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XANTHONES AND BIFLAVONOIDS FROM *GARCINIA DENSIVENIA* STEM BARK

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Key Word Index—*Garcinia densivenia*; Guttiferae; xanthones; pyranojacareubin; biflavonoids; morelloflavone; *O*-methyl fukugetin.

Abstract—The stem bark of a species of *Garcinia* (Guttiferae), provisionally identified as *G. densivenia*, has yielded a xanthone and two biflavonoids. The xanthone has been characterized as a novel 1,3,5,6-tetraoxygenated compound and has been assigned the trivial name pyranojacareubin (1,5-dihydroxy-6′,6′-dimethylpyrano (2′,3′:3,2)-6″,6″-dimethylpyrano (2″,3″:6,7)-xanthone). The biflavonoids were identified as morelloflavone and its methyl ether derivative, *O*-methyl fukugetin.

INTRODUCTION

The tropical plant family Guttiferae produces a wide range of secondary metabolites including xanthones, biflavonoids and 4-substituted coumarins (neoflavonoids) [1, 2]. In the course of an examination of the chemistry of the stem barks of sympatric species of Guttiferae from the Douala-Edea Forest Reserve of West Cameroun we have already reported the isolation of a wide range of neoflavonoids from *Mammea africana* Sabine [3], a biflavanone and polyisoprenyl benzophenone from *Garcinia mannii* Oliv. [4], a pentadecyl substituted α-pyrone from *G. conrauana* Engl. [5], and macluraxanthone and polyisoprenyl benzophenones from *G. ovalifolia* Oliv. [6]. All four of the above are common elements in the flora of the Reserve [7] and, in each case, they have yielded at least 0.5% of their major metabolite.

Among the rarer Guttiferae of the Reserve is a species of *Garcinia* characterized by the production of a pale cream latex, in contrast to the bright yellow product from other species. This taxon appears to be *Garcinia densivenia* Engl. but until the relationship between *G. densivenia* and *G. zenkeri* Engl., a taxon known only from the type collection and perhaps conspecific with *G. densivenia*, is resolved the identification must remain provisional. We now wish to report the isolation of small quantities of a novel dipyranoxanthone and much larger amounts of two flavone/flavanone biflavonoids from the stem bark of this species.

RESULTS AND DISCUSSION

Column chromatography of the petrol extract of the stem bark over silica gel, eluting with petrol containing increasing amounts of EtOAc, gave a mixture of sterols followed by a yellow crystalline solid (yield 0.012%), mp 224–227°. Accurate mass measurement gave an empirical formula $C_{23}H_{20}O_6$ indicative of a tetra-oxygenated xanthone substituted with two prenyl moieties. A base peak at M^+-15 suggested that the prenyl substituents were incorporated in 2,2-dimethylpyrano ring systems which can form stable benzopyrylium ions [8]. The UV spectrum was similar to that recorded for 1,3,5,6-oxygenated xanthones [9]. A bathochromic shift on addition of $AlCl_3$ indicated a chelated OH at C-1 whilst the absence of a comparable shift with NaOAc suggested that neither C-3 nor C-6 carried free OH substituents [9]. A positive Gibbs' test [10] was indicative that at least one position *para* to an OH lacked substitution.

The ^1H NMR spectrum was in agreement with the above observations and permitted partial resolution of the structure. A sharp singlet at δ 13.11, replaceable with D_2O, must be assigned to the C-1 OH whilst a broad signal for a second replaceable proton at δ 5.50 indicated another free OH. Signals for two aromatic protons were observed as singlets resonating at δ 7.50 and 6.26. The highly deshielded position of the former require that it be assigned to C-8 (*peri* to the carbonyl). The comparably shielded position of the second proton indicated its placement at either C-2 or C-4 of the phloroglucinol derived A-ring [11]. The remaining sixteen protons gave resonances typical of two 2,2-dimethylpyrano systems with two singlets (6H each) at δ 1.48 and 1.52 and two AB quartets (J 10 Hz) centred at 6.90 and 5.60 and at 6.46 and 5.73. The resonance positions of the olefinic protons were confirmed by spin decoupling experiments. The olefinic resonances at δ 6.90 and 5.60 are typical of a pyran ring undergoing modifying effects from the presence of a free OH *ortho* to the position of prenyl attachment [12, see also data in refs. 9 and 11]. In contrast the less widely separated chemical shifts for the second pyran indicate that the *ortho* position is in this case not substituted [9, 12].

