Pentacyclic Triterpenoids from Leaves of *Excoecaria agallocha*

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A new oleanane-type triterpenoid (1) and five known pentacyclic triterpenoids (2–6) were isolated from the leaves of *Excoecaria agallocha*. Their structures were elucidated by spectroscopic analyses. The new compound was characterized as 3 β -(2*E*,4*E*)-5-oxo-decadienyloxy]-olean-12-ene (1). Compounds 1–6 were found inactive *in vitro* against several human cancer cell lines.

Key words *Excoecaria agallocha*; mangrove; pentacyclic triterpenoid

Being one of non-viviparous true mangrove species, *Excoecaria agallocha* L. (Euphorbiaceae) is naturally distributed on wetland along the coastlines in China, which has been used as a dart poison and fish poison in Southeastern Asia. Previous studies of this plant have characterized the constituents mainly as daphnane diterpene ester and labdane diterpenoids.^{1–6} Some diterpenoids isolated have been found to possess anti-tumor promoting activity *in vivo* against mouse skin papillomas.^{7,8} In screening for anti-tumor biologically active compounds from Chinese mangrove plants, we have isolated and elucidated a new pentacyclic triterpenoid (1), along with five known triterpenoids, β -amyrin acetate (2),^{9,10} taraxerone (3),^{11,12} 3-epitaraxerol (4),^{13,14} 3-epilupeol (5),^{14,15} and taraxerol (6),^{11,12} from the leaves of *E. agallocha*. This paper deals with the elucidation of the new triterpenoid using spectroscopic methods.

Compound 1 was obtained as a white needle crystal, which was found to be unsaturated triterpenoid from the positive Libermann–Buchard test. The HR-EI-MS gave a molecular ion peak at m/z 590.4710 corresponding to the molecular formula C₄₀H₆₂O₃ (Calcd 590.4699). The IR spectrum exhibited conjugated carbonyl absorption bands at 1712 and 1695 cm⁻¹. The UV spectrum showed maximum wavelength at 272 nm suggesting the presence of considerable conjugation in the molecule. The EI-MS spectrum was characteristic of the olean-12-ene series of triterpenoids.¹⁶ The major fragment at m/z 218 was due to retro-Diels–Alder cleavage of the C(12)–C(13) bond, and which was accompanied by another fragment at m/z 203 resulting from the subsequent loss of a methyl group.

The ¹H-NMR spectrum revealed the presence of eight methyl singlets at δ 0.84 (Me-28), 0.88 (Me-29, 30), 0.90 (Me-24), 0.92 (Me-23), 0.98 (Me-25, 26), 1.14 (Me-27), one oxymethine proton at δ 4.61 (t, $J=8$ Hz, H-3), and one olefinic proton at δ 5.19 (brs, H-12). Moreover, in comparison with the ¹³C-NMR spectral data of compound 2, the results were consistent with an β -amyrin type triterpene. The ¹H-NMR spectrum showed four olefinic proton signals at δ 7.29 (dd, $J=11.0, 15.0$ Hz, H-3'), 7.18 (dd, $J=11.0, 15.0$ Hz, H-4'), 6.45 (d, $J=15.0$ Hz, H-5'), and 6.25 (d, $J=15.0$ Hz, H-2'), which suggested the presence of *trans*-butadiene group. The ¹³C-NMR spectrum and DEPT experiment indicated two carbonyl group signals at δ 200.2 (C-5') and 165.7 (C-1'). Together with the analysis of HMQC and HMBC, the sub-

stituent group was identified as (2*E*,4*E*)-5-oxo-decadienyl, which was attached to an oxygen atom at C-3 of the molecule. Compound 1 was therefore determined as 3 β -(2*E*,4*E*)-5-oxo-decadienyloxy]-olean-12-ene. This is the first report of its occurrence in nature.

Compounds 1–6 were evaluated for cytotoxic activity using MTT method. However, the results showed that all compounds were inactive (IC₅₀ >50 μ g/ml) against human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and human colon cancer (HCT-8) cell lines.

