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MANNIFLAVANONE, A NEW 3,8-LINKED FLAVANONE DIMER FROM THE STEM BARK OF *GARCINIA MANNII*

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Key Word Index—*Garcinia mannii*; Guttiferae; 3,8-linked biflavanones; manniflavanone; ^{13}C NMR; xanthoae; xanthochymol.

Abstract—A new biflavanone has been isolated from the stem bark of *Garcinia mannii* (Guttiferae) and identified as I-3′-II-3,3′-I-4′-II-4′-I-5-II-5-I-7-II-7-nonahydroxy-I-3-II-8-biflavanone. Structural assignments for this compound, which has been named manniflavanone, were made on the basis of spectral studies and simple degradation. The complex xanthone derivative xanthochymol has also been isolated.

INTRODUCTION

The large tropical genus *Garcinia* L. (Guttiferae), common in both Africa and Asia, is well known as a source of both xanthones [1] and biflavonoids [2]. The latter are commonly characterized by linkage of the monomers between C-3 and C-8 [3]. *Garcinia mannii* Oliv. is a rain forest tree of west tropical Africa [4] and is of particular note in the wet coastal forests of Cameroon where it often occurs in dense stands (Gartlan, J. S., unpublished data). The twigs and small adventitious roots have been observed to be used as chew-sticks (Waterman, P. G., personal observation) and the dried, powdered, root-bark is reported to be a cure for severe diarrhoea and dysentery [5]. As part of a chemical study of sympatric species of Guttiferae from the Douala-Edea Forest Reserve of West Cameroon [6], we have undertaken the analysis of the thick, lactiferous, stem-bark of this species. This has resulted in the isolation of the new biflavanone manniflavanone and the assignment of structure (**2a**) to this compound.

RESULTS AND DISCUSSION

Initial extraction of the ground bark with petrol followed by column chromatography of the extract over silica gel gave three compounds. Two of these, eluted from the column as a mixture, were identified as sitosterol and stigmasterol by GLC and MS. The third compound had an empirical formula $C_{38}H_{50}O_6$ and was found, on direct comparison with an authentic sample, to be identical with xanthochymol (**1**), a xanthone derivative previously isolated from the fruit of *Garcinia xanthochymus* [7, 8].

Further extraction of the bark with acetone yielded a yellow gum which, on repeated precipitation from acetone with diethyl ether, eventually gave a pale yellow solid that appeared homogeneous by TLC. This optically active material represented approximately 10% of the dry weight of the bark. Attempts to obtain accurate mass measurement of a molecular ion proved unsuccessful with EI-MS, presumably because of the low volatility of the compound. The results of FD-MS were confusing and suggested possible molecular ions at *m/e* 572, 588 or 590. Elemental analysis indicated an empirical formula $C_{30}H_{22}O_{13}$ (MW 590). These data suggested, in view of our knowledge of *Garcinia* chemistry, that the compound was probably a biflavonoid.

The simple UV spectrum which exhibited a single maximum at 293 nm was in close agreement with those previously recorded for biflavanones of the 'GB'-series (**2b–2e**) and kolaflavanone (**2f**) but not with mixed flavanone–flavone dimers [2, 9, 10]. On addition of alkali, the UV spectrum underwent a pronounced bathochromic shift indicating the general phenolic nature of the compound. A similar shift with NaOAc suggested free 7-hydroxy group(s) [11]. The intense IR absorptions at 3300 and 1640 cm^{-1} were also in close agreement with published data on biflavanones [2].

The 60 MHz ^1H NMR spectrum run at room temperature in Me_2CO-d_6 gave, for the most part, only ill-defined resonances. Salient features were two sharp singlets at δ 11.64 and 12.31 for two C-5 hydroxyl groups H-bonded to C-4 carbonyls, signals of 3H and 6H respectively for A- and B-ring aromatic protons, and two AB quartets ($J \simeq 12$ Hz) for the C-ring protons of C-3 substituted flavanones. The latter were partially hidden by a broad signal which could be attributed to some of the remaining hydroxyl groups.

