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THE BIFLAVONOIDS OF *GARCINIA VOLKENSII* (GUTTIFERAE)*

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Abstract—In addition to the known biflavonoids GB-1a, GB-2a and morelloflavone, the heartwood of *Garcinia volkensii* Engl. contains volkensiflavone, a new flavanone–flavone whose constituent units are naringenin and apigenin.

WE RECENTLY isolated a new series of biflavanones from *Garcinia buchananii* Baker and *G. eugeniifolia* Wall.[1] Since then, flavonoid dimers with the same carbon skeleton have been isolated from *G. morella*[2] and *G. spicata* Hook.[3] An examination of the extractives from the heartwood of *G. volkensii* Engl., again reveals the presence of flavonoid dimers.

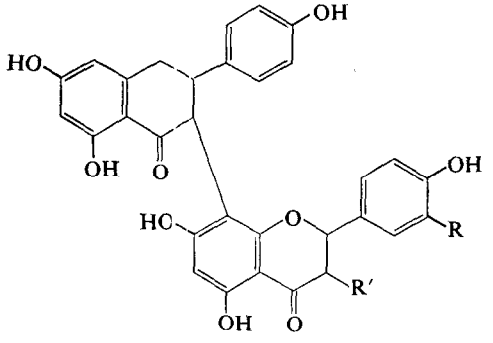
Soxhlet extraction of the heartwood with hot chloroform gave a residue which was chromatographed over silica gel. The known biflavanones GB-1a (I) and GB-2a (II) were isolated, purified by preparative TLC, and shown to be identical with authentic specimens.[1] Another fraction gave a flavanone–flavone, which was identical with morelloflavone (III) kindly supplied by Professor K. Venkataraman.[2]

Volkensiflavone (IV), a new flavanone–flavone, was also isolated by preparative TLC. Its u.v. spectral characteristics closely resemble those of morelloflavone (III) and show similarity with those expected for a combination of naringenin (V) and apigenin (VI), (see Table 1). The i.r. spectrum of the biflavonoid possesses a broad hydroxyl absorption at 3300 cm^{-1} and carbonyl bands at 1620–1640 cm^{-1}. The existence of the flavanone–flavone

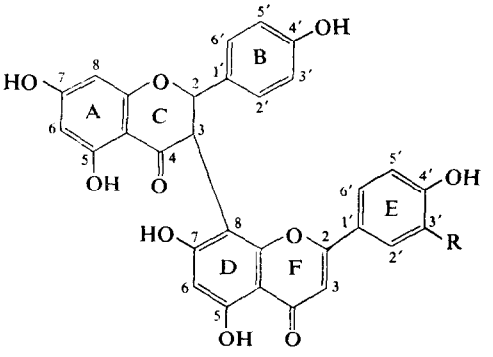
* Part XV in the series "Extractives from Guttiferae", for Part XIV see I. CARPENTER, H. D. LOCKSLEY and F. SCHEINMANN, *J. Chem. Soc.*, (*C*), 1969, in press.

[1a] B. JACKSON, H. D. LOCKSLEY, F. SCHEINMANN and W. A. WOLSTENHOLME, *Tetrahedron Letters* **9**, 787 (1967).
[1b] *Idem. ibid.*, **32**, 3049 (1967).
[1c] B. JACKSON, H. D. LOCKSLEY and F. SCHEINMANN, *Chem. Comm.* 1125 (1968); 1360 (1968).
[2] C. G. KARANJGAOKAR, P. V. RADHAKRISHNAN and K. VENKATARAMAN, *Tetrahedron Letters* **33**, 3195 (1967).
[3] M. KONOSHIMA, Y. IKESHIRO, A. NISHINAGA, T. MATSUURA, T. KUBOTA and H. SAKAMOTO, *Tetrahedron Letters* **2**, 121 (1969).

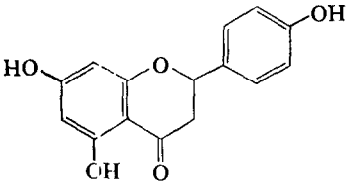
system is confirmed by the mass spectrum, which in addition provides some indication of the manner in which the two units are linked. Accurate mass measurement of the molecular ion (*m/e* 540) determines the molecular formula as $C_{30}H_{20}O_{10}$.



