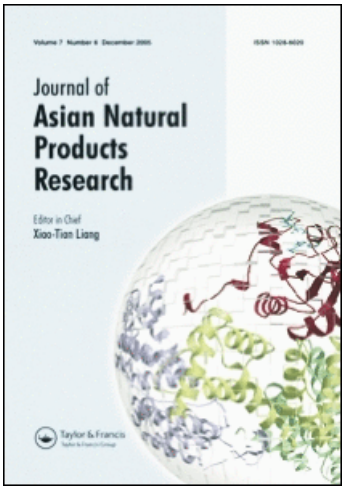

Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

A new isopimarane-type diterpene and a new natural atisane-type diterpene from *Excoecaria agallocha*

Jie Kang[a]; Ruo-Yun Chen[a]; De-Quan Yu[a]
[a] Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing, China

To cite this Article Kang, Jie , Chen, Ruo-Yun and Yu, De-Quan(2005) 'A new isopimarane-type diterpene and a new natural atisane-type diterpene from *Excoecaria agallocha*', Journal of Asian Natural Products Research, 7: 5, 729 — 734
To link to this Article: DOI: 10.1080/1028602042000324943
URL: http://dx.doi.org/10.1080/1028602042000324943

Journal of Asian Natural Products Research, Vol. 7, No. 5, October 2005, 729–734

A new isopimarane-type diterpene and a new natural atisane-type diterpene from *Excoecaria agallocha*

JIE KANG, RUO-YUN CHEN* and DE-QUAN YU

Institute of Materia Medica, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100050, China

(Received 9 July 2004; revised 21 July 2004; in final form 23 July 2004)

From the woods of *Excoecaria agallocha*, a new isopimarane-type diterpene, 3α,11β-dihydroxy-*ent*-isopimara-8(14),15-dien-2-one (**1**) and a new natural atisane-type diterpene, 16β-hydroxy-*ent*-atisan-3-one (**2**) were isolated together with three known compounds, ribenone (**3**), *ent*-labda-8(17),13*E*-diene-3β,15-diol (**4**), and *ent*-3β-hydroxybeyer-15-ene-2,12-dione (**5**). Their structures were determined by spectral data and x-ray crystallography evidence.

Keywords: *Excoecaria agallocha*; Isopimarane-type diterpene; 3α, 11β-Dihydroxy-*ent*-isopimara-8(14); 15-dien-2-one; Atisane-type diterpene; 16β-Hydroxy-*ent*-atisan-3-one

1. Introduction

The genus *Excoecaria* of Euphorbiaceae comprises 40 species which are distributed throughout tropical Africa, Asia and northwest Australia [1]. Some of them are very well known as extreme skin irritants and tumour promoters [2]. The bark and woods of the genus *Excoecaria* have been used in traditional medicines to treat flatulence in Thailand [3]. The resinous woods including the latex of *Excoecaria agallocha* has been used as a substitute for agarwood (Jinko) incense in Okinawa, Japan [3]. Our investigation on the woods of this plant has resulted in the isolation of a new isopimarane-type diterpene, 3α,11β-dihydroxy-*ent*-isopimara-8(14),15-dien-2-one (**1**) and a new natural atisane-type diterpene, 16β-hydroxy-*ent*-atisan-3-one (**2**), along with three known compounds, ribenone (**3**), *ent*-labda-8(17),13*E*-diene-3β,15-diol (**4**), and *ent*-3β-hydroxybeyer-15-ene-2,12-dione (**5**) (figure 1). In this paper, we report the isolation and structural elucidation of these constituents.