As had been previously observed for the 'GB'-series [9], a ^1H NMR spectrum run in DMSO-d_6 at elevated temperature (125°, 100 MHz) gave well-defined signals. The A-ring protons were resolved into an AB-quartet ($J = 2$ Hz) for *meta*-coupling between C-6 and C-8 together with a sharp singlet. The inference drawn from this observation was that one of the linkages between the monomers came through either C-6 or C-8. The B-ring protons formed a complex series of signals showing both *ortho*- and *para*-coupling but without the simplicity of the AA′XX′ pattern associated with *para*-substitution of flavonoids [2, 12]. It was therefore concluded that both B-rings were likely to carry at least two substituents. Of the two AB quartets for the C-rings that centred at δ 4.94 and 4.16 was in close agreement with anticipated resonances for a 3-hydroxyflavanone [2, 12]. The other quartet, centred at δ 5.54 and 4.55, can be assigned to a

C-ring in which linkage to the second monomer is occurring.

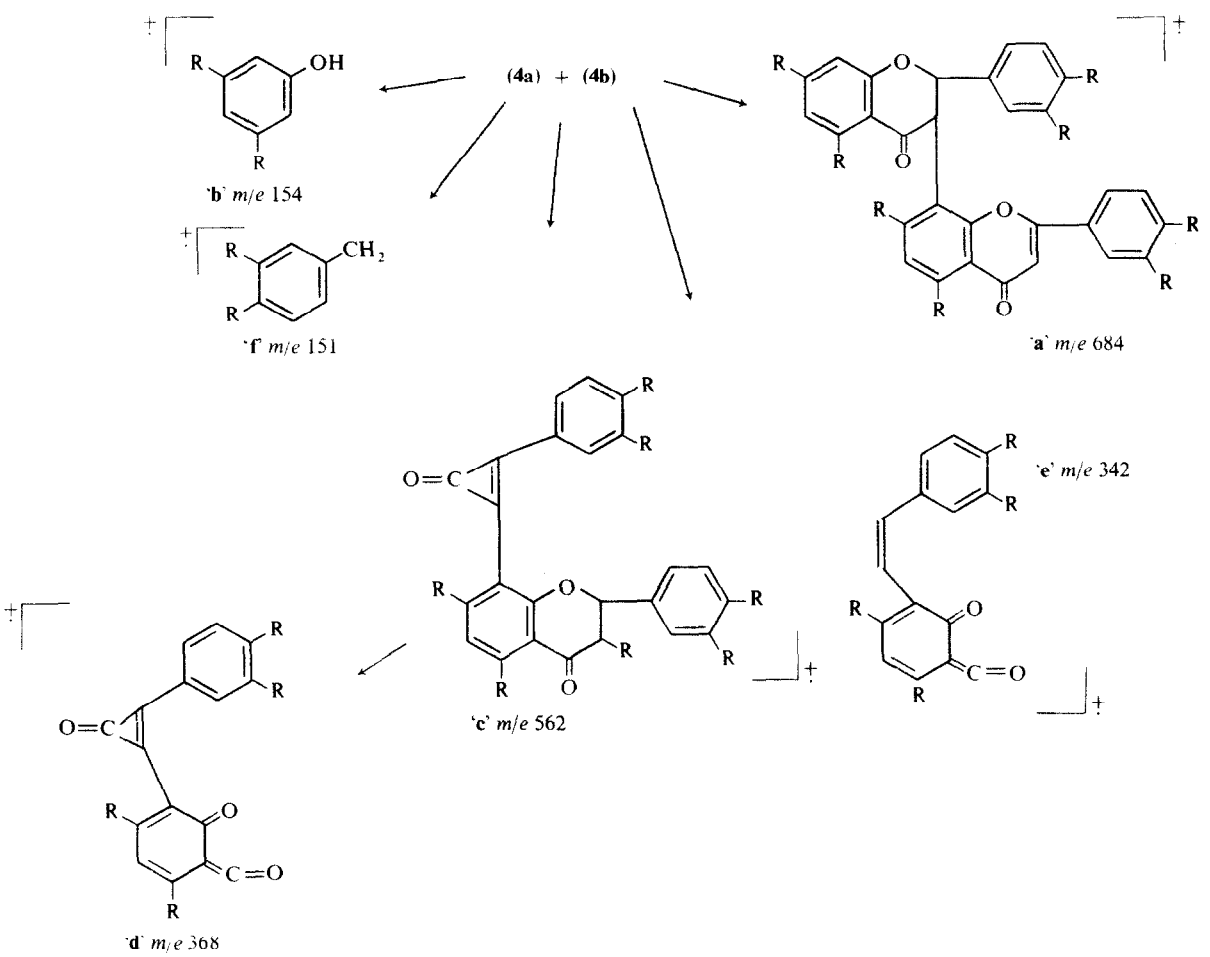
The conclusion from ^{1}H NMR analysis is therefore that the compound is a 3/8 (or 6)-linked biflavanone. Assuming from the elemental analysis the presence of nine hydroxyl groups, one must be assigned to II-3 and the others seem likely to occur symmetrically, two in each A-ring and two in each B-ring. This symmetry was substantiated by alkaline hydrolysis which yielded only phloroglucinol and 3,4-dihydroxybenzoic acid.

