most acidic of the flavonoid hydroxyl groups. Thus it is primarily to detect the presence of a free 7-hydroxyl groups.

NaOAC /H_3BO_3 bridges the two hydroxyls in an ortho-dihydroxy group and is used to detect their presence.

The "$AlCl_3$" and "$AlCl_3$/HCl" reagents form acid-stable complexes between hydroxyls and neighbouring ketones and acid-labile complexes with ortho-dihydroxyl groups such that the groups can be detected. The "$AlCl_3$" spectrum thus represents the sum effect of all complexes on the spectrum, while the "$AlCl_3$/HCl" spectrum represents the effect of only the hydroxyl-keto complexes.

1.6.2 ^{1}H -NMR spectroscopy

Typical applications of this technique to flavonoids include the definition of the oxygenation pattern (all three rings), determination of the number (and position) of methoxyl groups, distinction of isoflavones, flavanones and dihydroflavonols, determination of the number of sugars present (and whether α or β linked) and determination of hydrocarbon side chains such as C-linked CH_3 and C or O-linked prenyl.

The ^{1}H-nmr spectrum appears predominantly in the range 0-10 ppm downfield from the reference signal. Table 4 lists various flavonoid proton types.

Table 4. Approximate chemical shifts of various flavonoid proton types

Chemical shift ppm	Proton type
0	Tetramethylsilane (reference)
~1.0	Rhamnose C-CH_3 (broad doublet)
~1.7	Prenyl (-CH_2-CH=C (CH_3)$_2$) methyl groups (other protons 3.5 and 5.3 ppm)
~2.0	Acetate (-OCOCH_3) and aromatic methyl (C-CH_3)
2-3	H-3 of flavanones (two proton-multiplet)
3.5-4.0	Most sugar C-H, Methoxyls.
4.2-6.0	H-1 of sugars (also H-2 dihydroflanols, 5.0 ppm and H-2 of flavanones 5-5.5 ppm)
~6.0	Methylenedioxy (O-CH_2-O), singlet
6.0-8.0	A and B-ring protons
7.5-8.0	H-2 of isoflavones (singlet)
12-14	5-OH (observed only when solvent = DMSO-d_6)

1.6.3. ^{13}C-NMR spectroscopy

Typical applications include establishment of the total number of carbon atoms per molecule, the number of oxygenated carbons on the flavonoid nucleus and the number of carbons in the sugar moiety, identification of C-(and O-) linked sugars; determination of interglycosidic linkage points, identification of acyl substituents and the sites of acylation and C-linkages (e.g in C-glycosides, biflavonoids etc). Natural abundance of ^{13}C is only 1.1 per cent and it is this 1.1 per cent of any flavonoid sample that gives rise to the ^{13}C-nmr spectrum. Carbon-13 resonance occurs predominantly in the range 0-200 ppm downfield from tetramethylsilane (TMS), each different carbon being represented by one signal.

The position of a signal relative to the TMS reference is a good guide as to the type of carbon represented (See table 5).

As shown in table 5 this position (chemical shift) is affected markedly by nearby substitutents. Such effects are predictable in their extent and have led to the formulation of "substituent effect" data which defines the expected effect (on aromatic carbon resonances) of introducing a new substituent into an aromatic ring[71,72]. Table 6 demonstrates the effect of new substituents at the C-1 ortho-, meta- and para positions. Using this type of substituent effect data, it is possible to calculate

Table 5. Carbon-13 chemical shift ranges for various flavonoid carbon types

Carbon type	Usual chemical shift range (ppm from TMS)
Carbonyl (4-keto, acyl)	210 - 170
Aromatic and olefinic:	
a) oxygenated	165 - 155 (no o/p oxygenation)
	150 - 130 (with o/p oxygenation)
b) non-oxygenated	135 - 125 (no o/p oxygenation)
	125 - 90 (with o/p oxygenation)
Aliphatic:	
a) Oxygenated (sugars)	83 - 69 (C-1 of O-glycoside, ~100 ppm)
b) non-oxygenated (C-2,3 flavanones)	80 - 40 (epicatechin C-4, 28 ppm)
Methylenedioxy	~100 ppm
O - CH_3	55 - 63 (60-63=O-disubstituted)
C - CH_3, CO CH_3	~17 - 20
Isopropenyl ($-CH_2-CH=C(CH_3)_2$)	21 (CH_2), 122 (CH), 131 (C), 18 (CH_3)

Table 6: Shift data for the effect of new substituents at the C-1 ortho-, meta- and para positions (in ppm)

Group	C-1	ORTHO	META	PARA
Hydroxyl	+26.9	-12.7	+1.4	-7.3
Methoxyl	+31.4	-14.4	+1.0	-7.7
Methyl	+8.9	+0.7	-0.1	-2.9
Acetoxyl	+23.0	-6.4	+1.6	-2.3

with some accuracy the spectrum of an unknown flavonoid from that of a known similarly substituted flavonoid. To do this, it is necessary to have available a wide range of reference spectra and a number of such compilations have appeared[73,74,75,76].

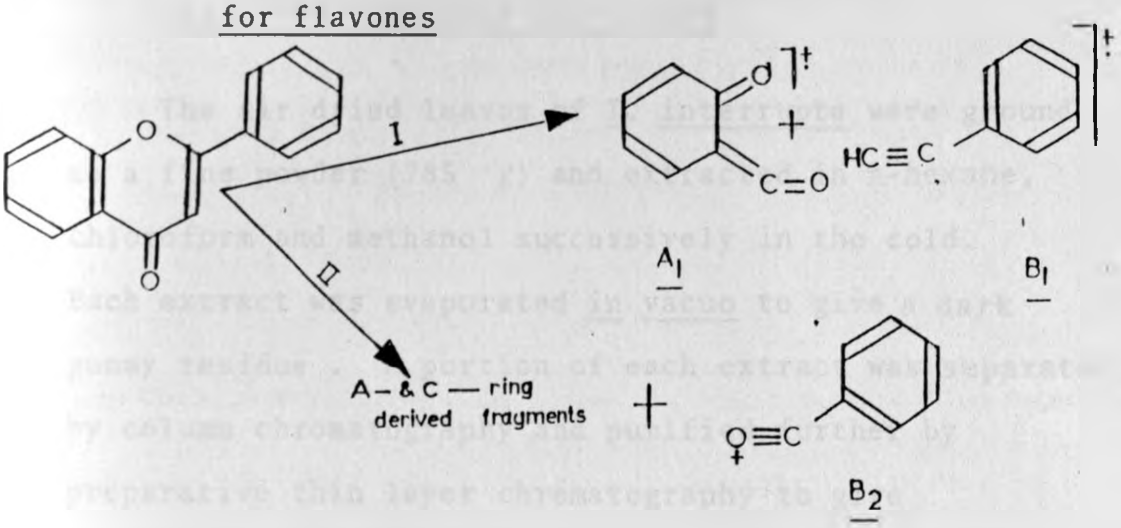
1.6.4 Mass spectroscopy

With respect to flavonoids, typical applications include determination of molecular weights, establishment of the distribution of substituents between A- and B-rings and determination of the nature and site of attachment of the sugars, in flavonoid C- and O-glycosides.

Fragmentation of flavonoids and their glycosides on electron impact occurs in a limited number of predictable ways[77,78].

Fission of the M^+ ion into A- and B-ring containing fragments often provides structurally useful information. These fragmentations usually involve one of two competing pathways, I (retro-Diels Alder) and II (Scheme 1).

Scheme 1. Diagnostic mass spectral fragmentation for flavones



The dominant pathway is determined by the aglycone type, although on occasions neither pathway produces detectable fragments. Flavones and isoflavones tend to produce A_1^+, $(A_1 + H)^+$ and B_1^+ fragments; flavonols, $(A_1 + H)^+$ and B_2^+ fragments, flavanones, A_1^+, $(A_1 + H)^+$ and $(B_1 + 2H)^+$ fragments, and dihydroflavonols, A_1^+ and $(B_1 + H_2O)^+$ fragments. Chalcones tend to produce fragments derived from cleavage of either side of the carbonyl, although 2'-hydroxy-chalcones may isomerize to flavanones and produce typical flavanone fragments instead[10].

CHAPTER 2

RESULTS AND DISCUSSION

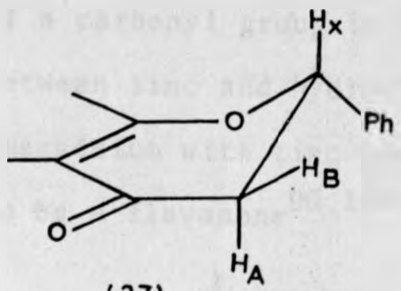
2.1 Isolation and characterisation of 5-methoxy-Isolonchocarpin (26) and Isopongaflavone (28) from Tephrosia interrupta

The air dried leaves of T. interrupta were ground to a fine powder (785 g) and extracted in n-hexane, chloroform and methanol successively in the cold. Each extract was evaporated in vacuo to give a dark gummy residue . A portion of each extract was separated by column chromatography and purified further by preparative thin layer chromatography to give 5-methoxy-isolonchocarpin (26) and isopongaflavone (28).

The dried pods (40g) were extracted with ethyl acetate at room temperature. The extract was concentrated to give a dark greenish oil (0.47 g). The dark greenish oil was separated by column chromatography using silica gel and a mixture of $CHCl_3$ and varying amounts of ethylacetate (20-100%) as the eluant. Further purification of the fractions from the column by preparative thin layer chromatography yielded 5-methoxy-isolonchocarpin (26) and isopongaflavone (28).

Compound 26 melted at 138-40°. The ultraviolet spectrum showed a major peak (Band II) at 269 nm and a low intensity peak at 295 nm. This suggested that compound 26 had a flavanone structure[10]. The characteristic ABX system in the ^{1}H NMR spectrum also agreed with a flavanone structure [79]. A one proton doublet of a doublet at δ5.43 and the two proton doubletsof doublets at δ 2.80 and 2.99 were assigned to H-2 and H-3 (cis) and H-3 (trans) of flavanone respectively. The C-2 proton of the flavanone which shows two doubletsof doublets J_{cis} = 3.50 Hz, J_{trans} = 12.63 Hz is due to the coupling of the C-2 proton with the two C-3 prtons. The C-3 protons couple with each other (J = 18.63 Hz) in addition to their spin-spin interaction with the C-2 proton (J = 12.63 Hz and 3.50 Hz) thus giving rise to two overlapping doubletsof doublets near δ2.99. The above coupling constants are consistent with a quasi-chair chromanone ring[80], with the phenyl group being in the equatorial position (Structure 27). A broad singlet at δ 7.42 that integrated for five protons (5H) suggested the presence of an unsubstituted B-ring.

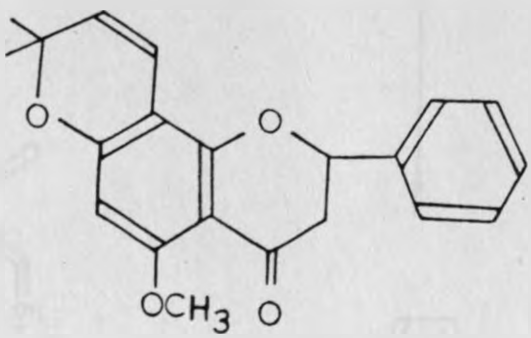
Two doublets at δ 6.63 (1H) and δ5.47 (1H) with J = 10.2 Hz and a singlet at δ 1.46 integrating for six protons indicated the presence of a chromene ring as a substituent on the A-ring[82,83]. A singlet at δ6.06 (1H) implied penta-substitution on ring A. Compound 26 had one methoxy group on ring A which was indicated by the singlet at δ 3.90 (3H).



(27)

Compound 26 showed a molecular weight of 336 in its mass spectrum. The ion at m/e 321 must have originated by loss of a methyl group. Ions at m/e 232 and 217 originated from the retro-Diels Alder (RDA) of M^+ and $M-15]^+$ respectively. The presence of an unsubstituted B-ring was suggested by the ^1H - NMR singlet (5H) at δ 7.42 and by the mass spectral ions at m/e 131, 104, 103 and 77.

Scheme 2 shows how these ions may arise. The ^{13}C - NMR spectral data of compound 26 was in excellent agreement with those reported for 5-methoxy-isolonchocarpin [81] (See Table 7). ^{13}C-DEPT spectrum indicated only one CH_2 carbon to be present in the compound. The other peaks were due to CH_3 and CH carbon atoms. These were ten in number, agreeing with the assigned structure (26). The IR spectrum suggested the presence of a carbonyl group in the molecule. The reaction between zinc and hydrochloric acid after replacing magnesium with zinc confirmed this compound to be a flavanone[99,109].



(26)

Scheme 2: Proposed mass spectral fragmentation of compound 26

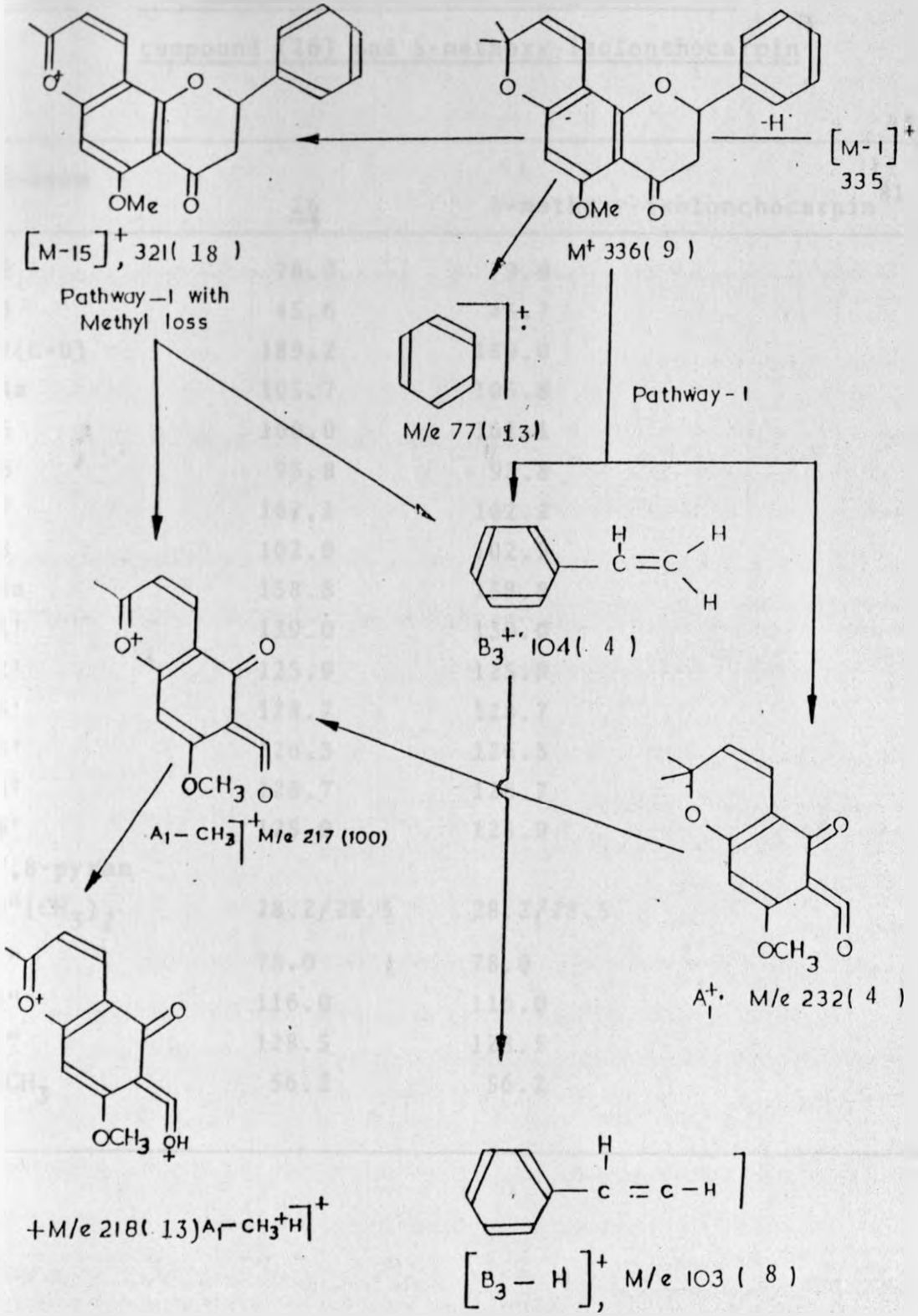


Table 7. ^{13}C Chemical shifts and assignments for compound (26) and 5-methoxy-isolonchocarpin

C-atom	26	5-methoxy-isolonchocarpin[81]
2	78.9	79.0
3	45.6	45.7
4(C=0)	189.2	189.0
4a	105.7	105.8
5	160.0	161.1
6	93.8	93.8
7	162.1	162.2
8	102.9	102.9
8a	158.8	158.8
1'	139.0	139.0
2'	125.9	125.9
3'	128.7	128.7
4'	126.3	126.3
5'	128.7	128.7
6'	125.9	125.9
7,8-pyran		
2"$(CH_3)_2$	28.2/28.5	28.2/28.5
2"	78.0	78.0
3"	116.0	116.0
4"	128.5	128.5
OCH_3	56.2	56.2

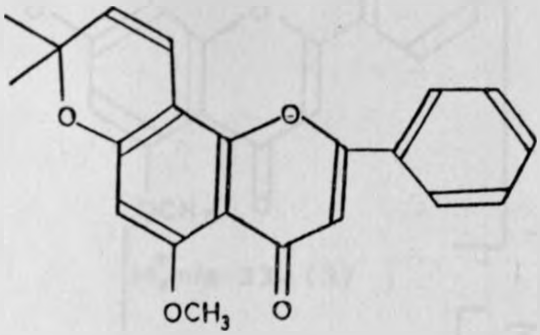
Irradiation of the doublet of a doublet at δ 5.43 resulted in the two doublets of doublets at δ 2.80 and 2.99 collapsing to two doublets. Similarly, irradiation of the two doublets of doublets at δ 2.80 and 2.99 resulted in the collapse of the doublet of doublet at δ 5.43 to a doublet. Thus, spin-spin coupling for the doublets of doublets at δ 5.43, 2.80 and 2.99 was confirmed.

The second compound from the chromatographic column was subjected to PTLC and shown to be a 7-oxygenated flavone. It has a C-8 prenyl unit that had undergone substitution and cyclization Its melting point was 213-17°c and its molecular mass was 334.

In the ^{1}H - NMR spectrum the flavone nucleus was evident from the low field singlet at δ 6.66 of the unsaturated C-ring proton[66]. The aromatic region exhibited two multiplets (δ 7.89-7.84, 2H; δ 7.55-7.48, 3H) accounting for the unsubstituted conjugated B-ring of a flavone[84,85]. Two olefinic doublets at δ 6.84 and δ 5.63 with splitting constant of 10 Hz and two saturated methyls at δ 1.51 (S,6H)

were characteristic of a 2,2-dimethylchromene ring[82]. The chromene ring must therefore be a substituent on the A-ring. The singlet at δ 3.96 (3H) could be assigned to a methoxy group on A-ring. Irradiation of the ^{1}H-NMR doublet at δ 5.63 converted the δ 6.84 doublet into a singlet. Thus, spin-spin coupling for the two doublets was confirmed.

The mass spectrum of this compound showed the loss of a methyl radical from the $[M]^+$ to give the pyrilium cation at m/e 319. This fragmentation is the most important as evidenced by the highest relative intensity of this ion (100%). This ion was a further confirmation of the presence of a 2,2-dimethylchromene ring. An ion at m/e 217 (74) in the mass spectrum was another suggestion of the presence of the chromene ring in the compound which must have arisen from the retro-Diels Alder (RDA) fragmentation of $[M-15]^+$. The other peaks which arise due to pathways I and II were observed at m/e 105, 102 and 77. On the basis of ^{1}H-NMR and EI-MS spectra, structure 28 was proposed and Scheme 3 tentatively proposed for its mass spectral fragmentation. The UV spectrum of compound 28 was consistent with the structure of a flavone having oxidation only in the A-ring[66].



(28)

The wavelength of band II of this compound was observed at 273 nm while that of band I was at 350 nm. No diagnostic shifts were observed on addition of shift reagents, suggesting that there was no hydroxylation in the molecule. This was confirmed by recording the ^{1}H - NMR spectrum of compound 28 in $CDCl_3$ and a drop of CD_3OD to determine the presence of exchangeable hydrogens. No effect was observed on the original spectrum indicating the absence of hydroxyl protons.

The IR absorption band at 1637 cm^{-1} was due to its carbonyl (C=O) group. The carbonyl group in the case of a flavone is conjugated with both A and B rings[86]. This gives rise to mesomerism[87,88] as illustrated in figure 5.

Scheme 3: Proposed mass spectral fragmentation of compound 28

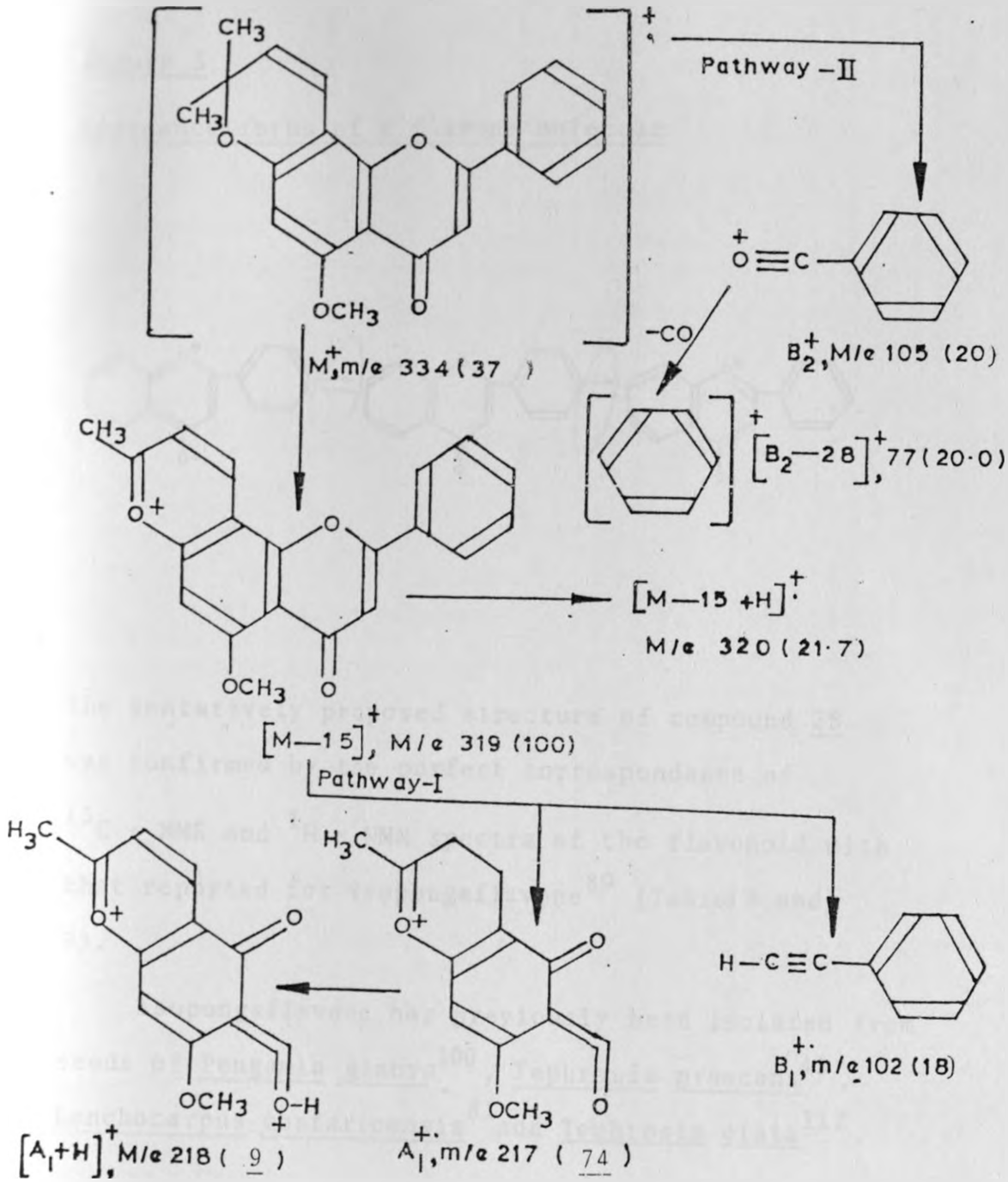
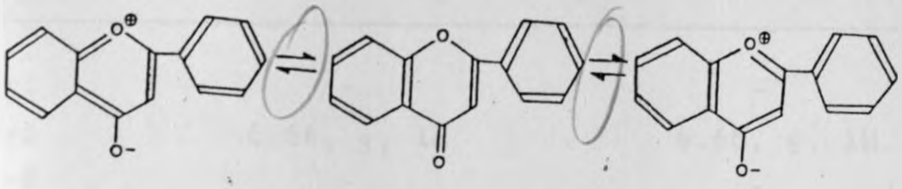


Figure 5

Resonance forms of a flavone molecule



The tentatively proposed structure of compound 28 was confirmed by the perfect correspondence of ^{13}C - NMR and ^{1}H - NMR spectra of the flavonoid with that reported for isopongaflavone[89] (Tables 8 and 9).

Isopongaflavone has previously been isolated from seeds of Pongamia glabra[100], Tephrosia praecans[45], Lonchocarpus costaricensis[81] and Tephrosia elata[112].

Table 8. ^{1}H-NMR - Chemical shifts and Assignments for Flavonoid (28) and Isopongaflavone[89].

	Compound (28)	Isopongaflavone[89]
H-2	-	-
H-3	6.66, s, 1H	6.66, s, 1H
H-4	-	-
H-4a	-	-
H-5	-	-
H-6	6.34, s, 1H	6.33, s, 1H
H-7	-	-
H-8	-	-
H-8a	-	-
OCH_3-5	3.96, s, 3H	3.95, s, 3H
$(CH_3)_2$-2"	1.51, s, 6H	1.50, s, 6H
H-3"	5.63, d, 1H, J=10Hz	5.62, d, 1H, J=9.8Hz
H-4"	6.86, d, 1H, J=10Hz	6.85, d, 1H, J=9.8Hz
H-1'	-	-
H-2'6'	7.89-7.84, m, 2H	7.90-7.80, m,2H
H-3',4',5'	7.55-7.48, m, 3H	7.53-7.46, m,3H

Table 9. Carbon-13-chemical shifts and Assignments for Flavonoid (28) and Isopongaflavone[81]

Carbon atom	Compound (28)	Isopongaflavone[81]
2	160.8	160.8
3	108.9	109.0
4	177.6	177.6
4a	108.9	109.0
5	154.0	154.0
6	96.6	96.7
7	160.2	160.2
8	102.9	102.8
8a	158.0	158.0
OCH_3	56.4	56.5
$(CH_3)_2$-2"	28.3	28.3
2"	78.1	78.1
3"	115.3	115.3
4"	127.5	127.6
1'	131.9	131.9
2'	125.9	125.9
3'	128.9	129.0
4'	131.1	131.2
5'	128.9	129.0
6'	125.9	125.9

2.2 Isolation and Identification of a prenylated chalcone (29) from Tephrosia interrupta

This was obtained both from the hexane and chloroform extracts of the stem of T. interrupta after subjecting them to column chromatography.