2. Results and discussion

Compound **1** was obtained as colourless needles, isolated from the alcohol extract of the wood by multiple column chromatography. The HREIMS of **1** gave an ion peak at *m/z*

*Corresponding author. Email: ruoyunchen@hotmail.com

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2005 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/1028602042000324943

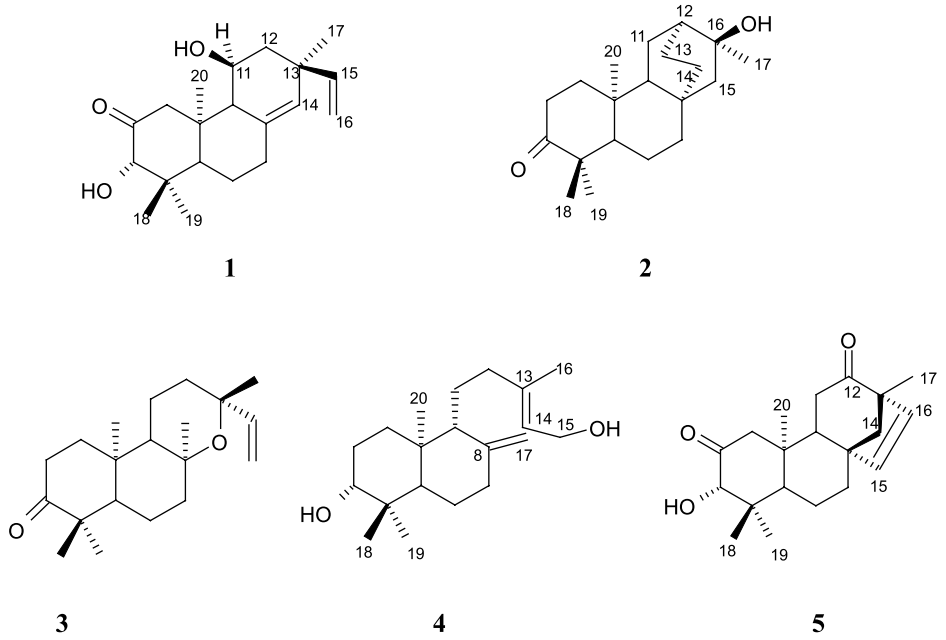

Figure 1. The structures of compounds **1–5**.

318.2180 $[M]^+$, corresponding to a molecular formula of $C_{20}H_{30}O_3$. IR spectrum revealed the presence of hydroxyl (3402 cm^{-1}), carbonyl (1701 cm^{-1}) and olefinic (1635, 1412, 1115, 910 cm^{-1}) groups in **1**. **1** showed a close resemblance with *ent*-3α,12α-dihydroxypimara-8(14),15-dien-2-one [4] in their ^1H NMR spectra. The only difference between the two diterpenes was observed in the position of the hydroxyl group. The ^{13}C NMR spectrum of **1** in $CDCl_3$ (table 1) indicated one carbonyl group [δ 211.0 (*s*)] and four olefinic carbons [δ 148.3 (*d*), 134.6 (*s*), 129.1 (*d*), 110.9 (*t*)]. Two oxygenated methine carbons resonating at δ 82.2 (*d*), 65.8 (*d*) in the ^{13}C NMR spectrum, along with its ^1H NMR data [δ 4.0 (*m*) (2H)], indicated that **1** had two oxygenated methine carbons. In the HMBC experiment (figure 2), H-12 showed long-range correlation with a methine group assignable to C-14, and H-12 also showed long-range correlation with a olefinic carbon signal of C-15.The above information suggested that one hydroxyl group might be located at C-11. The structure and relative configuration of **1** were confirmed by X-ray crystallographic analysis (figure 3), which indicated that an isopimarane skeleton for **1**. The CD spectrum of **1** showed a negative Cotton effect (286.6 nm) analogous to that of 16α-hydroxy-(-)-kauran-2-one [5] and excoecarins K [6]. Consequently, the structure and absolute configuration of **1** were assigned as 3α,11β-dihydroxy-*ent*-isopimara-8(14),15-dien-2-one.