To enable MS studies to be made the compound was subjected to exhaustive methylation. This process resulted in the formation of a number of compounds which were separated by column chromatography over silica gel. The major component ($C_{39}H_{40}O_{13}$) exhibited, in the ^{1}H NMR spectrum, a sharp singlet at δ 8.03. This resonance is typical of a chalcone and suggested that ring opening had occurred to yield the flavanone–chalcone dimer (**3**) which had then become fixed by methylation. The molecular ion at *m/e* 716 suggested that no methylation had been achieved at the II-C-3-OH substituent. No further investigations were performed on this compound.

The second derivative of methylation was a mixture, the ions at *m/e* 716 ($C_{39}H_{40}O_{13}$) and 702 ($C_{38}H_{38}O_{13}$) being attributed to the fully methylated (**4a**) and II-3-demethyl (**4b**) biflavanones, respectively. This was confirmed by a ^{1}H NMR spectrum which revealed a resonance at δ 3.55 for the shielded II-3-OMe of *ca* 40% of the required amplitude. The fragmentation of the mixture followed the pattern previously noted [2, 9] for biflavanones (Scheme 1). The major fragment (**a**) at *m/e* 684 may be the product of loss of water from (**4b**) or of methanol from (**4a**). Loss of ring I-A gave ions '**b**' and '**c**' with the latter then undergoing RDA to yield '**d**' in low intensity. Alternatively two RDA fragmentations on **4a** yielded ion '**e**'. The base peak '**f**' at *m/e* 151 further confirmed the disubstitution of both B-rings. These data were in agreement with those anticipated for structure (**2a**).

Direct FD-MS of unmodified (**2a**) gave results in agreement with those anticipated. The ions at *m/e* 588 and 572 must be formed by facile loss of 2H and H_2O from the parent. The major fragment normally observed was at *m/e* 126 (≡'**b**'). A series of fragments between *m/e* 464 and 446 can be attributed to formation of '**c**' followed by loss of 2H or H_2O. Ion '**e**' (at *m/e* 286) was also a major feature of the FD-MS spectrum.

Recently ^{13}C NMR of biflavanones have been reported for the first time [10, 13]. The spectrum of (**2a**) was obtained (Table 1) in the course of these investigations and found to be in close agreement with those published previously. The most significant feature was the occurrence

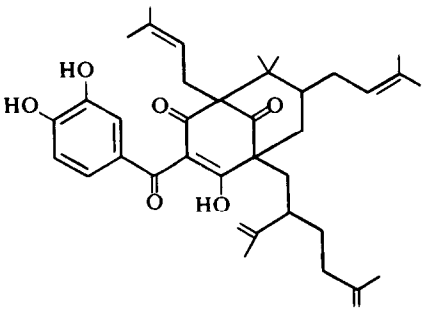


Scheme 1. EI-MS Fragmentation of nonamethylmanniflavanone (**4a**) and octamethylmanniflavanone (**4b**). R = OMe.