The compound crystallized from hexane as bright red needles with melting point 105°-108°C. On silica gel plate with solvent system D, the compound had an R_f value of 0.75. On illumination with a UV lamp, the same compound appeared yellow under short range and brown under long range. On fuming the thin layer plate with ammonia, the compound appeared yellow under short range and brown under long range.

Its ^1H - NMR spectrum showed a six-proton singlet at δ 1.46 and one proton doublets centred at δ 5.45 and δ 6.65 (J = 10.2 Hz) which were suggestive of a dimethylchromene system[82]. The ^1H - NMR showed the presence of an α,β -double bond with two protons represented as doublets at δ7.77 (J = 15.58 Hz) and δ 7.89 (J = 15.58 Hz) for the α and β protons respectively, correlating with published spectra[66]. This led to the suggestion that the compound could be a chalcone. Two multiplets centred at δ 7.41 and δ 7.61 integrating for five aromatic protons were assigned to those of an unsubstituted B ring of the chalcone.

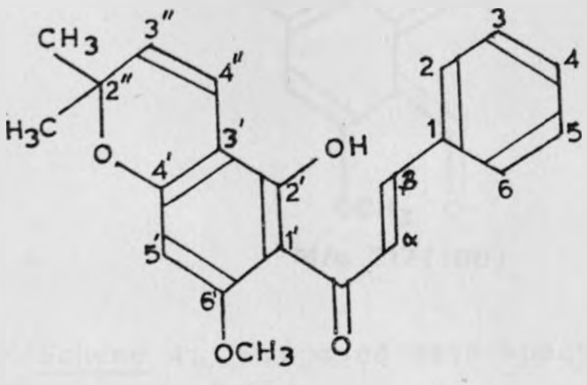
A three-proton singlet at δ 3.92 was assigned to the methoxyl group[77] and another one-proton singlet at δ 5.93 to the aromatic proton flanked by the two oxygen functions in ring-A.

The mass spectrum of the compound showed an $[m]^+$ peak at m/e 336 (15) and other fragment ions at m/e 321 [M-15], m/e 259 [M-77], m/e 232 [M-104], m/e 217 [M-15-104], m/e 244 [M-15-17], m/e 131 and 103. The fragment ion at m/e 321 was due to loss of a methyl radical. The peaks at m/e 259 [M-77] and m/e 232 [M-104] in its mass spectrum are due to the loss of a phenyl group and a neutral styrene molecule which indicates that ring B is unsubstituted. An ion at m/e 217 (100) must have come from the retro-Diels Alder (RDA) fragmentation of the $[M-15]^+$ fragment.

The arrangement of the three substitutents on ring A as suggested by the ^1H - NMR and mass spectra was determined by ultraviolet visible and infra-red spectroscopy studies. λ_{max} 230, 288, 299 and 344 nm were observed in methanol. The peak at 344 nm shifted to 376 nm in the presence of $AlCl_3$ indicating the presence of chelated hydroxyl group[90,91,107]. A broad band at ν_{max} 3400 cm^{-1} supported the idea that a chelated hydroxyl was present in the chalcone.

It was therefore concluded that the hydroxyl group was in position 2'.

The IR spectrum exhibited peaks at 1610 and 1330 cm^{-1} due to the presence of carbonyl and gem-dimethyl groups. On biogenetic consideration, the position of the chromeno ring could be considered similar to that of the furan ring in Lanceolatin-B[47,36] which has also been isolated from Tephrosia species. Assuming the compound to be an angular chromeno-chalcone the methoxyl could be fixed at C-5' or C-6' position. The singlet at δ 5.93 indicated that the proton at position C-5' was free and as such the methoxyl could be fixed at position C-6'. Thus the dimethylchromene system was attached at position 3' and 4'. The ^{1}H-NMR singlet at δ 14.50 was consistent with the 2' - chelated hydroxyl group of chalcones[113]. On this basis structure 29 was



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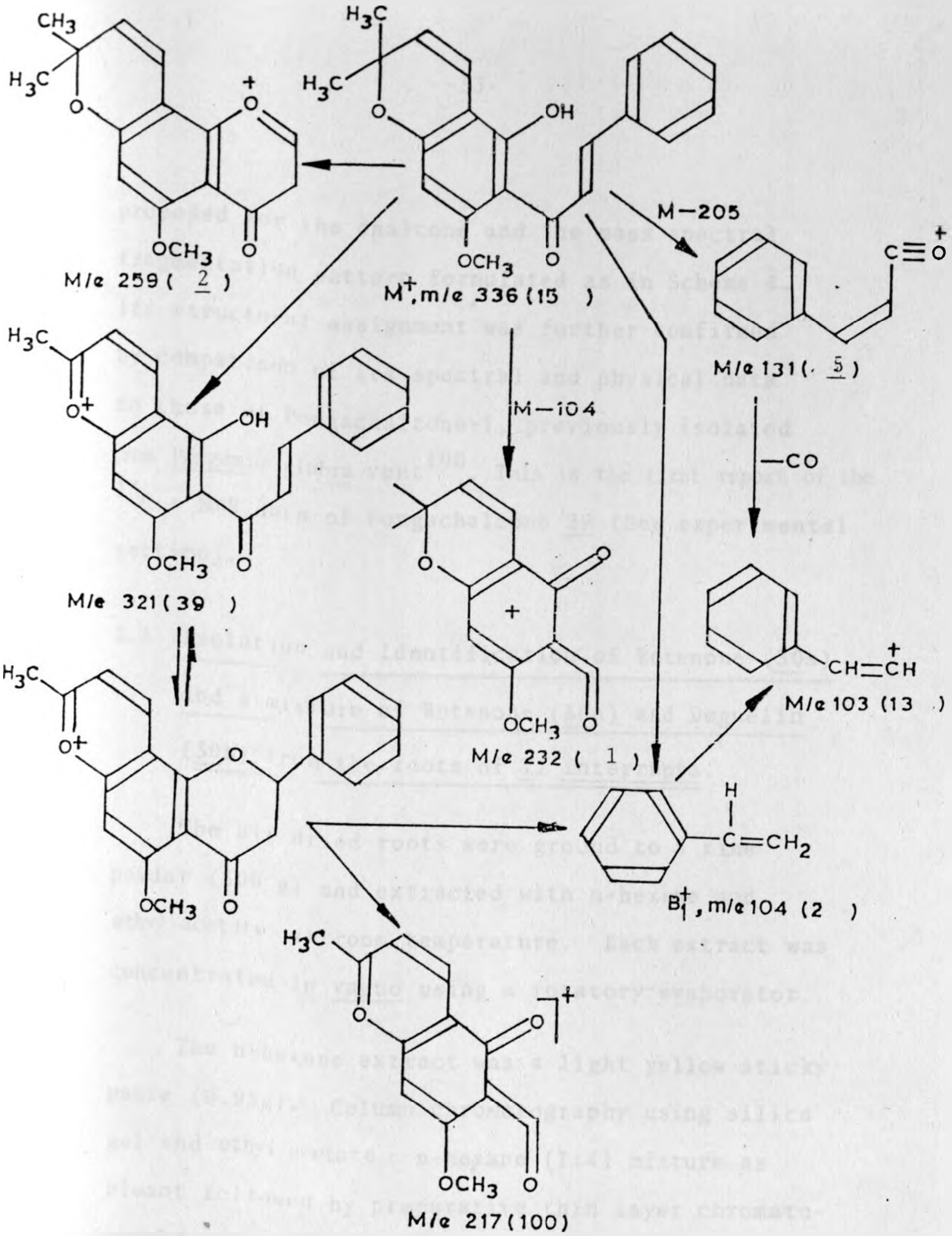


Scheme 4: Proposed mass spectral fragmentation of compound 29.

proposed for the chalcone and the mass spectral fragmentation pattern formulated as in Scheme 4. Its structural assignment was further confirmed by comparison of its spectral and physical data to those of Pongachalcone-I, previously isolated from Pongamia glabra vent[100]. This is the first report of the ^{13}C - NMR data of Pongachalcone 29 (See experimental section).

2.3 Isolation and Identification of Rotenone (30a) and a mixture of Rotenone (30a) and Deguelin (30b) from the roots of T. interrupta.

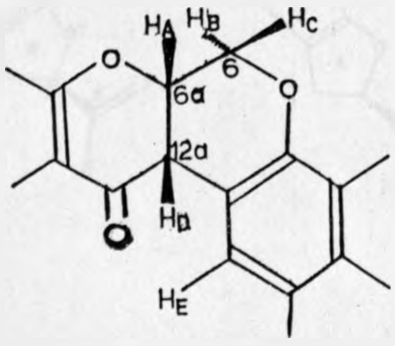
The air dried roots were ground to a fine powder (500 g) and extracted with n-hexane and ethyl acetate at room temperature. Each extract was concentrated in vacuo using a rotatory evaporator.

The n-hexane extract was a light yellow sticky paste (0.95g). Column chromatography using silica gel and ethyl acetate : n-hexane (1:4) mixture as eluant followed by preparative thin layer chromatography using ethyl acetate : chloroform (1:4) mixture gave Rotenone (30a) (16 mg) and compound 30 which was a mixture of rotenone (30a) and Deguelin (30b) (16 mg).

The ethyl acetate extract was a brown residue (9.8g). The extract was introduced onto the column and eluted using a mixture of n-hexane and varying amounts of ethyl acetate (20-100%) as the eluant.

Further purification of the fractions from the column by column and preparative TLC afforded compound 30a (18 mg) and a mixture of compounds 30a and 30b (48 mg).

The ^{1}H - NMR spectrum of compound 30a indicated the presence of an isopropenyl system, four aromatic protons, methoxy groups and an ABCD flavonoid ring characteristic of rotenoids due to the four protons in position 6, 6a and 12a[92,93]. (See structure 31 and also table 10).

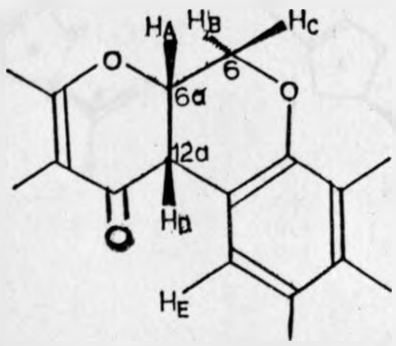


(31)

The ethyl acetate extract was a brown residue (9.8g). The extract was introduced onto the column and eluted using a mixture of n-hexane and varying amounts of ethyl acetate (20-100%) as the eluant.

Further purification of the fractions from the column by column and preparative TLC afforded compound 30a (18 mg) and a mixture of compounds 30a and 30b (48 mg).

The ^{1}H - NMR spectrum of compound 30a indicated the presence of an isopropenyl system, four aromatic protons, methoxy groups and an ABCD flavonoid ring characteristic of rotenoids due to the four protons in position 6, 6a and 12a[92,93]. (See structure 31 and also table 10).

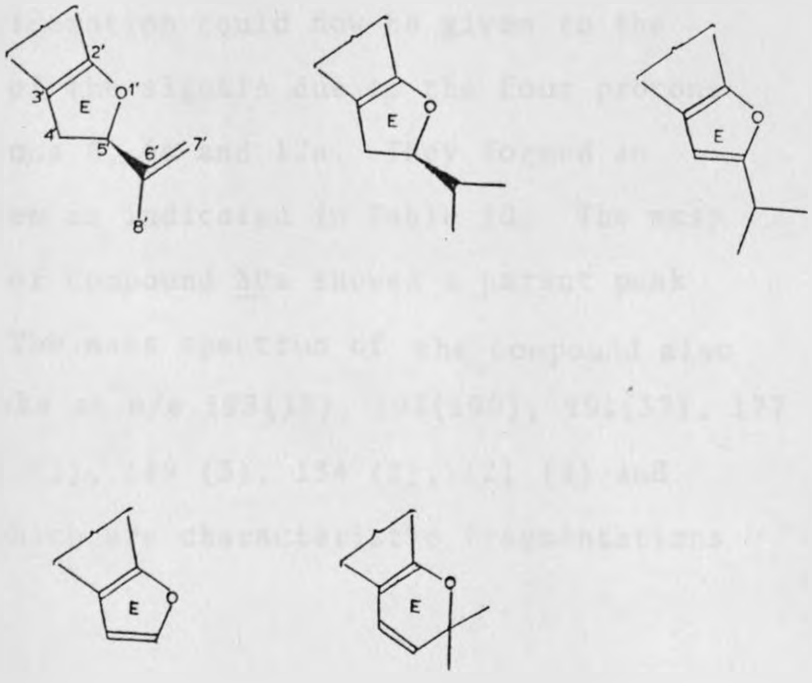


(31)

The various groups that are attached to ring E of the known rotenoids are the gem-dimethyl, isopropyl and isopropenyl groups (See Fig. 6). These can be readily distinguished by ^{1}H-NMR spectroscopy as there is no interfering absorption in appropriate regions of the spectrum[92]. The presence of two broadened singlets at δ 5.07 (1H) and 1.77 (1H), a triplet at δ 5.24 (1H) and two quartets (δ 3.31 (1H) and δ 2.97 (1H) led to the detection of isopropenyl dihydrofuran system as a substituent in the compound.

The doublet at δ 6.78 (J = 1.08 Hz, 1H) and the singlet at δ 6.47 (1H) were assigned to the H-1 and H-4 protons. The other pair of aromatic protons

Fig. 6: Possible c-methyl group variants attached to ring E in known rotenoids



formed an AB system (δ 7.82, doublet, J = 8.5 Hz, 1H and δ 6.51, doublet, J = 8.5 Hz, 1H) characteristic of ortho-related protons. The low field doublet at δ 7.82 could be assigned to the H-11 proton as it was deshielded by the adjacent carbonyl group. The other doublet at δ 6.51 was due to the H-10 proton which was adjacent to the ether oxygen of the isopropenyl dihydrofuran system[93].

Two three-proton singlets at δ 3.79 and 3.75 were assigned to methoxyl groups. However, the methoxy signals overlapped some peaks. All known rotenoids with methoxyl groups are known to have methoxyl at the 2- and the 3- positions[94].

Consideration could now be given to the analysis of the signals due to the four protons in positions 6, 6a and 12a. They formed an ABCD system as indicated in Table 10. The mass spectrum of compound 30a showed a parent peak at 394. The mass spectrum of the compound also showed peaks at m/e 193(13), 192(100), 191(37), 177 (16), 161 (2), 149 (3), 134 (2), 121 (4) and 106 (4) which are characteristic fragmentations

Table 10. Coupling constants deduced by the analysis of the ^{1}H-NMR spectra of compound 30a and Rotenone

Carbon proton	Coupling constant Compound 30a	Rotenone[95]
6eq, 6a	3.23	3.10
6ax, 6a	1.08	1.2
6, 6ax	12.10	12.10
6ax, 12a	4.02	4.00
10, 11	8.59	8.60
4',4"	15.71	15.90
4',5'	9.67	9.50
4",5'	8.27	8.20

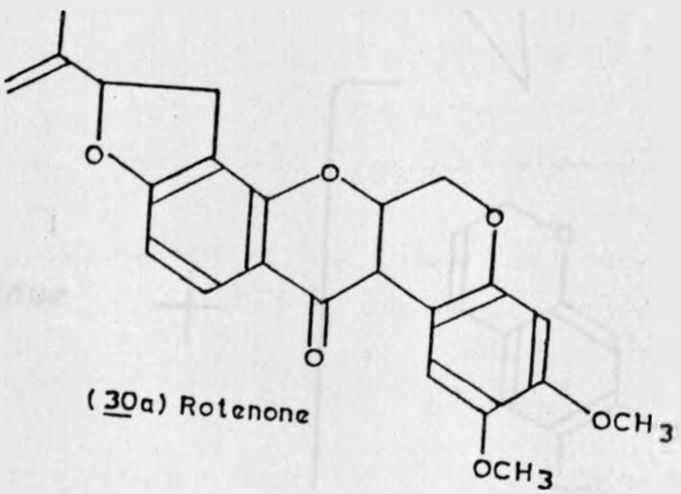
Note: H_A = 6a, H_B = 6eq , H_C = 6ax

H_D = 12a H_E = 1

Also H-4" is a proton that resonates at higher field than its identically numbered counterparts.

of the dimethoxy-chroman[94]. This group is characteric of the chromanochromanone ring systems[94]. The most abundant ion of the spectrum was at m/e 192 and may arise as shown in Scheme 5.

The ^{13}C NMR showed 23 signals corresponding to 23 carbon atoms. Off resonance decoupling gave the multiplicity of each signal. On the basis of this data the flavonoid was suspected to be Rotenone (30a). Indeed, ^{13}C NMR data was in full agreement with that previously reported for Rotenone[92] (table 11).



(30a) Rotenone

Scheme 5: Fragmentation of the dimethoxychromanone ring system

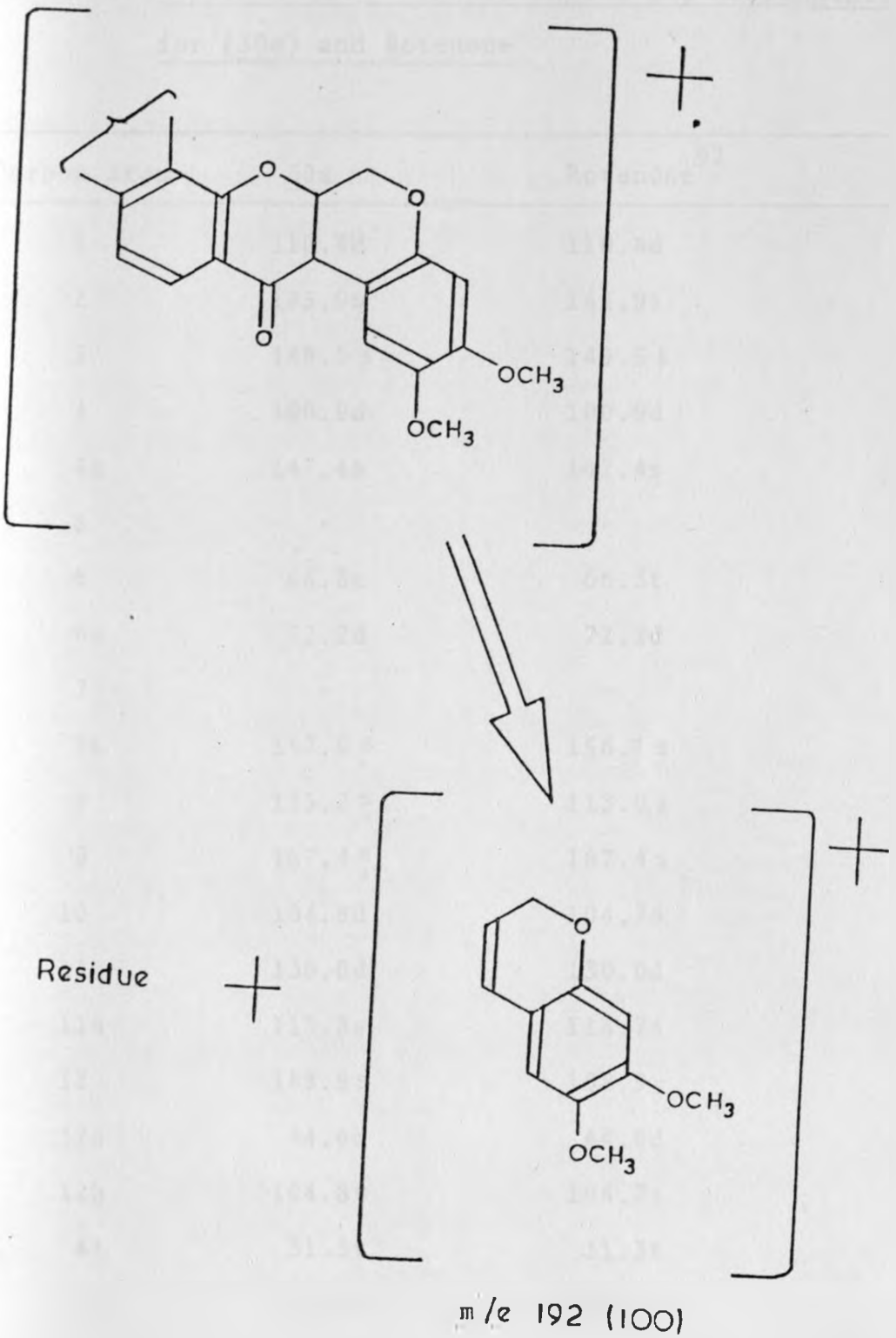


Table 11. Carbon-13 NMR Chemical Shifts and Assignments for (30a) and Rotenone

Carbon atom	30a	Rotenone[92]
1	110.4d	110.4d
2	143.9s	143.9s
3	149.5 s	149.5 s
4	100.9d	100.9d
4a	147.4s	147.4s
5	-	-
6	66.3t	66.3t
6a	72.2d	72.2d
7	-	-
7a	157.9 s	156.1 s
8	113.2 s	113.0 s
9	167.4 s	167.4 s
10	104.8d	104.7d
11	130.0d	130.0d
11a	113.3s	114.7s
12	188.9s	188.9s
12a	44.6d	44.6d
12b	104.8s	104.7s
4'	31.3t	31.3t
5'	87.8d	87.8d
6'	143.0s	143.0s
7'	112.5t	112.6t
8'	17.1q	17.2q
2-OCH_3	55.8q	55.8q
3-OCH_3	56.3q	56.3q

Further confirmation of its identity was obtained from ^{13}C-DEPT spectrum which displayed three CH_2 peaks up which agreed with the assigned structure. Finally, the melting point, UV and IR spectra of the crystalline compound (30a) were identical with those of Rotenone[96].

Compound 30 melted at 148° - 50°C. Its mass spectrum showed a molecular ion at m/e 394 and fragment ions were same as for compound (30a). The ^{1}H-NMR displayed all peaks due to Rotenone (30a) plus two singlets at δ1.45 (1H) and δ 1.39 (1H) with coupling constant 9.94 Hz and one doublet at δ 7.75 (J = 8.59 Hz, 1H). This suggested compound 30 to be a mixture of Rotenone (30a) and another compound. The two singlets at δ 1.45 (1H) and δ 1.39 (1H) and the two doublets centred at δ5.56 (1H) and δ 6.6 H (1H) with coupling constant 9.94 Hz led to the detection of dimethylchromene system[82] as part of the second compound in the mixture.

The doublet at δ7.75 (J = 8.59 Hz, 1H) suggested an AB system characteristic of ortho-related protons. As in the case of rotenone, this doublet was assigned to the H-11 proton.

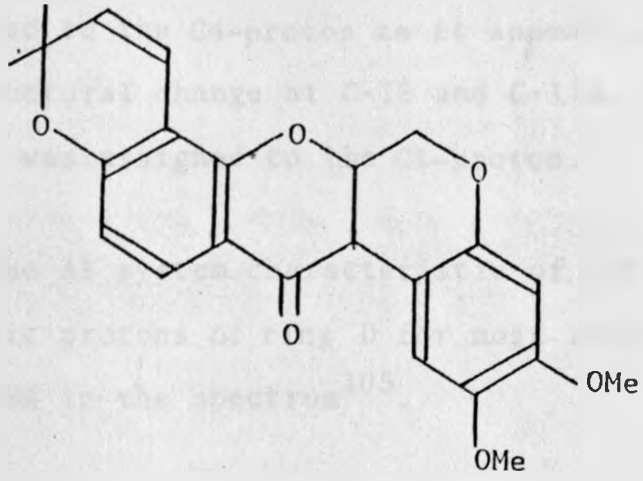
The spectrum of this mixture was run in deutero-benzene. The two singlets at δ1.45 (1H) and δ1.39 (1H) observed when the spectrum was run in deutero-chloroform shifted to δ 1.18 and δ 1.03 respectively. The two quartets centred at δ2.97 and δ 3.31 due to 4' proton of the isopropenyl dihydrofuran system in Rotenone were squashed to a doublet (δ 2.70, J = 9.52 Hz). This is as reported by Carlson et al[95]. A triplet at δ 4.06 in d_6 - benzene was assigned to H-6a of rotenone while the multiplet at δ 4.00 was assigned to H-6a of the second compound in the mixture. When the spectrum was run in d_5 - pyridine, the quartets centred at δ 2.97 and δ3.31 re-appeared. Two doublets due to the proton ussigned to the position 11 appeared this time at very low field δ 8.14 and 8.05. The peak at δ 8.14 must be due to the H-11 of rotenone (30a) and the peak centred at δ8.05 is due to the second compound in the mixture. From the above comparisons, available data suggested the second compound in the mixture to be Deguelin (30b) as summarized in table 12.

Table 12: ^{1}H NMR chemical shifts (δ) of Rotenone (30a) and Deguelin (30b) protons

Compound	Solvent	CH_3-8'	H-4'	H-4'	OCH_3-3	OCH_3-2	H-12a	H-6	H-6	H-6a	H-7'	H-5'	H-4	H-10	H-1	H-11
Rotenone	$CDCl_3$ Obtained	1.77bs	2.97q	3.31q	3.76s	3.79s	3.81s	4.16bd	4.59q	4.90s	5.07s	5.24t	6.46s	6.51d	6.78s	7.84d
	Literature	1.77bs	2.93q	3.32q	3.75s	3.79s	3.80s	4.15bd	4.59q	4.90s	5.06s	5.22t	6.45s	6.50d	6.77s	7.84d
	C_6D_6 Obtained	1.46bs	2.69d	2.69d	3.25s	3.39s	3.59s	3.5s	4.26q	4.06t	4.92	4.71	6.52s	6.37d	7.13s	8.14
	Literature	1.46bs	2.69d	2.69d	3.26s	3.39s	3.60s	3.5s	4.24q	4.06t	4.91	4.71	6.50s	6.38d	7.10s	8.11
	C_5D_5N Obtained	1.68bs	2.9q	3.1q	3.60s	3.63s	4.18s	4.29s	4.78	5.1s	5.1	5.2	6.70s	6.57	7.2	8.14
	Literature	1.67bs	2.9q	3.1q	3.61s	3.63s	4.14s	4.27s	4.78	5.1s	5.1	5.2	6.69s	6.58	7.2	8.09
Deguelin	$CDCl_3$ Obtained	1.38s 1.45s	6.63		3.76	3.79	3.81	4.16bd	4.62q	4.93m	-	5.55	6.43	6.43	6.79	7.73
	Literature	1.37s 1.44s	6.63		3.76	3.79	3.80	4.16bd	4.62q	4.90m	-	5.54	6.43	6.43	6.79	7.73
	C_6D_6 Obtained	1.03s 1.18s	6.64		3.22	3.37	3.53	3.48	4.22q	4.00m	-	5.10d	6.42s	6.43	7.13s	8.01d
	Literature	1.03s 1.18s	6.64		3.22	3.36	3.52	3.48	4.22q	4.00m	-	5.09d	6.42s	6.43	7.10s	8.04d

^{13}C NMR spectrum of the mixture revealed all peaks due to rotenone as assigned in table 11. Additional peaks were observed at 28.1/28.5, 115.6 and 128.5 ppm due to a dimethylchromene system and were assigned to 8' methyls, 4' and 5' carbons respectively. On this basis the proposed structure (30b) was confirmed. Commercially obtained rotenone was crystallized from CCl_4 followed by recrystallization from ethanol to give transparent plates melting point 162.5 - 164.0°c. Deguelin obtained from Tephrosia vogelii gave rectangular plates that melted at 155.5°c - 158°c. The mixture obtained from Tephrosia interrupta melted at 148-50°c.

Rotenone and deguelin have been isolated as mixtures from many species of Leguminosæ mainly Tephrosiae[50,58,96].



