

Chemical Structures of Excoecarins A, B and C: Three New Labdane-Type Diterpenes from Wood, *Excoecaria agallocha*

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Three new diterpenes, excoecarins A (2), B (3) and C (4) have been isolated from the wood of *Excoecaria agallocha* LINN. The structures of excoecarins A, B and C were established as (13*R*,14*R*)-*ent*-8 α ,13,14,15-diepoxy-13-*epi*-labda-3-one (2), (13*R*,14*S*)-*ent*-8 α ,13,14,15-diepoxy-13-*epi*-labda-3-one (3), (13*R*,14*R*)-*ent*-8 α ,13,14,15-diepoxy-13-*epi*-labda-3 β -ol (4), respectively, on the basis of spectroscopic data and chemical evidence.

Key words *Excoecaria agallocha*; Euphorbiaceae; labdane-type diterpene; excoecarin A; excoecarin B; structure elucidation

Excoecaria agallocha LINN (Euphorbiaceae) is known as a fish poison.¹⁾ Its constituents are known to be skin irritants and are used for caustic compounds, daphnane type diterpene esters²⁾ in latex, some triterpenes, hydrocarbons and fatty acids³⁾ as surface waxes, and cinnamoyl piperidine alkaloids in the stem wood,⁴⁾ which have been encountered so far only in the genus *Piper* (Piperaceae).

In phytochemical studies on the constituents of *Excoecaria agallocha* LINN, we have already reported on the isolation and structural elucidation of some new labdane-type diterpenes, *ent*-11 α -hydroxy-3-oxo-13-*epi*-manoyl oxide, *ent*-16-hydroxy-3-oxo-13-*epi*-manoyl oxide and *ent*-15-hydroxy-labda-8(17),13*E*-diene-3-one with three known compounds, ribenone (1), ribenol and *ent*-13-*epi*-manoyl oxide.⁵⁾ In addition to these compounds, three new labdane-type diterpenes, named excoecarins A (2), B (3) and C (4) have also been isolated.

In this paper, we describe in detail the structural elucidation of these three diterpenes.

Carbon Skeletons of Excoecarins A(2) and B(3) Compounds 2 and 3 were found to have the same molecular formula, C₂₀H₃₂O₃, on the basis of high-resolution fast atom-bombardment mass measurement (HR-FAB-MS). The infrared (IR) spectra of 2 and 3 showed absorption bands ascribable to a carbonyl group (2, 1707 cm⁻¹; 3, 1698 cm⁻¹) and an ether group (2, 3, 1103–1078 cm⁻¹). The ¹³C-NMR spectra showed 20 carbon signals in both compounds. In the distortionless enhancement by polarization transfer (DEPT) experiments, it was established that 2 and 3 were composed of five methyl, seven methylene, three methine and five quaternary carbons, including two carbons of oxygenic methine and methylene, with a carbonyl and two oxygenated quaternary carbons. The ¹³C-NMR data are listed in Table 1.

The mass spectral measurements and the ¹³C chemical shift data led us to presume that 2 and 3 were labdane-type diterpene analogues occurring along with ribenone (1) and ribenol.⁵⁾ Comparing the ¹³C chemical shift data of 2 and 3 with those of 1, carbon signals of C-9, -12, -13 and -16 attached to the ring C were observed in a somewhat high field in 2 and 3. Further, *sp*³ carbon signals at δ 57.5 and 47.5 in 2, in analogy with signals at δ 58.9 and 46.0 in 3 were observed instead of the *sp*² carbon signals of C-14 and 15 in 1.

Therefore, it could be concluded that compounds 1, 2 and 3 were on the same plane of a tricyclic structure (C-1–C-13). The ¹H-NMR signals of 2 and 3 were assigned by ¹H–¹³C correlation spectroscopy (¹H–¹³C COSY) and by double quantum filtered (DQF)-COSY, and were compared with those of 1. The assigned ¹H-NMR data are summarized in Table 2.