Previous assignments to C-1 and C-8 make it necessary to place one pyran substituent in the A-ring and the other in the B-ring. The A-ring pyran has, by virtue of the substitution pattern of that ring, to be affected by an *ortho* *O*-substituent but could be placed either linear or angular. The B-ring pyran can be unambiguously assigned to the linear position where it will be adjacent to the unsubstituted C-8 position.

The position of the A-ring pyran was resolved by use of the modifying effects of acetylation on the resonance positions of proximate protons [12]. Comparison of the

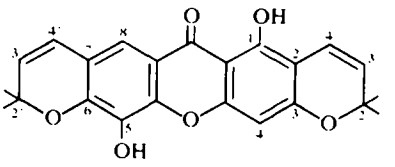
Table 1. Chemical shift differences (δ) between 1 and 1 diacetate

Proton	1	1 diacetate	$\Delta\delta$
4′	6.90	6.71	+0.19
3′	5.60	5.73	−0.13
4	6.26	6.47	−0.21
4″	6.44	6.41	−0.03
3″	5.73	5.72	+0.01
8	7.50	7.74	−0.24

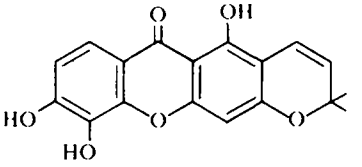
^{1}H NMR spectra of the original compound and the diacetate (Table 1) showed that the C-1 acetate exerted a diamagnetic effect on the C-4′ proton and a paramagnetic effect at C-3′ thus requiring linear attachment of the pyran to the A-ring and permitting assignment of structure **1** to the xanthone. The absence of significant shifts in the resonance positions of the pyran protons of the second substituent complies with the proposed structure as does the comparable paramagnetic shifts observed for C-4 and C-8 protons which indicate that both lie *para* to the acetylation sites of rings A and B [13]. A broadening of the signals for C-4 and C-4′ protons due to their long-range coupling [12] also agreed with the proposed structure. This xanthone appears to be novel. As it represents the only possible pyrano derivative of the common xanthone jacareubin (**2**) it has been assigned the trivial name pyranojacareubin.

From the Me_2CO soluble portion of the MeOH extract a mixture of two compounds was obtained by precipitation with Et_2O. Column chromatography of the Et_2O soluble material over silica gel gave, on elution with $CHCl_3$ containing 4% MeOH, two pale yellow compounds in yields of 0.1% and 0.53% respectively. The highly phenolic major compound failed to give a molecular ion by EI–MS but FD MS suggested a MW of 556 with major fragments at 430, 215 and 126. Both UV and IR spectra were typical of flavone/flavanone dimers [14]. Modifications of the UV spectrum by $AlCl_3$, NaOMe, NaOAc and H_3BO_3 were indicative of 5, 7, 3′ and 4′-hydroxy substituents. The ^{1}H NMR spectrum run at 125° (in DMSO-d_6) showed an AB-quartet (J 12 Hz) centred at δ 4.86 and 5.73 typical of C-3 and C-2 protons of a flavanone linked through C-3 to a flavone [15]. Exhaustive methylation of the compound gave the heptamethyl derivative ($C_{37}H_{34}O_{11}$) the ^{1}H NMR and MS of which proved to be identical with data previously recorded [16] for the heptamethyl ether of morelloflavone (**3**).

