Five New Labdane-Type Diterpenes from *Excoecaria agallocha.* IV[1)]

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Received March 9, 1998; accepted May 14, 1998

Five new labdane-type diterpenes were isolated from the resinous wood of *Excoecaria agallocha*. The structures of these compounds were established as *ent*-13-*epi*-8,13-epoxy-2-hydroxylabda-1,14-dien-3-one (1), *ent*-13-*epi*-8,13-epoxy-14*S*,15-dihydroxylabdan-3-one (2), *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-ene-2,3-dioic acid (3), *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-ene-2,3-dioic acid 3-methyl ester (4) and *ent*-13-*epi*-8,13-epoxy-2-oxa-3-oxolabd-14-ene-1*R*-carboxylic acid (5).

Key words *Excoecaria agallocha*; Euphorbiaceae; secolabdane-type diterpene; labdane-type diterpene; structure elucidation

The genus *Excoecaria* of Euphorbiaceae comprises 40 species which are distributed throughout tropical Africa, Asia and northwest Australia.[2,3)] They are very well known as extreme skin irritants and tumor promoters.[4)] The leaves and latex of *Excoecaria agallocha* L. have been used as a dart poison and fish poison in India,[5)] New Caledonia,[6)] and Malaysia[7)] and the bark and wood of this tree have been used in traditional medicines to that flatulence in Thailand.[4)] In Okinawa, the resinous wood including the latex of the so-called "Okinawa-jinko", has been used as a substitute for the incense of agarwood (Jinko).[8)] The piscicidal constituent of the twigs and bark of *E. agallocha* native to Okinawa has been characterized as the daphnane diterpene ester, excoecariatoxin.[6)] This diterpene ester and some related compounds have also been obtained from the latex of *E. agallocha* in Thailand.[4)] Daphnane- and tigliane-type diterpene esters are known as skin irritants and tumor promoters.[4)] Other constituents of *E. agallocha* are known, including triterpenes,[7,9)] hydrocarbons and fatty acids forming surface waxes of woods,[10)] and a cinnamoyl piperidine alkaloid in the stem wood, which have been encountered so far only in the genus *Piper* (Piperaceae).[5)] A novel phorbol ester acting as an anti-HIV principle, was recently isolated from the leaves and stems of *E. agallocha* collected in northwest Australia.[3)]

We previously reported the isolation and structure elucidation of eleven diterpenes from the resinous wood of *E. agallocha* collected in Okinawa.[1)] In this paper, we describe the isolation and structure elucidation of new diterpenes from the same source.

Results and Discussion

The more polar fraction of the ether extract of the resinous wood[1)] was purified by various chromatographic techniques to afford the labdane-type diterpenes **1**—**5** (Chart 1). The IR spectrum of **1** showed absorption bands ascribable to hydroxyl (3400 cm^{-1}), olefinic (1638, 985, 914, 843 cm^{-1}), carbonyl (1650 cm^{-1}) and ether (1145, 1089 cm^{-1}) groups. The high-resolution (HR)-FAB-MS of **1** indicated the molecular formula to be $C_{20}H_{30}O_3$ (M^+ at *m/z* 318.2176). The ^{1}H-NMR spectrum displayed signals due to five tertiary methyls, four methylene protons, mono- and trisubstituted olefin protons [δ 4.95 (d, J=11.0 Hz, 15-H), 5.02 (d, J=18.0 Hz, 15-H), 6.20 (dd, J=11.0, 18.0 Hz, 14-H), 6.46 (s, 1-H)]. Furthermore, the ^{13}C-NMR spectrum indicated the presence of the one carbonyl group [δ 201.0 (s)], two double bond groups [δ 127.2 (s), 144.1 (d), 109.9 (t), 147.2 (d)] and two oxygenated carbons [δ 73.9 (s), 76.0 (s)]. These data suggested **1** was a tricyclic diterpene with a labdane skeleton. From a comparison of the ^{13}C-NMR data (Table 1) of **1** with that of ribenone (**6**),[1,11)] it was deduced that **1** possesses olefinic carbon signals at δ 144.1 and 127.2 instead of the methylene carbon signals at δ 38.2 and 33.9 in **6**. This fact suggested that **1** was the 1,2-enolate of **6**, namely, 8,13-epoxy-2-hydroxylabda-1,14-diene-3-one.[12)] This was verified by ^{1}H-^{13}C chemical