Absolute Configurations of Excoecarins A (2) and B (3) In order to establish the absolute configurations of the new compounds, 2 and 3, especially at the chiral carbons on rings A, B and C, the epoxidation of 1 was carried out. Oxidation of a vinyl group in 1 with *m*-chloroperbenzoic acid gave a mixture of two epoxides which were separated by silica-gel chromatography to afford two colorless crystalline epoxides, 1a, mp 136.5–137 °C and 1b, mp 148–150 °C. Epoxides 1a and 1b were completely identical to 2 and 3, respectively, by comparison of physical data: IR, ¹H-NMR and circular dichroism (CD) spectra. In addition, the CD spectra of 2 and 3 showed a negative Cotton effect at 290 nm analogous to that of 1.⁵⁾ Therefore, the configurations at the chiral carbons on rings A, B and C of 2 and 3 were

Table 1. ¹³C (75.4 MHz)-NMR Spectral Data for Compounds 1–4 in CDCl₃

Carbon	1	2	3	4
1	38.2	38.0	38.1	37.4
2	33.9	33.8	33.9	27.2
3	217.4	217.2	217.4	78.8
4	47.3	47.3	47.3	38.8
5	54.6	54.6	54.7	55.2
6	20.8	20.8	20.8	19.6
7	42.2	41.9	42.1	42.8
8	75.5	74.5	74.8	74.8
9	57.7	56.6	56.9	57.1
10	36.4	36.4	36.4	36.6
11	16.4	16.8	17.0	16.5
12	34.8	31.0	32.0	30.9
13	73.6	71.5	71.9	71.3
14	147.4	57.5	58.9	57.6
15	109.8	47.5	46.0	47.5
16	32.7	29.2	28.7	29.2
17	23.4	23.5	22.2	24.0
18	26.7	26.7	26.6	28.0
19	20.9	21.0	20.9	15.7
20	15.5	15.3	15.3	15.2

Chemical shifts are given in δ (ppm) relative to internal tetramethylsilane (TMS).

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Table 2. ^1H (300 MHz)-NMR Spectral Data for Compounds 2–4 in CDCl_3

Proton	2	3	4
1	1.87 (ddd, 4.5, 7.5, 13.0)	1.88 (ddd, 4.0, 7.5, 13.0)	1.83 (m)
2 α	2.45 (ddd, 4.5, 7.5, 15.5)	2.43 (ddd, 4.0, 7.5, 16.0)	
β	2.54 (ddd, 7.5, 10.0, 15.5)	2.55 (ddd, 7.5, 10.0, 16.0)	
3			3.22 (ddd, 5.5, 5.5, 11.0)
7	1.86 (dd, 3.0, 9.0)	1.86 (dd, 3.0, 9.0)	
11	1.48 (ddd, 8.0, 10.0, 13.0)		
14	2.93 (ddd, 1.0, 3.0, 4.0)	3.12 (dd, 3.0, 4.0)	2.92 (ddd, 1.0, 3.0, 4.0)
15	2.80 (dd, 3.0, 5.0) 2.84 (dd, 4.0, 5.0)	2.51 (dd, 3.0, 5.0) 2.82 (dd, 4.0, 5.0)	2.79 (dd, 3.0, 5.0) 2.83 (dd, 4.0, 5.0)
16	1.22 (s)	1.12 (s)	1.21 (s)
17	1.32 (s)	1.46 (s)	1.28 (s)
18	1.11 (s)	1.10 (s)	0.99 (s)
19	1.03 (s)	1.03 (s)	0.77 (s)
20	0.86 (s)	0.90 (s)	0.76 (s)

Chemical shifts are given in δ (ppm) relative to internal TMS. Multiplicity and magnitude (Hz) of spin-coupling are listed in parentheses. s, singlet; dd, double doublet; ddd, double double doublet; m, multiplet.

