ADDIS ABABA UNIVERSITY
SCHOOL OF GRADUATE STUDIES

FLAVONOIDS OF THE SEED - PODS OF TEPHROSIA PUMILA

BEKELE DINKU

JUNE , 1987

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Flavonoids of the Seed-Pods of Tephrosia Pumila

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ACKNOWLEDGEMENTS

I wish to express my profound gratitude to my research advisor Dr. Ermias Dagne for his sustained interest, guidance and encoragment during the course of this investigation. I would also like to convey my sincere thanks to, Prof. Waterman University of Strathclyde, U.K for generating the ^{1}H NMR, ^{13}C NMR and MS data and for very helpful discussions, Mr. Michael Gilbert and Ato Masresha Fetene for identifying the plant material. My thanks also goes to Faculty staffs particularly to our research group who gave me technical assistance during the course of this work. I am also grateful for the financial assistance from the Swedish Agency for Research Co-operation with Developing Countries (SAREC) obtained through the Ethiopian Science and Technology Commission.

Abstract

Flavonoids of *Tephrosia pumila*

by

Bekele Dinku

Research Advisor Dr. Ermias Dagne

The Chloroform extract of *Tephrosia pumila* (Lam.) Pers. (Leguminosae) revealed the presence of eight flavonoids. Out of these three flavonoids were isolated. The major component was identified as the known praecansone A that requided structural revision based on ^{13}C NMR and other spectroscopic data. The second component was characterized as a new isoflavune that is given the trivial name pumilaisoflavone A.

Introduction

Tephrosia Pers. (Leguminosae - Papilionoideae) is a large tropical and sub-tropical genus estimated to contain about 300 species,[1] most of which are herbs. Several species are of importance in tropical agriculture as green manure crops, cover crops or for contour hedges to check soil erosion. Various Tephrosia species in Latin America, Africa and Asia beside their use in traditional system of medicine are also used as insecticides, arrow and fish poisons.[2,3] Some species are also reported to have toxic effects on goats.[4] About two dozen species have been recorded as used by natives in different parts of the world for stupefying and capturing fish.[3] Among the better known of of these are Tephrosia verginiana in North America, T.vogelii Hook.f. in Tropical Africa, T. macropoda Harv. in South Africa, T. toxicaria in Tropical America and T. piscatoria in Hawaii.

Some of the species in use as pesticides have been shown to possess marked insecticidal, pesticidal and anti-cancer properties.[5,6] Toxicity to insects is ascribed mainly to the presence of rotenones.[2]

Chemical studies on a number of taxa have revealed rotenoids, and a range of isoflavanones, chalcones, flavonols and flavones.[7] In Ethiopia so far 27 Tephrosia species are known to occur in places up to 2000m above sea level. Of these three

2. The Flavonoids

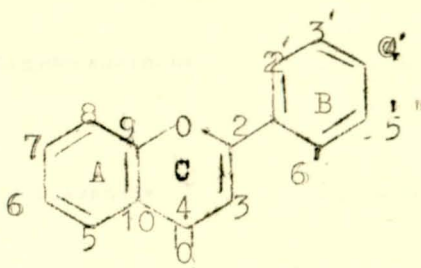
Flavonoids constitute one of the largest groups of naturally occuring phenols.[9] In plants flavonoids exist as aglycones (flavonoids with out attached sugar), glycosides, sulphates and biflavonoid.[10] Flavonoids are known to possess a wide arry of biological properties. Table 1 presents some selected bioactive flavonoid types.

Table 1 Some selected examples of bio active flavonoids.

Flavonoid Type	Source	Reported bio-activity	Ref.
Luteone	Lupinus albus	Anti-fungi	
Hispaglabridin A	Glycyrrhiza glabra	Anti-bacterial	
Hispaglabridin B	" "	" "	
glabridol	" "	" "	
glabridim	" "	" "	
Didimocarpin	Didymacarpus pedicellata	" "	
	" "	" "	
Isodidimocarpin	" "	" "	
6-hydroxy kaempferol 4-methylether 3,7-dirhamoside	Tephrosia candida	Anti-cancer	
neptrin		Radioprotective	
Sibrin and silymarin		Anti-hepatoxic	
Toxitolin		Anti-hypertensive	
Quarcetin		Anti-hypertensive	
Rutin		Inhibiting the aggregation of blood platelates	
Isopentenyl chalcone		Anti-peptic ulcer	

2.1. Flavonoid Types

Flavonoids occur in a varity of forms. All contain fifteen carbon atoms in their basic nucleus and these are arranged in a C_6-C_3-C_6 manner, that is, two aromatic rings linked by a three carbon unit which may or may not form a third ring.[10,] For convenience the rings are labelled as A, B and C and the individual carbon atoms are referred to by a numbering system which utilizes ordinary numerals for the A ring (1). For chalcones a modified numbering system is used(2).



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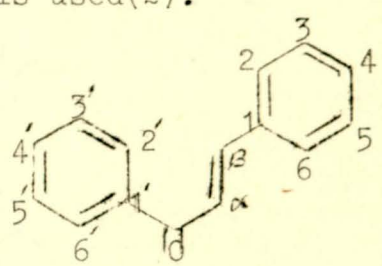


2

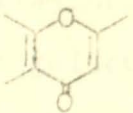
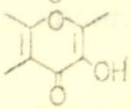
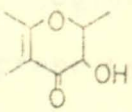
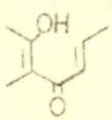
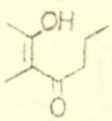
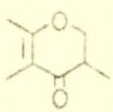
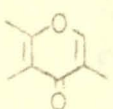
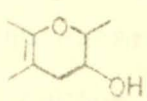
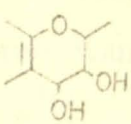
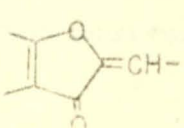
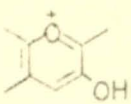
The various types of flavonoids differ from each other by the state of oxidation of the three carbon link, that is, ring C.

The main types of monomeric flavonoids are:

Flavones	Isoflavanones
Flavonols	Isoflavones
Flavanones	Catechines
Dihydroflavonols	Flavan-3, 4-diols
Chalcones	Aurones
Dihydrochalcones	Anthocyanins

Table 2

Structure of the 3-C portion of different flavonoid compounds[21,22]

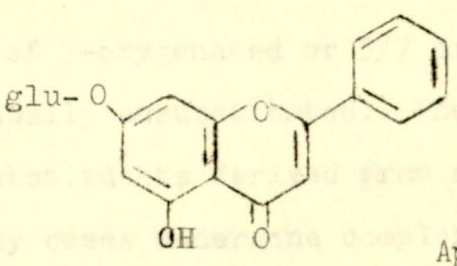
N A M E	Structure of 3-C portion
Flavone	
Flavonol	
Dihydroflavonol	
Chalcone	
Dihydrochalcone	
Isoflavanone	
Isoflavone	
Flavane-3-ol (catechins)	
Flavan-3,4-diols	
Aurone	
Anthocyanin	

2.1.1. Flavonoid glycosides

Flavonoids occur as flavonoid glycosides in which one or more sugar units are attached to the flavonoid nucleus. Glucose is the sugar most commonly involved; galactose, rhamnose, xylose and arabinose are uncommon but are known to occur.

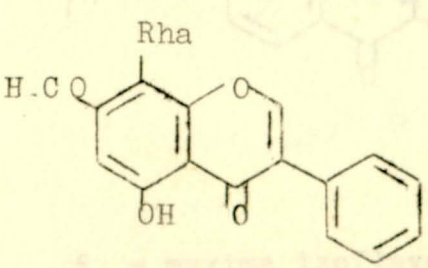
Flavonoid glycosides may be either O-glycosides or C-glycosides. O-glycosides such as the representative example compound (3) are distinguished by the ease with which they are hydrolysed by acids.

3



Apigenin 7-O-B-D glucopyranoside

C-glycosides are flavonoids in which sugars are attached directly to the aromatic ring by a carbon bond which is acid resistant. C-linked sugars have been found only at 6 or 8 positions on the flavonoid nucleus 4[10]



4

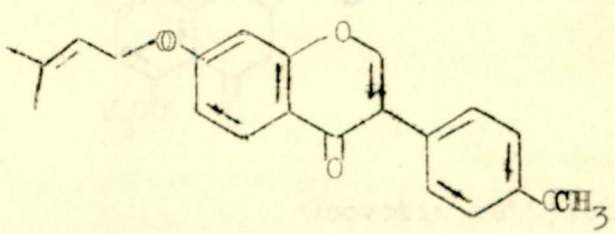
Volubilin[23]

2.1.2. Flavonoid sulphates

Flavonoid Sulphates contain one or more sulphate residues attached to a phenolic or sugar hydroxyl moiety. The occurenc of these compounds appears to be restricted to the angiosperms especially to those angiosperms which have an ecological asso-ciation with aquatic habitats.[10]

2.2. Tephrosia Flavonoids

The genus Tephrosia is distinguished by the production of a special group of 7-oxygenated or 5/7 oxygenated flavonoids. The B ring is usually unsubstituted. The C-8 or C-6 positions commonly bear substituents derived from a prenyl group,[7,24] which has in many cases undergone complex processes of furthe substitution and cyclization. O-prenylated flavonoids such as compound 5 are not common while C-prenyl substituted flavonoi are common in the genus and are likely to be potential taxono-mic markers.



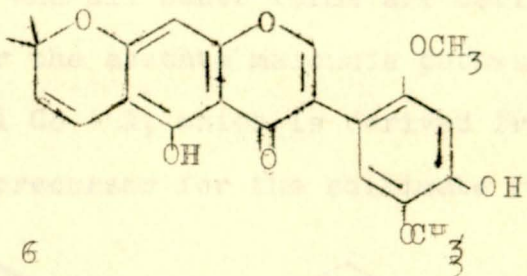
5 - maxima isoflavone J[25] (O-prenylated.)

At present it is possible to recognise three series of C-prenyl flavonoids.[7]

a) C-6 prenylated (6)

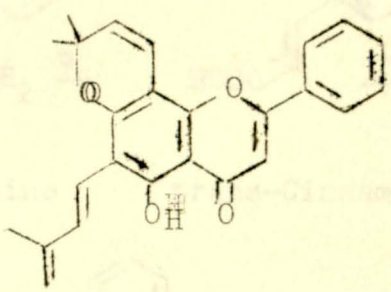
b) C-6/C-8 prenylated (7)

C) C-8 prenylated (8)



6

elongatin[26]



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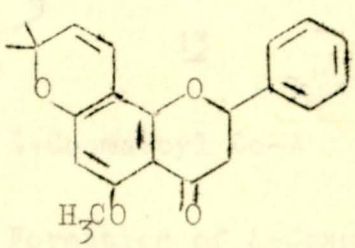
Fulvinervin B[27] (C-prenylated)

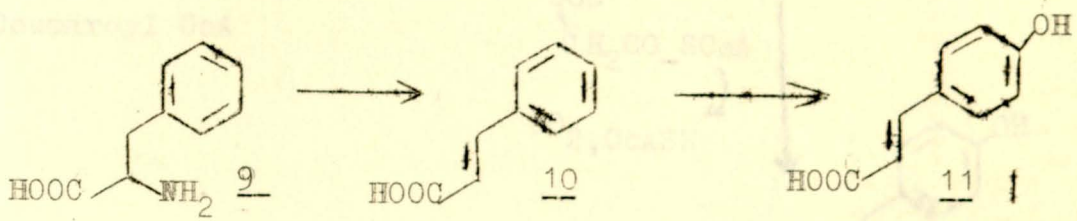


8

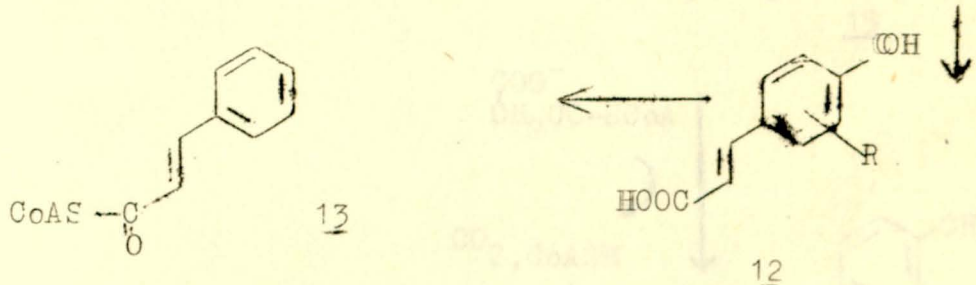
Obovatin methylether[28](C-prenylated

2.3 Biosynthesis of Flavonoids

The flavonoid variants are all related by a common biosynthetic pathway which incorporates precurrors from both the shikimate and acetate malonate pathwey.[24,29] The flavonoid initially formed in the biosynthesis is thought to be the chalcone and all other forms are derived from this. The precursor for the acetate malonate pathway is acetyl Co-A. 4 - Coumaroyl Co - A, which is derived from phenyl alanine, is the very precursor for the shikimate route.