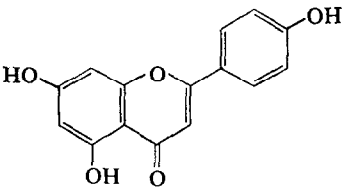
(I) R = R′ = H (GB-1a)
(II) R = OH, R′ = H (GB-2a)
(VII) R = H, R′ = OH (GB-1)
(VIII) R = R′ = OH (GB-2)



(III) R = OH (Morellofiavone)
(IV) R = H (Volkensiflavone)



(V) Naringenin



(VI) Apigenin

TABLE 1. ULTRAVIOLET SPECTRA OF VOLKENSIFLAVONE, MORELLOFLAVONE, AND RELATED MONOFLAVONOIDS

	λ_{max} (nm), (ε), in MeOH			
Volkensiflavone (IV)	330 (16,200)	289 (28,100)	275 (24,300)	225 (40,500)
Morelloflavone (III)	340 (18,100)	288 (27,200)	275s (24,500)	225 (38,400)
Naringenin (V)	330 (4,860)	288 (17,800)		
Apigenin (VI)	335 (25,700)		268 (23,800)	

s=shoulder.

As a general feature, it is known that 5,7-dihydroxy-2,3-diarylchromanones readily lose the elements of phloroglucinol under the influence of electron bombardment and/or thermolysis.[4] Thus, the loss of the elements of phloroglucinol from volkensiflavone (IV), a process observed also in the fragmentation of the GB biflavanones (I), (II), (VII) and VIII),[1a,b] and of morelloflavone (III),[2] gives a fragment ion at *m/e* 414 ($C_{24}H_{14}O_7$). It follows therefore that one phloroglucinol ring in volkensiflavone (IV) must be excluded from the linkage between

[4] B. JACKSON, H. D. LOCKSLEY, F. SCHEINMANN and W. A. WOLSTENHOLME, unpublished results.

the two flavonoid moieties. Metastable transitions indicate that the ion at *m/e* 414 (M-126) then undergoes further fragmentation by loss of CO followed by a retro Diels–Alder process, or by these processes in reverse order, to give ultimately an ion at *m/e* 268 ($C_{15}H_8O_5$) with the probable structure shown in the Scheme. The retro Diels–Alder process involves the loss of 118 mass units and corresponds to a hydroxyphenylacetylene fragment[1b, 5] (seen also as an ion at *m/e* 118) demonstrating the presence of an intact flavone moiety in the $(M\text{-}126)^+$ ion. The loss of a hydroxyphenylacetylene fragment shows that the flavanone–flavone linkage cannot involve rings E or F (see IV). Consequently, the linkage must lie between ring D and either ring B* or C. The NMR evidence only permits a linkage between rings C and D.

The NMR spectrum of volkensiflavone in hexadeuteriodimethylsulphoxide was recorded at room temperature and at 120°. Past experience has shown[4] that only at temperatures above 100°, are the 100 MHz NMR spectra of the natural biflavanoids well resolved. Therefore, the aliphatic and aromatic protons were analysed at the higher temperature in the presence of deuterium oxide to remove the interfering hydroxyl signals. The aliphatic protons appear as doublets ($J = 14$ Hz) at τ 4·3 (1 H) and τ 5·1 (1 H) (H-2 and H-3, ring C) characteristic of *trans*-diaxial hydrogens.[6a] The high field aromatic signals indicate[6] the presence of three phloroglucinol protons by a singlet at τ 3·7 (1 H) (H-8 or H-6, ring D) and a broad singlet at τ 3·9 to 4·0 (2 H) (H-6 and H-8, ring A). Thus the presence of only *two* aliphatic hydrogens and *three* high field aromatic signals necessitates linkage between ring C of the flavanone and the phloroglucinol ring D of the flavone unit as in (IV).

The remaining signals at low field are consistent with two AA'MM' systems corresponding to *p*-hydroxy substitution patterns in rings B and E:[6] the final hydrogen appearing at τ 3·45 can be attributed to the vinylic proton of ring F.[6b] For ring E, there are two broad doublets, one centred at τ 2·4 (corresponding to H-2′ and H-6′) and the other centred at τ 3·2 (corresponding to H-3′ and H-5′). For ring B, there are two broad doublets one centred at τ 2·9 (corresponding to H-2′ and H-6′) and the other centred at τ 3·4 (corresponding to H-3′ and H-5′).

