Phytochemistry, Vol. 21, No. 2, pp. 433–437, 1982.
Printed in Great Britain.

0031–9422/82/020433–05$03.00/0

ALKALOIDS, COUMARINS AND FLAVONOIDS OF *MICROMELUM ZEYLANICUM*

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(*Revised received* 5 *June* 1981)

Key Word Index—*Micromelum zeylanicum*; Rutaceae; leaves; stems; oxazole; carbazole; coumarin; flavone; *O*-methylhalfordinol; koenigine; micromelin; 6-formyl-7-methoxycoumarin; 5-hydroxy-3,3′,4′,7,8-pentamethoxyflavone; β-sitosterol.

Abstract—The leaves and stems of *Micromelum zeylanicum* have yielded a new oxazole alkaloid, *O*-methylhalfordinol, the coumarin micromelin and a flavone, 5-hydroxy-3,3′,4′,7,8-pentamethoxyflavone. In addition, the stems contained 6-formyl-7-methoxycoumarin, and the leaves contained the carbazole alkaloid koenigine and β-sitosterol. Chemical and spectral evidence are presented to support the proposed structures.

INTRODUCTION

In continuing our investigation [1] of Asian Rutaceae we have examined the leaves and young stems of *Micromelum zeylanicum* [2], a species restricted to Sri Lanka where it is common in the low country (dry zone). The name of the plant in Sinhalese is Walkarapincha (= wild *Murraya koenigii*) and its leaves are superficially similar to those of some *Murraya* species. From the leaves we have isolated an oxazole alkaloid which has been identified as *O*-methylhalfordinol (**1a**), a carbazole alkaloid which appears from the available data to be koenigine (**2a**), a coumarin (micromelin, **3**), 5-hydroxy-3,3′,4′,7,8-pentamethoxyflavone (**4a**) and β-sitosterol. The stems also contained **1a**, **3** and **4a** and, in addition, 6-formyl-7-methoxycoumarin (**5**).

RESULTS AND DISCUSSION

The leaves and young stems of *M. zeylanicum* were separately extracted successively with petrol and $CHCl_3$. Initial TLC examination of these extracts showed the presence of two alkaloids, A and B, in the leaves and of alkaloid A in the stems. In addition, the presence of several non-alkaloidal constituents was indicated. Concentration of the petrol extract of the leaves resulted in a waxy solid which was dissolved in boiling EtOH and filtered. The cooled filtrate deposited a crystalline solid C1 which was purified by further crystallization from EtOH. The evaporated mother liquors were partially purified by CC over Al_2O_3, the first fractions consisting of a mixture of β-sitosterol and alkaloid B, later fractions consisting of alkaloid A and C1 and the final fractions containing C1 and a flavone F. These compounds were purified by prep. TLC (Si gel, C_6H_6–EtOAc, 3:2). The $CHCl_3$ extract of the leaves had a similar composition on TLC and was treated in the same manner. The stems treated in the same way yielded A, C1, F and an additional coumarin C2.

Alkaloid A, mp 98–99°, was isolated from the petrol and $CHCl_3$ extracts of the leaves and young twigs as colourless needles. Accurate mass measurements on the M^+ indicated a molecular formula $C_{15}H_{12}N_2O_2$ (M^+ 252) and its alkaloidal nature was suggested by UV spectral shifts with dilute acid and also by its reaction with Dragendorff's reagent. The IR spectrum did not show any NH or OH absorptions and the UV spectrum did not show any shifts with NaOMe, indicating the absence of hydroxy groups. Initial inspection of the 60-MHz ^1H NMR spectrum suggested that the alkaloid might be related to a series of oxazole alkaloids occurring in *Halfordia scleroxyla* [3]. These alkaloids show characteristic downfield resonance due to the presence of a pyridine ring. Permanganate oxidation of the alkaloid gave anisic acid and nicotinamide and this, therefore, confirmed its oxazole nature and suggested that, due to the isolation of anisic acid, it might be *O*-methylhalfordinol (**1a**). The 100-MHz ^1H NMR spectrum supported structure **1a** for alkaloid A, the protons on all three rings producing well-resolved signals. The similarity of the NMR spectrum to that of myosmine (**6**) [4] gives further support to the structure **1a**.

