Excoecarins D, E, and K, from *Excoecaria agallocha*[1]

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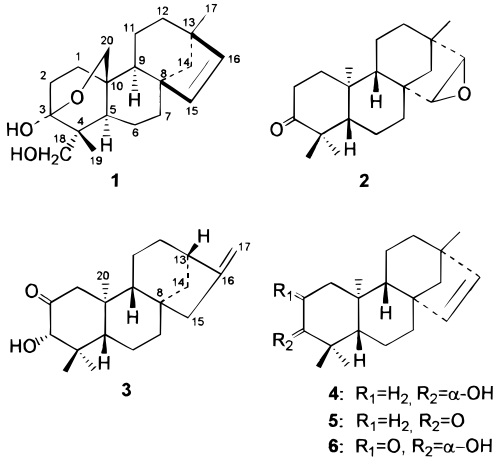
Three novel diterpenoids, excoecarins D, E, and K (**1**−**3**), were isolated from *Excoecaria agallocha*. The structures of **1**−**3** were established as 3α,18-dihydroxy-3*β*,20-epoxybeyer-15-ene, (15*R*,16*S*)-*ent*-15,16-epoxybeyeran-3-one, and *ent*-3*β*-hydroxykaur-16-en-2-one, respectively, on the basis of extensive NMR experiments and, in the case of **1**, by X-ray analysis.

Excoecaria species (Euphorbiaceae) are widely distributed in tropical Africa and East Asia.[2] The latex and leaves of *E. agallocha* L. have been used as a dart poison and fish poison in New Caledonia,[3] India,[4] and Malaysia,[5] and are used in traditional medicine in Thailand.[2] The piscicidal constituent, excoecariatoxin,[3] and some related daphnane diterpene esters that are known as skin irritants and tumor promoters, were obtained from the twigs, bark, and latex of *E. agallocha* in Japan and Thailand.[2,3] Recently, Erickson et al. reported a novel phorbol ester as an anti-HIV principle, which was isolated from the leaves and stems of *E. agallocha* collected in northwest Australia.[6] We previously reported on the isolation and structure elucidation of several diterpenoids from the same source.[1] In this paper, we describe the isolation and structure determination of three additional novel diterpenoids (**1**−**3**) and three known diterpenoids (**4**−**6**).[7]



Results and Discussion

The molecular formula of excoecarin D (**1**) was established by HRFABMS as $C_{20}H_{30}O_3$, and IR spectroscopy revealed the presence of hydroxyl, disubstituted double bond, and ether groups. The ^{13}C NMR and DEPT spectra of **1** in $CDCl_3$ (Table 1) showed 20 carbons with 28 directly attached protons for the diterpene nucleus. These spectra also indicated the presence of a double bond, four tertiary carbons, and three oxygenated carbons. The ^1H NMR spectrum exhibited two methyl groups as singlets (*δ* 1.14, 1.00), two olefinic protons (*δ* 5.48, 5.59) coupled to each other, and two sets of methylene protons (*δ* 3.28, 3.63 and *δ* 3.78, 3.86). The nature of the diterpene skeleton of **1** was established by means of 2D NMR experiments ($^1H-^1H$ COSY, HMQC, NOESY, and HMBC). These results allowed us to construct a structural formula based on an anthracene skeleton with epoxy rings at C-3 and C-20, *cis*-olefins at C-8 and C-13, and a hydroxyl methyl group at C-18 to complete a beyerane skeleton. The carbons of rings B, C, and D were similar to those of stachenone (**6**)[7] except for one methyl carbon (*δ* 12.9), four methylene carbons (*δ* 32.4, 30.1, 67.2, 70.7), three quaternary carbons (*δ* 43.9, 34.7, 98.0), and one methine carbon (*δ* 47.3) in the ^{13}C NMR spectrum of **1**.

