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FLAVONOIDS OF THE SEED - PODS
OF TEPHROSIA PUMILA

BEKELE DINKU

JUNE , 1987

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Abstract

Flavonoids of Tephrosia Pumila

by

Bekele Dinku

Research Advisor Dr. Ermias Dagne

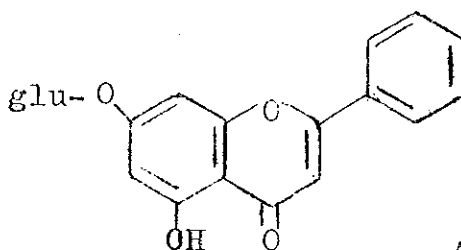
The Chloroform extract of Tephrosia pumila (Lam.) Pers. (Leguminosae) revealed the presence of eight flavonoids. Out of these three flavonoids were isolated. The major component was identified as the known praecansone A that required structural revision based on ^{13}C NMR and other spectroscopic data.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

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 hydrolyzed by acids.

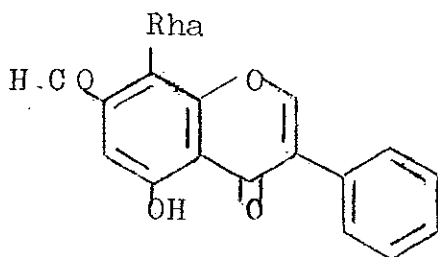
3



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 4¹⁰

4



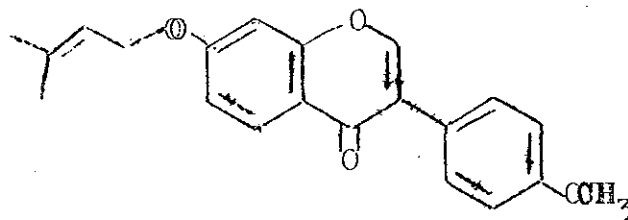
Volubilin²³

2.1.2. Flavonoid sulphates

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

2.2. Tephrosia Flavonoids

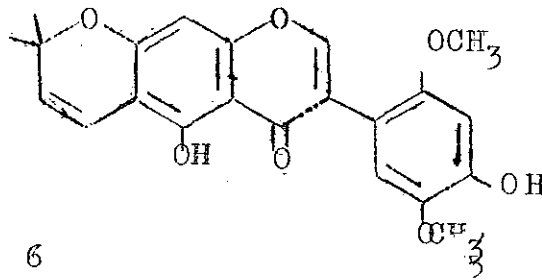
The genus Tephrosia is distinguished by the production of a special group of 7-oxygenated or 5/7 oxygenated flavonoids. The B ring is usually unsubstituted. The C-8 or C-6 positions commonly bear substituents derived from a prenyl group,^{7,24} which has in many cases undergone complex processes of further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



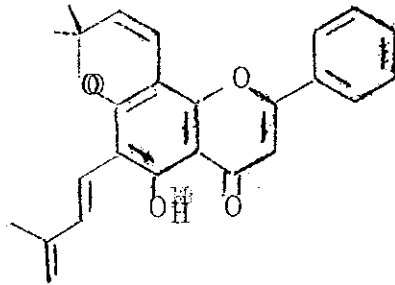
5 - maxima isoflavone J²⁵ (O-prenylated.)

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

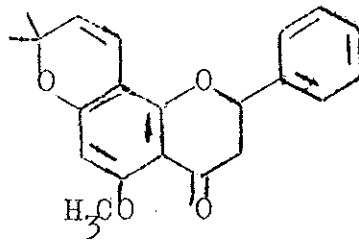
- a) C-6 prenylated (6)
- b) C-6/C-8 prenylated (7)
- c) C-8 prenylated (8)



6
—
elongatin²⁶



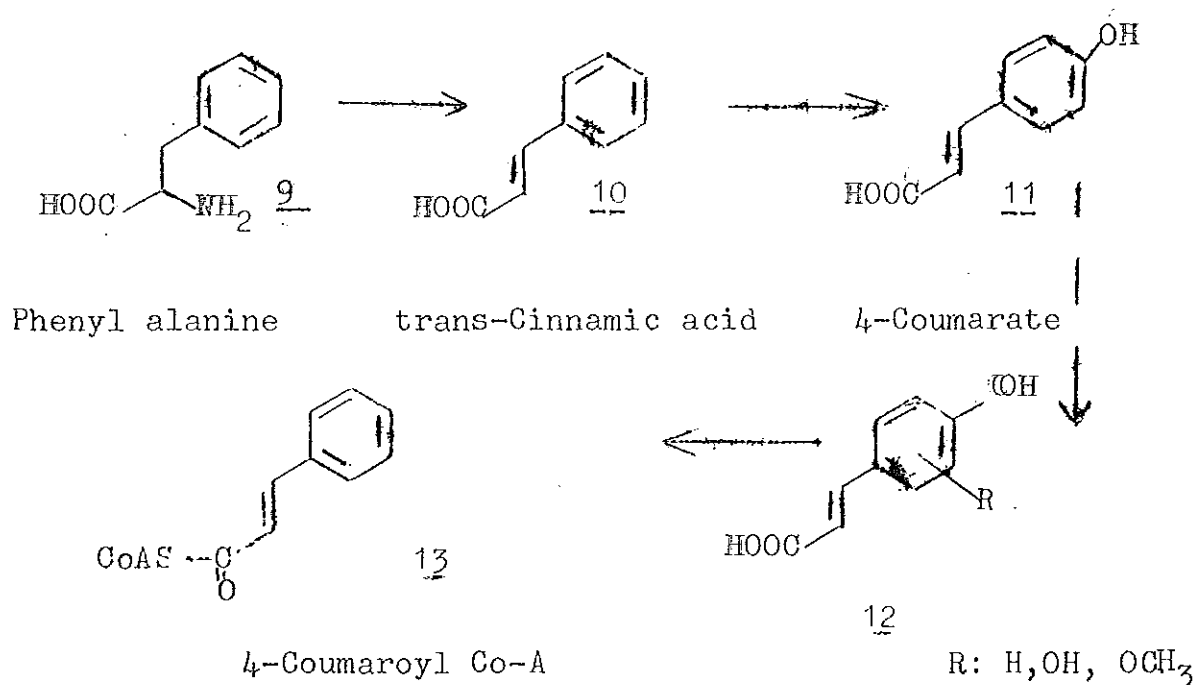
7
—
Fulvinervin B²⁷ (C-prenylated)



8
—
Obovatin methylether²⁸ (C-prenylated)

