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Cycloartane Triterpenoids Acid from *Garcinia eugenifolia*

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A study on the roots of *Garcinia eugenifolia* has resulted in the isolation of one cycloartane triterpenoid acid *i.e.*, magniferolic acid **(1)**. This is a first isolation and report on this compound from *Garcinia eugenifolia*. Three other triterpenes which are euphadienol **(2)**, β-sitosterol **(3)** and friedelin **(4)** were also obtained. Besides this, the ketone (3-hydroxyphenyl)3,4,5-trihydroxyphenyl)methanone **(5)** and two xanthones, 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2*H*)-one **(6)** and 1,3,6,8-tetrahydroxyxanthone **(7)** were also obtained from this plant.

Key Words: *Garcinia eugenifolia*, Guttiferae, Xanthones, Triterpenoids, Ketone.

INTRODUCTION

Garcinia (Clusiaceae) is widely available in Malaysia. Plants from this genus are traditionally used in treatments of abdominal pain, dysentery, diarrhea, suppuration, infected wound, leucorrhoea and chronic ulcer and gonorrhea[1]. The fruit of many species like *Garcinia mangostana*, *Garcinia xanthochymus* and *Garcinia multiflora* are edible. *Garcinia* have been reported to be rich in xanthones and triterpenoids[2-9]. In our continuing interest on Malaysian *Garcinia* plants, we carried out detail chemical studies on the roots of *Garcinia eugenifolia*. This study has led to the isolation and identification of four triterpenes, two xanthones and one ketone from the root extracts of the species. This paper reports the isolation and characterization of magniferolic acid **(1)**.

EXPERIMENTAL

The roots of *Garcinia eugenifolia* were collected from Sarawak Forestry Department, Semengok, Malaysia. The plant materials were identified and authenticated by a plant taxonomist at Forestry Research Centre (FRC), Sarawak. (Voucher Specimen No. UiTM3003).

General: Infrared spectra were measured using the universal attenuated total reflection (UATR) technique on a Perkin-Elmer 100 Series. EIMS were recorded on a Shimadzu GCMS-QP5050A spectrometer. NMR spectra were obtained using a Unity INOVA 500 MHz NMR/JEOL 400 MHz FT NMR spectrometer using tetramethylsilane (TMS) as internal standard. Ultraviolet spectra were recorded in $CHCl_3$ on a Shimadzu UV-160A, UV-visible recording spectrophotometer. Melting points were measured using Leica Galen III microscope, equipped with Testo 720 temperature recorder.

Extraction and isolation: The air-dried and powdered roots of *Garcinia eugenifolia* (2.3 kg) was extracted successively with hexane, ethyl acetate and methanol at room temperature. The extracts were evaporated to dryness under reduced pressure to yield 12 g of hexane extract, 45.7 g of ethyl acetate extract and 50.9 g of methanol extract. The hexane extract (12 g) was chromatographed on a silica gel (SiO_2) column using a stepwise gradient system (hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol) to give 20 fractions. The solid in fraction 8 yields stigmasterol (4 mg) **(3)** as fine white needles. Fraction 11 was purified by repeated column chromatography (SiO_2; hexane/ethyl acetate and chloroform/methanol gradient) to give friedelin (8 mg) **(4)**. The ethyl acetate extract (45.7 g) was fractionated by vacuum column chromatography (SiO_2; chlorofrom/methanol) to give 30 fractions. Fractions 3-5 were combined and further purified by column chromatography. Repeated purifications by column chromatography (SiO_2 and sephadex LH-20) resulted in 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2*H*)-one (5 mg) **(6)**. Fraction 8 was rechromatographed on a silica gel column (hexane/chloroform, chloroform/ethyl acetate and ethyl acetate/methanol gradient) to give 15 subfractions. Subfraction 7 yielded 1,3,6,8-tetrahydroxyxanthone **(7)** (5 mg). Fractions 9-10 were combined and further purified by column chromatography (SiO_2; chloroform/methanol gradient) to furnish 15 subfractions.

Fraction 3 afforded euphadienol **(2)** (10 mg) and fraction 7 afforded magniferolic acid **(1)** (12 mg). On the other hand, fractionation of the methanol extract (50.9 g) over a silica gel column (hexane/chloroform, chloroform-ethyl acetate and ethyl acetate-methanol gradient) provided 35 fractions. Fraction 15 afforded (3-hydroxyphenyl)(3,4,5-trihydroxyphenyl) methanone **(5)** (7 mg).