Table 1. ^{13}C NMR Spectral Data of Compounds **1**−**3** in $CDCl_3$

	1		**2**		**3**	
carbon	*δ*[a]	m[b]	*δ*	m	*δ*	m
1	32.4	t	38.2	t	53.4	t
2	30.1	t	34.3	t	211.0	s
3	98.0	s	217.1	s	82.8	d
4	43.9	s	47.6	s	45.3	s
5	47.3	d	55.5	d	55.4	d
6	21.1	t	19.5	t	20.1	t
7	35.5	t	35.3	t	39.2	t
8	48.4	s	44.1	s	45.5	s
9	45.6	d	55.5	d	55.4	d
10	34.7	s	37.0	s	44.3	s
11	21.8	t	20.8	t	18.4	t
12	32.4	t	32.6	t	32.8	t
13	44.0	s	39.1	s	43.7	d
14	60.2	t	46.5	t	40.5	t
15	132.9	d	60.0	d	48.7	t
16	137.5	d	55.6	d	154.9	s
17	24.6	q	21.4	q	103.6	t
18	70.7	t	26.3	q	29.7	q
19	12.9	q	21.7	q	18.6	q
20	67.2	t	15.5	q	16.4	q

[a] *δ* values are recorded in ppm. [b] Multiplicity is given from DEPT observations.

The relative stereochemistry of **1** was assigned on the basis of NOE correlations. The methylene protons at C-20 correlated with olefinic protons at C-15. Another NOESY experiment showed correlations between the methylene or methine protons at H-1, -2, and -5 with the H-18 protons. The optical rotation of **1** was negative, in contrast to that of **4**, **5**, and **6**. These data suggested 3,18-dihydroxy-3,20-epoxybeyer-15-ene as the structure for **1**. A suitable crystal of **1** was obtained for X-ray analysis. The crystal and molecular structures were determined from X-ray diffraction data, and the result is shown in Figure 1 (see Experimental Section). The 3,20-bridged hemiacetal-cyclic function of ring A had the same *β*-orientation as the unsaturated five-membered ring. We were able to assign the structure as 3α,18-dihydroxy-3*β*,20-epoxybeyer-15-ene.

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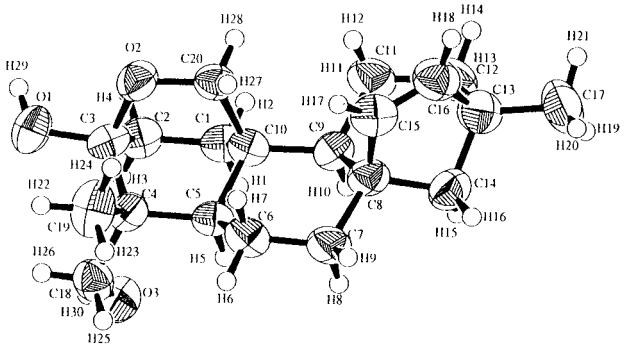

Figure 1. ORTEP drawing of **1**.

The molecular formula of **2** was established as $C_{20}H_{30}O_2$ on the basis of HRFABMS. IR spectroscopy revealed the presence of carbonyl and epoxy groups in **2**. The ^{13}C NMR spectrum of **2** in $CDCl_3$ (Table 1) indicated two oxygenated methine carbons and one oxygenated quaternary carbon. The ^1H NMR spectrum exhibited four methyl signals (δ 1.04, 1.08 × 2, 1.10) and oxocyclic methine protons at δ 3.06 and 3.42. On the basis of the above evidence and by comparison of the ^1H and ^{13}C NMR data of **2** with those for **4**,[7] the structure of **2** was assigned as 15,16-epoxybeyeran-3-one.