The first, minor, compound isolated from the column was obviously similar to **3**. FD MS indicated a molecular weight of 570 with major fragments at 444, 416, 223 and 126. UV characteristics differed from **3** only in failure to give a bathochromic shift with H_3BO_3. The ^{1}H NMR spectrum, run under identical conditions to **3**, exhibited an additional resonance at δ 3.76 for an OMe substituent. Exhaustive methylation gave a product identical to heptamethylmorelloflavone. The second biflavonoid must therefore be a monomethyl ether of **3** and, due to the lack of reactivity with H_3BO_3, the OMe must be assigned to the di-substituted B-ring. The position of the OMe was resolved by alkaline degradation to phloroglucinol and acetovanillone, thus confirming that this compound was *O*-methyl-fukugetin (**4**) [17].



1



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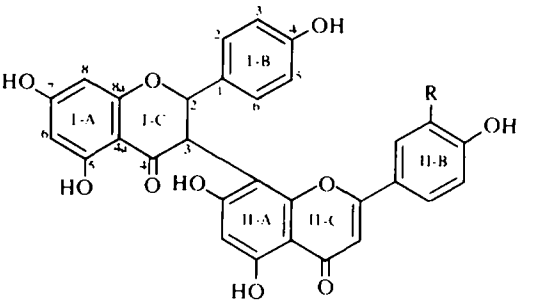

3 R = OH
4 R = OMe

Table 2. ^{13}C chemical shifts (ppm) for **3** and **4**

Carbon No.	3	4
I-3	50.1	49.8
I-2	82.3	80.9
I-8, I-6	96.1, 97.2	96.0, 97.2
II-6	99.6	99.4
II-3	103.8	101.4
I-4a, II-4a, II-8	101.6, 103.0, 103.9	103.0*
II-2′	114.2	116.1†
I-3′, I-5′	115.5	115.5
II-5′	116.5	116.2†
II-1′, II-6′	120.7, 123.5	121.6, 123.3
I-1′, I-2′, I-6′	129.8	129.2, 129.7
II-3′, II-4′	146.3, 150.0	148.8, 151.3
I-5, I-7, I-8a, II-2,	158.4, 162.4, 164.2,	158.4, 162.3, 164.2,
II-5, II-7, II-8a	164.9, 165.6, 165.8	165.4, 167.0
II-4	183.1	183.0
I-4	197.2	197.2
OMe	—	56.4

* Complex of three signals.
† Interchangeable.

In the course of these studies ^{13}C NMR spectra were obtained for both **3** and **4**. The data obtained are presented in Table 2. Chemical shifts were in general agreement with those noted for other biflavonoids [18, 19], the small differences observed from previously published data for **3** being attributable to different solvents.

CONCLUSION

Morelloflavone is a common metabolite in the Guttiferae, having been noted previously in at least twelve species of *Garcinia* and in *Allanblackia floribunda* Oliv. [20]. *O*-Methyl-fukugetin on the other hand has previously been found only in the Asian species *G. spicata* Hook. *f.* [17].

The isolation of pyranojacareubin and of macluraxanthone from *G. ovalifolia* [6] is of chemotaxonomic interest. Previously 1,3,5,6-oxygenated xanthones had been found quite commonly in the Calophylloideae subfamily of the Guttiferae (notably in *Calophyllum* L. itself) and more rarely in the Kielmeyeroideae and Moronoboideae. They had not been recorded from the Clusioideae, which includes *Garcinia*, in which 1,3,6,7-oxygenation appears to be the normal tetra-oxygenation pattern. However all previous data on *Garcinia* xanthones relate to Asiatic species and it may well be that there are patterns in xanthone chemistry that have a geographic rather than a phylogenetic basis. Gottleib [21] has previously observed similar chemo-geographic distinctions, notably among the Calophylloideae.

