INVESTIGATION OF FLAVONOIDS FROM *BERBERA* (MILLETTIA FERRUGINEA)

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Amha Bekele

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Abstract

Investigation of Flavonoids from Berbera

(Millettia ferruginea)

Amha Bekele

Research Advisor Dr. Ermias Dagne

Millettia ferruginea (Hochst) BAK., locally known as Berbera is a plant endemic to Ethiopia. Phytochemical investigation of the stem-bark of this plant resulted in the isolation of 10 compounds of which nine have been generated on the remaining one compound. The characterized compounds are 4-hydroxylonchocarpin(46), 4'hydroxyisolonchocarpin(47), flemichapparin-B(48), calopogonium isoflavone-B(49), isojamaicin(50), jamaicin(51), ichthynone(52) and 5-methoxy durmillone(53).

Three of the isolated compounds appear to be novel and are identified as 5-methoxy durmillone (5,6-dimethoxy-3',4'-methylenedioxy-2'',2''-dimethylpyrano [5'',6'':7,8] isoflavone), isojamaicin (3-methoxy-4,5-methylenedioxy- 2'',2''-dimethylpyrano [5'',6'':7,8] isoflavone) and 4'-hydroxyisolonchocarpin(4'-hydroxy-2'',2''-dimethylpyrano [5'',6'':7,8] flavanone).

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1. INTRODUCTION

Millettia (Leguminosae-Papilionoideae) is a large genus estimated to contain about 200 species in tropical and subtropical Africa, Asia and Australasia [1]. Although the number of Millettia species is very large, their phytochemical investigation has been limited only to a few cases. Previous examination of a number of species of Millettia have shown them to be a rich source of flavonoids and isoflavonoids typical of the Tephrosiae tribe of the Papilionoideae [2]. This is compatible with the various claims that some Millettia species show insecticidal and piscicidal activities [3].

The various reports on the genus show that a number of Millettia species are used as fish poisons in many parts of the world. The ground seeds of Millettia pachycarpa Benth. commonly known as "fish poisoning vines" in China [4], M. dura Dunn in Uganda [5], M. barteri Dunn in Gabon [6] and the root of Millettia taiwaniana Hayata in Formosa [7] have long been used to paralyze fish. Similar uses are ascribed to other leguminous plants belonging to the genera Derris, Lonchocarpus, Tephrosia and Mundulea [3]. Phytochemical investigation on the above mentioned genera have revealed the presence of rotenone, which is claimed to be responsible for their toxicity [3].

M. ferruginea (Hochst) Bak., locally known as Berbera, is the only species that is found in Ethiopia. This endemic

plant is widely used as fish poison in various parts of the Country. Two subspecies of Millettia ferruginea are known to occur [1]. These are subsp. ferruginea which is restricted to northern Ethiopia and subsp. darassana (Cuf) Gillet which occurs in the southern provinces, particularly in Sidamo. The hybrid of the two subspecies is believed to be distributed in the central and western parts of the Country.

Previous phytochemical study on the seeds of the plant by Clark [8] indicates the presence of three rotenoids namely rotenone, tephrosin and dehydrodeguelin. Highet and Highet [9] have also isolated two isoflavones i.e. ferrugone and durmillone. A gas chromatographic study by Ghigilione et al [6], of the extracts of the seeds showed the presence of steroids such as cholestane, β-sitosterol and stigmasterol.

The main objective of the present work is to investigate other parts of this plant namely the stem-bark, leaves and roots. The fact that only two isoflavones and three rotenoids were previously reported from this plant coupled with the recent increasing awareness of the biological significance of isoflavonoids and rotenoids prompted us to undertake a phytochemical study of this plant.

We report here the results of our phytochemical investigations on the stem bark which was collected in June and December of the 1987 from Sidamo (Aleta-Wondo) in September of 1987 from Addis Ababa and February of 1988 from

Gondar. The samples from Sidamo are believed to be the subsp. M.f./darassana, from Gondar the subsp. M.f./ferruginea and the samples from Addis Abeba could be the hybrid of the two forms. Using the various chromatographic techniques, it was possible to isolate a chalcone, a flavanone, a pterocarpene along with seven isoflavonoids. The complete characterization of these compounds by chemical as well as spectroscopic methods will be presented.

2. ISOFLAVONOIDS

In view of the fact that the genus Millettia is a good source of isoflavonoids, the salient features of the chemistry of isoflavonoids will be reviewed here albeit succinctly.

2.1 Distribution and Occurrence

Isoflavonoids are believed to be predominantly found in one group of plants, the subfamily Papilionoideae of the Leguminosae [10]. Their chief source of occurrence is in leguminous plants, some of which are very much in use as food materials. Moreover, leguminous plants sometimes exhibit remarkable physiological activities attributable to their isoflavone contents. Gyorgy and co-workers [11] found 4′,6,7-trihydroxy isoflavones to be antioxidative substances of fermented soyabean.

They also occur occasionally in the subfamily Caesalpiniodeae and in a few other families namely Rosaceae, Moraceae, Amaranthaceae, Iridaceae and Podocarpaceae [10].

2.2 Types of Isoflavonoids

Although the isoflavonoids are biogenetically related to the flavonoids, they constitute a distinctly separate class, because they contain a rearranged C_{15} skeleton as shown below.

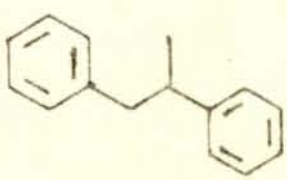
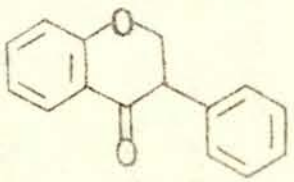
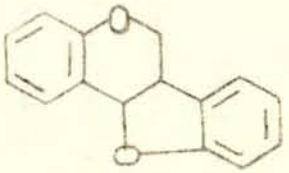


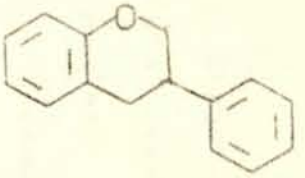
Fig-1 Structural variants of isoflavonoids



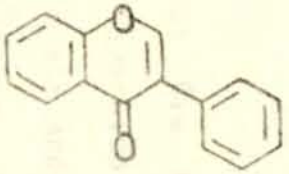
Isoflavanone



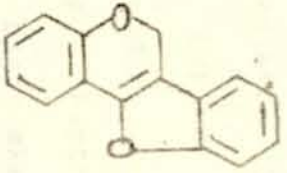
Pterocarpan



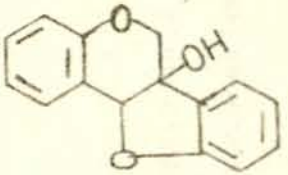
Isoflavan



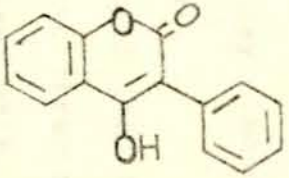
Isoflavone



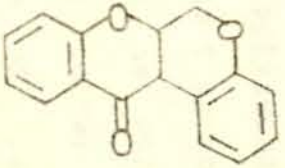
6a,11a-dehdropterocarpan



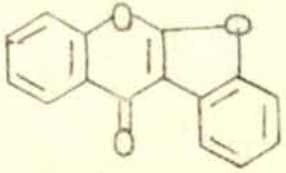
6a-hydroxy pterocarapan



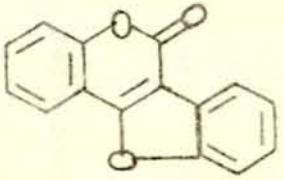
3-aryl-4-hdroxycoumarin



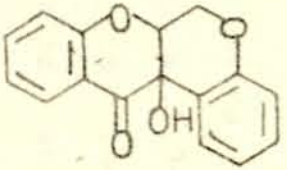
Rotenoid



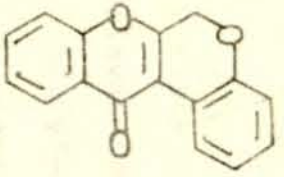
Coumaronochromone



Coumestan



12-hydroxyrotenoid



6a12a-dehydrorotenoid

Structurally isoflavonoids furnish a great variety of natural products such as the isoflavones, isoflavanones, coumestans, coumaronochromenes, isoflavans, 3-aryl-4-hydroxy-coumarins, pterocarpans, rotenoids and their hydroxy and dehydro variants [12].

2.3 Biosynthesis of Isoflavonoids

Isoflavonoids differ from the flavonoids in having a rearranged 1,2-diphenyl skeleton as opposed to a 1,3-diphenylpropane skeleton of the latter [13]. Like the flavonoids, the various types of isoflavonoids are determined by changes in the oxidation state of the heterocyclic ring. Further oxidation, reduction and alkylation of the basic skeleton may also occur to produce additional structural features [14].

The biosynthetic pathways which lead to the formation of isoflavonoids may be divided into three parts.

a) those early pathways shared with other secondary metabolites.

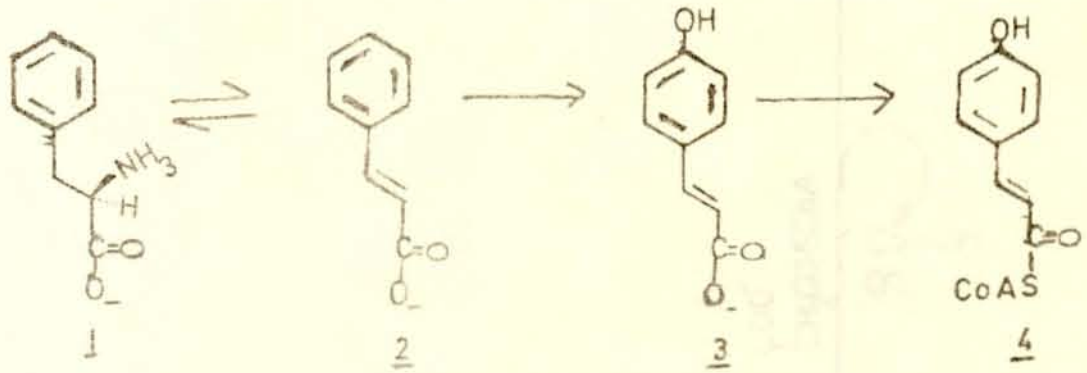
The essential C_{15} molecular framework of both flavonoids and isoflavonoids results from the convergence of the acetate / malonate and shikimic acid pathways. The precursor for the acetate / malonate pathways is acetyl Co-A while 4-coumaroyl Co-A (4) derived from phenyl alanine (1) as shown in Scheme-1 serves as the precursor of the shikimate route.

b) those steps common to flavonoid and isoflavonoid

biosynthesis.

The transformation leading to the formation of chalcones as shown in Scheme-2 consists of three successive condensation steps with acetate units which result in the elongation of the aliphatic side chain of 4-coumarate by six carbon atoms which then cyclizes to give the aromatic ring A.

Scheme - 1 Formation of 4-Coumaroyl Co-A

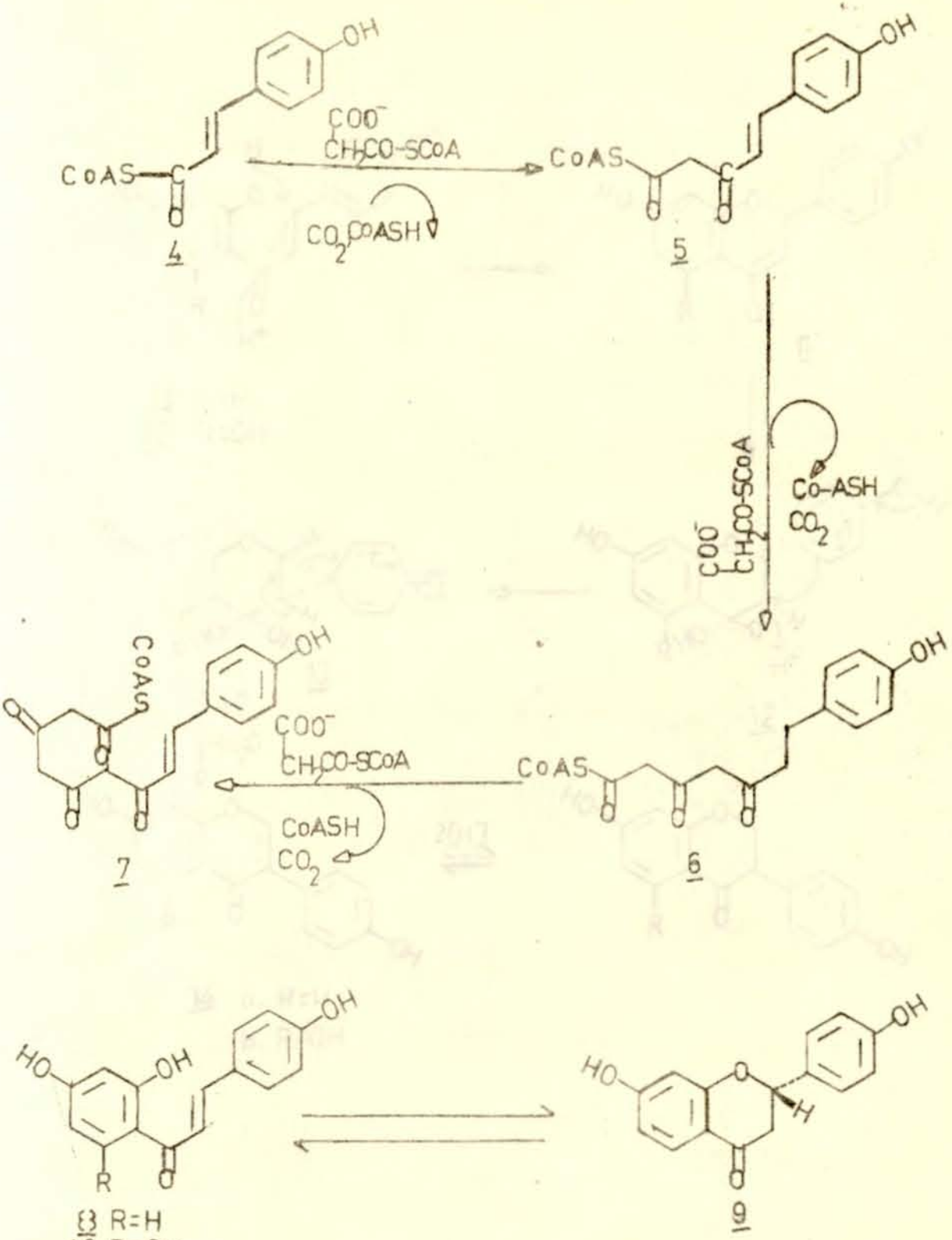


1 2 3 4

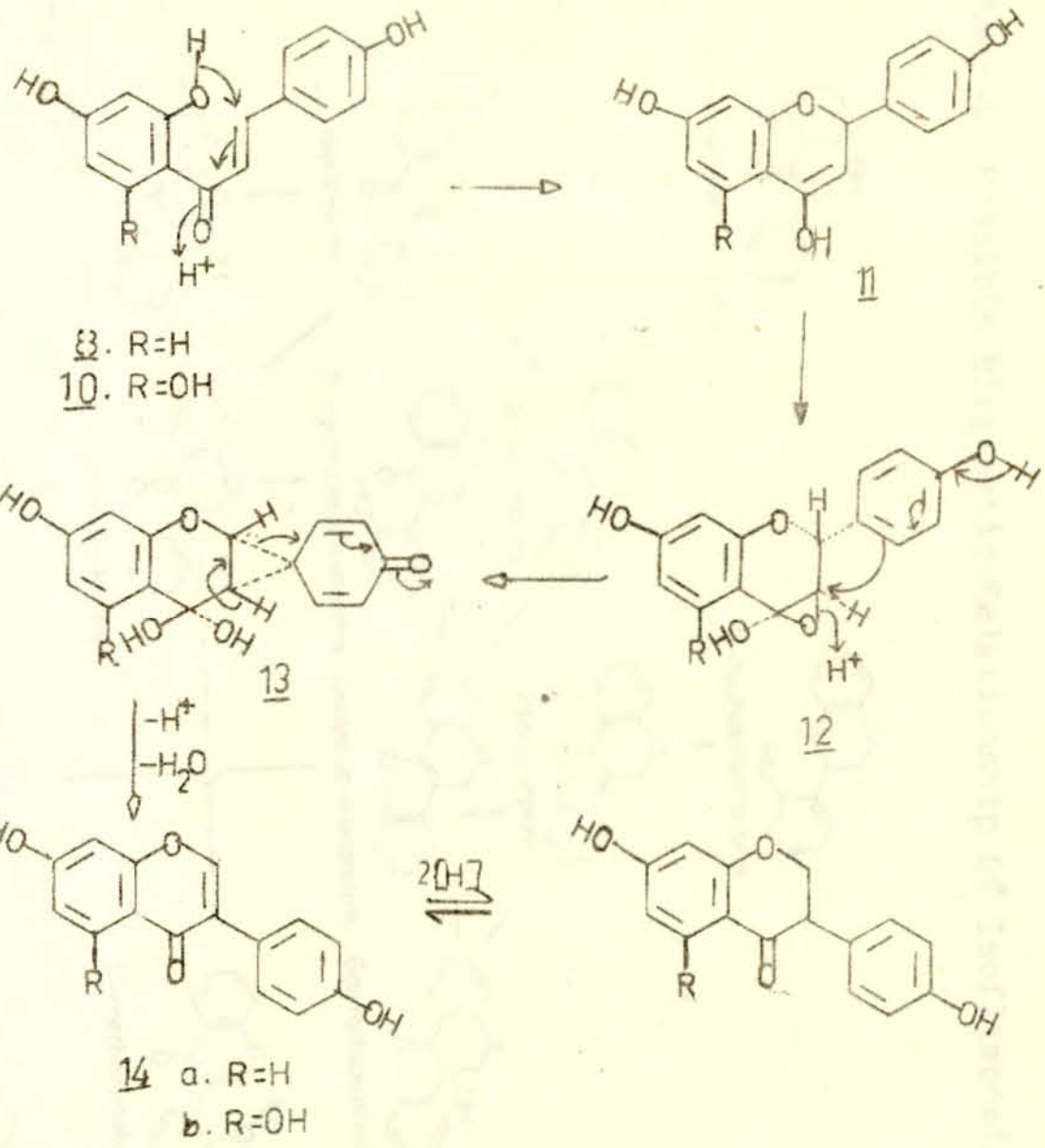
C) those reactions unique to the isoflavonoids.

