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MAJOR XANTHONES FROM *GARCINIA QUADRIFARIA* AND *GARCINIA STAUDTII* STEM BARKS

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Key Word Index—*Garcinia quadrifaria*; *G. staudtii*; Guttiferae; xanthones; 1, 3, 5-trihydroxy-4, 8-di(3, 3-dimethylallyl)xanthone; rheediaxanthone-A; biflavonoids; benzophenones.

Abstract—The stem bark of *Garcinia quadrifaria* has yielded the novel xanthone 1, 3, 5-trihydroxy-4, 8-di(3, 3-dimethylallyl)xanthone and the biflavonoids *O*-methylfukugetin and morelloflavone. The seeds contained the biflavonoids but not the xanthone. *G. staudtii* stem bark gave rheediaxanthone-A and the polyisoprenylated benzophenone xanthochymol.

INTRODUCTION

The large tropical genus *Garcinia* L. is well known as a source of xanthones [1], 3/8 linked biflavonoids [2], and polyisoprenylated benzophenones [3]. In continuation of our investigations of west African species of *Garcinia* [4–9] we now report on the constituents of two previously uninvestigated species, *G. quadrifaria* Baill. ex Pierre and *G. staudtii* Engl.

G. quadrifaria is a sub-canopy rain-forest tree found only in Cameroun and Gabon [10]. From a sample of stem bark collected in the Douala-Edea Forest Reserve in west Cameroun we have isolated the novel xanthone **1** and the known biflavonoids **2** and **3**. Seeds of this species yielded only **2** and **3**. *G. staudtii* is a shrub or small tree found only in Cameroun and south-east Nigeria [11]. Stem bark of this species, collected from the same area, yielded rheediaxanthone-A (**4**) and xanthochymol (**5**).

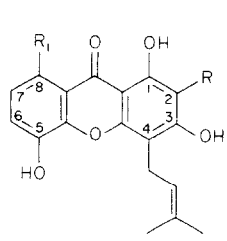
RESULTS AND DISCUSSION

CC of the acidic fraction of a petrol extract of *G. quadrifaria* stem bark over Si gel gave **1** in a yield of 0.004%. The UV spectrum was typical of a 4-substituted tri-oxygenated xanthone [12] and underwent a bathochromic shift with $AlCl_3$ indicating the presence of a 1-OH substituent. The lack of a shift on addition of NaOAc eliminated the possibility of a 1, 6-dihydroxy substitution pattern [13]. Accurate mass measurement suggested $C_{23}H_{24}O_5$, indicative of a tri-oxygenated xanthone substituted with two isoprenyl units. A base peak at $[M-43]^+$ and significant ions at $[M-55]^+$ and $[M-111]^+$ confirmed the presence of two non-cyclic prenyl units linked directly to the xanthone.

A sharp singlet at δ 11.73 (1H) confirmed the occurrence of 1-OH, hydrogen bonded to the carbonyl. Two other replaceable protons at δ 10.80 and 9.31 could be assigned to the other OH substituents. Signals in the regions δ 1.63–1.82 (12H), 3.59–4.00 (4H), and 5.40 (2H) were typical of two 3, 3-dimethylallyl units. The deshielded position of one of the 2H doublets (δ 4.00) indicated its placement at C-8, *peri* to the carbonyl [14]. The remaining three protons were observed in the aromatic region as a sharp singlet at δ 6.36 and an AB quartet for *ortho* coupled protons at δ 7.09 and 7.23. The former must be assigned to H-2 or H-4 of the 1, 3-oxygenated A-ring of xanthones with the second prenyl substituent occupying the other position. The other aromatic protons must be assigned to H-5 and H-6 or H-6 and H-7 with the remaining hydroxy substituent at either C-5 or C-7.

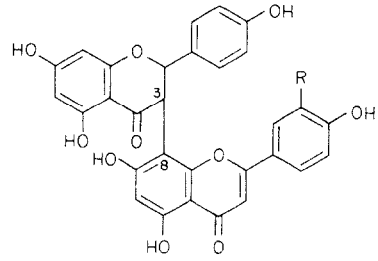
A Gibbs test on **1** was negative indicating that C-4 was substituted and that the A-ring proton must be at C-2. Support for placement of the B-ring protons at C-6 and C-7 was derived from the similarity of their resonances to those recorded for gartanin (**6**) [15] whereas in 7-hydroxyxanthones, such as thwaitesixanthone (**7**) and calabaxanthone (**8**), H-5 and H-6 are equivalent [16, 17]. Furthermore, the ^1H NMR spectrum of the triacetate of **1** showed a deshielding of H-2 of δ 0.58, typical of its placement between two acetate units [18], and well in excess of the shift in H-6. The absence of any deshielding on the methylene group of the 8-isoprene unit was also of value in confirming that the B-ring acetate was not adjacent to this moiety. On the basis of the above the xanthone can be assigned the structure **1**.

