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PHENOLIC COMPOUNDS FROM THE HEARTWOOD OF *GARCINIA MULTIFLORA*

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Key Word Index—*Garcinia multiflora* Champ.; Guttiferae; apigenin; 1,3,6,7- tetra-hydroxyxanthone; optically active biflavanoids; (−)-GB-1a; (+)-GB-2a; (+)-volkensiflavone; (+)-morelloflavone, i.e. (+)-fukugetin.

Plant. Garcinia multiflora Champ (Guttiferae). *Uses.* Furniture and cabinet work, yellow mordant dyestuff and medical. *Previous work.* On biflavanoids of stem barks [1]. On sister species; barks [1,2–5], heartwood [1,4,6–14], root [15], leaves [1,4], seed [16], and latex [17,18].

Present work. As part of a general screening programme of Formosan flora for antitumor compounds, we report the isolation and identification of seven phenolic compounds from the heartwoods of *G. multiflora*. This is the first reported isolation of apigenin and 1,3,6,7-tetrahydroxyxanthone from the subfamily Clusioideae.

The MeOH-extract of the dried heartwood-shavings was first extracted with C_6H_6, then followed by EtOAc. The EtOAc soluble part was chromatographed on a column of SiO_2 eluting with C_6H_6–EtOAc (1:2) giving three Fractions 1–3, then with EtOAc giving Fraction 4. Fraction 1 was rechromatographed on a polyamide column, eluting with 70% aq. MeOH giving four Fractions 1a–1d. As the Fraction 1b and 1c were shown to be a mixture by TLC, the Fraction 1b was rechromatographed on a column of cellulose, eluting with 40% aq. MeOH giving two Fractions $1b_1$ and $1b_2$. Fraction 1c was subjected to repeated preparative-scale TLC on silica gel with C_6H_6–pyridine–formic acid (20:5:1) yielding Fraction $1c_1$. Evaporation of each Fraction (1a, $1b_1$, $1b_2$, $1c_1$, 1d, 2 and 3) and recrystallization from MeOH afforded seven compounds (A–G) which were characterized as apigenin, 1,3,6,7-tetrahydroxyxanthone, GB-1a, GB-2a, (+)-volkensiflavone, (+)-morelloflavone and (±)-morelloflavone respectively. The constitutions of the Fraction 4 are under investigation. All the seven compounds (A–G) gave a red colour in the Mg–HCl test. The data of their UV spectra were shown in Table 1.

Compound A. Yellow crystals, m.p. 344–346°; triacetate, m.p. 183–185° [19], M^+ *m/e* 396; UV

Table 1. UV spectra and R_f values of compounds A–F from the heartwood of *Garcinia multiflora*

	Compound					
λ_{max} (nm)	A (Apigenin)	B (1,3,6,7-Tetra-hydroxyxanthone)	C (GB-1a)	D (GB-2a)	E (Volkensiflavone)	F (Morelloflavone)
MeOH (log ε)	222 (4·26) 266 (4·27) 295 sh (4·11) 335 (4·28)	237 (4·40) 254 (4·55) 312 (4·24) 361 (4·12)	231 (3·38) 292 (3·50) 329 sh (3·0)	226 (3·54) 255 sh (3·93) 291 (4·37) 335 sh (3·62)	215 (4·73) 275 sh (4·49) 289 (4·53) 330 sh (4·33)	208 (4·81) 225 (4·70) 261 (4·36) 274 (4·45) 286 (4·47) 337 (4·29)
$AlCl_3$, MeOH	226, 276, 301, 343, 383	210, 231, 266, 285 sh, 317, 351, 418	232, 314, 392	226, 255 sh, 310, 375 sh	224, 282 sh, 308, 345, 385	223, 275, 307, 428
$AlCl_3$/HCl, MeOH	227, 278, 300, 342, 385	204, 230, 262, 280 sh, 336, 402	230, 312, 390	218, 254 sh, 308, 340 sh	224, 283 sh, 304, 344, 384	231, 262, 284, 354, 390
NaOAc, MeOH	221, 269, 297, 343	235, 256, 316, 370	233, 293, 329	223, 256 sh, 290, 329	275 sh, 284, 318	256, 277, 320 387
NaOAc-H_3BO_3, MeOH	225, 268, 297 sh, 335	224 sh, 259, 317, 368	231, 292, 328 sh	226, 256 sh, 293, 340 sh	275 sh, 288, 330 sh	263, 286, 373
$R_f \times 100$	51, 07	36, 06	24, 47	18, 44	20, 34	09, 30

R_fs on Silica gel TLC, in C_6H_6–pyridine–HCO_2H (40:10:2), and PPC (Whatman No. 1), 15% HOAc.