In the mass spectrum, a prominent peak at *m/z* 135 ($C_8H_7O_2^+$) was evidently the stabilized ion **7** which would arise by fission of the oxazole ring. A substantial peak also arose by the loss of Me from the M^+, losing a further CO to form an ion of *m/z* 209. The remainder of the spectrum closely resembled that of halfordinol (**1b**) [3].

Finally, mild acid hydrolysis of alkaloid A (acetic acid + HCl) gave halfordinol (**1b**) (comparison with authentic sample) and methylation (MeI) of authentic halfordinol gave alkaloid A, thus confirming that alkaloid A was *O*-methylhalfordinol [2-(3-pyridyl)-5-(4-methoxyphenyl)-oxazole; **1a**].

Alkaloid B, mp 182–183°, was shown (mass spectrum, $M^+ = 309$) to have a molecular formula $C_{19}H_{19}NO_3$. It did not give a positive alkaloidal reaction with Dragendorff's reagent but produced a blue-violet colour with concentrated sulphuric acid, characteristic of carbazole alkaloids, and gave a blue

colour with methanolic $FeCl_3$ indicating the presence of an OH group. Its UV spectrum resembled those of koenigine (**2a**), koenimbine (**2b**), koenidine (**2c**) and koenine (**2d**); suggesting that all possessed a similar chromophoric system. Koenigine (**2a**) and alkaloid B showed similar methoxide shifts of band 1 (λ_{max} 301 nm). The IR spectrum and the 60-MHz ^1H NMR spectrum ($CDCl_3$) of alkaloid B were in close agreement with published data [5] and final confirmation that alkaloid B was koenigine came from TLC comparison with an authentic sample and undepressed mixed mp.

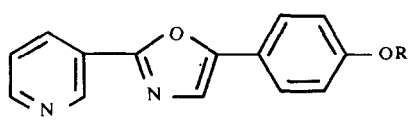
Compound C1 was obtained as colourless needles (mp 218–219°) from the leaves (0.85%) and stems (0.28%). The mass spectrum ($M^+ = 288$) gave a molecular formula $C_{15}H_{12}O_6$ and the IR spectrum showed strong bands at 1725 cm^{-1} (coumarin lactone) and 1770 cm^{-1} (γ-lactone). The UV spectrum was similar to those reported [6] for 7-oxygenated coumarins and had a striking similarity to the reported UV spectrum of micromelin (**3**), previously isolated from *M. minutum* [7], *M. pubescens* [8] (as micromelumin) and *M. integerrimum* [9]. The remainder of the IR spectrum (C–Me at 1360 cm^{-1}, epoxide at 1275 cm^{-1}) supported this, as did the ^1H NMR spectrum which was in agreement with published data [7, 8]. The mass spectrum showed a minor fragmentation ion at *m/z* 272 formed by loss of 16 mu from the M^+ as additional proof for the epoxide, and the loss of an unusual fragment (*m/z* 59) may be due to O=C–OMe arising through a rearrangement involving the OMe group and the γ-lactone system to form a Me ester (**8**) which would then lose COOMe. Other losses were as expected for the proposed structure (**3**). The data therefore supports the proposal that C1 is the coumarin micromelin (**3**).