The acetone extract of *G. quadrifaria* stem bark gave *O*-methylfukugetin (**2**) and morelloflavone (**3**) in yields of 0.15% and 0.32% respectively. On methylation both gave the same heptamethyl ether. They were both characterized by direct comparison with **2** and **3** isolated previously from *G. densivenia* [6]. A TLC analysis of the seeds of this species revealed the presence of **2** and **3** but not of **1**.



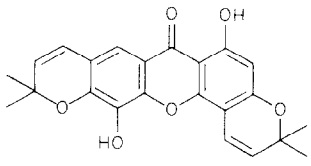
1 R = H; R_1 = $CH_2CH{:}C(Me)_2$

6 R = $CH_2CH{:}C(Me)_2$, R_1 = OH



2 R = OMe

3 R = OH



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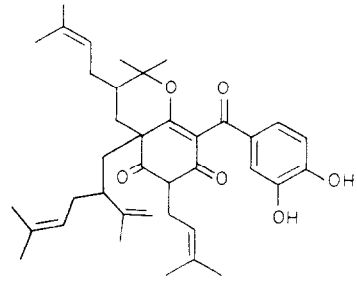

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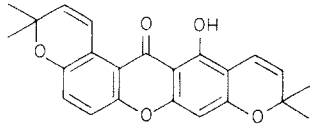

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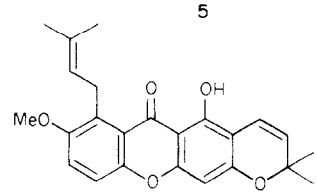

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From the petrol extract of *G. staudtii* stem bark two compounds were isolated by CC over Si gel. The major compound (yield 0.08%) was identified as xanthochymol (**5**) by direct comparison with authentic material [7]. The minor compound (yield 0.02%) analysed for $C_{23}H_{20}O_6$ and gave the UV spectral characteristics of a 1-hydroxyxanthone. The ^{1}H NMR spectrum revealed the presence of two hydroxy substituents, two aromatic protons, and two 2, 2-dimethylpyrano- substituents. The strongly deshielded nature of one of the aromatic protons (δ 7.45) required its placement at C-8 and the equally highly shielded nature of the other (δ 6.16) required placement at C-2 or C-4. All spectral and physical data obtained were in close agreement with that recently reported for rheediaxanthone-A (**4**) from *Rheedia benthamiana* Planch. et Triana [19].

The identity of the isolated xanthone as **4** was confirmed by synthesis of the diacetate. The ^{1}H NMR spectrum showed shifts for the olefinic protons of the A-ring pyrano substituent (δ ±0.12) consistent with its attachment *para* to the 1-acetoxy group [19]. Both the less shielded and deshielded positions of the olefinic protons of the B-ring pyrano substituent and the absence of any shift in the acetylated compound required that this pyrano ring was linear and with the olefinic protons adjacent to the non-oxygenated C-8.

These are the third and fourth west African *Garcinia* species that we have found to contain xanthones. The combination of xanthone and biflavonoid occurs in *G. quadrifaria* and *G. densivenia* Engl. [6] whilst in *G. staudtii* and *G. ovalifolia* Oliv. [7] the xanthones are accompanied by benzophenones. The oxygenation patterns of the xanthones, 1, 3, 5- in the case of **1** and 1, 3, 5, 6- in all other examples, are not those usually found among the simple xanthones of *Garcinia* and are more typical of the genus *Calophyllum* L. [1]. To date it has only proved possible to isolate one xanthone from each source due to the relatively small amounts of material available. Whilst further xanthones are certainly present in each species, yields, even of the isolated and therefore major compound of this class, have always been very low.

EXPERIMENTAL

UV: EtOH; IR: KCl discs; ^{1}H NMR: 90 MHz, $CDCl_3$, TMS as int. standard unless otherwise stated; EIMS: 70 eV and elevated temp. Mps are uncorr. Petrol refers to the bp 40–60° fraction unless otherwise stated.