Feeding* experiments by Grisebach and collaborators [15,16] demonstrated that the isoflavone skeleton results from the flavonoid precursors by a 1,2-aryl migration of the B-ring. This shift is believed to take place after the formation of the C-15 chalcone (8) intermediate. This was established by feeding experiments that showed the chalcone rather than the isomeric flavonone (9) acted as the substrate for aryl migration [17,18]. Thus the proposal that chalcones are the more immediate precursors of isoflavones enjoys general acceptance. Indeed, it is probable that only two chalcones 4, 2, 4' - trihydroxychalcone (isoliquiritigenin) and

Scheme-2 Biosynthesis of chalcones



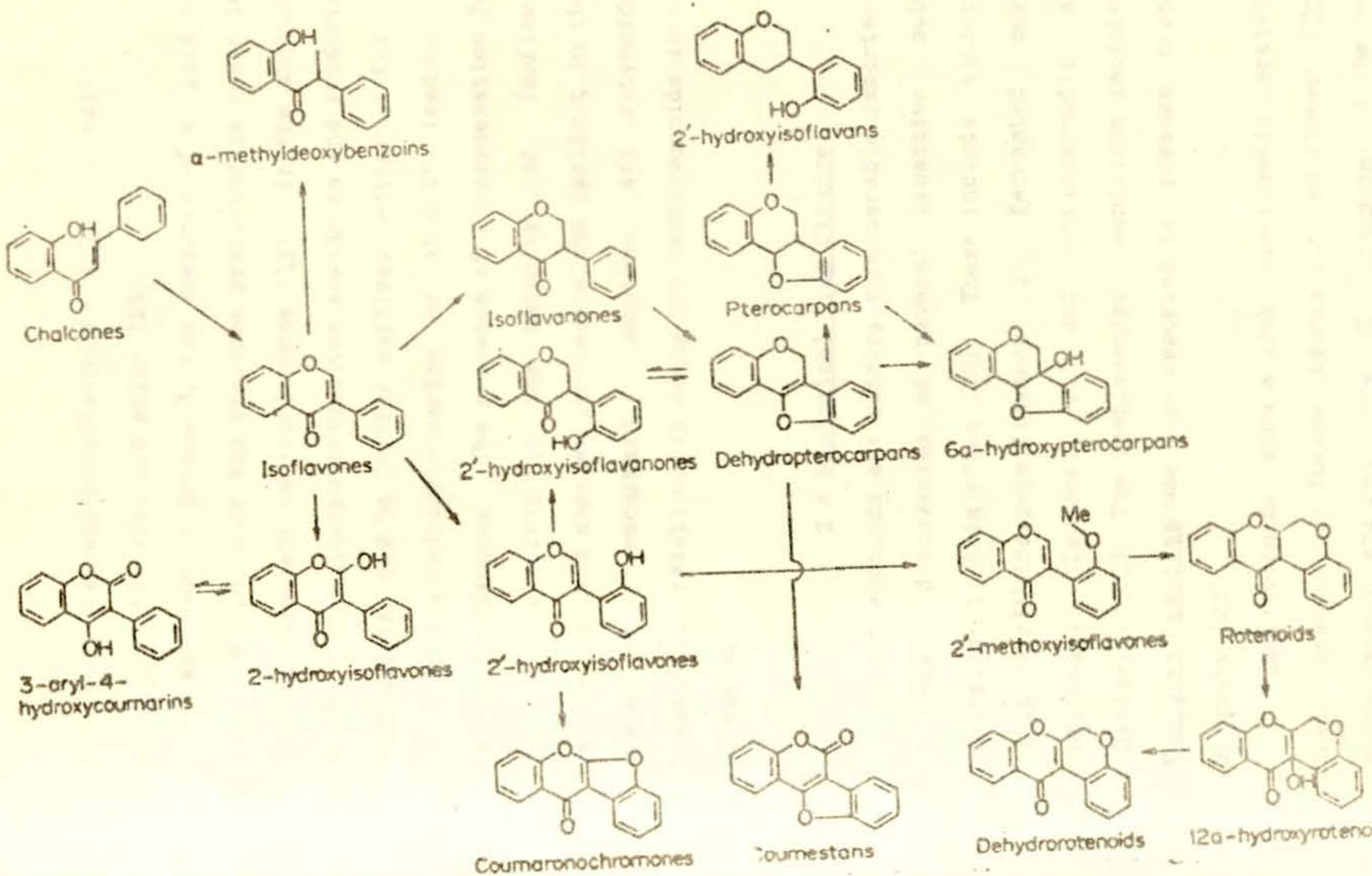
Scheme-3 Proposed pathway for 1,2-aryl migration



9. R=H
10. R=OH

14 a. R=H
b. R=OH

Scheme-4 Possible Biogenetic Relationship Of Isoflavonoids



4,2',4',6'-tetrahydroxychalcone (10) normally act as substrates for aryl migration [19].

As shown in Scheme-3, the presence of a para hydroxyl group on ring B of the chalcone precursor is essential for the rearrangement to isoflavones (14). In the absence of a 4'-OH the chalcones themselves appear as end products [2]. Isoflavones may be further oxidized either at C-2 leading to 3-aryl-4-hydroxycoumarins or at C-2' leading to 2'-hydroxyisoflavones. The presence of 2'-oxygenation function [12] is explicit in the majority of isoflavanones, isoflavans, and complex isoflavans and implicit in the ring system of pterocarpans, coumestans, and rotenoids. The biogenetic relationship among the isoflavonoids is shown in Scheme-4.

2.4 Biological Significance

Isoflavonoids are gaining considerable importance due to recent discoveries of several bioactive substances belonging to this class [20]. These include antioxidative (4',6,7-trihydroxy-isoflavones in fermented soyabean), antifungal (Leutone) [21] and antispasmodic activity (Daidzein) [22]. The isoflavoniod, mundalone isolated from Mundulea sericea was also reported to possess hypotensive activity [23].

Isoflavonoids show a weak oestrogenic activity and their presence in forage legumes such as clover (Trifolium subterraneum) and red clover (T. pratense) is the cause of

infertility sometimes occurring in animals grazing on these pasture species [24]. Formononetin, geinistein and biochanin A are the main oestrogenic isoflavones of subterranean clover [12,24]. Coumestrol and other coumestans are also oestrogenic agents found especially in clover and alfalfa [14].

Rotenoid bearing plants of the related genera Derris, Lonchocarpus, Tephrosia, Mundulea, and Millettia have been used by people of Asia, Africa, and South America as fish poison [3]. The insecticidal and piscicidal property of rotenone and related compounds have also been known for a long time. The major effect of rotenoids on insects as well as fish is a remarkable decrease in the oxygen uptake which finally results in death [12].

Some isoflavonoids and in particular pterocarpans are sometimes produced as phytoalexins under attack by viral or fungal agents by plants. The first known phytoalexin isoflavonoids are pisatin from pea (Pisum sativum) and phaseollin from bean (Phaseolus vulgare) [13]. In addition to the first known phytoalexins, 6a-hydroxyphaseollin, medicarpin and maackiain are also known to occur in soyabean, alfalfa and red clover respectively [13]. The antifungal activity of the prenylated isoflavone, leutone, found in immature fruits of L. albus has been recently demonstrated and may serve to highlight an important role for isoflavonoids in plant protection [13].

2.5 Spectral Properties

Recent developments in the structural study of isoflavonoids have centered largely on the application of spectroscopic methods. The main steps generally followed in structure elucidation of an isoflavonoid are: recognition of the class to which the compound belongs and determination of the nature and orientation of substituent groups in the aromatic rings. These steps are well served by the application of UV, IR. NMR and MS techniques.

2.5.1 UV Absorption Spectroscopy

UV absorption spectroscopy is a useful spectral techniques used in the structural elucidation of natural products. The UV spectra of naturally occurring isoflavonoids exhibit two absorption peaks [25.26]. These absorption peaks are usually referred to as band I (300-550 nm) and band II (240-285 nm).

Table-I UV Absorption of Isoflavonoids [10]

Isoflavonoids	Band I (nm)	Band II (nm)
Isoflavones	300-340 (sh)	245-275
Isoflavanones	~ 310	~ 270
Isoflavans	-	~ 281 and 287
Pterocarpans	-	" "
Coumestans	340-350 intense	-
	300-310 less intense	-
Rotenoids	~ 315 (sh)	

Although coumestans do have absorption peaks in the above mentioned UV range (Table I), their most characteristics property is an intense blue to violet flourescence under UV light.

The characteristic feature of an isoflavone is a strong absorption at band II and rather weak absorption at band I. The latter band often escapes resolution and appears as a shoulder. Variation within these ranges will depend on the hydroxylation pattern, degree and type of substitution of the hydroxyls. Increased hydroxylation of isoflavones in the B-ring does not usually have a significant effect in the absorption of band II in: 5,7-dihydroxyisoflavone (259 nm): 5,7,4'-trihydroxyisoflavone (261 nm) and 5,7,3',4'-tetrahydroxyisoflavone (262 nm), however band II is shifted bathochromically by increased oxygenation in the A-ring as shown in Table II [25].

Table-II Band II in the UV Spectra of Isoflavones Differing in their A-ring Oxidation Pattern

Isoflavone	Oxidation Pattern		Band II
	A-ring	B-ring	(nm)
Daidzein	7	4'	240
Genistein	5,7	4'	261
6-hydroxygenistein	5,6,7	4'	270

Methylation of either 7- or 4'-hydroxyl group in isoflavones has little effect, while substitution or loss of a 5-hydroxyl group causes band shifts to shorter wavelength. Additional information on the substitution pattern of the hydroxyl group can be made using shift reagents whenever necessary.

2.5.2 ^{1}H NMR Spectroscopy [25]

NMR spectroscopy can be considered as the most powerful spectroscopic techniques for the structural elucidation of natural products. Chemical shifts as well as splitting patterns and coupling constant values provide valuable information about the substitution of an isoflavonoid.

In 5,7-oxygenated isofolavones, the protons located at C-6 and C-8 of ring-A usually gives rise to two doublets due to meta coupling (J-2.5 Hz). As a result of the ortho shielding effect of the two oxygenated substituent the signal for H-6 is usually observed at higher field (6.0-6.2 ppm) than the H-8 (6.3-6.5 ppm).

If an isoflavone is oxygenated at the 7- position of the A-ring, the C-5 proton appear as a doublet due to ortho coupling (J = 9 Hz) with the C-6 proton and occurs at lower field (8 ppm) than most aromatic protons are observed. this is due to the ortho deshielding effect of the C-4 keto group. In a fully substituted A-ring except at C-5, where the 6-position is oxygenated the C-5 proton appears as a singlet in the range 7.5-7.8 ppm. In this case, the ortho

deshielding effect is minimized to certain extent by the ortho shielding effect of the C-6 oxygenated substituent.

The protons of ring-B usually appear in the range 6.7 -7.0 ppm. Which is down field from the region where the A-ring aromatic protons absorb. The signal pattern for the B-ring is characteristic for the substitution pattern of the ring. If the B-ring is oxygenated at C-4', a 4H resonance of two doublets (J = 8.5 Hz) is observed. The C-3' and C-5' protons will be shielded by the C-4' oxygenated substituent as a result the signal appears upfield in the range (6.65-7.1 ppm) from the C-2' and C-6' protons (7.2-7.5 ppm).

For a C-3',4' oxygenated B-ring the NMR spectra will be more complex than the C-4' oxygenated isoflavones. In such a case multiplet of ABX pattern in the region (6.7-7.1 ppm) is observed.

A singlet that appears at ca. 7.8 ppm representing the C-2 proton of ring C is a characteristic feature of an isoflavone. The presence of methoxyl and methylenedioxy groups is readily recognized by the appearance of strong signals at 3.5-4.1 ppm and at about 6.0 ppm respectively.

2.5.3 ^{13}C NMR Spectroscopy

^{13}C NMR spectroscopy has not as fully applied to the study of isoflavones as it has been for the flavones. The carbonyl signals of both flavones and isoflavones resonate 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 [27]. In the flavones the C-2 signal appear 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, whereas in isoflavones they occur as a doublet at 149.8-155.4 and that of C-3 as a singlet at 122.3-125.9 ppm.

A carbonyl resonance of 181 + 1 ppm clearly indicates the presence of a hydroxyl moiety at C-5. The deshielding of about 6 ppm relative to all other substituted isoflavones arise from intramolecular hydrogen bonding between C-5 hydroxyl and the C-4 carbonyl [20,23].

A 4'-substituted isoflavones (OH, OCH_3) is well characterized by signal sequences 123 + 2 ppm, 130 + 1 ppm (strong), 114 + 1 ppm (strong) and 152 + 2 ppm.

Table-III Chemical Shift of C-5 Hydroxylated and C-4' Substituted Isoflavones.

Isoflavone	C-shifts (ppm)					
	4	1'	2'&6'	3'&5'	4'	Ref.
4',5,7-trihydroxy-isoflavone (Genistein)	181.37	122.90	130.97	115.82	158.20	20
5-hydroxy-4',7-dimethoxy isoflavone	180.52	122.91	130.13	113.74	159.43	20
Wighteone	180.40	121.50	130.30	115.20	151.50	29

An off-resonance quartet at 55.1 + 0.1 ppm is very likely due to a 4'-methoxy group while all other methoxy groups of isoflavones resonates near 56 ppm. In case of 6,7-dimethoxy derivates two closely spaced methoxy signals at 55.9 (C-6-OCH_3) and 56.2 ppm (C-7-OCH_3) will be observed most likely [20]. In a polyoxygenated isoflavones, a resonance observed at 59-64 ppm will most likely be due to di-O-substituted methoxy groups [10].

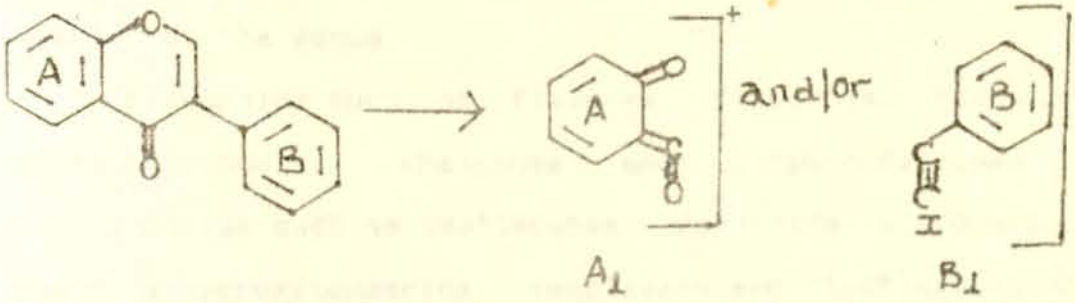
Table - IV Methoxy carbon shifts of isoflavones and flavones

		5	6	7	8	2'	3'	4'	5'	6	Ref
1	4',6-dimethoxy isoflavone	56.31						55.09			20
2	4',7- " "			55.88				55.09			20
3	6,7 " "		55.82	56.28							20
4	7,8 " "			56.43	60.98						20
5	4',7,8-trimethoxy isoflavone			56.37	60.95			55.06			20
6	3',4',5'- " "						56.00	60.13	56.00		20
7	5,7-dihydroxy-6,2',4',5'-tetramethoxy isoflavone		60.1			55.7		56.2			28
8	4',5-dihydroxy-3',6,7-trimethoxy flavone		60.0	56.3			56.0				29
9	3',4',5,6,7-pentamethoxy flavone	62.1	61.5	56.3			56.1	56.1			29
10	3',5'-dihydroxy-2',7,8-trimethoxy flavone			56.5	61.0	60.2					29

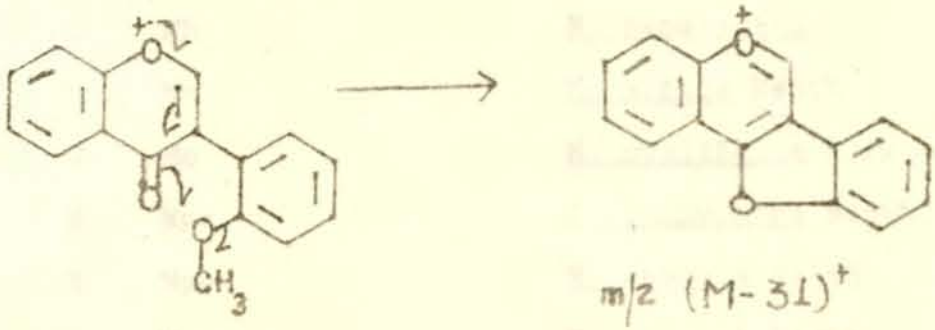
2.5.4 Mass Spectrometry [30]

EIMS distinguishes easily isoflavones bearing 2'-hydroxyl or 2'-methoxyl substituents from those that do not.

a) Isoflavones without a 2'-substituent give a relatively abundant $M^{+\cdot}$ along with $(M-H)^{+}$, $(M-CH_3)^{+}$ and M^{2+}. Furthermore fragment derived by RDA, cleavage are usually present and these greatly aid in the identification of the substitution pattern [31]



b) Isoflavones with 2'-hydroxyl and 2'-methoxyl substituent give some of the above mentioned ions in addition to certain fragments that are unique for the 2'-substitution pattern. In RDA process a hydrogen transfer from the 2'-methoxyl moiety to the A-ring fragment occurs. The 2'-methoxyl group can also be eliminated to give an $(M-31)^{+}$ ion as shown below [32].



3. SECONDARY METABOLITIES OF MILLETTIA

Phytochemical investigation of a number of Millettia species have shown them to be a rich source of flavonoids and isoflavonoids typical of the Tephrosieae tribe of Papilionoidea [2]. In addition to the flavonoids, the presence of steroids have also been reported from some species of the genus.

Flavonoids such as flavones, flavonols, flavanone, dihydroflavonols, chalcones and dihydrochalcones and isoflavonoids such as isoflavones, rotenoids, pterocarpans, 3-aryl-4-hydroxycoumarins, isoflavans and isoflavanquinone are known to occur in this genus.

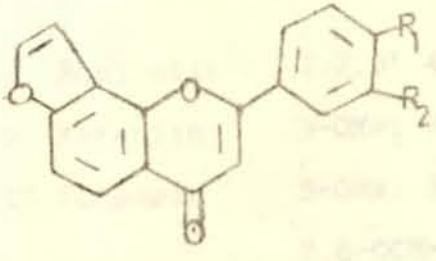
Secondary metabolites so far known from the Millettia species listed below are summarized in Table 5-12 according to their structural types.