Compund C2 was isolated from the petrol and $CHCl_3$ extracts of the stem (0.14%). It could not be detected in the leaf extracts. CC (Al_2O_3 eluting with C_6H_6–MeOH, 3:1 and MeOH) followed by prep. TLC (Si gel, C_6H_6–EtOAc, 3:2) and crystallization (EtOH) yielded a white solid, mp 252–253°. The mass spectrum ($M^+ = 204$) indicated a molecular formula $C_{11}H_8O_4$ and the IR spectrum showed the presence of a coumarin carbonyl (1745 cm^{-1}) and an additional carbonyl absorption (1680 cm^{-1}) which was possibly due to an aromatic aldehyde. The ^1H NMR spectrum (60 MHz) showed a downfield proton (δ 10.82) which did not exchange with D_2O and which was assigned to an aromatic CHO. The olefinic protons of the coumarin (C-3, C-4) appeared as an AB quartet (or a pair of doublets), the doublet at δ 7.65 ($J = 9.5$ Hz) was assigned to the C-4 proton and the doublet at δ 6.3 ($J = 9.5$ Hz) to the C-3 proton. A methoxy group absorption occurred at δ 4.0, and two singlets each equivalent to one proton and exhibiting *para*-coupling ($J = 0.5$ Hz) were seen at δ 8.06 and 6.92. These were assigned to the C-5 and C-8 protons respectively of the aromatic ring. The aldehyde and methoxy groups must therefore be in the 6- and 7-positions of the coumarin nucleus and the data were correct for 6-formyl-7-methoxy coumarin (**5**) which has previously been reported as a constituent of *Zanthoxylum dominianum* [3], *Boenninghausenia albiflora* [10] (and named angelical), *Glycomis cyanocarpa* [6] and *Hesperethusa crenulata* [11] (and named crenulatin).

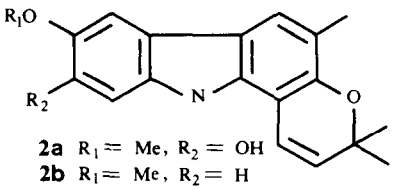
C2, in ethanolic solution, fluoresced light blue-green under UV light and its UV spectrum showed marked shifts of all bands on addition of NaOH or NaOMe, presumably due to the ready opening of the lactone ring in the presence of base. In the mass spectrum the usual coumarin loss of $M^+ - 28$ to give *m/z* 176 was observed.

Compound F crystallized from EtOH as yellow needles, mp 162–163°, and had a molecular formula $C_{20}H_{20}O_8$ ($M^+ = 388$). The spectral data showed it to be a flavone and the presence of a 5-hydroxyl group was supported by the green $FeCl_3$ reaction [12] and the bathochromic shifts of both bands in the UV spectrum in the presence of $AlCl_3$ [13]. Addition of NaOMe also caused bathochromic shifts in the UV spectrum, again suggesting the presence of a hydroxy group in the molecule. The IR spectrum indicated the presence of a hydroxy group (3400 cm^{-1}) hydrogen bonded with a (pyrone) carbonyl group of low stretching frequency (1656 cm^{-1}). Further data from acetyl and methyl derivatives showed that the molecule had only one hydroxy group which must be H-bonded since it did not methylate with diazomethane, whereas under more drastic conditions (prolonged refluxing with MeI) a mono-methyl derivative was formed. The 100-MHz ^1H NMR spectrum ($CDCl_3$) showed downfield signals around δ 7.84 (*dd*, $J = 2$ Hz, 9 Hz) and 7.79 (*d*, $J = 2$ Hz) assignable to flavone 6′ and 2′ protons respectively, and a multiplet at δ 3.86–3.96 due to five methoxy groups. A sharp singlet at δ 12.41 which disappeared on addition of D_2O confirmed the presence of a OH group at C-5. A doublet a δ 7.01 ($J = 9$ Hz) was assigned to the 5′ proton, and a sharp singlet at δ 6.4 could have been due to a proton at any of positions 6, 7 or 8. The signal at δ 6.4 was moved considerably downfield by addition of TFA to the solution showing that the proton was in ring A. This technique has been shown [14] to be useful in the structural determination of flavonoids where in TFA the carbonyl group is protonated, with some positive charge distribution in ring A causing a downfield shift of the A-ring protons. This technique established the presence of only one proton in the A-ring of compound F and the chemical shift (δ 6.4) of this proton suggested that it was at C-6 or C-7. In addition, the hydroxyl resonance was moved to a higher field in TFA, confirming its presence at C-5.

