heptamethyl ether (7) were studied for solutions containing increasing concentrations of perdeuteriobenzene in deuteriochloroform. All the seven methoxy signals were moved upfield (44, 49, 54, 55, 58, 61 and 68 Hz) as with cupressuflavone hexamethyl ether [8] and amentoflavone hexamethyl ether [9] indicating that each methoxyl group has at least one free *ortho*-position. Hence SA5 heptamethyl ether must have a C–C linkage at the B-3′-D-8″ position. For a B-3′-D-6″ linkage, the D-5″ methoxy group, with two adjacent substituents, should not undergo any solvent induced shift on addition of perdeuteriobenzene [10–12].

The above observation of SA5 heptamethyl ether with regard to the biphenyl linkage has thus given an unambiguous proof that the interflavonoid linkage in the parent compound, jeediflavanone, must be at the B-3′-D-8″ position as originally postulated [1] and not at the B-3′-D-6″ position.

From the foregoing chemical and spectral studies, the assignment of structure **5** for the biflavone SA5 is taken as confirmative evidence for the structure of jeediflavanone (**1**).

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FLEMIPHYLLIN, AN ISOFLAVONE FROM STEMS OF *FLEMINGIA MACROPHYLLA*

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Key Word Index—*Flemingia macrophylla*; Leguminosae; stems; isoflavone; 5,7,4′-trihydroxy-3′,5′,8-tri(3-methylbut-2-enyl) isoflavone; flemiphyllin.

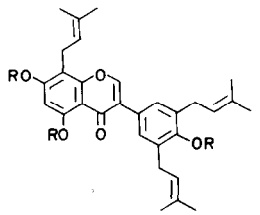
Abstract—A new isoflavone, designated flemiphyllin, was isolated from a petrol extract of the stems of *Flemingia macrophylla*. Its structure was established as 5,7,4′-trihydroxy-3′,5′,8-tri(3-methylbut-2-enyl) isoflavone on the basis of physical and chemical evidence.

INTRODUCTION

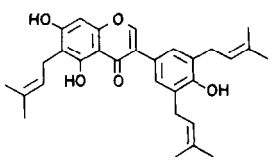
Earlier we have communicated the isolation and structural elucidation of fleminone a new flavanone from the petrol extract of the stems of *Flemingia macrophylla* [1]. The petrol–benzene (1 : 1) elutions (fractions 34–60) from the main column chromatography [1] on purification furnished a product, designated as flemiphyllin. The present paper deals with the structure determination of this compound.

RESULTS AND DISCUSSION

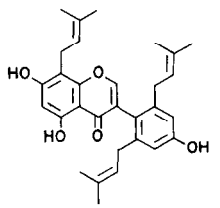
Flemiphyllin (**1**), mp 172°, $C_{30}H_{34}O_5$, $[M]^+$ 474 gave a green colour with alcoholic ferric chloride solution indicating the presence of a chelated hydroxyl group. It is also soluble in alkali indicating its phenolic nature. It did not give a Shinoda test [2] indicating that it is not a flavone or a flavanone. The IR spectrum [ν_{KBr}^{max} 1625 cm^{-1} (C=O) and ν_{KBr}^{max} 3300 cm^{-1} (OH)] and a low field singlet at δ 7.72 in ^{1}H NMR spectrum (100 MHz, recorded in



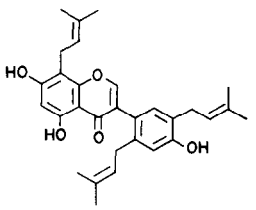
1 R=H
2 R=COMe



3



4



5

$CDCl_3$) indicated the isoflavone nature [3] of **1**. The UV data [λ_{max}^{MeOH} 268 nm (log ε 4.55)] is similar to that of 5,7,4′-trihydroxy isoflavone, genestein [3]. The UV absorption maximum (λ_{max}^{MeOH} 268 nm) suffered a bathochromic shift by 8 nm in the presence of sodium acetate suggesting the presence of a 7-hydroxyl of isoflavones [3]. Furthermore the absorption maximum also suffered a bathochromic shift by 13 nm in aluminium chloride–hydrochloric acid which is characteristic of the 5-hydroxyl of isoflavones [3].