Compound **2** was obtained as colourless needles. Its molecular formula was determined to be $C_{20}H_{32}O_2$ based on HREIMS at *m*/*z* 304.2397 $[M^+]$. The IR spectrum of **2** showed hydroxyl (3458 cm^{-1}) and carbonyl (1701 cm^{-1}) groups. The ^1H NMR spectrum of **2** in $CDCl_3$ (table 1) contained the four methyl signals at δ 1.06, 1.10, 1.13 and 1.32. The ^{13}C NMR spectrum of **2** in $CDCl_3$ (table 1) indicated the presence of carbonyl carbon [δ 217.4 (*s*)] and oxygenated carbon [δ 72.0 (*s*)]. The ^{13}C NMR spectral data of **2** and *ent*-16*S*,17-dihydroxyatisan-3-one [7] are very similar, except for the presence of methyl group (C-17), instead of hydroxymethene group (C-17). The chemical shift of each carbon of **2** was assigned by a comparative study of its spectral

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds 1-2 in $CDCl_3$.

	1		***2***	
No.	^{1}H	^{13}C	^{1}H	^{13}C
1	2.68 *s* (2H)	52.2	1.38 *m*, 1.86 *m*	38.0
2		211.0	2.35 *m*, 2.60 *m*	34.0
3	4.0 *s*	82.2		217.4
4		45.3		47.6
5	1.68 *m*	53.3	1.31 *m*	55.7
6	1.73 *m* (2H)	22.3	1.41 − 1.52 *m* (2H)	19.6
7	2.13 *m*, 2.35 *m*	35.2	1.18 *m*, 1.41 *m*	38.8
8		134.6		33.6
9	2.02 *d J* = 5.7 Hz	59.1	1.28 *m*	50.3
10		44.7		37.1
11	4.0 *m*	65.8	1.22 *m*, 2.01 *m*	23.2
12	1.63 *m* (2H)	44.0	1.59 *m*	37.9
13		37.8	1.52 *m*, 1.70 *m*	23.9
14	5.37 *s*	129.1	0.88 *m*, 1.86 *m*	27.0
15	5.83 *dd J* = 10, 17.8 Hz	148.3	1.23 *m*, 1.38 *m*	57.2
16	4.94 *d J* = 10 Hz, 5.00 *d J* = 17.8 Hz	110.9		72.0
17	1.05 *s* (3H)	26.5	1.32 *s* (3H)	30.5
18	1.19 *s* (3H)	29.3	1.10 *s* (3H)	26.2
19	0.69 *s* (3H)	16.4	1.06 *s* (3H)	21.6
20	0.80 *s* (3H)	16.5	1.13 *s* (3H)	13.5

data with those of *ent*-16*S*,17-dihydroxyatisan-3-one and also by its HMQC analysis. Accordingly, the structure of **2** was assigned as 16β-hydroxy-*ent*-atisan-3-one, which was synthesised by Francis in 1983 [8].

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Reichert Nr-229 micromelting point apparatus and are uncorrected. The optical rotations were measured on a Perkin-Elmer 241 digital polarimeter. CD spectrum was recorded using a JASCO-712 spectropolarimeter in $CHCl_3$ at 25°C. IR spectra were recorded on an IMPACT 400 spectrometer. ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), DEPT, HMQC and HMBC spectra were run on an INOVA-300 spectrometers with TMS as internal standard. HR-mass spectra were performed on VG-Autospec-300 mass spectrometer. Silica gel (160-200, 200–300 mesh) (Qingdao) was used for column chromatograph and silica gel GF-254 (Qingdao) for TLC.

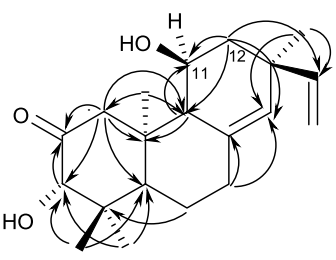

Figure 2. The key HMBC correlations for **1**.

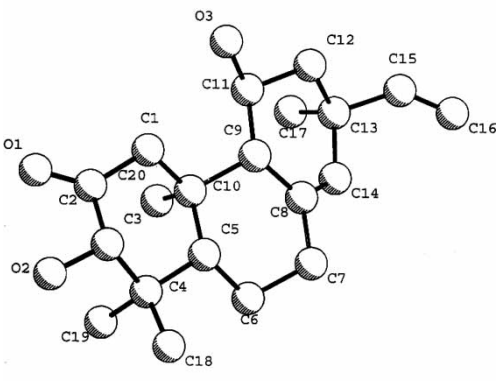

Figure 3. X-ray crystallographic structure of **1**.