1.	Ma	M. auriculata Bak., ex Brand
2.	Mdi	M. dielsiana Harms
3.	Mdu	M. dura Dunn
4.	Mf	M. ferruginea (Hochst) Bak.
5.	Mh	M. hemsleyana
6	Mn	M. nitida Benth
7.	Mo	M. ovalifolia kurz
8.	Mpa	M. pachycarpa Benth
9.	Mpe	M. pendula Benth
10.	Mpu	M. pulchra Benth
11.	Mra	M. racemosa Benth

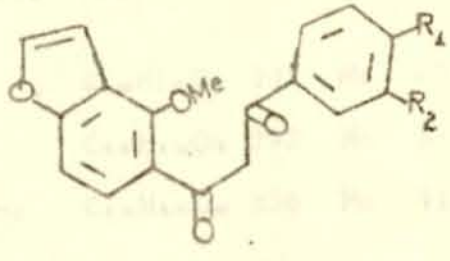
12.	Mre	M. reticulata Benth
13.	Mru	M. rubiginosa (Wt and Arn)
14.	Ms	M. stuhlmannii Taub
15.	Mta	M. taiwaniana Hayata
16.	Mth	M. thonningii (Schuma and Thonn) Bak.

3.1 Flavones and Flavonols

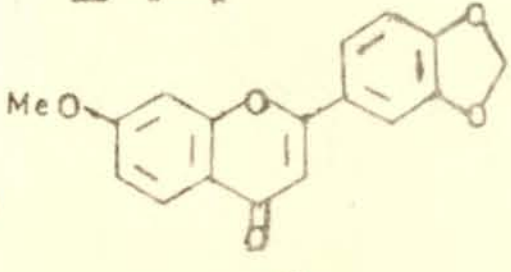
The flavones and flavonols from this genus show the absence of a 5-hydroxyl group (Table-5). Furanoflavones, pongaglabrone and kanjone and furanoflavonols, karanjin and pongapin which seem to be restricted to the genera Pongamia [33,34,35] and lanceolatin B to Tephrosia [36] are also reported to occur in this genus as well. Moreover, pongaglabrone (15), lanceolatin B (16) from M. ovalifolia [32] and 3',4'-methylenedioxy-7-methoxyflavone (17) from M. hemsleyana [38] can be considered as the cyclized forms of the demethylated 8-hydroxychalcones, ovalitenone (18), pongamol (19) and milletenone (20) respectively.



15. $R_1+R_2 = -OCH_2O-$
16. $R_1=R_2=H$



18 $R_1+R_2 = -OCH_2O-$
19 $R_1=R_2=H$



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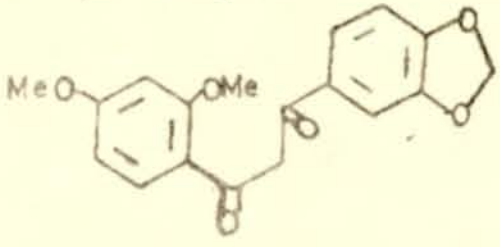

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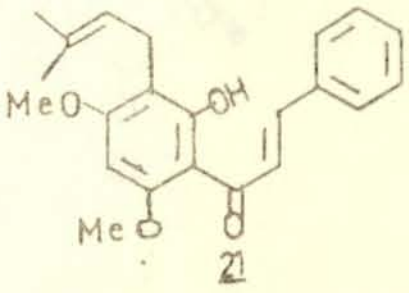
Table-5 Structures of Flavones and Flavonols

No	Flavone	Substitution	Mol.for	M.W.	Sou.	Ref.
1	Lanceolatin-B	7,8-OCH=CH-	$C_{17}H_{10}O_3$	262	Mo Mh	37 38
2		7-OMe;3',4'-OCH_2O-	$C_{17}H_{12}O_5$	296	Mh	38
3	Pongaglabrone	7,8-OCH=CH-;3,4-OCH_2O-	$C_{18}H_{10}O_5$	306	Mo	39
4	Kanjone	6-OMe;7,8-OCH=CH-	$C_{18}H_{12}O_4$	292	Mo	39
5	Milletenin C	6,7-$(OMe)_2$;3,4-OCH_2O-	$C_{18}H_{14}O_6$	326	Mo	37
6	Pongaflavone	3-OMe; 7,8-$OCMe_2C$=CH-	$C_{21}H_{18}O_4$	334	Mh	38
7	Ovalifolin	6-OCH_2CH=CMe_2; 7,8-OCH=CH-	$C_{22}H_{18}O_4$	346	Mo	37
	Flavonols					
8	Robinetin	3,7,3',4',5'-$(OH)_5$	$C_{16}H_{10}O_7$	302	Ms	40
9	Karanjin	3-OMe; 7,8-OCH=CH-	$C_{18}H_{12}O_4$	292	Mo	39
10	Pongapin	3-OMe; 3',4'-OCH_2O-; 7,8-OCH=CH-	$C_{19}H_{12}O_6$	336	Mo	41

3.2 Chalcones and Dihydrochalcones

Table 6 summarizes chalcones and dihydrochalcones that are so far known to occur in the genus. In this series the furano derivaties seem to be more common. Thus, ovalitenin-A ocurrs in M. ovalifolia seeds along with other flavonoids having the same A-ring substitution, namely ovalitenin-B, ovalitenin-C, ovalitenone and pogamol.

Ovalichalcone (21) and ovalichalcone-A (22) isolated from M. ovalifolia [42,43] can be considered as having a phloroglucinol A-ring pattern, where C-prenylation and O-metylation has taken place. Ring closure of (21) could result in pongachalcone-I (23) isolated from M. ovalifolia [44].



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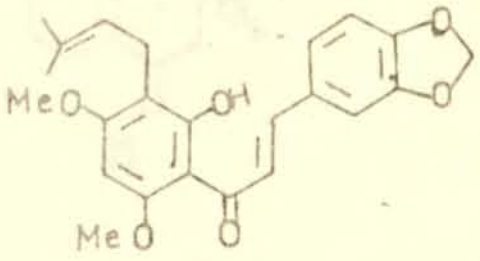

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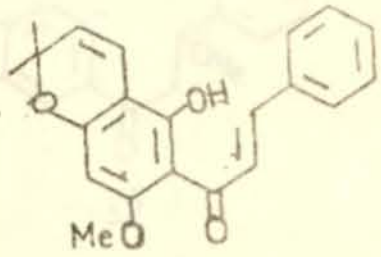
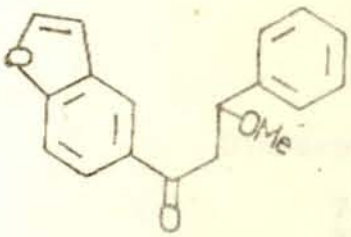


23

Dihydrochalcones having β-hydroxyl function have been reported from this genus. Ovalitenin-B (24) occuring in M. ovalifolia [45] seems to arise from pogamol (19) as a result of reduction of the β-keto-function followed by O-methylation. Dihydromilletenone methyl ether (25) and the dihydroisomilletenone ether (26) are also reported to occur along with the β-hydroxychalcone milletenone (20) in M. hemsleyana [38].



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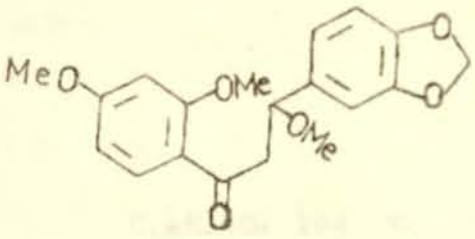

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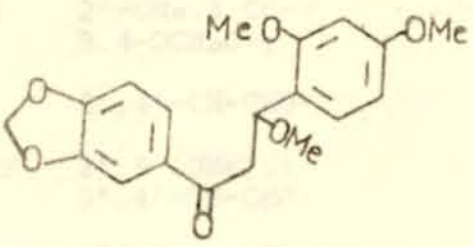

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Table-6 Structures of Chalcones and dihydrochalcones

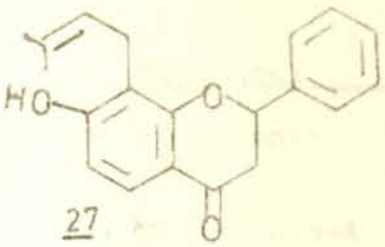
No	Chalcone	Substitution	Mol.for	M.W.	Sou.	Ref.
11	Ovalitenin-A	2'-OMe;3',4'-CH=CHO-	$C_{18}H_{14}O_5$	278	Mo	45
12	Ovalitenin-C	2-OMe;3,4-OCH_2O-; 3',4'-CH=CHO-	$C_{19}H_{14}O_5$	322	Mo	46
13		2'-OH;4-OMe: 3.4-CH=$CHCMe_2O$-	$C_{21}H_{20}O_4$	336	Mpa	47
14	Pongachalcone-I	2-OH; 6'-OMe; 3',4'-CH=$CHCMe_2O$-	$C_{21}H_{20}O_4$	336	Mo	44
15	Ovalichalcone	2'-OH;4',6'-$(OMe)_2$; 3'-$CH_2CH=CMe_2$	$C_{22}H_{24}O_4$	352	Mo	42
16	Ovalichalcone-A	2-OH;4',6'-$(OMe)_2$; 3.4-OCH_2O-; 3'-$CH_2CH=CMe_2$	$C_{23}H_{24}O_4$	396	Mo	43
		Dihydrochalcone				
17	Pongamol	2'-OMe;β-CO-; 3',4'-CH=CHO-	$C_{18}H_{14}O_4$	294	Mo Mo	41 37
18	Milletenone	2',4'-$(OMe)_2$;β-CO-; 3',4'-OCH_2O	$C_{18}H_{16}O_6$	328	Mh	38
19	Ovalitenone	2'-OMe;β-CO-; 3.4-OCH_2O-; 3',4'-CH=CHO-	$C_{19}H_{14}O_6$	388	Mo	45
20	Ovalitenin-B	2',β-$(OMe)_2$: 3',4'-CH=CHO-	$C_{19}H_{18}O_4$	310	Mo	45
21	Dihydromilleno-ne methylether	2',4',β-$(OMe)_3$; 3.4-OCH_2O-	$C_{19}H_{20}O_6$	344	Mh	38
22	Dihydroisomill-etenone methyl ether.	2,4,β-$(OMe)_3$; 3',4'-OCH_2O-	$C_{19}H_{20}O_6$	344	Mh	38

3.3 Flavanones and Dihydroflavonols

A complex series of C-alkylated flavanones

a) lacking B-ring substitution

b) possessing the 3',4'-methylenedioxy function occuring along with the dimethylchromeno flavanones in the seeds of M. ovalifolia [39,41,44,46]. This suggests that isolonchocarpin (29) and ovalichromene-B (33) are intramolecular cyclization products derived from ovaliflavanone-B (27) and ovaliflavanone-C (31) respectively. Ovalichromene (30) and ovalichromene-A (34) can be considered as the cyclized products of the C-6 O-methylated precursors.



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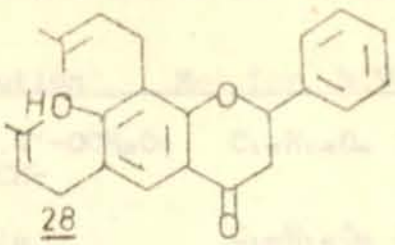

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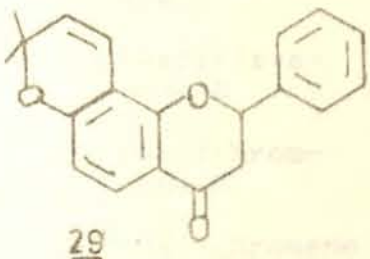

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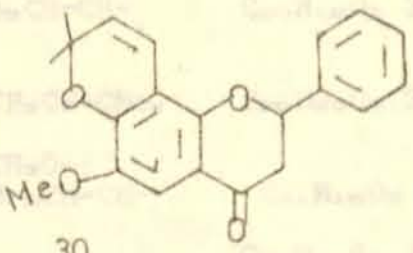

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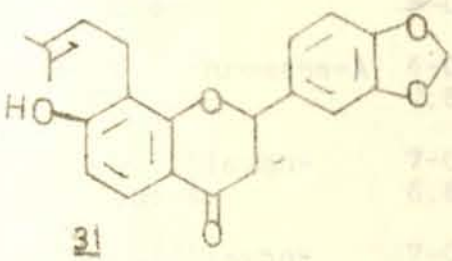

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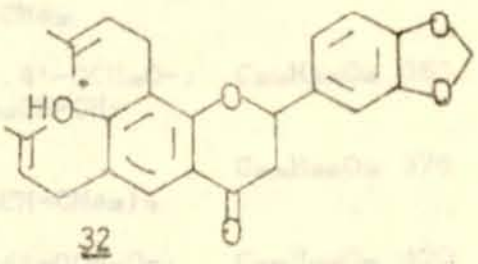

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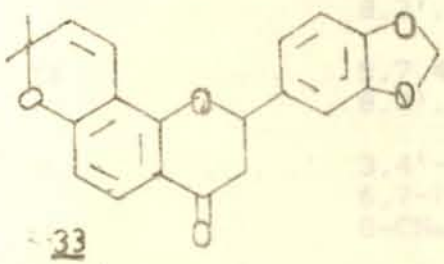

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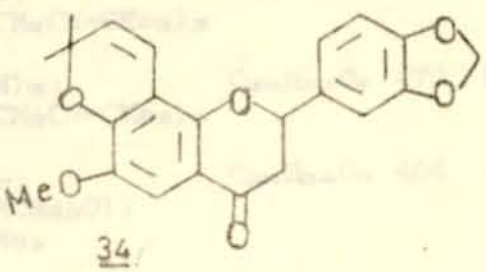

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Table-7 Structures of Flavanones and dihydroflavonols

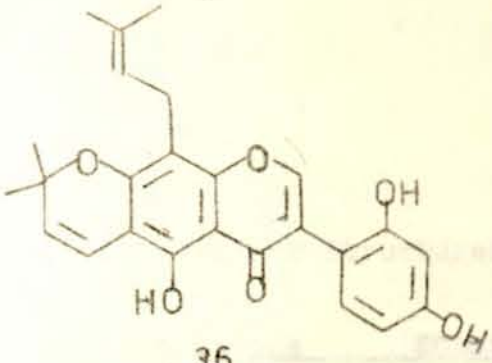
No	Flavanone	Substitution	Mol.for	M.W.	Sou.	Ref.
23	Milletenin	6-OMe:3',4'-OCH_2O- 7,8-OCH=CH-	$C_{19}H_{14}O_6$	338	Mo	37
24	Milletenin-A	6,7-$(OMe)_2$ 3',4'-OCH_2O-	$C_{18}H_{14}O_5$	312	Mo	37
25	Isolonchocarpin	7,8-$OCMe_2CH$=CH-	$C_{20}H_{18}O_5$	306	Mo	47
26	Ovaliflavanone-B	7-OH:8-CH_2CH=CMe_2	$C_{20}H_{20}O_5$	308	Mo	39
27	Ovalichromene-B	3',4'-OCH_2O-; 7,8-$OCMe_2CH$=CH-	$C_{21}H_{18}O_5$	350	Mo	41
28	Ovalichromene	6-OMe; 7,8-$OCMe_2CH$=CH-	$C_{21}H_{20}O_4$	336	Mo	41
29	Ovliflavanone-C	7-OH;3',4'-OCH_2O; 8-CH_2CH=CMe_2	$C_{21}H_{20}O_5$	352	Mo	46
30	Ovlichromene-A	6-OMe;3',4'-OCH_2O-; 7,8-$OCMe_2CH$=CH-	$C_{22}H_{20}O_6$	380	Mo	44
31	Ovliflavanone-A	7-OH; 6,8-$(CH_2CH$=$CMe_2)_2$	$C_{25}H_{28}O_5$	376	Mo	39
32	Ovliflavanone-D	7-OH;3',4'-OCH_2O-; 6,8-$(CH_2CH$=$CMe_2)_2$	$C_{25}H_{28}O_5$	420	Mo	44
33	Sophoranone	7,4'-$(OH)_2$; 8,3',5'-$(CH_2CH$=$CMe_2)_3$	$C_{30}H_{32}O_4$	457	Mpu	49
34		5,7,4'-$(OH)_3$; 8,3',5'-$(CH_2CH$=$CMe_2)_3$	$C_{30}H_{32}O_4$	473	Mpu	49
35		3,4'-$(OH)_2$; 6,7-(CH=$CHCMe_2O$); 8-CH_2CH=CMe_2	$C_{25}H_{26}O_5$	406	Mpa	50
36		3,5,4'-$(OH)_3$; 8-CH_2CH=CMe_2; 6,7-CH=$CHCMe_2O$	$C_{25}H_{26}O_6$	422	Mpa	51
37		3,7,4'-$(OH)_3$; 8,3',5'-$(CH_2CH$=$CMe_2)_3$	$C_{30}H_{32}O_5$	473	Mpu	40

3.4 Isoflavonoids

Table 8-11 summarizes the various forms of isoflavonoids from Millettia species. Among the reported islfavonoids from the genus. the isoflavones are the most common.

The co-occurrence of a rotenoid (sumatrol-35-) the 2'-hydroxyisoflavones (auriculatin-36-) and isoauriculatin (37) and a coumaronochromene (millettin-38-) along with other isoflavones within the seeds of M. auriculata is biogenetically interesting and is consistent with the biogenetic scheme suggested by ollis [52,53] that 2'-hydroxy-isoflavones are precursors for rotenoids and coumarino-chromones.

The natural co-occurrence of the 3-aryl-4-hydroxycoumarin (robustic acid (39)) with the structurally corresponding isoflavone (alpinum-isoflavone (40)) was reported from the seeds of M. thonningii [54] by Olivares et. al. This co-occurrence is certainly of biogenetic interest and there can be little doubt that the biosynthesis of isoflavones and 3-aryl-4-hydroxy-coumarins follow similar pathways.