Magniferolic acid (1): White amorphous solid. UV (EtOH) λ_{max} nm (log ε): 255 (0.23), 308 (0.56). IR (KBr, ν_{max}, cm^{-1}): 1686, 1643, 1452, 1378. EI-MS m/z (rel. int.): 456(8), 438 (20), 423 (42), 395 (24), 369 (14), 316 (28). ^{1}H NMR (400 MHz, $CDCl_3$): δ: 6.90 (1H, t, *J* = 6.9), 3.29 (1H, m), 2.28 (1H, m), 2.13 (1H, m), 1.84 (1H, s), 0.97 (1H, s), 0.89 (1H, s), 0.81 (1H, s), 0.56 (1H, d, *J* = 4.6), 0.33 (1H, d, *J* = 3.7). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 173.3(C-26), 145.8 (C-24), 126.6 (C-25), 78.9 (C-3), 52.2 (C-17), 48.8 (C-14), 48.0 (C-8), 47.1 (C-5), 45.3 (C-13), 40.4 (C-4), 36.0 (C-20), 35.5 (C-15), 34.8 (C-22), 32.9 (C-12), 31.9 (C-1), 30.3 (C-2), 29.8 (C-19), 28.2 (C-16), 28.1 (C-7), 26.4 (C-11β), 25.9 (C-11α), 25.9 (C-10), 25.9 (C-23), 25.4 (C-28), 21.1 (C-6), 19.9 (C-9), 19.3 (C-30), 18.1 (C-18), 18.1 (C-21), 14.0 (C-29), 12.0 (C-27). Spectral data are in agreement with published data[10].

Euphadienol (2): White amorphous solid. UV (EtOH) λ_{max} nm (log ε): 236 (0.75), 320(0.89). IR (KBr, ν_{max}, cm^{-1}): 3392, 2945, 1706, 1650, 1456, 1375, 1025. EI-MS m/z (rel. int.): 426 (7), 411 (24), 393 (12), 259 (8). ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.11 (1H, t, *J* = 7.1), δ 3.25 (1H, dd, *J* = 11.4, 4.8 Hz), 2.09 (1H, m), 1.97 (1H, m), 1.76 (1H, m), 1.97 (1H, m), 0.81-1.70 (24 Hs, 8 CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 134.0 (C-8), 133.5 (C-9), 130.9 (C-25), 125.2 (C-24), 79.0 (C-3), 51.3 (C-5), 50.0 (C-14), 49.6 (C-17), 44.1 (C-13), 38.9 (C-4), 37.2 (C-10), 35.8 (C-20), 35.4 (C-22), 35.2 (C-1). 30.9 (C-15), 29.8 (C-16), 28.2 (C-12), 28.1 (C-29), 27.9 (C-7), 27.7 (C-2), 25.8 (C-27), 24.7 (C-23), 24.5 (C-28), 21.5 (C-11), 20.1 (C-19), 18.9 (C-6), 18.9 (C-21), 17.7 (C-26), 15.5 (C-30). Spectral data are in agreement with published data[11].

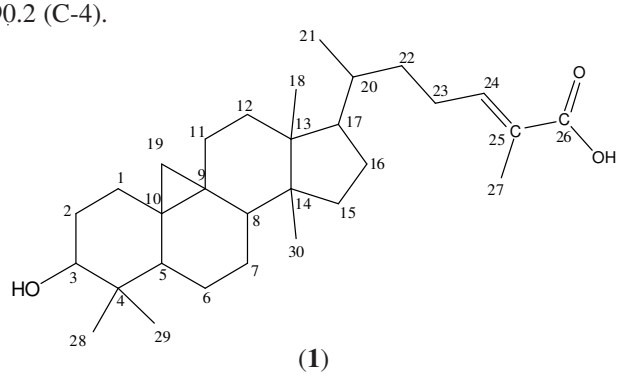
β-Sitosterol (3): White crystals with a melting point of 132-134 ºC. UV (EtOH) λ_{max} nm (log ε): 213, 236. IR (KBr, ν_{max}, cm^{-1}): 3424 (broad, OH), 2942 (C-H), 1628 (C=C). EI-MS m/z (rel. int.): 414 (M^+, 55), 396 (24), 381 (17), 354 (5), 329 (34), 303 (23), 273 (10), 255 (23), 231 (15), 213 (27), 199 (12), 187 (10), 173 (15), 159 (28), 145 (34), 133 (29), 119 (29), 107 (48), 95 (48), 81 (56), 57 59), 43 (100) and 41 (56). ^{1}H NMR (400 MHz, $CDCl_3$): δ: 5.35 (1H, d, *J* = 5.1 Hz, H-6), 3.52 (1H, m, H-3), 0.68-1.07 (18H, s, 6 × CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 140.7 (C-5), 121.7 (C-6), 71.8 (C-3), 56.7 (C-14), 56.0 (C-17), 50.1 (C-9), 45.8 (C-24), 42.3 (C-4), 42.2 (C-13), 39.7 (C-12), 37.2 (C-1), 36.5 (C-10), 36.1 (C-20), 33.9 (C-22), 31.9 (C-7), 31.9 (C-8), 31.6 (C-2), 29.1 (C-25), 28.2 (C-16), 26.0 (C-23), 24.3 (C-15), 23.0 (C-28), 21.1 (C-11), 19.8 (C-26), 19.4 (C-19), 19.0 (C-27), 18.8 (C-21), 12.0 (C-29), 11.8 (C-18).