Phenyl alanine trans-Cinnamic acid 4-Coumarate

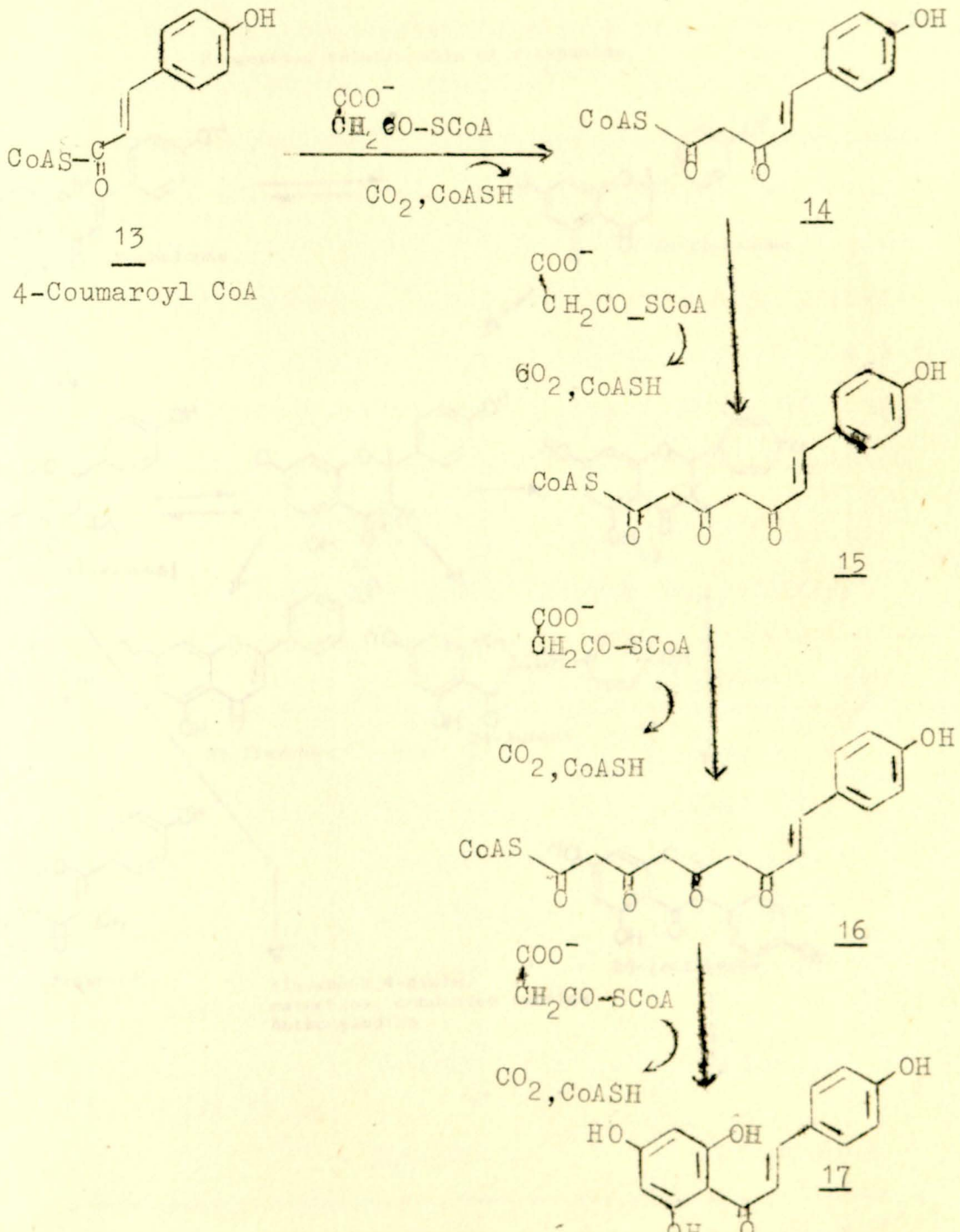


4-Coumaroyl Co-A R: H,OH, OCH

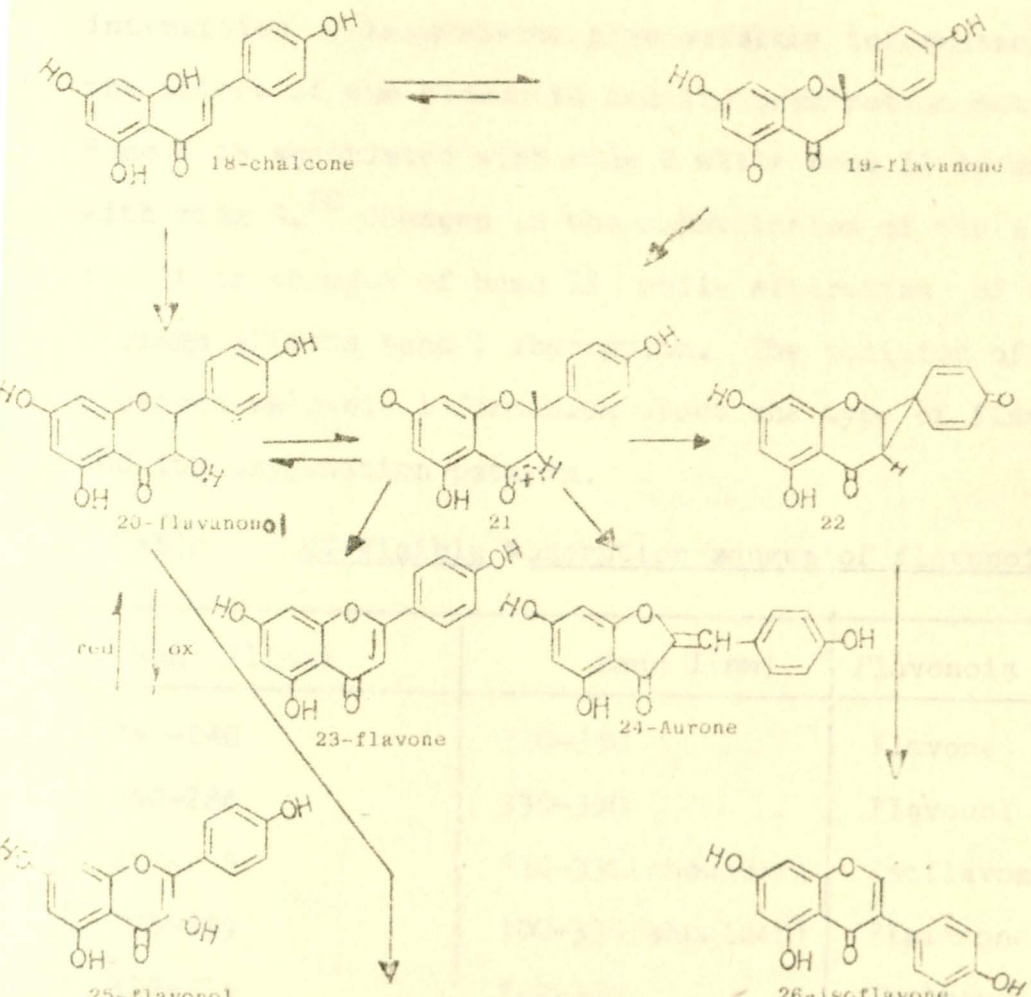
Scheme 1: Formation of 4-Coumaroyl Co- A[24]

The transformations leading to formation of chalcones, consist of 3 successive condensation steps with acetate units which result in the elongation of aliphatic side chain of 4-coumarate by six carbon atoms which then cyclizes to give the aromatic ring A.[24,29]

The isoflavonoids share a common biosynthetic pathway with the other flavonoids as far as chalcone intermediates, but then a 1,2 aryl migration occurs to give the characteristic rearranged skeletom.



Biogenetic relationship of flavonoids



2.4. Spectral properties of Flavonoids

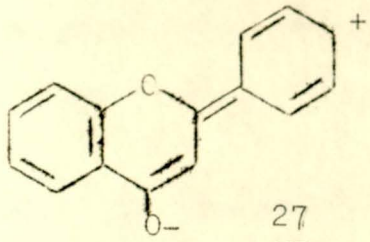
2.4.1 Ultraviolet-Visible Absorption Spectroscopy

UV absorption spectroscopy is one of the most useful tachniques for flavonoid structure analysis. The spectrum typecally consists of two absorption maxima in the region 300-500nm (Band I) and 240-285 nm (Band II).[10,30] The position and intensities of these maxima give valuable information about the nature of the flavonoid and its oxygenation pattern. Band I is associated with ring B while band II is associated with ring A.[30] Changes in the substitution of the A ring result in changes of band II while alteration of the B and C rings affects band I absorption. The position of band I provides valuable information about the type of flavonoid and its oxygenation pattern.

Table 3 UV-Visible absorption ranges of flavonoids.[10]

Band II(nm)	Band I(nm)	Flavonoid Types
250-280	310-350	Flavone
250-280	330-360	Flavonol
247-275	310-330(shoulder)	Isoflavone
275-295	300-330(shoulder)	Flavanone
230-275 (low intensity)	340-390	Chalcone
230-270 (low intensity)	340-390	Aurone
270-280	465-360	Anthocyanins

Band I is considered to be due to absorption of the B ring cinnamoyl system(27)and band II with absorption involving the A ring benzoyl system 28



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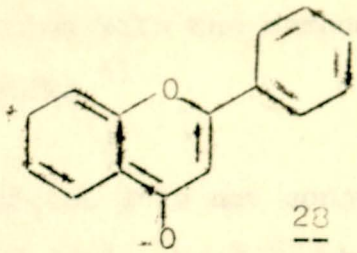

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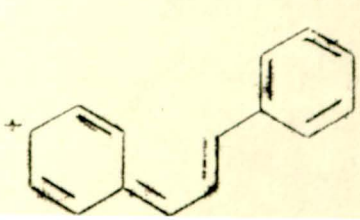
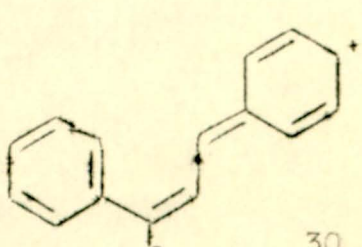
The introduction of electron donating groups such as hydroxyl in the B ring increases the relative resonance contribution of 27 and consequently produce considerable bathochromic shifts of band I (see Table 4)

Table 4 Band I in the UV spectra of flavonols differing in their B ring oxygenation pattern.[39]

Flavonol	Oxygenation pattern		Band I (nm)
	A and C ring	B ring	
Galangin	3,5,7	---	359
Kaempferol	3,5,7	4'	367
Quercetin	3,5,7	3',4'	370
Myricetin	3,5,7	3',4',5'	374

The chief chromophoric systems in chalcones the benzoyl and cinnamoyl groupings, are shown by structure 29 and 30 respectively.





30

Substitution of hydroxyl or methoxyl groups on "B" ring produces bathochromic shifts on band I. Methylation of a 2' hydroxyl group prevents its chelation with the carbonyl group and results in hypsochromic shifts.[31]

In isoflavone the phenyl ring at position 3 is not conjugated with the carbonyl group. Consequently, band I, which in flavones is associated with conjugated "B" ring, is either absent or considerably diminished in intensity.[30] Isoflavones therefore, show one intensity maxima at 250-270 nm band II and a peak or inflection of very low intensities for band I.