The hydroxy groups are visible in the NMR spectrum when it is measured at room temperature in the absence of deuterium oxide: the C-5 hydrogen bonded hydroxyls of rings A and D are at τ −2·7 and τ −2·1 while the other hydroxyls appear as a broad hump between τ −0·5 and τ 1·2.

The above evidence supports structure (IV)† for volkensiflavone but to confirm this assignment, GB-1a (I) of proven structure,[1] was converted into volkensiflavone (IV) by partial dehydrogenation with iodine and potassium acetate in acetic acid.[7] The flavanone–

* The appearance of an ion at *m/e* 107 is probably due to a hydroxybenzyl fragment (or its tropylium equivalent) from ring B of a flavanone unit.[1a,b] Therefore, it is unlikely that ring B of volkensiflavone is involved in the linkage.

† This structure for volkensiflavone, and those for the related biflavanones, GB-1, GB-1a, GB-2 and GB-2a, are presented with the reservation that the linkage may be alternatively between C-3 (ring C) and C-6 (ring D). No entirely unambiguous means has been found to distinguish isomers of this type [cf. A. J. BIRCH, C. J. DAHL and A. PELTER, *Australian J. Chem.* **22**, 423 (1969)].

[5] A. PELTER, P. STAINTON and M. BARBER, *J. Heterocyclic Chem.* **2**, 262 (1965); H. AUDIER, *Bull. Soc. chim., France*, 2892 (1966); see also J. W. CLARK-LEWIS, *Australian J. Chem.* **21**, 3025 (1968).

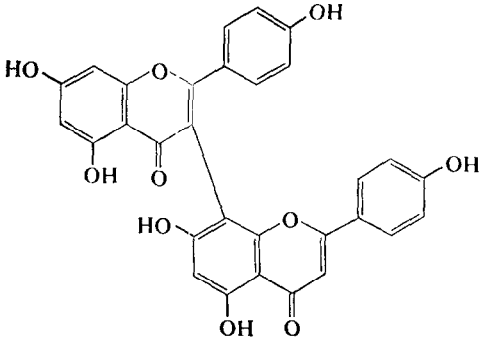
[6a] J. W. CLARK-LEWIS, *Australian J. Chem.* **21**, 2059 (1968).

[6b] J. MASSICOT, J.-P. MARTHE and S. HEITZ, *Bull. Soc. chim., France*, 2712 (1963).

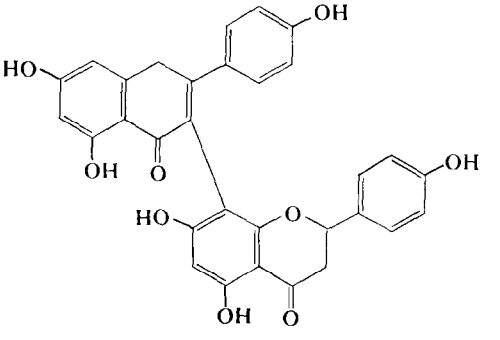
[7] T. R. SESHADRI, in *The Chemistry of Flavonoid Compounds* (edited by T. A. GEISSMAN), p. 160. Pergamon Press, Oxford (1962).

flavone (IV) was separated from the biflavonyl (IX)[1a] by preparative TLC, and shown to be identical with volkensiflavone (IV). The alternative flavanone–flavone structure (X), which would have spectral characteristics typical of a 2,3-diarylchromone (e.g. structure XI)[4] and possess a quite different pattern of aliphatic signals in the NMR is probably not formed in the dehydrogenation reaction.

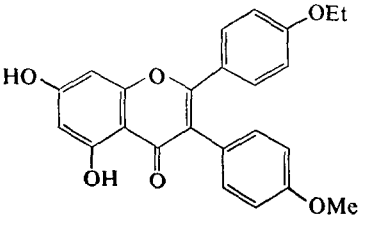
Thus the biflavonoids, which have been exclusively isolated from five species of *Garcinia*,[1,2,3] all contain the same carbon skeleton, and this feature may have taxonomic value.† The co-occurrence of biflavanones GB-1a (I) and GB-2a (II) with volkensiflavone (IV) and morelloflavone (III) is interesting. Previously GB-1a (I) and GB-2a (II) have been found together with their 3-hydroxy co-metabolites GB-1 (VII) and GB-2 (VIII). In both cases the



(IX)



(X)



(XI)

co-metabolites of GB-1a and GB-2a differ only in the state of oxidation of ring F in accordance with the suggestion that a flavanone[8] nucleus (or its chalcone[9]) is a precursor to both flavones and 3-hydroxyflavanones.