(Table 1) identified as apigenin confirmed by comparison with an authentic sample (m.m.p., TLC, PPC, IR and NMR).

Compound B. Yellow crystals, m.p. >300°. Red with Mg–HCl and green with alcoholic $FeCl_3$. IR: 3300 (OH), 1650 (conj. CO), 1620, 1580, 1510 and 1485 cm^{-1} (arom.). UV (Table 1) indicates a xanthone bearing 1 or 8-OH and an *ortho*-dihydroxy group. NMR (DMSO-d_6) showed four OH groups at δ 13·30 (*s*, 1H) (OH-1) and 11·03–9·72 (*br*, 3H) (3 × OH); two isolated aromatic protons at δ 7·50 (*s*, 1H) (H-8) and 7·00 (*s*, 1H) (H-5); two aromatic protons as *meta*-coupled doublets (*J* 2 Hz) at δ 6·45 (1H) (H-4) and 6·27 (1H) (H-2). The above evidence suggested that B was 1,3,6,7-tetrahydroxyxanthone. This was supported by its acetate, m.p. 192–194°, M^+ *m/e* 428; NMR ($CDCl_3$); four acetoxyl protons appeared at δ 2·47 (*s*, 3H) (OAc-1) and 2·34 (*s*, 9H) (3 × OAc); four aromatic protons at δ 8·04 (*s*) (H-8), 7·42 (*s*) (H-5), 7·27 (*d*, *J* 2 Hz) (H-4) and 6·87 (*d*, *J* 2 Hz) (H-2). The structure was confirmed by comparison with an authentic sample and its acetate [20] (TLC, PPC, IR and NMR).

Compound C. Pale yellow crystals, m.p. 210–212°, $[\alpha]_D^{20}$ −8·0°. $C_{30}H_{22}O_{10}$, M^+ *m/e* 542. IR at 3300 (OH), 1640 (conj. CO), 1615 and 1520 cm^{-1} (arom.). The UV (Table 1), showed a flavanone with OH groups at 5- and 7-positions. NMR (acetone-d_6) showed protons of OH groups at δ 12·62 (*s*, 1H) (OH-5″), 12·40 (*s*, 1H) (OH-5) and 8·17–9·17 (*br*, 1H); 8 aromatic protons as two sets of A_2B_2 doublets (*J* 9 Hz) at δ 7·33 (2H) (H-2″′, 6″′), 6·90 (2H) (H-3″′, 5″′) and δ 7·25 (2H) (H-2′, 6′), 6·80 (2H) (H-3′, 5′); two aromatic protons as *meta*-coupled doublets (*J* 3 Hz) at δ 6·03 (1H) (H-8) and 5·93 (1H) (H-6); one isolated aromatic protons at δ 5·95 (*s*, 1H) (H-6″); two protons in the ring 1-C as *trans*-coupled doublets (*J* 12 Hz) at δ 5·77 (1H) (H-2) and 4·77 (1H) (H-3); three protons in the ring 2-C at δ 5·47 (*br*, 1H) (H-2″) and 3·07–2·63 (*m*, 2H) (H-3″). On methylation with Me_2SO_4–K_2CO_3 in dry acetone C gave a hexamethyl ether, m.p. 131–134°, M^+ *m/e* 626. IR 2990, 2930, 2900, 2830 (OMe); 1675 (flavanone CO), 1600, 1570 and 1515 cm^{-1} (arom.), and was transparent around 1650 cm^{-1} indicating no chalcone, although isomerization of flavanone to chalcone during methylation has been reported [10]. NMR $CDCl_3$ showed six OMe groups at δ 3·90 (*s*, 6H), 3·83 (*s*, 6H) and 3·80 (*s*, 6H); eight aromatic protons as two sets of A_2B_2 doublets (*J* 8 Hz) at δ 7·28 (2H) (H-2′, 6′), 6·82 (2H) (H-3′, 5′), 7·32 (2H) (H-2″′, 6″′), 6·90 (2H) (H-3″′, 5″′); 2 aromatic protons as *meta*-coupled doublets (*J* 2 Hz) at δ 6·17 (1H) (H-8), 6·08 (1H) (H-6); one isolated aromatic proton at δ 6·15 (*s*, 1H) (H-6″); two protons in the heterocyclic ring 1-C of a flavanone unit as doublets (*J*, 12 Hz) at δ 5·73 (1H) (H-2) and 4·70 (1H) (H-3); 3 protons in the ring 2-C of another flavanone unit as multiplets at δ 5·28 (1H) (H-2″) and 2·72 (2H) (H-3″). From the above data the structure of the *C* must be a binaringenin with a 3-6″ or 3-8″ linkage between the ring 1-C and the ring 2-A, which was confirmed as GB-1a (3-8″ linkage) by comparison with an authentic sample [10] (m.m.p., TLC, PPC, IR and NMR).