3.2 Plant material

The woods of *Excoecaria agallocha* were collected in Sanya, Hainan province, People's Republic of China in September 2002. The plant material was identified by Professor Shi-Man Huang, Hainan University. A voucher specimen has been deposited at the herbarium of this institute.

3.3 Extraction and isolation

The air-dried and powdered woods of *Excoecaria agallocha* (7.5 kg) were extracted with 95% EtOH three times at reflux temperature. The combined extract was concentrated in vacuo. Then, the residue (334 g) was suspended in H_2O, and extracted with $CHCl_3$. The $CHCl_3$ fraction (111 g) was chromatographed on silica-gel column using petroleum ether—EtOAc as gradient eluent to afford pure compound **3** (100 mg) (petroleum ether—EtOAc = 9:1) and compound **4** (70 mg) (petroleum ether-EtOAc = 1:1). The other crude $CHCl_3$ fraction was chromatographed repeatedly on a silica-gel column (petroleum ether—$CHCl_3$ = 9:1) to give compounds **1** (40 mg), **2** (7 mg), and **5** (60 mg).

3.3.1 3α,11β-Dihydroxy-*ent*-isopimara-8(14),15-dien-2-one (1). White needles. mp 174–175°C. $[\alpha]_D^{25}$ +47.3 (*c* 0.11, $CHCl_3$). CD: $[\theta]_{299}$ −3899, $[\theta]_{286}$ −6530, $[\theta]_{265}$ −2244, $[\theta]_{207}$ +84747. EIMS *m/z*: 318 [M^+] (100), 303 (40), 285 (30), 274 (11), 227 (35), 213 (25), 199 (20), 171 (20). HREIMS: 318.2180 [M^+] (calcd for $C_{20}H_{30}O_3$, 318.2195). IR (KBr) ν_{max} (cm^{-1}): 3402, 2949, 1701, 1635, 1412, 1115, 1028, 910. ^{1}H NMR ($CDCl_3$): δ 5.83 (1H, *dd*, J = 10, 17.8 Hz, H-15), 5.37 (1H, *s*, H-14), 5.00 (1H, *d*, J = 17.8 Hz, H-16), 4.94 (1H, *d*, J = 10 Hz, H-16), 4.00 (1H, *s*, H-3), 4.00 (1H, *m*, H-11), 2.68 (2H, *s*, H-1), 2.35 (1H, *m*, H-7), 2.13 (1H, *m*, H-7), 2.02 (1H, *d*, J = 5.7 Hz, H-9), 1.73 (2H, *m*, H-6), 1.68 (1H, *m*, H-5), 1.63 (2H, *m*, H-12), 1.19 (3H, *s*, H-18), 1.05 (3H, *s*, H-17), 0.80 (3H, *s*, H-20), 0.69 (3H, *s*, H-19). ^{13}C NMR ($CDCl_3$): δ 211.0 (C-2), 148.3 (C-15), 134.6 (C-8), 129.1 (C-14), 110.9 (C-16), 82.2 (C-3), 65.8 (C-11), 59.1 (C-9), 53.3 (C-5), 52.2 (C-1), 45.3 (C-4), 44.7 (C-10), 44.0 (C-12), 37.8 (C-13), 35.2 (C-7), 29.3 (C-18), 26.5 (C-17), 22.3 (C-6), 16.5 (C-20), 16.4 (C-19).