Plant material. Stem bark and seeds of *Garcinia quadrifaria* were collected in the Douala-Edea Forest Reserve, west Cameroun, in the summer of 1976. A voucher, P. G. Waterman and D. McKey 802, has been deposited at the Herbarium of the Royal Botanic Gardens, Kew. Stems of *Garcinia staudtii* were collected from the Marienberg Road by the River Sanaga and opposite the Reserve in the summer of 1979. A voucher, D. McKey 270, has been deposited at the Herbarium of the Royal Botanic Gardens, Kew.

Isolation of compounds from G. quadrifaria *stem bark.* The ground bark (300 g) was extracted successively with petrol and Me_2CO. The concentrated petrol extract was shaken with 5% Na_2CO_3 and the aq. layer acidified and extracted into EtOAc. CC of the EtOAc fraction over Si gel gave **1** (12 mg) on elution with EtOAc–petrol (3:47). The

Me_2CO extract was concentrated and ppted with Et_2O (50% dilution). The supernatant was concentrated and subjected to CC over acid washed Si gel. Elution with MeOH–$CHCl_3$ (1:49) gave **2** (460 mg) identical in all respects (UV, IR, ^1H NMR, ^{13}C NMR, TLC) with an authentic sample [6]. Further elution with MeOH–$CHCl_3$ (3:47) gave **3** (945 mg) similarly identical with an authentic sample [6]. The identity of **2** and **3** was further confirmed by MeI–K_2CO_3 methylation to morelloflavone heptamethyl ether which was again identical with an authentic sample (EIMS, ^1H NMR).

Identification of 1,3,5-*trihydroxy*-4,8-*di*-(3′,3′-*dimethyl*-*allyl*)*xanthone* (**1**). Pale yellow needles from petrol–EtOAc, mp 168–169°. Found: $[M]^+$ 380. 1616; $C_{23}H_{24}O_5$ requires 380.1624. UV λ_{max} nm (log ϵ): 251 (4.43), 260 *sh* (4.39), 273 *sh* (4.31), 330 (4.10); (+$AlCl_3$) 258, 273 *sh*, 295, 358; IR ν_{max} cm^{-1}: 3380, 2920, 1635, 1580, 1410; ^1H NMR (Me_2CO-d_6): δ 1.63 (3H, *s*, =C–Me), 1.72 (6H, *s*, 2×=C–Me), 1.82 (3H, *s*, =C–Me), 3.59 (2H, *d*, J = 10 Hz, H-1′), 4.00 (2H, *d*, J = 10 Hz, H-1″), 5.40 (2H, *t*, J = 10 Hz, H-2′ and H-2″), 6.36 (1H, *s*, H-2), 7.09, 7.23 (2H, AB*q*, J = 9 Hz, H-6 and H-7); EIMS *m/z* (rel. int.): 380 $[M]^+$ (59), 337 $[M-C_3H_7]^+$ (100), 325 $[M-C_4H_7]^+$ (12), 269 $[M-C_8H_{15}]^+$ (17); Gibbs test [20]—negative.

Acetylation of **1**. **1** (6 mg) was dissolved in pyridine (1 ml) and treated with Ac_2O (1.5 ml) at 40° for 12 hr. Normal work-up gave the triacetate as an amorphous white solid from petrol (bp 60–80°), mp 152–155°. Found: $[M]^+$ 506.1942; $C_{29}H_{30}O_8$ requires 506.1940; UV λ_{max} nm: 243 *sh*, 283, 332; IR ν_{max} cm^{-1}: 2960, 2940, 1680, 1635, 1600, 1540; ^1H NMR: δ 1.72 (3H, *s*, =C–Me), 1.76 (6H, *s*, 2×=C–Me), 1.83 (3H, *s*, =C–Me), 2.34, 2.38, 2.45 (3×3H, 3×*s*, 3×COMe), 3.60 (2H, *d*, H-1′), 4.04 (2H, *d*, H-1″), 5.38 (2H, *t*, H-2′ and H-2″), 6.94 (1H, *s*, H-2), 7.30, 7.48 (2H, AB*q*, J = 9 Hz, H-7 and H-6).

Isolation of compounds from G. staudtii *stem bark*. The ground bark (48 g) was extracted with petrol, then Me_2CO. Prep. TLC of the concd petrol extract on Si gel (petrol–EtOAc 7:3) gave **4** (R_f 0.57, 8 mg) and **5** (R_f 0.33, 37 mg). The identity of **5** was confirmed by direct comparison (UV, IR, ^1H NMR, EIMS, mmp) with an authentic sample [7].