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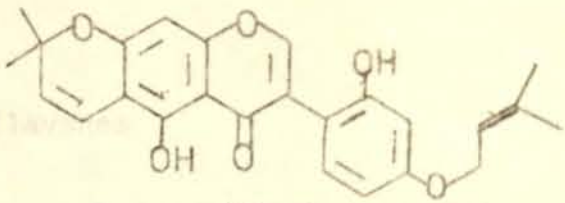

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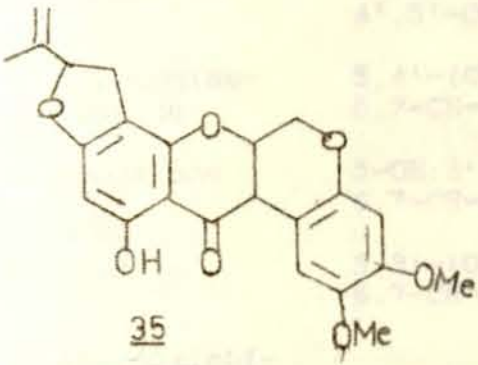

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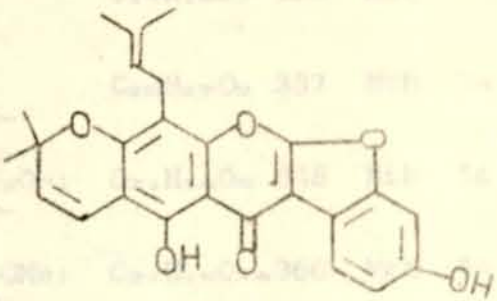

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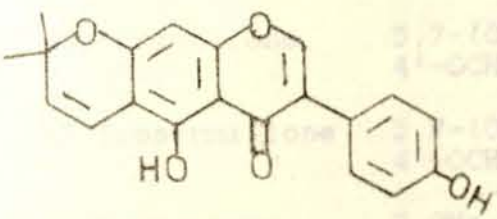

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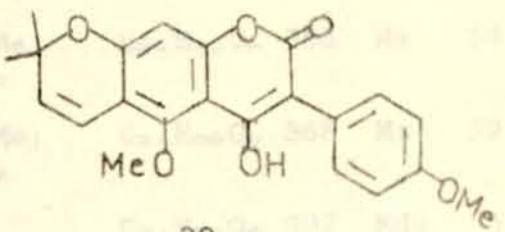

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Table-8 Structures of Isoflavones

No	Isoflavone	Substitution	Mol.for	M.W.	Sou.	Ref
38	Afformosin	7-OH;6,4'-$(OMe)_2$	$C_{17}H_{16}O_5$	300	Mre	55
39		7-OH;8,4'-$(OMe)_2$	$C_{17}H_{16}O_5$	300	Mre	55
40	Mildurone	6,7,2'-$(OMe)_3$; 4',5'-OCH_2O-	$C_{19}H_{16}O_7$	356	Mdu	5
41	Alpinumisoflavone	5,4'-$(OH)_2$; 6,7-$CH=CHCMe_2O$-	$C_{20}H_{17}O_5$	337	Mth	54
42	Robustone	5-OH;3',4'-OCH_2O-; 6,7-$CH=CHCMe_2O$-	$C_{21}H_{16}O_5$	348	Mth	56
43		5,3'-$(OH)_2$;4'-OMe; 6,7-$CH=CHCMe_2O$-	$C_{21}H_{18}O_{16}$	366	Mth	54
44	Alpinumisoflavone-4'-methylether	5-OH; 4'-OMe; 6,7-$CH=CHCMe_2O$-	$C_{21}H_{19}O_5$	351	Mth	54
45		5-OH;4'-OMe; 7,8-$OCMe_2CH=CH$-	$C_{21}H_{20}O_5$	352	Mpa	57
46	Aurimillone	5,7-$(OH)_2$;8-OMe; 4'-$OCH_2CH=CMe_2$	$C_{21}H_{20}O_6$	368	Ma	58
47	Isoaurmillone	5,7-$(OH)_2$;6-OMe; 4'-$OCH_2CH=CMe_2$	$C_{21}H_{20}O_6$	368	Ma	59
48	Durlettone	7-OMe; 4'-$OCH_2CH=CMe_2$	$C_{21}H_{21}O_4$	337	Mdu	5
49	Durmillone	6-OMe;3,4'-OCH_2O-;			Mf	9
					Mdu	5
		7,8-$OCMe_2CH=CH$-	$C_{22}H_{21}O_6$	378	Mru	60
50	Alpinumisoflavone-dimethylether	5,4'-$(OMe)_2$; 6,7-$CH=CHCMe_2O$-	$C_{22}H_{21}O_5$	365	Mth	54

Table-8 contd.

No	Isoflavone	Substitution	Mol.for	M.W.	Sou.	Ref.
51	Ferrugone	2',5'-$(OMe)_2$; 3',4'-OCH_2O- 7,8-$OCMe_2CH$=CH-	$C_{23}H_{20}O_7$	408	Mf	9
52	Ichthynone 4',5'-OCH_2O-	6,2'-$(OMe)_2$; 4'5'-OCH_2O- 7,8-$OCMe_2CH$=CH-	$C_{23}H_{20}O_7$	408	Mru	60
53	Auriculatin	5,2',4'-$(OH)_3$; 8-CH_2CH=CMe_2; 6,7-CH=$CHCMe_2O$-	$C_{25}H_{24}O_6$	420	Ma	61
54	Isoauriculatin	5,2'-$(OH)_2$; 4'-OCH_2CH=CMe_2; 6,7-CH=$CHCMe_2O$-	$C_{25}H_{24}O_6$	420	Ma	62
55	Auriculasin	5,3',4'-$(OH)_3$; 8-CH_2CH=CMe_2; 6,7-CH=$CHCMe_2O$-	$C_{25}H_{24}O_6$	420	Ma	63
56	Isoauricula-sin	5,3'-$(OH)_2$; 4'-OCH_2CH=CMe_2; 6,7-CH=$CHCMe_2O$-	$C_{25}H_{24}O_6$	420	Ma	63
57	Pomiferin	5,3',4'-$(OH)_3$; 6-CH_2CH=CMe_2; 7,8-$OCMe_2CH$=CH-	$C_{25}H_{24}O_6$	420	Mpa	57
58	6,8-Di(dimet-hylallyl) genistein	5,7,4'-$(OH)_3$; 6,8-$(CH_2CH$=$CMe_2)_2$	$C_{25}H_{26}O_5$	406	Mpa	50
59	6,3'-Di(dimet-hylallyl) genistein	5,7,4'-$(OH)_3$; 6,3'-$(CH_2CH$=$CMe_2)_2$	$C_{25}H_{24}O_6$	406	Mpa	50
60	6,8'-Di(dimet hylallyl) orobol	5,7,3',4'-$(OH)_4$; 6,8-$(CH_2CH$=$CMe_2)_2$	$C_{25}H_{26}O_6$	422	Mpa	55

Table-8 contd.

No	Isoflavone	Substitution	Mol.for	M.W.	Sou.	Ref
61		5,7,2',4'-(OH)$_4$; 6,3'-(CH_2CH=CMe_2)$_2$	$C_{25}H_{26}O_6$	422	Mpu	49
62	Auriculin	5,2'-(OH)$_2$;4'-OMe; 8-CH_2CH=CMe_2	$C_{26}H_{25}O_6$	433	Ma	61 62
63		5,7,4'-(OH)$_3$: 2'-OMe; 6,3'-(CH_2CH=CMe_2)$_2$	$C_{26}H_{28}O_6$	436	Mpa	49
64		4'-OH;3'-OMe; 5,6-$OCMe_2CH_2CH_2$-; 7,8-$OCMe_2CH_2CH_2$-	$C_{26}H_{28}O_6$	436	Mpa	51
65		2'-OH;4'-OMe; 5,6-$OCMe_2CH_2CH_2$-; 7,8-$OCMe_2CH_2CH_2$-	$C_{26}H_{28}O_6$	436	Mpa	51
66		5-OH;3',4'-(OMe)$_2$; 8-$CH_2CH_2C(OH)Me_2$; 6,7-$CH_2CH_2CMe_2O$-	$C_{27}H_{32}O_7$	468	Mpa	51
67		5-OH;2',4'-(OMe)$_2$; 8-$CH_2CH_2C(OH)Me_2$; 6,7-$CH_2CH_2CMe_2O$-	$C_{27}H_{32}O_7$	468	Mpa	51

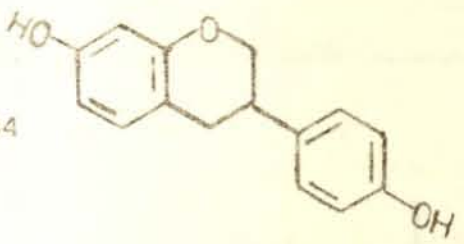
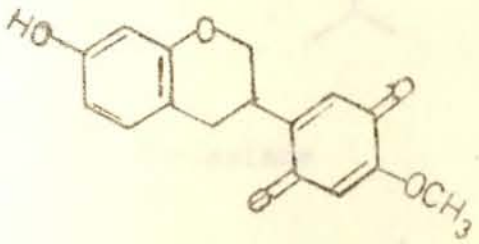
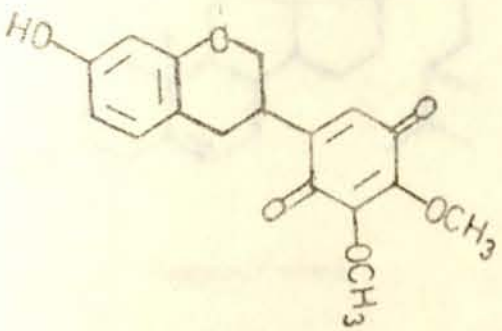
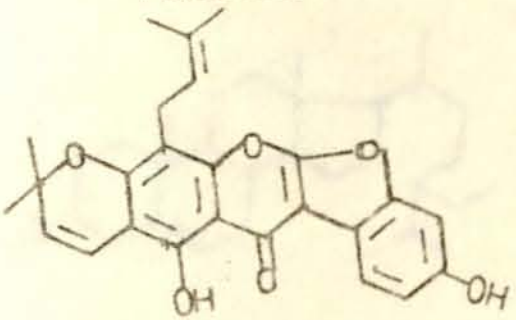
Table-9 Structures of Rotenods.

No	Rotenoid	Substitution	Mol.for	M.W.	Sou.	Ref
68	Milletone	2,3-OCH_2O-; 8,9-CH=$CHCMe_2O$-	$C_{22}H_{18}O_6$	378	Mdu	5
69	Rotenone	2,3-$(OMe)_2$; 8,9-$CH_2CH(MeC=CH_2)O$-	$C_{23}H_{22}O_6$	394	Mdu	5
70	Sumatrol	1,1-OH;2,3-$(OMe)_2$; 8,9-$CH_2CH(MeC=CH_2)O$-;	$C_{23}H_{22}O_7$	410	Ma	61
71	Rot-2-enonic-acid	9-OH;2,3-$(OMe)_2$ 8.-CHCH=CMe_2	$C_{23}H_{24}O_6$	396	Mpa	64
	12-Hydroxyrotenoids					
72	Millettosin	12a-OH;2.3-OCH_2O-; 8-CH=$CHCMe_2O$-	$C_{22}H_{20}O_7$	396	Mdu	5
73	12a-hydroxy-rotenone	12a-OH;2.3-$(OMe)_2$; 8,9-$CH_2CH(MeC=CH_2)O$-	$C_{23}H_{22}O_7$	410	Mpa	64
74	Tephrosin	12a-OH;2,3-$(OMe)_2$; 8,9-$CH_2CH=CHCMe_2O$-	$C_{23}H_{22}O_7$	410	Mdu Mf	5 9
75	12a-hydroxy-rot-2-enonic acid	9,12a-$(OH)_2$; 2.3-(OMe); 8-$CH_2CH=CMe_2$	$C_{23}H_{24}O_7$	412	Mpa	64
	Dehydrorotenoid					
76	Dehydrodeguelin	6a 12a-dehydro; 23-$(OMe)_2$; 8,9-CH=$CHCMe_2O$-	$C_{23}H_{20}O_6$	392	Mdu Mf	5 9

Table-10 Structures of Petrocarpans & 3-aryl-4-hdroxy-coumarins

No	Rotenoid	Substitution	Mol.for	M.W.	Sou.	Ref
77	Maackiain	3-OH;8,9-OCH_2O-	$C_{16}H_{12}O_5$	284	Mpe Mpu	65 49
78	Pterocarpin	3-OMe;8,9-OCH_2O-	$C_{17}H_{14}O_5$	298	Mpu	49
79	6α-methoxy-hemopteroc-arpin	3,6,-$(OMe)_2$; 8,9-OCH_2O-	$C_{18}H_{16}O_5$	312	Mpu	49
80	6α-methoxy-homopteroc-arpin	3,6,9-$(OMe)_3$	$C_{19}H_{18}O_5$	314	Mpu	49
	3-aryl-4-hydroxycoumarin					
81	Roubstic acid	4-OH;5,4'-(OMe); 6,7-CH=CHCMe$_2$O-	$C_{22}H_{20}O_6$	380	Mth	56
82	Thonningii A	4-OH;5,8-$(OMe)_2$; 3',4'-OCH_2O-	$C_{23}H_{18}O_8$	422	Mth	56
83	Thonningii B	4-OH;5,8,4'-$(OMe)_3$; 6,7-CH=C(MeC=CH_2)O-	$C_{23}H_{20}O_7$	408	Mth	56

Table-11 Isoflavan, Isoflavanquinone & Coumaronochromene

No	Compound	M.f	M.w	Sour	Ref.
84	 Equol	$C_{15}H_{14}O_3$	242	M.pe	65
85	 Claussequinone	$C_{16}H_{14}O_5$	286	M.pe	65
86	 Pendulone	$C_{17}H_{14}O_6$	316	M.pe	65
87	 Millettin	$C_{25}H_{22}O_6$	418	M.a	66

2.5 Steroids

Table-12 Steroids

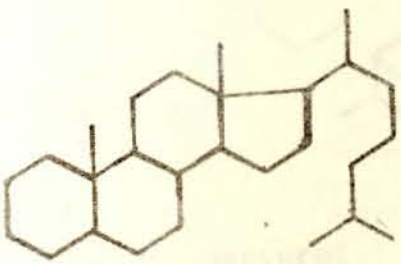
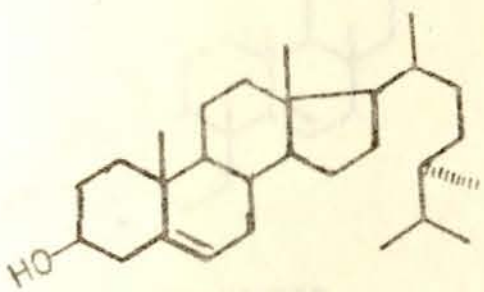
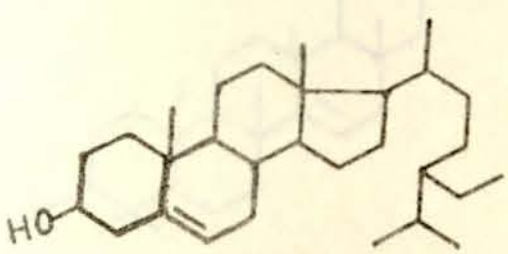
No	Compound	M.f	M.w	Sour.	Ref.
88		$C_{27}H_{48}$	372	M.f	6
	Cholestane				
89		$C_{28}H_{48}O$	400	M.di M.n	67 67
	Campesterol				
90		$C_{29}H_{50}O$	414	M.di M.f M.n M.pa M.pe M.ra	67 6 67 67 68 60
	β-Sitosterol				

Table-12 Contd.

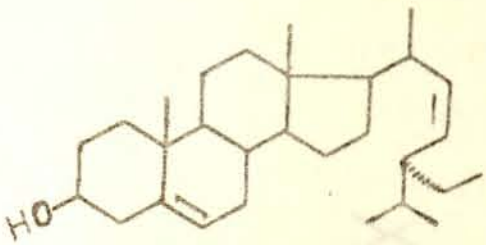
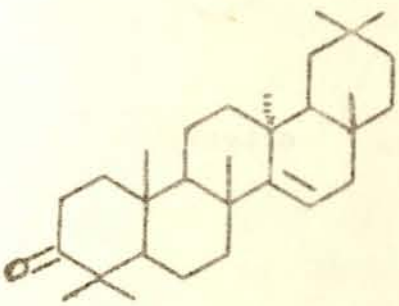
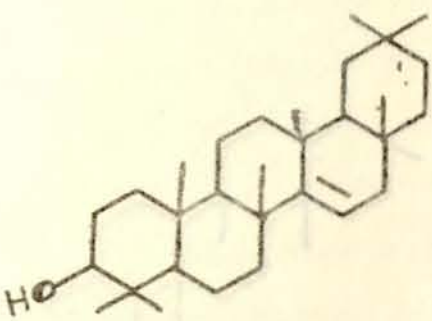
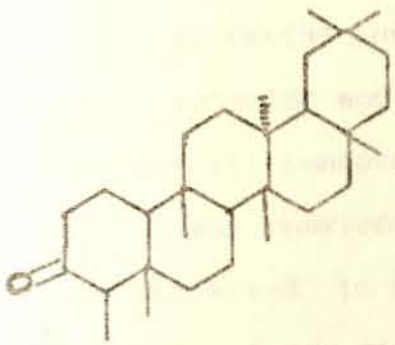
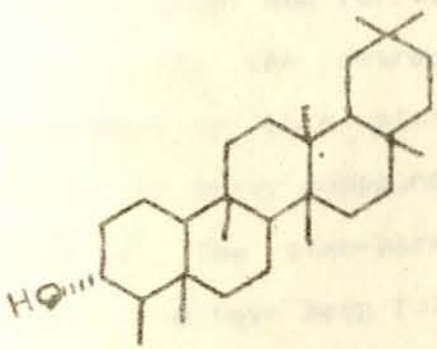
No	Compound	M.f	M.w	Sour.	Ref.
91	 Stigmasterol	$C_{29}H_{48}O$	412	M.di M.f M.n M.pa M.pe M.re	67 6 67 67 68 60
92	 Taraxerone	$C_{30}H_{48}O$	424	M.di M.n	67 67
93	 Taraxerol	$C_{30}H_{50}O$	426	M.n	67

Table-12 Contd.

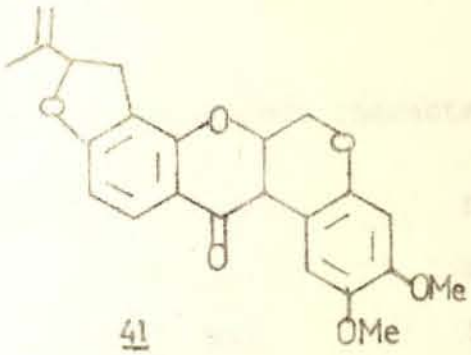
No	Compound	M.f	M.w	Sour.	Ref.
94	 Friedelin	$C_{30}H_{50}O$	426	M.di M.n M.pa	67 67 67
95	 Friedelinol	$C_{30}H_{52}O$	428	M.di M.n M.pa	67 67 67

4. Results and Discussion

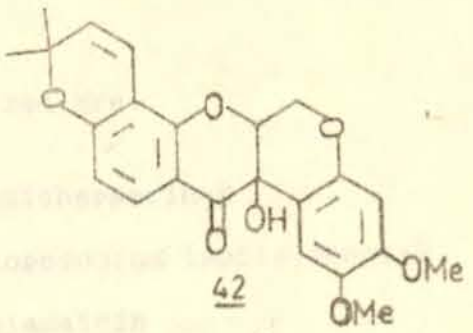
Several reports exist in the literature on chemical studies of Millettia species. To date over 25 flavonoids. 50 isoflavonoids. 12 chalcones and 8 miscellaneous compounds have been reported from this genus alone. The genus is well known for elaborating interesting secondary metabolites such as the isoflavonoids and rotenoids. An attempt has been made here to list all compounds so far isolated from Millettia species. To our knowledge so far only 16 Millettia species have been subjected to phytochemical study. The compounds so far isolated are given in Tables 5-12 indicating the source, species and the corresponding reference.