3.3.2 16β-Hydroxy-*ent*-atisan-3-one (2). Colourless needles. mp 157–158°C. $[\alpha]_D^{25}$ −33.0 (*c* 0.10, $CHCl_3$). ESIMS *m/z*: 631 ([M × 2 + Na]$^+$). HREIMS: 304.2397 [M^+]

(calcd for $C_{20}H_{32}O_2$, 304.2402). IR (KBr) ν_{max} (cm^{-1}): 3458, 2981, 2898, 1701, 1394, 1122, 1078. ^1H NMR ($CDCl_3$): δ 1.32 (3H, *s*, H-17), 1.13 (3H, *s*, H-20), 1.10 (3H, *s*, H-18), 1.06 (3H, *s*, H-19). ^{13}C NMR ($CDCl_3$): δ 217.4 (C-3), 72.0 (C-16), 57.2 (C-15), 55.7 (C-5), 50.3 (C-9), 47.6 (C-4), 38.8 (C-7), 38.0 (C-1), 37.9 (C-12), 37.1 (C-10), 34.0 (C-2), 33.6 (C-8), 30.5 (C-17), 27.0 (C-14), 26.2 (C-18), 23.9 (C-13), 23.2 (C-11), 21.6 (C-19), 19.6 (C-6), 13.5 (C-20).

3.3.3 Ribenone (3) [9]. White needles. mp 109–110°C. EIMS *m/z*: 304 [M^+] (20), 303 (40), 289 (40), 85 (70), 83 (100). IR (KBr) ν_{max} (cm^{-1}): 2941, 2858, 1705, 1458, 1383, 1082, 1007. ^1H NMR ($CDCl_3$): δ 6.15 (1H, *dd*, $J = 12$, 18 Hz, H-14), 4.90–5.00 (2H, *m*, H-15), 2.53 (1H, *ddd*, $J = 7.5$, 9.3, 15.5 Hz, H-2α), 2.45 (1H, *ddd*, $J = 3.9$, 8.1, 15.5 Hz, H-2β), 2.24 (1H, *dd*, $J = 3$, 9 Hz, H-12α), 1.90 (1H, *ddd*, $J = 4.5$, 7.5, 13 Hz, H-1α), 1.81 (1H, *dd*, $J = 3$, 9 Hz, H-7α), 1.25 (3H, *s*, H-17), 1.14 (3H, *s*, H-16), 1.08 (3H, *s*, H-18), 1.00 (3H, *s*, H-19), 0.84 (3H, *s*, H-20). ^{13}C NMR ($CDCl_3$): δ 217.5 (C-3), 147.3 (C-14), 109.7 (C-15), 75.5 (C-8), 73.6 (C-13), 57.6 (C-9), 54.6 (C-5), 47.3 (C-4), 42.1 (C-7), 38.2 (C-1), 36.4 (C-10), 34.7 (C-12), 33.8 (C-2), 32.6 (C-16), 26.6 (C-18), 23.3 (C-17), 20.9 (C-19), 20.7 (C-6), 16.3 (C-11), 15.5 (C-20).

3.3.4 *ent*-Labda-8(17),13*E*-diene-3β,15-diol (4) [9]. White needles. mp 167 − 168°C. EIMS *m/z*: 306 [M^+] (0.5), 291 (10), 288 (5), 273 (20), 255 (15), 187 (8), 175 (10), 135 (100). IR (KBr) ν_{max} (cm^{-1}): 3317, 2943, 1645, 1456, 1381, 1034, 891. ^1H NMR ($CDCl_3$): δ 5.40 (1H, *m*, H-14), 4.85 (1H, *s*, H-17), 4.53 (1H, *s*, H-17), 4.15 (2H, *d*, $J = 6.9$ Hz, H-15), 3.27 (1H, *dd*, $J = 8$, 15 Hz, H-3), 1.67 (3H, *s*, H-16), 0.99 (3H, *s*, H-18), 0.77 (3H, *s*, H-19), 0.69 (3H, *s*, H-20).^{13}C NMR ($CDCl_3$): δ 147.9 (C-8), 140.4 (C-13), 123.0 (C-14), 106.7 (C-17), 78.8 (C-3), 59.4 (C-15), 56.0 (C-9), 54.5 (C-5), 39.3 (C-10), 39.1 (C-4), 38.3 (C-7), 38.1 (C-12), 37.0 (C-1), 28.2 (C-18), 27.9 (C-2), 24.0 (C-6), 21.9 (C-11), 16.3 (C-16), 15.4 (C-19), 14.5 (C-20).