2.3 Biosynthesis of Flavonoids

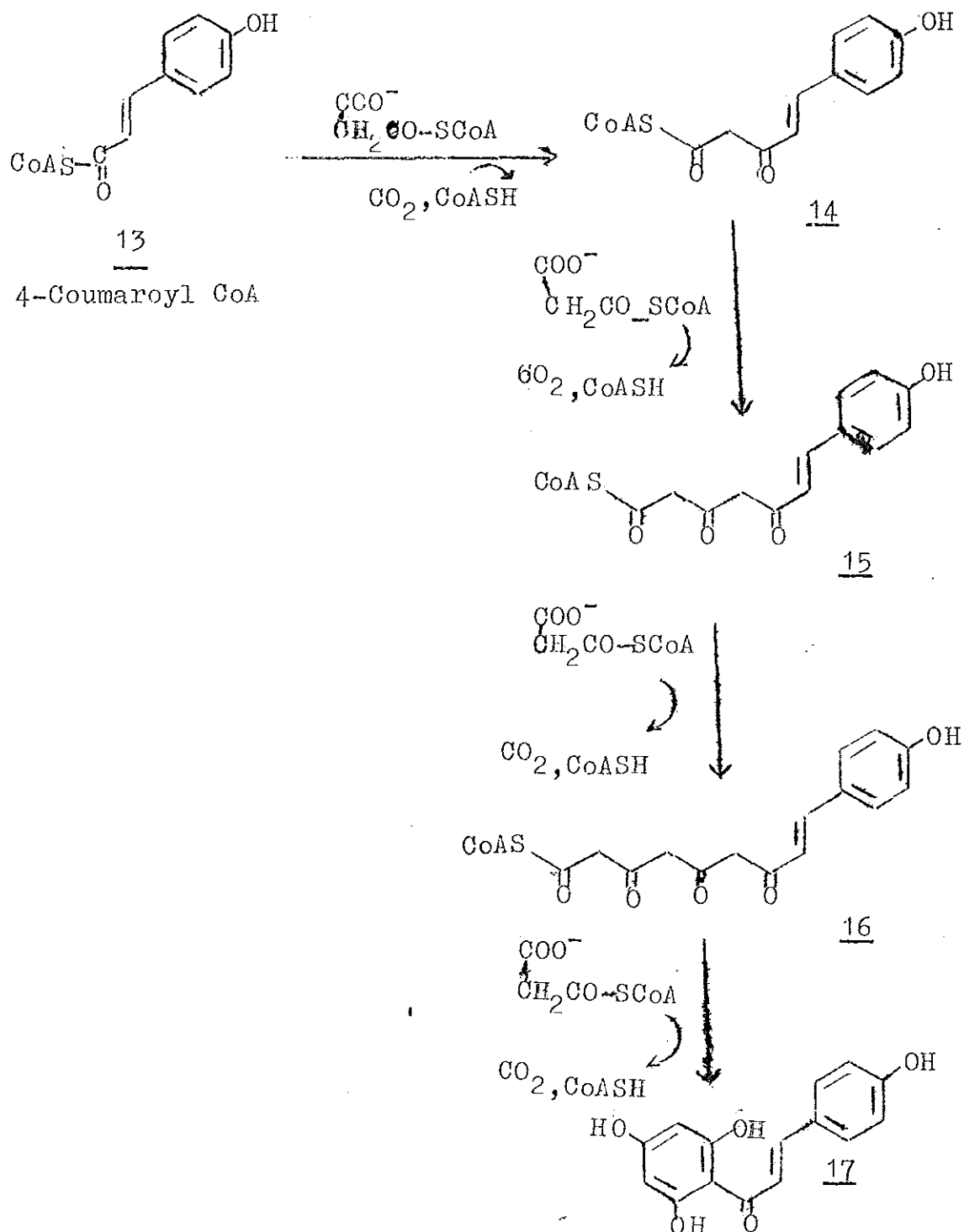
The flavonoid variants are all related by a common biosynthetic pathway which incorporates precursors from both the shikimate and acetate malonate pathway.^{24,29} The flavonoid initially formed in the biosynthesis is thought to be the chalcone and all other forms are derived from this. The precursor 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.



Scheme 1: Formation of 4-Coumaroyl Co-A²⁴

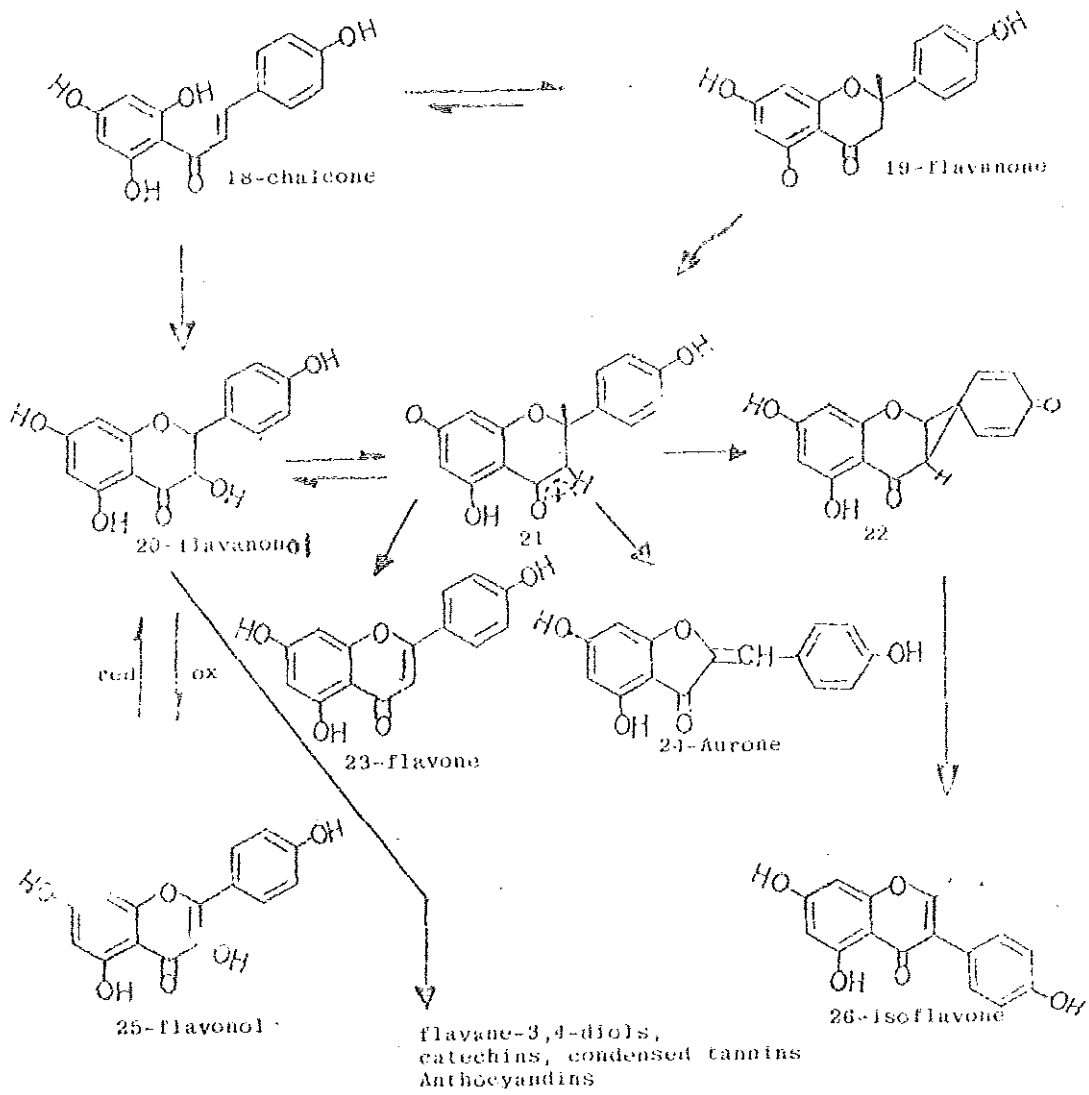
The transformations leading to formation of chalcones, consist of 3 successive condensation steps with acetate units which result in the elongation of aliphatic side chain of 4-coumarate by six carbon atoms which then cyclizes to give the aromatic ring A.^(24,29)

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 skeleton.



Scheme 2: The biosynthesis of chalcones.²⁴

Biogenetic relationship of Flavonoids



2.4. Spectral properties of Flavonoids

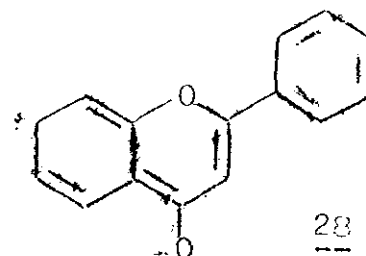
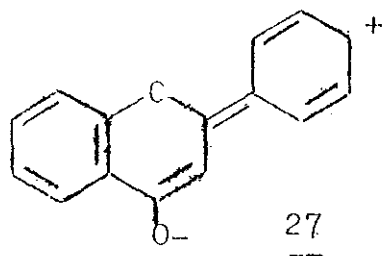
2.4.1 Ultraviolet-Visible Absorption Spectroscopy

UV absorption spectroscopy is one of the most useful techniques for flavonoid structure analysis. The spectrum typically 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

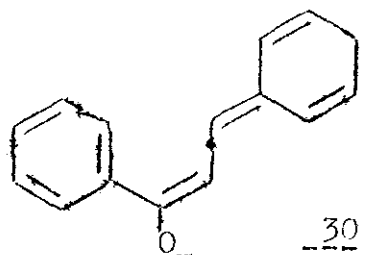
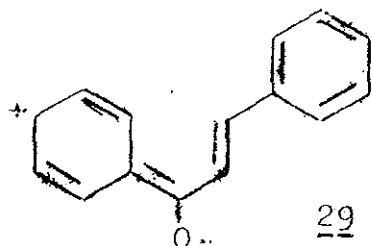


The introduction of electron donating groups such as hydroxyl in the B ring increases the relative resonance contribution of 27 and consequently produce considerable bathochromic shifts of band I (see Table 4)

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.



