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8-*C*-PRENYLFLAVONOIDS FROM THE SEED OF *TEPHROSIA BRACTEOLATA*

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Key Word Index—*Tephrosia bracteolata*; Leguminosae; 8-C-prenyl-5,7-dioxygenated flavonoids; obovatin methyl ether; isopongaflavone; *trans*-tephrostachin; *trans*-anhydrotephrostachin.

Abstract—Four flavonoids in the seeds of *Tephrosia bracteolata* were identified as the flavanone obovatin methyl ether and the flavones isopongaflavone, *trans*-tephrostachin and *trans*-anhydrotephrostachin.

INTRODUCTION

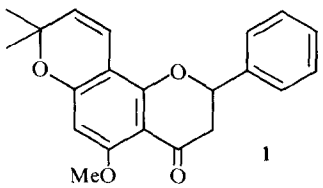
Tephrosia bracteolata Guill. et Perr. is an erect undershrub common throughout savanna areas of tropical Africa [1]. As part of a study of the flavonoids of the Tephrosieae [2], we now report the results of an investigation of the seeds of this species.

RESULTS AND DISCUSSION

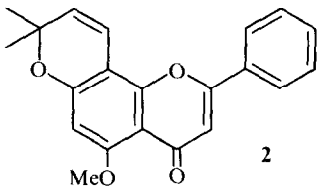
Extraction of the powdered seeds with petrol and then $CHCl_3$, followed in each case by column chromatography and preparative TLC over silica gel, gave four flavonoids. Two of these were identified on the basis of a comparison with published spectral data as obovatin methyl ether (**1**) and isopongaflavone (**2**). **1** had previously been isolated from *T. obovata* Merr. [3] and *T. praecana* Brummitt [4], and **2** from *Pongamia glabra* Vent. [5].

The major compound analysed for $C_{22}H_{22}O_5$. Both UV and IR spectra were typical of a flavone [6], and the latter indicated the presence of an OH substituent. The ^{1}H NMR spectrum permitted assignment of all twenty-two protons. Two multiplets centred at δ 7.55 (3H) and 7.90 (2H) could be attributed to an unsubstituted B-ring [2]. Four singlets, at δ 3.96 and 4.01 (3 H each) and 6.42 and 6.68 (1 H each), were indicative of a 5,7-dimethoxyflavone nucleus substituted at either C-6 or C-8. The remaining nine protons were observed as an AB quartet (2 H, J = 17 Hz) centred at δ 6.75 and 6.90, a singlet at 1.90 (replaceable with D_2O), and a 6 H singlet at 1.49. These resonances are typical of the unusual 3-hydroxy-3-methyl-*trans*-but-1-enyl side-chain [2, 7], and permit assignment of structure **3** or **4** to this flavonoid. The position of the C_5-substituent on the A-ring was resolved from a study of the resonance positions of the OMe carbons in the ^{13}C NMR spectra. Both were observed at 56.3 ppm. Since in **3** the C-5 methoxyl would be forced, by steric hindrance, to exist outside the plane of the ring, it would resonate at least 2 ppm further downfield than this [8]. Structure **4** must therefore be assigned. **4** is the hitherto unrecorded *trans*-isomer of tephrostachin (**5**), reported from *T. polystachyoides* E. Mey [9], and the 5-methoxy derivative of lanceolatin-A (**6**) known from *T. lanceolata* Gamb [10], *T. purpurea* Pers. [11] and *T. apollinea* Link [2]. It seems most appropriate to assign to it the trivial name of *trans*-tephrostachin.

The fourth flavonoid analysed for $C_{22}H_{20}O_4$. The UV spectrum was similar to that of **4** but the IR spectrum differed both in the absence of OH absorption and in exhibiting a weak band at 3100 cm^{-1}, typical of $=CH_2$. The ^{1}H NMR spectrum again indicated a 5,7-dimethoxyflavone with C-8 substitution. The seven protons of the C-8 substituents once more included an AB quartet exhibiting *trans*-coupling together with broad



1



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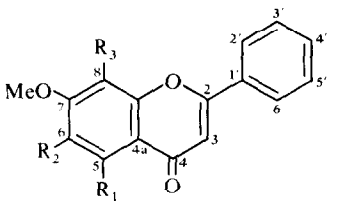

	R_1	R_2	R_3
3	OMe	*t* CH:CHC(OH)Me$_2$	H
4	OMe	H	*t* CH:CHC(OH)Me$_2$
5	OMe	H	*c* CH:CHC(OH)Me$_2$
6	H	H	*t* CH:CHC(OH)Me$_2$
7	OMe	H	*t* CH:CHC(Me):CH$_2$

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singlets at δ 2.08 (3 H) and 5.12 (2 H) for Me and $=CH_2$ groups. These data are typical of the 3-methyl-*trans*-but-1,3-dienyl sidechain [7] and permit the assignment of structure **7** to this flavonoid. Compound **7**, to which we have assigned the trivial name *trans*-anhydrotephrostachin, appears to occur naturally rather than as an artefact from the dehydration of **4** [7].

A further blue fluorescent compound isolated from the MeOH extract of the seeds was identified as 6,7-dihydroxycoumarin (aesculetin) by direct comparison with an authentic sample. TLC analysis of this extract also indicated the occurrence of aesculin, the 6-*O*-glucoside of this coumarin.