Compound D. Pale yellow powder, m.p. 214–216° (dec.), $[\alpha]_D^{20}$ +28° $C_{30}H_{22}O_{11}$, M^+ *m/e* 558, IR at 3300 (OH), 1645 (conj. CO), 1610 and 1520 cm^{-1} (arom.). NMR (acetone-d_6) showed seven OH groups at δ 12·43 (*s*, 1H) (OH-5″), 12·27 (*s*, 1H) (OH-5), 10·37–9·03 (*br*, 2H) and 8·87–7·83 (*br*, 3H); ten aromatic protons at δ 7·50–6·97 (7H) (H-2′, 6′, 5′, 2″′, 6″′, 3″′, 5″′) and 6·07 (*bs*, 3H) (H-6,8,6″); 2 protons in the heterocyclic ring 1-C as doublets (*J* 12 Hz) at δ 5·83 (1H) (H-2) and 4·83 (1H) (H-3); 3 protons in the ring 2-C at 5·43 (*br*, 1H) (H-2″) and 2·83 (*br*, 2H) (H-3″). Methylation gave a heptamethyl ether, m.p. 128–130°, M^+ *m/e* 656. IR 2970, 2930, 2900, 2830 (OMe), 1680 (flavanone CO), 1605, 1575 and 1520 cm^{-1} (arom.). NMR ($CDCl_3$) showed 7 OMe groups at δ 3·93 (*s*, 6H), 3·92 (*s*, 3H), 3·82 (*s*, 6H) and 3·78 (*s*, 6H); 3 protons in the heterocyclic ring 2-C of a flavanone unit at δ 2·83 (*m*, 2H) and 5·30 (*m*, 1H); 2 protons in the ring 1-C of another flavanone unit at δ 4·70 (*d*, *J* 12 Hz, 1H) and 5·73 (*d*, *J* 12 Hz, 1H); 3 aromatic protons at δ 6·05 (*d*, *J* 2 Hz, 1H) (H-6) and 6·13 (*b*, 2H) (H-8 and 6″); 7 protons in two phenyl rings at δ 7·25–6·67 (*m*, 7H). From these data the compound D was suggested to be GB-2a [10] (naringenin-3→8″-eriodictyol) which was confirmed by comparison with an authentic sample (TLC, PPC, IR and NMR).

GB-1a and GB-2a were previously isolated from the heartwoods [10] of *G. buchananii* and *G. eugeniifolia*, barks [1] of *G. spicata*, *G. multiflora* and *G. linii*, heartwoods and leaves of *G. xanthochymus* [1], but no information on their optical properties is available. It is noteworthy that GB-1a

and GB-2a isolated in the present investigation are (−) and (+) rotatory respectively.