Irradiation of the H-20 and H-17 signals gave enhancements of the H-15 and H-16 signals, respectively, in NOE difference experiments. The equatorial proton on C-14 resonated upfield at δ 0.55 under the influence of the epoxy ring. Thus, the configurations at C-15 and C-16 were assigned as *R* and *S*, respectively. The CD spectrum of **2** gave a Cotton effect similar to that of **5**.[7] Consequently, the structure of **2** was assigned as (15*R*,16*S*)-*ent*-15,16-epoxybeyeran-3-one.

Excoecarin K (**3**) gave IR absorption bands indicating hydroxyl, carbonyl, and exomethylene groups. The molecular formula of **3** was established as $C_{20}H_{30}O_2$ by HR-FABMS. The ^{13}C NMR spectrum of **3** indicated three methyl groups, seven CH_2 units, three sp^3 quaternary carbons, two oxygenated carbons (δ 82.8 and 211.0), and an exomethylene group (δ 103.6 and 154.9). Its ^1H NMR spectrum exhibited three tertiary methyl groups, a methine proton-bearing carbon attached a hydroxyl group at δ 3.87, two downfield protons at δ 4.77 and 4.83, and an allylic proton at δ 2.67. In NOE difference NMR experiments, irradiation at H_3-20 enhanced the signal intensity of H-14. NOEs were also observed between H-13 and H_3-17 and between H_3-20 and H_3-19. These data indicated that **3** was a kaur-16-ene diterpenoid with the orientation in the C and D rings opposite to compounds with a beyerane skeleton. The hydroxyl group was considered to be equatorial from comparison of the coupling pattern of the H-3 proton (δ 3.87) with that of **6**.[7] In NOE experiments, irradiation of the H-3 signal gave enhancements of the H_3-18 and H_{ax}-1 signals. CD spectral data of **3** showed a negative Cotton effect similar to those of *ent*-16-hydroxykauran-2-one[8] and *ent*-3β-hydroxykaur-16-ene.[9] These results indicated that **3** was an *ent*-kaurene-type diterpenoid, and **3** was concluded to be *ent*-3β-hydroxykaur-16-en-2-one. (See Figure 2.)

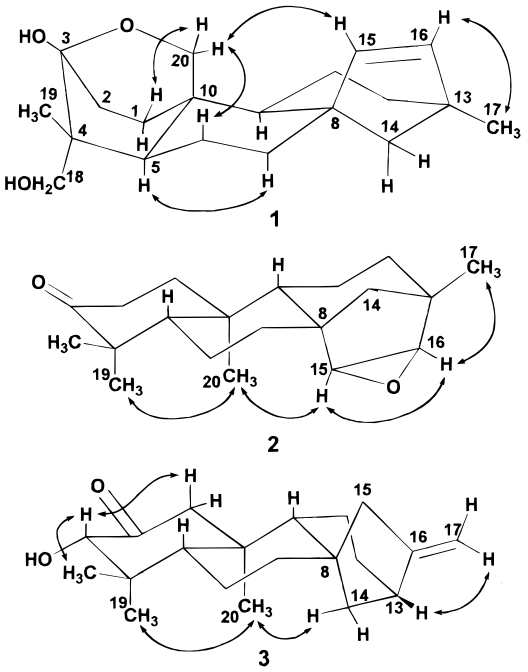

Figure 2. NOE correlations for compounds **1**−**3**.

Experimental Section

General Experimental Procedures. Melting points were measured with a Yanagimoto micromelting-point apparatus and are uncorrected. Optical rotations were recorded using a Horiba digital polarimeter. IR spectra were recorded using a Shimadzu 100A spectrometer with KBr pellets. CD spectra were recorded using a JASCO J-500C spectropolarimeter in $CHCl_3$ or MeOH at 24 °C. ^1H NMR (300 and 500 MHz) and ^{13}C NMR (75.4 and 125 MHz) spectra were recorded on Varian XL-300 and JEOL JNM-LA500 spectrometers in $CDCl_3$ with TMS as internal standard, respectively. MS were obtained with a JEOL LMS-SX-120A-QQ mass spectrometer. HPLC was carried out on a LC-09 instrument with JAIGEL-310 column (500 × 20.0 mm i.d.). For column chromatography, Si gel (Merck), Lichroprep reversed-phase C_{18} (20 × 1.0 mm i.d.) (Merck), and Sephadex LH-20 (Pharmacia) were used.