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 strongly in the 270-290 nm (band II), but band I is an inflection of low intensity.

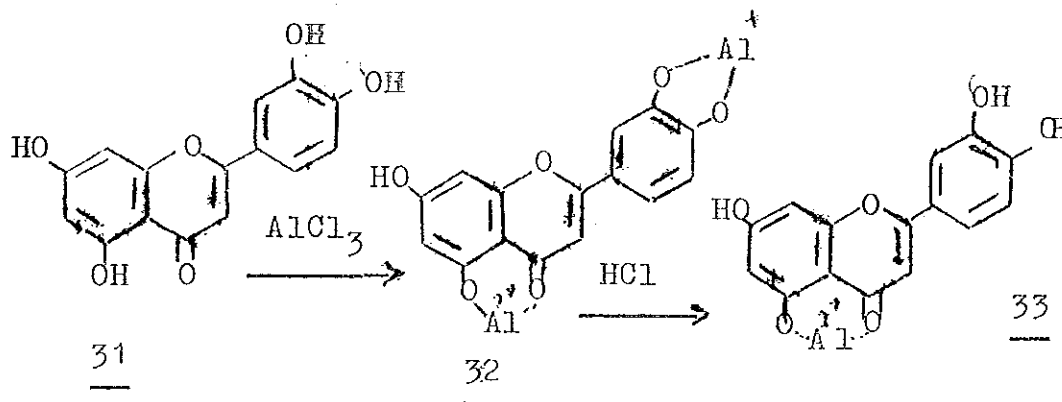
2.4.2 Shift reagents in UV-Visible study of flavonoids

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 groups¹⁰ as shown below.

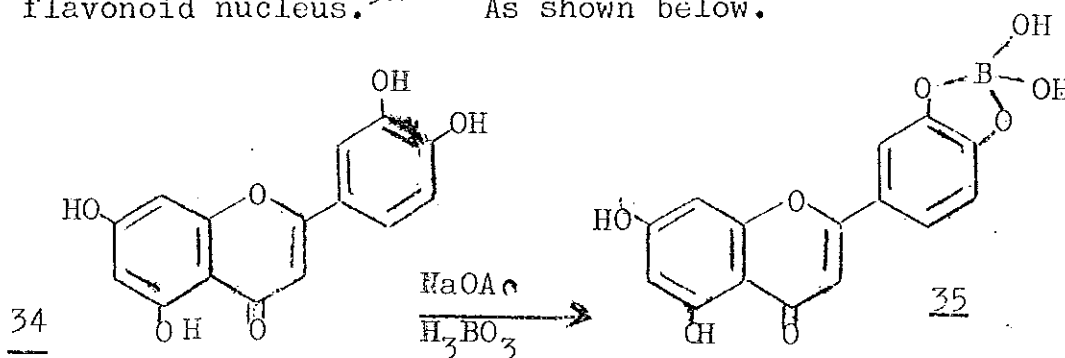


The reagents can therefore be used to detect the presence of both groupings on the flavonoid nucleus. The $AlCl_3$ spectrum represents the sum effects of all complexes on the spectrum, that is, a bathochromic shift due to all of the complexes formed. In the $AlCl_3/HCl$ spectrum, however, only the effect of the acid stable complexes, i.e the hydroxy-keto complexes, will be observed because the acid labile complexes (complexes formed by ortho 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 bathochromic shift of the flavonoid spectrum will be observed.

NaOAc/H₃BO₃ 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

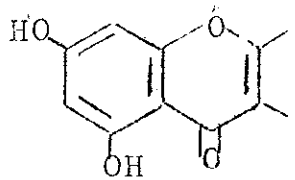
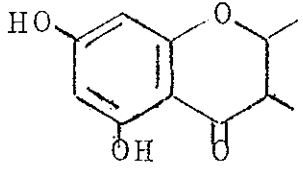
Proton NMR spectroscopy is one of the most important tools for the structural elucidation of organic compounds.

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 meta 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. Attachment of sugar residue at C-7 causes a down field shift for H-6 and H-8 signals.

Table 5 ¹H NMR Chemical shift ranges of H-6 and H-8 in flavones, isoflavones and flavonols.³¹

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 ppm³¹ 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 likewise 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 ppm³¹.

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	160.5-165.1(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)
Aarones	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 8 C^{13} NMR of unsubstituted chalcones, flavones, flavones and 7-Ome Isoflavone

	1'	2'	3'	4'	5'	6'	C	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10
Flavone	151.5	125	125.6	131.5	128.5	125	155	107.5	178	145.4	104.3	153.5	117.4	156	123.7
Flavone	135.6	126	128.7	128.7	128.7	126	74.5	44.6	141.6	126.3	124.5	155	117.9	151.5	126.6
7-Ome Isoflavone	127.9	128.3	126.8	131.6	123.6	128.3	154	125.1	175.5	147.6	111.4	153.8	110.0	157.7	126.3
	0-1	0-2	0-3	0-4	0-5	0-6	0-7	0-8	0-9	0-10	0-11	0-12	0-13	0-14	0-15
Chalcone	134.7	128.3	126.3	130.5	126.3	128.3	144.4	121.7	169.5	136.0	126.7	128.7	122.6	128.5	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 - 191.6³¹ppm except when a 5-OH group is present that shifts the peak to lower field due to hydrogen bonding. The C - 3 methylene carbon signal is characteristic and is at 42.8 - 44.6 where as the C - 2 oxymethine carbon resonates at 75 - 80³²ppm. 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 identify the unfragmented molecular ion and then to relate other major fragments to it by rationalizing the loss in molecular weight using recognized fragmentation path way.

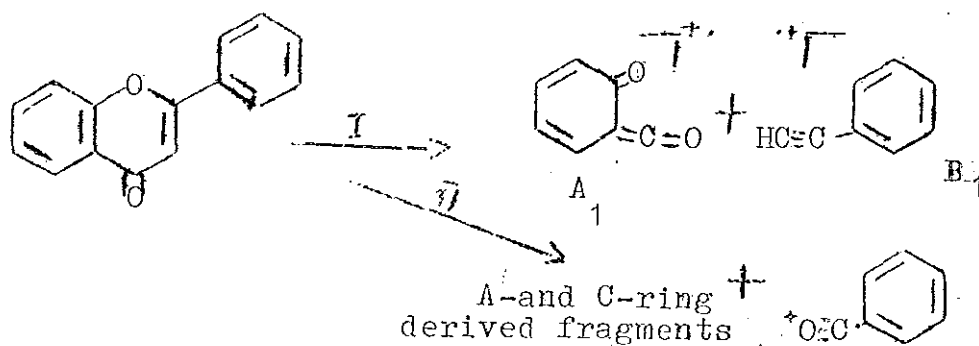
Molecular ion (M^+). The molecular ion normally appears as a major peak in the MS of aglycones. Therefore it is simple to get the elemental composition of aglycones. ($M - 1$)⁺ which is loss of hydrogen is common in most flavonoids.⁽³³⁾

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 II



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!, 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 CDCl₃ with tetramethyl silane as an internal standard. ¹³C NMR were measured on Bruker · 90.13MHz.

The mass spectra were recorded on a high resolution AEI MS 902 spectrometer. Analytical thin layer chromatography (TLC) were run on a 0.2mm thick layer of silica 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 silica gel and the product was detected by their UV fluorescence. Column chromatography were performed on silica gel 60(70 - 230 mesh) and Sephadex 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 petroleum ether followed by chloroform. The petroleum ether 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 silica 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 chlorophyll and fats using solvent system 2 as eluent. A total of fifteen fractions each 50 ml were collected. The first few fractions contained only chlorophyll 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 separately 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 using 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 punila 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.