Friedelin (4): Colourless crystals with a melting point of 263-265 ºC. IR (KBr, ν_{max}, cm^{-1}): 2930 (CH-stretching), 2868 (CH-stretching), 1716 (C=O), 1458 (CH_3), 1388 (CH_2). EI-MS m/z (ret. int.): 426 M^+ (20), 411 (6), 341 (6), 302 (19), 273 (39), 246 (30), 205 (39), 109 (80), 95 (96), 69 (100). ^{1}H NMR (400 MHz, $CDCl_3$): δ: 2.38 (m, 1H, H-4), δ 2.24 (m, 2H, H-2), 0.73-1.18 (24 Hs, 8 CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 213.2 (C-3), 59.5(C-4), 58.3 (C-10), 53.1 (C-8), 42.8 (C-18), 42.2 (C-5), 41.5 (C-2), 41.3 (C-1), 39.7 (C-14), 39.3 (C-19), 38.3 (C-13), 37.5 (C-9), 36.0 (C-12), 35.7 (C-6), 35.4 (C-22), 35.0 (C-21), 32.8 (C-16), 32.5 (C-11), 32.1 (C-29), 31.8 (C-15), 30.5 (C-7), 30.0 (C-17), 22.3 (C-24), 20.3 (C-28), 18.7 (C-30), 18.3 (C-27), 18.0 (C-26), 14.7 (C-25), 16.8 (C-23).

(3-Hydroxyphenyl)(2,4,6-trihydroxyphenyl)methanone (5): Yellow amorphous solid. UV (EtOH) λ_{max} nm (log ε): 212 (3.40), 261 (2.71), 307 (3.08). IR (KBr, ν_{max}, cm^{-1}): 3250, 1594, 1453, 1298, 1233, 1165. EI-MS m/z (rel. int.): 246 (67), 245 (92), 229 (41), 153 (100). ^{1}H NMR (300 MHz, $CDCl_3$): δ: 7.17 (1H, t, *J* = 7.8Hz), 7.04 (1H, dt, *J* = 7.5 and 1.4 Hz), 6.97 (1H, m), 6.88 (1H, ddd, *J* = 8.1, 2.7 and 1.2 Hz), 5.97 (2H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 199.0 (C=O), 164.8 (C-3), 162.9 (C-5), 156.5 (C-3'), 142.5 (C-1'), 142.5 (C-4), 129.0 (C-2), 129.0 (C-5'), 119.4 (C-6'), 118.3 (C-4'), 114.6 (C-2'), 104.9 (C-1), 95.3 (C-6).

5,9-Dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one (6): Yellow gum. UV $\lambda^{EtOH\ max\ nm}$ (log ε): 243.5 (0.52), 316.5 (0.35). IR (KBr, ν_{max}, cm^{-1}): 3384 (broad OH), 2934 (C-H stretching), 1604 (C=O). EIMS m/z (ret. int.): 408 (3), 393 (3), 380 (2), 365 (5), 353 (7), 310 (16), 295 (100), 271 (10), 229 (21), 147 (14). ^{1}H NMR (400 MHz, $CDCl_3$): δ: 13.7 (s, 1H, OH-5), 6.84 (s, 1H, H-10), 6.74 (d, *J* = 10.1 Hz, 1H, H-4), 6.25 (s, 1H, H-12), 5.56 (d, *J* = 10.1 Hz, 1H, H-3), 5.26 (t, *J* = 6.4 Hz, 1H, H-2'), 4.10 (d, *J* = 6.4 Hz, 2H, H-1'), 3.81 (s, 3H, 8-OMe), 1.83 (s, 3H, H-4'), 1.69 (s, 3H, H-5'), 1.46 (s, 6H, 2CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ: 181.9 (C-6), 159.9 (C-12a), 157.9 (C-5), 156.3 (C-11a), 155.7 (C-10a), 154.6 (C-9), 142.6 (C-8), 136.9 (C-7), 132.2 (C-3'), 127.2 (C-3), δ 123.1 (C-2'), 115.7 (C-4), 112.2 (C-6a), 104.5 (C-4a), 103.7 (C-5a), 101.7 (C-10), 94.1 (C-12), 77.9 (C-2), 62.0 (8-OMe), 28.3 (2-CH_3), 26.5 (C-1'), 25.8 (C-4'), 18.2 (C-5'). Spectral data are in agreement with published data[12].