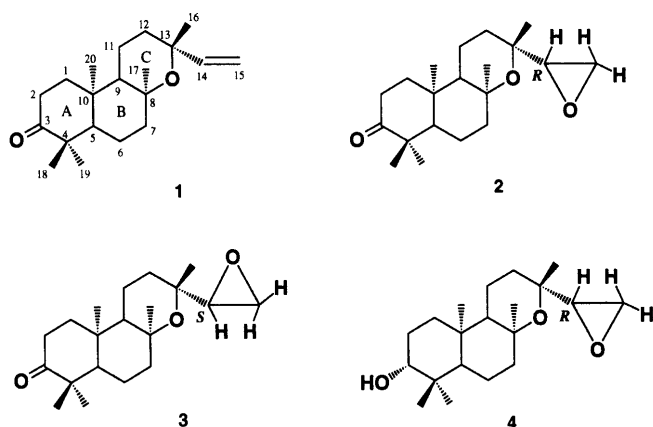
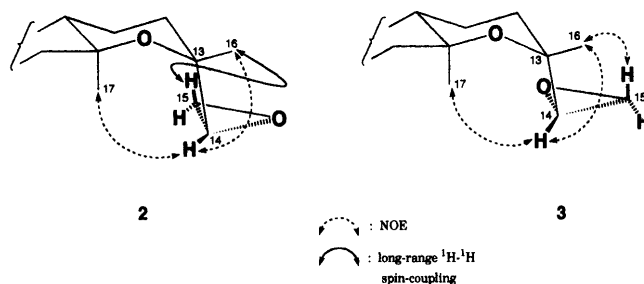


Chart 1. Structures and Numbering of 1, 2, 3 and 4

the same as those of 1, and it was presumed that the structural difference between 2 and 3 results from the configuration at C-14. The structures of 2, 3 and 1 are shown in Chart 1.

For determination of the configuration at C-14 in 2 and 3, nuclear Overhauser effect (NOE) difference and spin decoupling spectra were measured and the data are summarized in Fig. 1. As shown in Fig. 1, NOE enhancements were observed between 16- H_3 , 17- H_3 and 14- H for both 2 and 3. On the irradiation of 16- H_3 , NOE enhancement was observed on the 15- H signal at δ 2.82 in 3, while long-range couplings were observed between 16- H_3 and 15- H signals at δ 2.84 in 2. Based on the NOEs and the stable conformations for 2 and 3 calculated by using the molecular mechanics program (MM2) of CSC Chem 3D/plus, the absolute configuration at C-14 was determined to be an *R* configuration for 2 and an *S* for 3. Consequently, the structures of 2 and 3 were determined to be (13*R*,14*R*)-*ent*-8 α ,13,14,15-diepoxy-13-*epi*-labda-3-one and (13*R*,14*S*)-*ent*-8 α ,13,14,15-diepoxy-13-

Fig. 1. NOE and Long-Range ^1H - ^1H Spin-Coupling Data for 2 and 3

epi-labda-3-one, respectively.

Compound 4 was obtained as colorless needles, mp 183–185°C, $[\alpha]_D -30.7^\circ$. Its molecular formula was determined to be $\text{C}_{20}\text{H}_{34}\text{O}_3$ on the basis of HR-FAB-MS. Instead of absorption due to the carbonyl group in 2 and 3, the IR spectrum of 4 showed absorptions at 3400, 1105 and 1043 cm^{-1} due to hydroxyl and ether groups. The ^{13}C -NMR spectrum (Table 1) of 4 was similar to that of 2 except for some signals affected by the hydroxyl group at C-3. The ^1H -NMR spectrum (Table 2) of 4 showed a signal due to a methine proton on the carbon bearing a hydroxyl group at δ 3.22 ($J=5.5, 5.5, 11.0$ Hz) as double-double-doublets (ddd). These findings led us to presume that 4 is a 3-hydroxyl analogue of 2. The configuration of the 3-hydroxyl group for 4 was confirmed to be an equatorial form by comparison of the NMR spectroscopy with that of ribenol.⁶⁾

Oxidation of 4 with CrO_3 -pyridine complex yielded a ketone derivative, mp 137–138°C, $[\alpha]_D -49.7^\circ$, which was identical with 2 on the basis of melting points and IR spectral comparisons.

Therefore, the structure of 4 was established as (13*R*,14*R*)-*ent*-8 α ,13,14,15-diepoxy-13-*epi*-labda-3 β -ol.