Experimental

General Experimental Procedures Melting points were determined on an XT-4 micro melting point apparatus and are uncorrected. Specific rotations were measured with Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Nicolet Impact 400 FT-IR spectrophotometer. UV spectra were measured with a Shimadzu UV-260. NMR spectra were recorded in CDCl₃ with INOVA 500 NMR spectrometer, using visual CDCl₃ resonances (¹H δ 7.26, ¹³C δ 77.0) for internal reference. EI-MS and HR-EI-MS were performed with an Autospec-Ultima ETOF spectrometer. Column chromatography was carried out on silica gel (300–400 mesh). TLC analysis was carried out with glass precoated silica gel GF₂₅₄ plates; and detection was performed by spraying with 5% H₂SO₄ in ethanol, followed by heating at 110 °C for 5 min.

Plant Material The leaves of *Excoecaria agallocha* were collected at Guangxi province, P.R. China, in March 2005. A voucher specimen (No. 2005E06) was deposited in Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, P.R. China.

Extraction and Isolation Air-dried leaves of *E. agallocha* (245 g) were

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extracted with acetone (31×3) at room temperature for 1 month. The filtrate was concentrated under reduced pressure at 40 °C to obtain the acetone extract (20 g). The extract was chromatographed over silica gel to afford 18 fractions, eluting with petroleum ether (60–90 °C)–acetone (100:0 to 0:100, gradient increasing). The fractions 1–3 (2 g) were repeatedly purified by silica gel column chromatography using petroleum ether–acetone (100:1), to yield compounds **1** (6.8 mg), **2** (106 mg), **3** (14 mg), **4** (57 mg), and **5** (168 mg). The fractions 4 and 5 (700 mg) were chromatographed over silica gel using petroleum ether–acetone (98:2) to afford compounds **6** (78 mg), and β -sitosterol (41 mg).

3 β -[(2*E*,4*E*)-5-Oxo-decadienoyloxy]-olean-12-ene (**1**): White needle crystal (acetone); mp 186–188 °C; $[\alpha]_D^{23} +81.1^\circ$ ($c=0.11$, CHCl₃); UV $\lambda_{\max}$ (MeOH) nm (log ϵ): 272 (4.51); IR (KBr) cm⁻¹: 2951, 1712, 1695, 1601, 1259, 1238, 1142, 1014, 1000; EI-MS m/z (rel. int.) 590 (M)⁺ (7), 408 (6), 257 (5), 218 (100), 203 (72), 189 (31), 175 (10); HR-EI-MS m/z 590.4710 (M)⁺ (Calcd for C₄₀H₆₂O₃: 590.4699); ¹H-NMR (CDCl₃) δ : 7.29 (1H, dd, $J=11.0$, 15.0 Hz, H-3'), 7.18 (1H, dd, $J=11.0$, 15.0 Hz, H-4'), 6.45 (1H, d, $J=15.0$ Hz, H-5'), 6.25 (1H, d, $J=15.0$ Hz, H-2'), 5.19 (1H, br s, H-12), 4.61 (1H, t, $J=8$ Hz, H-3), 2.60 (2H, t, $J=7.5$ Hz, H-7'), 1.61 (2H, m, H-8'), 1.36 (2H, m, H-9'), 1.14 (3H, s, Me-27), 0.98 (6H, s, Me-25, 26), 0.93 (3H, t, $J=6.5$ Hz, Me-10'), 0.92 (3H, s, Me-23), 0.90 (3H, s, Me-24), 0.88 (6H, s, Me-29, 30), 0.88 (1H, overlapped, H-5), 0.84 (3H, s, Me-28); ¹³C-NMR (CDCl₃) δ : 38.2 (C-1), 23.6 (C-2), 81.6 (C-3), 37.9 (C-4), 55.2 (C-5), 18.3 (C-6), 32.6 (C-7), 39.8 (C-8), 47.6 (C-9), 36.8 (C-10), 23.5 (C-11), 121.6 (C-12), 145.2 (C-13), 41.7 (C-14), 26.1 (C-15), 26.9 (C-16), 32.5 (C-17), 47.2 (C-18), 46.8 (C-19), 31.1 (C-20), 34.7 (C-21), 37.1 (C-22), 28.1 (C-23), 16.8 (C-24), 15.6 (C-25), 16.8 (C-26), 25.9 (C-27), 28.4 (C-28), 33.3 (C-29), 23.7 (C-30), 165.7 (C-1'), 129.6 (C-2'), 141.1 (C-3'), 138.3 (C-4'), 135.3 (C-5'), 200.2 (C-6'), 41.0 (C-7'), 26.1 (C-8'), 22.3 (C-9'), 13.9 (C-10').

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