As with isoflavones, the B ring of flavanones is not conjugated with carbonyl group. Consequently flavanones absorb most strogly in the 270-290 nm (band II), but band I is an inflection of low intensity.

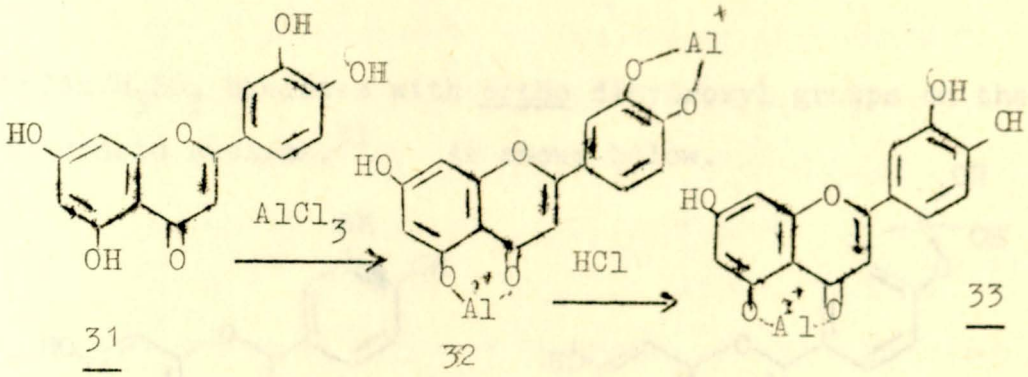
2.4.2 Shift reagents in UV-Visible study of flavonoids

The oxygenation pattern of a flavonoid nucleus may be established by adding " Shift reagents" to the sample solution and observing the resultant shifts in the absorption peaks.[10,30,31]

Some of the shift reagents used are:

- $AlCl_3$/HCl
- Sodium acetate.
- Boric acid - Sodium acetate

$AlCl_3$ has the ability of forming acid stable complexes between hydroxyls and neighbouring keto group and acid labile complexes with ortho dihydroxy groups[10] as shown below.



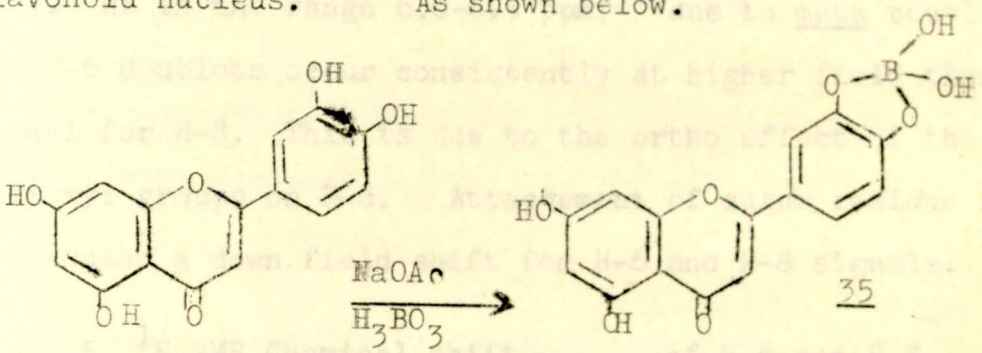
31 32 33

The reagents can therefore be used to detect the presence of both groupings on the flavonoid nucleus. The $AlCl_3$ spectrum represents the sum effects of all complexes on the spectrum, that is, a bathochromic shift due to all of the complexes formed. In the $AlCl_3$/HCl spectrum, however, only the effect of the acid stable complexes, i.e the hydroxy-keto complexes, will be observed because the acid labile complexes (complexes formed by <u>ortho</u> hydroxy groups) are destroyed by the acid. The $AlCl_3$/HCl spectrum therefore indicates the presence of 3 or 5 hydroxy groups in the flavone nucleus and 2' or 6' hydroxyl groups in chalcones resulting in bathochromic shifts.

Sodium acetate is a weaker base than NaOMe and ionizes the most acidic of the flavonoid hydroxyl groups,[10] that is, hydroxyl groups at position 3, 7 and 4' of the flavone nucleus, and is consequently used to confirm presence of hydroxyl groups at these positions. Due to the ionization

of the hydroxyl groups the availability of the lone pair of electrons on the oxygen atom to enter in the conjugation system increases and a bathochomic shift of the flavonoid spectrum will be observed.

$NaOAc/H_3BO_3$ chelates with ortho dihydroxyl groups on the flavonoid nucleus.[31] As shown below.



Due to this chelate formation a bathochromic shift on the spectrum will be observed. Therefore, the reagent is used to detect the presence of ortho dihydroxyl groups on the flavonoid nucleus.

NaOMe is a strong base capable of ionizing all phenolic hydroxyl groups.[10,30,31] It is therefore generally a good "finger print" indicator for the presence of free hydroxyl groups on a flavonoid nucleus.

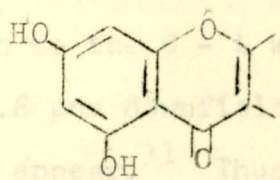
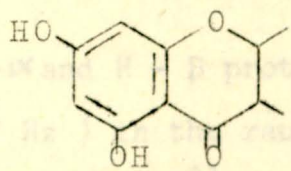
2.4.3 Proton NMR spectroscopy of Flavonoids

Proton NMR spectroscopy is one of the most important tools for the structural elucidation of organic compounds. Present structure elucidation of flavonoids also rely on NMR techniques to a considerable extent. Chemical shifts as well as coupling patterns and coupling constant values

provide information about the substitution pattern of flavonoids.

The protons of ring A located at C-6 and C-8 of flavones, flavonols and isoflavones, which contain the common 5,7 - dihydroxy substitution pattern give rise to two doublets (J:2.5 Hz in the range 6.0-6.5 ppm)[31] due to <u>meta</u> coupling. The H-6 doublets occur consistently at higher field than the signal for H-8. This is due to the ortho effect of the two hydroxyl groups on H-6. Attachement of sugar residue at C-7 causes a down field shift for H-6 and H-8 signals.

Table 5 <u>^{1}H NMR Chemical shift ranges of H-6 and H-8 in flavones, isoflavones and flavonols.[31]</u>

Type structure	H-6 ppm	H-8 ppm
	6.0-6.2	6.3-6.5
	5.75-5.95	5.9-6.1

The protons of ring B usually appear in the range 6.7-7.9 ppm[31] which is down field from the region where the A ring protons absorb. The signal pattern observed for the B ring

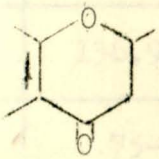
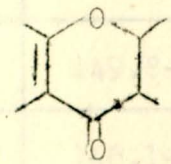
protons is characteristic for the substitution of the ring. In addition it suggests the oxygenation level of ring C. If the B ring is in conjugation with a double bond of ring C as in the case of isoflavone, flavone and chalcones H-2', and H-6' appear together and likwise H-3', and H-4' appear with H-5'. If ring B is oxygenated at C-4' a typical four-peak pattern of two doublets (J: 8.5Hz) is observed.[31] The doublets for H-3' and H-5' always appear upfield from the H-2' and H-6' protons because they will be shielded by the C-4' oxygen substitution.

Considerable variation is found in the chemical shifts of the C-ring protons among the different flavonoid classes depending upon the oxygenation level of the C-ring. The C - 3 proton in flavones gives a sharp singlet near 6.3 ppm while the C- 2 proton in isoflavone which is in the beta position to the C - 4 keto function, occurs in the range 7.6 - 7.8 ppm downfield from where most aromatic proton signals appear.[31] Thus ring C proton chemical shift values aid in differentiating flavone from isoflavone.

The H - α and H - β protons of chalcones occur as doublets (J = 17 Hz) in the range 6.4 - 7.4 ppm (H - α) and 7.3 - 7.4 ppm (H - β).[31] The aurone benzylic proton appears as a singlet between 6.5 - 6.7 ppm[31].

The signal for H - 2 proton of flavanones appears as a double doublets with Jcis 5 Hz and J trans 11 Hz near 5.2 ppm. The geminal protons of C - 3 usually appearing at 2.8 ppm (J = 17 Hz) in addition to their spin spin interaction with H-2.

Table 6 Chemical shifts of C - 2 and C - 3 protons in flavanones and dihydroflavonols.[31]

	H-2	H-3
	5-5.5[a] ppm	2.8[b]
	4.8-5[c] ppm	4.1-4.3[c]

a : quartet J = 5, 11

b : two quartets J = 17, 5 ; J = 17, 11

c : doublet J = 11

2.4.4. ^{13}C NMR of Flavonoids

The different types of aglycones are not distinguishable on the basis of the aromatic carbon resonance alone, but chemical shifts for the central three carbon units are often quite distinctive.[32]

Table 7 ^{13}C NMR Chemical shifts of the central 3 Carbon atoms in Flavonoids.[24]

Types of Flavonoid	C - 2 (ppm)	C - 3 (ppm)	C=O (ppm)
Chalcones	136.9-145.4(d)	116.6-128.1(d)	188.6-194.6(s)
Flavanones	75-80.3(d)	42.8-44.6(t)	189.5-195.5(a)
Flavones	160.5-165(s)	103.0-111.8(d)	176.3-184(s)
Isoflavones	149.8-155.4(d)	122.3-125.9(s)	174.5-181(s)
Aurones	146.1-147.7(d)	=CH- 111.6-111.9(d)	182.5-182.7
In chalcones	C - 2 and C - 3 are C-β and C-α respectively.		

Table 3 C^{13} NMR of unsubstituted chalcones,[24] flavones[23] flavanones and 7-OMe Isoflavone

	C 1'	2'	3'	4'	5'	6'	C 2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10
Flavone	131.9	126	128.8	131.3	128.9	126	163	107.3	178	125.4	124.9	133.5	117.4	156	123.7
Flavanone	138.6	126	128.7	128.7	128.7	126	74.9	44.6	191.6	126.9	124.9	136	117.9	161.3	126.8
7-OMe Isoflavone	127.9	128.3	128.8	131.8	128.8	128.3	153	125.1	175.3	127.6	114.4	163.8	100.0	157.7	118.3
	C-1	C-2	C-3	C-4	C-5	C-6	C-α	C-β	C=O	C-1'	C-2'	C-3'	C-4'	C-5'	C-6'
Chalcone	134.7	128.3	128.3	130.3	128.3	128.3	144.4	121.7	189.8	138.0	128.7	128.7	132.6	128.7	128.3

To assign chemical shifts in C^{13} NMR spectrum of new flavonoids. One can use the additive principle using the C^{13} NMR spectrum of known flavonoids.

Flavanones

In the ^{13}C NMR spectra of flavanones the carbonyl signal comes in the region 189.5 - 191.6[31]ppm except when a 5-OH group is present that shifts the peak to lower field due to hydrogen bonding. The C - 3 methylene carbon singnal is characteristic and is at 42.8 - 44.6 where as the C - 2 oxymethine carbon resonates at 75 - 80[32]ppm. The aliphatic carbon signals of flavanones are easily identified and the characteristic chemical shifts of C - 2 and C - 3 can be used to distinguish it from other types of flavonoids such as isoflavones and flavones.

Flavones and Isoflavones

The carbonyl carbon signals of both flavones and isoflavones come in the region 174.5 - 178.6 ppm but C -2 and C - 3 are sufficiently different in the two series to permit an immediate distinction. In the flavones the C - 2 signal appears as a singlet (in the off-resonance decoupled spectrum) at 160.5 - 163.2 ppm and that of C - 3 as a doublet at 104.7-111.8 ppm where as in isoflavones the C - 2 resonance is seen as a doublet at 149.8 - 155.4 and that of C - 3 as a singlet at 122.3 - 125.9ppm (in the off resonance decoupled spectrum).[33]

Chalcones

In the ^{13}C NMR spectra of chalcones the carbonyl carbon signals come between 188.6 - 194.6ppm. The presence of a

2' hydroxy- group moves this signal downfield due to hydrogen bonding. The α and β Carbon atoms give rise to signals at 116.6 - 128.1 and 136.7 - 145.4 ppm respectively.[32]

2.4.5 Mass spectroscopy of flavonoids

The first objective in interpreting a flavonoid MS is to identity the unfragmented molecular ion and then to relate other major fragments to it by rationalizing the loss in moleculer weight using recognized fragmention path way.