Flemiphyllin formed a triacetate (**2**), mp 117–118°, $C_{36}H_{40}O_8$. Its ^{1}H NMR spectrum (100 MHz, $CDCl_3$) revealed the presence of an acetoxyl group at a relatively low field, (δ 2.46, 3H) characteristic of a 5-*O*-acetyl group [4], and the other two acetoxyl signals at (δ 2.32, 3H) and (δ 2.38, 3H) are characteristic of 4′-*O*-acetyl and 7-*O*-acetyl groups, respectively [4]. Therefore the presence of three phenolic hydroxyls is indicated in **1**.

The ^{1}H NMR spectrum of **1** revealed a set of peaks [δ 3.37, *m*, 6H, 3- × C–$\underline{CH_2}$; δ 5.25, *m*, 3H, 3 × =$\underline{CH}$ and δ 1.8, *m*, 18H, 3 × =C$\begin{matrix}\diagup\underline{Me}\\ \diagdown\underline{Me}\end{matrix}$] suggesting the presence of three C-3-methylbut-2-enyl groups [5]. The spectrum also revealed the presence of two proton singlets at δ 7.1 which may be assignable to H-2′ and H-6′. It may be mentioned that in 3′,4′,5′-trisubstituted flavonoids, if 3′ and 5′ are substituted with identical groups, the H-2′ and H-6′ are equivalent and appear as a two proton singlet [6, 7]. In sophoranone where the 4′-OH-3′,5′-di-(3-methylbut-2-enyl) system is present, the H-2′ and H-6′ protons appear as a two proton singlet [7]. A relatively up field aromatic proton at (δ 6.24, *s*, 1H) is characteristic of H-6 rather than H-8. For example it was observed that H-6 in 5,7,8-trisubstituted flavonoids reasonate up field in comparison to that of H-8 in isomeric 5,6,7-trisubstituted flavonoids [8, 9]. To be specific the H-6 of vitexin resonates upfield at δ 6.31 while H-8 in isomeric sapona-retin resonates at δ 6.58 [8]. A chelated phenolic hydroxyl at δ 10.92 (1H, C-5-OH) and two phenolic hydroxyls at δ 5.5 (2H, *br*, 2 × OH) were also observed in the ^{1}H NMR spectrum.

In the ^{1}H NMR spectrum of the triacetate, in addition to the three acetoxyl signals, the spectrum also revealed three C-3-methylbut-2-enyl groups [δ 3.26, *m*, 6H 3 × =C–$\underline{CH_2}$; δ 5.2, *m*, 3H, 3 × =$\underline{CH}$; δ 1.74, *m*, 18H, 3 × =C$\begin{matrix}\diagup\underline{Me}\\ \diagdown\underline{Me}\end{matrix}$] [5], a low field singlet (δ 7.77, *s*, 1H, H-2) and a two proton aromatic singlet (δ 7.12, *s*, 2H, H-2′ and H-6′) and one single aromatic singlet (δ 7.18, *s*, 1H, C_6-H). The two proton singlet due to H-2′ and H-6′ in **1** suffered a negligible down field shift (δ 0.02) in its triacetate (**2**) suggesting these protons are *meta* to a hydroxyl [10]. On the other hand the one proton singlet due to H-6 shifted considerably down field, δ 0.94 in **2** indicating that the proton is *ortho* and/or *para* to a hydroxyl [3, 4, 6, 10]. These results suggest the structure **1** for flemiphyllin although structure **3** could not be ruled out.

Flemiphyllin gave a negative Gibb's test [11] suggesting that the *para* position to the 5-hydroxyl (i.e. C-8) is substituted. Therefore one C-3-methylbut-2-enyl group is placed at C-8.

In the mass spectrum of **1**, the $[M]^+$ at *m/z* 474 is the base peak. The prominent ions due to $[M-C_4H_7]^+$ (92%) to give a stable *ortho* quininoid oxonium ion, $[M-C_4H_7-C_4H_8]^+$ (45%) and $[M-C_3H_7]^+$ (50%) which all are characteristic fragments due to the *O*-hydroxy-C-3-methylbut-2-enyl system [12–14] are observed. Furthermore the spectrum revealed RDA ions [*m/z* 220 (5%) and *m/z* 254 (4%)] and $[M-1]^+$ (8%) which are characteristic of the isoflavone skeleton [14]. The prominent ions at *m/z* 165 (45%) and *m/z* 229 (7%) further support the placement of one C-3-methylbut-2-enyl group on a benzenoid ring and two C-3-methylbut-2-enyl groups on the side phenyl ring at the 3′,5′-position.