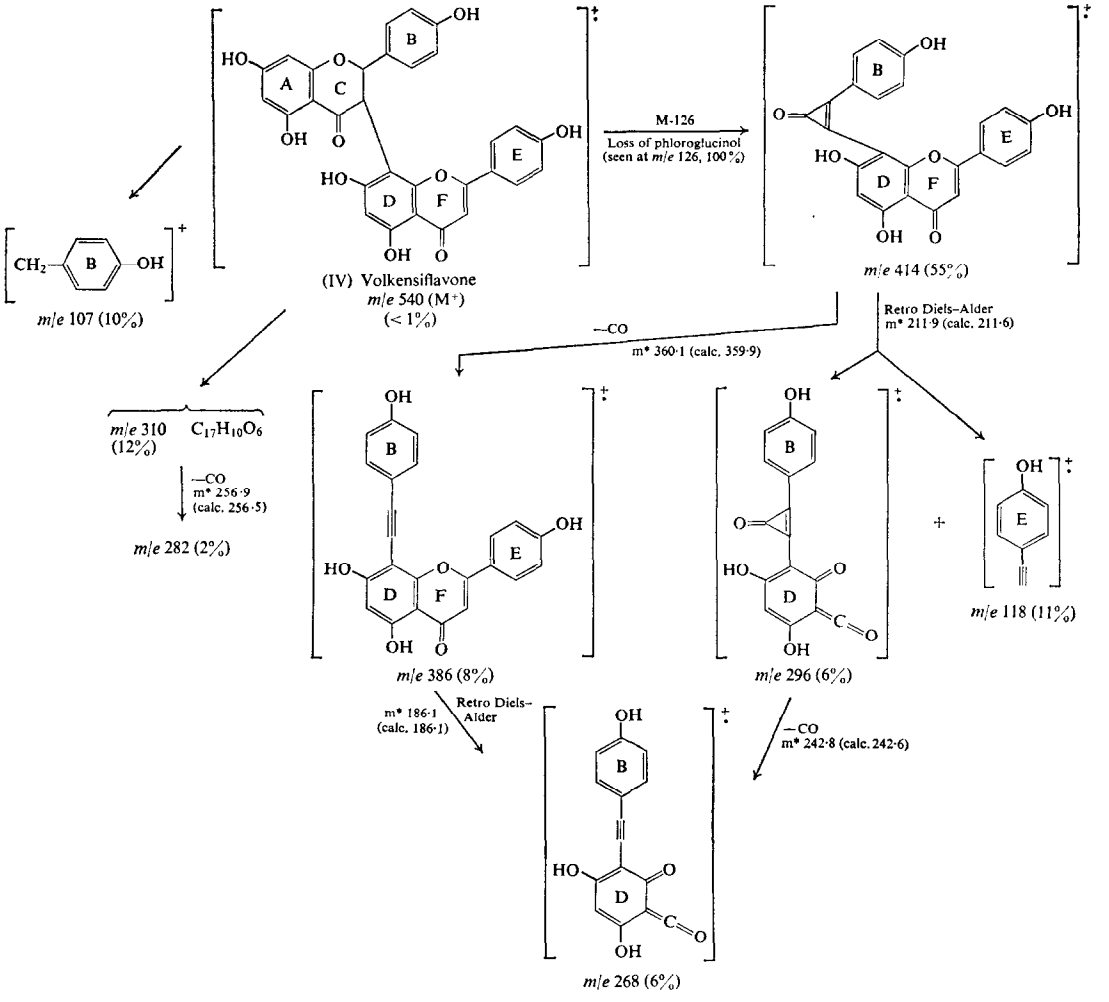
EXPERIMENTAL

Ultraviolet spectra were recorded on an Unicam SP800 recording spectrophotometer, i.r. spectra, as Nujol mulls, on a Perkin–Elmer 257 grating instrument, and NMR spectra on Varian A60 and HA100 instruments. Mass spectra were obtained with A.E.I. MS12 single focusing and MS902 double focusing instruments at an ionisation potential of 70 eV. Analytical and preparative TLC were carried out using silica gel, Stahl (Merck).