1,3,6,8-Tetrahydroxyxanthone (7): Brown powder with a melting point of 276-278 ºC. UV (EtOH) λ_{max} nm (log ε): 330.0 (0.49), 255.5 (0.87). IR (KBr, ν_{max}, cm^{-1}): 434 (broad, OH), 2926 (C-H stretching), 1638 (C=O). EI-MS m/z (rel. int.): 260 [M^+, 100], 231 (8), 203 (8), 152 (10), 116 (15), 69 (14), 51 (13). ^{1}H NMR (400 MHz, Me_2CO-d_6): δ: 13.67 (1H, s, OH-1), 13.9 (1H, s, OH-8), 6.42 (1H, d, *J* = 1.80 Hz, H-4), 6.38 (1H, d, *J* = 1.80 Hz, H-5), 6.20 (1H, d, *J* = 1.80 Hz, H-2, H-7). ^{13}C NMR (100 MHz, Me_2CO-6): δ : 180.5 (C-9), 162.2 (C-1), 162.1 (C-3), 161.3 (C-8), 160.9 (C-6), 154.5 (C-4a), 153.9 (C-10a), 99.8 (C-8a, C-9a), 94.9 (C-2, C-7), 90.7 (C-5), 90.2 (C-4).



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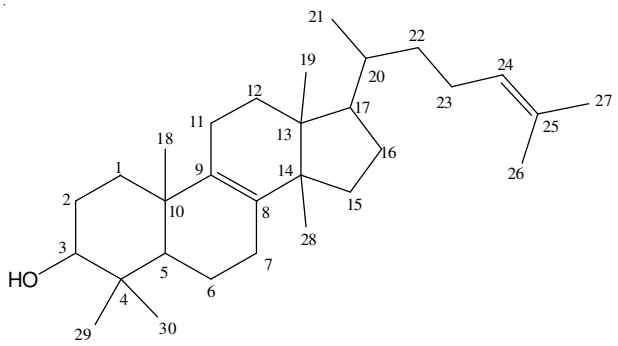

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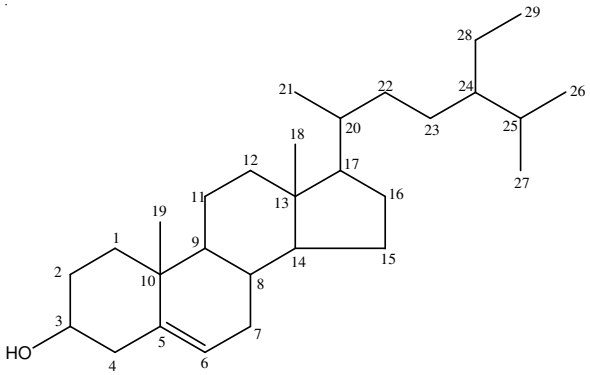

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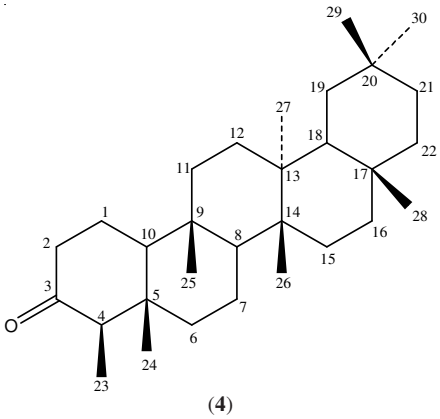

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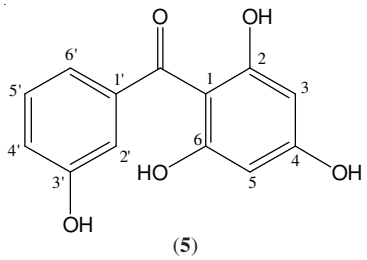