(30b)

2.4 Isolation and characterisation of Toxicarol (32) from Tephrosia linearis pods

The pods of Tephrosia linearis were extracted with ethyl acetate. The extract was then subjected to column chromatography. Fraction 2 and 3 were pooled and the main component (R_f 0.30 solvent system B) isolated by PTLC using solvent system D to give yellow needles of pure compound (32) with melting point 104 - 106°c.

The ^1H - NMR spectrum of this compound displayed an ABCD pattern characteristic of rotenoids due to the four protons in position 6, 6a and 12a[93]. Two doublets at δ 5.47 (1H) and δ 6.55 (1H) with coupling constant J = 10 Hz and two singlets at δ 1.37 (3H) and δ 1.43 (3H) led to the detection of a 2,2-dimethylchromene system as the carbon-methyl group attached to ring E[92]. Two aromatic sharp singlets were observed at δ6.86 and δ 6.46. These were characteristic of aromatic protons of ring A of rotenoids[105]. The one at δ 6.46 was assigned to the C4-proton as it appears less susceptible to structural change at C-12 and C-12a. The singlet δ 6.86 was assigned to the C1-proton.

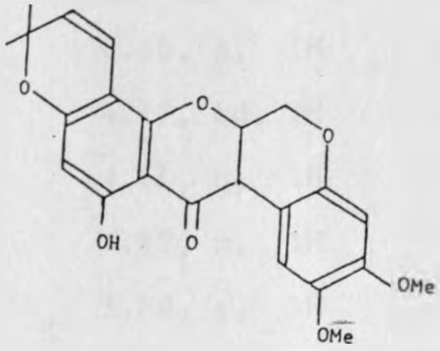
The AB system characteristic of ortho related aromatic protons of ring D for most rotenoids was not observed in the spectrum[105].

Instead, two singlets were observed at δ 5.95 (1H) and δ12.19 (1H). The peaks at δ 3.79 (3H) and δ 3.82 (3H) were assigned to methoxyl groups which occur at positions 2 and 3 respectively in most natural rotenoids[105].

The infrared spectrum of the compound showed carbonyl absorption at 1625 cm^{-1} but no hydroxyl absorption, a phenomenon observed by Flett[106] in 1-hydroxylanthraquinones. This suggested that if a hydroxyl group was part of this molecule then it was ortho to the keto group and thus it must be hydrogen bonded. This further suggested that the hydroxyl group was in position 11 of the molecule thus explaining the lack of an AB system characteristic of ortho related aromatic protons of ring D. Thus the peak at δ12.19 (1 H) could be due to a hydroxyl group at postion 11 and that at δ5.95 (1H) could be due to a proton at position 10.

The mass spectrum of this compound showed a parent peak at m/e 410. Like in the case of Rotenone (30a) the peaks at m/e 193 (13) 192(100), 191(35), 177(16), 121(4) and 106(4) were marked in the mass spectrum and were observed fragmentations of the dimethoxychroman group[94]. This led to the confirmation of the methoxyl groups being in positions 2 and 3.

On this basis therefore, structure 32 was proposed.



(32)

Ferric chloride test gave red colouration thus confirming the presence of a phenol hydroxyl group[98]. The proposed structure for compound 32 was found to be identical to Toxicarol from the correspondence of the ^{1}H NMR spectrum of compound 32 .reported for toxicarol[95] (table 13).

With respect to this compound, the infra-red spectrum displayed λ_{max} values 230, 270, 294, 308 sh and 360 in methanol. The peak at 294 nm shifted to 324 nm in the presence of aluminium trichloride indicating the presence of a chelated hydroxyl group[90,91].

Table 13: ^{1}H - NMR Chemical shifts and assignments for compound (32) and Toxicarol.

Hydrogen-atom	Compound (32)	Toxicarol[95]
1	6.86, bs, 1H	6.86, bs, 1H
4	6.46, s, 1H	6.44, s, 1H
6	4.17, bd, 1H	4.14, bd, 1H
	4.61, q, 1H	4.60, q, 1H
6a	4.85, m, 1H	4.84, m, 1H
12a	3.80, s, 1H	3.80, s, 1H
2-OCH_3	3.82, s, 3H	3.80, s, 3H
3-OCH_3	3.79, s, 3H	3.79, s, 3H
10	5.95, s, 1H	5.94, s, 1H
11	12.19, s, 1H	-
4'	6.55, d, 1H	6.54, d, 1H
5'	5.47, d, 1H	5.47, d, 1H
8'-CH_3	1.37, s, 3H	1.36, s, 3H
	1.43, s, 3H	1.43, s, 3H

The spectrum taken in $AlCl_3$/HCl solution did not show any changes in the absorption bands. This indicated that only a hydroxy-keto complex was formed. This compound was recently obtained from Derris malaccensis. It also occurs in Crotalaria burchia. It was also isolated from T. obovata merril[44], T. toxicaria (Sw.) pers.[56,57] and T. virginiana (L.) pers. (Cracca virginiana)[61,62]. Toxicarol isolated from Derris malaccensis was greenish-yellow needles that crystallized from ethyl acetate / ethanol with mp 125 - 7°c [108].

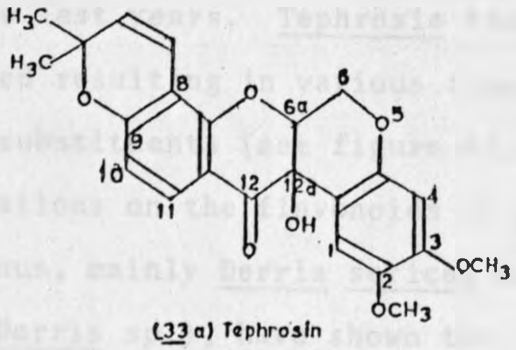
2.5 Isolation and characterisation of a mixture of Tephrosin (33a) and 12a-hydroxyrotenone (33b) from the roots of Tephrosia linearis

The roots of T. linearis were extracted with hexane and ethyl acetate. The extracts were subjected to column chromatography separatelyfollowed by PTLC to give two separate mixtures; Rotenone (30a) and Deguelin (30b), and Tephrosin (33a) and 12a-hydroxyrotenone (33b).

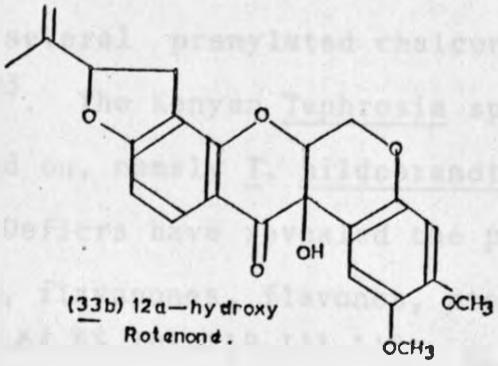
The mass spectrum of Tephrosin (33a)and 12a-hydroxyrotenone (33b) mixture gave a parent peak at m/e 410. Unlike in the case of Rotenone (30a) where the base peak was observed at 192 a.m.u, the peak was observed at 208 a.m.u. The compound gave no

colouration with ferric chloride suggesting absence of phenolic hydroxyl groups in the compound. Peaks due to chromano-chromanone ring system having methoxyl groups at the 2- and the 3-position were observed[94]. The ^1H - NMR spectrum gave peaks at δ 3.73 (s, 3H) and δ 3.81 (s,3H) which suggested the presence of a dimethoxychroman group that is characteristic of most chromanochromanone ring systems. The intense peak at m/e 207 (47) in the mass spectrum of compound 33 (a+b) could be due to loss of a proton from the m/e 208 fragment. The peak at m/e 202 was not observed. Instead there was one at m/e 203 (10) which could be due to protonation of the m/e 202 peak. Another characteristic peak was observed at m/e 187 (6.0) which might have arisen from loss of a methyl radical from the m/e 203 peak. The ^1H NMR did not show the characteristic 12a peak which is normally overlapped by the methoxyl signals. This led to the suggestion that the 12a position was substituted. A broad singlet was observed at δ 4.51. This is a rare peak in most naturally occurring rotenoids. It is characteristic of 12a hydroxy rotenoids. The 7.73 doublet (J = 8.76 Hz) and the δ 7.83 doublet (J = 8.67 Hz) were due to H-11 proton and an H-10 proton respectively.

The presence of peaks at δ 1.76 (S, 3H) and δ 5.08 (S, 1H), a triplet at δ5.24 (1H) and two quartets at δ2.96 (1H) and δ3.32 (1H) led to the detection of isopropenyl dihydrofuran system as part of the compound. Similarly, the presence of peaks at δ 5.56 (1H) and δ 6.65 (1H) with coupling constant J = 10 Hz and two singlets at δ 1.39 (3H) and δ 1.45 (3H) led to the detection of another system, the dimethylchromene ring. Thus, compound (33) was a mixture of two compounds namely, Tephrosin (33a) and 12a - hydroxy-rotenone (33b).



(33 a) Tephrosin



(33b) 12a—hydroxy Rotenone.

2.6 Comment and Conclusion

As earlier described[20] taxonomic opinion has favoured a circumscription of Tephrosieae to include, among other genera, Derris, Lonchocarpus, Millettia, Mundulea, Piscidia, Pongamia, Tephrosia and Wisteria. Work is already in progress towards using a chemotaxonomic approach to clarify the taxonomic situation of the tribe Tephrosieae[24].

The occurrence in nature of a number of prenylated phenols, coumarins and xanthones has been described in the last years. Tephrosia flavonoids may be prenylated resulting in various types of prenyl derived substituents (see figure 4). Recent investigations on the flavonoids of the Lonchocarpus genus, mainly Derris sericea and Cordoa piaca (Derris sp.), have shown the presence of several prenylated chalcones and flavanones[113]. The Kenyan Tephrosia species so far worked on, namely T. hildebrandtii vatke and T. elata Deflers have revealed the presence of rotenoids, flavanones, flavones, pterocarpans and flavans[6,64,65,101,110,111,112]. Earlier phytochemical screening of a number of species of Tephrosia did not show the presence of flavans.

Thus this calls for continued research to explore the entire constituents of the Tephrosia species.

Results from studies of Tephrosia species examined in this project support the suggestion that the majority of Tephrosia flavonoids are prenylated[113]. The occurrence of the chromano-chalcone, Pongachalcone-I (29); the flavanone, 5-methoxy-isolonchocarpin (26), the flavone, Isopongaflavone (28) and the Rotenoids, Rotenone (30a), Deguelin (30b), Tephrosin (33a) and 12a-hydroxyrotenone (33b) gives further support that chalcones, flavanones, flavones and rotenoids are biogenetic precursors in the biosynthesis of prenylated flavonoids.[113]

CHAPTER 3

3. EXPERIMENTAL

3.1. General Experimental Procedures

Melting points (m.p.) were determined on an electrochemical melting point apparatus.

Infrared (IR) spectra were recorded on a Perkin-Elmer 467 infrared spectrophotometer. The spectra were measured on samples dispersed in KBr and pressed into pellets. Occasionally samples were examined as solutions in chloroform.

Proton and carbon-13 nuclear Magnetic Resonance (^{1}H and ^{13}C) spectra were obtained at 200 MHz and 50.3 MHz respectively using varian XL-300 and Joel FX-200 spectrometers. The spectra were recorded in deuterochloroform ($CDCl_3$) unless otherwise specified. Tetramethylsilane (TMS) was used as an internal standard. Chemical shifts (δ) are reported in parts per million (ppm) relative to TMS and multiplicities are indicated by s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, bd = broad doublet, bs = broad singlet and dd = doublet of a doublet.

Spin-spin coupling constants (J) are given in Hertz (Hz). For some compounds, off resonance decoupled ^{13}C NMR spectra were used to assign the different atoms.

Mass spectra (MS) were recorded with a Mass Lab 12-250 gas chromatograph-mass spectrometer (GC-MS) system at an ionization potential of 70eV, and are given as mass to charge ratios (m/e) in atomic mass units (a.m.u.), with relative ion intensities in parentheses.

Ultraviolet-Visible (UV) spectra were determined using a Perkin-Elmer Lambda-3 spectrophotometer and a Shimadzu UV - 200 spectrometer.

For thin layer chromatography (TLC) and in some cases, preparative thin layer chromatography (PTLC), precoated silica gel plates (Merck, $60GF_{254}$, 2.5 cm x 7.5 cm, 0.25 mm layer thickness) were used. In some cases, PTLC was carried out using plates that were prepared by spreading a slurry of silica gel in water (2mm thickness) on 10 cm x 20 cm and 20 cm x 20 cm glass plates.

They were air dried for at least 24 hrs followed by heating in an oven for 1 hour at 120°C before they were used. TLC spots were visualized under UV light and by developing in an iodine chamber.

Chromatographic solvent systems used were:- chloroform, ethyl acetate , hexane, methanol, ethyl-acetate :methanol (9:1), ethyl acetate :hexane (1:9) (solvent system A); ethyl acetate :chloroform (1:4) (Solvent system B);ethyl acetate :hexane (2:3) (Solvent system C);ethyl acetate :hexane (1:4) (Solvent system E), dichloromethane. All solvent mixtures were determined on a volume/volume basis.

Preparation of shift reagents:-

Sodium methoxide: Metallic sodium (2.5 g) was added cautiously to methanol (100 ml). The resultant solution was stored in a plastic-stoppered glass bottle.

Sodium acetate: Powdered, anhydrous, analytical grade sodium acetate was used.

Aluminium chloride: Fresh, dry Aluminium chloride (5g) was added cautiously to analytical grade methanol (100 ml). Residual insoluble material dissolved with time. The resultant solution was stored in a plastic stoppered bottle.

Hydrochloric acid: Concentrated reagent grade HCl (50 ml) was added to distilled water (100 ml). The resultant solution was stored in a plastic stoppered bottle.

Boric acid: Anhydrous, powdered, analytical reagent grade was used.

3.2 Tephrosia Interrupta

Plant Material

The roots, stem, leaves and pods of Tephrosia interrupta were collected from Ngon'g Hills, south of Nairobi, Kenya. A voucher specimen No. 1/86 is deposited in the University Herbarium, Department of Botany, University of Nairobi.

3.3.0 Extraction of T. interrupta leaves

The leaves were air dried in the shade and then ground to a fine powder. The ground leaves (785 g) were successively extracted with n-hexane, chloroform and methanol at room temperature. Each extract was concentrated *in vacuo*. The extracts were all dark, gummy residues, 14.0, 19.2 and 9.3g respectively.

3.3.1 Separation and Purification of hexane extract of T. interrupta leaves

A column was packed with silica gel (300 g) in chloroform and a portion of the hexane extract (5.1g) introduced onto the column and eluted with chloroform. Nine fractions were collected.

Thin layer chromatography of fraction 2 using chloroform as eluant showed a purple spot when the plate was visualized under short range UV light and fluoresced blue under long range illumination. This fraction was concentrated to yield a black residue which was eluted through a small silica gel column using chloroform as eluant.

Four fractions were collected. Fraction 3 showed a single spot on the TLC plate. This was evaporated to yield an oil which gave a white precipitate on addition of methanol. The supernatant was decanted to leave a white precipitate which was purified by PTLC (solvent system B) to give 5-methoxy-isolonchocarpin (26) (50mg).

5-Methoxy-Isolonchocarpin (26). White powder, R_f 0.69 (solvent system B), m.p. 138-40° (Methanol) (Lit: 135°[89], 125-27°[45,50], 155°[81]) UV λ_{max} 260 sh, 269, 295 and 345 nm. IR: ν_{max} 2960, 2920, 2845, 1677, 1630, 1600, 1570, 1480, 1460, 1343, 1195, 1140, 1113, 1100, 1070, 910, 895, 880, 815, 760, 744, 700 and 640 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ ppm 7.36 - 7.48 (5H, m, H-2' - H-6'), 6.63 (1H, d, J = 10.2 Hz, H - 4"), 6.06 (1H, s, H-6), 5.47 (1H, d, J = 10.2 Hz, H - 3"), 5.43 (1H, dd, J_{cis} = 3.5 Hz, J_{trans} = 12.6 Hz, H-2) 3.90 (3H, s, OCH_3), 2.92 (1H, dd, J = 18.6 Hz, J = 12.6 Hz, H - 3_{trans}), 1.46

(6H, s, 2" - $C(CH_3)_2$. ^{13}C-NMR δ ppm 28.2/28.5 (C-2"$(CH_3)_2$), 45.6 (C-3), 56.2 (5-OCH_3), 78.0 (C-2"), 78.9 (C-2), 93.8 (C-6), 102.9 (C-8), 105.7 (C-4a), 116.0 (C-3"), 125.9 (C-2', C-6'), 126.3 (C-4'), 128.5 (C-4"), 128.7 (C-3', C-5'), 139.0 (C-1'), 158.8 (C-8a), 160.0 (C-5), 162.1 (C-7), 189.2(C-4). EI-MS, m/e (relative intensity): 336 (9.0, M^+), 321 (18.0, M-15]$^+$), 232 (4.0, RDA from M^+), 218 (13.0), 217 (100, RDA from M-15]$^+$), 202 (8.0), 175 (3.0), 174 (3.0), 146 (3.0), 104 (4.0), 103 (8.0), 77 (13.0).

Fractions 3 and 4 resulting from column chromatography of the hexane extract were pooled together after they showed similar spots on TLC plate on illumination with a UV lamp (the predominant spot was purple under short range and intense yellow under long range). Fractions 3 and 4 were separated further by column chromatography using solvent system B as eluant followed by PTLC using chloroform to give Isopongaflavone (28)(24 mg).

Isopongaflavone (28). Pale yellow crystals m.p. 213-7° (methanol) (Lit: 203°[81], 201-5°[89],

206-8°[45], 212-4°[101], 215-6°[100]). UV: λ_{max} 270, 350 nm. IR: ν_{max} 1637, 1595, 1560, 1480, 1445, 1370, 1295, 1240, 1195, 1150, 1110, 845, 770 and 690 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 7.89-7.84 (2H, m, H-2', 6'), 7.55-7.48 (3H, m, H-3', 4', 5'). 6.84 (1H, d, J = 10 Hz, H-4") 6.67 (1H, s , H-3) 6.34 (1H, s, H-6), 5.63 (1H, d, J = 10Hz, H-3"), 3.96 (3H, s , OCH_3), 1.51 (6H, s, 2"-$C(CH_3)_2$. ^{13}C -NMR δ ppm: 28.3 (C-2"$(CH_3)_2$), 56.4 (5-OCH_3), 78.1 (C-2"), 96.6 (C-6), 102.9 (C-8), 108.9 (C-3, C-4a), 115.3 (C-3"),125.9 (C-2', C-6'), 127.5 (C-4"), 128.9 (C-3', C-5'), 131.1 (C-4'), 131.9 (C-1'), 154.0 (C-5), 158.0 (C-8a), 160.2 (C-7), 160.8 (C-2), 177.6(C-4). EI-MS m/e (rel. int.): 334 (37), 320 (22), 319 (100), 305 (8), 303 (17), 290 (10), 289 (5), 218 (87), 217 (74), 202 (19), 187 (9), 167 (2), 159 (7), 153 (5), 146 (10), 145 (10), 144 (12), 105 (20), 102 (18), 77 (20).

Shinoda test for 5-methoxy-isolonchocarpin.(26). To compound 26 (3 mg) in absolute ethanol (3ml) was added magnesium granules and a drop of concentrated hydrochloric acid. A deep red colour formed. Magnesium was then replaced by zinc in the above test. No colour change was observed.

3.3.2 Separation and purification of the chloroform extract of T. interrupta leaves

After extracting T. interrupta leaves with n-hexane, the residue was dried for 24 hrs and then extracted with chloroform in the cold for 21 days. Evaporation of the solvent from the filtrate in vacuo gave a dark gummy residue. (19.2g)

A portion of the extract (5.7 g) was separated by column chromatography using solvent system B. PTLC of fraction 6 using chloroform as eluant yielded 5-methoxyisolonchocarpin (26) (49 mg). (See pg. 79 for spectral data).

The main component of fraction 5 was contaminated with green colouring matter believed to be chlorophyll. This was removed by adding one spatula of activated carbon followed by PTLC using solvent system C to give 5-Methoxy-Isolonchocarpin 26 (123 mg)(See pg. 79 for spectral data).

Fractions 9-11 showed similar spots on TLC. They were pooled together and concentrated to dryness. Crystallization of the resulting oil yielded isopongaflavone (28) (42 mg) (See pg. 80 for spectral data).

3.3.3 Separation and Purification of the Methanol extract of T. interrupta leaves

After extracting T. interrupta leaves with chloroform, the residue was extracted in the cold using methanol for 17 days. Filtration followed by evaporation in vacuo of the extract gave a dark residue (9.3g).

A portion of the residue (5.3g) was introduced onto a chromatographic column and eluted with ethyl acetate followed by an ethyl acetate: methanol (9:1) mixture.

Fractions 1 and 2 were combined, decolourized using activated charcoal and subjected to PTLC using solvent system B to given 5-methoxy isolonchocarpin (26) (40 mg) (See pg 79 for spectral data).

Fractions 3-5 were also combined, decolourized using activated charcoal and subjected to PTLC using solvent system B to give Isopongaflavone (28) (50 mg) (See pg 80 for spectral data).

Fractions 7-14 were pooled together and concentrated. The concentrate was a precipitate which was filtered off and washed with ethyl acetate

followed by drying under vacuum. This resulted in a pure compound (7 mg) whose identity has not been ascertained.

3.4.0 Extraction of T. interrupta Roots

The air dried roots were ground to a fine powder (500 g). The powder was placed in a large conical flask and extracted successively with n-hexane and ethyl acetate at room temperature. Each extract was concentrated *in vacuo* using a rotary evaporator. The n-hexane extract gave a light yellow sticky paste (0.95g) and the ethyl acetate extract (9.8g) was brown.

3.4.1 Separation and Purification of n-hexane extract of T. interrupta roots

A column was packed with silica gel in pure n-hexane. The n-hexane extract (0.95 g) was separated by column chromatography using solvent system D. A total of 23 fractions of varying amounts (25 - 100 ml) were collected.

Fractions 10-13 from column chromatography of the hexane extract were combined, concentrated

and purified by PTLC using solvent system B to yield Isopongaflavone (28) (4 mg) (See pg. 80 for spectral data.)

Fractions 18-12 showed one main spot on illumination with a UV lamp. PTLC using solvent system B resulted in compound 30 (16 mg) which was identified as a mixture of Rotenone (30a) and Deguelin (30b).

Rotenone (30a). White crystals R_f 0.63 (Solvent system B), m.p. 160 - 62° (Methanol), (Lit: 162.5-164.0°[95], 163-164°[93], 167-168°[41]): UV: λ_{max} 206, 232, and 290 nm. IR: ν_{max} 1669, 1595, 1502, 1460, 1343, 1305, 1235, 1210, 1190, 1140, 1089, 1005, 950, 905, 861 and 813 cm^{-1}. ^1H NMR ($CDCl_3$): δ ppm 1.77 (3H, s, 8'-CH_3), 2.97 (1H, q, H-4'), 3.31 (1H, 1, H-4'), 3.76 (3H, s, 3-OCH_3), 3.79 (1H, s, 2-OCH_3), 3.81 (1H, s, H-12a), 4.16 (1H, bd, H-6), 4.59 (1H, q, H-6), 4.90 (1H, s, H-6a), 5.07 (1H, s, H-7'), 5.24 (1H, t, H-5'), 6.46 (1H, s, H-4), 6.51 (1H, d, J = 8.6 Hz, H-10), 6.78 (1H, s, H-1), 7.84 (1H, d, J = 8.6 Hz, H-11). ^{13}C NMR δ ppm: 110.4 (C-1), 143.9 (C-2), 149.5(C-3), 100.9 (C-4), 147.4 (C-4a), 66.3 (C-6), 72.2 (C-6a), 157.9(C-7a), 113.0(C-8),

167.4 (C-9), 104.8 (C-10), 130 (C-11), 113.3 (C-11a), 188.9 (C-12), 44.6 (C-12a), 104.8 (C-12b), 31.3 (C-4'), 87.8 (C-5'), 142.9 (C-6'), 112.5 (C-7'), 17.1 (C-8'), 55.8 (C-2-OCH_3), 56.3 (C-3-OCH_3). EI-MS (rel. int.): 394 (15, M^+), 203 (3), 192 (100), 191 (37), 187 (1), 177 (16), 161 (2), 149 (3), 134 (2), 131 (3), 121 (4), 107 (2), 106 (4), 95 (1), 77 (9).

Having made spectroscopic assign ment due to Rotenone (30a,) it became possible to make assignments due to Deguelin (30b) as below:

Deguelin (30b). ^{1}H-NMR ($CDCl_3$): δ 1.38 (3H, s, H-8'), 1.45 (3H, s, H-8'), 3.76 (3H, s, 3-OCH_3), 3.79 (3H, s, 2-OCH_3), 3.81 (1H, s, H-12a), 4.93 (1H, m, H-6a), 5.55 (1H, d, J = 10Hz, H-5'), 6.43 (1H, d, J = 8.6 Hz, H-10), 6.43 (1H, s, H-4), 6.63 (1H, d, J = 10 Hz), H-4'), 6.79 (1H, s, H-1), 7.73 (1H, d, J = 8.6 Hz, H-11).

The ^{1}H-NMR of the mixture 30 was run in d_6-benzene to give the following results:

Rotenone (30a): ^{1}H NMR (C_6D_6): δ ppm 1.46 (3H, s, H, H-8'), 2.69 (2H, d, J = 9.50 Hz, H-4'), 3.25 (3H, s, 3-OCH_3), 3.39 (3H, s, 2-OCH_3),

3.50 (1H, s, H-6), 4.70 (1H, , H-7'), 4.71 (1H, s, H-5'), 4.06 (1H, t, H-6a), 4.25 (1H, q, H-6), 4.92 (1H, bs, H-7'), 3.59 (1H, s, H-12a), 6.52 (1H, s, H-4), 7.13 (1H, s, H-1), 6.37 (1H, d, J = 8.55 Hz, H-10), 8.14 (1H, d, J = 8.55 Hz, H-11).

Deguelin (30b). ^{1}H-NMR (C_6D_6): δ ppm 1.03 (1H, s, CH_3-8'), 1.18 (1H, s, CH_3-8'), 3.22 (3H, s, 3-OCH_3), 3.37 (3H, s, 2-OCH_3), 3.48 (1H, s, H-6), 3.53 (1H, s, H-12a), 4.00 (1H, m, H-6a), 4.25 (1H, q, H-6), 5.10 (1H, d, J = 10Hz, H-5'), 6.42 (1H, , H-4), 6.43 (1H, bd, J = 8.55 Hz, H-10), 6.64 (1H, bd, J = 10 Hz, H-4'), 7.13 (1H, s, H-1), 8.04 (1H, d, J = 8.55 Hz, H-11).

3.4.2 Separation and Purification of the ethyl acetate extract of T. interrupta roots

After extracting with n-hexane the roots were dried and then subjected to repeated extractions with ethylacetate in the cold until the extract was colourless. The combined extracts were concentrated in vacuo to give a brown residue (9.8g).

A large column was packed with silica gel in hexane. The extract was introduced onto the column and eluted with solvent systems D, E and ethyl acetate in that order. Each fraction that was collected was monitored by TLC using solvent system E.

Fractions 4 and 5 were combined and concentrated to give a brown oil (450 mg). This was introduced onto another chromatographic column and eluted using solvent system E. Six fractions were collected.

Fraction 1 showed one main spot with traces of impurities on illumination of the TLC plate with UV light. PTLC using solvent system E gave compound 30 which was a mixture of rotenone (30a) and deguelin (30b) (48 mg) (See pg. 85 for spectral data).

