Pentacyclic Triterpenoids from Leaves of *Excoecaria agallocha*

Jian-Hua Zou,[a,b] Jungui Dai,*[a,b] Xiaoguang Chen,[a,b] and Jing-Quan Yuan[c]

[a] *Key Laboratory of Bioactive Substances and Resources Utilization of Chinese Herbal Medicine (Peking Union Medical College), Ministry of Education; Beijing, 100050 P.R. China:* [b] *Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College; Beijing 100050, P.R. China: and* [c] *Guangxi Affiliated Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences & Guangxi Institute of Medicinal Plant; Nanning 530023, P.R. China.* Received February 22, 2006; accepted March 22, 2006

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 3β-[(2*E*,4*E*)-5-oxo-decadienoyloxy]-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 $C_{40}H_{62}O_3$ (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 ^{1}H-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 (br s, H-12). Moreover, in comparison with the ^{13}C-NMR spectral data of compound **2**, the results were consistent with an β-amyrin type triterpene. The ^{1}H-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 ^{13}C-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 substituent group was identified as (2*E*,4*E*)-5-oxo-decadienoyl, which was attached to an oxygen atom at C-3 of the molecule. Compound **1** was therefore determined as 3β-[(2*E*,4*E*)-5-oxo-decadienoyloxy]-olean-12-ene. This is the first report of its occurrence in nature.

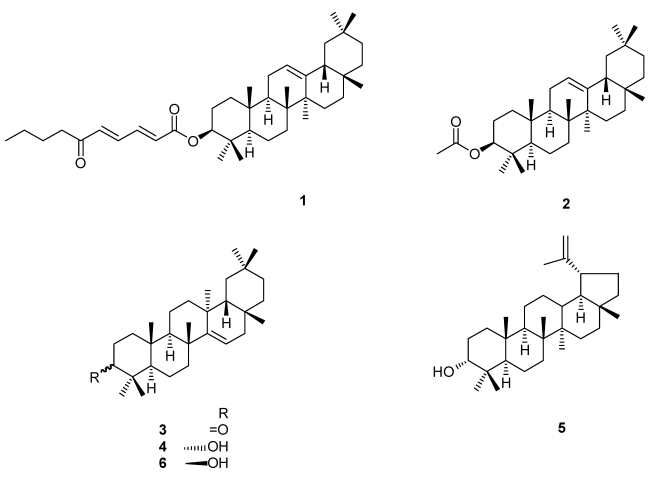
Compounds **1**—**6** were evaluated for cytotoxic activity using MTT method. However, the results showed that all compounds were inactive (IC_{50} >50 μg/ml) against human cancer cell lines including lung adenocarcinoma (A549), stomach cancer (BGC-823), breast cancer (MCF-7), hepatoma (Bel7402), and 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 $CDCl_3$ with INOVA 500 NMR spectrometer, using visual $CDCl_3$ resonances (^{1}H δ 7.26, ^{13}C 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 GF_{254} plates; and detection was performed by spraying with 5% H_2SO_4 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



* To whom correspondence should be addressed. e-mail: jgdai@imm.ac.cn

extracted with acetone (3 l×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).

3β-[(2*E*,4*E*)-5-Oxo-decadienoyloxy]-olean-12-ene (**1**): White needle crystal (acetone); mp 186–188 °C; $[\alpha]_D^{23}$ +81.1° (c=0.11, $CHCl_3$); UV λ_{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 $C_{40}H_{62}O_3$: 590.4699); ^{1}H-NMR ($CDCl_3$) 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); ^{13}C-NMR ($CDCl_3$) 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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