The seeds of Millettia ferruginea have been investigated by Clark in 1943 [8] and Highet and Highet in 1967 [9] and resulting in the isolation and characterization of retenone (41), tephrosin(42), dehydrodeguelin (43), durmillone(44) and ferrugone(45)

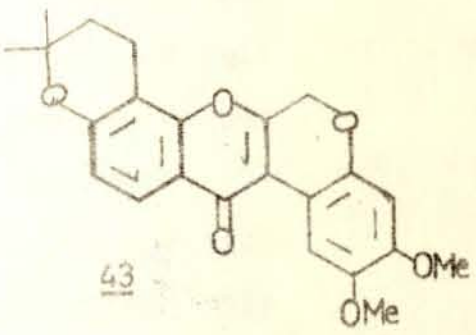
During the course of our chemical study on the steam-bark of this plant except for ferrugone (45) even traces of other compounds present in the seed could not be detected. The stem-bark contains at least ten compounds of which nine have been fully characterized and considerable data has been generated on the remaining one compound. The compounds were named as compound A,B,....,I,J.



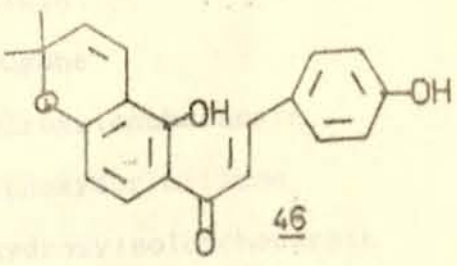
41



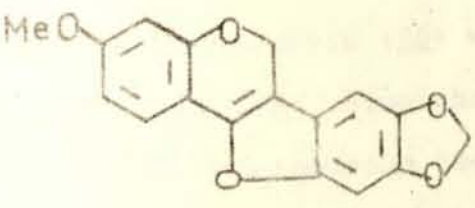
42



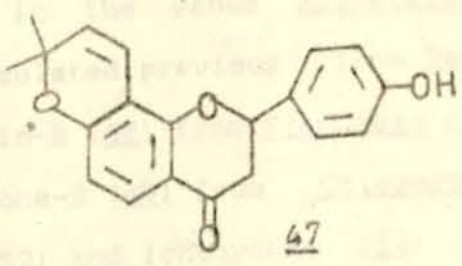
43



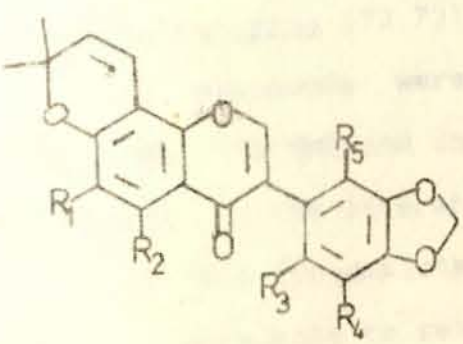
46



48



47



44. R_1=OMe; R_2=R_3=R_4=R_5=H
45. R_4=R_5=OMe; R_1=R_2=R_3=H
49. R_1=R_2=R_3=R_4=R_5=H
50. R_4=OMe; R_1=R_2=R_3=R_5=H
51. R_3=OMe; R_1=R_2=R_4=R_5=H
52. R_3=R_5=OMe; R_1=R_2=R_4=H
53. R_1=R_2=OMe; R_3=R_4=R_5=H

The compounds characterized are:

compound-A (48)	flemichapparin-B
compound-B (49)	calopogonium isoflavonone-B
compound-C (50)	lsojamaicin
compound-D (51)	jamaicin
compound-E (45)	ferrugone
compound-F (46)	4-hydroxylonchocarpin
compound-G (53)	5-methoxydurimillone
compound-H (47)	4'-hydroxyisolonchocarpin
compound-I (52)	ichthynone

The known compounds (46.48.49.51.52) are described here for the first time to occur in the genus Millettia. 4-hydroxylonchocarpin (46) was isolated previously from Derris flouribunda [69], flemichapparin-B (48) from Flemingia chapar [80], calopogonium isoflavone-B (49) from Calopogonium mucconoides [71], jamaicin (52) and ichthynone (52) from Piscidia erythrina [72,73].

All compounds were characterized by spectrscopic methods MS, ^{1}H NMR and in some cases ^{13}C NMR and comparison with data in the literature. Data in some of the known compounds are incomplete in the literature and therefore attempts were made to generate the missing data. Thus ^{13}C NMR data were generated for the first time for 4-hydroxylonchocarpin(46), ferrugone(45), jamaicin(51) and ichthyone (52). However, for 49, it was not possible to generate full spectral data due to the limited amounts of compound

obtained. The characterizatin of this compound was based mainly on MS and ^{1}H NMR data. Comparsion with literature was not possible due to lack of access to the original sources.

The remaining two isoflavones and one flavanone were characterized as novel and have been assigned the trivial names isojamaicin (50), 5-methoxydurmillone (53) and 4'-hydroxyisolonchocarpin (47).

4.1 Characterization of 4-hydroxylonchocarpin (46)

The HRMs for this yellow compound analyzes as $C_{20}H_{18}O_4$ (M.W. 322.1205). The bands in the IR spectrum at 1590,1520 and 1490 cm^{-1} suggest the precence of an aromatic moiety [74]. The singlet at 14.67 ppm in the ^{1}H NMR and the band that appears at 1640 cm^{-1} in the IR spectrum suggest a carbonyl group chelated with OH.

The UV spectrum of this compound showed maxima at 226,272 and 368 nm suggesting a chalcone with a ring-A fused 2,2-dimethylpyranyl ring [54,75,76]. The MS provided evidence for the presence of the dimethylpyranyl ring because of the characterstic ion resulting from the loss of CH_3 from the molecular ion (m/z 307) as shown in **Scheme-5**. Moreover, the base peak at m/z 187 clearly shows a 2,2-dimethylpyrano ring fused with ring A.

The presence of a band at 3250 cm^{-1} in the IR spectrum and the fact that addition of NaOMe causes a bathochromic shift of 64 nm of band I with increased peak intensity of

its UV spectrum indicates a chalcone containing a free 4-hydroxy group [77].

The two ortho coupled doublets at 8.06 and 6.36 ppm (J=9 Hz) suggest a substituted A ring except the C-5' and C-6' positions. The doublets at 7.74 and 6.92 ppm (J=8.5 Hz) integrating for two protons indicates the B-ring is symmetrically substituted.

Based on the above spectropic observations, structure 46 was assigned to this compound. This structural assignment was fully supported by the ^{13}C NMR spectrum (Table-13) 46 turned out to be the known natural product 4-hydroxylonchocarpin which was isolated from Derris flouribounda [69]. The UV, IR, ^{1}H NMR and MS are in good agreement with those reported for 4-hydroxylonchocarpin (46).

Scheme-5 MS spectral fragmentation of 4-hydroxylonchocarpin

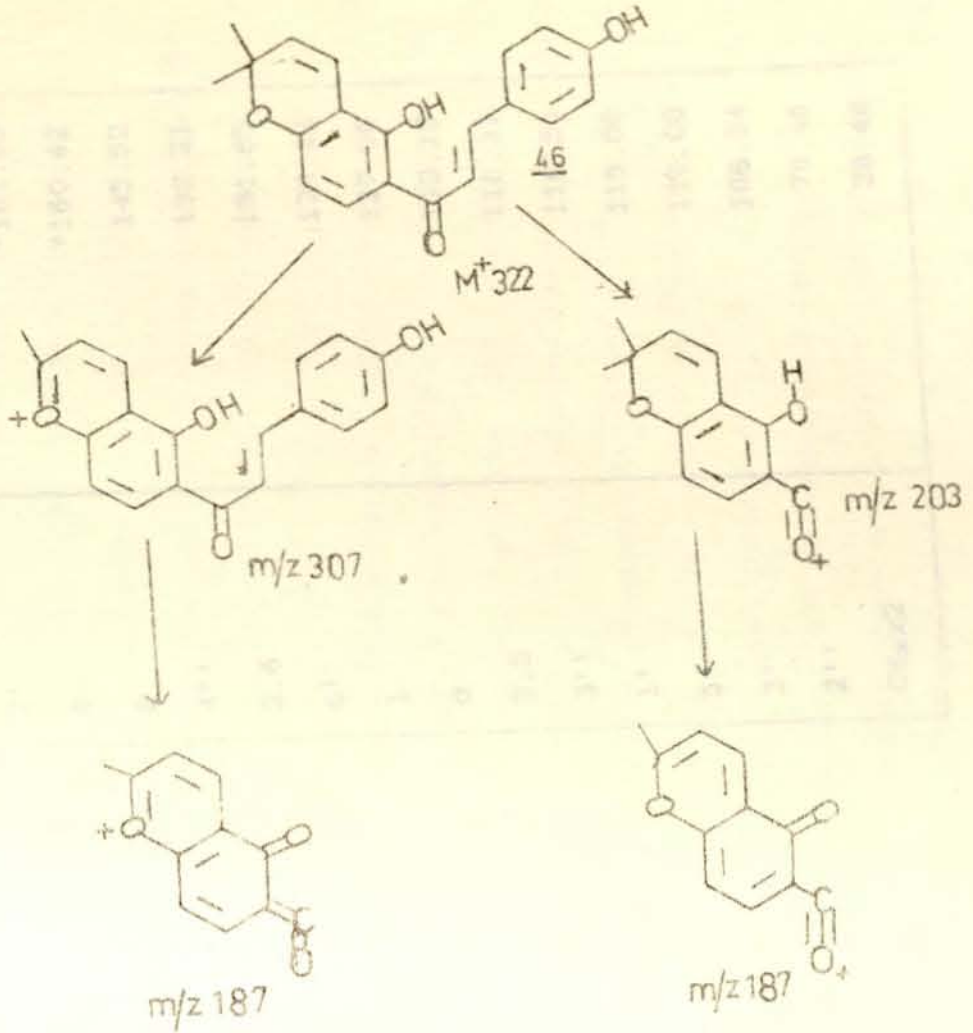


Table 13 ^{13}C NMR [$(CD_3)_2CO$] of 4-hydroxylonchocarpin

Carbon	(ppm)
C=O	193.23
4'	*161.71
2'	*161.08
4	*160.42
B	145.52
4''	132.21
2.6	131.85
6'	129.22
1	127.56
α	118.15
3,5	116.31
3''	116.30
1'	115.00
5'	110.00
3'	108.84
2''	78.46
CH_3x2	28.48

* Interchangeable.

4.2 Characterization of 4'-hydroxyisolonchocarpin (47)

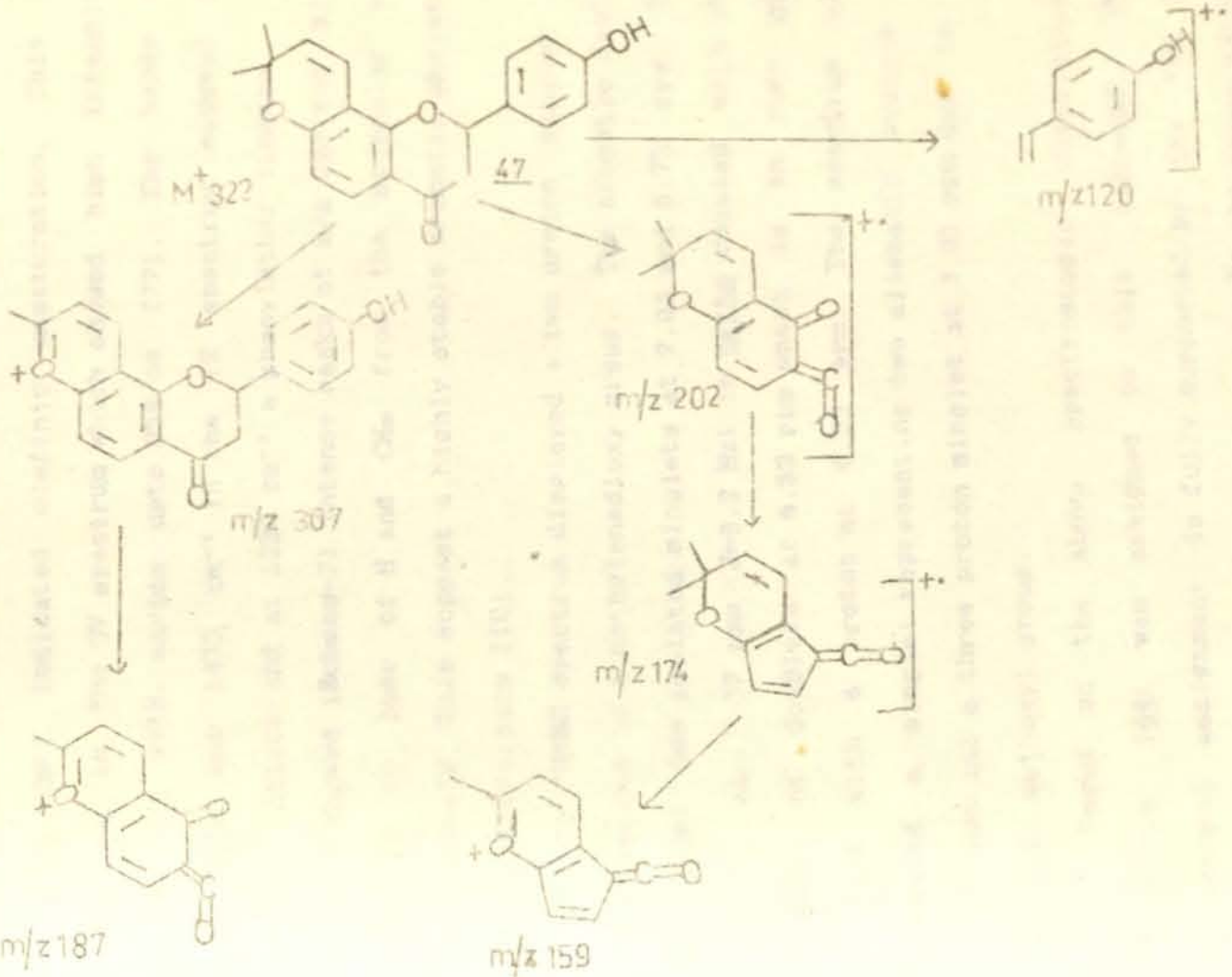
The HRMS analyzes for this compound $C_{20}H_{18}O_4$ as its molecular formula. This suggests a $C_{15}O_2$ nucleus of a flavonoid in association with a C_5-residue.

The ^1H NMR spectrum showed the resonances typical of the ABX spin system of the flav none saturated ring [25] which are clearly resolved into an AMX system allowing first order analysis at 5.40 ppm (1H,dd,$J_{H-2ax/H-3ax}$=13.1 Hz. $J_{H-2ax/H-3eq}$=3.1 Hz), 3.01 ppm (1H, dd, $J_{H-3ax/H-3eq}$=16.8 Hz, $J_{H-3ax/H-2ax}$=13.0 Hz), 2.80 ppm (1H, dd, $J_{H-3eq/H-3ax}$=16.8 Hz, $J_{H-3eq/H-2ax}$=3.2 Hz).

The doublets that appear at 5.56 and 6.62 ppm (J=10 Hz) equivalent for one proton each and two singlets at 1.47 and 1.44 ppm equivalent for three protons each are highly characterstics of a cis olefinic and gem-dimethyl group of a 2,2-dimethyl - chromene structure. The MS further provides evidence for the presence of the dimethyl-chromene ring because of the characterstic ion resulting from the molecular ion (m/z 307) as shown in **Scheme-6**. The base peak at m/z 187 cleary shows a 2,2-dimethyl-chromene ring fused with ring A. The doublets at 7.74 and 6.49 ppm integrating for one proton each indicate that the C-5 and C-6 positions of ring A are unsubstituted. This clearly established that the chromene ring is angularly fused with ring A at 7 and 8 positions.

The presence of a four peak pattern of two doublets at 7.36 and 6.89 ppm (J=8.8 Hz) integrating for two protons each suggest a symmetrically oxygenated B-ring. 4'-hydroxy-isolonchocarpin (47) was deduced to be the angularly fused pyranoflavanone 4'-hydroxy-2'',2''-dimethylpyrano [5'', 6'': 7,8] flavanone.

Scheme-6 MS spectral fragmentation of 4'-hydroxyisolonchocarpin



4.3 Characterization of Flemichapparin-B (48)

The HRMS revealed a molecular ion peak at m/z 296 consistent with the molecular formula $C_{17}H_{12}O_5$. The IR spectrum showed neither -OH nor carbonyl absorption. A band at 1660 cm^{-1} indicated olefinic unsaturation. This is reflected in the UV spectrum which showed high intensity absorption with maxima upto 358 nm [77]. The bands at 1610,1570 and 1470 cm^{-1} in the IR spectrum suggest an aromatic moiety and at 1230 cm^{-1} a phenylether linkage.

MS showed (**Scheme-7**) intense peaks at m/z 295 and m/z 281 due to loss of H and CH_3 from the molecular ion respectively. This suggest a highly stable aromatic skeleton of a pterocarpene [10].

The ^{1}HNMR spectrum displayed a two proton singlet at 6.00 ppm due to a methylenedioxy group. The aromatic region contained two isolated singlets at 7.02 and 6.73 ppm. The doublet at 7.37 ppm (J=8.2 Hz) is ortho coupled with the doublet of doublets at 6.53 ppm which is in turn meta coupled with a proton at 6.53 ppm. The spectrum also revealed a singlet representing two aliphatic protons at 5.52 ppm and a three proton singlet at 3.81 ppm due to an aromatic methoxyl group.

Based on the above spectroscopic observations, structure (48) was assigned to this compound. This structural assignment is fully supported by the ^{13}C NMR spectrum (Table-14). 48 truned out to be the known natural

product Fleminga chappar [70]. The IR, ^{1}H NMR, MS and UV data are in good agreement with those reported for flemichapparin B.