Fractions 2-5 were pooled together and concentrated. The concentrate was a thick brown oil. Trituration with methanol gave a white precipitate. The supernatant was decanted and the remaining solid recrystallized from methanol to give Rotenone (30a) (18 mg)

3.5.0 Extraction of T. interrupta Pods

These were cut into small pieces and then dried. The dried pods (40g) were extracted with ethyl acetate for 28 days in a conical flask at room temperature. The extract was filtered and concentrated leaving a dark greenish oil (0.47g).

3.5.1 Separation and Purification of the ethyl acetate extract of T. interrupta Pods

A small column was packed with silica gel (40 g) in solvent system B. The extract (0.47 g) was introduced onto the column and eluted with solvent system B followed by ethyl acetate. Ten fractions were collected.

Fraction 1 from the column was concentrated to dryness to give a dark greenish oil (258 mg) that was separated by PTLC using chloroform as eluant to give 5-methoxy isolonchocarpin (26) (16.9 mg) (See pg. 79 for spectral data).

Fractions 2-4 were pooled together after they showed similar spots on the TLC plate on illumination

with UV light. PTLC on the concentrated material using solvent system B gavea pale yellow solid that was found to be Isopongaflavone (28) (12 mg) (See pg. 80 for a spectral data).

Fraction 10 was collected after flashing the column with ethyl acetate PTLC using ethyl acetate resulted in a compound (2mg) that was not fully characterised.

3.6.0 Extraction of T. interrupta stem

The dry powdered stem material (749.7g) of T. interrupta was extracted with 3.5 litres of methanol at room temperature in a large conical flask. The extract was concentrated to give a dark gummy residue (54g). This was dissolved in 20 ml methanol and partitioned between chloroform and water. Some brown insoluble material resulted which was filtered off leaving chloroform and the aqueous layers. The chloroform fraction was then partitioned between aqueous methanol (1:4) and hexane. A brown insoluble material was observed again and removed as previously.

3.6.1 Separation and Purification of the hexane extract of T. interrupta stem

The hexane extract from the above process was concentrated in vacuo to give a black gummy residue (1.03 g). This was dissolved in hexane (10 ml), spotted and eluted on a TLC plate using chloroform. The spot with R_f value of 0.75 predominated.

Column chromatography of this extract was carried out using chloroform as eluant.

Fraction 4 which appeared as a yellow band on the column was also observed as a yellow solution. The solvent was evaporated by bubbling nitrogen gas through the solution. The solid material that remained in the test tube dissolved in methanol on slight warming. On cooling, some white suspension formed. This was filtered off, washed with cold methanol and was not examined further. The filtrate was concentrated, spotted and eluted using chloroform on a TLC plate. Two spots were observed with R_f values 0.75 and 0.81. Column chromatography of the filtrate using solvent system A as eluant afforded a yellow needlelike crystalline compound, Pongachalcone-I (29) (32 mg).

Pongachalcone-I (29). Yellow needlelike crystals, R_f 0.75 (Chloroform), m.p. 105-8° (Methanol), (Lit: 105°[104], 108°[103], 109-11°[102]). UV: λ_{max}^{MeOH} 230, 288, 299 and 344 nm, $\lambda_{max}^{MeOH + AlCl_3}$ 224, 286sh, 299 and 376 nm, $\lambda_{max}^{MeOH + AlCl_3 + HCl}$ 224, 286sh, 299 and 376 nm, $\lambda_{max}^{MeOH + NaOCH_3}$ 238sh, 288 and 348 nm, $\lambda_{max}^{MeOH + NaOAC}$ 224, 286, 299 and 344 nm, $\lambda_{max}^{MeOH + NaOAC + H_3BO_3}$ 224, 286, 299 and 344 nm IR: ν_{max} 3400, 2920, 1635, 1550, 1445, 1420, 1130, 1190, 1140, 1120, 805, 760 and 695 cm^{-1}. ^1H - NMR ($CDCl_3$): δ ppm 1.46 (6H, s, $C(CH_3)_2$), 3.92 (3H, s, OCH_3), 5.47 (1H, d, J = 10.2 Hz, H-3"), 5.93 (1H, s, H-5'), 6.65 (1H, d, J = 10.2 Hz, H-4"), 7.38 - 7.44 (3H, m, H-3, 4, 5), 7.48 - 7.63 (2H, m, H-2, 6), 7.77 (1H, d, J = 15.58 Hz, H - α), 7.89 (1H, d, J = 15.58 Hz, H -β) and 14.5 (1H, s, 2' -OH). ^{13}C - NMR ($CDCl_3$) δ ppm 193 (C=O), 128.9 (C-2,C-6), 128.3 (C-3, C-5), 130.2 (C-4), 106.1 (C-1'), 162.6 (C-2'), 91.5 (C-3'), 162.5 (C-4') 125.4 (C-5'), 160.3 (C-6'), 116.1(C-3"), 127.7(C-4"), 78.8 (C-2"), 28.4(2$(CH_3)_2$, 130.0 (C-1), 55.9 (OCH_3), 116.1 (C- α) and 135.6 (C- β).

EI-MS, m/e (rel. int): 336 (15, M^+), 321 (39, M-15), 217 (100, (M-15)-104), 202 (10), 160 (17), 152 (4), 131 (5), 115 (4), 104 (2), 103 (13), 91 (5), 77 (17).

3.6.2 Separation and Purification of the chloroform extract of T. interrupta stem

Evaporation of the methanol from the aqueous methanol fraction and subsequent extraction of the residue with chloroform gave rise to a black gummy residue (2.4g).

Column chromatography using chloroform followed by solvent system B as eluant was undertaken.

The first twelve fractions from the column showed only one intense yellow spot on the TLC plate with R_f value 0.75, using chloroform as the eluant. PTLC on the combined and concentrated residue gave Pongachalcone-I (29)(23 mg).

Fractions 13-18 were combined after they had shown similar spots on TLC plate. The spot with R_f value 0.36 (solvent system B) predominated.

Preparative thin layer chromatography resulted in Isopongaflavone 28 (36.3 mg) (See pg 80 for spectral data).

3.7 TEPHROSIA LINEARIS

Plant material

The pods and roots of Tephrosia linearis were collected at Fourteen Falls Thika. A voucher specimen No. 1/87 is deposited in the University Herbarium, Department of Botany, University of Nairobi.

3.8.0 Extraction of T. linearis pods

These were ground to a powder (90g) that was extracted with ethyl acetate in the cold for two weeks.

3.8.1 Separation and Purification of the ethyl acetate extract of T. linearis pods

The extract was concentrated in vacuo to give a dark black gum (2.32g). This was introduced onto the column packed with silica gel (138 g) in chloroform and eluted with the same solvent followed by solvent system B, chloroform:ethyl acetate (1:1) and ethyl acetate in that order.

Fractions 2 and 3 were separately concentrated, spotted and eluted on a TLC plate using solvent system B. Each fraction showed two spots with the predominant one having R_f value 0.30. The two were pooled and purified by PTLC using solvent system D to give Toxicarol 32 (11.6 mg).

Toxicarol (32). m.p. 104-6°(methanol). UV: $\lambda_{max}^{CH_3OH}$ 230, 270, 294, 308sh and 360 nm; $\lambda_{max}^{CH_3OH + NaOCH_3}$ 278, 300sh and 380 nm; $\lambda_{max}^{CH_3OH + AlCl_3}$ 220sh, 270sh, 280, 320 and 420 nm, $\lambda_{max}^{CH_3OH + AlCl_3 + HCl}$ 220, 270sh, 278, 320 and 420 nm, $\lambda_{max}^{CH_3OH + NaOAc}$ 278, 294sh and 360 nm and $\lambda_{max}^{CH_3OH + NaOAc + H_3BO_3}$ 278, 294sh, 308sh and 360 nm. IR: ν_{max} (CCl_4 solution, NaCl cell) 1625, 1505 1400, 1340, 1300, 1190, 1105 and 950 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.37 (3H, S, CH_3-8'), 1.43 (3H,s, CH_3-8), 3.79 (3H, s, OCH_3), 3.80 (1H, s, H-12a), 3.82 (3H, s, OCH_3 , 4.17 (1H, bd, J = 12.10 Hz, H-6), 4.61 (1H, q, H-6), 4.85 (1H, m, H-6a), 5.47 (1H, d, J = 10 Hz, H-5'), 5.95 (1H, s, H-10), 6.46 (1H, s, H-4), 6.65 (1H, d, J = 10 Hz, H-4'), 6.86 (1H, bs, H-1), 12.19 (1H, s, OH-11). EI-MS m/e (rel. int.): 410 (15),

395 (10), 219 (3), 218 (2), 217 (8), 203 (13), 197 (10), 193 (13), 192 (100), 191 (35), 179 (23), 177 (16), 121 (4), 106 (4), 93 (6), 91 (5), 77 (9), 69 (12), 53 (4) and 43 (8).

3.9.0 Extraction of T. linearis roots

A powdered sample of the roots (129g) of T. linearis were extracted at room temperature in a 2 litre conical flask using hexane and ethyl acetate. Hexane extraction was done for three weeks and on evaporation of the solvent, a light yellow residue (0.95 g) was obtained. Ethyl acetate extraction was done for 28 days at room temperature and on evaporation gave a yellow residue (6.9 g).

3.9.1 Separation and Purification of the hexane extract of T. linearis roots

A small column was packed with silica gel (50g) in chloroform. The hexane extract (0.95 g) was introduced onto the column and eluted using chloroform.

Fraction 3 from the column of the hexane extract showed two main spots R_f values of 0.66 and 0.54 on the TLC plate on illumination with UV light.

This fraction was concentrated to give a crude oil (106 mg). PLTC using ethylacetate: dichloromethane (1:9) mixture led to the isolation of a mixture of Rotenone (30a) and Deguelin (30b) (9.4 mg) (physical and spectral data as on pg. 85) and a mixture of Tephrosin (33a) and 12a-hydroxyrotenone (33b).

Mixture of Tephrosin (33a) and 12a-hydroxyrotenone(33b) (17.2 mg). UV: λ_{max} 236, 270 and 294 nm, $\lambda_{max}^{CH_3OH + NaOCH_3}$ 270 and 290 nm; $\lambda_{max}^{CH_3OH + AlCl_3}$ 236, 270 and 294; $\lambda_{max}^{CH_3OH + NaOAC}$ 270 and 290 nm; $\lambda_{max}^{CH_3OH + NaOAC + H_3BO_3}$ 270 and 290 nm; ^{1}H-NMR ($CDCl_3$): δ 1.39 (3H, s), 1.45 (3H, s). δ 1.76 (3H, s), 2.96 (1H, q), 3.32 (1, s), 3.73 (3H, s), 3.79 (3H, s), 3.81 (3H, s), 4.19 (1H, bd, J = 11.95 Hz), 4.60 (1H, q), 4.93 (1H, m), 5.08 (1H, s), 5.24 (1H, t), 5.56 (1H, d, J = 9.89 Hz), 6.46 (1H, s), 5.51 (1H, d, J = 8.43 Hz), 6.65 (1H, d, J = 10.14 Hz), 6.77 (1H, s), 6.79 (1H, s), 7.73 (1H, d, J = 8.76 Hz), 7.83 (1H, d, J = 8.67 Hz) 7.83 (1H, d, J = 8.67 Hz). EI-MS, m/e (rel. int): 410 (12), 209 (13), 208 (100), 207 (47), 203 (10), 197 (2), 193(5), 192(2), 191 (3), 187(6), 181(5), 165(8), 161(2), 151(2), 109 (5), 105 (4),

95(4), 93(4), 91(5), 77(7), 69(7), 57(5), 55(13) and 43(10).

Fractions 4 and 5 were pooled after they showed similar spots on a TLC plate at R_f values of 0.54 and 0.66 (chloroform). PTLC using ethyl acetate dichloromethane (1:9) mixture yielded a mixture of Rotenone (30a) and Deguelin (30b)(7 mg) and a mixture of Tephrosin (33a) and 12-hydroxyrotenone (33b) (6 mg).

3.9.2 Separation and Purification of the ethyl acetate extract of T. linearis roots

After extracting the powdered roots of T. linearis with hexane, the material was left to dry. Ethyl acetate was then introduced and left to extract for 28 days in the cold. The extract was filtered and concentrated in vacuo to give a yellow residue (6.9g). On spotting and eluting using chloroform, the components with R_f values 0.54 and 0.66 predominated.

A large column was packed with silica gel in hexane. The sample (6.9g) was introduced onto the column and eluted using chloroform and ethyl acetate respectively.

Fractions 7,8 and 9 showed similar spots and were thus pooled together. Spots with R_f values 0.54 and 0.66 (chloroform) predominated. The combined fractions were purified by further column chromatography using an ethyl acetate:hexane (1:1) mixture.

Fraction 1 collected from column chromatography of the pooled fractions 7,8 and 9 was concentrated to give a brown gum (102 mg). PTLC yielded compound 30a (16 mg) and Deguelin (30b) (physical and spectral data as on pg 85).

Fractions 3 and 4 were also pooled and concentrated to give a brown gum (92 mg). PTLC yielded compound 33 (7 mg) which was a mixture of Tephrosin (33a) and 12-hydroxyrotenone (33b) (spectral data as on pg 97).

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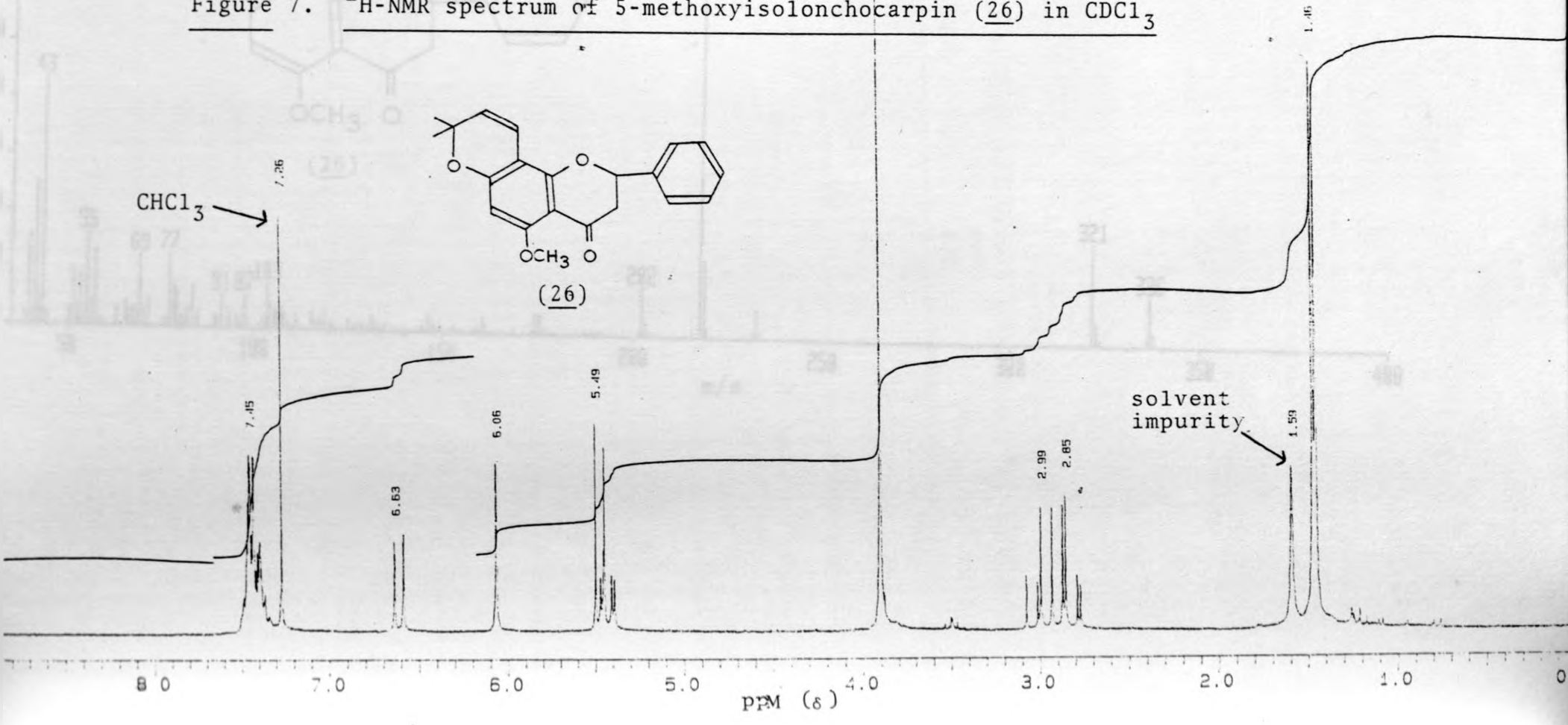
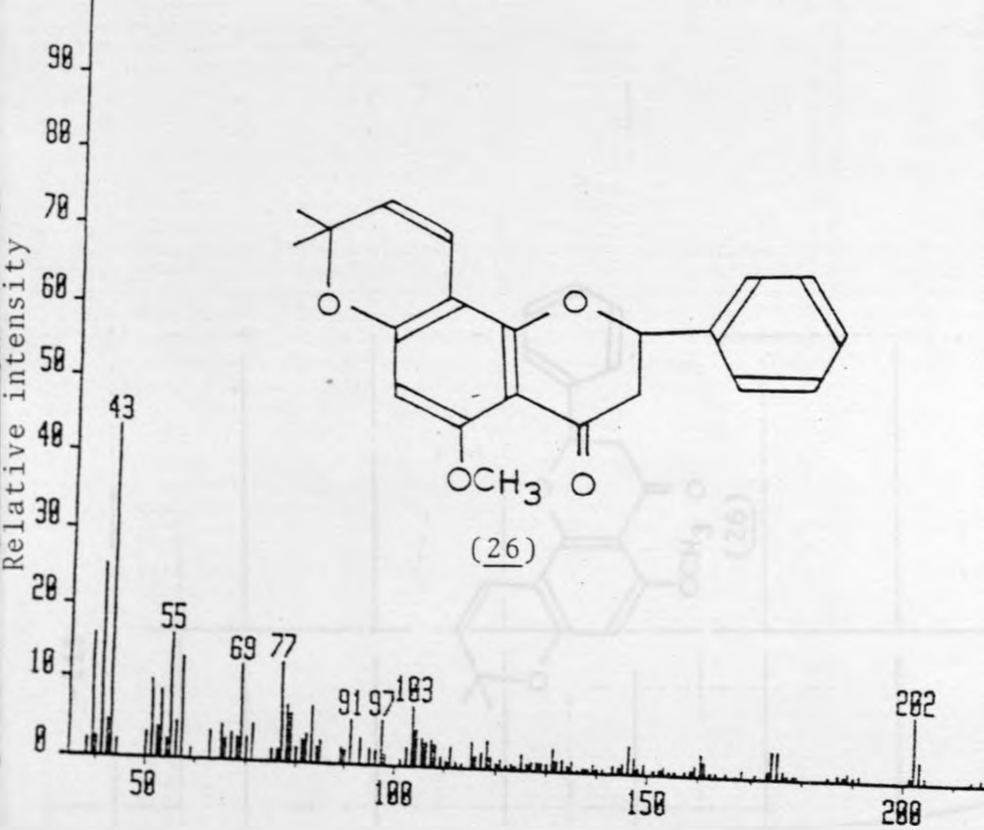
Figure 7. ^{1}H-NMR spectrum of 5-methoxyisolonchocarpin (26) in $CDCl_3$

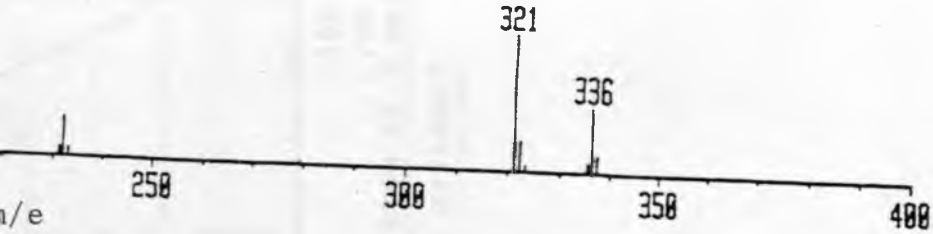


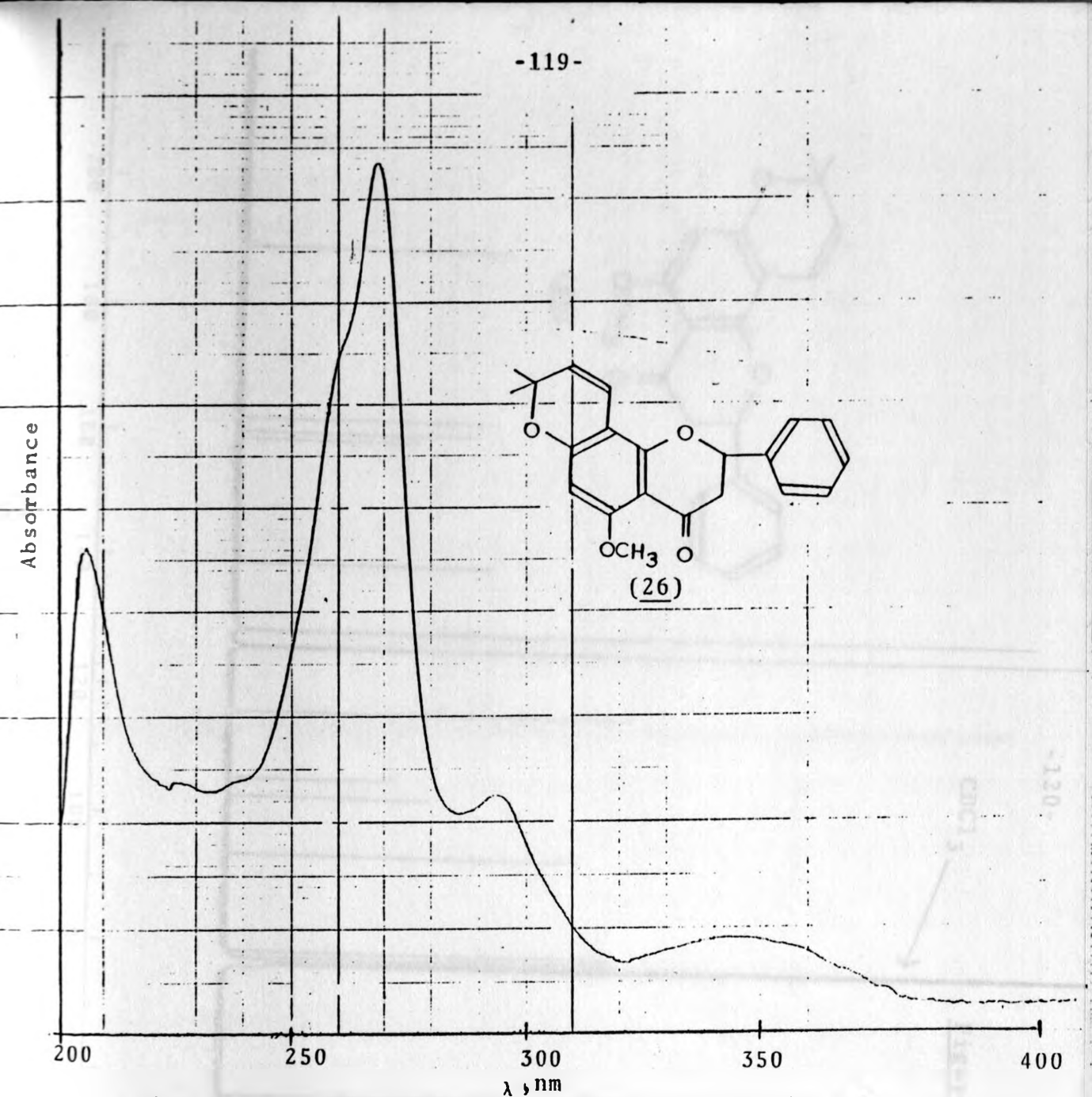
(26)


Relative intensity
90
80
70
60
50
40
30
20
10
0
43
55
69
77
91
97
183
282
50
100
150
200
OCH3
O
O
O

(26)

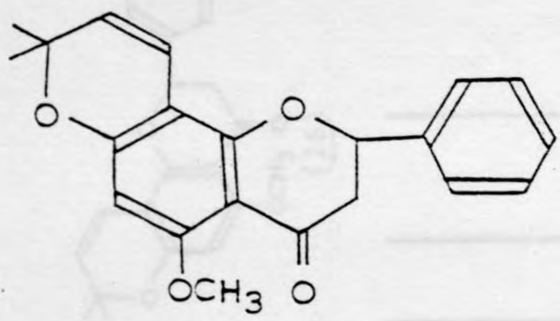
Figure 8. Mass spectrum of 5-methoxyisolon-carpin (26).





(26)

Figure 9. UV spectrum of 5-methoxyisoloncarpin (26) in methanol.