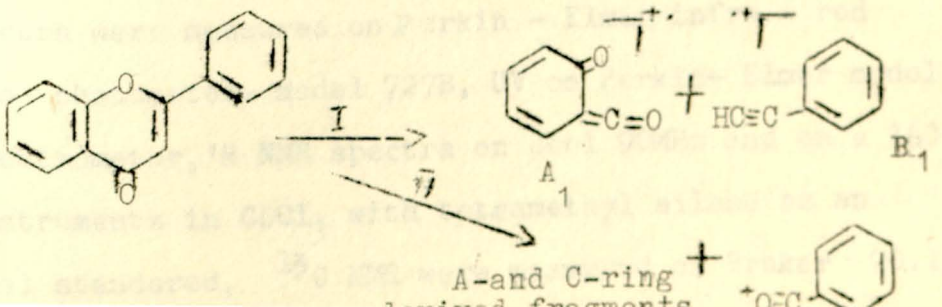
Molecular ion (M^{+}). The molecular ion normally appears as a major peak in the MS of aglycones. Therefore it is simple to get the elemental composition of aglycones. $(M - 1)^{+}$ which is loss of hydrogen is common in most flavonoids.(33)

A and B ring Fragmentation

Fission of the M^{+}ion into A and B ring containing fragments allows identification of the substitution pattern of the A and B ring. The fragmentation usually involves.(34,35,36,37)

I) Retro-Diels-Alder fission.

II) Path I1



3. Experimental

3.1. Plant material

Literature search on the Tephrosia species of Ethiopia revealed that a number of species have not been examined for their chemical composition at all. Because of the potential economic importance of the genus and the recent world wide revival of interest in plants known to contain flavonoids, we set out to study the chemistry of Tephrosia of Ethiopia. This is the first of a series of such studies and we would be reporting here the results of our investigation with one of the indigeneous Tephrosia species namely Tephrosia pumila.(Lam.) Pers. This plant, although known to occur in many parts of Tropical Africa, Asia and Central America, has so far not been investigated at all. T.pumila is found in abundance in the Gibey valley and is also reported to occur in the provinces of Tigria[8], Gondar, Wellega, Hararghe, Illubabor, Gamu Gofa and Sidamo[8]. The pods leaves and roots were collected from Gibey valley in August 1986. The plant was identified by Mr Michael Gilbert and a voucher sample was kept in the National Herbarium, Addis Ababa.

3.2 Materials and apparatus

Melting points were determined on Kofler hot stage apparatus. IR spectra were measured on Perkin - Elmer infra - red spectro- photometer- model 727B, UV on Perkin- Elmer model 555 spectrometer, ^1H NMR spectra on Jeol 90MHz and on a 360 MHz instruments in $CDCl_3$ with tetramethyl silane as an internal standared. ^{13}C NMR were measured on Bruker 90.13MHz.

The mass spectra were recorded on a high resolution AEI MS 902 spectrometer. Analytical thin layer chromatography (TIC) were run on a 0.2mm thick layer of silca gel and the products were detected by their UV fluorescence and by spraying with 0.5% fast blue salt B solution followed by 0.1N NaOH.[38] Preparative layer chromatography were run on 1 mm thick layer of silca gel and the product was detected by their UV fluorescence. Column chromatography were performed on silca gel 60(70 - 230 mesh) and Sephade LH - 20.

Table 9

Solvent system used for column, thin and preparative layer chromatography.

No	Solvent System	Ratio
1	Toluene : Hexane : EtOAc	3: 4: 3
2	MeOH: $CHCl_3$	1: 1
3	Benzene : EtOAc	9: 1
4	Benzene : EtOAc	3: 2
5	Hexane : $CHCl_3$	1: 1

3.3. Extraction, fractionation and isolation

Pods of T. pumila (300g) were extracted on Soxhlet apparatus using petether followed by chloroform. The petether extract gave 6g of greenish sticky product (T-1) while the chloroform yielded 6g of crude extract (T-2). All of the latter extract (T-2) was applied on a silca gel (100g) column and eluted first with benzene/EtOAc 9:1.

Five fractions, each 100 ml, that were eluted with solvent system 3 were combined based on their TLC similarities and concentrated to give 2g of greenish oil (T - 3). Three fractions, each 100ml, that were eluted with solvent system 4 were combined to give a greenish oil (T - 4).

T - 3 was re-chromatographed, on Sephadex LH-20, to remove chloropyll and fats using solvent system 2 as eluent. A total of fifteen fractions each 50 ml were collected. The first few fractions contained only chlorophyl and fats. Fractions 4-6 were combined and concentrated to give 70mg of yellowish oil (T-5) which on addition of methanol gave a precipitate (T-6). The filtrate afforded a yellow oil (T-7).

Chromatography of T-4 on sephadex LH - 20 using solvent system 2 afforded reddish oil (T-8).

T-6, T-7 and T-8 separatly and repeatedly were column filtered to remove fats and other impurities using solvent system 5 as eluent. Finally they were purified using prep TLC developed by solvent system 1 and final purification by Sephadex LH-20 usimg solvent system 2.

T-8 yielded 6 mg of as yet unknown compound designated as Tp-5, T-6 gave 11mg of the novel compound that we named pumila iso-flavone-A36, T-7 gave 50 mg of an oil identified initially by spectroscopic means as praecansone A which was

first isolated from Tephrosia praecans by Camele et al 1980. Based on the spectroscopic data, particularly ^{13}C NMR and MS, arguments for revising the structure of praecansone-A to 53 is presented in section 4.2.

It was not possible from TLC of the crude extract to establish the total number of flavonoids present in the pods, due mainly to masking by the chlorophyll and the fats. However after the fractionation it was possible to determine that 8 flavonoids were present labelled as Tp-1 to Tp-8.

Table 10

Rf value of the components using solvent system L

Spots	Rf	Compound
Tp-1		
Tp-2	0.64	Pumila-isoflavone-A
Tp-3	0.46	Praecansone A
Tp-4		
Tp-5	0.31	Unknown
Tp-6		
Tp-7		
Tp-8		

Spots of Tp-3 and Tp-4 were not sensitive to the spraying reagent while the other spots developed colours when sprayed.

4. Results and discussion

4.1. Characterization of pumila-isoflavone-A

Table 11

Summary of Physicochemical data of Pumila-isoflavone-A

I TLC	Single spot, Rf 0.64 using solvent system 1
2 Colour reaction	$FeCl_3$ (greenish), NaOH (yellow, Shinoda test (-ve) and conc.-H_2SO_4 (yellow)
3 MS	$C_{27}H_{28}O_7M^+$: 464, major fragments at m/z (relint.) 464(9) 449(5), 396 (M - $C_5H_8)^+$- 086), 381(100), 203(4), 177(2),142 (2)
4 Mp	197 - 200°c
5 NMR, UV, IR	See text.

Pumilaisoflavone-A36: The UV spectrum of pumila-isoflavone-A (Fig.1) gave single absorption peak at 280nm which is characteristic of an isoflavone nucleus. The $AlCl_3$ and $AlCl_3$/HCl spectra showed bathochromic shift indicating the presence of OH at position 5 of the flavonoid nucleus. The phenolic nature of the compund was further confirmed by the +ve reaction with 1% $FeCl_3$ solution.

The IR spectrum (Fig.2) shewed no OH absorption but a chelated keto group at 1630cm^{-1} showing that the 5 hydroxyl group may be chelated.

The ^{1}H NMR spectrum (Fig 3) showed a deshielded phenolic proton singlet at 13:13 ppm. Signals at 6.8(d) and 5.5 ppm(d) which integrate for one proton each, correspond to the vinylic protons H-4" and H-3" respectively.[40] A singlet apeared at 7.8ppm which is characteristic of an isoflavone proton, H-2. Three dd's at 6.22 (J=17.5, 10.8Hz), 5.1 (J=17.5, 10.8Hz) and at 4.98ppm (J=10.8, 1.2Hz) of H-3", H-3"' (trans) and H-2" (cis) respectively support the -dimethyl allyl nature of the prenyl side chain. A singlet at 3.81ppm (6H) indicated the presence of two equivalent methoxy groups. Two singlets at 1.47 and 1.46 ppm (6 protons each) indicated the presence of 2Me groups on the chromene ring and two on the prenyl side chain. Two equivalent aromatic protons at 6.71 ppm suggested the B ring to be symmetrically substituted.

The high resolution MS of this compound yielded $C_{27}H_{28}O_7$ confirming an isoflavonoid nucleus, attached to two prenyl units three methoxy and one hydroxy groups. Furthermore the ion at m/z 177, resulting from Retro Diels Alder fission of the A and B rings, suggested the attachment of the two methoxy and the C-prenyl side chain to be on the B ring. A major peak at m/z 395 ($M^{+}-C_5H_9$) can be best accounted for if the prenyl group is ortho to the methoxy groups. This is because the cleavage of