Experimental

The instruments used for obtaining physical data and the conditions for chromatography were the same as described in the preceding paper.⁵⁾ Silica-gel (Merck) and octadecyl silica (ODS) (Lichroprep Rp-18, Merck) were used for column chromatography. Preparative recycled HPLC was performed using a gel partition column (JAI-GEL GS-310, MeOH).

Isolation of Compounds 2, 3 and 4 The finely chipped wood of *Excoecaria agallocha* (665 g) was extracted with ether at room temperature three times. The combined ether extract was concentrated to a brown syrup and then the syrup was subjected to column chromatography on silica-gel with a binary solvent system to obtain ten fractions, fr. 1–10.⁵⁾ Fraction 5 (1.18 g) was subjected to ODS column chromatography and eluted with $\text{MeOH}-\text{H}_2\text{O}$ (5:1), rechromatographed on a silica-gel column using $\text{CHCl}_3-\text{MeOH}$ (20:1) as an eluate, and purified on a recycled HPLC to give 2 (70.5 mg), 3 (24.5 mg) and 4 (28 mg).

Excoecarin A (2): Colorless needles from MeOH, mp 137.5–138°C, $[\alpha]_D^{21.7} -50.5^\circ$ ($c=0.2$, CHCl_3). CD ($c=6.0 \times 10^{-5}$, CHCl_3) $\Delta\epsilon^{26}$ (nm): 0 (320), -1.26 (290), 0 (250). IR (KBr) cm^{-1} : 1707 (C=O), 1103, 1078, 981, 962, 862, 816. EI-MS m/z : 305 ($\text{M}-\text{CH}_3$) $^+$, 277 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$, 259 ($277-\text{H}_2\text{O}$) $^+$, 217 ($\text{C}_{15}\text{H}_{21}\text{O}$) $^+$, 207 ($\text{C}_{14}\text{H}_{23}\text{O}$) $^+$, 151 ($\text{C}_{10}\text{H}_{15}\text{O}$) $^+$, 43 ($\text{C}_2\text{H}_3\text{O}$) $^+$. FAB-MS m/z : 321 ($\text{M}+\text{H}$) $^+$, 277 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$. HR-FAB-MS m/z : Calcd for $\text{C}_{20}\text{H}_{33}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 321.2429. Found: 321.2446. ^{13}C -NMR (CDCl_3): Table 1. ^1H -NMR (CDCl_3): Table 2.

Excoecarin B (3): Colorless needles from MeOH, mp 148–151°C, $[\alpha]_D^{25} -53.4^\circ$ ($c=1.0$, CHCl_3). CD ($c=5.5 \times 10^{-5}$, CHCl_3) $\Delta\epsilon^{25}$ (nm): 0 (320), -0.78 (290), 0 (250). IR (KBr) cm^{-1} : 1698 (C=O), 1103, 1078, 991, 964, 866, 819. EI-MS m/z : 305 ($\text{M}-\text{CH}_3$) $^+$, 277 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$, 259 ($277-\text{H}_2\text{O}$) $^+$, 217 ($\text{C}_{15}\text{H}_{21}\text{O}$) $^+$, 207 ($\text{C}_{14}\text{H}_{23}\text{O}$) $^+$, 151 ($\text{C}_{10}\text{H}_{15}\text{O}$) $^+$, 43 ($\text{C}_2\text{H}_3\text{O}$) $^+$. FAB-MS m/z : 321 ($\text{M}+\text{H}$) $^+$, 277 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$. HR-FAB-MS m/z : Calcd for $\text{C}_{20}\text{H}_{33}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 321.2430. Found:

321.2452. ^{13}C -NMR (CDCl_3): Table 1. ^1H -NMR (CDCl_3): Table 2.

