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Xanthones and triterpenoids from the bark of *Garcinia vilersiana*

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Abstract

The hexane extract of the bark of *Garcinia vilersiana* from Vietnam contained four triterpenoids (olean-12-ene-3β,11α-diol, lupeol, β-amyrin and oleanolic acid), and six xanthones (globuxanthone, subelliptenone H, subelliptenone B, 12b-hydroxy-des-D-garcigerrin A, 1-*O*-methylglobuxanthone and symphoxanthone). The structure of 1-*O*-methylglobuxanthone, the only novel compound, was determined using 1D and 2D NMR techniques and by correlation with globuxanthone.

Keywords: *Garcinia vilersiana*; Guttiferae; Xanthones; Triterpenoids; Isolation; Structure determination

1. Introduction

Garcinia vilersiana Pierre is a medium size tree, which can grow up to 15 m, and is indigenous to Indo-China. The bark is greenish yellow, and exudes a yellow resin. A preliminary study (Douk, 1965) of the leaves and trunk bark of this species reported that the leaves contained flavanones, whereas the trunk bark contained xanthones. However, none of the metabolites was identified.

2. Results and discussion

This examination of the hexane extract of the bark of *Garcinia vilersiana* led to the isolation of four widely occurring triterpenoids, olean-12-ene-3β,11α-diol (Ikuta, 1992), lupeol (Pereira, Domingues & Silva, 1996), β-amyrin (Knight, 1974) and oleanolic acid (Lin, Chung, Gan & Chiang, 1987), and five known xanthones, globuxanthone (**1**) (Iinuma, Ito, Tosa & Tanaka, 1996), subelliptenone H (**2**) (Iinuma, Tosa, Tanaka, Asai & Shimano, 1995), subelliptenone B (**3**) (Iinuma, Tosa, Tanaka, Shimano, Asai & Yonemori, 1994), 12b-hydroxy-des-D-garcigerrin A (**4**) (Iinuma et al., 1996), and symphoxanthone (**5**) (Minami, Kuwayama, Yoshizawa & Fukuyama, 1996).

1-*O*-Methylglobuxanthone (**6**), $C_{19}H_{18}O_5$, was obtained as pale yellow needles from acetone–hexane, mp. 145–146°C. A positive test with alcoholic ferric chloride revealed its phenolic nature. The ^{1}H- and ^{13}C-NMR spectra of (**6**) (see Section 3) had resonances for two free hydroxyl groups [δ_H 8.83 and 8.11 (1H each, *br s*, exchangeable with D_2O, 5-OH and 2-OH, respectively)], a 1,2,3-trisubstituted benzene ring [δ_H 7.66 (1H, *dd*, J = 7.8 and 1.7 Hz, H-8), 7.31 (1H, *dd*, J = 7.8 and 1.7 Hz, H-6) and 7.19 (1H, *t*, J = 7.8 Hz, H-7); δ_C 124.1 (*d*, C-7), 119.8 (*d*, C-6) and 116.9 (*d*, C-8)], an isolated aromatic proton [δ_H 7.40 (1H, *s*, H-3); δ_C 122.3 (*d*, C-3)], a methoxyl group [δ_H 3.90 (3H, *s*, 3-OMe); δ_C 62.2 (*q*, 3-OMe)], a 1,1-dimethylprop-2-enyl group [δ_H 6.46 (1H, *dd*, J = 17.0 and 10.6 Hz, H-12), 5.18 (1H, *dd*, J = 17.0 and 1.1 Hz, H-13*E*), 5.05 (1H, dd, J = 10.6 and 1.1 Hz, H-13Z) and 1.67 (6H, *s*, H_3-14 and H3-15); δ_C 148.7 (*d*, C-12), 111.5 (*t*, C-13), 41.6 (*s*, C-11) and 27.8 (*q* × 2, C-14 and C-15)]. There were also signals due to a conjugated carbonyl group [δ_C

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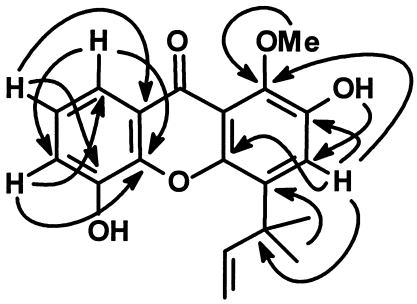


Fig. 1. HBMC correlations for (**6**).