Plant Material. Wood of *E. agallocha* was collected in February 1994, from Okinawa Island, Japan. A voucher specimen (KPU 001949) is deposited in the Herbarium of the Department of Pharmaceutical Sciences of Natural Resources, Kyoto Pharmaceutical University, Japan.

Extraction and Isolation. Chopped wood of *E. agallocha* (588 g) was extracted three times with diethyl ether at room temperature. The combined extracts were evaporated to give a brown syrup (103 g). This syrup (30 g) was adsorbed onto Si gel and column chromatographed, eluting with binary solvent systems (hexane−EtOAc gradient and $CHCl_3$−MeOH gradient) and preparative recycling HPLC on a gel column eluting with MeOH to afford **1** (20.5 mg), **2** (15.0 mg), **3** (8.3 mg), **4** (25.6 mg), **5** (111.5 mg), and **6** (46.3 mg).

Excoecarin D (1): mp 177.5−179.5 °C; $[\alpha]^{25}_D$ −37.8° (*c* 1.0, $CHCl_3$); IR (KBr) ν_{max} 3360, 1670, 1458, 1387, 1317, 1178, 1136, 1111, 1051, 1034, 744 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.91 (1H, ddd, J = 1.3, 3.9, 12.9 Hz, H-9), 0.98 (1H, d, J = 9.7 Hz, H-14), 1.00 (3H, s, H-17), 1.07 (1H, dd, J = 4.2, 6.8 Hz, H-5), 1.08 (1H, dd, J = 6.8, 12.2 Hz, H-6), 1.14 (3H, s, H-19), 1.17 (1H, m, H-1), 1.17 (1H, m, H-12), 1.24 (1H, m, H-12), 1.28 (1H, ddd, J = 3.6, 13.5, 13.5 Hz, H-7), 1.48 (1H, dd, J = 2.4, 9.7 Hz, H-14), 1.54 (1H, ddd, J = 3.7, 7.0, 13.4 Hz, H-11), 1.60 (1H, m, H-6), 1.60 (1H, ddd, J = 3.1, 6.1, 13.5 Hz, H-7), 1.66 (1H, ddd, J = 3.0, 13.4, 13.4 Hz, H-11), 1.80 (1H, ddd, J = 6.4, 13.4, 13.4 Hz, H-2), 2.12 (1H, ddd, J = 3.9, 3.9, 13.4 Hz, 1-H), 2.14 (1H, ddd, J = 3.9, 3.9, 13.4 Hz, H-2), 3.28 (1H, d, J = 11.0 Hz, 18-H), 3.63 (1H, d, J = 11.0 Hz, H-18), 3.78 (1H, dd, J = 1.5, 9.0 Hz, H-20a), 3.86 (1H, dd, J = 3.0, 9.0 Hz, H-20b), 5.48 (1H, d, J = 5.8 Hz, H-16), 5.59 (1H, d, J = 5.8 Hz, H-15); ^{13}C NMR ($CDCl_3$), Table 1; FABMS *m/z* 319 $[M + H]^+$, 341 $[M + Na]^+$, 637 $[2 \times M + H]^+$; HRFABMS *m/z* 319.2266 $[M + H]^+$ (calcd for $C_{20}H_{31}O_3$, 319.2259).