(26)

Figure 10. ^{13}C-NMR spectrum of 5-methoxy-isolonchocarpin (26) in $CDCl_3$

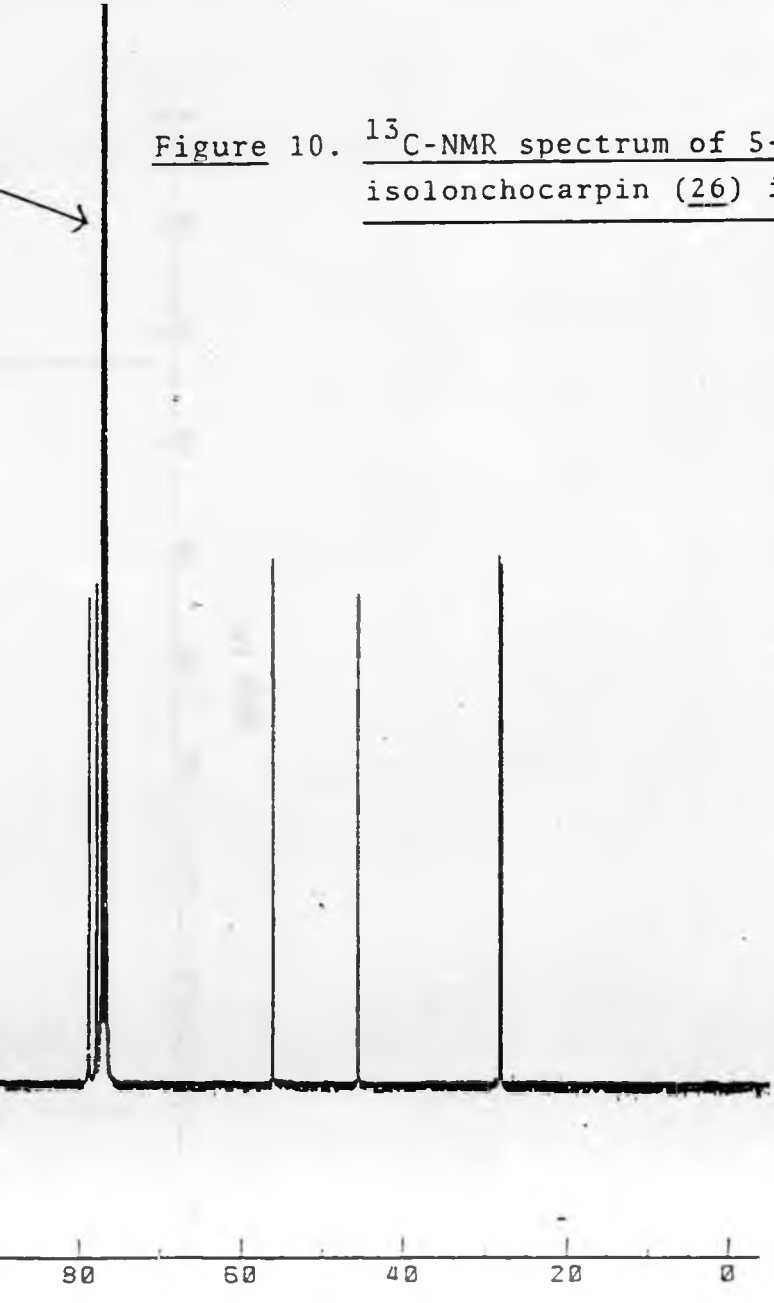
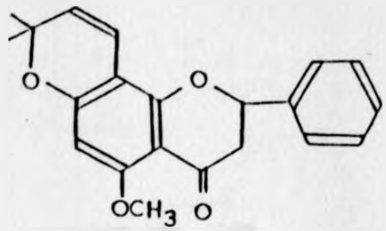
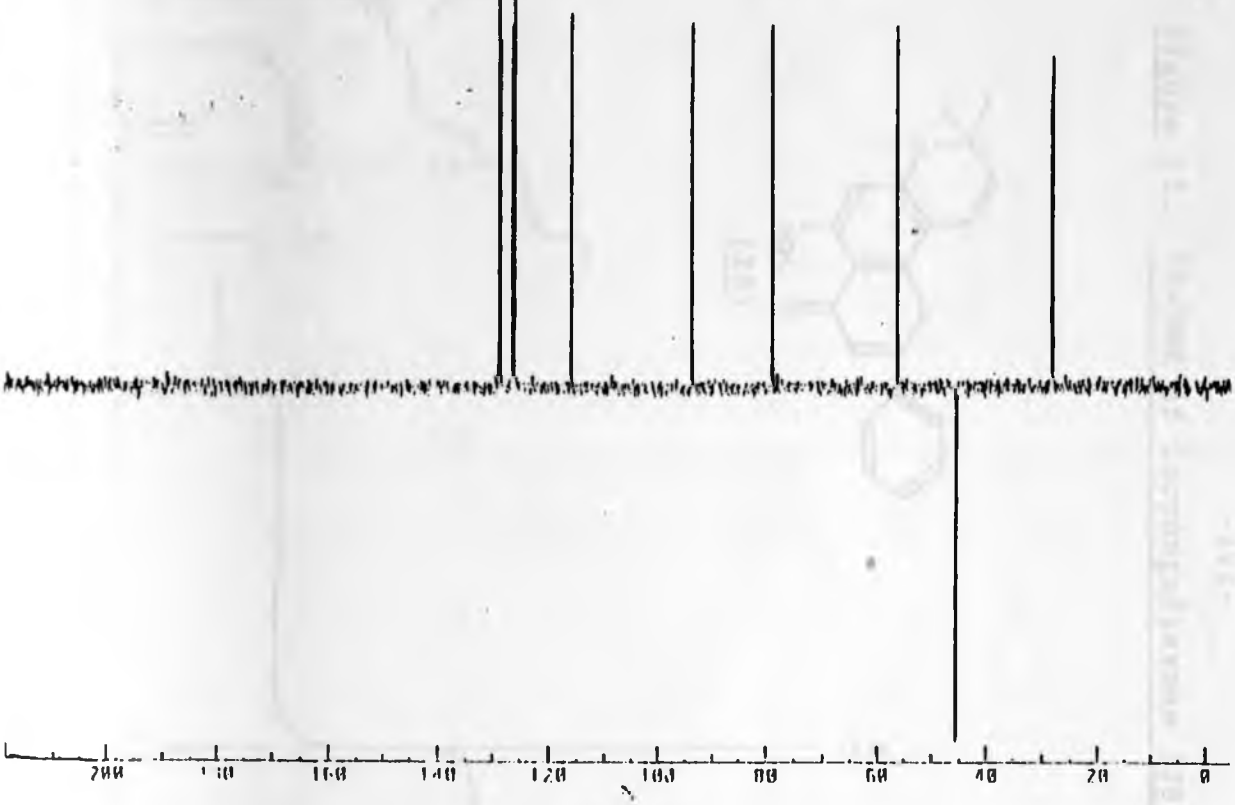


Figure 11. DEPT. spectrum of 5-methoxy-isolonchocarpin (26) at 135°C.

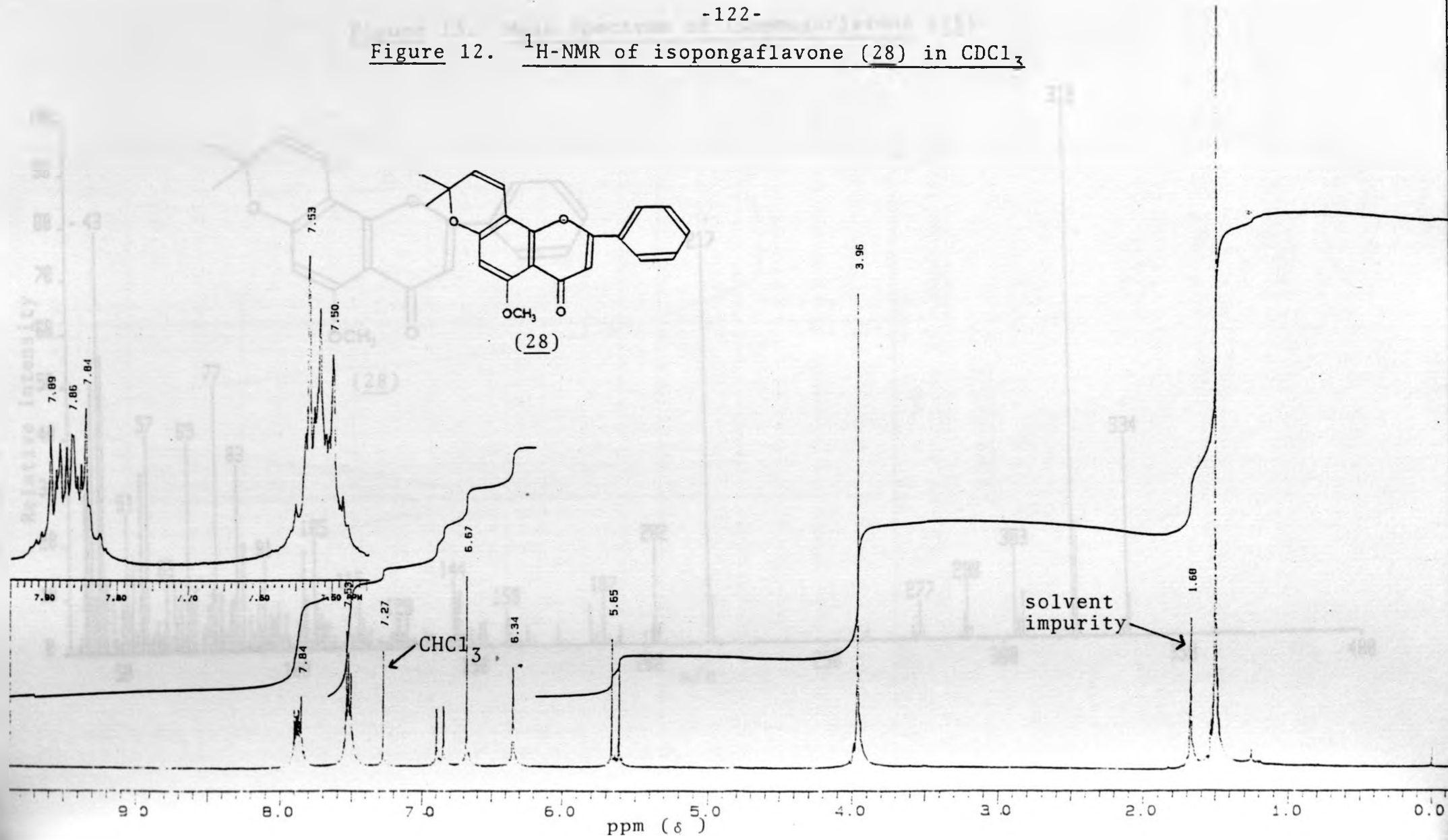


(26)



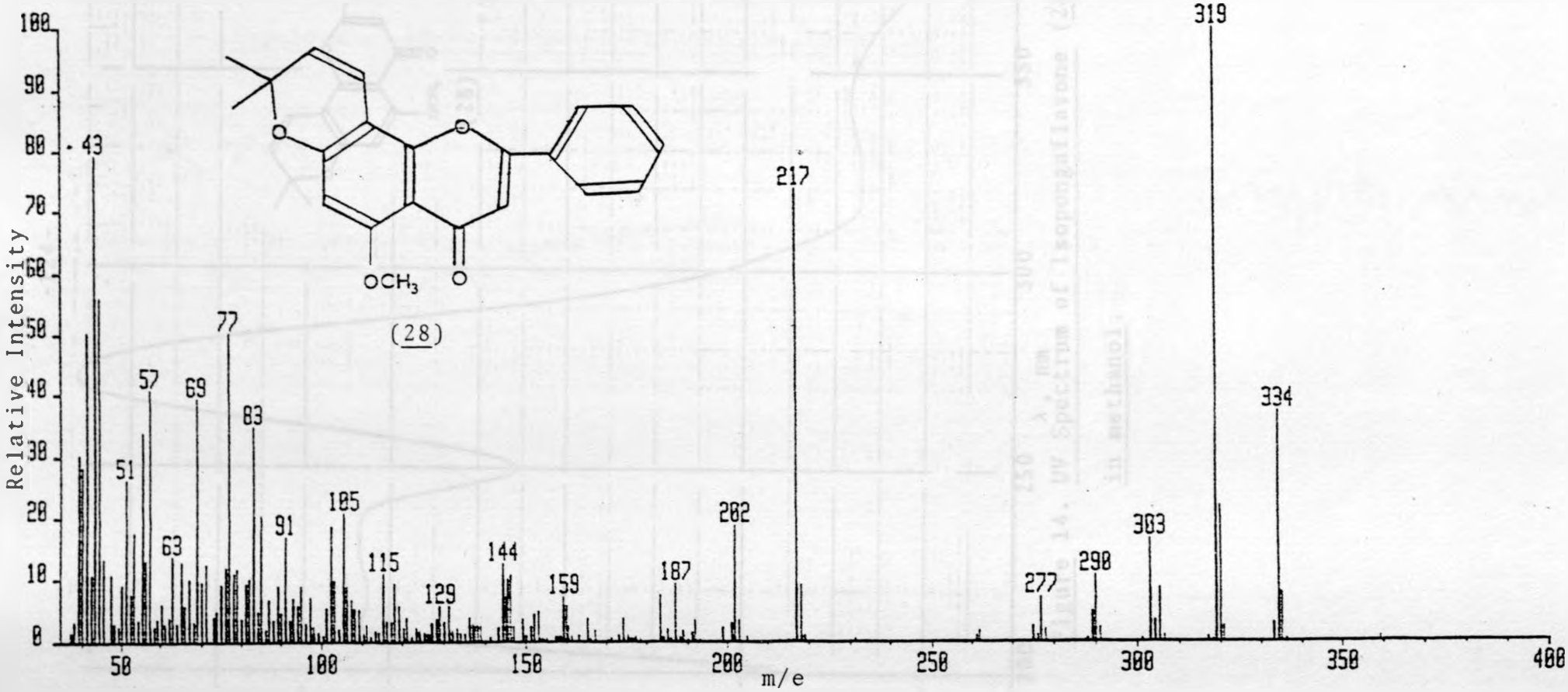
ppM (δ)

Figure 12. ^{1}H-NMR of isopongaflavone (28) in $CDCl_3$



(28)

Figure 13. Mass spectrum of Isopongaflavone (28)



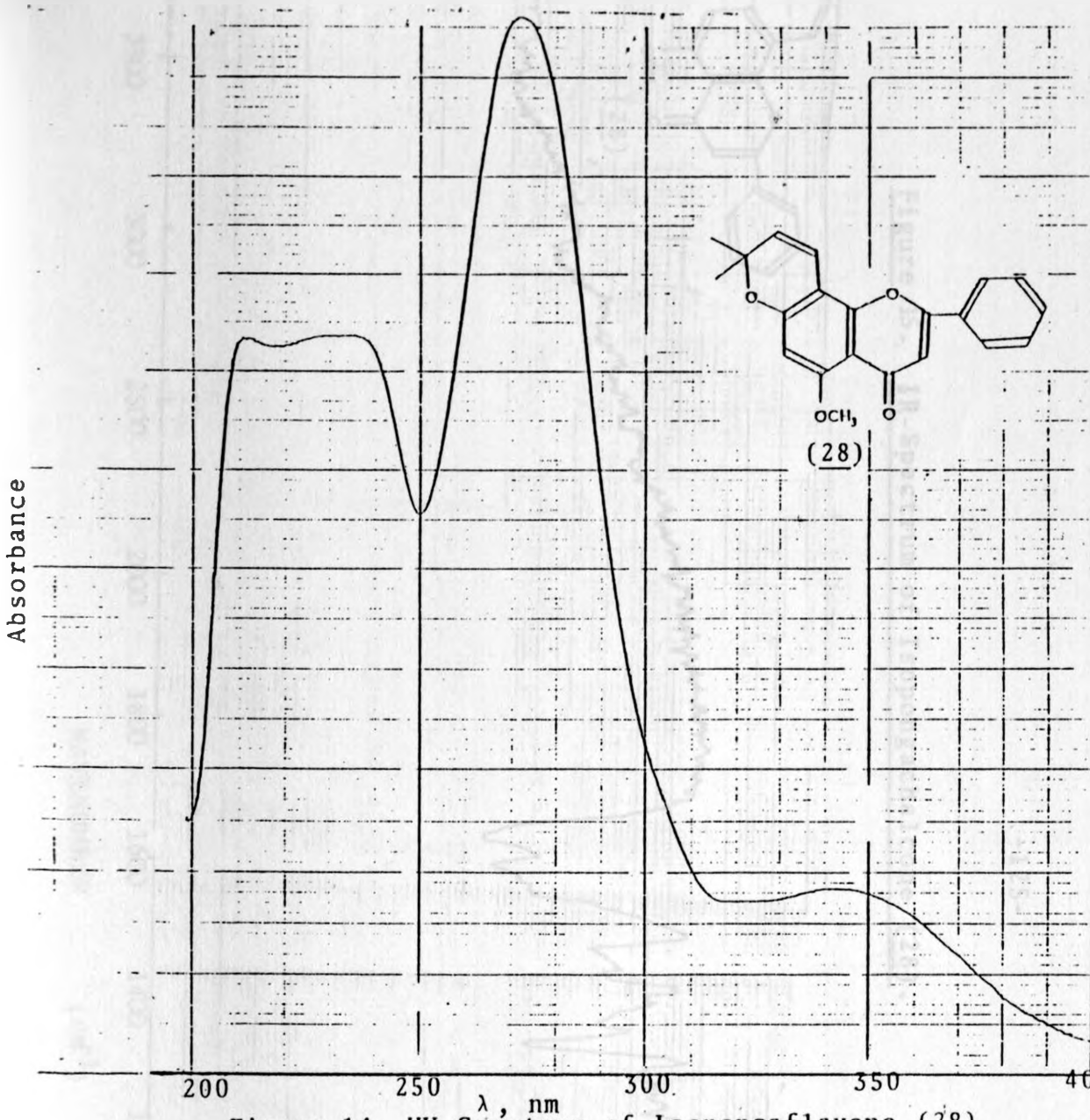


Figure 14. UV Spectrum of Isopongaflavone (28) in methanol.

Figure 15. IR-Spectrum of Isopongachalcone (28).

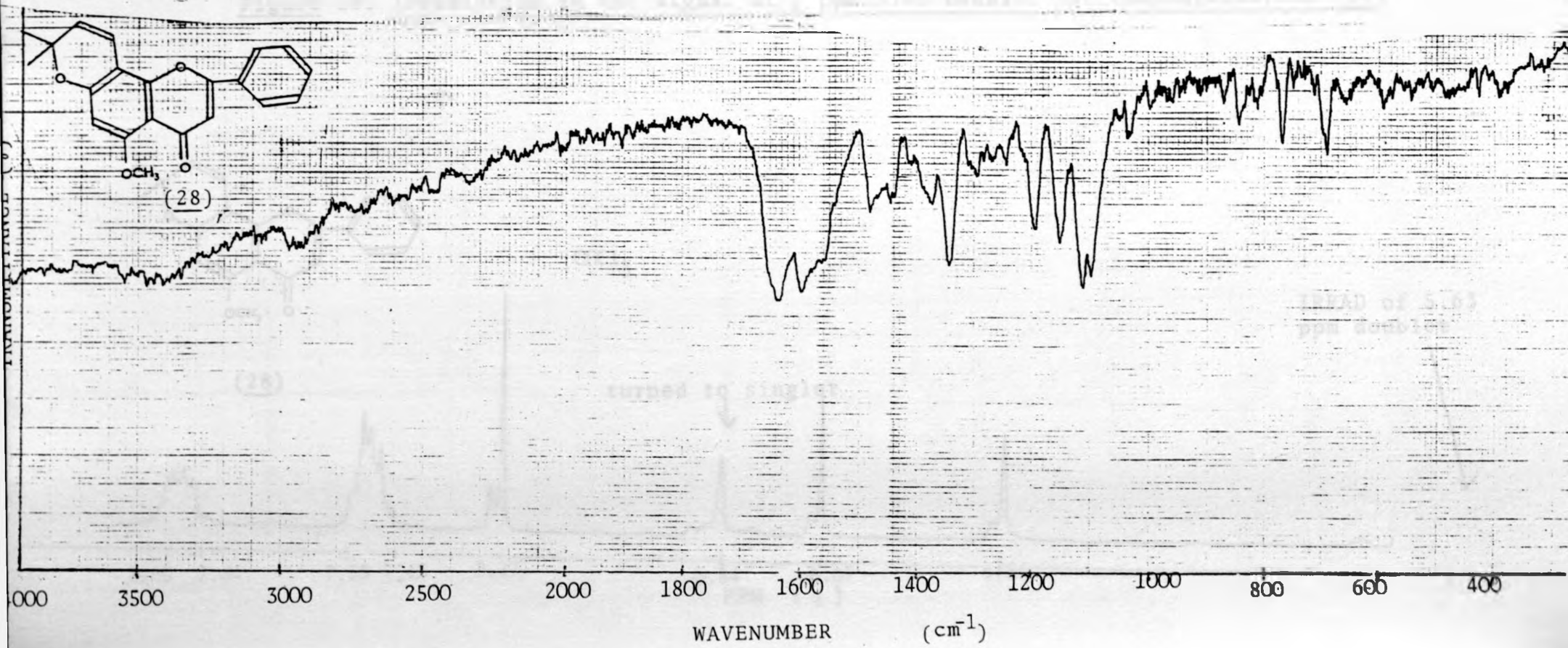


Figure 16. Irradiation on the signal at δ ppm 5.63 doublet for Isopongaflavone (28)

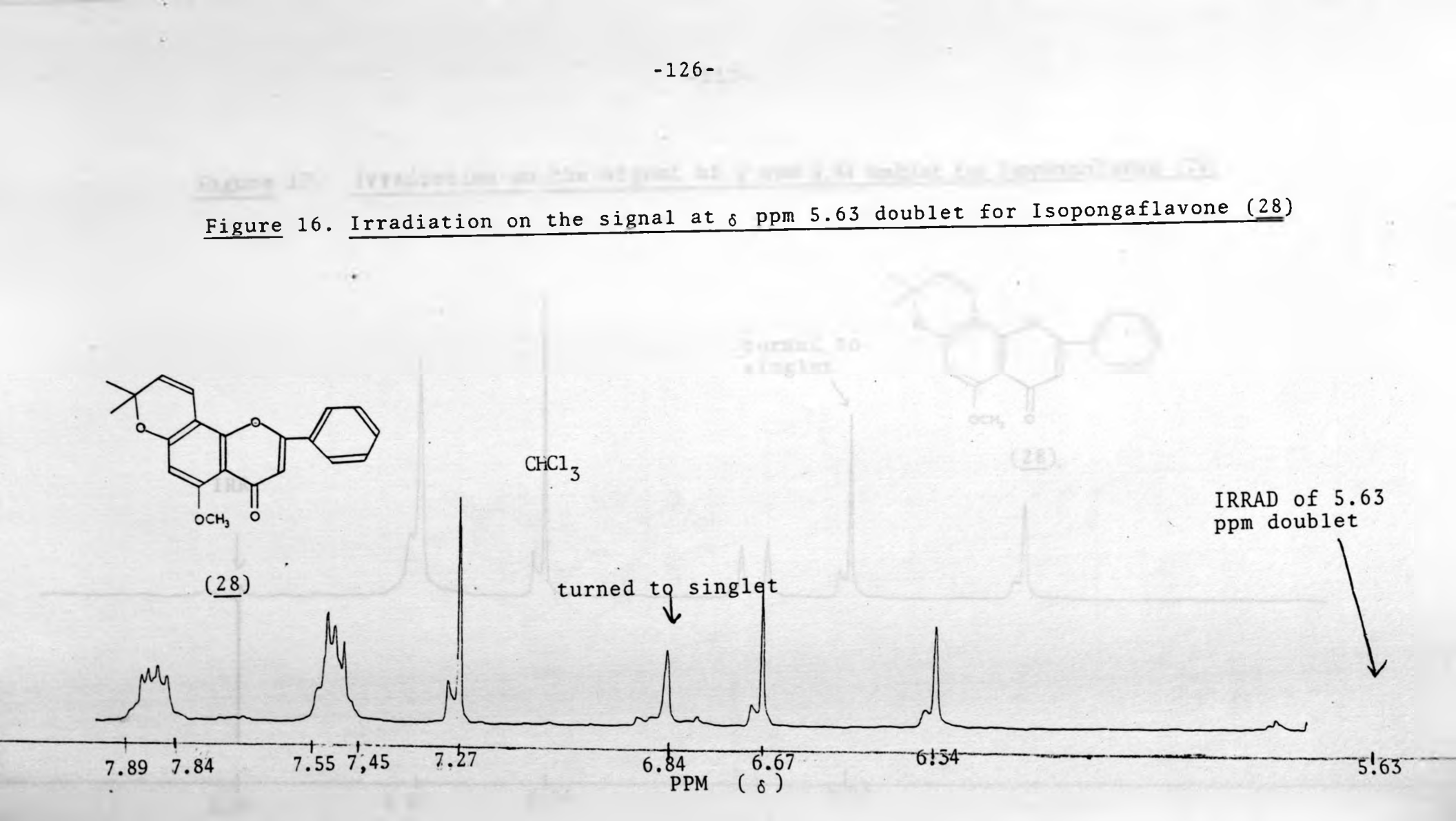


Figure 17. Irradiation on the signal at δ ppm 6.84 doublet for Isopongaflavone (28)

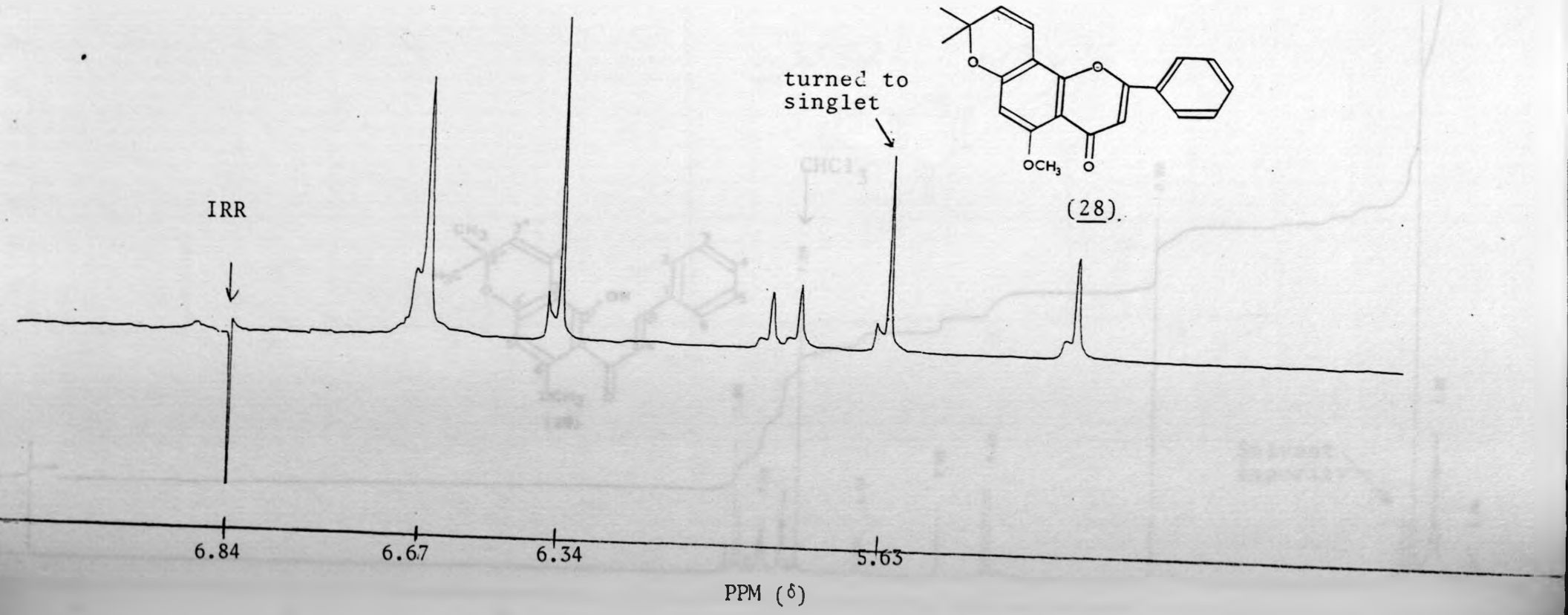


Figure 18. ^{1}H - NMR of Pongachalcone-I (29) in $CDCl_3$

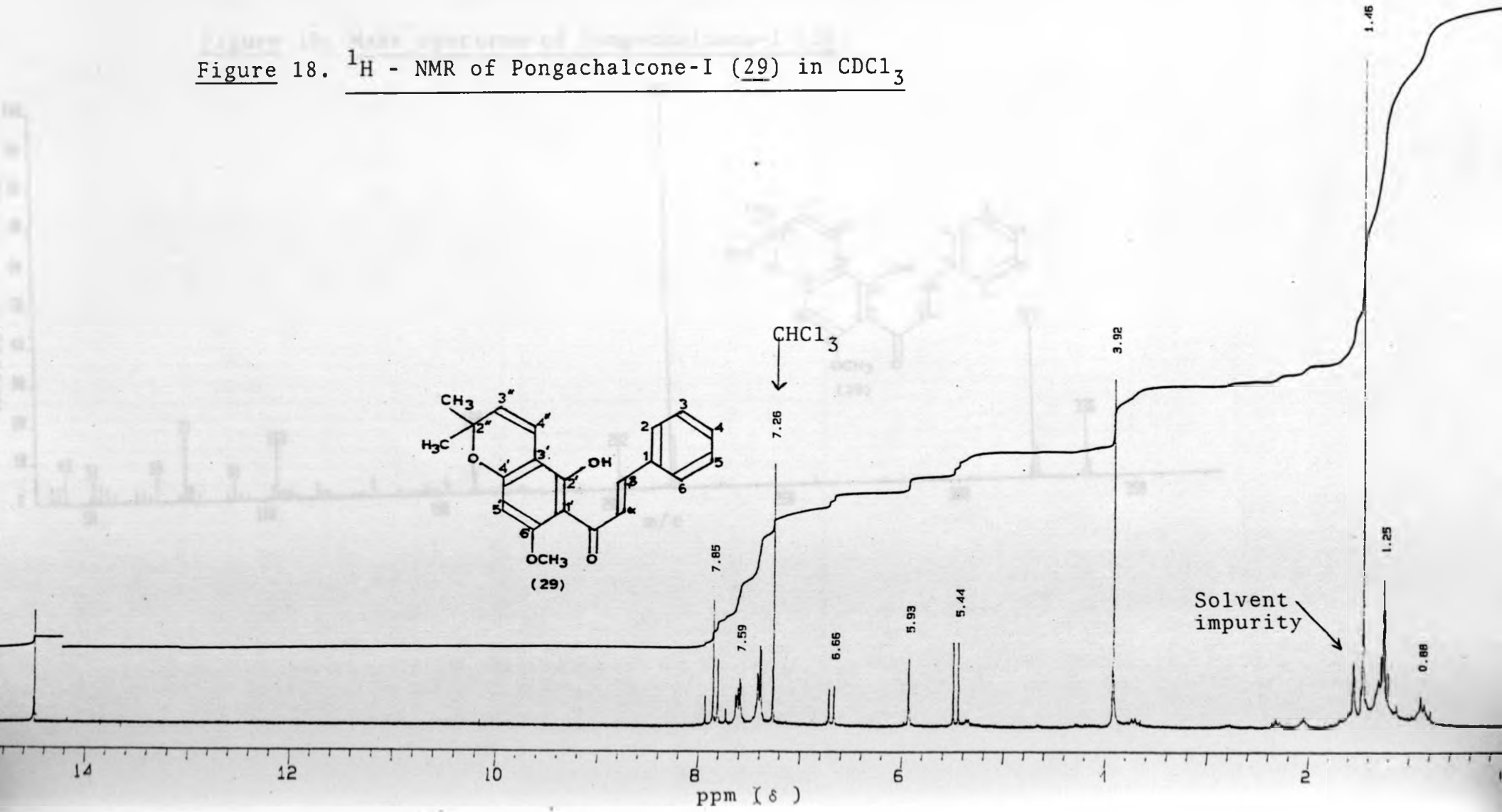


Figure 19. Mass spectrum of Pongachalcone-I (29)