EXPERIMENTAL

UV spectra were run in EtOH and IR spectra as KCl discs. ^1H NMR spectra were run at 90 MHz or 100 MHz (elevated temp.) in stated solvents. ^{13}C NMR spectra were run at 25.1 MHz in DMSO-d_6 using FT. TMS was employed as internal standard except at elevated temp when DSS was used. EI–MS were obtained at 70 eV and elevated temp; FD–MS at 80° using an accelerating voltage of 3 kV. Mps are uncorrected. Petrol refers to the bp 40–60° fraction unless otherwise stated.

Plant material. Stem bark of *Garcinia densivenia* Engl. was collected in the Douala-Edea Forest Reserve, west Cameroun, in the summer of 1976. A voucher, P. G. Waterman and D. McKey 869, has been deposited at the Herbarium of the Royal Botanic Gardens, Kew. Further vouchers: D. McKey and J. S. Gartlan 92 and 170, Gartlan 12, and D. W. Thomas 285, all collected from the same locality are also representative of this taxon.

Isolation of compounds. Ground stem bark (320 g) was extracted with petrol and then MeOH. The conc petrol extract yielded a yellow ppt. which on recrystallization from petrol (bp 80–90°) gave **1** (11 mg). Column chromatography over Si gel, eluting with $CHCl_3$, gave further **1** (24.5 mg). The conc MeOH extract was refluxed with Me_2CO for 24 hr and the Me_2CO soluble fraction then precipitated with Et_2O. The conc supernatant was then subjected to column chromatography over Si gel and on elution with $CHCl_3$ containing 4% MeOH gave **4** (309 mg) followed by **3** (1.7 g).

Identification of isolated compounds. Pyranojacareubin (**1**). Yellow needles from petrol (bp 80–90°), mp 224–227°. Found: M^+ 392.1255; $C_{23}H_{20}O_6$ requires: 392.1260. UV λ_{max}nm: 220, 278, 334, 380 sh; (+ NaOH) 270 sh, 286, 315 sh, 345; (+ $AlCl_3$) 223, 269 sh, 287, 312 sh, 355. IR ν_{max}cm^{-1}: 3450 (OH), 1675 (C=O), 1650, 1580, 1505, 1495, 1345, 1140. ^1H NMR (90 MHz, $CDCl_3$): δ 13.04 (1H, *s*, replaceable by D_2O, 1-OH), 7.50 (1H, *s*, 8-H), 6.90, 5.60 (2H, AB*q*, J = 8 Hz, 4′-H and 3′-H), 6.44, 5.73 (2H, ABq, J = 8 Hz, 4″-H and 3″-H), 6.26 (1H, *s*, 4-H), 5.46 (1H, *s*, replaceable by D_2O, 5-OH), 1.53, 1.48 (2 × 6H, 2 × *s*, 2′-Me_2 and 2″-Me_2). MS: *m/e* (rel. int.): 392 (24), 377 (100), 361 (5), 347 (3).

Pyranojacareubin diacetate. **1** (5 mg) was dissolved in C_5H_5N (2 ml) and acetic anhydride (0.5 ml) added. The reaction mixture was maintained at 40° for 12 hr and after normal work up gave the diacetate (4.9 mg), mp 173°. Found: M^+ 476.1478; $C_{27}H_{24}O_8$ requires 476.1471. IR: ν_{max}cm^{-1}: 1735, 1700, 1565. ^1H NMR (90 MHz, $CDCl_3$): δ 7.74 (8-H), 6.71, 5.73 (4′-H and 3′-H), 6.47 (4-H), 6.41, 5.72 (4″-H and 3″H), 2.43 (2 × Ac), 1.49, 1.46 (2′-Me_2 and 2″-Me_2). MS: *m/e* (rel. int.): 476 (21), 434 (71), 419 (100), 391 (13), 377 (61), 361 (17).