Table 1. ^{13}C chemical shifts of manniflavanone

Signal (ppm)	Assignments
48.90	I-C-3
73.11	II-C-3
82.63, 84.03	I-C-2, II-C-2
95.68, 96.83	I-C-6, I-C-8, II-C-6
102.41, 102.66	I-C-4a, II-C-4a, II-C-8
115.45, 115.70	I-C-2′, I-C-5′, II-C-2′, II-C-5′
120.25	I-C-6′, II-C-6′
129.47	I-C-1′, II-C-1′
145.00, 145.18, 145.97	I-C-3′, I-C-4′, II-C-3′, II-C-4′
161.56, 163.99, 164.90, 165.20, 166.78	I-C-5, I-C-7, I-C-8a, II-C-5, II-C-7, II-C-8a
197.72	I-C-4, II-C-4

Solvent: Me_2CO-d_6.



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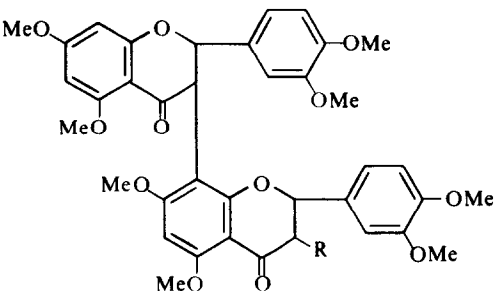
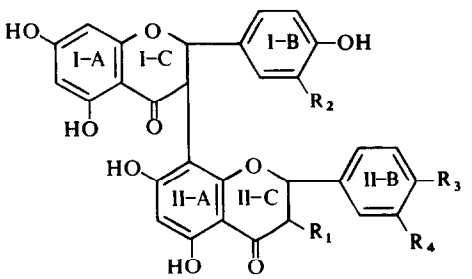
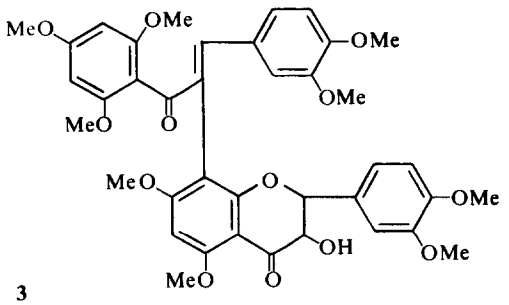


4a R = OMe
4b R = OH



Manniflavanone (**2a**) $R_1 = R_2 = R_3 = R_4 = OH$
GB-1 (**2b**) $R_1 = R_3 = OH$; $R_2 = R_4 = H$
GB-1a (**2c**) $R_3 = OH$; $R_1 = R_2 = R_4 = H$
GB-2 (**2d**) $R_1 = R_3 = R_4 = OH$; $R_2 = H$
GB-2a (**2e**) $R_3 = R_4 = OH$; $R_1 = R_2 = H$
Kolaflavanone (**2f**) $R_1 = R_4 = OH$; $R_3 = OMe$; $R_2 = H$



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of a series of resonances between 145 and 146 ppm confirming the presence of *ortho*-substituted aromatic carbons. This, together with characteristic resonances for 2′, 5′ and 6′ carbons of the B-rings [14], again confirmed the 3′,4′-dihydroxy substitution patterns of those rings.

The final problem of whether the linkage between the monomers occurred through C-6 or C-8 was also resolved by ^{13}C NMR. It has been shown [13, 15] that the resonances of sterically hindered OMe substituents of flavonoids occur above 59.5 ppm as compared with 55–57 ppm for normal substituents. In **4a** all aromatic OMe substituents were observed below 57 ppm. If linkage had been through C-6 then the 5-OMe group of that monomer would have been sterically hindered and consequently deshielded. The linkage must therefore be through C-8, thus permitting the structure of manniflavanone to be finally formulated as **2a**.