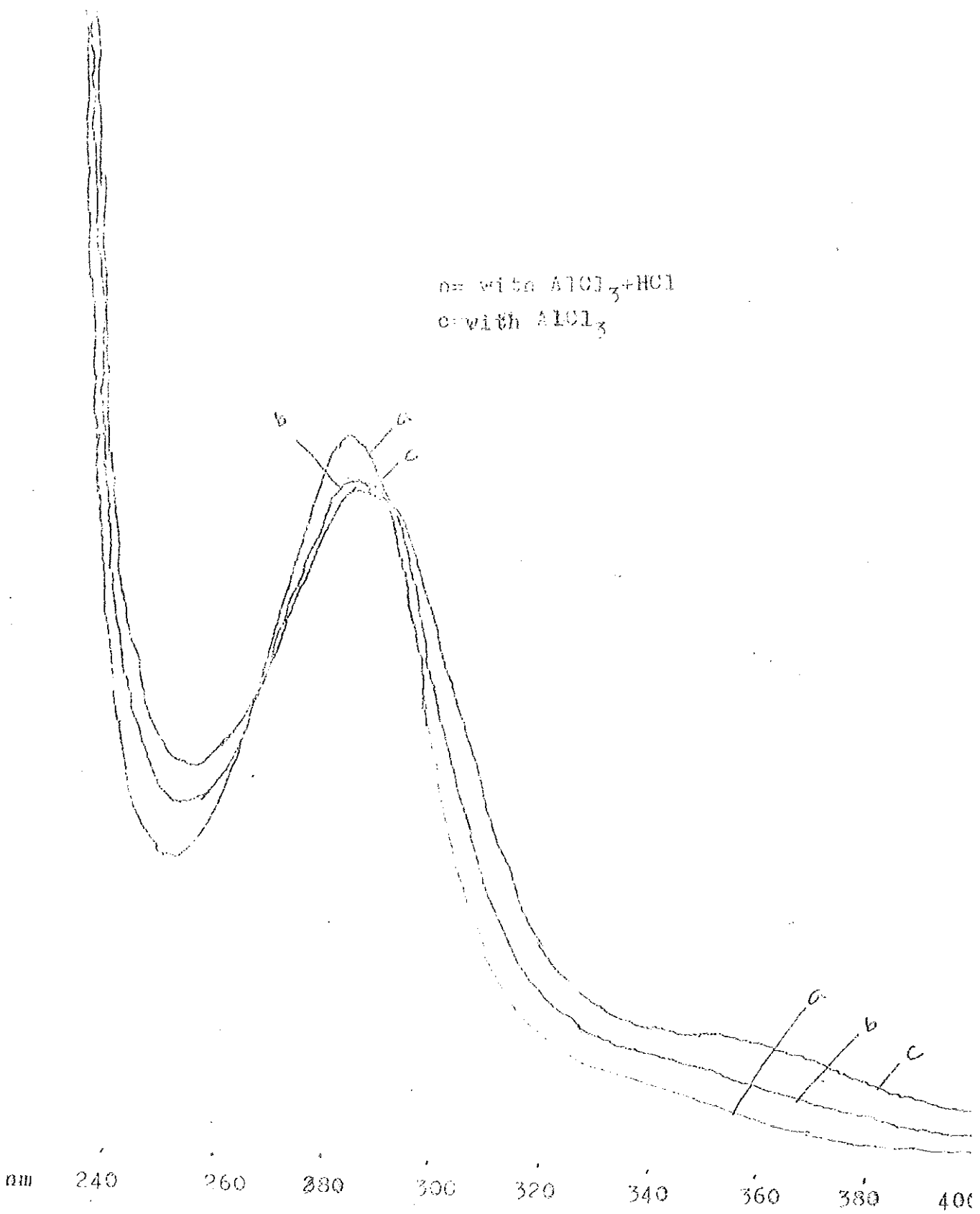
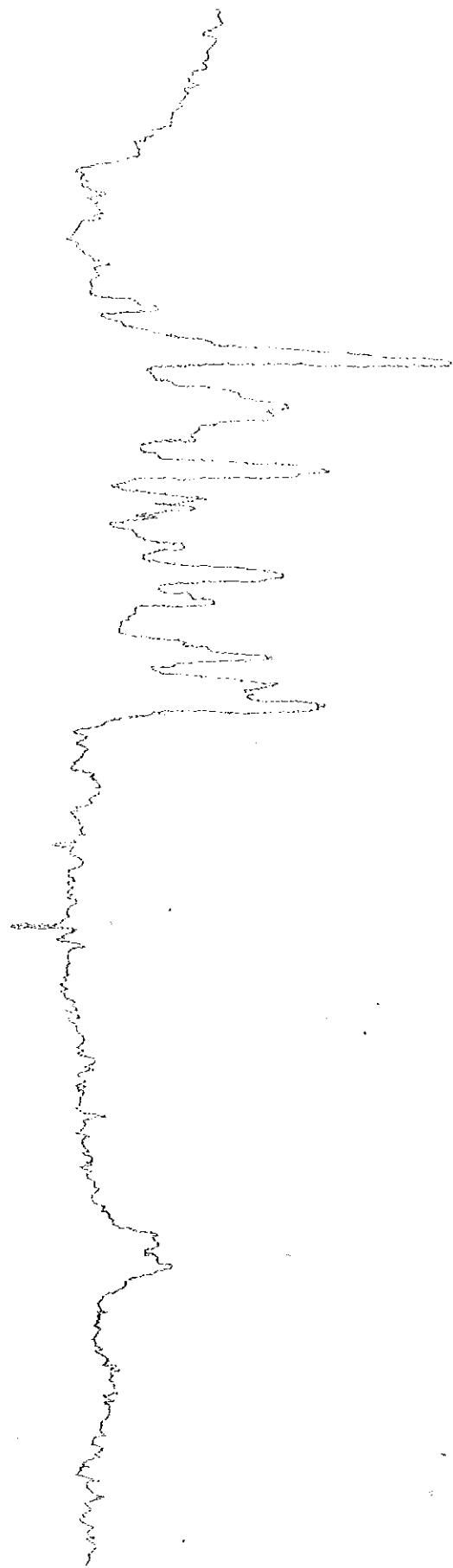


Fig.1. UV absorption of pumilaisoflavone-A



3600 2800 2400 2000 1800 1600 1400 1200 1000 800 600
frequency (cm⁻¹)