Scheme-7 MS spectral fragmentation of flemichapparin-B

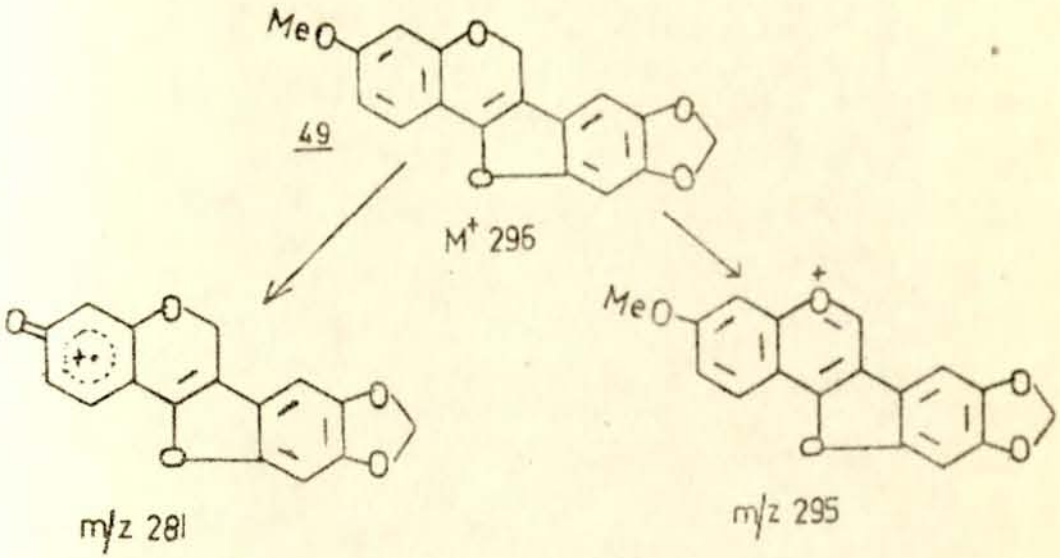


Table - 14 ^{13}C NMR of Flemichapparin-B

Carbon	C-Shift in (ppm)
1	120.74
2	107.08
3	160.69
4	94.11
4a	154.77
6	65.30
6a	106.25
6b	119.03
7	102.39
8	144.75
9	145.61
10	93.87
10a	150.35
11a	147.60
11b	109.81
OCH_2O	101.27
OMe	55.26

4.4 Characterization of Calopogogoniun Isoflavone-B (49)

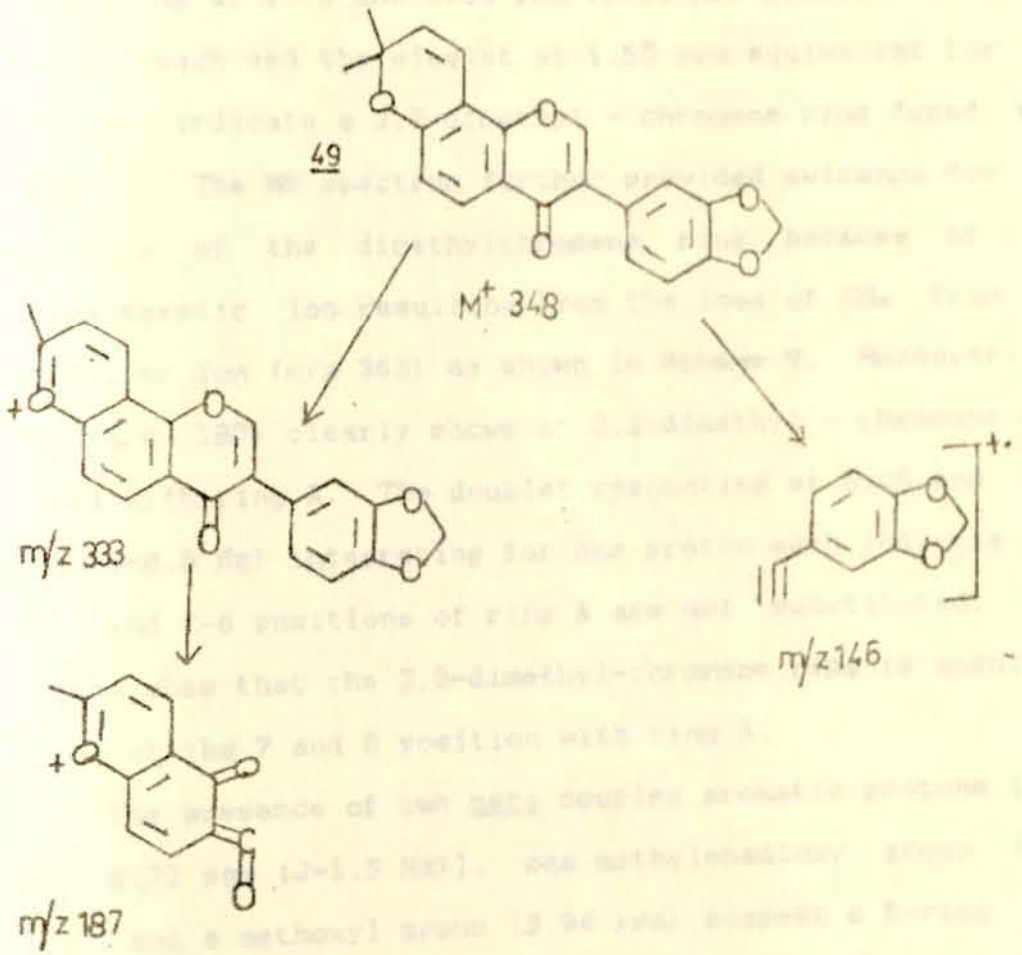
The HRMS analyzes $C_{21}H_{16}O_5$ as the molecular formula for this compound. The ^{1}HNMR spectrum showed a singlet resonating at 7.93 ppm equivalent for one proton. This suggests that the compound is an isoflavone (Table 15). The doublets at 6.81 and 5.72 ppm (J=10 Hz) integrating for one proton each and a singlet at 1.50 ppm equivalent for six protons suggest the presence of a 2,2-dimethyl - chromene ring fused with ring A [5]. Furthermore, the MS provide evidence for the presence of a 2,2-dimethyl - chromene ring fused with ring A from the fragment m/z 187 derived from the $(M-CH_3)^+$ ion as a result of RDA ring fission as shown in **Scheme-8**. The ortho coupled doublets that appear at 8.05 and 6.86 ppm (=8.8 Hz) integrating for one proton each suggest a C-5 and C-6 unsubstituted A-ring. This will place the 2,2-dimethyl - chromene ring at C-7 and C-8 positions fused with ring -A.

A singlet resonating at 5.99 ppm equivalent for two protons indicates a methylenedioxy group. The signals with an ABX pattern resonating at 7.09 (d,J=1.7 Hz) 6.97 (dd,J=8 ,1.7 Hz) and 6.86 ppm (d,j=8 Hz) integrating for one proton each suggest a 3',4' substituted B-ring.

Based on the MS and ^{1}H NMR spectroscpy, structure (49) was assigned for the compound. 49 turned out to be the

known natural product calopogonium isoflavone-B which was isolated from Calopogonium mucconoides [71]. Comparison of data with the literatute was not possible due to lack of access to original source.

Scheme-8 MS spectral fragmentation of calopogoniumisoflovne- B

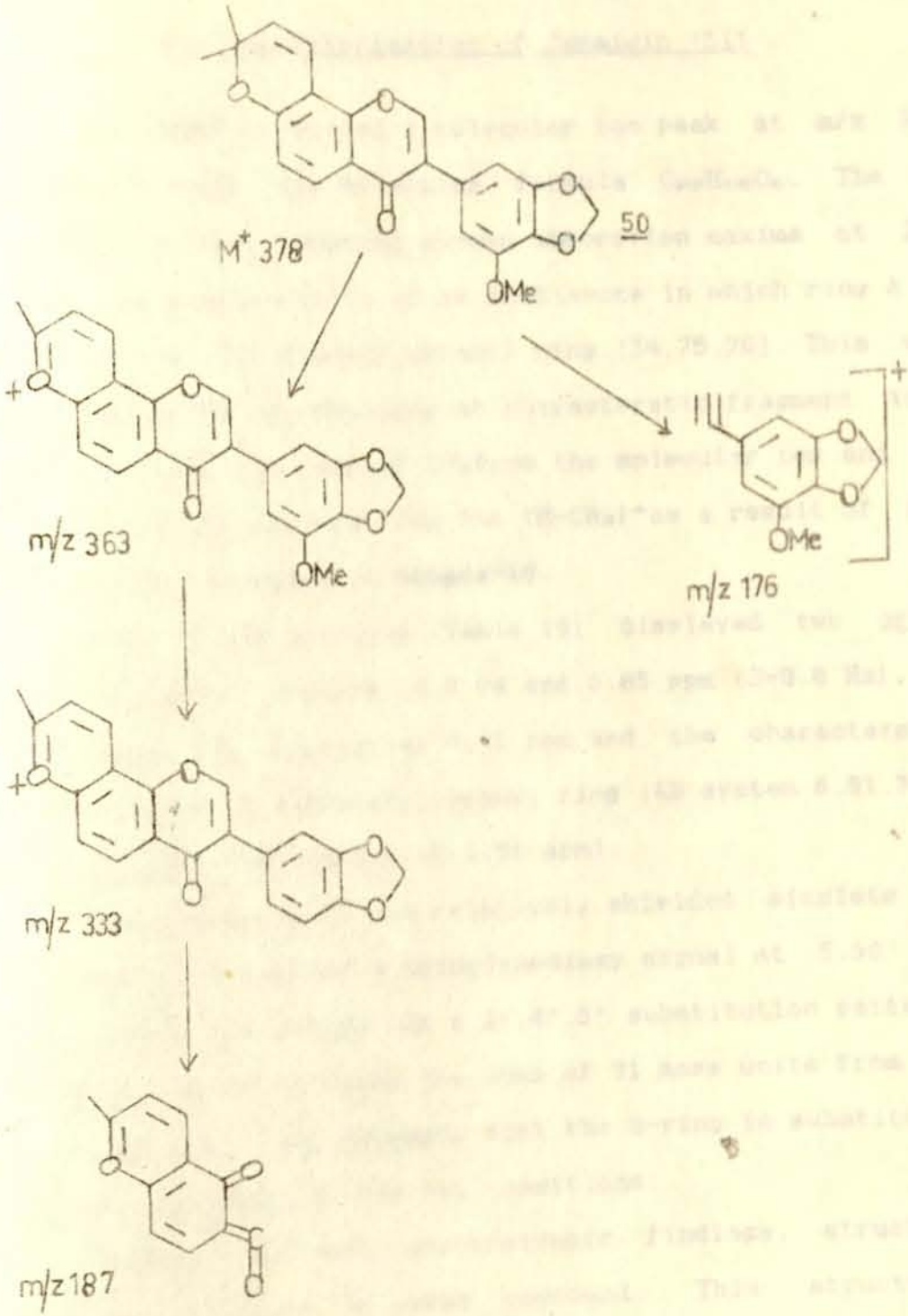


4.5 Characterization of Isojamaicin(50)

The HRMS analyzes for this compound $C_{22}H_{18}O_4$ as its molecular formula. In the ^{1}H-NMR spectrum, the singlet resonating at 7.95 ppm (Table 15) that integrates for one proton is characterstic of an isoflavone (H-2). The doublets resonating at 5.72 and 5.80 ppm (J=10 Hz) equivalent for one proton each and the singlet at 1.50 ppm equivalent for six protons indicate a 2,2-dimethyl - chromene ring fused with ring A. The MS spectrum further provided evidence for the presence of the dimethylchromene ring because of the characterstic ion resulting from the loss of CH_3 from the molecular ion (m/z 363) as shown in **Scheme 9**. Moreover, ion at (m/z 187) clearly shows a 2,2-dimethyl - chromene ring fused with ring A. The doublet resonating at 8.05 and 6.87 ppm (J=8.8 Hz) integrating for one proton each indicate the C-5 and C-6 positions of ring A are not substituted. This establishes that the 2,2-dimethyl-chromene ring is angularly fused at the 7 and 8 position with ring A.

The presence of two meta coupled aromatic protons [6.80 and 6.72 ppm (J=1.5 Hz)], one methylenedioxy group (6.00 ppm) and a methoxyl group (3.94 ppm) suggest a B-ring with 3',4',5'-trioxygenation pattern, and isojamaicin was deduced to be the angularly fused pyranoisoflavone 3'-methoxy-4',5'-methylenedioxy-2'',2''-dimethylpyrano [5'',6'':7,8] isoflavone

Scheme-9 MS spectral fragmentation of isojamaicin



4.6 Characterization of Jamaicin (51)

The HRMS revealed a molecular ion peak at m/z 378 consistent with the molecular formula $C_{22}H_{18}O_6$. The UV spectrum of this compound showed absorption maxima at 228 and 261 nm characterstic of an isoflavone in which ring A is fused with a 2,2-dimethylpyranyl ring [54,75,76]. This was supported by MS observations of characterstic fragment ions resulting from the loss of CH_3 from the molecular ion and an ion at m/z 187 derived from the $(M-CH_3)^+$ as a result of RDA ring fission as shown in **Scheme-10**.

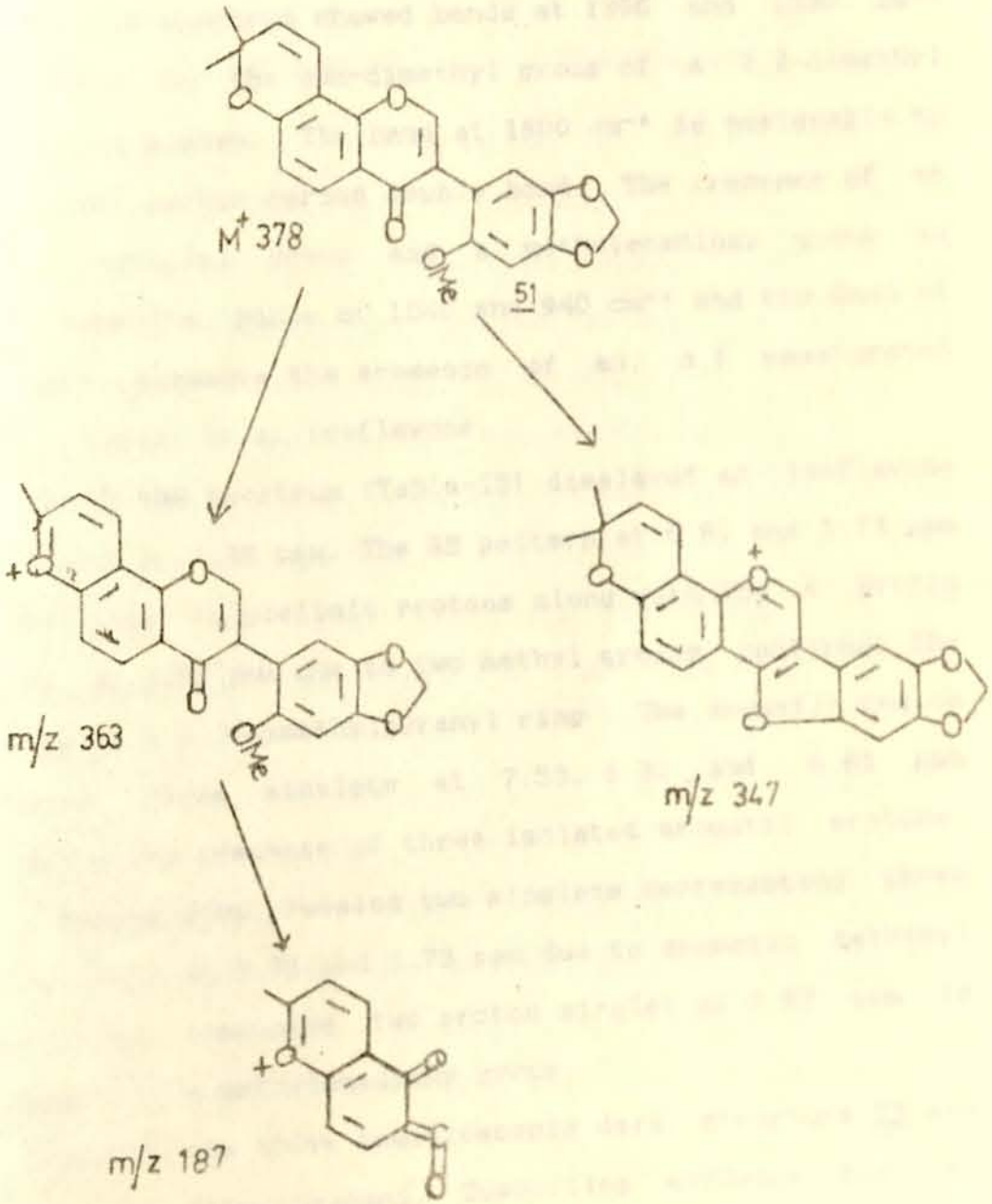
The ^1H NMR spectrum (Table 15) displayed two ortho coupled aromatic protons at 8.04 and 6.85 ppm (J=8.8 Hz), an isoflavone H-2 singlet at 7.91 ppm and the characterstic signals for a 2,2-dimethylpyranyl ring [AB system 6.81,5.71 ppm (J=10 Hz): 6H singlet at 1.50 ppm].

The presence of two relatively shielded singlets at 6.62 and 6.85 ppm and a methylenedioxy signal at 5.96 ppm suggest that the B-ring has a 2',4',5' substitution pattern. The MS spectrum indicates the loss of 31 mass units from the molecular ion. This suggests that the B-ring is substituted by a methoxy group at the C-2' positions.

Based on the above spectroscopic findings, structure (51) was assigned to this compound. This structural assignment was fully supported by the ^{13}C NMR spectrum

(Table-16). (51) is the known natural product jamaicin which was isolated from root bark of Piscidia erythrina [72]. The ^{1}H NMR. MS. IR and UV data are in good agreement with those reported for jamaicin (51).

Scheme-10 MS spectral fragmentation of jamaicin



4.7 Characterization of Ichthynone (52)

The UV spectrum of this compound showed absorption maxima at 229 and 260 nm characterstic of an isoflavone with ring A fused to a 2,2-dimethylpyranyl ring [54,75,76]

The IR spectrum showed bands at 1390 and 1330 cm^{-1} attributable to the gem-dimethyl group of a 2,2-dimethyl pyranyl ring system. The band at 1600 cm^{-1} is assignable to a styrenoid carbon-carbon double bond. The presence of an aromatic methoxyl group and a methylenedioxy group is derived from the bands at 1040 and 940 cm^{-1} and the band at 1640 cm^{-1} suggests the presence of an, α,β unsaturated carbonyl group of an isoflavone.