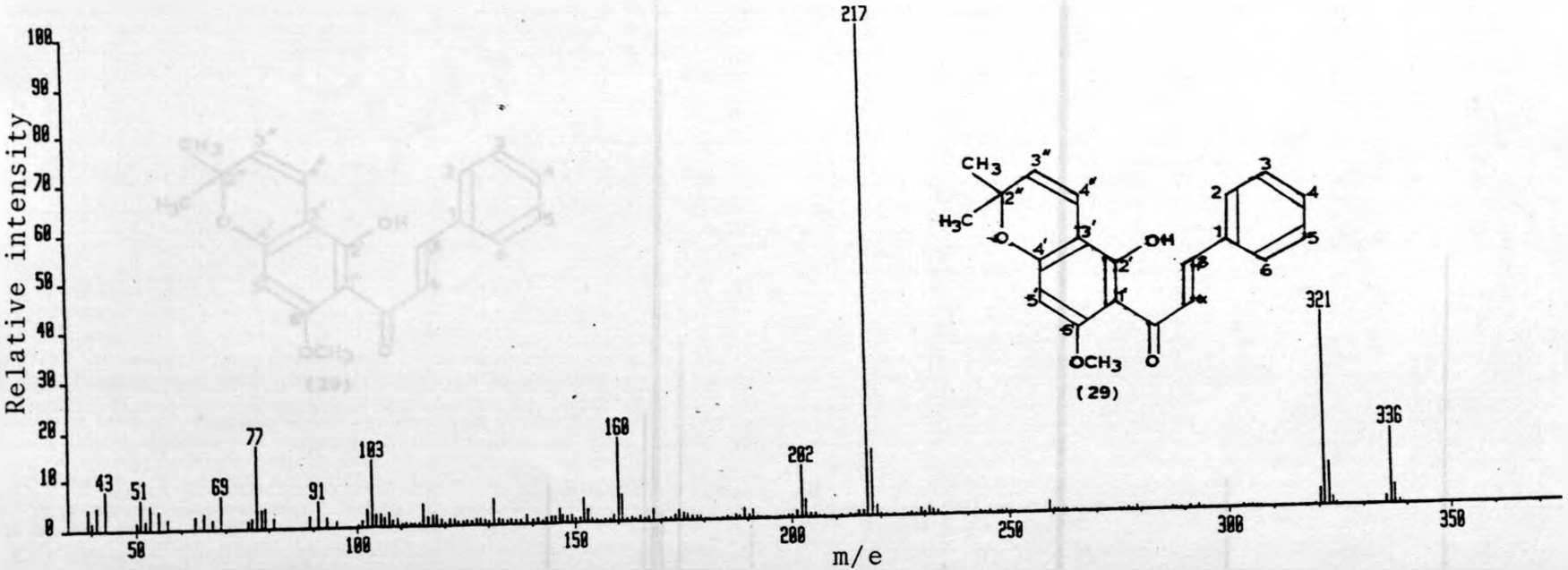
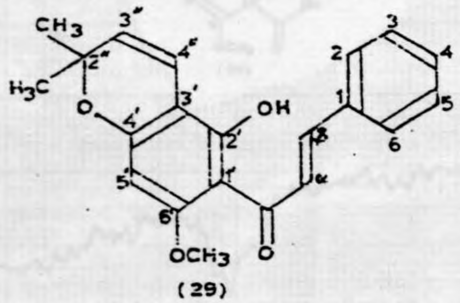


Figure 20. ^{13}C-NMR Spectrum of Pongachalcone-1 (29)



(29)

Figure 21. IR Spectrum of Pongachalcone-I (29)

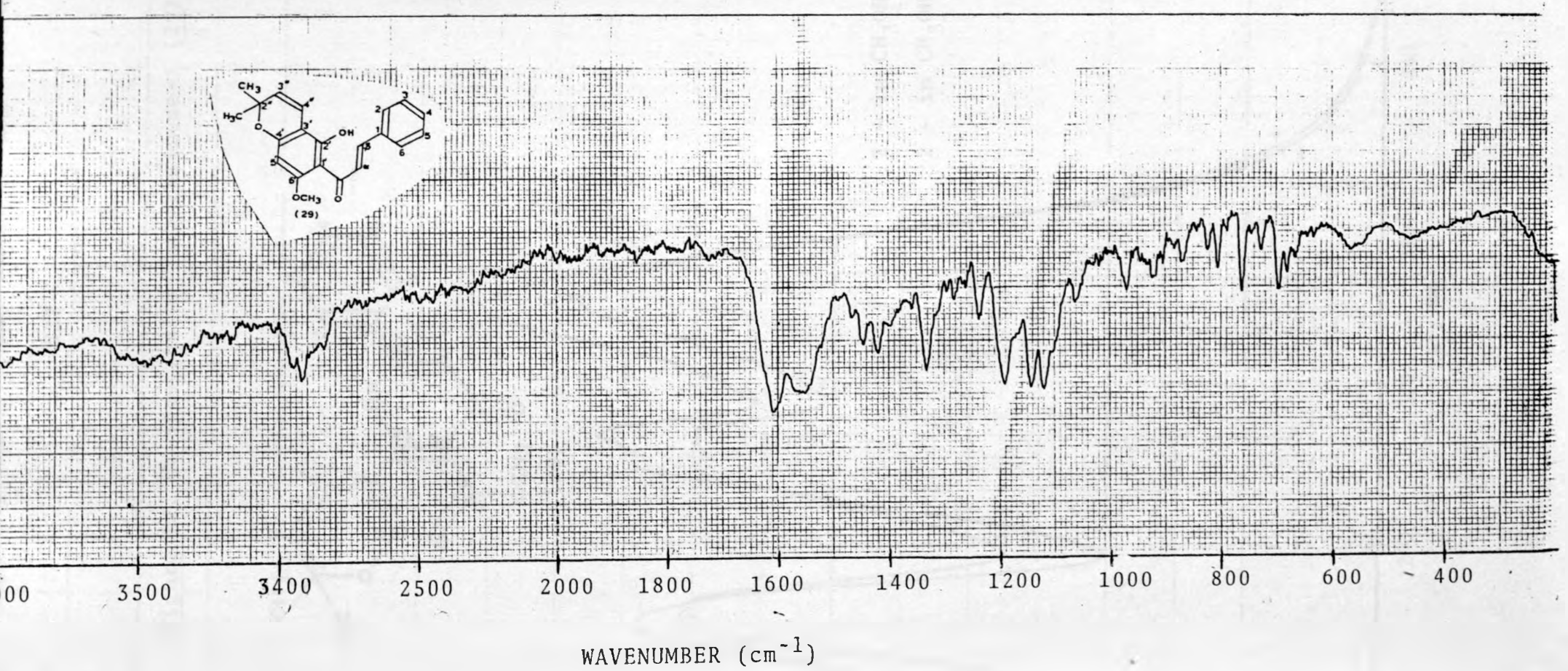
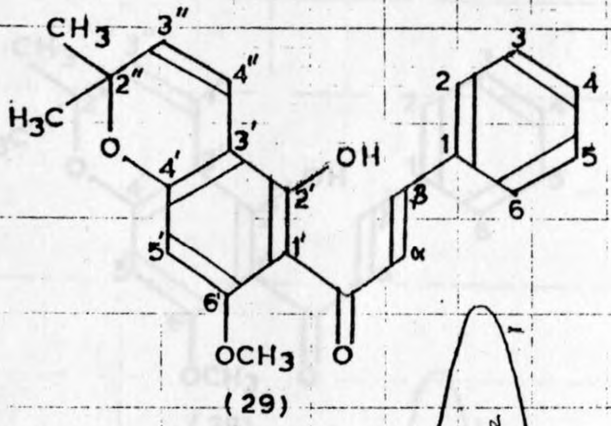


Figure 22. UV-spectrum of Pongachalcone-I (29)



(29)

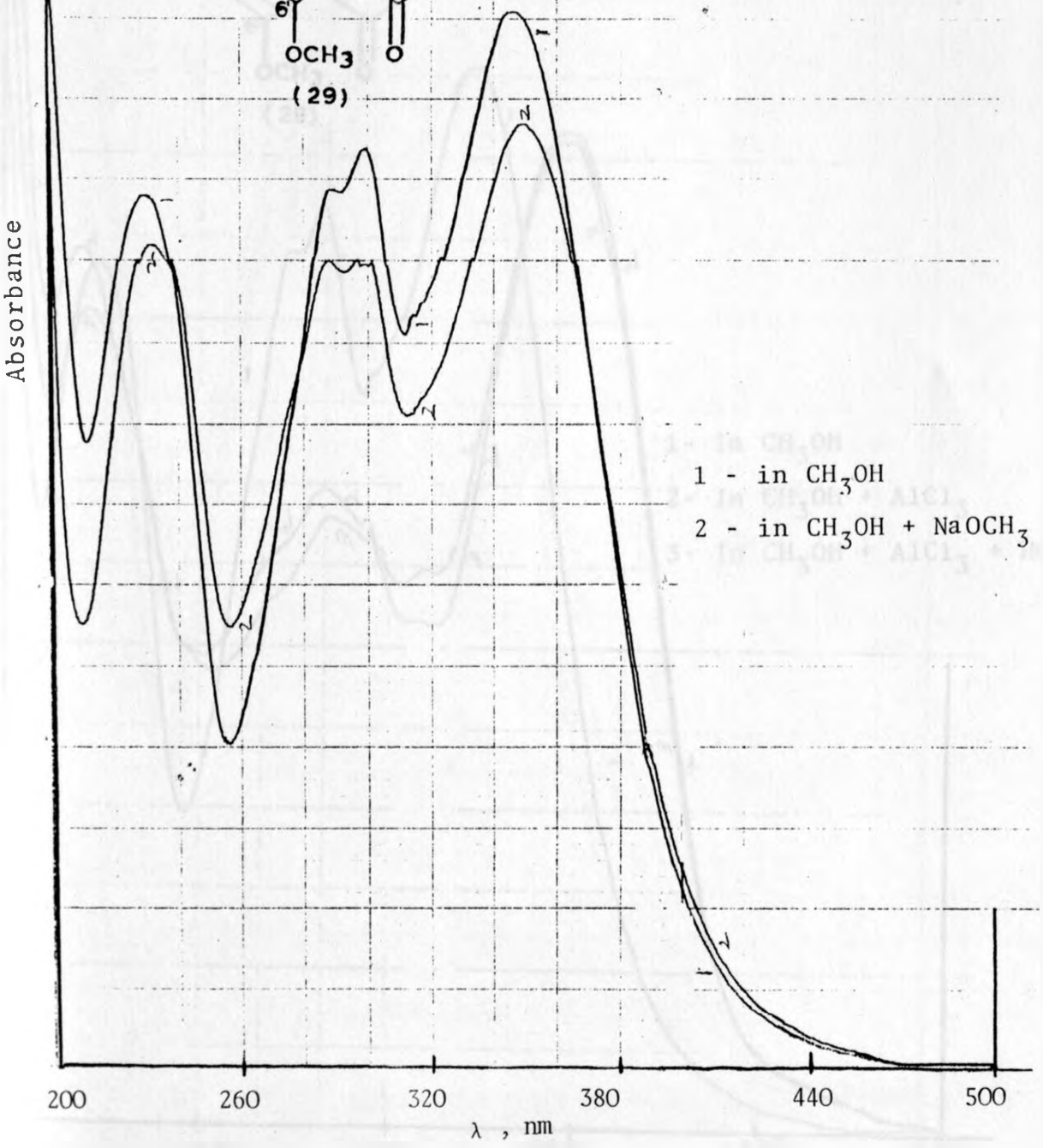
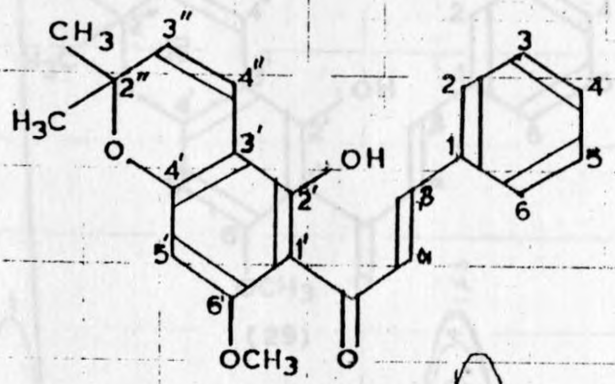


Figure 23. UV-spectrum of Pongachalcone-I (29)



(29)

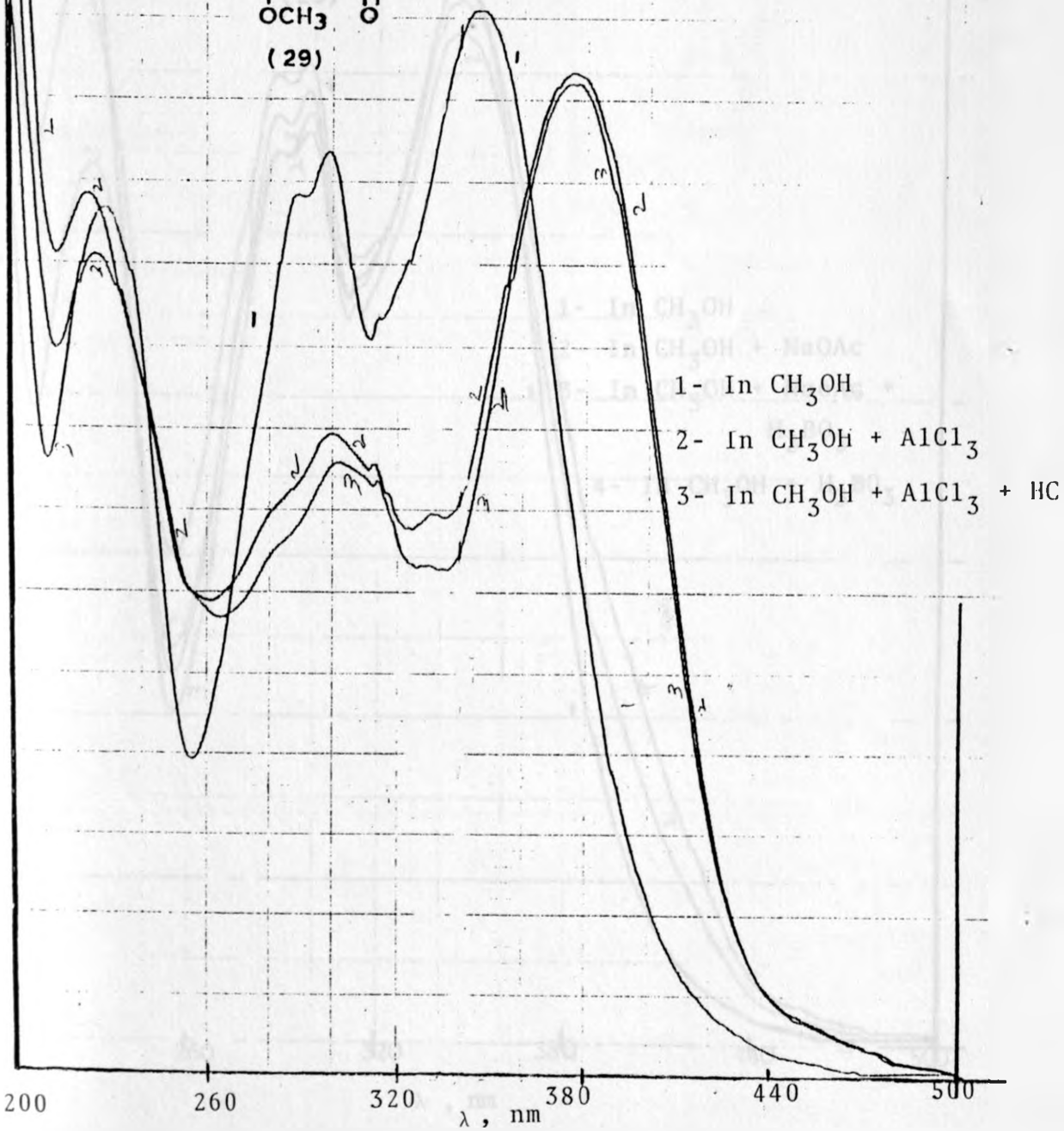
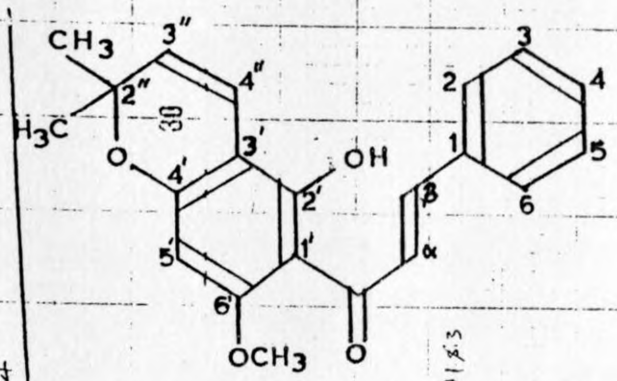


Figure 24. UV-spectrum of Pongachalcone-I (29)



(29)

1- In CH_3OH

2- In CH_3OH + NaOAc

3- In CH_3OH + NaoAc + H_3BO_3

4- In CH_3OH + H_3BO_3

Absorbance

200 260 320 380 440 500

λ , nm

Figure 25. Irradiation on the signal at δ ppm6.65 doublet for Pongachalcone-I[29]

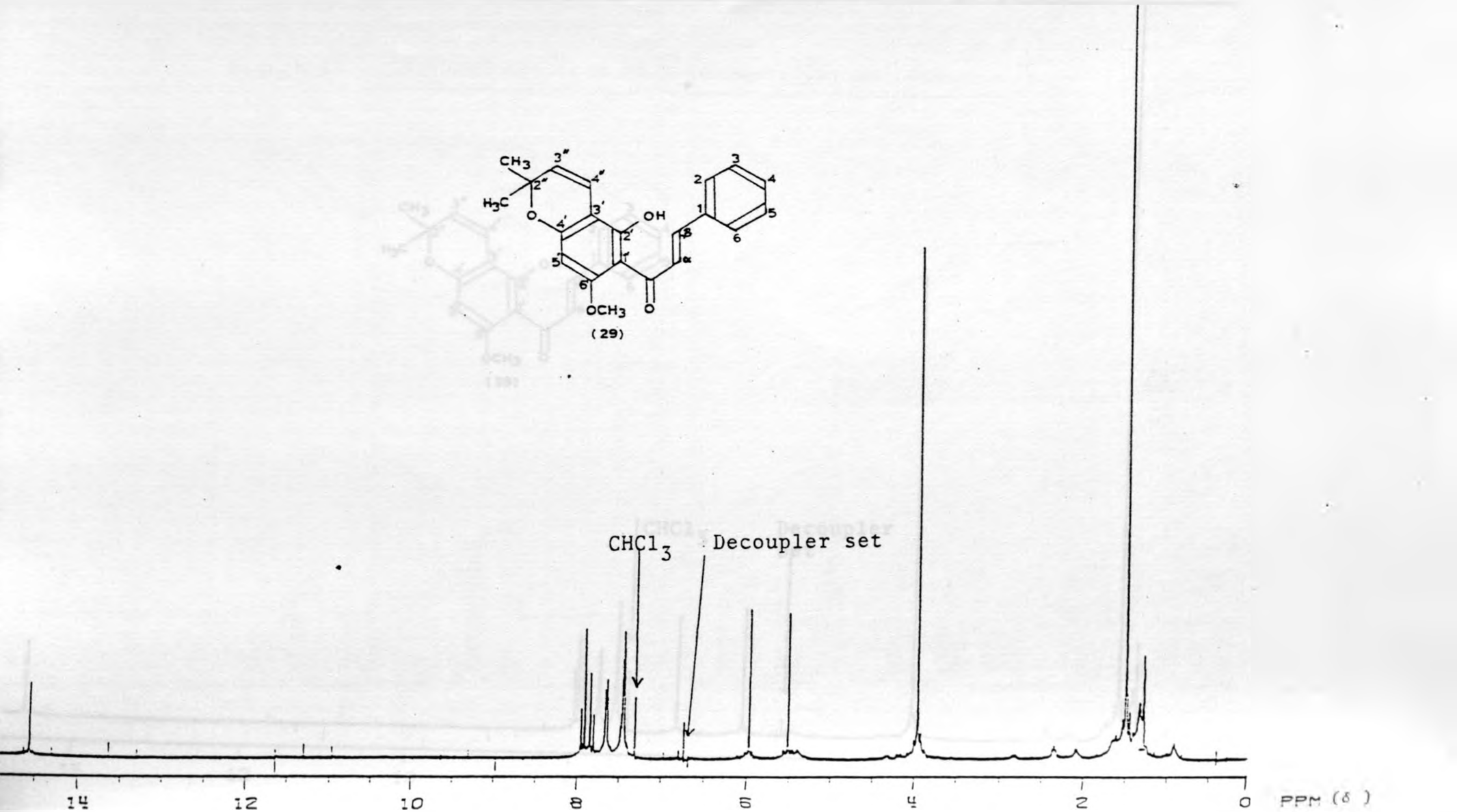


Figure 26. Irradiation on the signal at δ ppm 5.47 doublet for Pongachalcone (29)

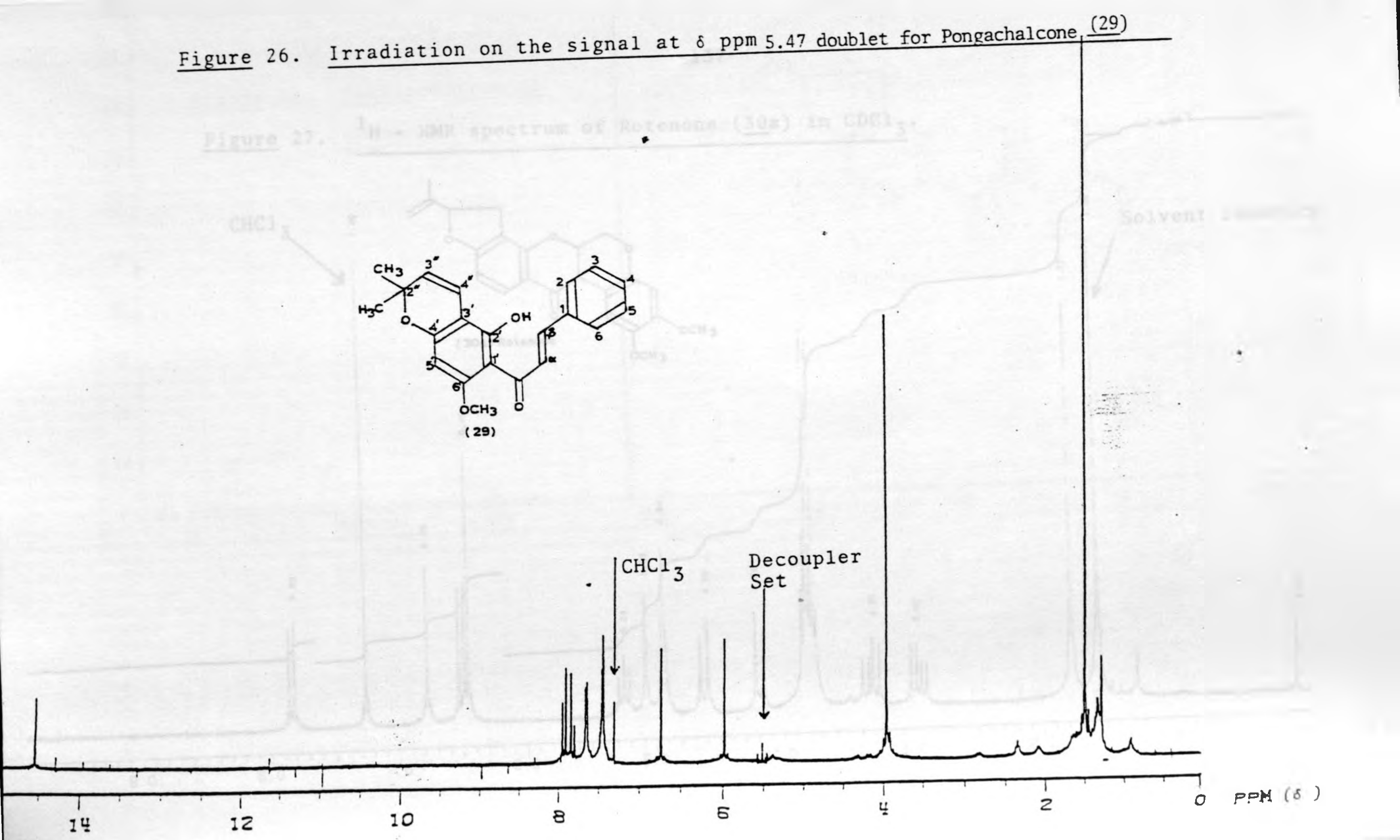


Figure 27. ^{1}H - NMR spectrum of Rotenone (30a) in $CDCl_3$.