Fig.2. IR spectrum of pumilaflavone-A

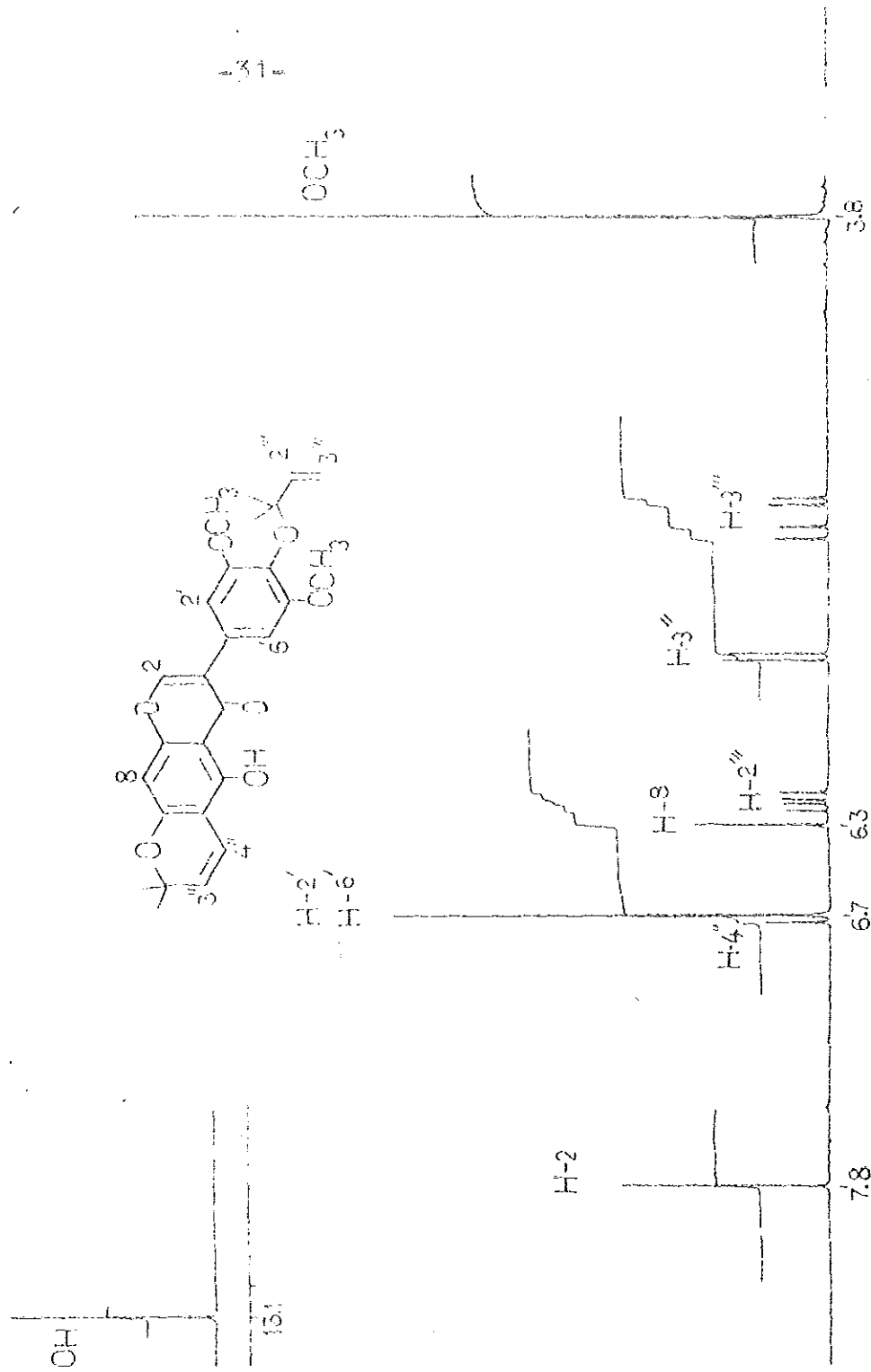


Fig. 5. Partial ^1H NMR of pumilaisoflavone-4

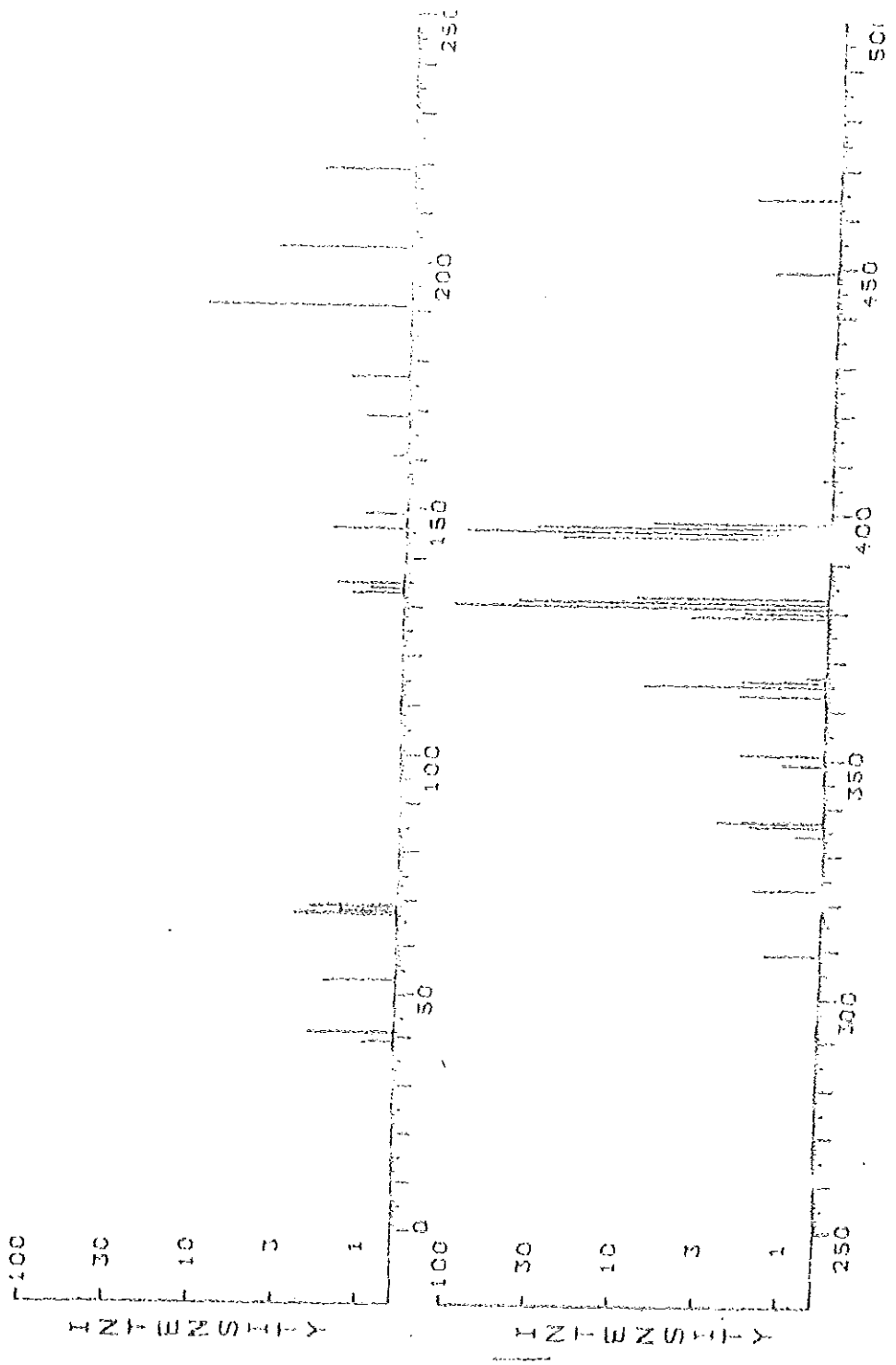
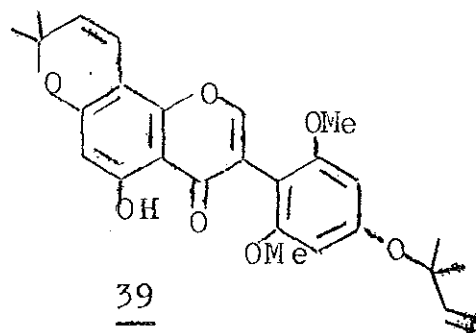
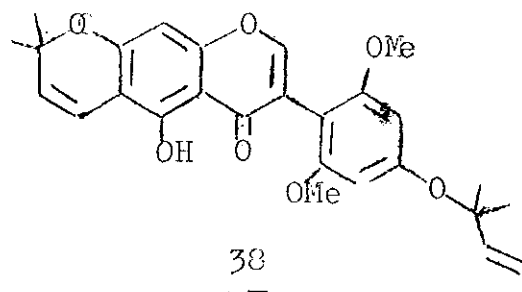
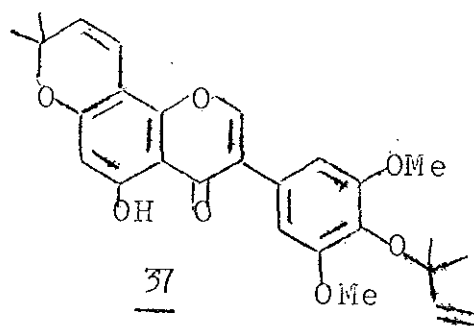
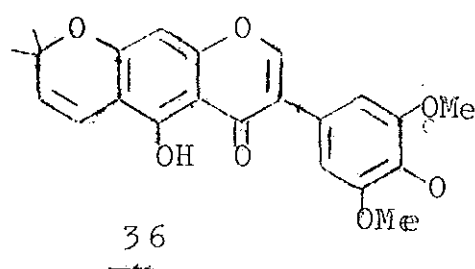


Fig.4. Mass spectrum of cumilaisoflavone -4

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 chromone 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.



The ^{13}C NMR of this compound showed, presence of 7CH of which 2 are equivalent, 6 CH_2 , 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 confirms the O-prenyl side chain is ortho to the two methoxy groups.