3.3.5 *ent*-3β-Hydroxybeyer-15-ene-2,12-dione (5) [4]. White needles. mp 161–162°C. EIMS *m/z*: 316 [M^+] (100), 243 (40), 201 (38), 119 (37), 105 (35), 93 (90), 59 (50), 135 (100). IR (KBr) ν_{max} (cm^{-1}): 3525, 2939, 2873, 1705, 1388, 1111, 1092, 762. ^1H NMR ($CDCl_3$): δ 6.01 (1H, *d*, $J = 5.7$ Hz, H-15), 5.65 (1H, *d*, $J = 5.7$ Hz, H-16), 3.90 (1H, *s*, H-3), 1.20 (3H, *s*, H-18), 1.11 (3H, *s*, H-17), 0.74 (3H, *s*, H-20), 0.71 (3H, *s*, H-19). ^{13}C NMR ($CDCl_3$): δ 211.0 (C-2), 210.1 (C-12), 138.5 (C-15), 136.8 (C-16), 82.6 (C-3), 58.0 (C-14), 57.3 (C-13), 54.6 (C-9), 53.7 (C-5), 50.9 (C-1), 49.4 (C-8), 45.6 (C-4), 43.5 (C-10), 36.0 (C-11), 35.6 (C-7), 29.2 (C-18), 17.0 (C-17), 16.5 (C-19), 17.0 (C-6), 14.8 (C-20).

3.4 X-ray diffraction structure determination for 1

Intensity data were collected at a detector to crystal distance of 10 cm, using the $\omega - 2\theta$ scan technique ($2\theta_{max} = 50°$) at a constant speed of 1.5°/min. Crystal data: $C_{20}H_{30}O_3$; crystal size (mm), $0.01 \times 0.10 \times 0.20$; crystal system, monoclinic; space group, $P2_1$; unit cell dimensions, $a = 11.216(2)$Å, $b = 6.220(1)$Å, $c = 13.028(3)$Å; volume, 904.8 (3)Å^3; $Z = 2$; formula weight (calcd), 318.46; density (calcd), 1.169 g/cm^3. There were 1799, of which 1303 were observed ($|F|^2 \geq 8\delta|F|^2$).

References

[1] T. Konishi, T. Konoshima, T. Maoka, Y. Fujiwara. *Tetrahedron Lett.*, **41**, 3419–3422 (2000).
[2] T. Konishi, Y. Fujiwara, T. Konoshima, S. Kiyosawa. *Chem. Pharm. Bull.*, **46**, 1393–1398 (1998).
[3] T. Konishi, T. Konoshima, Y. Fujiwara, S. Kiyosawa, K. Miyahara, M. Nishi. *Chem. Pharm. Bull.*, **47**, 456–458 (1999).
[4] T. Sakai, T. Nakagawa. *Phytochemistry*, **27**, 3769–3779 (1998).
[5] C.M. Chen, T. Murakami. *Tetrahedron Lett.*, **16**, 1121–1124 (1971).
[6] T. Konishi, T. Konoshima, Y. Fujiwara, S. Kiyosawa. *J. Nat. Prod.*, **63**, 344–346 (2000).
[7] K.R. Gustafson, M.H.G. Munro, J.W. Blunt, J.H. Cardellina, J.B. McMahon, R.J. Gulakowski, G.M. Cragg, P.A. Cox, L.S. Brinen, J. Clardy, M.R. Boyd. *Tetrahedron*, **47**, 4547–4554 (1991).
[8] F.J. Schmitz, D.J. Vanderah, K.H. Hollenbeak, C.E.K. Enwall, Y. Gopichand. *J. Org. Chem.*, **48**, 3941–3945 (1983).
[9] T. Konishi, M. Azuma, R. Itoga, S. Kiyosawa, Y. Fujiwara, Y. Shimada. *Chem. Pharm. Bull.*, **44**, 229–231 (1996).