Compound E. Pale yellow crystals m.p. 290–292°, $[\alpha]_D^{20}$ +1·6°. $C_{30}H_{20}O_{10}$, M^+ *m/e* 540. IR 3545, 3150 (OH), 1640 (conj. CO), 1610, 1590, 1560 and 1510 cm^{-1} (arom.). UV (Table 1) NMR (DMSO-d_6) showed 6 OH groups at δ 12·47 (*d*, 1H) (OH-5), 13·27 (*d*, 1H) (OH-5″), 11·83–10·0 (*br*, 3H) and 9·50 (*bs*, 1H); 8 aromatic protons as two sets of A_2B_2 doublets (*J* 9 Hz) at δ 7·13 (2H) (H-2′, 6′), 6·63 (2H) (H-3′, 5′) and 7·70 (2H) (H-2‴, 6‴), 6·87 (2H) (H-3‴, 5‴); 3 aromatic protons at δ 6·30 (*d*, *J* 2 Hz, 1H) (H-8), 6·22 (*bs*, 2H) (H-6, 6″); one isolated proton at δ 6·50 (*s*, 1H) (H-3″); 2 protons in the ring 1-C as doublets (*J* 12 Hz) at δ 5·80 (1H) (H-2) and 4·90 (1H) (H-3).

Methylation afforded hexamethyl ether, m.p. 258–260°, M^+ *m/e* 624. IR 2990, 2950, 2850 (OMe), 1680 (flavanone CO), 1645 (flavone CO), 1600, 1580, 1510 and 1490 (arom.). NMR of E (DMSO-d_6) revealed the presence of six OH groups at δ 13·27 (*d*, 1H), 12·47 (*d*, 1H), 11·83 10·0 (*br*, 3H), 9·50 (*bs*, 1H), whereas the signals of the aromatic regions were not readily resolved. NMR of E methyl ether ($CDCl_3$) showed 6 OMe groups at δ 3·93 (*s*, 3H), 3·87 (*s*, 3H), 3·83 (*s*, 6H), 3·77 (*s*, 3H) and 3·67 (*s*, 3H); 8 aromatic protons as 2 sets of A_2B_2 doublets (*J* 9 Hz) at δ 7·13 (2H) (H-2′, 6′), 6·63 (2H) (H-3′, 5′), 7·70 (2H) (H-2‴, 6‴) and 6·87 (2H) (H-3‴, 5‴); 2 aromatic protons as *meta*-coupled doublets (*J* 2 Hz) at δ 6·30 (1H) (H-8), 6·22 (1H) (H-6); one isolated aromatic protons at δ 6·23 (*s*, 1H) (H-6″); one proton in the pyranone of a flavone unit at δ 6·50 (*s*, 1H) (H-3″); 2 protons in the heterocyclic ring of a flavanone unit as doublets (*J* 12 Hz) at δ 5·80 (1H) (H-2) and 4·90 (1H) (H-3). From these data E was suggested to be volkensiflavone [12,15] (naringenin-3 → 8″-apigenin) which was confirmed by comparison with an authentic sample by the courtesy of Dr Scheinmann. The optical rotation of volkensiflavone, isolated from *G. volkensii* [12], *G. talboti* [15], *G. livingstonii* [4], and *G. xanthochymus* [1] as m.p. 250°, 300°, 267–268° and 290–293° respectively, was not mentioned except the last one which was reported to be optically inactive. Volkensiflavone isolated in this investigation is (+).