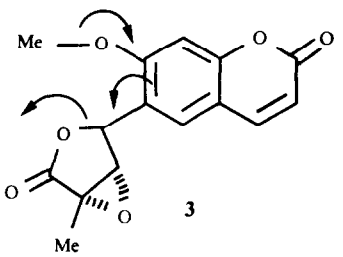
Double resonance studies on the ^1H NMR spectrum in C_6D_6 confirmed the 3′, 4′-substitution of the B-ring. Decoupling of the 5′-resonance (at δ 6.56) caused the collapse of the 6′ double doublet (δ 7.89, $J = 2$ Hz, 9 Hz) to a weak doublet (δ 7.92, $J = 2$ Hz). The mass spectrum of F showed an abundant M^+, the base peak at $M^+ - 15$ indicating the facile loss of a methyl group, typical of 3-, 6- or 8-methoxy flavones [15] and which can be ascribed to well-stabilized quinoid cations when oxygenated substituents are present in these positions. The compound appeared therefore, to be 5-hydroxy-3,3′,4′,7,8-pentamethoxyflavone (**4a**) and this was confirmed by UV and mp comparison with an authentic sample. Methylation of F produced a mono-methyl derivative whose ^1H NMR spectrum was identical with that of hexa-*O*-methylgossypentin (**4b**), and giving further proof of the structure of F as **4a**, a flavone previously



1a R = Me
1b R = H



2a R_1 = Me, R_2 = OH
2b R_1 = Me, R_2 = H
2c R_1 = Me, R_2 = OMe
2d R_1 = R_2 = H



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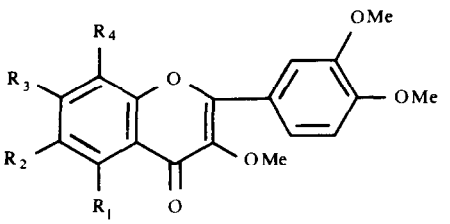
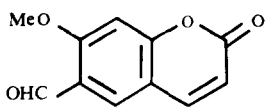


4a R_1 = OH, R_2 = H, R_3 = R_4 = OMe
4b R_1 = R_3 = R_4 = OMe, R_2 = H
4c R_1 = OCOMe, R_2 = H, R_3 = R_4 = OMe



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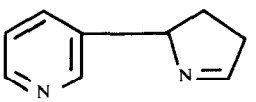

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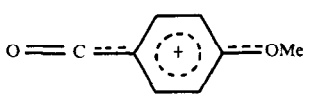

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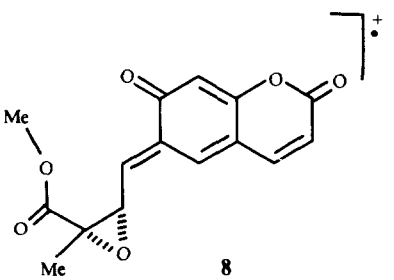

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isolated from *Citrus sinensis* [10] and *Ricinocarpus stylosus* [16].

From the petrol and $CHCl_3$ extracts of the leaves a white solid was obtained (mp 138–140°). The mass spectrum gave a M^+ at *m/z* 414 for $C_{29}H_{50}O$. The fragmentation pattern and IR spectrum were identical with those of an authentic sample of β-sitosterol.

BIOLOGICAL SIGNIFICANCE

One of the richest sources of carbazole alkaloids is *Murraya koenigii* Spreng. [17, 15] and a carbazole alkaloid has also been isolated from *Murraya exotica* Linn. [18] (= *M. paniculata* L. Jack). Other species found to produce carbazole alkaloids are *Glycosmis pentaphylla* [19] *Glycosmis mauritania* [20], *Clausena anisata* [21], *Cl. heptaphylla* [22] and *Cl. indica* [23]. Thus, it appears that these alkaloids are limited to taxonomically related genera of the Auran-tiodeae–Clauseneae (Rutaceae). The occurrence of koenigine in *Murraya koenigii* and *Micromelum zeylanicum*, and the very restricted distribution of the 3-methylcarbazoles, offers strong support for Swingle's revision [24] of the Aurantioideae, in which *Micromelum*, *Clausena*, *Glycosmis* and *Murraya* form the tribe Clauseneae.