The ^{13}C spectral analysis is particularly useful in distinguishing from the two remaining proposed structures 36 and 37. The linear structure 36 is preferred 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 angustone²⁴ 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

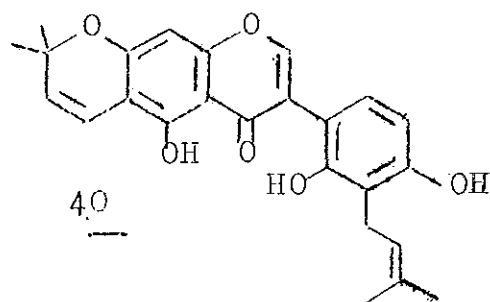


Table 13

¹H NMR assignment of pumila-isoflavone-A 36

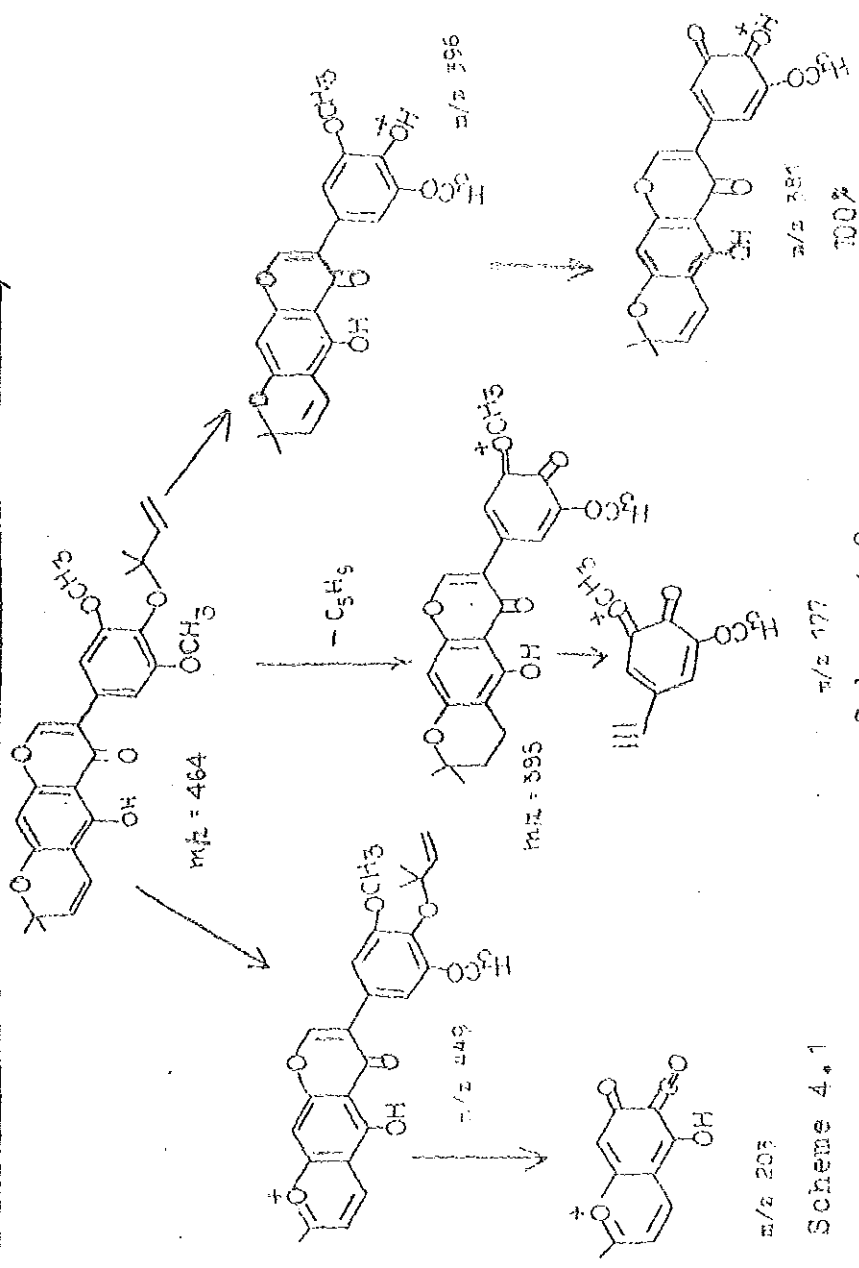
Proton	ppm
5-OH	13.13 (1H, s)
H-2	7.86 (1H, s)
H-4''	6.72 (1H, d, J=10.1)
H-3''	5.61 (1H, d, J=10.1)
H-2', H-6'	6.71 (2H, s)
H-8	6.53 (1 H, s)
H-2'''	6.22 (1 H, dd, J=17.5, 10.8Hz)
H-3''' (trans)	5.11 (1 H, dd, J=17.5, 1.2 Hz)
H-3''' (cis)	4.98 (1 H, dd, J=10.8, 1.2Hz)
3'-OMe, 5'-OMe	3.82 (6H , s)
2''-Me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

Table 14

¹³C NMR assignment of pumlila-isoflavone -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

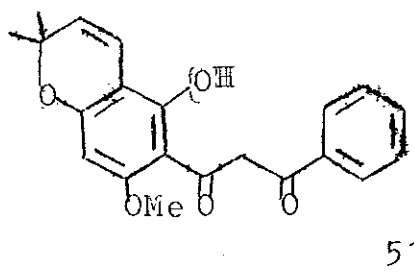
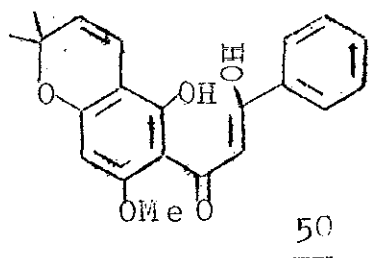
Scheme 4.2

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 ¹HNMR 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 Lonchocarpus constaricensis (leguminosae), a B-hydroxy chalcone 50, which in chloroform was found to equilibrate with the diketo tautomer⁴⁴ 51.



Tentative assignments were given to the ¹³C 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 ¹H NMR 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 al⁴³ during a study of the ¹³C 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 ¹³C 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 data on praecansone A.

Mp	Light yellow oil
colour reaction	FeCl ₃ (-ve), NaOH (Redish conc-- H ₂ SO 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)
UV, IR, NMR	see text

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

<u>1H NMR spectral data of praecansone A (53)</u>	
H-2, H-6	7.81 (2H, 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, $J = 9.6$)
E- 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)