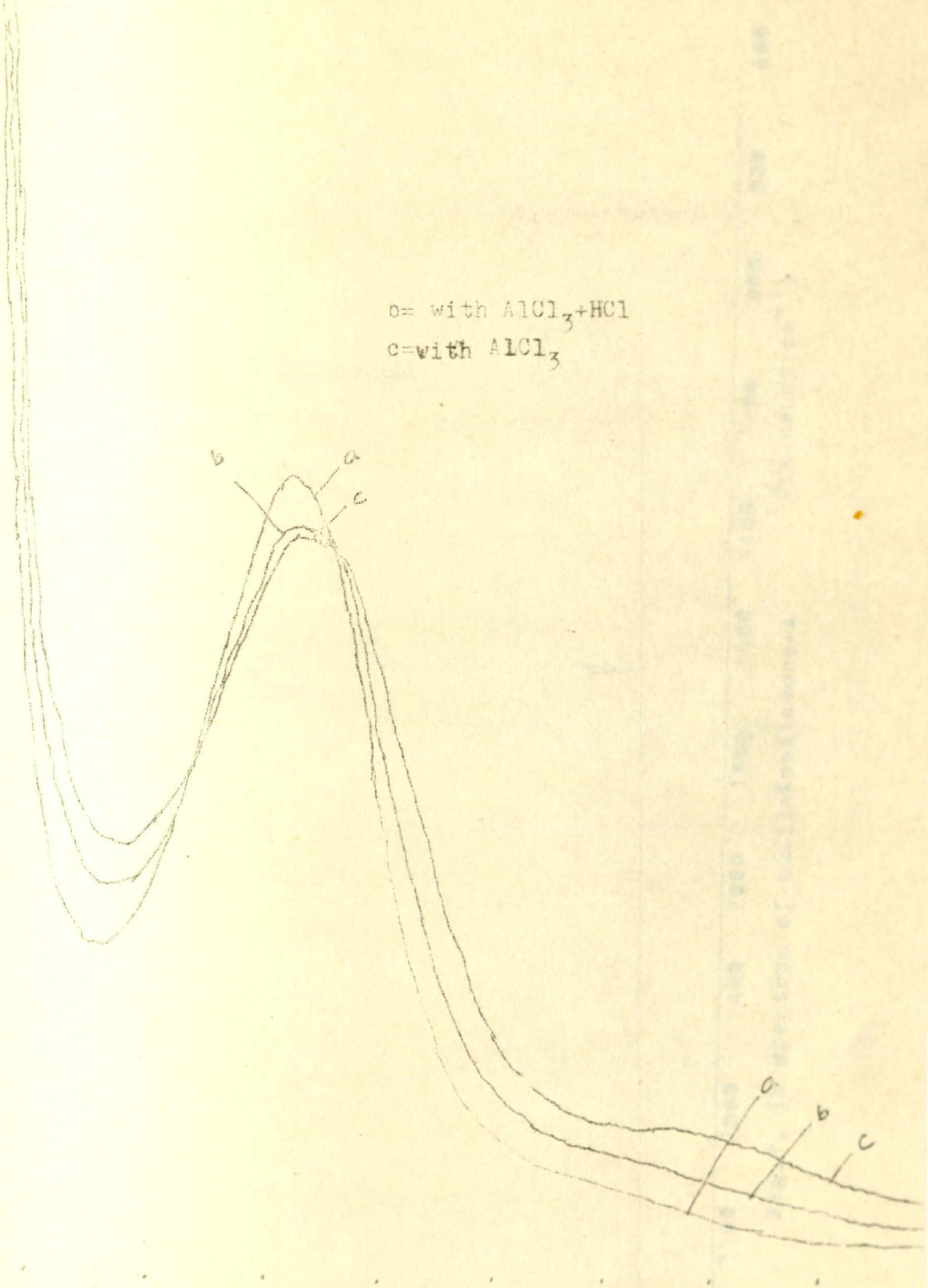


Fig. 1. UV absorption of pumilaisoflavone-A

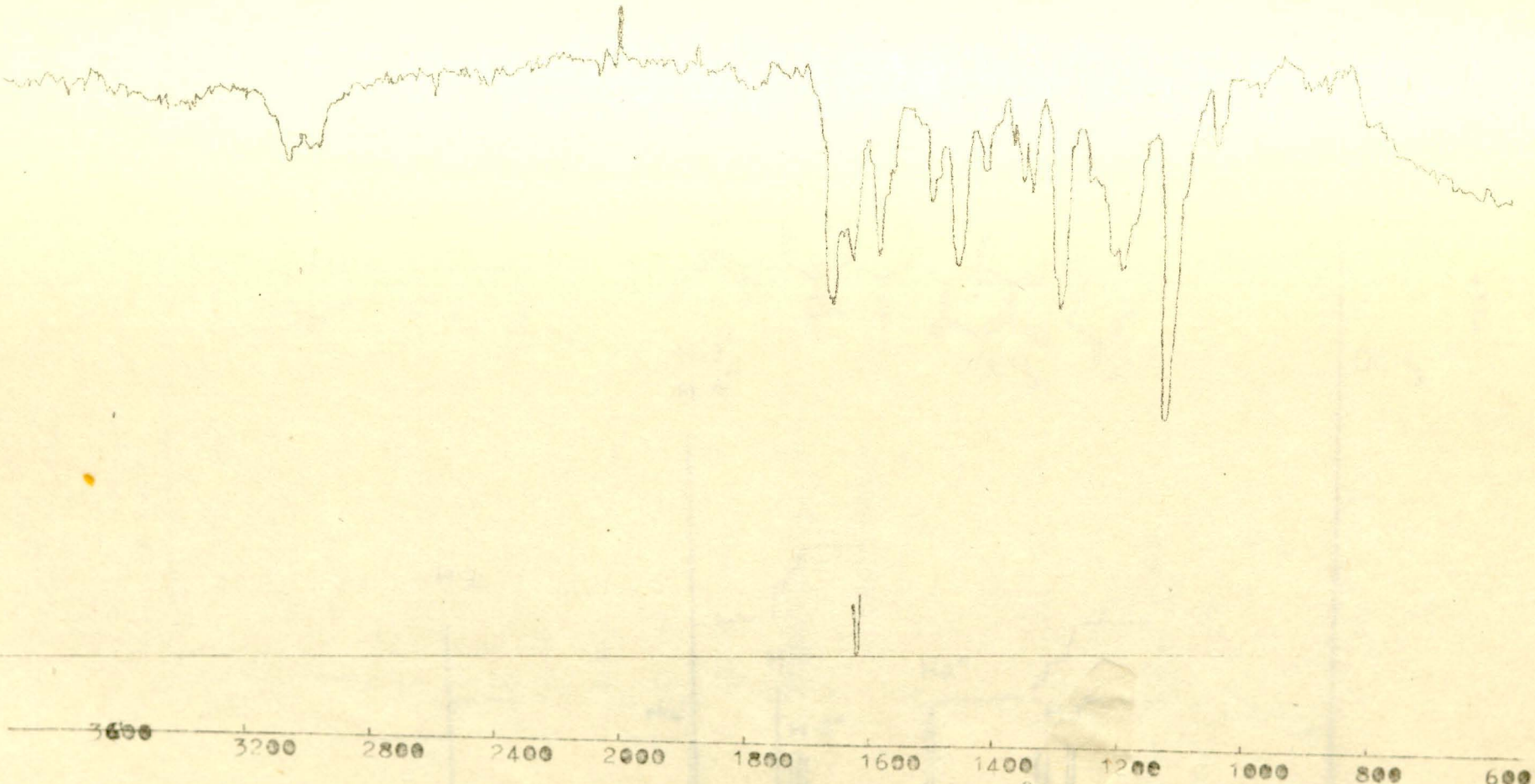


Fig.2. IR spectrum of pumilaisoflavone-A

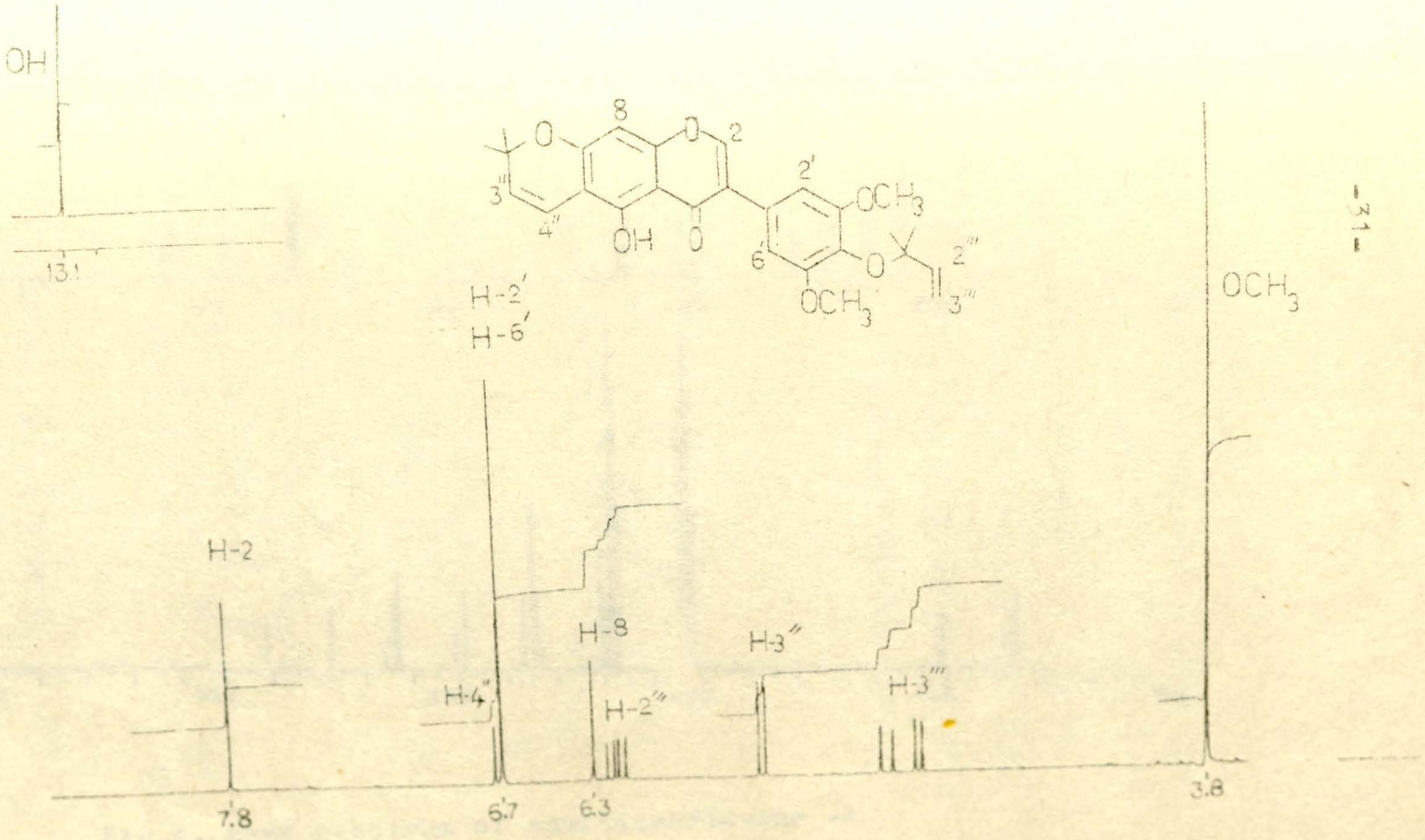


Fig.3. Partial ^{1}H NMR of pumilaisoflavone-A

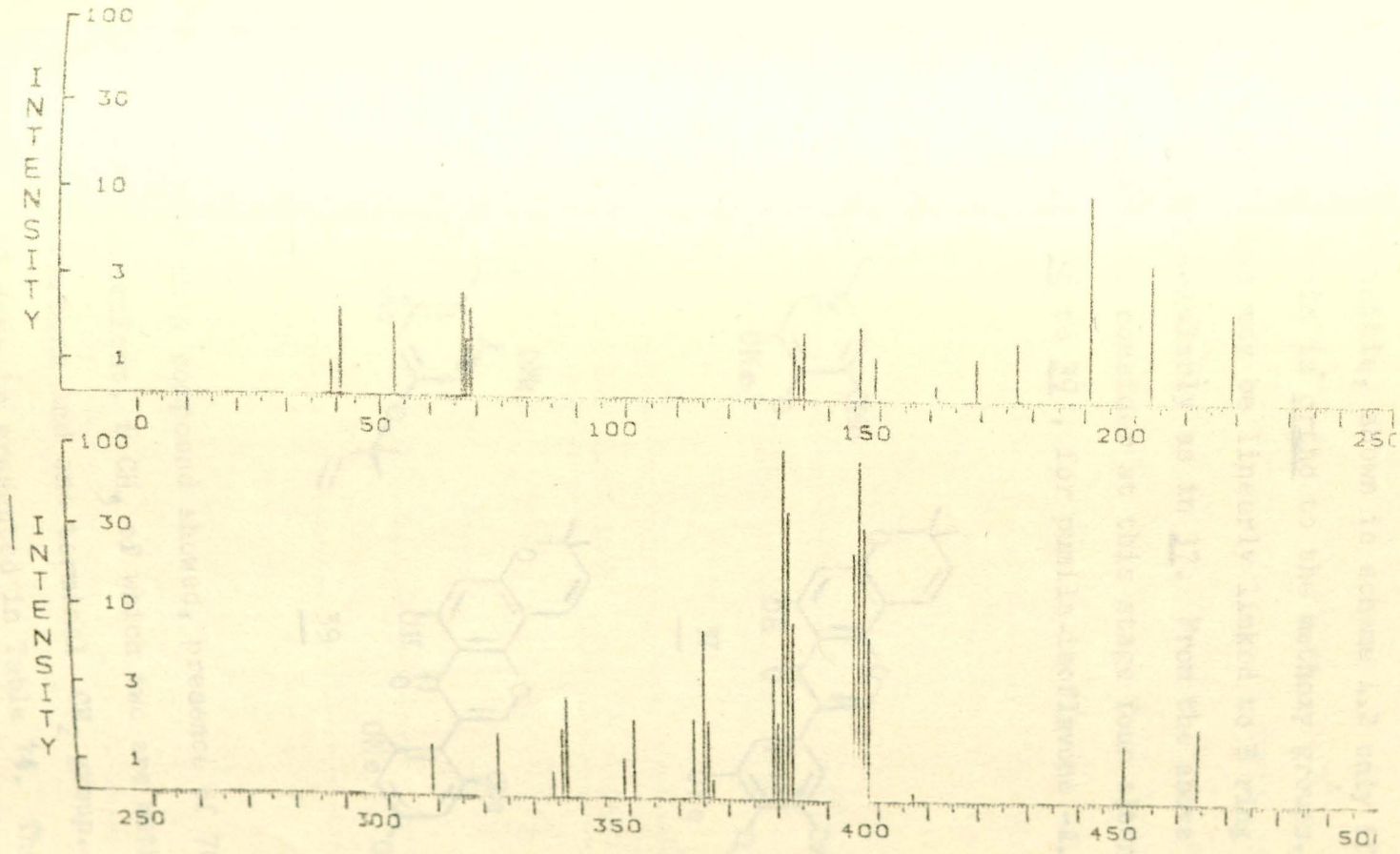
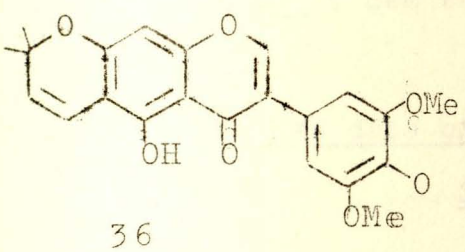


Fig.4. Mass spectrum of pumilaisoflavone -A

a C_5H_9 group is possible, shown in scheme 4.2 only if the O-prenyl side chain is ortho to the methoxy groups.[41,42] The chromene ring may be linearly linked to B ring as shown in 36 or angularly as in 37. From the above data it is possible to consider at this stage four alternative structures i.e 36 to 39 , for pumila-isoflavone -A.