The ^1H NMR spectrum (Table-15) displayed an isoflavone H-2 singlet at 7.90 ppm. The AB pattern at 6.81 and 5.73 ppm (J=10Hz) due to olefinic protons along with the 6 proton singlet at 1.50 ppm due to two methyl groups confirms the presence of a 2,2-dimethylpyranyl ring . The aromatic region contained three singlets at 7.53, 6.82 and 6.61 ppm indicating the presence of three isolated aromatic protons. The spectrum also revealed two singlets representing three protons each at 3.95 and 3.73 ppm due to aromatic methoxyl groups. The remaining two proton singlet at 5.97 ppm is assignable to a methylenedioxy group.

Based on the above spectroscopic data, structure 52 was assigned to this compound. Supporting evidence for the

structure was provided by the ^{13}C NMR spectrum (Table-16). 52 was found th be the known natural product ichthynone which was isolated from the root bark of Piscidia erythrina [17]. The UV. IR. and ^{1}H NMR data are in good agreement with those reported for ichthynone 52.

4.8 Characterization of Ferrugone (45)

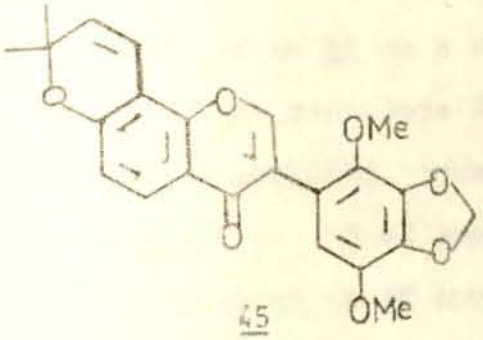
The HRMS revealed a molecular ion peak at m/z 408 consistent with the molecular formula $C_{23}H_{20}O_7$. The UV spectrum of this compound showed absorption maxima at 229 and 260 nm. characterstic of an isoflavne with ring A fused to a 2,2-dimethylpyranyl ring [54,75,76].

The MS (Scheme-11) also provided evidence for the presence of a dimethylpyranyl ring because of the characterstic ion at m/z 393 resulting from the loss of CH_3 from the molecular ion and an ion at m/z 187 derived from the retro Diels-Alder ring fission of rings A and B from the $(M-CH_3)^+$ ion.

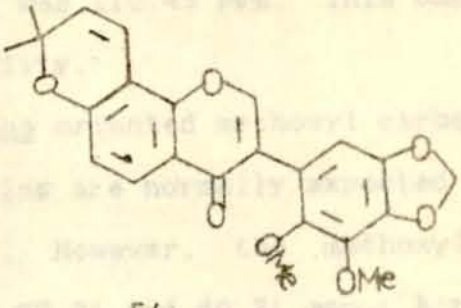
The IR spectrum showed bands at 1390.1375. and 1330 cm^{-1} attributable to the gem-dimethyl group of a 2,2-dimethylpyranyl ring system. The band at 1600.1580 and 1510 suggest an aromatic moiety. The band at 1280.1210.1160 and 1040 cm^{-1} suggest the presence of a phenyl ether linkage while the band at 1640 cm^{-1} is due to an α,β - unsaturated carbonyl group.

The ^{1}H NMR spectrum (Table-15) showed an isoflavone H-2 singlet at 7.91 ppm. The presence of a 2,2-dimethylpyranyl ring was deduced from the AB pattern at 6.81 and 5.73 ppm (J=10 Hz) due to the cis olefinic protons and the 6 proton singlet at 1.50 ppm due to the gem-dimethyl group. The aromatic region of the spectrum revealed one isolated singlet at 6.52 ppm and two ortho coupled doublets at 8.05 and 6.86 ppm (J=8.8 Hz). The spectrum also displayed two aromatic methoxyl groups at 3.87 and 3.85 ppm and a methylenedioxy group at 6.02 ppm.

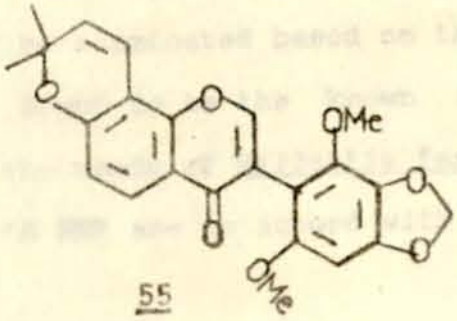
Based on the above spectroscopic data, three alternative structures 45, 54 and 55 can be assigned to the compound.



45



54



55

Structure 45 was finally assigned to this compound based on evidences obtained from ^{13}C NMR spectrum. Particularly helpful in the structural assignment was the comparison made between the ^{13}C NMR spectra of 46 and that of jamaicin (52) (Table-16). The C-3 carbon in jamaicin resonates at 95.55 ppm. If according to structure 56 there were a methoxyl substituent para to the C-3' carbon, one would expect an upfield shift of the C-3'carbon resonance as compared to that of jamaicin. However, the resonance for the unsubstituted carbon in 46 was 110.45 ppm. This observation eliminates 56 as a possibility.

The resonance for ortho oriented methoxyl carbons in a polysubsituted aromatic ring are normally expected in the range of 59-64 ppm [29]. However, the methoxyl carbon resonances in 45 appear at 57.01 and 60.21 ppm. Alternative structure 54, where the methoxyl groups are ortho to each other can thus be eliminated based on the above argument.

45 was found to be the known isoflavone ferrugone isolated from the seeds of Millettia ferruginea [9]. The UV, IR, MS and ^{1}H NMR are in accord with those reported for ferrugone.

Table - 15 ^{1}H NMR Shifts (ppm) of Isoflavones (45, 49-52)

	Calopognium Isoflavone B(49) ($CDCl_3$)	Isojamaicin(50) (C_5D_5N)	Jamaicin(51) ($CDCl_3$)	Ichthynone(52) ($CDCl_3$)	Ferrugone(45) ($CHCl_3$)
	7.90(s)	7.95(s)	7.91(s)	7.90(s)	7.91(s)
5	8.05(d,J=8.8H_z)	8.06(d,J=8.8H_z)	8.04(d,J=8.8H_z)	7.53(s)	8.05(d,J=8.8H_z
6	6.86(d,J=8.8H_z)	6.87(d,J=8.8H_z)	6.86(d,J=9H_z)	-	6.86(d,J=8.8H_z)
	7.39(d,J=1.7H_z)	8.80(d,J=1.8H_z)	-	-	-
3'	-	-	6.67(s)	6.61(s)	-
5'	6.86(d,J=8, H_z)	-	-	-	-
6'	6.97(dd,J=8,1.7H_z)	6.72(d,J=1.8H_z)	6.83(s)	6.82(s)	6.52(s)
3	5.72(d,J=10H_z)	5.71(d,J=H_z)	5.71(d,J=10H_z)	5.72(d,J=10.2H_z)	5.73(d,J=10H_z)
4	6.81(d,J=10H_z)	6.80(d,J=10H_z)	6.81(d,J=8H_z)	6.81(d,J=10.2H_z)	6.91(d,J=10H_z)
OCH_2O-	5.99(s)	6.00(s)	5.96(s)	5.97(s)	6.02(s)
OMe	-	3.94(s)	3.73(s)	3.95(s)	3.87(s)
OMe	-	-	-	3.73(s)	3.85(s)
CH_3-C-	1.50(s)	1.50(s)	1.50(s)	1.50(s)	1.50(s)

Scheme-11 MS spectral fragmentation of ferrugone

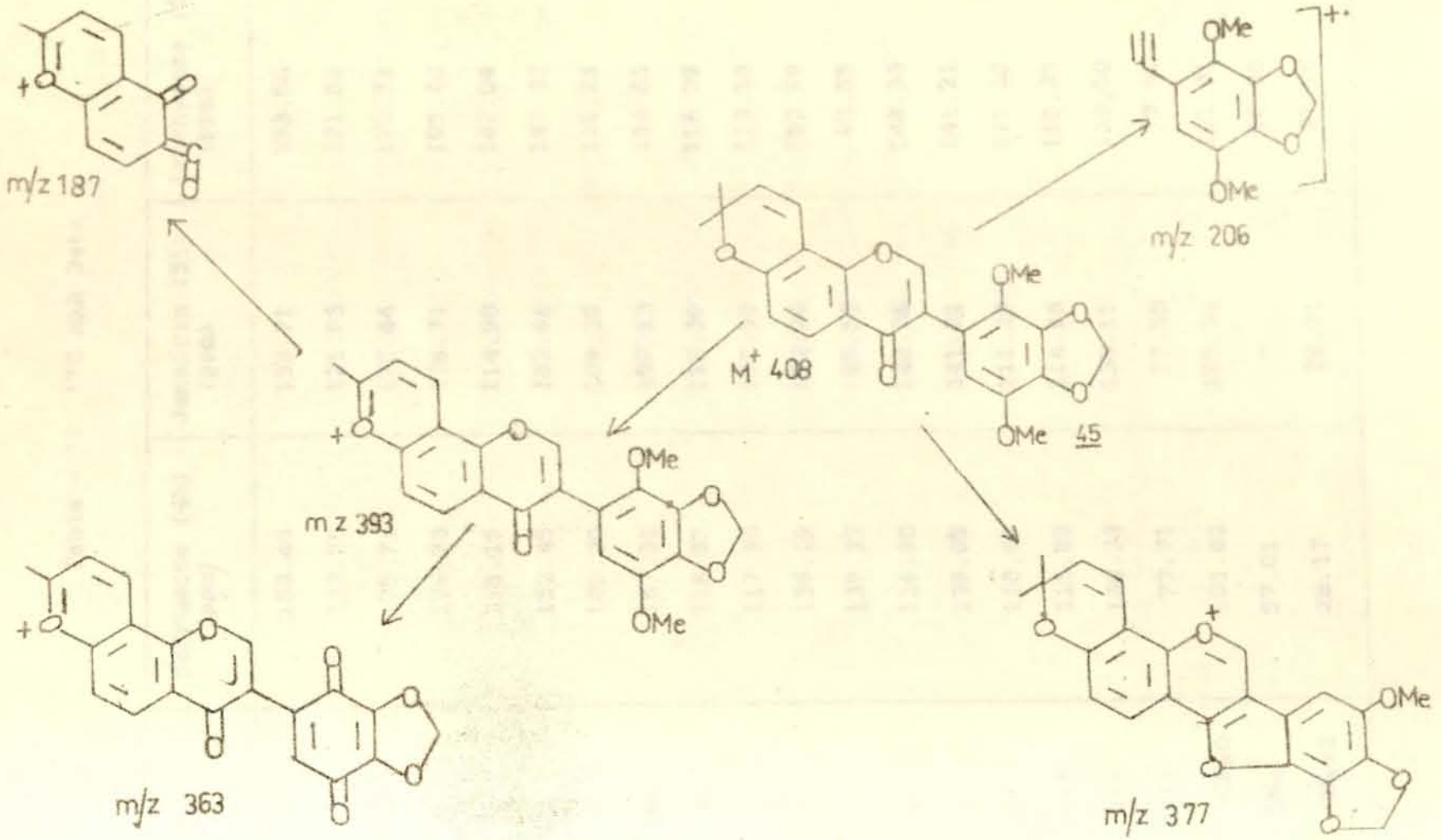


Table - 16 ^{13}C NMR Data

Carbon	Ferrugone (45) (ppm)	Jamaicin (51) (ppm)	Ichthynone (52) (ppm)
2	153.40	153.71	153.50
3	122.08	121.93	121.52
4	175.71	175.64	175.32
5	126.73	126.71	105.40
6	115.15	114.98	147.04
7	152.45	152.96	147.37
8	109.30	109.20	110.25
8a	157.31	157.13	154.00
4a	118.37	118.39	118.39
1'	117.98	112.90	113.18
2'	139.19	152.96	152.99
3'	137.22	95.53	95.55
4'	136.90	148.36	148.33
5'	139.05	141.21	141.21
6'	110.45	111.20	111.20
3''	115.03	114.98	115.25
4''	130.29	130.15	130.50
2''	77.71	77.58	77.99
OCH_2O	101.82	101.30	101.31
OMe	57.01	-	56.30
CH_3x2	28.17	28.01	21.95

4.9 Characterization of 5-methoxy durmillone

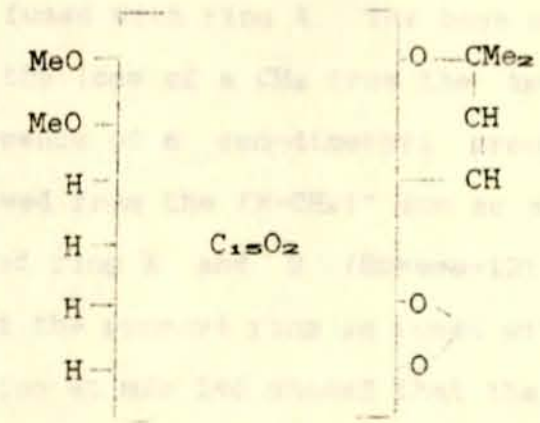
Table - 17 Summary of Physiochemical data on the novel Compound

1. TLC	Single spot, R_f 0.64. Solvent: Tol: Hex: EtOAc (3:4:3)
2. M.P.	142-143°C
3. Colour reaction	1% alcoholic $FeCl_3$(-Ve)
4. UV λ_{max}^{MeOH} nm	224,263,294sh.320sh remain unchanged by addition of NaOMe
5. IR ν_{max}^{KBr} cm^{-1}	1660,1630,1530,1510.1425.1420. 1375.1360.1290.1180.1075
6. MS	$C_{23}H_{22}O_7$(M^+=408).$C_{21}H_{14}O_6$(11%) $C_{20}H_{19}O_7$(100%).$C_{20}H_{13}O_6$(12%) $C_{11}H_5O_5$(1%). $C_9H_8O_5$(11%) $C_9H_6O_2$(100%).
7. NMR	see text

The HRMS spectrum showed a molecular ion peak at m/z 408 constitent with the molecular formula $C_{23}H_{22}O_7$. The presence of a carbonyl group is derived from the absorption band at 1660 cm^{-1} in the IR spectrum. Based on biogenetic grounds. the molecular formula $C_{21}H_{14}O_5(OMe)_2$ is possibly

compatible with the presence of a $C_{15}O_2$ nucleus of a flavonoid or an isoflavonoid in association with an isoprenoid C_5-residue.

The absence of an absorption band above 3000 cm^{-1} in the IR spectrum and the fact that the UV spectrum was unchanged by base suggests the absence of phenolic hydroxyxl group in 5-methoxy durimillone. The UV spectrum of this compound showed absorption maxima at 224 and 263 nm. characterstic of an isoflavone with ring A fused to a 2.2-dimethylpyranyl ring [54,75,76]. This led to the consideration of the partial formula 56 for this compound which immediately indicated that the remaining carbon atom in the structure of 5-methoxy durmillone might well be present as a methylenedioxy group.



56

The nature of the groups present in the structure of 5-methoxy durmillone are indicated by its ^1H NMR spectrum (Table-18). The high field singlets at 3.90 and 3.96 ppm integrating for three protons each can be assigned to two

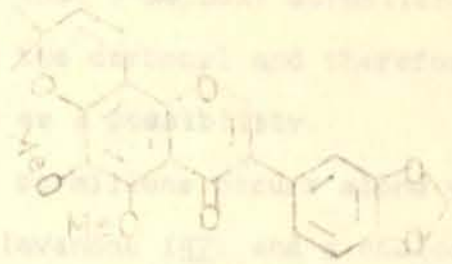
methoxy groups. A singlet at 5.98 ppm integrating for two protons suugests the presence of one methylenedioxy group. Moreover, the two doublets at 5.68 and 6.76 ppm (J=10 Hz) integrating for one proton each and the singlet at 1.55 ppm equivalent to six protons are characterstic of the cis-olefinic and gem-dimethylpyranyl ring [73]. The low field singlet at 7.81 ppm is characterstic of an isoflavone H-2.

The IR spectrum further showed some characterstic features of a 2.2-dimethylpyranyl system. The bands at 1375 and 1360 cm^{-1} are attributable to the gem-dimethyl group and the bands at 1600, 1425 and 1420 cm^{-1} are expected for styrencid carbon-carbon double bond. The bands at 1290, 1180 and 1075 cm^{-1} suggest the presence of an aromatic ether.

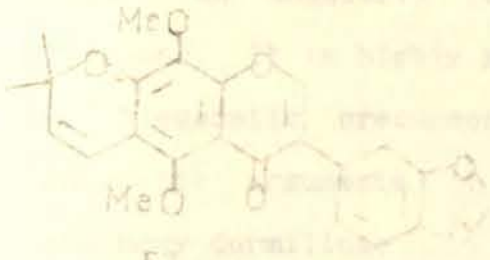
The MS provide further evidence for the presence of a dimethyl pyranylring fused with ring A. The base peak at m/z 393 originates by the loss of a CH_3 from the molecular ion indicating the presence of a gem-dimethyl group. The peak at m/z 217 is derived from the $(M-CH_3)^+$ ion as a result of RDA ring fission of ring A and B (**Scheme-12**). This clearly established that the pyranyl ring is fused with ring A. Further more, the ion at m/z 146 showed that the B-ring is only substituted by a methylenedioxy group. This was supported by the ^1H NMR which showed an aromatic ABX system at 6.93 (dd,J=8.1.6 Hz), 6.85(d,J=8 Hz) and 7.08(d,J=1.7 Hz) ppm.

Based on the MS and ^1H NMR data, it can be deduced that the A-ring of 5- methoxy durmillone is completely

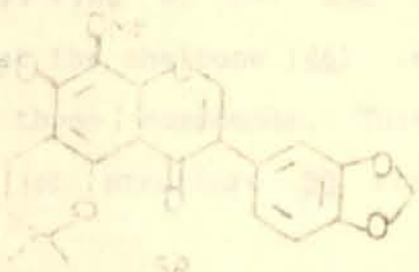
substituted [illegible] Thus leaves three options
open of which one has a 6,7-oxygenation pattern 53 and the
other two 5,7-oxygenation patterns [illegible] and 52.



53

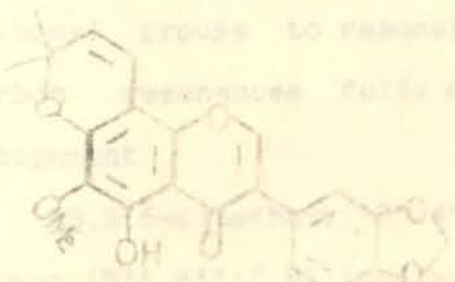


57

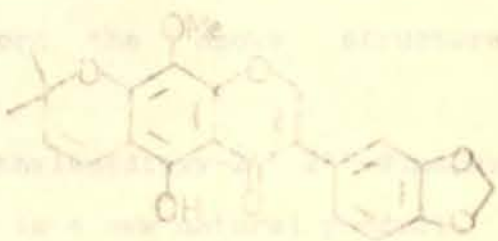


58

5-methoxy derivative was done using 48% HBr.
The ^1H NMR spectra of the product 59 or [illegible] showed the
disappearance of one of the methoxyl groups and the
appearance of a chelated hydroxyl (Table 3).