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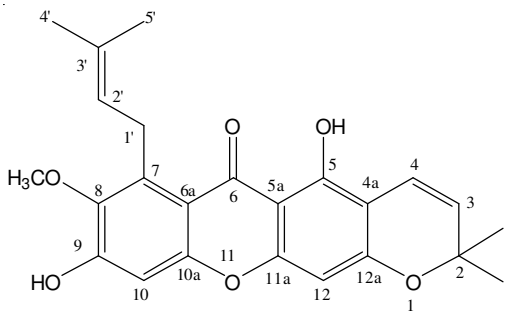

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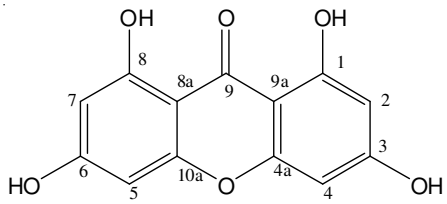

(**7**)

RESULTS AND DISCUSSION

Compound **1** was isolated as a white amorphous solid with a molecular ion peak at m/z 456 in the mass spectrum and corresponding to the molecular formula $C_{30}H_{48}O_3$. Fragment peaks at m/z 316 (M-$C_8H_{12}O_2$) is indicative of a cycloartane skeleton with a hydroxyl group and C8 side chain. The UV spectrum exhibited characteristic absorption bands of a triterpene at 255 and 308 nm. The FTIR spectrum exhibited a carbonyl group (1686 cm^{-1}), stretching of the C-H bonds (1452 and 1378 cm^{-1}) and conjugated C=C (1643 cm^{-1}).

The ^1H and ^{13}C NMR spectra gave a total of 30 carbons and 48 hydrogens in the molecule. The DEPT experiment indicated 6 methine, 11 methylene, 6 methyl and 7 quarternary carbons. The ^1H and ^{13}C chemical shift values for the C-3 position were observed as δ 3.29 (m) and δ 78.9, respectively indicating the hydroxyl group to be attached to this position. This was confirmed by comparing these values with the related compound[10]. Also observed are typical signals of the methylene protons of the cyclopropane ring in a cycloartane [δ_H 0.56 and 0.33 (1H each, d, J = 4.2 Hz, H_2-19); δ_C 29.8 (C-19)]. The compound was therefore deduced to be a cycloartane acid.

In the HMBC spectrum, H-24 gave cross-peaks to C-27, C-23, C-22, C-25 and a carbonyl acid carbon (δ_C 173.3) which had to be C-26. H_3-23 correlated to C-22, C-24 and a fully substituted olefinic carbon [δ_C 126.6 (C-25)]. The second carbon of this double bond, also the last olefinic carbon of the molecule [δ_C 145.8 (C-24)] was a mono substituted carbon, indicating that the carbon-carbon double bond was located at C-24/C-25. Hence the structure of **1** was elucidated to be magniferolic acid. This is the first isolation of a cycloartane acid from *Garcinia eugenifolia*. The HMBC correlations of **1** are shown in Fig. 1.

Compound **2-7** were identified as euphadienol **(2)**, friedelin **(3)**, β-sitosterol **(4)**, (3-hydroxyphenyl)(3,4,5-trihydroxyphenyl)methanone **(5)** 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2H)-one **(6)** and 1,3,6,8-tetrahydroxyxanthone **(7)**. Spectral data are in agreement with previous reports.

Conclusion

Magniferolic acid **(1)** was isolated for the first time from the stem bark of *Garcinia eugenifolia* together with six other compounds *i.e.*, euphadienol **(2)**, friedelin **(3)**, β-sitosterol **(4)**, (3-hydroxyphenyl)(3,4,5-trihydroxyphenyl)methanone **(5)** 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)pyrano[3,2-b]xanthen-6(2*H*)-one **(6)** and 1,3,6,8-tetrahydroxyxanthone **(7)**.

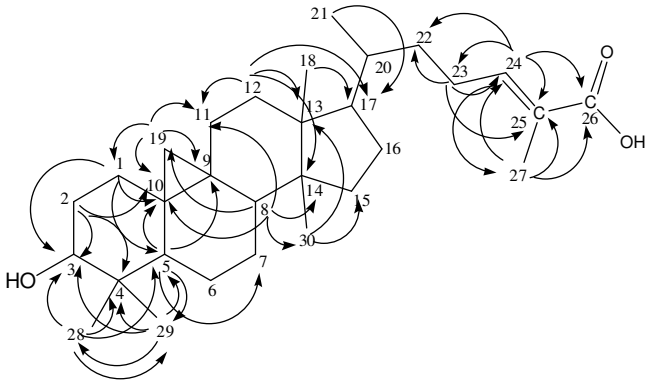

Fig. 1. HMBC ^{2}J and ^{3}J correlations between ^{1}H and ^{13}C in magniferolic acid **(1)**

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