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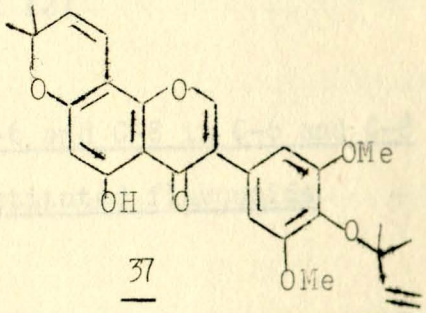

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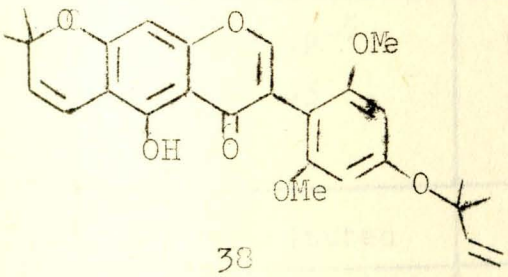

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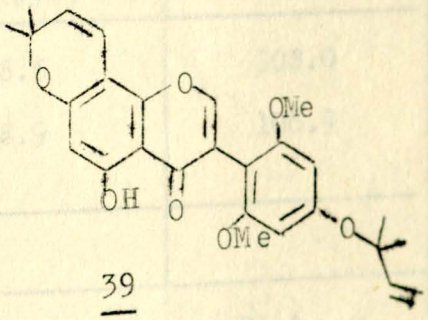


39

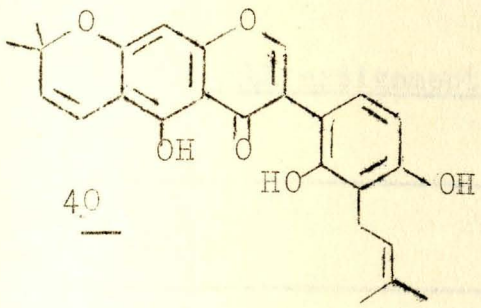
The ^{13}C NMR of this compound showed, presence of 7CH of which 2 are equivalent, 6 CH_3 of which two are methoxy, 13 quaternary carbons and one terminal CH_2 group. The ^{13}C measurement data is presented in Table 14. The relatively shielded position of C-4' at 134.7 ppm requires ortho oxygenation at C-3' and C-5'. This also confirme

the two methoxy groups.

The ^{13}C spectral analysis is perticularly useful in distinguishing from the two remaining proposed structures 36 and 37. The linear structure 36 is prefered by the presence of shielded aromatic tertiary carbon at 94.7 ppm in the ^{13}C NMR of the compound which is typical of C-8 in C-6 substituted 5,7 oxygenated flavonoids, like that of angustone[24] C. 40 (See table 12)

Table 12 ^{13}C NMR data of C-6 and C-8 in C-6 and C-8 Substituted flavonoids

C-8 Substituted	C-6(ppm)	C-8(ppm)
Moracenin B[45]	98.5	108.0
Kuwanon C[45]	98.9	106.9
C-6 Substituted		
Angustone C[24]	128.9	94.6
Wighteone[24]	111.2	93
Luteone	111.2	93



40

Table 13

^{1}H NMR assignment of pumila-isoflavone-A 36

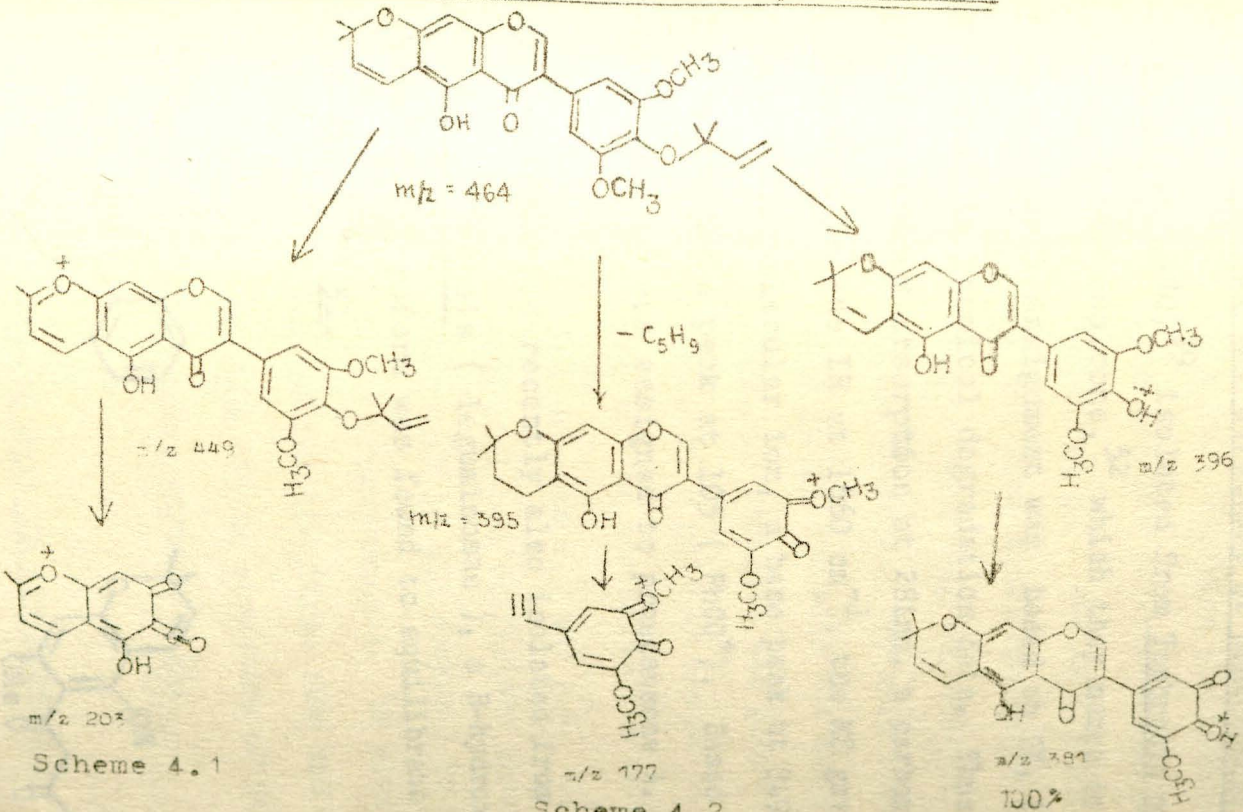
Proton	ppm
5-OH	13.13 (1H, s)
H-2	7.86 (1H, s)
H-4"	6.72 (1H, d, J=10.1)
H-3"	5.61 (1H, d, J=10.1)
H-2', H-6'	6.71 (2H, s)
H-8	6.53 (1 H, s)
H-2"'	6.22 (1 H, dd, J=17.5, 10.8Hz)
H-3"' (trans)	5.11 (1 H, dd, J=17.5, 1.2 Hz)
H-3"' (cis)	4.98 (1 H, dd, J=10.8, 1.2Hz)
3'-OMe, 5'-OMe	3.82 (6H , s)
2"-Me_2, 1"'-Me_2	1.47, 1.46 (2 x 6H, 2s)

Table 14

^{13}C NMR assignment of pumlila-isoflawone -A

Carbon	ppm
1'''-Me_2, 2''' = Me_2	26.4, 28.2
3'-OMe, 5'-OMe	55.9
C - 3'''	111.9
C - 8	94.7
C - 2' C - 6'	106.3
C - 3''	115.3
C - 4''	128.1
C - 2'''	144.1
C - 2	152.9
C - 2''	78.0
C - 1'''	82.5
C - 10, C -6	105.7, 106.0
C - 3	123.7
C - 1'	126.2
C - 4'	134.7
C - 3', C-- 5'	155.1
C - 5, C - 9	156.9, 157.1
C - 7	159.7
C - 4	180.6

Major Fragments in MS of Pumila isoflavone



Scheme 4.1

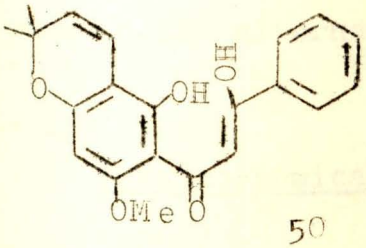
Scheme 4.2

Scheme 4.3

4.1. Revision of the structure of praecansone A

Camele et al in 1980,[39] isolated from Tephrosia praecans. (Leguminosae) the chalcone,[52] which they named as praecansone-A. The structural assignment was based on UV, IR, MS and ^{1}HNMR as well as chemical degradation data. Thus the UV displayed a maximum absorption at 284nm, a carbonyl absorption appeared in the IR at 1660 cm^{-1}, the MS gave in addition to the molecular ion, a base peak at 349(M-31) and interestingly a peak at 105 ($PhCO^+$). Based on these data structure 52 was assigned to praecansone A.

Waterman and Mahmoud recently also isolated from Loncho-carpus constaricensis (leguminosae), a B-hydroxy chalcone 50, which in chloroform was found to equilibrate with the diketo tautomer[44] 51.



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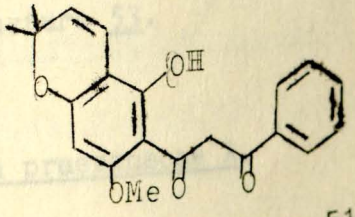


51

Tentative assignments were given to the ^{13}C resonances of 50 and 51. Accordingly most of the resonances assigned were:

	1	2/6	3/5	4	1'
50	135.0	129.7	127.5	134.0	103.4
51	137.9	129.6	128.9	132.9	103.8

An interesting observation reported for compound 51 was the chemical shift of the methoxy group in the ^{1}HNMR which was 4.50 ppm as opposed to 3.90. Waterman and Mahmoud did not elaborate how they ruled out the alternative structure (not shown here) for 50, Pelter et al[43] during a study of the ^{13}C NMR of chalcone 54 and some model synthetic compounds, 55 and 56 raised questions regarding the validity of the proposed structure of praecansone-A, by only reinterpreting the data given by Camele et al and with out studying the compound itself. Particular emphasis was given by these workers to the appearance of a peak in the MS at m/z $(M-31)^+$ and 105, both these can not be accounted for by structure 52, as can be surmised from schemes 4.5 and 4.6. We have during the course of this work isolated sufficient amount of a compound which was identical in all respects to that reported by Camele et al for praecansone A. We have generated ^{13}C data on the compound for the first time and these enable use to suggest a revision of the structure of the title compound to the alternative structure 53.

Table 15

Physicochemical date on praecansone A.

Mp colour reaction	Light yellow oil $FeCl_3$(-ve), NaOH (Redish conc-- H_2SO_4(deep yellow), Shinoda test(-ve)
TLC	Rf, 0.46 (solvent system 1)
MS	380(M^+) , 365 (rel. int 34), 350(24), 349(100), 105(15), 77(11)

The UV spectrum showed as in in ref. 39, absorption at 284 nm and did not change upon addition of $AlCl_3$, indicating absence of OH groups at the 2' and 6' positions of the chalcone, further confirmed by the negative $FeCl_3$ test.