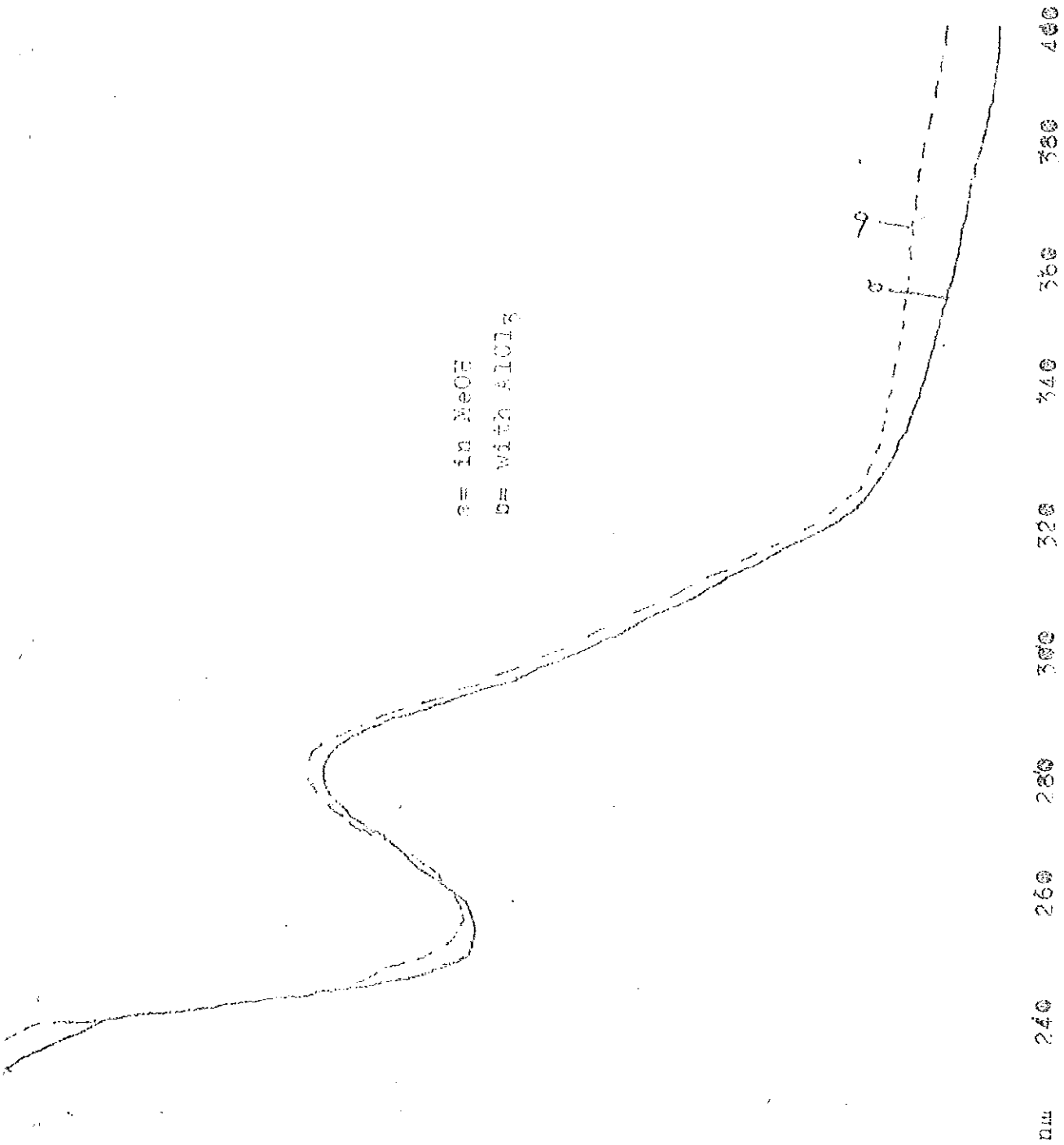


Fig.5. UV absorption spectrum of praecansone-A

1000

Fig. 5. IR-spectrum of trapezoidal-A

3000 2500 2000 1500 1000 500 400



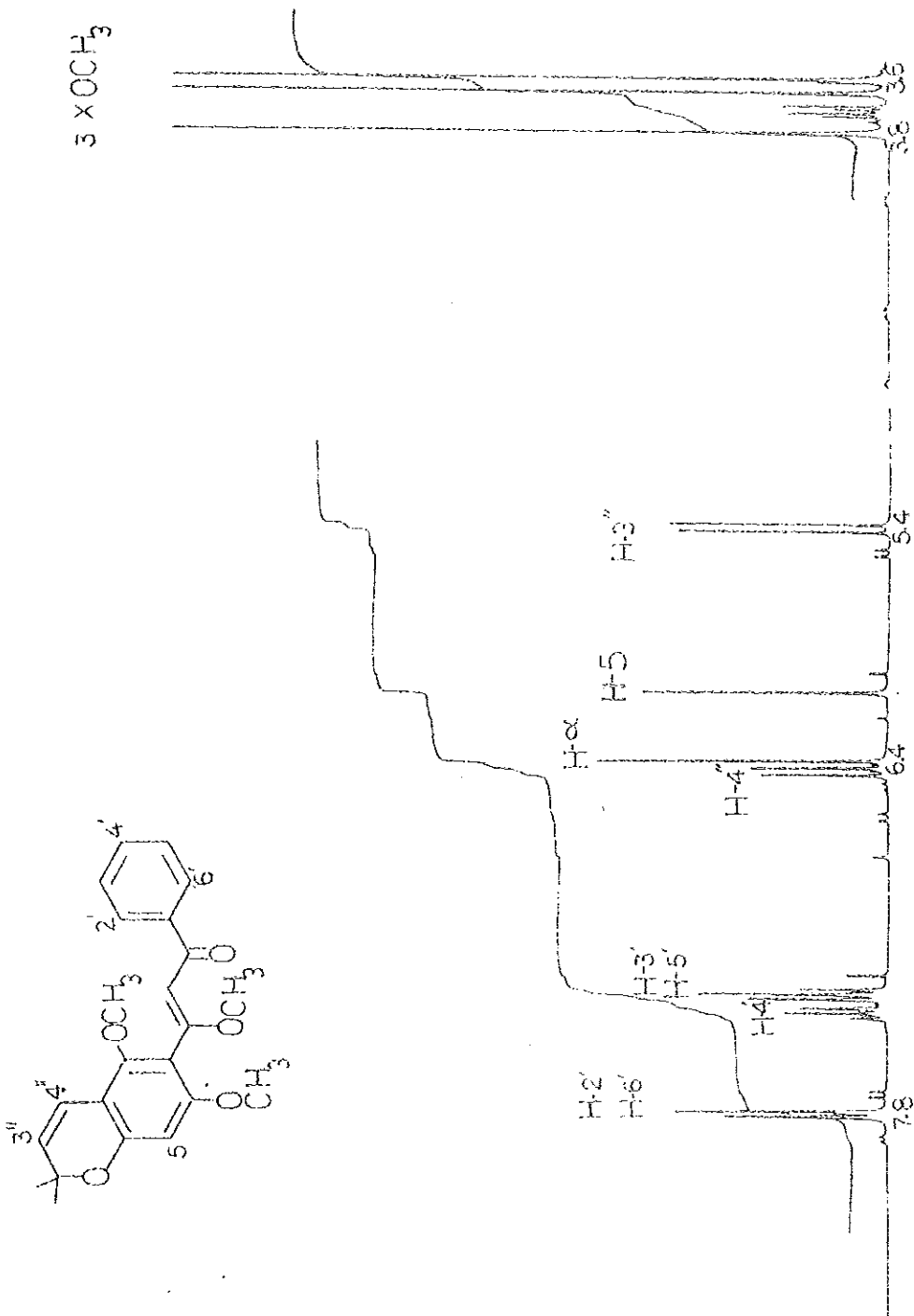
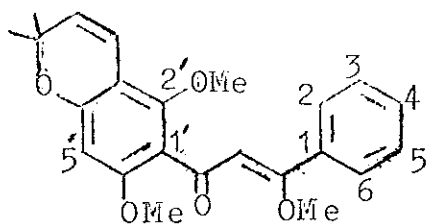


Fig.7. partial ¹H NMR spectrum of praecansone-A

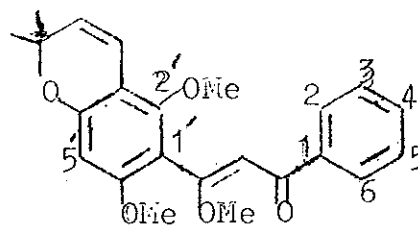
The peaks in the MS, worthy of note, are the signals at m/z 105 and 349 which could arise only if the carbonyl is attached to the B ring, rather than, as suggested originally, to the A ring. The fragmentation patterns are shown Scheme 4.5 and 4.6. Further evidence in support of structure 53 for praecansone-A 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.

Felter 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.

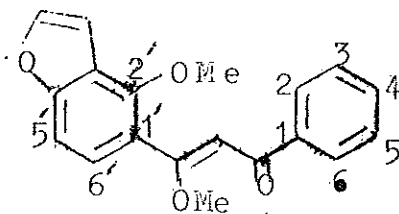
The ^{13}C resonance values of praecansone-A can be accounted for best if the carbonyl is attached to the phenyl ring as in 53 and differs considerably from values for the alternative models.



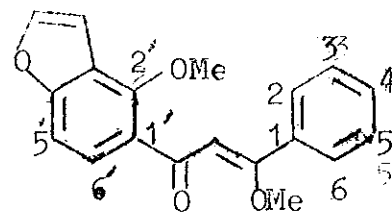
52



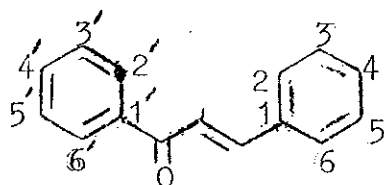
53



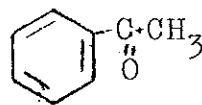
54



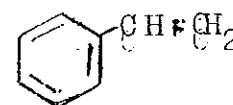
55



56



57



58

Table 18

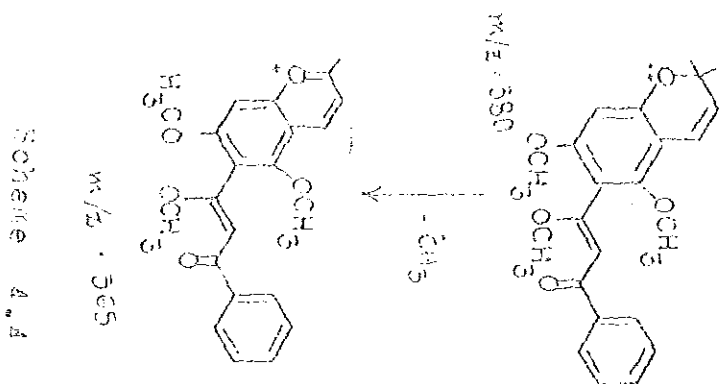
Chemical shifts of 53, 54, 55, 56, 57, 58.