O-Methylhalfordinol (**1a**) isolated in this study is a reasonable biogenetic intermediate to the more highly oxidized halfordinol derivatives occurring in *Halfordia* [3, 25], *Aegle* [26–28] and *Aeglopsis* [29] species. *O*-methylhalfordinol (**1a**) appears to be the first simple oxazole-ether whose natural occurrence has been reported; it cannot be an artefact, as has been suggested [28] for the isolation of halfordinol (**1b**). It is remarkable that Rutaceae alone accounts for the major share of the total number of known naturally occurring oxazole alkaloids; the only reports other

than from Rutaceae are from one plant, *Lolium multiflorum* [30], and as a fermentation product of *Streptomyces pimprina* [31]. The occurrence of oxazole alkaloids in *Micromelum* may provide additional support for the suggestion [32] of a biochemical link between the sub-families Aurantiodeae (oxazoles also in *Aegle* and *Aeglopsis*) and Toddalioideae (oxazoles in *Halfordia*). Since little is known, however, of the biogenetic pathways leading to these alkaloids it is not possible to elaborate further on their significance in these sub-families.

The widespread occurrence of coumarins and flavonoids in the Aurantioideae makes taxonomic assessment of these results difficult. 7-oxygenated coumarins commonly occur in the Rutaceae, and their presence in *Micromelum* is of little taxonomic interest. The presence of C-6 prenylated coumarins in this genus, however, may be more significant, especially since many species of *Micromelum* are able to modify the prenyl side-chains in unusual ways. In several instances these coumarins are similar to those occurring in the Umbelliferae [33] and recently an umbelliferous coumarin has been isolated [34] from *Micromelum zeylanicum* although this compound was not detected during our own investigations.

EXPERIMENTAL

Coarsely powdered leaves of *M. zeylanicum* Wight (1.2 kg) were extracted separately and successively with petrol (60–80°) and $CHCl_3$ (Soxhlet, 7 l. of each). Evaporation of the petrol extract yielded a waxy mass which dissolved in boiling EtOH and on filtration and cooling deposited a crystalline, non-alkaloidal solid C1 which was separated by filtration and purified by further crystallization from EtOH. The mother liquor was evaporated to dryness and the residue dissolved in C_6H_6 (150 ml) and partially purified by CC (Al_2O_3, activity II, 800 g), eluting successively in 250-ml fractions with C_6H_6 (fractions 1–20), C_6H_6–MeOH, 3:1 (fractions 21–40) and MeOH (fractions 41–50). The eluates were monitored by Si gel TLC (C_6H_6–EtOAc, 3:2) and similar fractions were combined. Fractions 9–20 contained β-sitosterol and alkaloid B, fractions 21–30 alkaloid A and coumarin C1, fractions 31–40 C1 and flavone F. Further purification of these mixtures was achieved by several prep. TLC separations (C_6H_6–EtOAc, 3:2). The $CHCl_3$ extract of the leaves contained a similar mixture to the petrol extract and the same stages for isolation and purification of the constituents were followed. The leaves therefore yielded alkaloid A (0.009%), alkaloid B (0.00125%), coumarin C1 (0.85%), flavone F (0.1%) and β-sitosterol (0.2%). The young stems (1 kg) treated in a similar manner yielded alkaloid A (0.006%), C1 (0.28%) and a second coumarin C2 (0.14%).