Morelloflavone (**3**). Yellow crystals from $CHCl_3$–MeOH, mp 285° (decomp.) (Lit. [14] 298° decomp.). UV λ_{max}nm: 257 sh, 276, 344; (+ NaOH) 245 sh, 272, 285, 326, 412; (+ $AlCl_3$) 280, 303 sh, 428; (+ NaOMe) 240 sh, 273, 282, 322, 407; (+ NaOAc) 275 sh, 284, 323, 404; (+ H_3BO_3) 273, 287, 374. IR ν_{max}cm^{-1}: 3400 (OH), 1655, 1620 (C=O), 1525, 1375, 1260, 1170. ^1H NMR (100 MHz, 125°, DMSO-d_6): δ 12.90, 12.15 (2H, 2 × *s*, replaceable by D_2O, I-A-5-OH and II-A-5-OH), 7.72 (1H, *d*, J = 2 Hz, II-B-2′), 7.19 (1H, *dd*, J_1 = 9 Hz, J_2 = 2 Hz, II-B-6′), 7.08 (2H, *d*, J = 9 Hz, I-B-2′ and 6′), 6.80 (1H, *d*, J = 9 Hz, II-B-5′), 6.50 (2H, *d*, J = 9 Hz, I-B-3′ and 5′), 6.43 (1H, *s*, II-C-3), 6.20 (1H, *s*, II-A-6), 5.97 (2H, *s*, I-A-6 and 8), 5.73, 4.86 (2H, AB*q*, J = 12 Hz, I-C-2 and 3). ^{13}C NMR (see Table 2). FD MS: *m/e* 556, 430, 215, 126. Heptamethylmorelloflavone. **3** (250 mg) was dissolved in dry Me_2CO (250 ml) and MeI (5 ml) and fused K_2CO_3 (5 g) added. The mixture was refluxed for 24 hr with addition of further MeI (1.25 ml) and K_2CO_3 (2.5 g) after 8 hr. The reaction mixture was filtered and concd. Prep. TLC of the concentrate on Si gel (solvent: $CHCl_3$–Me_2CO, 6:4) gave heptamethylmorelloflavone (90 mg). Found: M^+ 654.2098; $C_{37}H_{34}O_{11}$ requires 654.2101.

O-Methyl-fukugetin (**4**). Yellow crystals from $CHCl_3$–MeOH, mp 167°, decomp. (Lit. [17] 178–180°, decomp.). UV λ_{max}nm: 226, 255 sh, 277 sh, 288, 335; (+ NaOH) 245 sh, 286, 325, 400; (+ $AlCl_3$) 283, 296 sh, 354; (+ NaOMe) 245 sh, 284, 323, 410; (+ NaOAc) 245 sh, 286, 324, 404. IR ν_{max}cm^{-1}: 3450 (OH), 1650, 1635 (C=O), 1525, 1370, 1265. ^1H NMR (100 MHz, 125°, DMSO-d_6): δ 13.13, 12.40 (2H, 2 × *s*, I-A-5-OH and II-A-5-OH), 7.33 (2H, *m*, II-B-2′ and 6′), 7.07 (2H, *d*, J = 9 Hz, I-B-2′ and 6′), 6.84 (1H, *d*, J = 9 Hz, II-B-5′), 6.62 (1H, *s*, II-C-3), 6.55 (2H, *d*, J = 9 Hz, I-B-3′ and 5′), 6.19 (1H, *s*, II-A-6), 5.98 (2H, *s*, I-A-6 and 8), 5.92, 4.92 (2H, AB*q*, J = 12 Hz, I-C-2 and 3), 3.76 (3H, *s*, OMe). FD MS: *m/e* 570, 444, 416, 222, 126. Exhaustive methylation of **4** (75 mg) using the procedure described above gave heptamethylmorelloflavone (17 mg), identical in all respects with the product obtained from **3**. Alkaline degradation of **4**. **4** (25 mg) was refluxed, under N_2, with 75% aq. KOH (10 ml) containing MeOH (0.5 ml) for 16 hr. The reaction mixture was acidified with conc HCl and extracted with Et_2O. Phloroglucinol and acetovanillone were identified in the Et_2O extract by direct comparison with authentic samples.

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