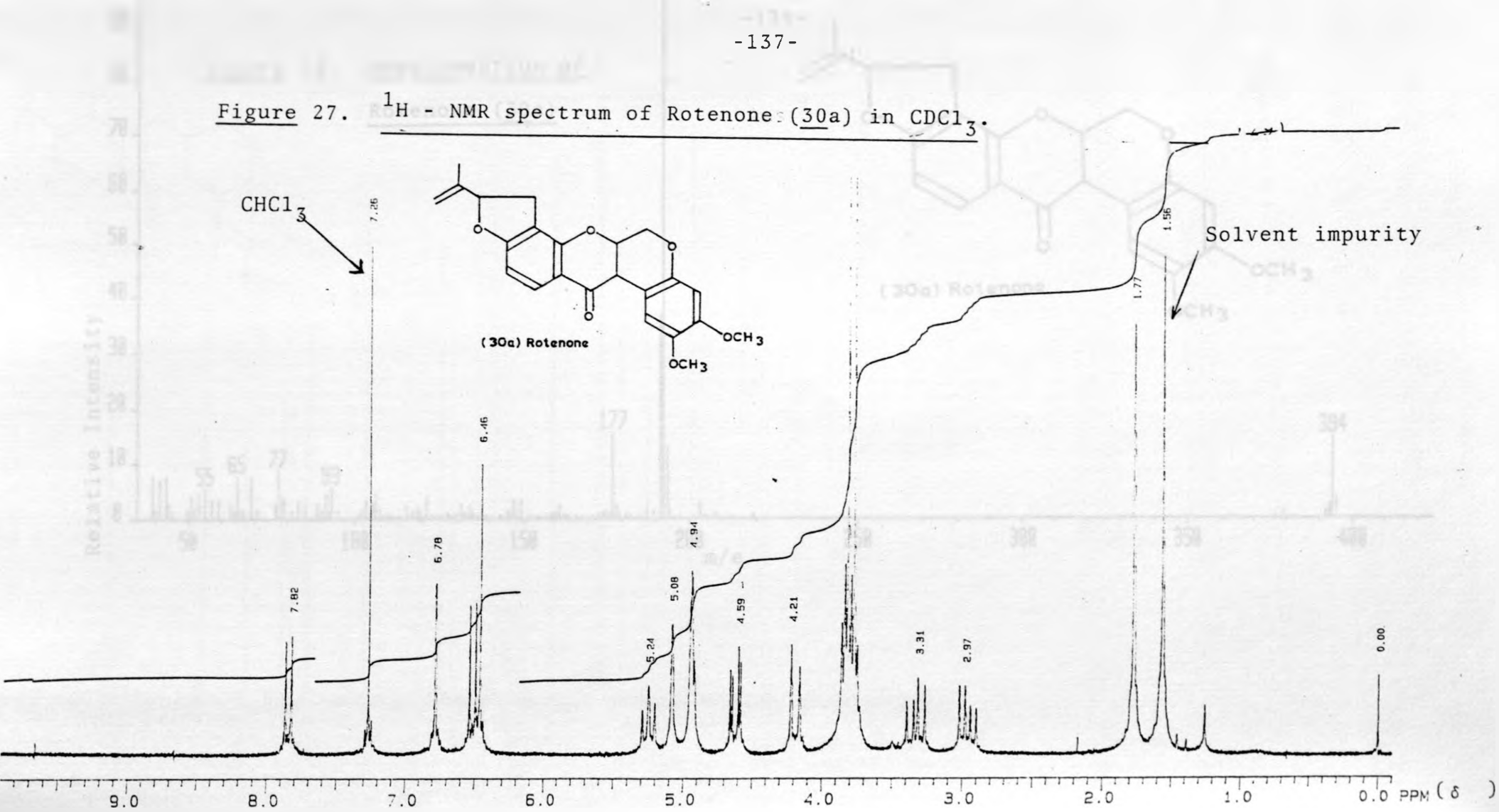
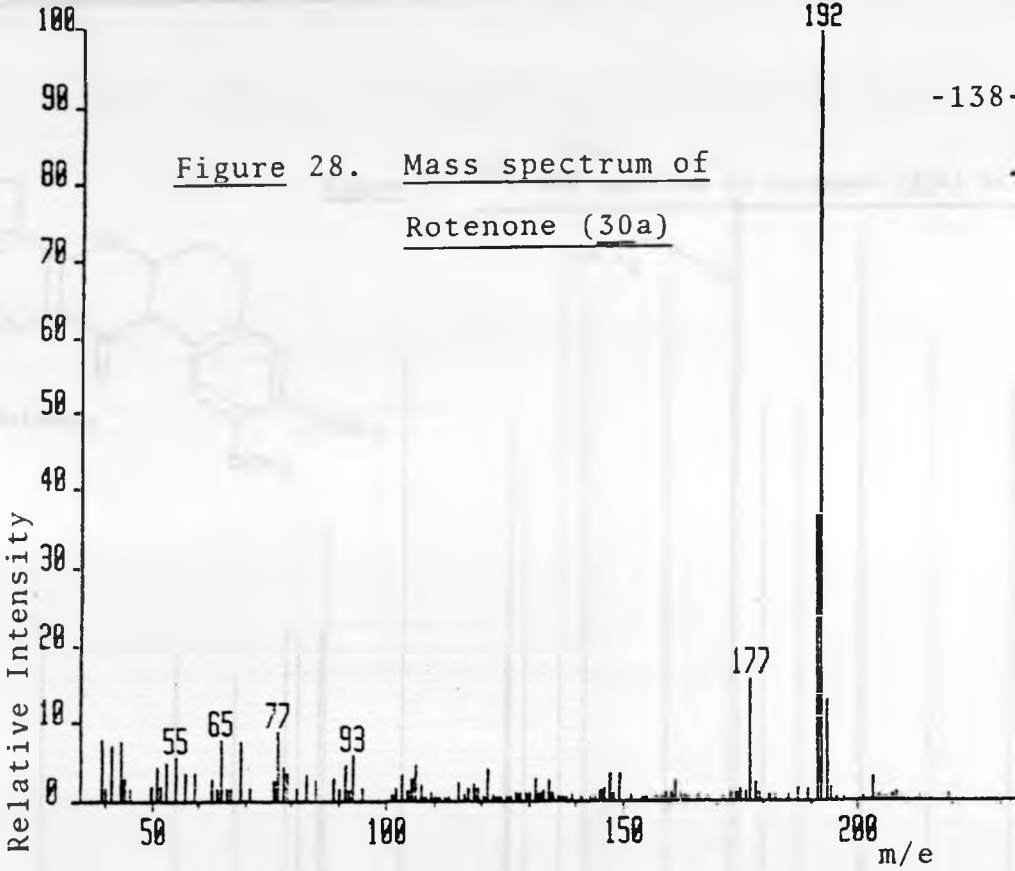
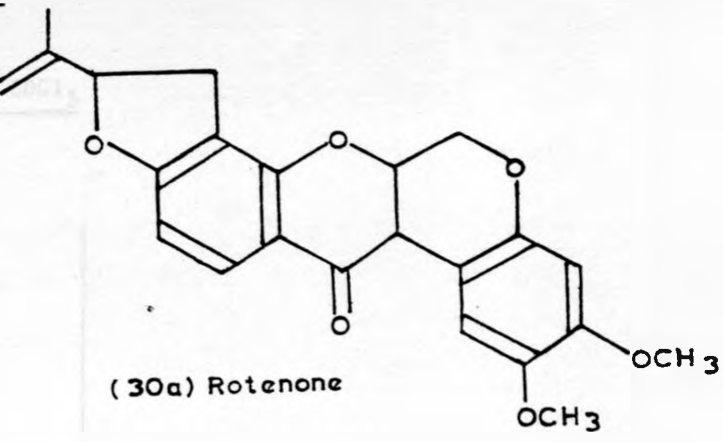


Figure 28. Mass spectrum of Rotenone (30a)





(30a) Rotenone

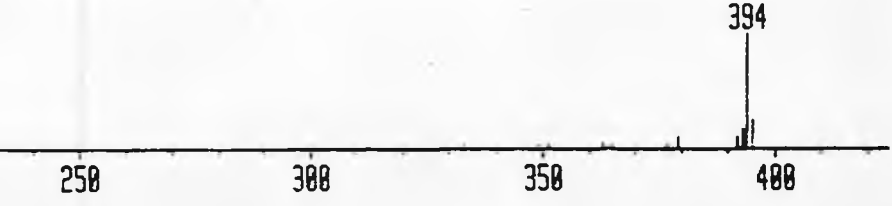


Figure 29. ^{13}C-NMR spectrum of Rotenone (30a) in $CDCl_3$

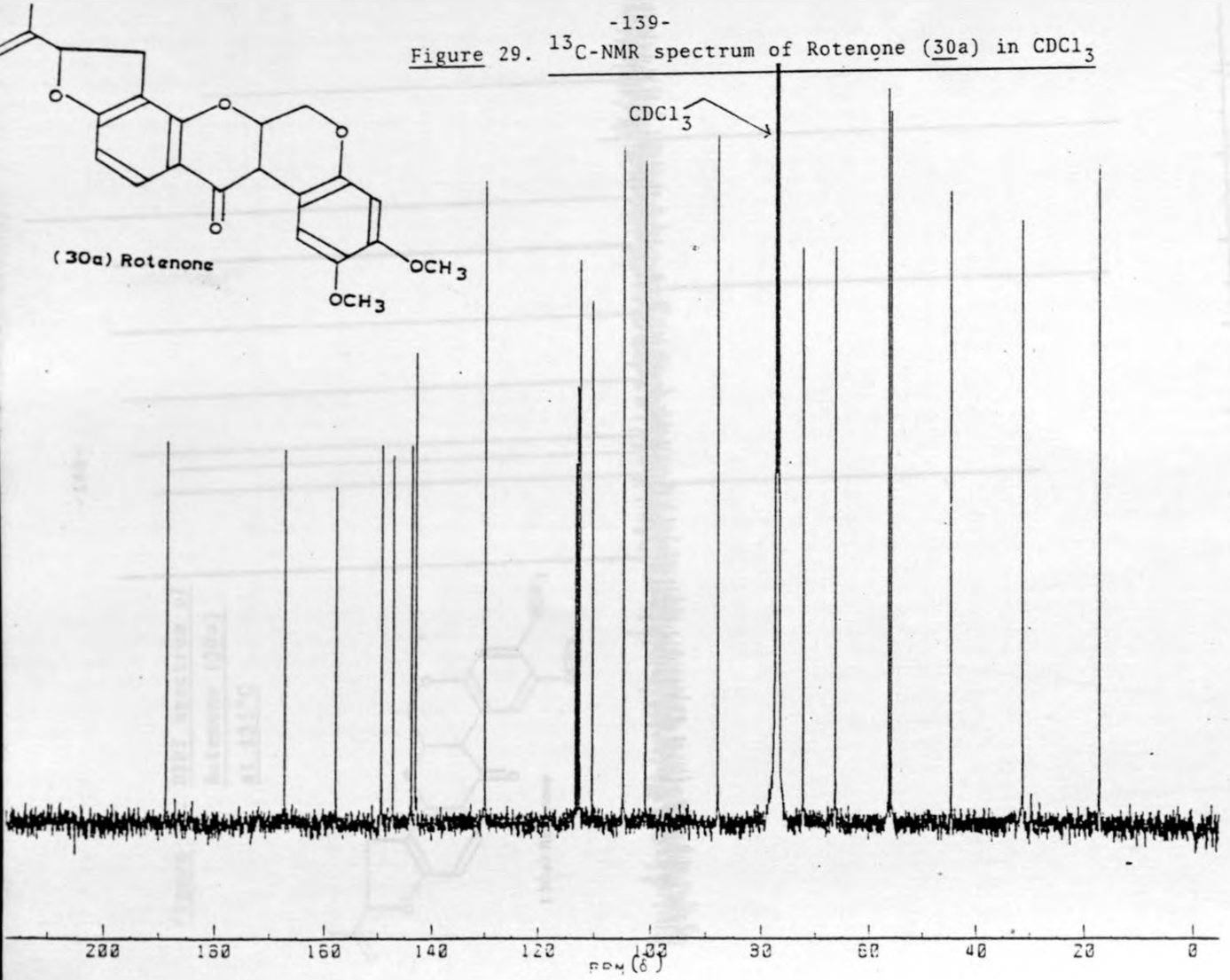
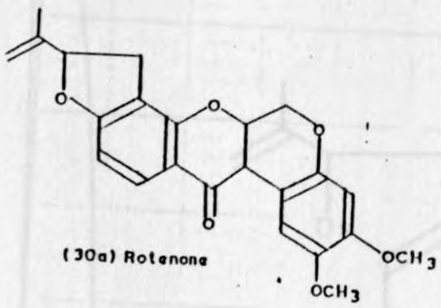
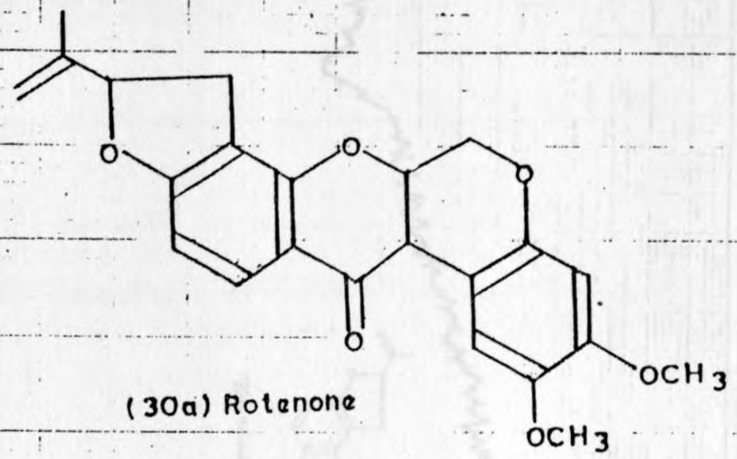


Figure 30. DEPT spectrum of Rotenone (30a) at 135°C



(30a) Rotenone

Figure 31. UV-spectrum of Rotenone (30a)



(30a) Rotenone

FIGURE 32. IR Spectrum of Rotenone (30a)

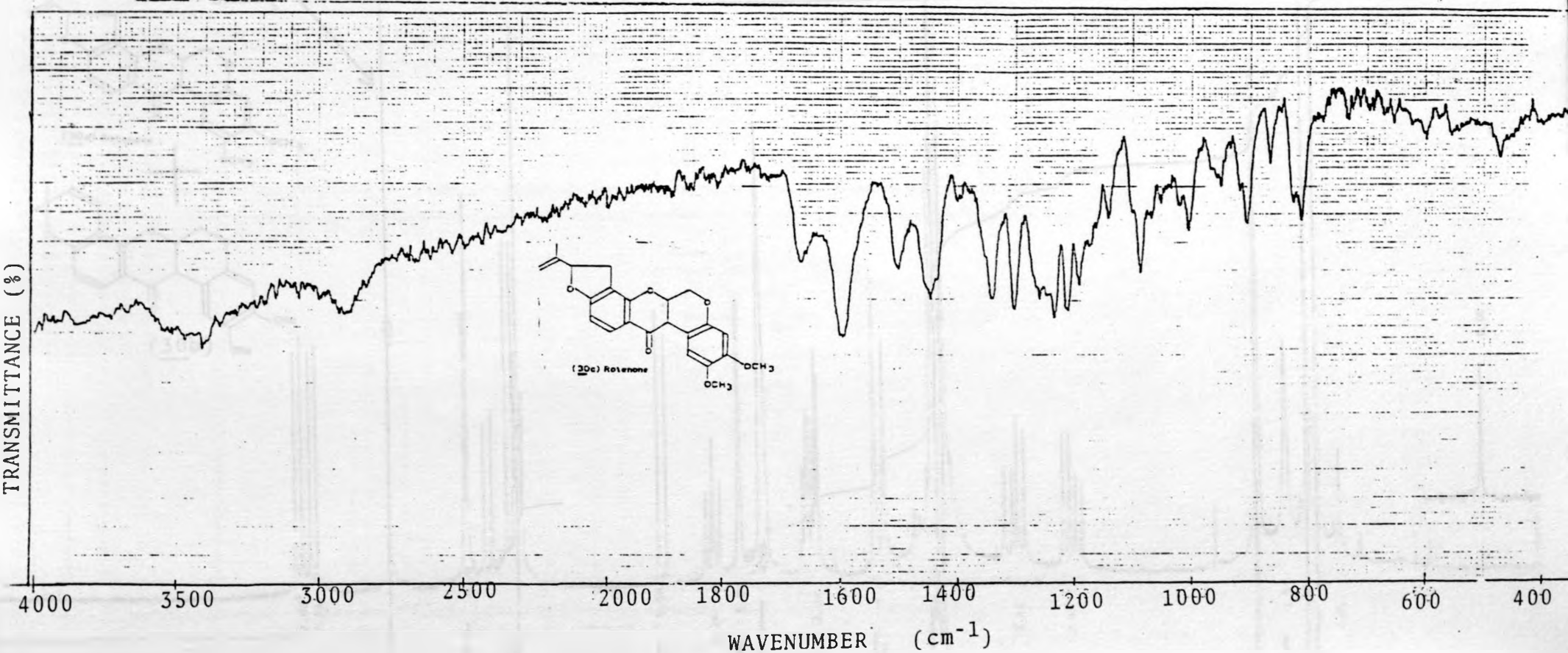
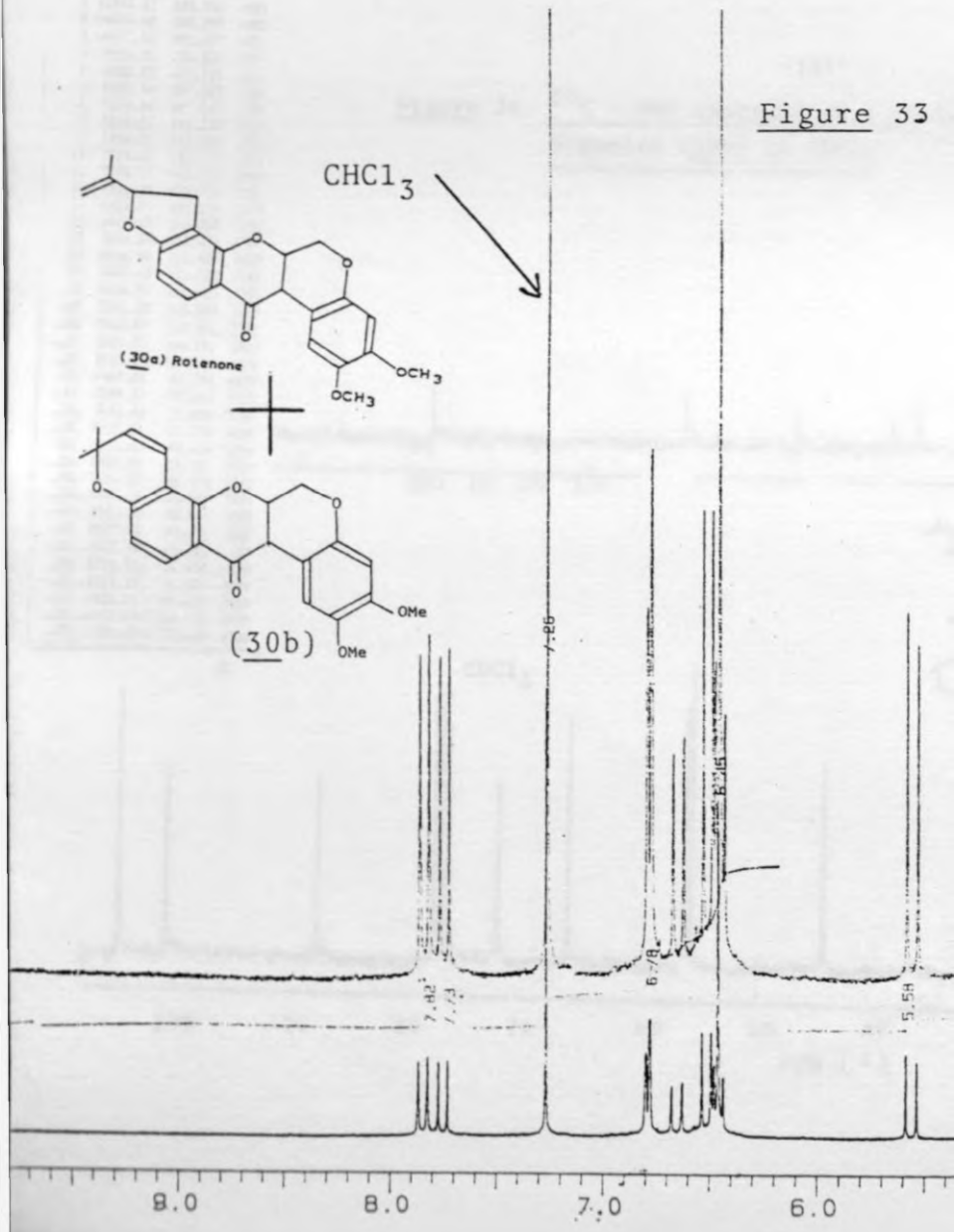


Figure 33



^{1}H-NMR Spectrum of a mixture of Rotenone (30a) and Deguelin (30b) in $CDCl_3$

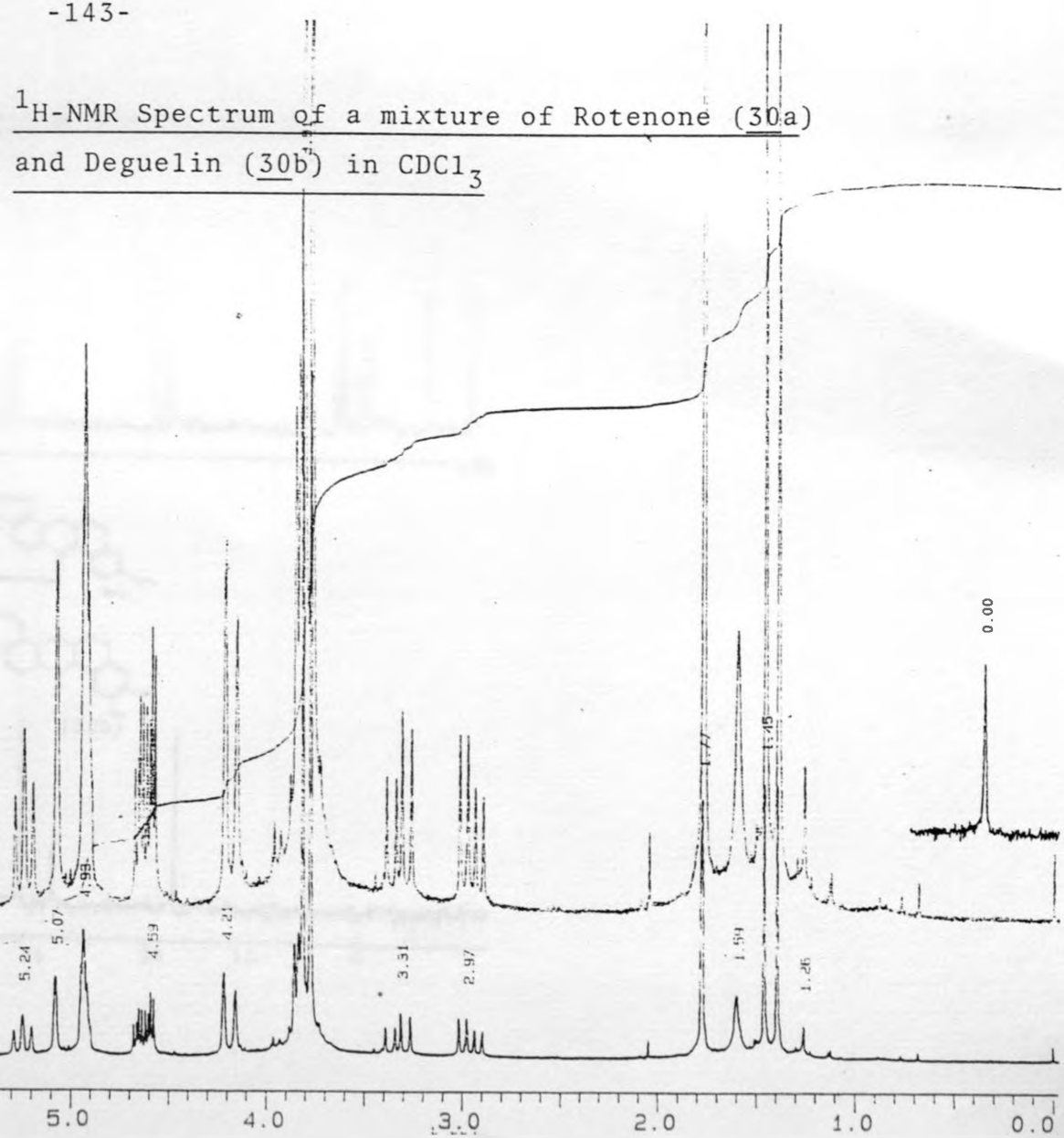


Figure 34. ^{13}C - NMR spectrum of a mixture of Rotenone (30a) and Deguelin (30b) in $CDCl_3$

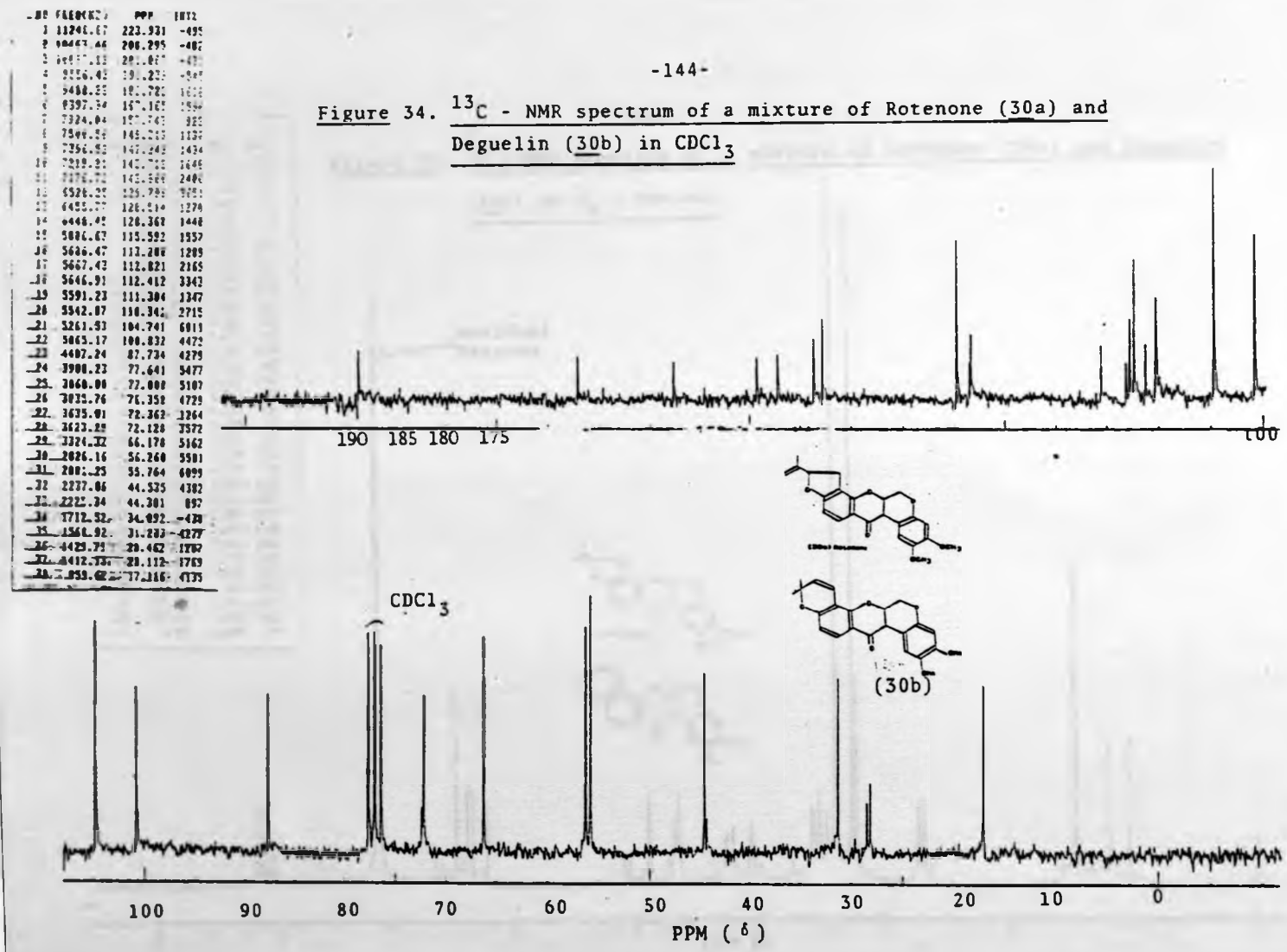
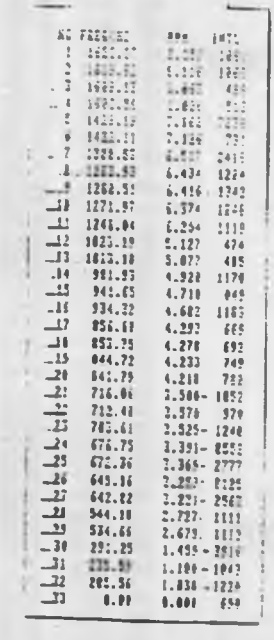