Alkaloid A. (*O*-Methylhalfordinol; 2-(3-pyridyl)-5-(4-methoxyphenyl)-oxazole; **(1a)** crystallized (EtOH) as colourless needles mp 98–99°, R_f (Me_2CO–petrol–Et_2NH, 2:7:1) 0.33, R_f (C_6H_6–EtOAc, 3:2) 0.22, R_f ($CHCl_3$–MeOH, 19:1) 0.67, R_f (C_6H_6–EtOH, 9:1) 0.43, R_f (C_6H_6–EtOAc–MeOH, 40:10:1) 0.47, R_f (*n*-BuOH–HOAc–H_2O, 6:1:1) 0.78; λ_{max}^{EtOH} nm: 266 (log Σ 3.92), 306 (*sh*, 3.90), 326 (4.14), 348 (3.61); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1618, 1600, 1500, 1460, 1412, 1300, 1260, 1180, 1120, 1080, 1060, 1025, 958, 838, 740, 710; NMR δ ($CDCl_3$, 100 MHz) 3.83 (3H, *s*, OMe), 6.95 (2H, *d*, J_{2-3} = 8.5 Hz, phenyl C-3, 5), 7.32 (1H, *s*, oxazole C-4), 7.33 (1H, *dd*, J_{5-6} = 5 Hz, J_{4-5} = 8.5 Hz, pyridyl C-5), 7.63 (2H, *d*, J_{2-3} = 8.5 Hz, phenyl C-2, 6), 8.30 (1H, *d*, J_{4-5} = 8 Hz, pyridyl C-4), 8.64 (1H, *d*, J_{5-6} = 5 Hz, pyridyl C-6), 9.28 (1H, *s*, pyridyl C-2). Found: M^+ 252.0902, $C_{15}H_{12}O_2N_2$ requires: 252.0898. MS (*m/z*) 252 (M^+, 100), 373 (86), 224 (20), 209 (68), 197 (82), 182 (82), 167 (52), 154 (62), 146 (32), 135 (78), 126 (50), 117 (32), 112 (49), 92 (65), 78 (65), 63 (65), 51 (62). M* 154 for 252→197, M* 222.9 for 252→237.

Synthesis of Halfordinol **(1b)**. (a) *From O-methylhalfordinol* **(1a)**. A soln of alkaloid A (8 mg) in HOAc (2 ml) containing 5 drops of conc HCl was heated for 15 min at 100°. The mixture was cooled, diluted with Na_2CO_3 soln and extracted with EtOAc. The EtOAc extract was washed with 5% Na_2CO_3 soln, dried (Na_2SO_4) and the solvent removed. The residue crystallized from EtOH as white needles, mp 255–256°, found to be identical (mmp, TLC) with an authentic sample of halfordinol prepared from *N*-methylhalfordinium chloride.

(b) *From N-methylhalfordinium chloride.* *N*-Methylhalfordinium chloride (2 g) was heated under red. pres. for 45 min. When the decomposition was almost complete the gummy residue was dissolved in MeOH and halfordinol was isolated by prep. TLC (C_6H_6–EtOAc, 3:2). The halfordinol (22 mg) thus obtained was purified by crystallization from MeOH, resulting in white needles, mp 253–255° (lit. [3] 255–256°).

Synthesis of O-methylhalfordinol **(1a)**. Halfordinol (12 mg) in MeOH (4 ml) was stirred at room temp. for 2 days with excess CH_2N_2–Et_2O. The solvent was removed *in vacuo* and *O*-methylhalfordinol **(1a)** was obtained by prep. TLC (C_6H_6–EtOAc, 3:2) as colourless needles, mp 99–100°. This compound was found to be identical (mmp, TLC) with the natural alkaloid from *M. zeylanicum*.