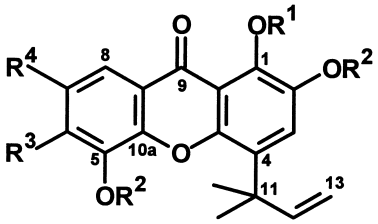
176.8, (*s*, C-9)] and eight substituted aromatic carbons [144.5 (C-1), 146.3 (C-2), 134.5 (C-4), 149.6 (C-4a), 147.4 (C-5), 124.2 (C-8a), 117.8 (C-9a), 145.6 (C-10a)], five of which were oxygenated. These data suggested that **6** was a dihydroxymethoxyxanthone with a 1,1-dimethylprop-2-enyl side chain. This was supported by the UV maxima at 248 (sh), 260, and 380 nm.

HMQC, HMBC and NOE spectroscopy were used in establishing the substitution pattern as well as the ^{1}H- and ^{13}C-NMR assignments for **6**. The carbonyl carbon [δ_C 176.8] was more shielded than usual [$\delta_C > 180$] and this was ascribed to the presence of a methoxyl at C-1. The same effect has been observed in other xanthone derivatives, e.g. 2,5-dihydroxy-1- methoxyxanthone [δ_C 175.4] and 2,5,6-trihydroxy-1-methoxy-4-(1,1-dimethylprop-2-enyl)xanthone [δ_C 175.3] (Ikeya, Sugawa, Okada & Mitsuhashi, 1991). The ^{13}C shift of the methoxyl group [δ_C 62.2] showed it to be *ortho*-disubstituted (Chaudhuri, Zymalkowski & Frahm, 1978) and that C-2 was therefore hydroxylated or alkylated. The HMBC correlations (see Fig. 1) were sufficient to show that the isolated aromatic proton was *ortho* to a hydroxyl and the 1,1-dimethylallyl group and that it was meta to the methoxyl. The other hydroxyl group was therefore attached to C-5. The question of the position of the alkyl group could not be solved by consideration of the observed HMBC correlations. Irradiation of the methoxyl protons gave rise to no NOE enhancement of any other signals, whilst saturation of the methyl protons of the 1,1-dimethylprop-2-enyl group caused enhancement of only H-3 (9%), H-12 (4%) and H-13*E* (4%). This suggested that the compound was 1-*O*-methylglobuxanthone (**6**), which is a novel natural product. Confirmation of the substitution pattern was obtained by methylation of **6** and of globuxanthone (**1**) to give, in each case, 1,2,5-tri-*O*-methylglobuxanthone (**7**).

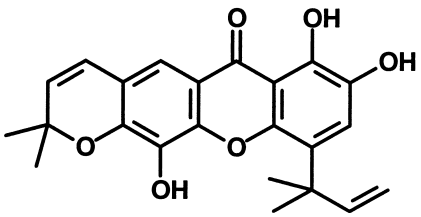
3. Experimental

3.1. General conditions

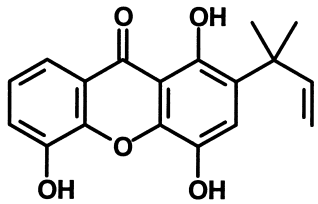
(Nguyen & Harrison, 1999; Leong, Harrison & Powell, 1999); GPC: Sephadex LH-20 ($CHCl_3$–MeOH, 1:1).



(1) R^1=R^2=R^3=R^4=H
(3) R^1=R^2=R^3=H, R^4=3-methylbut-2-enyl
(5) R^1=R^2=R^4=H, R^3=OH
(6) R^1=Me, R^2=R^3=R^4=H
(7) R^1=R^2=Me, R^3=R^4=H



(2)



(4)

3.2. Plant material

The bark (500 g) of *Garcinia vilersiana* was collected in south Vietnam and identified by Prof. Le Cong Kiet, Department of Botany, University of Ho Chi Minh City. A voucher specimen is retained in the Chemistry Department, National University of Singapore.