59



[illegible]

This suggests that 5-methoxy durmillone contains a methoxyl group peri to the carbonyl and therefore structure 58 could be eliminated as a possibility.

5-methoxy durmillone occurs along with other isoflavones (49-52), a flavanone (47) and a chalcone (46) in the steam-bark of this plant. All the isoflavones and the flavanone contain an angularly fused pyranyl ring at C-7 and 8 positions. It is highly probable that the chalcone (46) is the biogenetic precursor for all these compounds. This biogenetic arguments is in favour of structure 53 for 5-methoxy durmillone.

Further support in favour of structure 53 for 5-methoxy durmillone was obtained from the ^{13}C NMR spectrum (Table-19). The methoxyl carbon resonances appear at 61.33 and 62.10 ppm. Such resonance are expected for Ortho oriented methoxyl groups [29]. If the methoxyl groups were para oriented as in structure 57, one would not expect both methoxyl groups to resonate above 59 ppm. The remaining carbon resonances fully support the above structural assignment.

53,5,6-dimethoxyl-3',4'-methylenedioxy-2'',2''-dimethyl pyrano [5'',6'':7,8] isoflavone is a new natural product.

MS spectral fragmentation of 5-methoxy durmillone

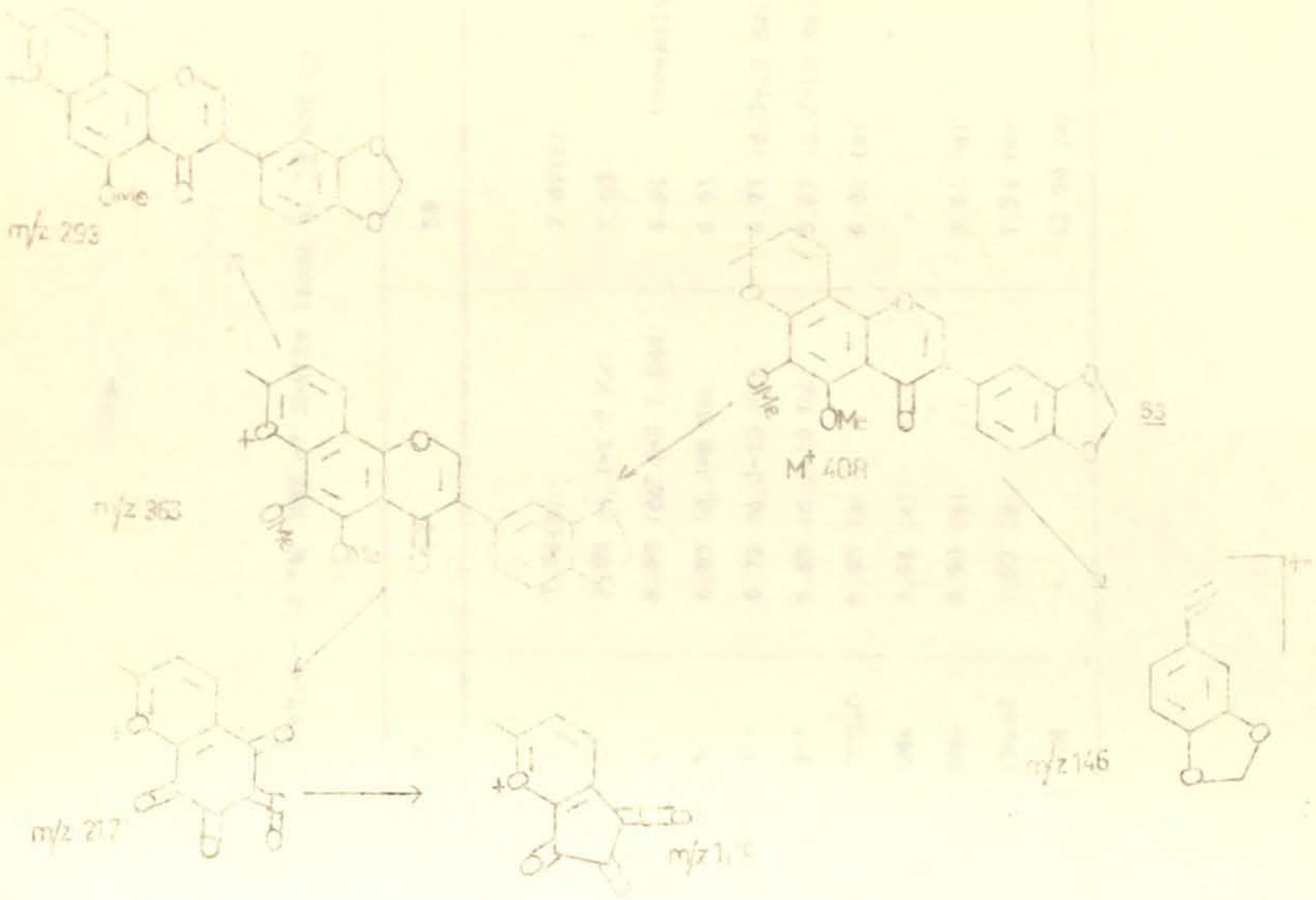


Table - 18 ^1H NMR of Shifts (ppm) of 53 and 59

H	53	59
2	7.81(s)	7.89(s)
2'	7.08 (d,J=1.7 Hz)	7.03
6'	6.93 (dd,J=8.1.6Hz)	6.91 Unresolved
5'	6.85 (d,J=8 Hz)	6.91
4''	6.76 (d,J=10 Hz)	6.71 (d,J=10 Hz)
3''	5.68 (d,J=10 Hz)	5.62 (d,J=10 Hz)
OCH_2O	5.93 (s)	6.00 (s)
OMe	3.96 (s)	-
OMe	3.90 (s)	3.91 (s)
CH_3x2	1.55 (s)	1.54 (s)
-OH	-	12.98 (s)

Table - 19 ^{13}C NMR of 5-methoxy durmillone

Carbon	C-Shift in (ppm)
2	150.21
3	125.30
4	175.02
5	151.01
6	139.97
7	149.05
8	113.10
8a	153.01
4a	106.54
1'	125.64
2'	109.96
3'	147.50
4'	147.50
5'	108.17
6'	122.46
3''	114.94
4''	129.02
6-OCH_3	62.10
5-OCH_3	61.33
OCH_2O	100.00
CH_3x2	28.01

5. Experimental

5.1 Plant Material

The stem-bark of Millettia ferruginea (Hochst) Bak. used in this study was collected from three different localities, in June and December from Sidamo (Aletawondo) in September 1987 from Addis Ababa and in February 1983 from Gondar. According to M.Thulin [1] the samples collected from Sidamo is believed to be the subsp darassana. whereas the sample from Gondar is the subsp ferruginea and the sample from Addis Ababa is most likely the hybrid of the two forms.

5.2 Material and Apparatus

Melting points were determined using Thomas Hoover capillary melting point apparatus. UV spectera were recorded on Perkin-Elmer model 555 UV spectrometer. IR on Perkin-Elmer model 727 Infra red spectrometer, ^{1}H NMR: 90, 250 and 360 MHz instruments in $CDCl_3$ with tetramethylsilane as an internal standard. ^{13}C NMR were recorded on 22.5 and 90 MHz instrument in $CDCl_3$. The mass spectra were recorded on a high resolution mass spectrometer. Analytical thin layer chromatography (TLC) was run on a 0.2 mm thick layer silica gel and the spots were detected by their UV flourescence and by spraying with 0.5% fast blue salt B solution followed by 0.1 N NaOH [78]. Circular preparative layer chromatography was run on a 1 mm silica gel. while preparative thin layer chromatography on a 1 mm thick silica gel and bands were

detected by their UV fluorescence. Column chromatography was done on silica gel 60 (70-230 mesh) and sephadex LH-20. The pet. ether used through out the study had B.p (60-80)°C.

Table-20 Solvent Systems 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	Pet. ether: EtOAc	95:5
4	Pet. ether: EtOAc	90:10
5	Pet. ether: EtOAc	85:15
6	Pet. ether: EtOAc	80:20
7	Pet. ether: EtOAc	75:25
8	Pet. ether: EtOAc	70:30

5.3 Extraction, Fractionation and Isolation

5.3.1 Millettia ferruginea (bark

400 g of grounded bark of M.ferruginea collected from Addis Ababa was first extracted with cold per.ether followed by chloroform on a Soxhlet apparatus yielding 3 and 10 g of yellowish oily products respectively.

a) The crude pet-ether extract was applied on Sephadex LH-20 column (70g) and eluted with solvent system 2. A total of 30 fractions each 7 ml were collected. The first 20 fractions contain fatty material. while the remaining fractions were combined and concentrated yielding 10 mg that showed by TLC the presence of four compounds. The separation of the mixture was made by circular preparative TLC using solvent system 1. This resulted in 3 mg of compound-B. 1.5 mg compound-C. 3 mg compound-F and 1 mg compound-H.

b) 5 g of the crude chloroform extract was applied on Sephadex. LH-20 (70g) and eluted with solvent system 2. A total of 45 fractions 7 ml each were collected. The first 15 fractions were found to contain chlorophyll and fatty material and were not examined further. The remaining fractions were combined and concentrated. The above mentioned procedure was repeated with another 5 g of crude

chloroform extract and combined yielding 5 g of a yellowish sticky substance. 6 g of the residue was applied on a silica gel column (250 g). It was eluted with increasing polarities of pet.ether and EtOAc mixtures. A total of 25 fractions each 100 ml were collected.

Fractions (3-7), eluting with solvent system 3 gave 120 mg of an orange colured copmpound-F which was purified by column filteration and crystallization from methanol (MP 189-192°C).

Fractions (8-12), eluting with solvent system 4, gave 40 mg of compound-D which crystallizes from methanol (159-160°C). Recrystallization of compound-D (40 mg) from methanol raised its melting point (MP 189-191°C).

Fractions (13-18), eluting with solvent system 5, resulted in a mixture of compound-D and E. Repeated chromatography of the mixture gave 45 mg of compound-E that crystallizes in methanol (MP 164-166°C).

Fractions (19-25), eluting with solvent system 5 gave a mixture containing one compound as a major and two other in trace amounts. crystallization of the mixture from methanol resulted in compound-G as a colourless needle (MP 142-143°C).

5.3.2 Millettia dorassana(bark)

5 kg of grounded bark of M.dorassana was socked in 8 liters of ethanol for 15 days. The extract was filtered and upon removal of the solvent under reduced pressure 149 g of a dark yellow sticky, substance was obtained. The residue was fractionated with chloroform and methanol. resulting in 90 and 50 g of extracts respectively. The methanol soluble portion contained highly polar substances and was not examined further.

A 22 g portion of the chloroform extract was applied on a silica gel column (600 g). It was thoroughly washed with pet.ether to remove the fatty material. It was then eluted with increasing polarities of pet. ether ans EtOAc mixtures. A total of 120 fractions each 200 ml were collected.

Fractions (1-59). eluting with solvent system 3 and 4 contained only fatty substances and were not examined further.

Fractions (60-63). eluting with solvent system 5 gave a yellowish oily and upon addition of methanol resulted a precipitats. The filterate afforded 50 mg of a colourless compound-A (MP 174-175°C).

Fractions (64-79). eluting with solvent 6 gave 400 mg of a mixture of compound-D and E.

Fractions (80-99), eluting with solvent system 7 gave 1.5 g of compound-G as a major compound 1 which crystallizes from methanol as a colourless needles (MP 142-143°C).

Fractions (100-120), eluting with solvent system 8 resulted in compound-I, J and chlorophyll. The mixture was chromatographed on Sephadex LH-20 using solvent system 2.

A total of 10 fractions each 25 ml were collected. The first five fractions contain chlorophyll and the remaining fractions were combined. After removal of the solvent, the residue was applied on a preparative TLC using solvent system 1. This resulted in 40 mg of compound-I (MP 201-202°C) and 10 mg of compound-J (MP 230-231°C) crystallizing from methanol and acetone respectively.

5.4 Demethylation of Compound-G (79,80)

In a 50 ml round bottom flask containing compound-G (50 mg), a 10% excess of 48% hydrobromic acid (23 ml) was added. The reaction mixture was refluxed for 3 hours, untill the evolution of methyl bromide ceased. The excess hydrobromic acid was removed under reduced pressure. The demethylated product was then purified by chromatography on silica-gel (10 g) eluting with solvent system 4, to give 6 mg of a yellowish crystalline compound, MP 205-207°C (MeOH).

5.5 Identification of Compounds

Compound-A (Flemichapparin-B) (48) R.f 0.93 (solvent system 1) needles from MeOH. M.P (174-174°C)(lit(70) 179-180°C.

Found: $[M]^+$ 296.0690; $C_{17}H_{12}O_5$ requires: 296.0685

$UV^{MeOH}_{\lambda max}$ nm = 230 int, 250.292.339.358.

$IR^{KBr}_{\nu max}$ cm^{-1}: 1660,1570.1470,1230

^1H NMR ($CDCl_3$) 7.37 (1H,d,J = 8.2 Hz,H-1), 7.02(1H,S,H-7), 6.72(1H,S,H-10), 6.53 (2H,M,H-2,4), 6.00 (2H,S,O-CH_2-O), 5.52(2H,S,H-6),3.81(3H,S,3-OMe)

EIMS m/z (rel.int): 296[M]$^+$(100), 295(60.8), 281(9.7)

^{13}C NMR (see text).

Compound-B (Calopogoniumisoflavone-B) (49)

R.f 0.84 (solvent system 1)

Found [M]$^+$ 348.1103: $C_{21}H_{16}O_5$ requires: 348.098

^1H NMR ($CDCl_3$) see text.

EIMS m/z (rel.int.) 348[M]$^+$(98.7), 33(100).187(45.3).

Compound-C (Isojamaicin) (50)

R.f 0.77 (solvent system 1)

Found [M]$^+$ 378.114: $C_{22}H_{18}O_6$ requires: 378.1103

^1H NMR see text.

EIMS m/z (rel.int.) 378[M]$^+$(66), 363(100),333(25).206(35).

187(35).

Compound-D (Jamaicin) (51)

R.f 0.70 (solvent system 1)

Colourless needles from MeOH MP(189-192°C)(lit[72]193-194°C)

Found [M]$^+$ 378.112; $C_{22}H_{18}O_6$ requires: 378.1103

$UV^{MeOH}_{\lambda max}$ nm = 226. 261.308

$IR^{KBr}_{\nu max}$ cm^{-1}: 1640,1600.1580.1510.1500.1430.1400.1380.1330

EIMS m/z (rel.int.) 378[M]$^+$(100).363(62).347(44). 187(12.0)

Compound-E (Ferrugone) (45)

R.f 0.69 (solvent system 1)

Colourless needles from MeOH MP(164-166°C)

Found [M]$^+$ 408.1220: $C_{23}H_{20}O_7$ requires: 408.1209.

$UV^{MeOH}_{\lambda max}$ nm 229.260.310 sh. 320 sh

$IR^{KBr}_{\nu max}$ cm^{-1}: 1640.1610.1590.1510.1475.1440.1405.1390.1375
1360.1320.1280.1240.1205.1160.1110.1140.

^1H, ^{13}C NMR see text

EIMS m/z (rel.int.) 408[M]$^+$(100).393(98).377(30). 363(16)
206(87).187(13.6).

Compound-F (4-hydroxylonchocarpin) (47)

R.f 0.68 (solvent system 1)

Orange needles from MeOH MP(189-192°C)

Found [M]$^+$ 322.1210: $C_{20}H_{18}O_4$ requires: 322.1205.

$UV^{MeOH}_{\lambda max}$ nm 226,272.305 sh, 368

$UV^{MeOH+NaOMe}_{\lambda max}$ nm 226 sh, 270.432

$IR^{KBr}_{\nu max}$ cm^{-1}: 3250.1640,1620.1590.1520.1490.,1380,1330

^1H, ^{13}C NMR ($(CO_3)_2CO$) 14.07(1H,S.2'-OH),8.06(1H.d.J=9 HZ.
6'-H),7.86(1H.d.J=15.4 HZ.-H.
7.77(1H.d.J=15.4 HZ. -H).7.74(2H.d
,J=8.5 Hz, H-2.6),6.92(2H.dJ=8.5 Hz
H-3.5),6.69(1H.d,J=10 HZ. H-4''),
6.36(1H.d,J=8.8 HZ,H-5'),
5.71(1H.d.J=10 HZ,H-3''), 1.44(6H.S
2''-CH_3).

^{13}C NMR see text.

EIMS m/z (rel.int.) 322[M]$^+$(53),307(67).187(100).

Compound G (5-methoxy durmillone) (53)

See text

Compound-H (4'Hydroxyisolonchecarpin). (47)

R.f. 0.62 (solvent system-1)

Found [M]$^+$ 322.1128: $C_{20}H_{18}O_4$ requires: 322.1205

^1H NMR ($CDCl_3$) S 7.74(1H,d,J=8.5 HZ.H-5).7.36(2H.d.J=8.3 HZ.
H-2',6') ,6.89(2H.d.J=8.8 Hz. H-3',5').

6.62(1H.d.J=10 Hz. H-4''),6.49(1H.d.
J=8.8.Hz.H-6).5.56(1H.d.J=10.3 HZ.H-3'',
5.40(1H.dd.J=13.1,3.1 HZ.H-2).3.01(1H,
dd.J=16.8.13.0 HZ.H-3_{ax}).2.80(1H.dd,
J=16.8.3.2 Hz.H-3_{eq}).1.47(3H.S.2''-CH_3)
.144(3H.S,2''-CH_3).

EIMS. m/z (rel.int.) 322[M]$^+$(67.1),307(86.6),187(100).
120(36.5).

Compound-I (Ichthynone) (53)

R.f 0.44 (solvent system 1)

Colourless needle from MeOH MP(201-202°C)(lit[73]203-204°C)

$UV^{MeOH}_{\lambda max}$ nm 229.260.306.330.344 sh

$IR^{KBr}_{\nu max}$ cm^{-1}: 1640,1600.1580.1540,1390.1330,1280.1200.1110.
1040

^1H, ^{13}C NMR see text.

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DECLARATION

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

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