Compound F. Yellow powder, m.p. 249–250°, $[\alpha]_D^{20}$ +17°. $C_{30}H_{20}O_{11}$, M^+ *m/e* 556. UV (Table 1). IR 3350 (OH), 1645 (conj. CO), 1615, 1570, 1550 and 1510 cm^{-1} (arom.). NMR (DMSO-d_6) revealed the presence of seven OH groups at δ 12·43 (*s*, 1H), 13·27 (*s*, 1H), 11·53 (*br*, 1H), 10·97 (*br*, 1H), 10·0 (*br*, 1H), 9·65 (*br*, 1H) and 9·42 (*br*, 1H), whereas the signals of the aromatic and aliphatic regions were not readily resolved. These difficulties have been oversome by using acetone-d_6 as solvent, but in this case the signals of all the OH groups except the two hydrogen bonded OH-5 (δ 13·23 and 12·45) were not visible; four aromatic protons appeared as a set of A_2B_2 doublets (*J* 8 Hz) at δ 7·30 (2H) (H-2′, 6′) and 6·63 (2H) (H-3′, 5′); three aromatic protons in the 1,3,4-trisubstituted benzene ring at δ 7·52 (*m*, 2H) (H-2‴, 6‴) and 6·68 (*d*, *J* 8 Hz, 1H) (H-5‴); two aromatic protons as *meta*-coupled doublets (*J* 2 Hz) at δ 6·38 (1H) (H-8) and 6·10 (1H) (H-6); two isolated protons at δ 6·55 (*s*, 1H) (H-3″) and 6·08 (*s*, 1H) (H-6″); two protons in the ring 1-C as *trans*-coupled doublets (*J* 12 Hz) at δ 5·87 (1H) (H-2) and 5·07 (1H) (H-3). Methylation gave a heptamethyl ether, m.p. 212–215°. M^+ *m/e* 654. IR 1680 (flavanone CO) and 1645 (flavone CO). NMR ($CDCl_3$) showed seven methoxyl groups as singlets at δ 4·00 (3H), 3·97 (3H), 3·90 (6H), 3·85 (3H), 3·80 (3H), 3·73 (3H); four protons in the 1,4-disubstituted benzene ring as a set of A_2B_2 doublets (*J* 8 Hz) at δ 7·23 (2H) (H-2′, 6′) and 6·73 (2H) (H-3′, 5′); three protons in 1,3,4-trisubstituted benzene ring at δ 7·63 (*q*, *J* 9 Hz, 2 Hz, 1H) (H-6‴), 7·30 (*d*, *J* 2 Hz, 1H) (H-2‴) and 6·93 (*d*, *J* 9 Hz, 1H) (H-5‴); two protons in a flavanone unit as doublets (*J* 12 Hz) at δ 5·96 (1H) (H-2) and 5·03 (1H) (H-3); two aromatic protons as *meta*-coupled doublets (*J* 2 Hz) at δ 6·40 (1H) (H-8) and 6·27 (1H) (H-6); two isolated aromatic protons at δ 6·60 (*s*, 1H) (H-3″) and 6·30 (*s*, 1H) (H-6″). From these data F was suggested to be morelloflavone (naringenin-3→8″-luteolin) i.e. fukugetin [3,14]. The NMR, TLC and PPC of F were identical with those of an authentic sample of morelloflavone kindly supplied by Dr. Scheinmann, but its IR spectrum (KBr) was different which may be explainable on its being optically active [3]. The NMR, IR, TLC and m.m.p. of the heptamethyl ether of F were identical with those of an authentic sample kindly supplied by Professor Venkataraman.

Compound G. Yellow crystals, m.p. 280–281°, M^+ *m/e* 556, its UV and NMR spectra were identical with those of the above compound F. Thus it

was seen to be a mixture of (±) and (−)-morelloflavone.

EXPERIMENTAL

Extraction of Garcinia multiflora. A section of the heartwood of *G. multiflora* was collected from the No. 43 Compartment, Tawu, located near Taitung at height of 700 m by Prof. J. C. Liao, on 28 Jan., 1970.

The dried heartwood (shaving, 2·8 kg) was extracted 4× boiling MeOH. The extract (35 l.) was evaporated to yield a brown oily matter which was extracted with C_6H_6. The insoluble part was extracted with EtOAc. The EtOAc yielded a light brown solid (23 g) which was chromatographed on SiO_2 (500 g) eluting with C_6H_6–EtOAc (1:2) to give Fractions 1 (4 g), 2 (0·3 g) and 3 (1·7 g), and then eluting with EtOAc to give 4 (3·3 g). Fraction 1 was then rechromatographed on a column of polyamide (nylon 66, 200 g) eluting with 70% aq. MeOH to give Fractions 1a (0·1 g), 1b (0·6 g), 1c (0·35 g) and 1d (0·5 g). As the Fraction 1b contained two compounds shown by TLC, it was rechromatographed on a column of cellulose (30 g) eluting with 40% aq. MeOH to give Fractions $1b_1$ (0·15 g) and $1b_2$ (0·4 g). The Fraction 1c was separated by preparative TLC (silica gel) with C_6H_6–pyridine–formic acid (40:10:2) gave Fraction $1c_1$.

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