3.3. Extraction and isolation

The powdered material was extracted using continuous percolation with hot hexane. Concentration of the solution afforded 22 g of crude extract. The extract was fractionated using silica gel CC (step gradient of EtOAc in hexane) to give 13 frs. Frs which contained predominantly triglycerides (^{1}H-NMR) or which failed to give distinct spots on TLC were not further studied. Fr 4 (940 mg) was subjected to VLC (silica gel, hexane to 5% EtOAc-hexane as eluant) to furnish 6 frs. The major fr was separated by a combination of RP CC (C-18, MeOH–H_2O) and HPLC (C-18, Me_2CO–H_2O) to give olean-12-ene-3β,11α-diol (7.6 mg), lupeol (6.6 mg) and β-amyrin (10.3 mg). Fr 6 (1.9 g) was subjected to GPC followed by flash column chromatography (silica gel, 5–10% acetone–hexane) and HPLC (C-18, 80% MeOH–H_2O) to afford globuxanthone (**1**) (9.4 mg) and subelliptenone H (**2**) (6.2 mg). Fr 7 (1 g) was subjected to GPC, CC (silica gel, 15% acetone–hexane) and HPLC (silica gel, 15% acetone–hexane) to give subelliptenone B (**3**) (2.8 mg). Fr 8 (1.8 g) was separated by GPC to afford three frs. Fr 8A (365 mg) was purified by flash column chromatography (silica gel, $CHCl_3$) to give oleanolic acid (252 mg). Fr 8C was subjected to CC (silica gel, 20% EtOAc-hexane) followed by HPLC (silica gel, 20% EtOAc-hexane) to afford 12b-hydroxy-des-D-garcigerin (**4**) (12.1 mg). Fr 10 (3.9 g) was subjected to GPC followed by CC (silica gel, 2–5% EtOAc–C_6H_6) and HPLC (DIOL, 25% EtOAc-hexane) to furnish 1-*O-methylglobuxanthone* (**6**) (1.2 mg). CC (silica gel, $CHCl_3$) of fr 11 (3.2 g) followed by GPC gave symphoxanthone (**5**) (2.8 mg).

3.4. 1-O-Methylglobuxanthone (*6*)

Pale yellow needles, mp. 145–146°C (acetone–hexane); HREI-MS m/z 326.1176 ($C_{19}H_{18}O_5$ requires m/z 326.1154); UV λ_{max} nm (log ε): 248 (sh), 260 (4.3), 380 (3.4); EIMS m/z (rel. int.): 326 ($[M]^+$, 92), 308 ($[M-H_2O]^+$ 100), 379 (88), 285 (99), 239 (38), 137 (69), 69 (84), 43 (97); ^{1}H- and ^{13}C-NMR (see text).

3.5. 1,2,5-Tri-O-methylglobuxanthone (*7*)

Methylation of either **1** or **6** with MeI/K_2CO_3/Me_2CO gave 1,2,5-tri-*O*-methylglobuxanthone (**7**) as pale yellow needles, mp. 124–126°C (CH_2Cl_2–hexane), Lit. (Locksley, Moore & Scheinmann, 1966), 125.5°C. HREI-MS m/z 354.1463 ($C_{21}H_{22}O_5$ requires 354.1467). EI-MS m/z (rel. int.): 354 ($[M]^+$, 96), 339 (100), 325 (76), 309 (64), 243 (23), 151 (21), 69 (34), 41 (19). ^{1}H-NMR: 7.83 (1H, *d*, J =7.9 Hz, H-8), 7.40 (1H, *s*, H-3), 7.23 (1H, *t*, J =7.9 Hz, H-7), 7.16 (1H, *d*, J =7.9 Hz, H-6), 6.31 (1H, *dd*, J =17.4 and 10.7 Hz, H-12), 5.15 (1H, *br d*, J =17.4 Hz, H-13*E*), 5.07 (1H, *br d*, J =10.7 Hz, H-13*Z*), 4.00 (3H, *s*, 5-OMe), 3.99 (3H, *s*, 1-OMe), 3.95 (3H, *s*, 2-OMe), and 1.67 (6H, *s*, H_3-14 and H_3-15). ^{13}C-NMR: 177.2 (*s*, C-9), 149.6 (*s*, C-4a), 148.8 (*s*, C-5), 148.1 (*s*, C-2), 147.3 (*s*, C-10a), 146.6 (*d*, C-12), 145,5 (*s*, C-1), 133.2 (*s*, C-4), 123.0 (*d*, C-7), 122.9 (*s*, C-8a), 119.8 (*d*, C-3), 117.4 (*s*, C-9a), 117.2 (*d*, C-6), 114.6 (*d*, C-8), 111.4 (*t*, C-13), 61.6 (*q*, 1-OMe), 57.6 and 56.3 (*q* each, 2-OMe and 5-OMe), 41.0 (*s*, C-11), and 27.1 ($q \times 2$, C-14 and C-15); NOE: 5-OMe [H-6 (10%)], and 2-OMe [H-3, (10%)].

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