Excoecarin C (**4**): Colorless needles from aqueous MeOH, mp 183–185°C, $[\alpha]_D^{21} -30.7^\circ$ ($c=0.97$, CHCl_3). IR (KBr) cm^{-1} : 3400 (OH), 1105, 1043, 997, 974, 829, 808. EI-MS m/z : 322 (M^+), 307 ($\text{M}-\text{CH}_3$) $^+$, 279 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$, 261 ($279-\text{H}_2\text{O}$) $^+$, 243 ($261-\text{H}_2\text{O}$) $^+$, 153 ($\text{C}_{10}\text{H}_{17}\text{O}$) $^+$, 135 ($\text{C}_{10}\text{H}_{15}$) $^+$, 43 ($\text{C}_2\text{H}_3\text{O}$) $^+$. HR-FAB-MS m/z : Calcd for $\text{C}_{20}\text{H}_{35}\text{O}_3$ ($\text{M}+\text{H}$) $^+$: 323.2586. Found: 323.2613. ^{13}C -NMR (CDCl_3): Table 1. ^1H -NMR (CDCl_3): Table 2.

Epoxidation of Ribenone (1) A solution of **1** (100 mg) and *m*-chloroperbenzoic acid (110 mg) in CHCl_3 (15 ml) was stirred at room temperature for 40 h. The reaction mixture was diluted with CHCl_3 (30 ml), washed with aqueous Na_2CO_3 , dried with Na_2SO_4 and evaporated under reduced pressure.

The residue was purified by column chromatography to obtain **1a** (10.8 mg) and **1b** (32.6 mg) as colorless needles, which were identified as **2** and **3** by direct comparisons with authentic samples, respectively.

1a: Colorless needles from MeOH, mp 136.5–137°C, $[\alpha]_D^{22} -49.5^\circ$ ($c=0.3$, CHCl_3). CD ($c=6.0 \times 10^{-5}$, CHCl_3) $\Delta\epsilon^{25}$ (nm): 0 (320), -1.10 (290), 0 (250). IR (KBr) cm^{-1} : 1707 (C=O), 1100, 1080, 981, 962, 864, 816. EI-MS m/z : 305 ($\text{M}-\text{CH}_3$) $^+$, 277 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$, 259 ($277-\text{H}_2\text{O}$) $^+$, 217 ($\text{C}_{15}\text{H}_{21}\text{O}$) $^+$, 207 ($\text{C}_{14}\text{H}_{23}\text{O}$) $^+$, 151 ($\text{C}_{10}\text{H}_{15}\text{O}$) $^+$, 43 ($\text{C}_2\text{H}_3\text{O}$) $^+$. ^1H -NMR (CDCl_3) spectrum coincided with that of **2**.

1b: Colorless needles from MeOH, mp 147–148.5°C, $[\alpha]_D^{22} -50.5^\circ$ ($c=0.3$, CHCl_3). CD ($c=5.3 \times 10^{-5}$, CHCl_3) $\Delta\epsilon^{25}$ (nm): 0 (320), -0.70 (290), 0 (250). IR (KBr) cm^{-1} : 1700 (C=O), 1103, 1078, 990, 965, 866, 819. EI-MS m/z : 305 ($\text{M}-\text{CH}_3$) $^+$, 277 ($\text{M}-\text{C}_2\text{H}_3\text{O}$) $^+$, 259 ($277-\text{H}_2\text{O}$) $^+$, 217 ($\text{C}_{15}\text{H}_{21}\text{O}$) $^+$, 207 ($\text{C}_{14}\text{H}_{23}\text{O}$) $^+$, 151 ($\text{C}_{10}\text{H}_{15}\text{O}$) $^+$, 43

($\text{C}_2\text{H}_3\text{O}$) $^+$. ^1H -NMR (CDCl_3) spectrum coincided with that of **3**.

Oxidation of 4 A pyridinium dichromate complex (30 mg) was added to a stirred solution of **4** (10 mg) in acetone (10 ml) and the resulting mixture was stirred for 10 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated. The residue was purified by column chromatography eluted with hexane–AcOEt (6:1) to obtain **2** (mp 135–136.5°C, $[\alpha]_D^{22} -49.7^\circ$, 4.9 mg) as colorless needles. This product was identified by comparison of its melting point and IR spectrum with those of excoecarin B (**2**).

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