Manniflavanone is only the sixth 3/8-linked biflavanone to be reported and the first to carry this degree of oxidation. To date these compounds are restricted in their distribution to genera of the Guttiferae *sensu strictu*. The extremely high yield of this compound demonstrates the trend, noted previously in *Mammea africana* Sabine (Guttiferae) [6] and *Popowia cauliflora* Chipp (Annonaceae) [15], for species from the impoverished soils of the Douala-Edea Forest Reserve to be particularly rich in secondary metabolites. This phenomenon, which has been noted on a community basis for tannins [16], is in agreement with Janzen's hypothesis that impoverished soils will tend to lead to an increased emphasis on secondary metabolites [17]. In the case of *G. mannii*, the

'apparency' [18] caused by its clustering habit may also have influenced optimum levels of secondary metabolites.

EXPERIMENTAL

UV spectra were run in EtOH and IR spectra as KCl discs. ^{1}H NMR spectra were run at 60 MHz in Me_2CO-d_6 or at 100 MHz (elevated temp.) in DMSO-d_6. ^{13}C NMR spectra were run at 25.1 MHz in DMSO-d_6 using FT. TMS was employed as internal standard for all NMR studies. EI-MS were obtained at 70 eV and elevated temp.; FD-MS at 80° using an accelerating voltage of 3 kV. Mps are uncorr. Petrol refers to the bp 40–60° fraction unless otherwise stated.

Plant material. Bark of *Garcinia mannii* Oliv. was collected in the Douala-Edea Forest Reserve, Cameroon, in the summer of 1976. A voucher, P. G. Waterman and D. McKey 877, has been deposited at the Herbarium of the Royal Botanic Gardens, Kew.

Isolation of compounds. Ground bark (432 g) was extracted with petrol and then Me_2CO. The conc petrol extract was applied to a column of Si gel and eluted with petrol (bp 60–80°) containing increasing amounts of EtOAc. Eluates were checked by TLC. Elution with 1% EtOAc yielded a mixture of sitosterol and stigmasterol (5 mg). Further elution with 2% EtOAc yielded a third component which fluoresced yellow on Si gel and gave a colour reaction with $FeCl_3$. This material was purified by prep. TLC (Si gel, 1 mm, petrol (bp 60–80°)–EtOAc 3:2) to give xanthochymol (**1**) (36.4 mg). On concn, the Me_2CO extract gave a gum which, after repeated precipitation from Et_2O, finally gave from the ethereal solution a yellow solid which appeared, by TLC, to be pure manniflavanone (**2a**). The yield of 44.93 g represented approximately 10.4% of the dry weight of the bark.

Identification of isolated compounds. Sitosterol and stigmasterol. MS gave M^+ 414.3847; $C_{29}H_{50}O$ requires 414.3861 for sitosterol and M^+ 412.3697; $C_{29}H_{48}O$ requires 412.3705 for stigmasterol. Both compounds were identical by TLC and GLC (SE-30 column at 240°) with authentic samples.

Xanthochymol (**1**). Pale yellow crystals, mp 76–80°. MS: Found; M^+ 602.3610; $C_{38}H_{50}O_6$ requires 602.3607. Identical with an authentic sample of xanthochymol [9] by UV, IR, MS and TLC.