Identification of rheediaxanthone-A (**4**). Yellow clusters from petrol–EtOAc, mp 245–247° (lit. [18] 259–261°). Found: $[M]^+$ 392.1249; $C_{23}H_{20}O_6$ requires 392.1260; UV λ_{max} nm: 268 *sh*, 278, 300 *sh*, 333; (+NaOH) 269 *sh*, 287, 313, 347 (+$AlCl_3$) 268 *sh*, 286, 314 *sh*, 356; IR ν_{max} cm^{-1}: 3380–3340, 1645, 1460, 1440; ^1H NMR: δ 1.46 (6H, *s*, CMe_2), 1.49 (6H, *s*, CMe_2), 5.73, 6.94 (2H, AB*q*, J = 8 Hz, H-3′ and H-4′), 5.90, 6.54 (2H, AB*q*, J = 8 Hz, H-3″ and H-4″), 6.16 (1H, *s*, H-2), 7.45 (1H, *s*, H-8); EIMS *m/z* (rel. int.): 392 $[M]^+$ (55), 377 $[M-CH_3]^+$ (100); Gibbs test [20]—negative.

Acetylation of **4**. **4** (4 mg) was acetylated by the same procedure as **1** and gave the diacetate as an oil. Found: $[M]^+$ 476.1486; $C_{27}H_{24}O_8$ requires 476.1471; IR ν_{max} cm^{-1}: 1725, 1638. ^1H NMR: δ 1.46 (6H, *s*, CMe_2), 1.49 (6H, *s*, CMe_2), 5.90, 6.82 (2H, AB*q*, J = 8 Hz, H-3′ and H-4′), 5.91, 6.51 (2H, AB*q*, J = 8 Hz, H-3″ and H-4″), 6.49 (1H, *s*, H-2), 7.71 (1H, *s*, H-8).

Analysis of the seeds of G. quadrifaria. The ground seeds (1 g) were extracted with petrol then Me_2CO. TLC of the Me_2CO extract (3 systems [21]) revealed the presence of **2** and **3**.

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REFERENCES

1. Rezende, C. M. da M. and Gottlieb, O. R. (1973) *Biochem. Syst. Ecol.* **1**, 111.
2. Locksley, H. D. (1973) *Forschr. Chem. Org. Naturst.* **30**, 207.
3. Rama Rao, A. V., Venkatswamy, G. and Pendse, A. D. (1980) *Tetrahedron Letters* 1975.
4. Crichton, E. G. and Waterman, P. G. (1979) *Phytochemistry* **18**, 1553.
5. Waterman, P. G. and Crichton, E. G. (1980) *Phytochemistry* **19**, 1187.
6. Waterman, P. G. and Crichton, E. G. (1980) *Phytochemistry* **19**, 2723.
7. Waterman, P. G. and Crichton, E. G. (1980) *Planta Med.* **40**, 351.
8. Hussain, R. A., Owegby, A., Parimoo, P. and Waterman, P. G. (1981) *Planta Med.* **44**, 78.
9. Hussain, R. A. and Waterman, P. G. (1982) *Phytochemistry* **21**, 1393.
10. Oliver, D. (1868) *Flora of Tropical Africa*, Vol. 1, p. 164. London.
11. Hutchinson, J. and Dalziel, J. M. (1954) *Flora of West Tropical Africa*, Vol. 1, p. 290. Crown Agents, London.
12. Owen, P. J. and Scheinmann, F. (1974) *J. Chem. Soc. Perkin Trans.* 1, 1018.
13. Mesquita, A. A. L., Correa, D. D. B., Gottlieb, O. R. and Magalhaes, M. T. (1968) *Analyt. Chim. Acta* **42**, 322.
14. Sen, A. K., Sarkar, K. K., Majumder, P. C., Banerji, N., Uvsvuori, R. and Hase, T. A. (1980) *Phytochemistry* **19**, 2223.
15. Govindachari, T. R., Kalyanaraman, P. S. and Muthukumaraswamy, N. (1971) *Tetrahedron Letters* 3919.
16. Dahanayaka, M., Kitagawa, I., Somanathan, R. and Sultanbawa, M. U. S. (1974) *J. Chem. Soc. Perkin Trans.* 1, 2510.
17. Somanathan, R. and Sultanbawa, M. U. S. (1972) *J. Chem. Soc. Perkin Trans.* 1, 1935.
18. Markham, K. R. (1964) *Tetrahedron* **20**, 991.
19. Delle Monache, F., Botte, B., Nicoletti, M., Coelho, J. S. de B. and Lyra, F. D. de A. (1981) *J. Chem. Soc. Perkin Trans.* 1, 484.
20. Gibbs, H. D. (1927) *J. Biol. Chem.* **72**, 649.
21. Hussain, R. A. (1981) Ph.D. thesis, University of Strathclyde.