† Morelloflavone (III) and volkensiflavone (IV) have recently been isolated (H. D. Locksley, I. G. Murray and F. Scheinmann, unpublished results) from the heartwood of *Allanblackia floribunda*. This is the first isolation of such biflavonoids outside *Garcinia* species, but it should be noted that the genera *Allanblackia* and *Garcinia* are close relatives: both belong to the Clusioideae subfamily of the Guttiferae.

[8] H. GRISEBACH, *Chemistry and Biochemistry of Plant Pigments* (edited by T. W. GOODWIN), p. 279, Academic Press, London (1965).

[9] E. WONG, *Chem. Comm.* 395 (1968).



SCHEME. INTERPRETATION OF THE MASS SPECTRAL DATA FOR VOLKENSIFLAVONE (IV).

(High resolution mass spectral results: Found, *m/e* 107·0500; C_7H_7O requires, *m/e* 107·0496. Found, *m/e* 414·0740; $C_{24}H_{14}O_7$ requires, *m/e* 414·0740. Found, *m/e* 310·0454; $C_{17}H_{10}O_6$ requires *m/e* 310·0477. Found, *m/e* 126·0316; $C_6H_6O_3$ requires *m/e* 126·0316. Found, *m/e* 268·0377; $C_{15}H_8O_5$ requires *m/e* 268·0372).

Extraction Procedure

The sample of *Garcinia volkensii* Engl., (Voucher No. 14047, identical with Herbarium Specimen No. 3035, on the authority of Dr. J. B. Gillett, Botanist in Charge, East African Herbarium, Nairobi) was collected at a point 3½ miles from Kieni Forest Station on the Mangu-South Kinangop Road, Kenya.

Soxhlet extraction of the powdered heartwood (1 kg) of *G. volkensii* Engl., with hot $CHCl_3$ for 4 days and subsequent concentration of the extract yielded a brown oily residue (8 g) which was chromatographed on silica gel. The eluate from the column was then separated into three fractions on the basis of the constituents revealed from accompanying TLC.

Fraction 1 (eluted with $CHCl_3$) contained xanthones (which are currently under investigation) and oily material.

Fraction 2 (eluted with $CHCl_3$/acetone 4:1) (4·5 g) contained three components (i)–(iii), a portion of which was separated using preparative TLC to give,

(i) GB-1a (I) as a colourless amorphous solid identical (i.r. and mass spectrum) with an authentic sample.[1a]
(ii) GB-2a (II) as a colourless amorphous solid identical (i.r. and mass spectrum) with an authentic sample.[1c]
(iii) Volkensiflavone (IV) as a yellow amorphous solid of diffuse m.p. (finally decomposing at ~250°).

γ_{max} 3300 (OH) and 1640–1620 cm^{-1} (C=O groups of flavone and flavanone). [Found: *M*, (mass spectrum) 540·1049. $C_{30}H_{20}O_{10}$ requires 540·1056]. The relative abundance of GB-1a (I), GB-2a (II) and volkensiflavone (IV) is in the approximate ratio of 65:25:10.

Fraction 3 (eluted with $CHCl_3$/acetone 7:3), after purification by preparative TLC, yielded morelloflavone (III) as a yellow amorphous solid identical (TLC, i.r., and mass spectra) with an authentic sample.[2]

Oxidation of GB-1a

GB-1a (I) (200 mg), KOAc (1·6 g) and I_2 (100 mg) were heated together in boiling acetic acid (15 ml) for 5 hr. The solution was poured into water, to give a precipitate which was extracted with ether. Evaporation of the ether left a solid whose constituents were separated and purified using preparative TLC (in MeOH/$CHCl_3$ (15:85)). This procedure yielded a light yellow solid (15 mg) which was identical (TLC and mass spectra) with an authentic specimen of volkensiflavone (IV). Also present in the reaction mixture was unreacted GB-1a (I), and the biflavone (IX).[1a]

Acknowledgements—We thank the S.R.C. for an equipment grant and for the award of finance for an Experimental Officer (to B.J.). Dr. J. B. Gillett, Botanist in Charge, East African Herbarium, Nairobi, is thanked for his kind assistance in identifying *Garcinia volkensii* Engl. We are indebted to Professor K.Venkataraman for an authentic sample of morelloflavone, obtained from *G. morella*.