Figure 35. ^{1}H - NMR Spectrum of a

(30b) in d_6 - benzene



mixture of Rotenone (30a) and Deguelin

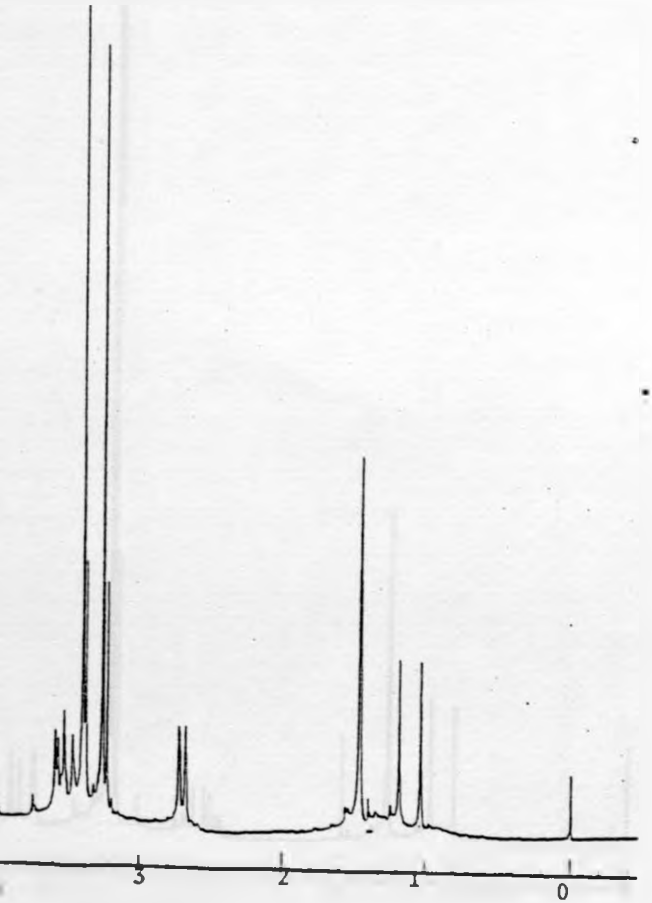
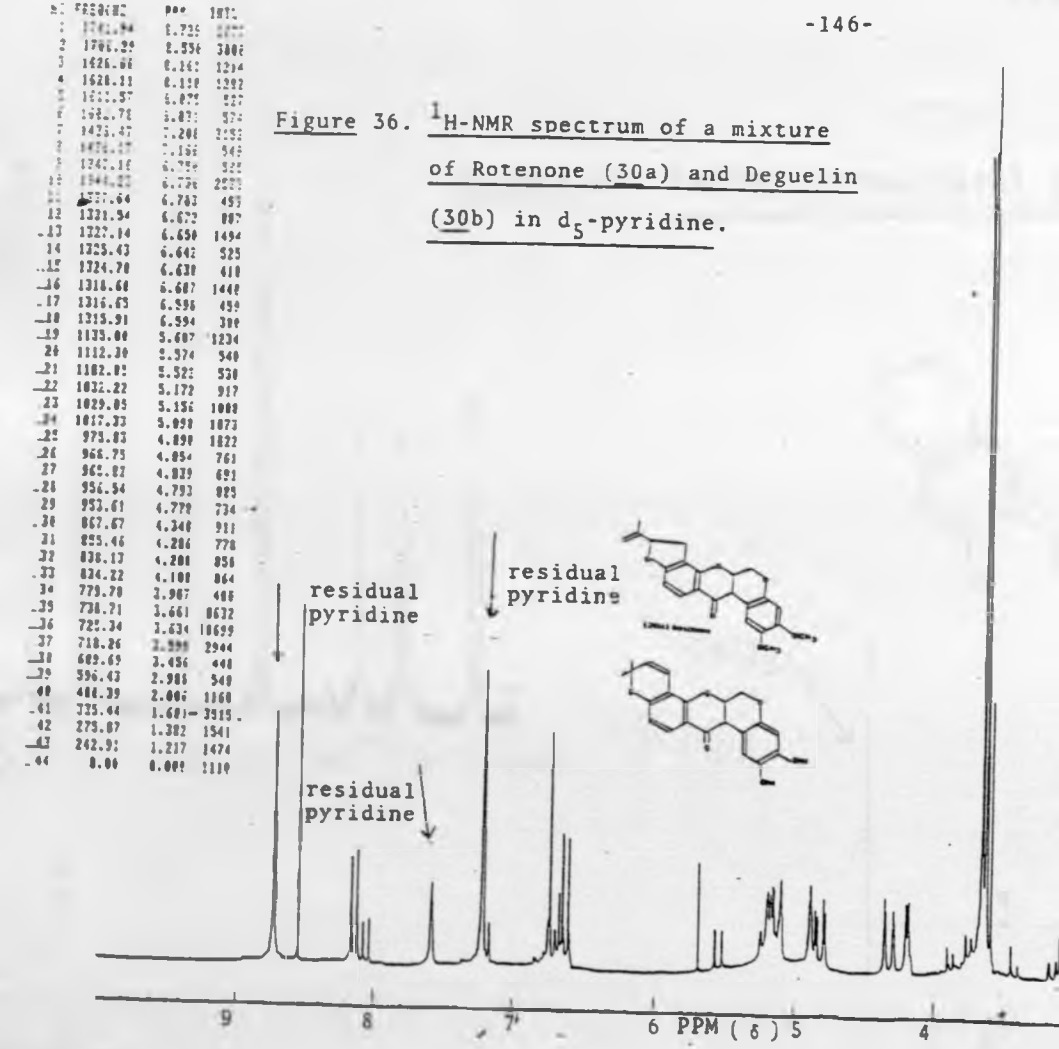


Figure 36. ^{1}H-NMR spectrum of a mixture of Rotenone (30a) and Deguelin (30b) in d_5-pyridine.



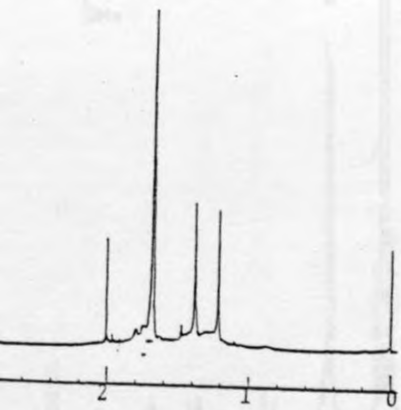
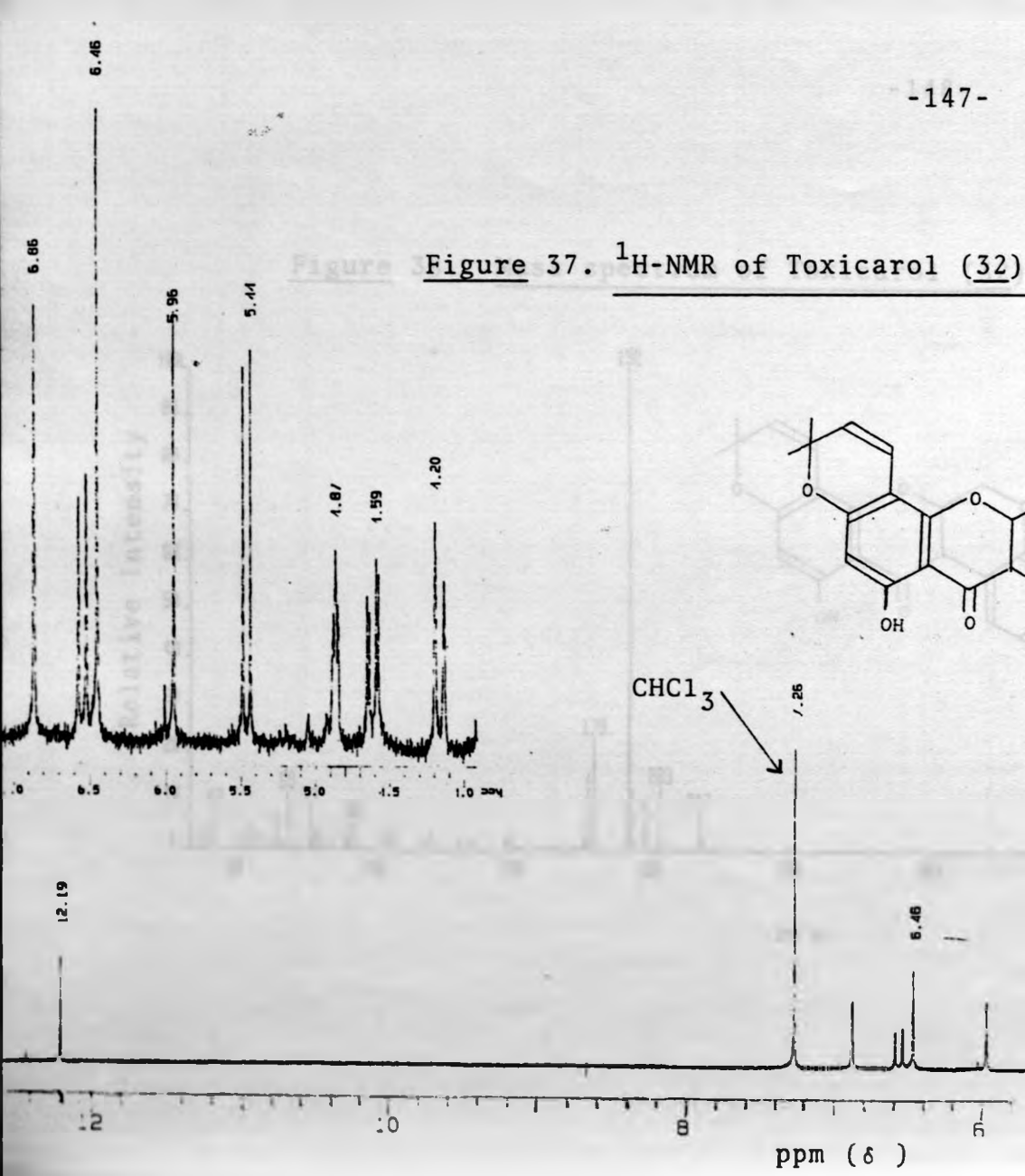

2
1
0

Figure 37. ^{1}H-NMR of Toxicarol (32)



in $CDCl_3$

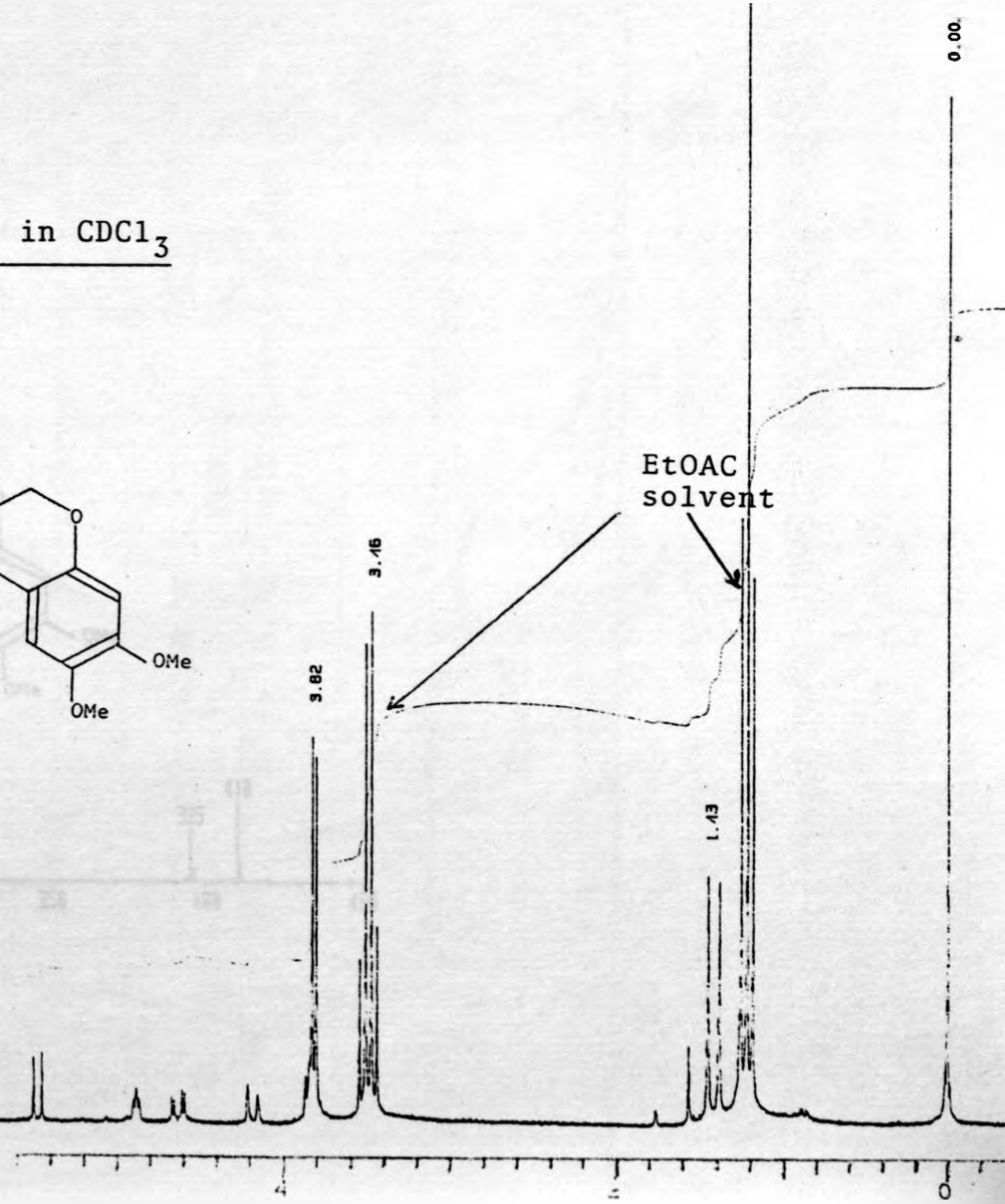


Figure 38. Mass spectrum of Toxicarol (32)

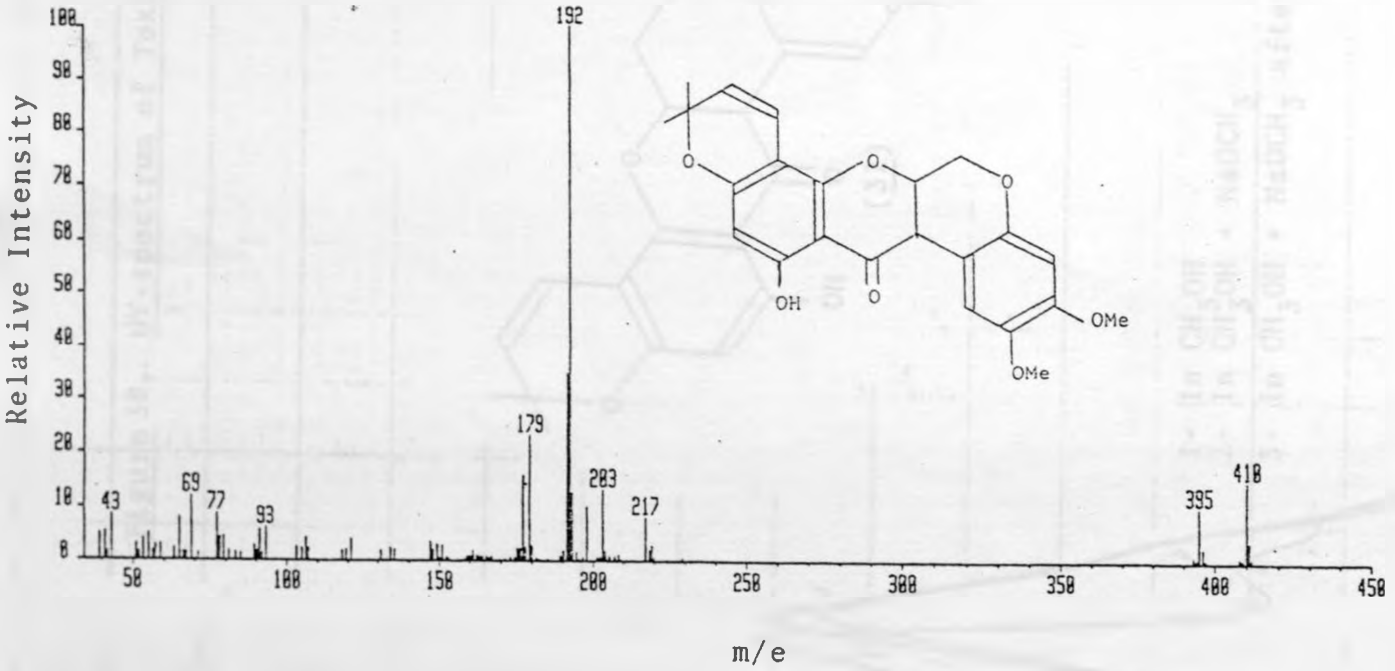
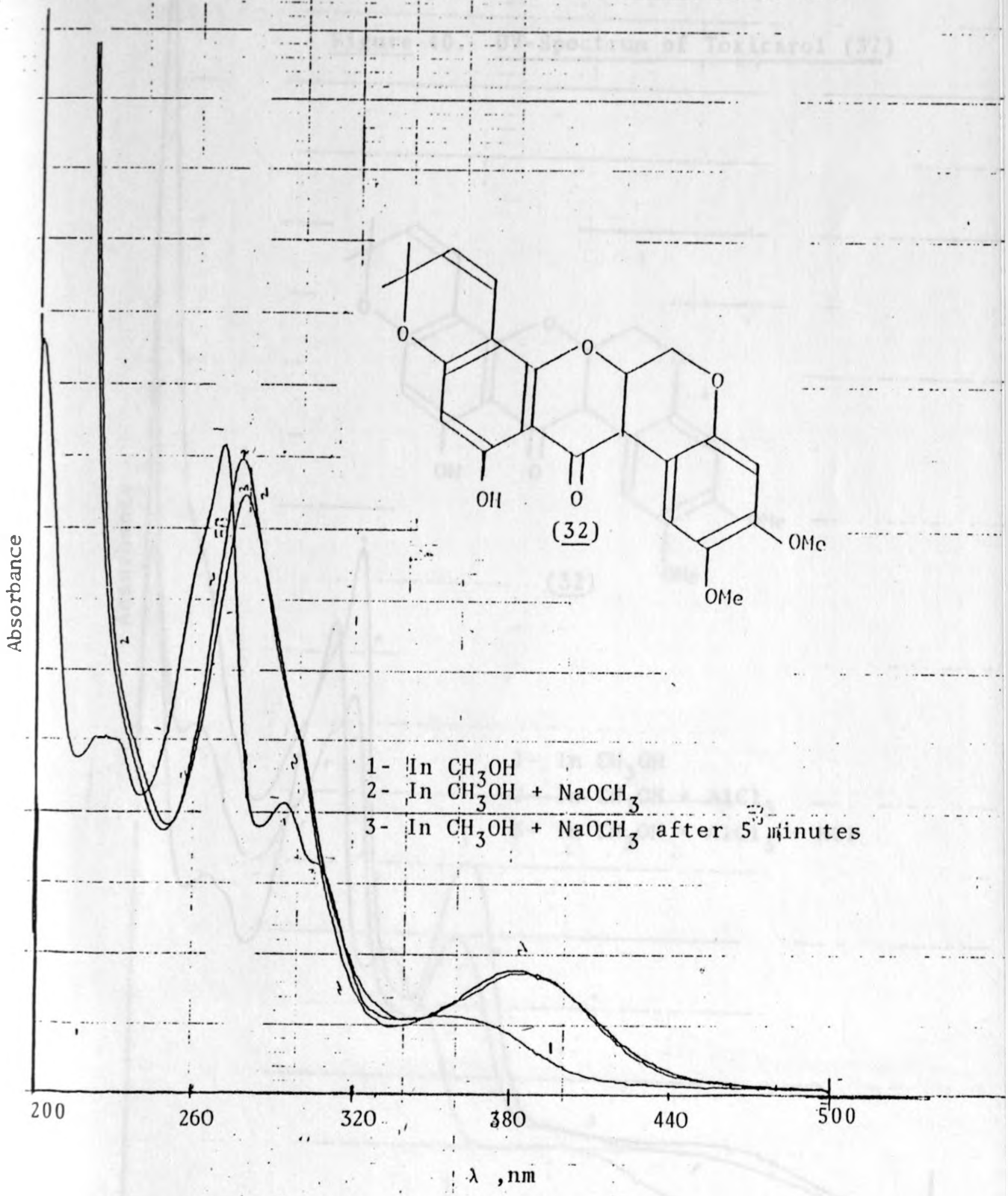
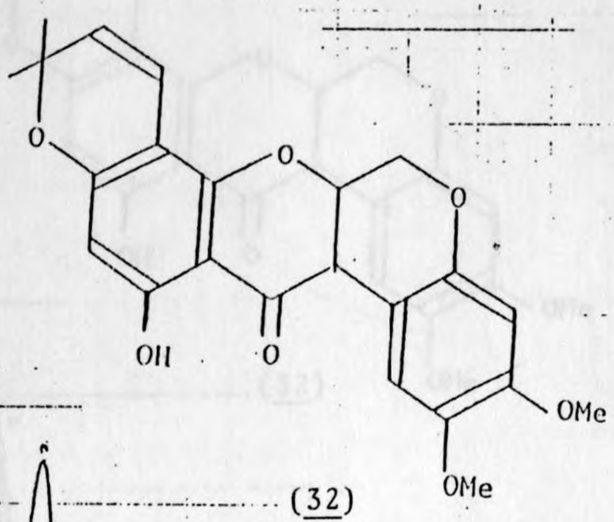


Figure 39. UV-spectrum of Toxicarol (32)



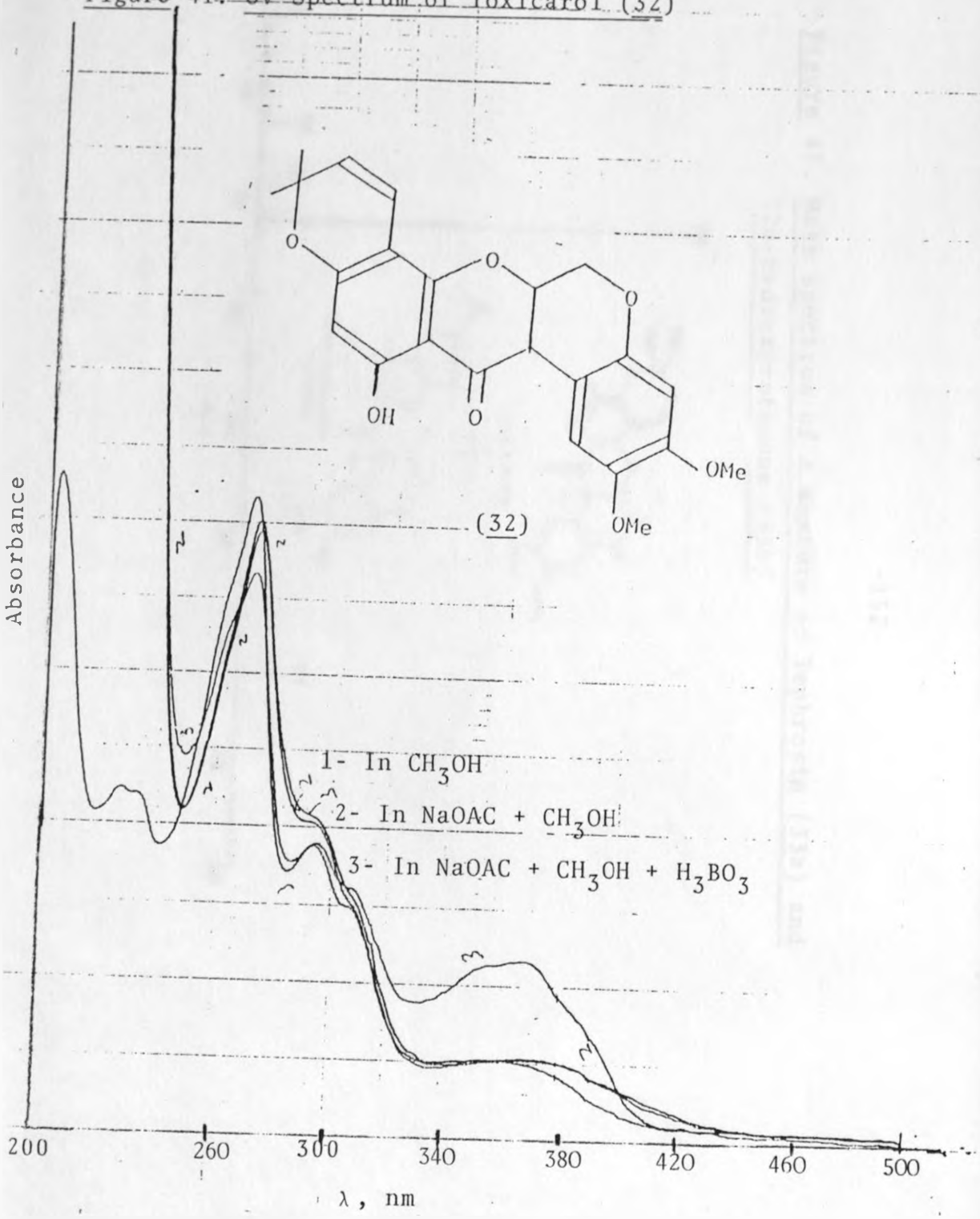
(32)

Figure 40. UV-Spectrum of Toxicarol (32)



(32)

Figure 41. UV-Spectrum of Toxicarol (32)



(32)

Figure 42. Mass spectrum of a mixture of Tephrosin (33a) and 12a-hydroxyrotenone (33b)