X-ray Diffraction Structure Determination for 1.[10] A single crystal of **1** was obtained by recrystallization from

MeOH. Crystal data of **1**: $C_{20}H_{30}O_3$; Mr = 318.46, colorless needles, space group *C*2(No. 5), a = 13.416(2) Å, b = 6.119(2) Å, c = 21.203(2) Å, V = 1753.6(7) Å^3, Z = 4, D_{calc} = 1.219 g/cm^3, μ(Cu Kα) = 6.30 cm^{-1}. The R (R_w) value of **1** was 0.049 (0.062). The data were collected on a Rigaku AFC7R diffractometer at 23 ± 1 °C using graphite-monochromated Cu Kα (λ = 1.54178 Å) radiation. The structure was solved by direct methods (MITHRIL84[11]). The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. Neutral atoms scattering factors were taken from Cromer and Waber.[12] All calculations were performed using the teXsan[13] crystallographic software package of Molecular Structure Corporation.

Excoecarin E (2): mp 117−118 °C; $[\alpha]^{26}_D$ + 2.7°(*c* 1.5, $CHCl_3$); IR (KBr) ν_{max} 1705, 1244, 978, 924, 895, 846 cm^{-1}; CD ($CHCl_3$) (λ ext) 307 ($\Delta\epsilon$ 0), 285 (−0.39), 250 nm(0); ^{1}H NMR ($CDCl_3$) δ 0.55 (1H, d, J = 11.0 Hz, H-14), 1.04 (3H, s, Me-17), 1.08 (6H, s, Me-19, -20), 1.10 (3H, s, Me-18), 1.20 (1H, d, J = 11.0 Hz, H-5), 1.21 (1H, d, J = 11.0 Hz, H-14), 1.91 (1H, ddd, J = 3.7, 7.0, 13.0 Hz, H-1), 1.94 (1H, ddd, J = 3.0, 3.0, 13.0 Hz, H-12), 2.37 (1H, ddd, J = 3.7, 7.0, 16.0 Hz, H-2), 2.56 (1H, ddd, J = 7.0, 12.0, 16.0 Hz, H-2), 3.06 (1H, d, J = 3.0 Hz, H-16), 3.42 (1H, d, J = 3.0 Hz, H-15); ^{13}C NMR ($CDCl_3$), Table 1; FABMS *m/z* 303 $[M + H]^+$; HRFABMS *m/z* 303.2343 $[M + H]^+$ (calcd for $C_{20}H_{31}O_2$, 303.2324).

Excoecarin K (3): mp 111−116 °C; $[\alpha]^{26}_D$ −50.8° (*c* 0.89, MeOH); IR (KBr) ν_{max} 3526, 1713, 1655, 885 cm^{-1}; CD ($CHCl_3$) (λ ext) 313 ($\Delta\epsilon$ 0), 285 (−2.35), 250 nm(0); ^{1}H NMR ($CDCl_3$) δ 0.70 (3H, s, H-19), 0.98 (3H, s, Me-20), 1.19 (3H, s, Me-18), 1.91 (1H, d, J = 12.0 Hz, H-14), 2.03 (1H, dd, J = 1.5, 12.0 Hz, H-1), 2.11 (2H, dd, J = 2.0, 3.0 Hz, H-15), 2.67 (1H, m, H-13), 2.69 (1H, d, J = 12.0 Hz, H-1), 3.41 (1H, d, J = 5.0 Hz, OH-3), 3.87 (1H, dd, J = 1.5, 5.0 Hz, H-3), 4.77 (1H, br s, H-17), 4.83 (1H, br s, H-17); ^{13}C NMR ($CDCl_3$), Table 1; FABMS *m/z* 303 $[M + H]^+$; HRFABMS *m/z* 303.2309 $[M + H]^+$ (calcd for $C_{20}H_{31}O_2$, 303.2324).

Stachenol (4): colorless needles; physical and spectral data were in agreement with literature values.[7]

Stachenone (5): colorless needles from hexane; physical and spectral data were in agreement with literature values.[7]

***ent*-3*β*-Hydroxy-15-beyeren-2-one (6)**: colorless needles from aqueous MeOH; physical and spectral data were in agreement with literature values.[7]

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References and Notes

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