The IR spectrum showed absorption at 1650 cm^{-1} attributed to the presence of an alpha, beta unsaturated carbonyl group. Outstanding signals in the ^1H NMR: Two doublets at 6.45 and 5.43 ppm characteristic resonance for the two olefinic protons of the chromene ring, H-4" and H-3" respectively; three each three proton singlets at 3.85, 3.69 and 3.64 due to three methoxy groups; a two proton dd at 7.81 due to H-2 and H-6 would resonate at such low fields only if they are adjacent to a carbonyl functionality as in 53 but not 52.

Table 16

^1H NMR spectral data of praecansone A (53)

H-2, H-6	7.81 (2M, dd, J 8, 2Hz)
H-3, H-4, H-5	7.41-7.3 (3H-m)
H-4"	6.45 (1H, d, J = 9.6)
H.3"	5.43 (1H, d, J=9.6)
H- α	6.4 (1H, s)
H-5'	6.13 (1H, s)
2'-OMe, 6'-OMe, β-OMe	3.85, 3.69, 3.64 (3 x s)
2" - Me_2	1.39 (6H,s)

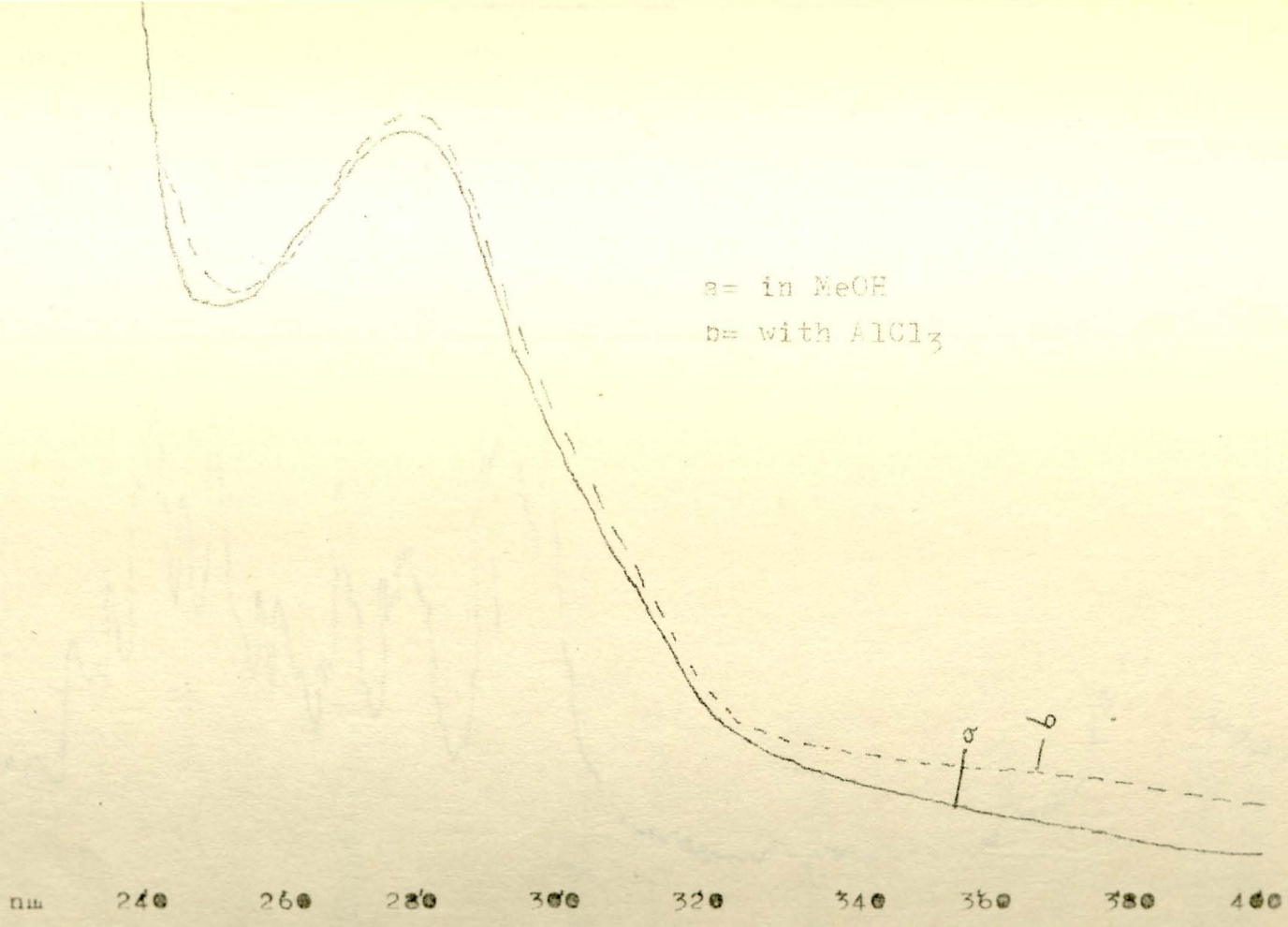


Fig.5. UV absorption spectrum of praecansone-A

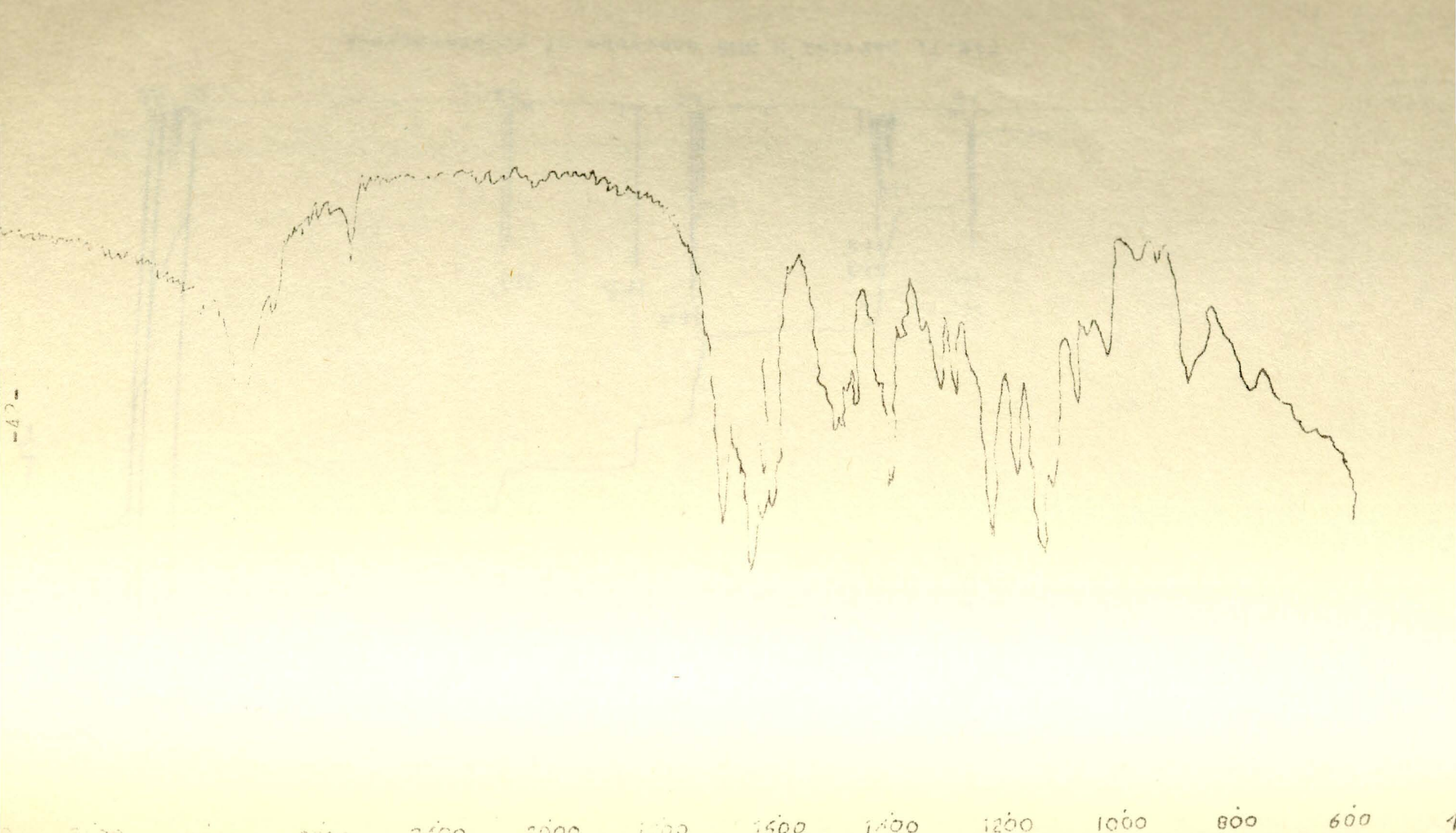


Fig 6 IR-spectrum of praecansone-A

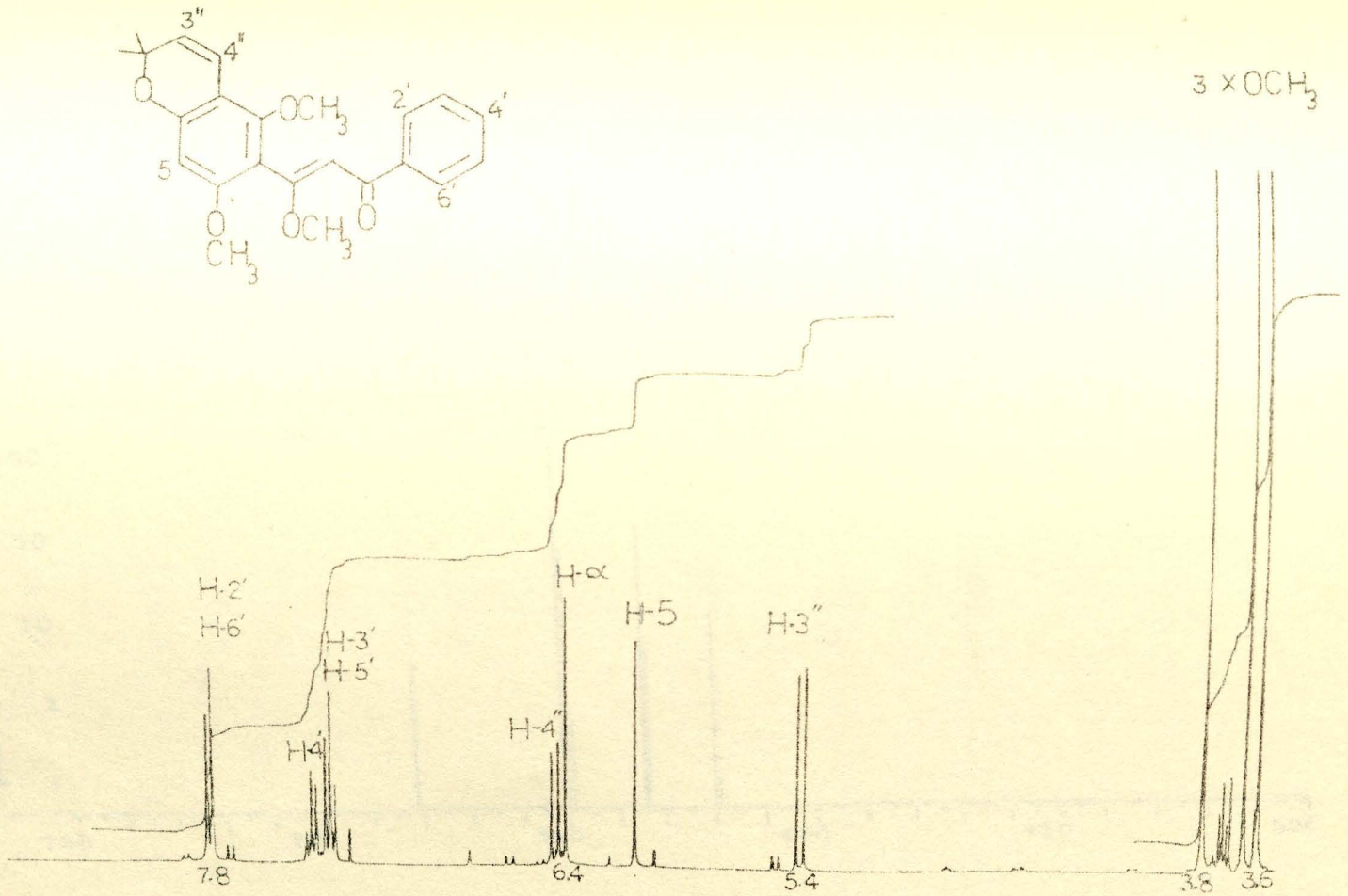


Fig.7. partial ^{1}H NMR spectrum of praecansone-A

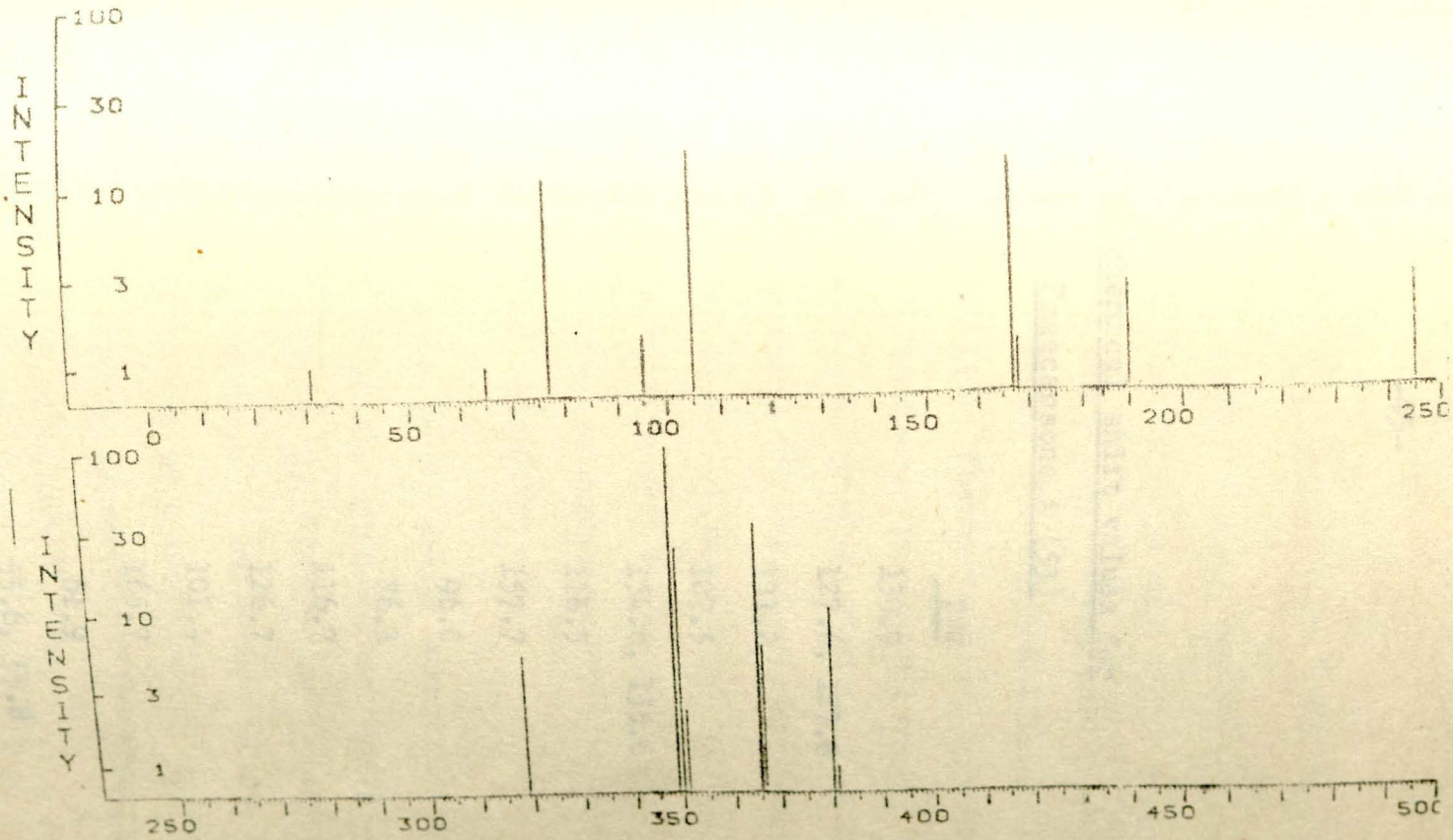


Fig.8. Mass spectrum of praecansone-A

Table 17

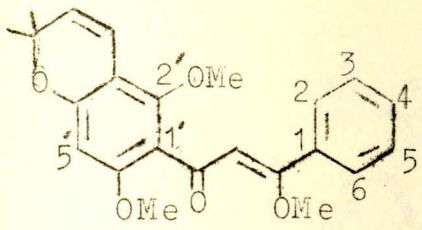
^{13}C NMR chemical shift values for Praecansone A (53)

Carbon	ppm
1'	139.9
2',3',5', 6'	127.6, 127.8
4'	131.2
1	107.5
2, 6	154.6, 155.6
3	114.5
4	157.7
5	96.0
2"	76.3
3"	116.7
4"	126.7
α	101.4
β	165.7
2'-OMe	61.9
6'-OMe, β-OMe	55.6, 55.8
2"-Me_2	27.8
C = O	189.9

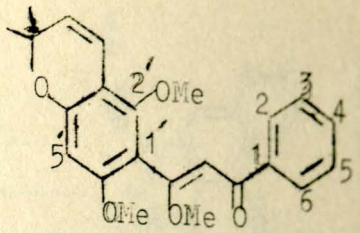
The peaks in the MS, worthy of note, are the signals at m/z 105 and 349 which could arise only if the carbonyl is attached to the B ring, rathar than, as suggested originally, to the A ring. The fragmentation patterns are shown Scheme 4.5 and 4.6. Further evidence in support of structure 53 for praecansone-A is provided by the ^{13}C NMR spectrum. The ^{13}C data of praecansone-A is given in Table 17. The DEPT. programmed ^{13}C NMR of this compound showed the presence of 5 methyl groups of which three are methoxy, 9 CH (two of the peaks each represent 2 equivalent CH) and 9 quaternary carbons. C-1 resonates at 139-9ppm which is due to the carbonyl group attached to it.

Pelter et al have shown that a phenyl carbon directly attached to a carbonyl (as in acetophenone) is expected to have a chemical shift 4-5 ppm at lower field than the one attached to an olefin (as in styrene). The effect is also transmitted to some extent to the para carbon and a corresponding difference of 1 or 2ppm is expected for the above two types of compounds for their resonance at C-4.