Alkaloid B (*koenigine*, **2a**). This compound, after prep. TLC of the column eluent, was further purified by TLC ($CHCl_3$–MeOH, 19:1) and required several TLC purifications to achieve separation from sterols which moved close to it in most of the solvent systems used. Alkaloid B crystallized (EtOH) as a white powder mp 182–183° (lit. [5] 183–185°). UV, IR and ^1H NMR data consistent with published [5] values. MS (*m/z*) 309 (M^+, 96), 294 (100), 279 (67), 264 (27), 250 (28), 236 (12), 234 (14), 222 (16), 154 (11), 146 (32), 139 (36), 83 (38). M^+ 309.1362, $C_{19}H_{19}NO_3$ requires 309.1364. Identical (mmp, TLC) with an authentic sample of koenigine.

Coumarin C1 (*micromelin*, *micromelumin*, **3**) C1 crystallized from EtOH as colourless needles (10.2 g, 0.85%, from leaves), mp 218–219°. UV, IR and ^1H NMR data were in agreement with published values [7, 8]. MS (*m/z*) 288 (M^+, 100), 272 (5), 260 (3), 243 (22), 229 (95), 214 (64), 213 (89), 203 (58), 186 (72), 175 (32), 158 (62), 145 (31), 131 (33), 115 (32), 102 (32), 89 (40), 77 (32). Found M^+ 288.0637, $C_{15}H_{12}O_6$ requires 288.0633.

Coumarin C2 (*6-formyl-7-methoxycoumarin*, **5**). C2 crystallized from EtOH as a white solid, mp 252–253°, (0.14%), R_f (C_6H_6–EtOAc, 3:2) 0.56, R_f ($CHCl_3$–MeOH, 19:1) 0.77, R_f (C_6H_6–EtOH, 9:1) 0.65, R_f (*n*BuOH–HOAc–H_2O, 6:1:1) 0.75, λ_{max}^{EtOH} nm 265 (log Σ 4.52), 308 (4.22), 330 (4.20); $\lambda_{max}^{EtOH-NaOMe}$ nm 285 (4.68), 330 (4.36), 360 (4.22); ν_{max}^{KBr} cm^{-1} 1745 (α-pyrone), 1720, 1680 (aromatic aldehyde), 1620 (C=C), 1500, 1440, 1387, 1340, 1298, 1220, 1138, 1010, 860, 840; NMR δ (60 MHz, $CDCl_3$) 4.0 (3H, *s*, OMe), 6.30 (1H, *d*, J = 9.5 Hz, C-3), 6.96 (1H, *d*, J = 0.5 Hz, C-8), 7.65 (1H, *d*, J = 9.5 Hz, C-4), 8.06 (1H, *d*, J = 0.5 Hz, C-5), 10.82 (1H, *s*, CHO). M^+ 204.0419, $C_{11}H_8O_4$ requires 204.0419. MS (*m/z*) 204 (M^+ 100), 187 (68), 175 (86), 159 (86), 145 (72), 133 (72), 116 (74), 105 (95).