EXPERIMENTAL

Plant material. Fruiting material of *Tephrosia bracteolata* Guill. et Perr. was collected in Kwabenya-Accra, Ghana, during December 1979. A voucher specimen, Enti FE-1959, has been deposited in the Herbarium of the Royal Botanic Gardens, Kew.

Extraction and separation. Powdered seeds (80 g) were extracted separately with petrol (bp 60–80°), then $CHCl_3$, then 70% aq. MeOH. Concn of the petrol extract followed by column chromatography over Si gel gave, on elution with petrol–EtOAc (49:1), impure **2**. Prep. TLC of this material (Si gel, solvent—petrol–EtOAc, 4:1) yielded pure **2** (56 mg). Further elution of the column with petrol–EtOAc (3:7) gave impure **7**, subsequently purified by prep. TLC (Si gel, solvent—C_6H_6–EtOAc 3:2) to yield 46 mg. Concn of the $CHCl_3$ extract gave, on standing, **4** (211 mg). Column chromatography of the residue of extract over Si gel, eluting with petrol–EtOAc (4:1), with subsequent prep. TLC of the resulting eluate (Si gel, solvent—C_6H_6–EtOAc, 3:2) gave **1** (61 mg). Concn of the MeOH extract and prep. TLC of the EtOAc-soluble fraction (Si gel, solvent—$CHCl_3$–MeOH, 17:1) gave 6,7-dihydroxycoumarin (27 mg).

Obovatin methyl ether (**1**). Needles from petrol–EtOAc, mp 135° (lit. [4] 125–127°). Found: M^+ 336.1369; $C_{21}H_{20}O_4$ requires: 336.1361. UV, IR and ^{1}H NMR as lit. [3]. MS *m/z* (rel. int.): 336 (36), 321 (38), 218 (12), 217 (100).

Isopongaflavone (**2**). Plates from $CHCl_3$–MeOH, mp 201–205° (lit. [5] 215–216°). Found: M^+ 334.1216; $C_{21}H_{18}O_4$ requires: 334.1205. UV and IR as lit. [5]. ^{1}H NMR ($CDCl_3$): δ 1.49 (6 H, *s*, 2″-Me_2), 3.94 (3 H, *s*, 5-OMe), 5.66, 6.84 (2 H, AB*q*, J = 10 Hz, 3″ and 4″-H), 6.33 (1 H, *s*, 6-H), 6.67 (1 H, *s*, 3-H), 7.45–7.55 (3 H, *m*, 2′,4′,6′-H), 7.80–7.95 (2 H, *m*, 3′,5′-H). MS *m/z* (rel. int.): 334 (48), 320 (16), 217 (23).

trans-Tephrostachin (**4**). Needles from $CHCl_3$, mp 215–220°. Found: M^+ 366.1469; $C_{22}H_{22}O_5$ requires: 366.1467. UV λ_{max} nm: 265, 345. IR ν_{max} cm^{-1}: 3350, 1640. ^{1}H NMR ($CDCl_3$): δ 1.49 (6 H, *s*, 3″-Me_2), 1.90 (1 H, *s*, replaceable by D_2O, 3″-OH), 3.96, 4.01 (2 × 3 H, 2 × *s*, 5 and 7-OMe), 6.42 (1 H, *s*, 6-H), 6.68 (1 H, *s*, 3-H), 6.75, 6.90 (2 H, AB*q*, J = 17 Hz, 2″ and 1″-H), 7.47–7.62 (3 H, *m*, 2′,4′6′-H), 7.84–7.98 (2 H, *m*, 3′,5′-H). ^{13}C NMR (DMSO-d_6): ppm 30.2 (3″-Me_2), 56.3 (5 and 7-OMe), 70.4 (C-3″), 93.1 (C-6), 107.3, 108.0 (C-4a, C-8), 108.5 (C-3), 114.7 (C-1″), 126.7, 126.8 (C-2′, C-6′), 129.7 (C-3′, C-5′), 132.1 (C-4′, C-1′), 143.7 (C-2″), 155.9 (C-8a), 160.0, 161.1 (C-5, C-2), 162.2 (C-7), 177.6 (C-4). MS *m/z* (rel. int.): 366 (20), 351 (11), 349 (38), 348 (100), 320 (21), 319 (81), 317 (27), 287 (31), 225 (13).

trans-Anhydrotephrostachin (**7**). Clusters from EtOAc, mp 128–134°. Found: M^+ 348.1364; $C_{22}H_{20}O_4$ requires 348.1361. UV λ_{max} nm: 264, 277, 350. IR ν_{max} cm^{-1}: 3100, 1640. ^{1}H NMR ($CDCl_3$): δ 2.08 (3 H, *s*, 3″-Me), 3.92 (6 H, *s*, 5 and 7-OMe), 5.12 (2 H, *s*, 3″- $=CH_2$), 6.49 (1 H, *s*, 6-H), 6.62 (1 H, *s*, 3-H), 6.89, 7.31 (2 H, AB*q*, J = 17 Hz, 2″ and 1″-H), 7.47–7.60 (3 H, *m*, 2′,4′,6′-H), 7.85–8.00 (2 H, *m*, 3′,5′-H). MS *m/z* (rel. int.): 348 (69), 319 (16), 310 (27), 309 (15), 295 (27), 230 (16), 125 (15), 95 (100).

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