All natural isoflavones invariably are 4′-oxygenated on biogenetic considerations [15]. Further natural phenolic compounds invariably possess C-3-methylbut-2-enyl groups *ortho* to the phenolic hydroxyl again on biogenetic considerations [16]. Therefore, alternative structures **4** and **5** in the side phenyl ring are not tenable. Other natural flavonoids like sophoradin and sophoranone [7, 17] possess two C-3-methylbut-2-enyl groups on either side of the 4′-hydroxyl of the side phenyl ring.

Thus based on chemical, spectral and biogenetic considerations 5,7,4′-trihydroxy-8,3′,5′-tri(3-methylbut-2-enyl) isoflavone structure was assigned to flemiphyllin.

EXPERIMENTAL

Fractions 34–60 eluted with petrol–C_6H_6 (1:1) (obtained during the isolation of the flavanone, fleminone) [1] on concn under red. pres. furnished a light reddish yellow semi-solid (1.2 g). The semi-solid material was taken up in EtOH (150 ml), shaken well and centrifuged. The residue was further washed with (2 × 25 ml) cold EtOH when a colourless solid (0.5 g) was obtained. It was found to be aliphatic in nature.

The EtOH soln (centrifugate) was concd under red. pres. from which a semi-solid product was obtained (0.7 g). To this material a large excess of petrol (500 ml) was added, shaken well and left overnight at room temp.

The petrol insoluble solid (85 mg) on two successive crystallizations from petrol–$CHCl_3$ furnished cream coloured feathery crystals (15 mg) mp 172°. The product was homogeneous on TLC (silica gel G, R_f 0.82 in C_6H_6). (Found: C, 75.90; H, 7.2, $C_{30}H_{34}O_5$ requires C, 75.95; H, 7.18%. $[M]^+$ 474). The product gave a green colour with $FeCl_3$ in EtOH and did not respond to either the Shinoda or Gibbs [2, 11] tests.

Flemiphyllin triacetate (2). Flemiphyllin (7 mg) was dissolved in 0.7 ml of Ac_2O, pyridine (3 drops) added and the mixture gently refluxed on an oil bath for 6 hr and poured into crushed ice (50 g) while shaking. After 2 hr the product was filtered, washed throughly with H_2O and crystallized from petrol–C_6H_6, mp 117–118° (Found: C, 71.85; H, 6.68, $C_{36}H_{40}O_8$ requires C, 71.99; H, 6.66%).

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BROUSSONETINE, A BISQUINOLYL-γ-BUTYROLACTONE FROM *BROUSSONETIA ZEYLANICA**

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Kew Word Index—*Broussonetia zeylanica*; Moraceae; wood; quinoline alkaloid; broussonetine.

Abstract—The wood of *B. zeylanica* (Moraceae) contains a new alkaloid broussonetine, identified as 3,4-bis(8-hydroxyquinolin-4-yl)-γ-butyrolactone.

The only *Broussonetia* species occurring in Sri Lanka is *B. zeylanica* (Thw.) Corner (= *Allaeanthus zeylanicus* Thw.) which is endemic to the country. The wood contains 4-formyl-8-hydroxyquinoline (**1**) [1] and 3,4′-dihydroxy-2,3′-bipyridine (**2**) [2]. Further investigation has revealed another minor quinoline alkaloid, broussonetine (**3**).

Broussonetine, $C_{22}H_{16}N_2O_4$, is soluble in dil. hydrochloric acid and dil. sodium hydroxide (yellow), gives positive tests with ferric chloride and Dragendorff's reagent and, like 8-hydroxyquinoline, forms a fluorescent complex with Mg^{2+} ions [3]. The UV spectrum shows λ_{max} at 252 and 333 nm. The presence of two 8-hydroxyquinoline moieties in the alkaloid is evident from the ^{13}C NMR spectrum; this reveals signals for 18 aromatic carbons which can be assigned as shown (Table 1) and bear a close resemblance to those of **1**. The ^1H NMR spectrum (DMSO-d_6) includes a 2H singlet at δ 9.71 (2 × OH), and in the aromatic region two overlapping doublets at δ 8.89 (H-2′ and H-2″, J = 4.6 Hz) coupled to

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