Flavone F (*5-hydroxy-3,3',4',7,8-pentamethoxyflavone* **4a**). Compound F was purified by prep. TLC (C_6H_6–EtOAc, 3:2) and crystallized from EtOH (1.2 g, 0.10%) as orange needles, mp 162–163°; ν_{max}^{KBR} cm^{-1} 3400 (*br* chelated OH), 1656 (C=O), 1605 (aromatic system), 1560, 1500, 1460, 1440, 1380, 1360, 1315, 1295, 1265, 1220, 1200, 1180, 1150, 1138, 1100, 1045, 1025, 1010; NMR δ (100 MHz, $CDCl_3$) 3.86, 3.90, 3.92 (each 3H, *s*, OMe). 3.94 (6H, *s*, 2×OMe), 6.4 (1H, *s*, C-6), 7.01 (1H, *d*, $J = 9$ Hz, C-5), 7.79 (1H, *d*, $J = 2$ Hz, C-2'), 7.84 (1H, *dd*, $J = 9$ Hz, and 2 Hz, C-6'), 12.41 (1H, *s*, exchanging with D_2O, OH, C-5). NMR δ (100 MHz, C_6D_6) 3.2 (3H, *s*, OMe), 3.36 (3H, *s*, OMe), 3.53 (3H, *s*, OMe), 3.74 (3H, *s*, OMe), 3.76 (3H, *s*, OMe), 6.28 (1H, *s*, C-6), 6.56 (1H, *d*, $J = 9$ Hz, C-5'), 7.78 (1H, *d*, $J = 2$ Hz, C-2'), 7.89 (1H, *dd*, $J = 2$ Hz and 9 Hz, C-6'), 13.22 (1H, *s*, OH). NMR δ (60 MHz, $CDCl_3$ + 10% TFA): identical to shifts in $CDCl_3$ except the downfield shift of singlet at δ 6.39–7.89 and upfield shift of singlet at δ 12.39–10.62. MS (*m/z*) 388 (M^+, 85), 373 (100), 360 (6), 358 (35), 357 (37), 345 (42), 343 (36), 331 (22), 330 (32), 329 (33), 328 (27), 315 (35), 301 (29), 194 (46), 186 (47), 181 (41), 178 (41), 169 (20), 165 (58), 153 (45), 137 (30), 131 (29), 83 (60). Found C, 61.92; H, 5.15. $C_{20}H_{20}O_8$ requires: C, 61.85; H, 5.15. Identical (UV and mmp) with an authentic sample of 5-hydroxy-3,3',4',7,8-pentamethoxyflavone.

Acetylation of compound F (*preparation of 5-acetoxy-3,3',4',7,8-pentamethoxyflavone*, **4c**). Compound F was acetylated (Ac_2O–pyridine) to yield a monoacetate (cream needles from EtOH, mp 171–172°). NMR δ (60 MHz, $CDCl_3$) 2.45 (3H, *s*, COMe), 3.79 (3 H, *s*, OMe), 3.95 (12 H, *s*, 4×OMe), 6.62 (1H, *s*, C-7), 6.95 (1H, *d*, $J = 8.5$ Hz, C-5'), 7.75 (1H, *d*, $J = 3$ Hz, C-2'), 7.82 (1H, *dd*, $J = 2$ Hz and 9 Hz, C-6').

Methylation of compound F (*preparation of hexa-O-methylogossypetin*; *3,3',4',5,7,8-hexamethoxyflavone*, **4b**). Methylation of F by prolonged refluxing with MeI produced the 5-*O*-methyl derivative as cream needles (EtOH) mp 152–153°. NMR δ (60 MHz, $CDCl_3$) 3.83 (3H, *s*, OMe), 3.90 (3H, *s*, OMe) 3.92 (3H, *s*, OMe), 3.95 (9H, *s*, 3×OMe), 6.35 (1H, *s*, C-6), 6.95 (1H, *d*, $J = 8,5$ Hz, C-5'), 7.75 (1H, *d*, $J = 2$ Hz, C-2'), 8.2 (1H, *dd*, $J = 2$ Hz and 9 Hz, C-6'), identical (UV, NMR) with an authentic sample of hexa-*O*-methylgossypetin. β-Sitosterol was isolated from the column eluates by prep. TLC (C_6H_6–EtOAc, 3:2) and crystallized from EtOH, mp 136–137°. M^+ 428.3651, identical with an authentic sample.

Acknowledgements—We thank Dr. J. R. Lewis for advice and the provision of MS and 100-MHz ^1H NMR data, Prof. M. U. S. Sultanbawa and Dr. N. Balasubramaniam, University of Sri Lanka, for collecting, authenticating and supplying plant materials, Prof. W. D. Crow for an authentic sample of *N*-methylhafordinium chloride, Dr. J. H. Tatum and Prof. R. M. Horowitz for samples of hexa-*O*-methylgossypetin, Prof. P. R. Jeffries for a sample of 5-hydroxy-3,3',4',7,8-pentamethoxyflavone and Prof. T. J. Mabry for samples of related flavones. We also thank Sunderland Polytechnic for the provision of a research assistantship (to K.P.W.C.P.).

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