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	---	---

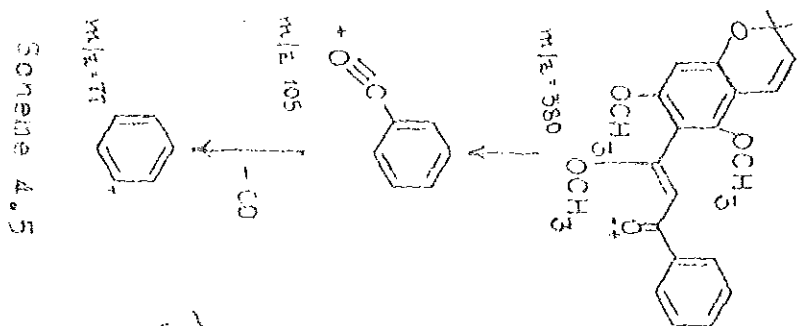
[†]For the sake of simplicity the numbering system of chalcones is not followed for the compounds.

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

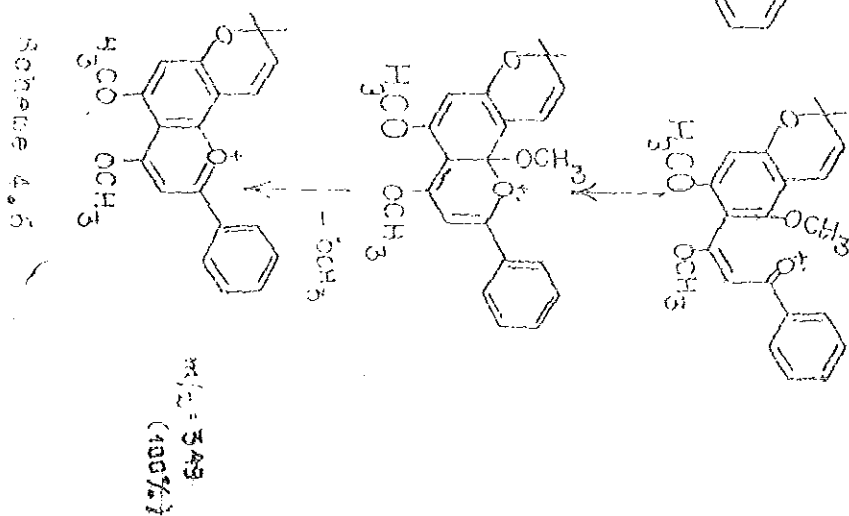
Major Fragments in MS of Procaconone A



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 designated 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 FeCl₃ (greenish), conc, H₂SO₄
(yellow) Shinoda test (-ve); NaOH
(yellow)

The class of this compound 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 chelated OH group can be substantiated further by the deshielded singlet at 13.13 ppm.

5. References

1. Willis, J.C. (1973) A Dictionary of the Flowering plants and Ferns. (Revised by H.K. Airy-Shaw) 8th edn. Cambridge University press, Cambridge
2. Watt, J.M. and Breyer, M.G (1962) The Medicinal and poisnous plants of South and Eastern Africa, 2nd end. London.
3. Holman, H.J.(1940) A Survey of Insecticidal Materials of Vegetable Origin, London.
4. Zemtsove, G.H. and Bandyukova, V.A. (1982) Chem. Abs. 97, 3417e
5. Gomez, F., Calderon, J.S., Quijanao, L., Dominguez, M., and Rios, T. (1985) Phytochemistry 24, 1126.
6. Barnawov, O.D., Mancheva, O.A., Zapesochayaya, G.G., Shelyuto, V.L. And Glyzin, V.V. (1982) Chem. Abs. 96, 142934J.
7. Waterman, P.G. and Khalid, S.A. (1980) Phytochemistry 19, 909.
8. Thulin, M. (1983) Leguminosae of Ethiopia, Academic press, Copenhagen.
9. Harborne, J.B. (1964) Biochemistry of Phenolic Compounds, Academic press, London.
10. Markham, K.R. (1982) Techniques of Flavonoid Identification, Academic press, London.

11. Harborne, J.B., Ingham, J.L., King, L. and Payne, M.
(1976) Phytochemistry 15, 1485.
12. Milcher, L.A., Park, Y.H. and Clorrk, D. (1980) J. Nat. Prod. 43, 259
13. Biswas, P., Bhattacharyya, S., Bose, P.C., Mukherjee, N.
and Adityacha, U.N. (1981) Chem. Abs. 95, 19854c
14. Sarin, J.P.S, Sigh, S., Carg, H.S., Khamma, N.M. and
Dhar, M.M. (1976) Phytochemistry 15, 232.
15. Agarwal, O.P. and Nagaratnam, A. (1981) Chem. Abs. 94,
167981Y.
16. Trease, G.E. and Evans, W. C. (1978) Pharmacognosy
11th edn. London, 403.
17. Griffith, J.Q., Krewson, C.F. and Naghskin, C.F.
(1955) Rutin and Related Flavonoids, Easton.
18. Younes, M.J. and Siegers, C.P. (1981) Planta Medica
43, 240
19. Beretz, Alain, Gazenave, Jean pierr, Antoni and Robert
(1982) Chem. Abs. 97,84775V.
20. Dhar, D.N. (1981) The Chemistry of Chalcones and related
Compounds, Newyork, 224
21. Nakanishi, K., Goto, T., Ito, S. Natori, S. and Nozoe,
S. (1975) Natural products chemistry, Vol. 2. Academic
press, New York, 218.

22. Goodwin, T.W. (1965) Chemistry and Biochemistry of Plant Pigments, Academic press, New York, 211
24. Herborne, J.B, and Mabry, T.J. (1982) The Flavonoids: Advances in Research, London.
25. Nurthy M.S.R. and Red, E.W. (1985) J. Nat. Prod. 48,967.
26. Smalberger, T.M., Vleggaar, R. and Weber, J.C. (1965) Tetrahedron 24,2429
27. Rao, E.V., Venkataratnam, G. and Vilainc (1965) Phytochemistry 24,2427
28. Khalid, S.A. and Waterman, P.G. (1981) Phytochemistry 20,1719
29. Bernfels, P. (1963) Biogenesis of Natural Compounds, Pergamon Press, New York
30. Geissman, T.A. (1962) The Chemistry of Flavonoid Compounds, Pergamon press, Oxford.
31. Mabry, T.J., Narkham, K.K. and Thomas, M.B. (1970) The Systematic Identification of Flavonoid compounds, New York,
32. Pelter, A., Ward, R.S. and Longray, T. (1976) J. Chem. Soc., Perkin I, 249.,
33. Kingbury, A.C. and Looker, H.J. (1975) J. Org. Chem. 40,1120.

34. Valesi, A.G., Rodriguez, E., Velde, G.V. and Mabry, T.J. (1972) Phytochemistry 11,2821
35. Audier, P.M. (1966) Bull Soc. Chem. France, 2892
36. Birch, A.J. and Salahuddin, M. (1964) Tetrahedron Letters, 2211
37. Schmid, R.D. Nues, R., McCreynolds, J.H., Velde, G.V., Nakatani, N., Rodriguez, E. and Mabry, T.J. (1973) Phytochemistry 12,2765.
38. Stahl, C. (1969). Thin layer Chromatography, 2nd edn. New York, 874
39. Camele, G., Delle Monache, F., Delle Monache, G. and Marini-Bettolo, G.B. (1980) Phytochemistry 19,707
40. Smalberger, T.M., Vleggaar, R. and Weber, J.C. (1974) Tetrahedron 30,3927
41. Kaouadji, M., Agban, A. and Mariotte, A.M. (1986) J. Nat. Prod. 49, 231.
42. Williams, D.H. and Fleming, I. (1973) Spectroscopic methods in Organic Chemistry, 2nd edn. London, 167.
43. Pelter, A., Ward, R.S., Fao, E.V. and Raju. N.R. (1981) J. Chem. Soc, Perkin I, 249.
44. Waterman, P.G. and Mahmoud, E.N. (1985) Phytochemistry 24,571
45. Oshima, Y., Konno, C. at Hikino, H. (1980) Tetrahedron letters 21, 3381.