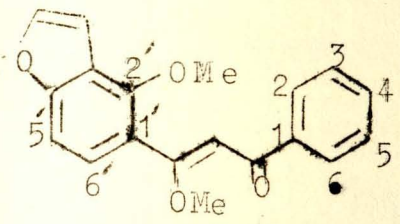
The ^{13}C resonance values of praecansone-A can be accounted for best if the carbonyl is attached to the phenyl ring as in 53 and differs considerably from values for the alternative models.



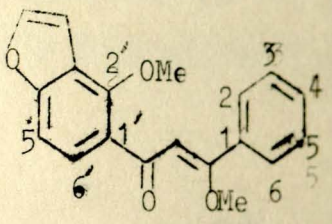
52



53



54



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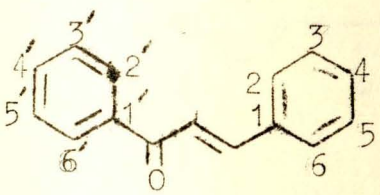

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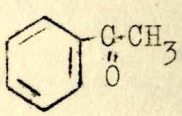

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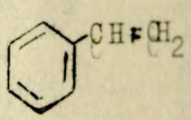


58

Table 18

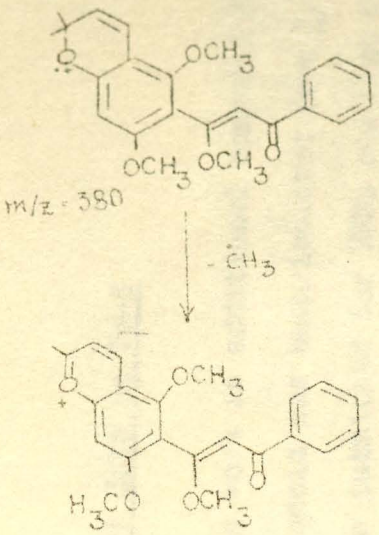
Chemical shifts of 53, 54, 55, 56, 57, 58.

C	53+	54+	55	56	57	58
1	139.9	139.76	135.48	134.7	137.1	137.8
4	131.2	131.84	129.61	130.1	132.9	127.6
1'	107.5	118.48	119.13	138.0	---	----

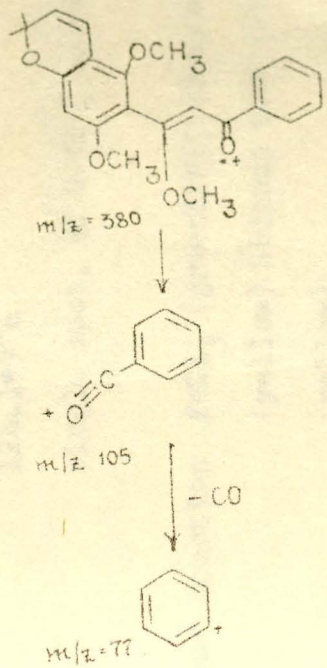
+For the sake of simpilicity the numbering system of chalcones is not followed for the compounds.

The MS and ^{13}C NMR data are thus in accord with structure 53 for praecansone-A, hence our suggestion to revise its

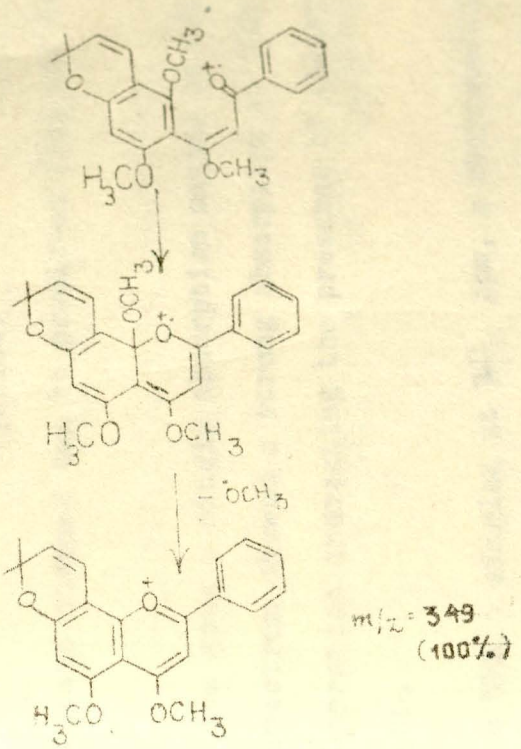
Major Fragments in MS of Praecansone A



Scheme 4.4



Scheme 4.5



Scheme 4.6

4.3 Preliminary data on Tp-5

In the course of this work an additional compound disignated here as Tp-5 isoflavonoid has been isolated from T. pumila. The compound has been submitted for HRMs and high resolution NMR studies and conclusive results, it is hoped, will be forth coming:

MP	126.1•9°c
TLC	single spot, Rf 0.31 (solvent system)
Colour reaction	$FeCl_3$ (greenish), conc, H_2SO_4 (yellow) Shinoda test (-ve), NaOH (yellow)

The class of this compund can be predicted from the data collected.

The UV spectrum gave a single absorption maxima at 280 nm and the IR spectrum showed a strong absorption at 1620 cm^{-1} and no OH absorption indicating the presence of chelated carbonyl group.

In the proton NMR a singlet at 7.8 ppm, a characteristic absorption of an isoflavone proton, H - 2, strongly suggests Tp-5 to be an isoflavonoid, bearing a hydroxyl at C-5. The chelacted OH group can be substantiated further by the deshieled singlet at 13.13 ppm.

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D E C L A R A T I O N

L the undersigned, declare, that this thesis is my work and that all sources of material used for the thesis have been duly acknowledged.

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