Manniflavanone (**2a**). Obtained as a yellow amorphous solid, becoming sticky and melting between 220 and 225°. UV λ_{max} nm: 293, 335 (sh) (log ε 4.73); λ_{max}^{NaOH} nm: 242 (sh), 335; λ_{max}^{NaOAc} nm: 335. IR ν_{max} cm^{-1}: 3500–3000 (broad, OH); 1640 (C=O); 1520, 1455. ^{1}H NMR (60 MHz): δ 3.60–4.80 (broad OH resonance), 4.84, 5.63 (2H, ABq, J = 12 Hz, I-2-H and I-3-H), 4.25, 4.99 (2H, ABq, J = 12 Hz, II-2-H and II-3-H), 6.01 (3H, ABq overlaid by *s*, A-ring), 6.72–7.03 (6H, *m*, B-ring), 11.64, 12.31 (2H, 2 × *s*, both replaceable by D_2O, I-5-OH and II-5-OH): (100 MHz, 125°): δ 4.55, 5.54 (2H, ABq, J = 12 Hz, I-H-2 and I-H-3), 4.16, 4.94 (2H, ABq, J = 12 Hz, II-H-2 and II-H-3), 5.88 (2H, ABq, J = 2 Hz, I-6-H and I-8-H), 5.95 (1H, *s*, II-H-6), 6.55–6.93 (6H, *m*, B-ring). (Found: C, 59.66; H, 4.53. $C_{30}H_{22}O_{13}$ H_2O requires: C, 59.21; H, 3.95%). EI-MS: no parent ion, *m/e* 126 ($C_6H_6O_3$) was the only significant fragment. FD-MS: *m/e* 590, 588, 572, 464, 462, 448, 446, 436, 434, 326, 286, 224, 223, 126, 110. ^{13}C NMR: see Table 1. $[\alpha]_D^{24}$ −13.48° (*c* 1.00, Me_2CO).

Methylation of manniflavanone. **2a** (600 mg) was dissolved in 600 ml dry Me_2CO and MeI (12 ml) and K_2CO_3 (12 g) added. The mixture was refluxed for 24 hr with the addition of further MeI (3 ml) and K_2CO_3 (6 g) after 8 hr. The mixture was filtered and the residue washed with hot Me_2CO. Isolation of products was achieved by column chromatography over Si gel eluting with $CHCl_3$ containing increasing quantities of Me_2CO. This yielded the chalcone derivative **3** (100 mg) followed by a mixture of nonamethyl- and octamethylmanniflavanone (**4a** and **4b** respectively, 120 mg).

Chalcone (**3**). A cream amorphous solid melting between 135 and 140°. UV λ_{max} nm: 228, 285, 315. IR ν_{max} cm^{-1}: 1650 (C=O). MS: Found: M^+, 716.2447: $C_{39}H_{40}O_{13}$ requires 716.2469. ^{1}H NMR (60 MHz): δ 8.03 (1H, *s*, α-H).

Nonamethylmanniflavanone (**4a**) *and octamethylmanniflavanone* (**4b**). A pale yellow solid. UV λ_{max} nm: 228, 284, 315 (sh), not changed by addition of alkali. IR ν_{max} cm^{-1}: 3450 (sharp, OH), 1685, 1620, 1525, 1470. MS: Found, M^+ 716.2471; $C_{39}H_{40}O_{13}$ requires 716.2469 (for **4a**) and M^+ 702. 2286, $C_{38}H_{38}O_{13}$ requires 702.2312 (for **4b**). ^{1}H NMR (60 MHz): δ 3.60 (OMe at II-3), 3.78–3.84 (OMe), 4.20–4.50 (*m* for H at II-3 of **4a** and **4b**), 5.02 (*d* for II-2-H, J = 12 Hz), 4.70–5.70 (2H, ABq, J = 12 Hz, I-2-H and I-3-H), aromatic H as before. MS: *m/e* 716 (1.4%), 702 (0.2), 684 (60.1), 562 (32.6), 516 (30.6), 504 (72.5), 369 (12.5), 368 (1.0), 342 (13.6), 195 (45.8), 181 (99.3), 154 (55.1), 151 (100). ^{13}C NMR (25.1 MHz): δ 56.18, 56.55 (OMe).

Alkaline degradation of manniflavanone. **2a** (126 mg) was refluxed, under N_2, with 10 ml of a soln of 75% aq. KOH plus 0.25 ml MeOH for 16 hr. The reaction mixture was cooled, acidified with conc HCl and extracted into Et_2O (4 × 15 ml). The ethereal extract was shaken with 8% aq. Na_2CO_3 (3 × 20 ml) and the bulked aq. extract acidified and re-extracted into Et_2O. Phloroglucinol was identified (direct comparison with authentic sample) in the final aq. extract. 3,4-Dihydroxybenzoic acid was similarly confirmed in the final ethereal extract.

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