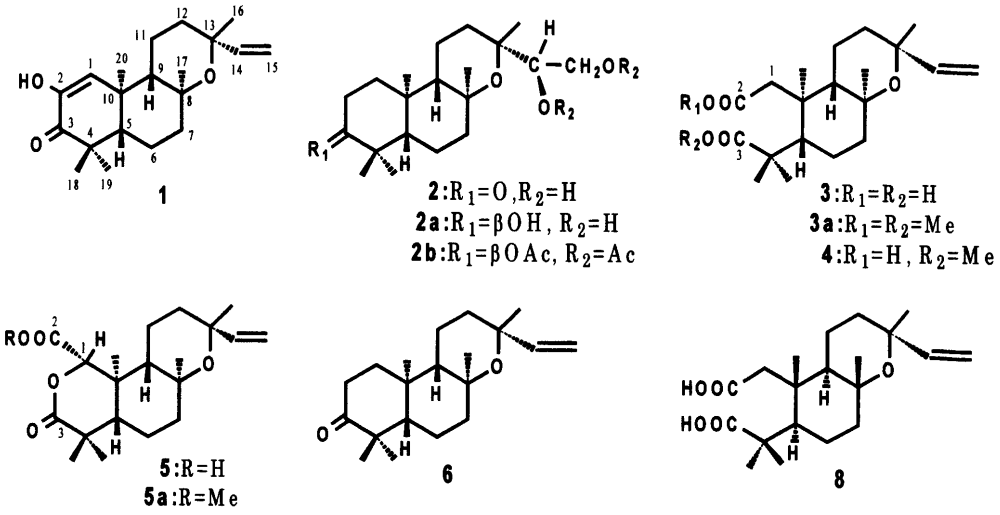

Chart 1. Chemical Structures of **1**—**6** and **8**

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shift correlation spectroscopy (COSY) NMR. The relative stereochemistry of **1** was established by nuclear Overhauser effect (NOE) difference spectra measurements (Fig. 1). The irradiation of methyl group resonance at δ 1.04 (20-H_3) produced NOE enhancements for the signals of 1-H, 17-H_3 and 19-H_3. Furthermore, an NOE was detected between 17-H_3 and 14-H, confirming the *trans*-orientation of the two methyl groups, 16 and 17-CH_3. These observations showed that the relative stereochemistry of **1** was the same as **6**. The circular dichroism (CD) spectrum of **1** showed a negative Cotton effect at 278 nm, analogous to that of **6**. Therefore, the absolute stereostructure of **1** was determined to be *ent*-13-*epi*-8,13-epoxy-2-hydroxylabda-1,14-dien-3-one.

Table 1. ^{13}C-NMR Spectral Data for Compounds **1**—**6** (δ in $CDCl_3$)

Carbon	1	2	3	4	5	6
1	144.1	37.7	40.5	41.3	77.2	38.2
2	127.2	33.8	177.7	175.7	176.2	33.9
3	201.0	216.8	187.0	180.1	176.5	217.4
4	44.0	47.3	45.2	46.0	40.3	47.3
5	53.9	55.0	48.6	48.0	52.6	54.6
6	19.9	20.8	20.5	21.8	20.2	20.8
7	42.3	42.9	42.3	41.6	41.3	42.2
8	76.0	75.1	75.7	75.7	75.4	75.5
9	53.9	53.3	49.6	50.6	52.5	57.7
10	38.1	36.6	41.0	41.5	41.1	36.4
11	16.3	14.7	16.5	16.7	18.0	16.4
12	34.6	29.8	34.4	34.6	34.8	34.8
13	73.9	75.7	73.3	73.4	73.8	73.6
14	147.2	75.7	147.3	147.4	147.8	147.4
15	109.9	63.5	109.8	109.7	109.4	109.8
16	32.5	25.2	32.3	32.4	32.7	32.7
17	24.2	24.6	23.8	23.0	23.4	23.4
18	27.0	26.4	30.1	28.1	29.7	26.7
19	21.4	21.1	32.3	23.4	22.4	20.9
20	20.0	14.7	20.8	19.6	10.4	15.5
OMe				52.1		

Compound **2** was obtained as colorless needles, mp 98—99 °C, and showed $[\alpha]_D$ −34.0°. The IR spectrum of **2** showed a hydroxyl (3457 cm^{-1}), carbonyl (1699 cm^{-1}) and ether (1076, 989, 958 cm^{-1}) groups. Its molecular formula was determined by HR-FAB-MS and elemental analysis to be $C_{20}H_{34}O_4$. The ^{1}H-NMR spectrum of **2** contained the five methyl signals at δ 0.94, 1.03, 1.10, 1.12 and 1.34, methine and methylene protons on each carbon bearing a hydroxyl group [δ 3.58 (dd, J=3.0, 6.0 Hz, 14-H), δ 3.64 (dd, J=3.0, 11.0 Hz, 15-H), 3.72 (dd, J=6.0, 11.0 Hz, 15-H)]. The ^{13}C-NMR spectrum indicated the presence of carbonyl carbon (δ 216.8) and oxygenated carbons [δ 75.1 (s), 75.7 (s), 75.7 (d), 63.5 (t)]. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **6**, except for some signals due to the side-chain [**2**: δ_C 75.7, δ_H 3.58 (1H, dd, J=3.0, 6.0 Hz, 14-H), 63.5, 3.64 (1H, dd, J=3.0, 11.0 Hz, 15-H), 63.5, 3.72 (1H, dd, J=6.0, 11.0 Hz, 15-H)]. The 14,15-diol structure of **2** was characterized on the basis of its ^{1}H–^{13}C COSY, distortionless enhancement by polarization transfer (DEPT) and NOE differenced spectra measurements. NOE correlations were observed between the 17-H_3 and 14-H, 17-H_3 and 20-H_3, 16-H_3 and 14-H, and 19-H_3 and 20-H_3 signals. These findings led us to presume that **2** is a 14,15-diol of **6**. The reduction of **2** with $NaBH_4$ provided the 3-equatorial OH derivative (**2a**, 75%) whose ^{1}H-NMR spectrum showed a signal due to the 3-axial proton at δ 3.20 (1H, dd, J=6.0, 11.0 Hz, 3-H) (Chart 2). Furthermore, acetylation of **2a** with acetic anhydride in pyridine yielded the triacetate (**2b**, 52%), whose ^{13}C-NMR spectrum showed acetylation shifts around the 3, 14, 15-positions of **2a**. Garcia-Granados *et al.* reported the assignment of the C-14 stereochemistry of 8,13-epoxy-labdane diterpenes with a C-14 or C-14, -15 oxygenated side-chain could be determined by the variation of chemical shifts at C-12 and C-16 in the ^{13}C-NMR spectra of these acetylated derivatives.[13] In the 14*S*-configuration, acetylation of the C-14 hydroxyl group causes a large down-

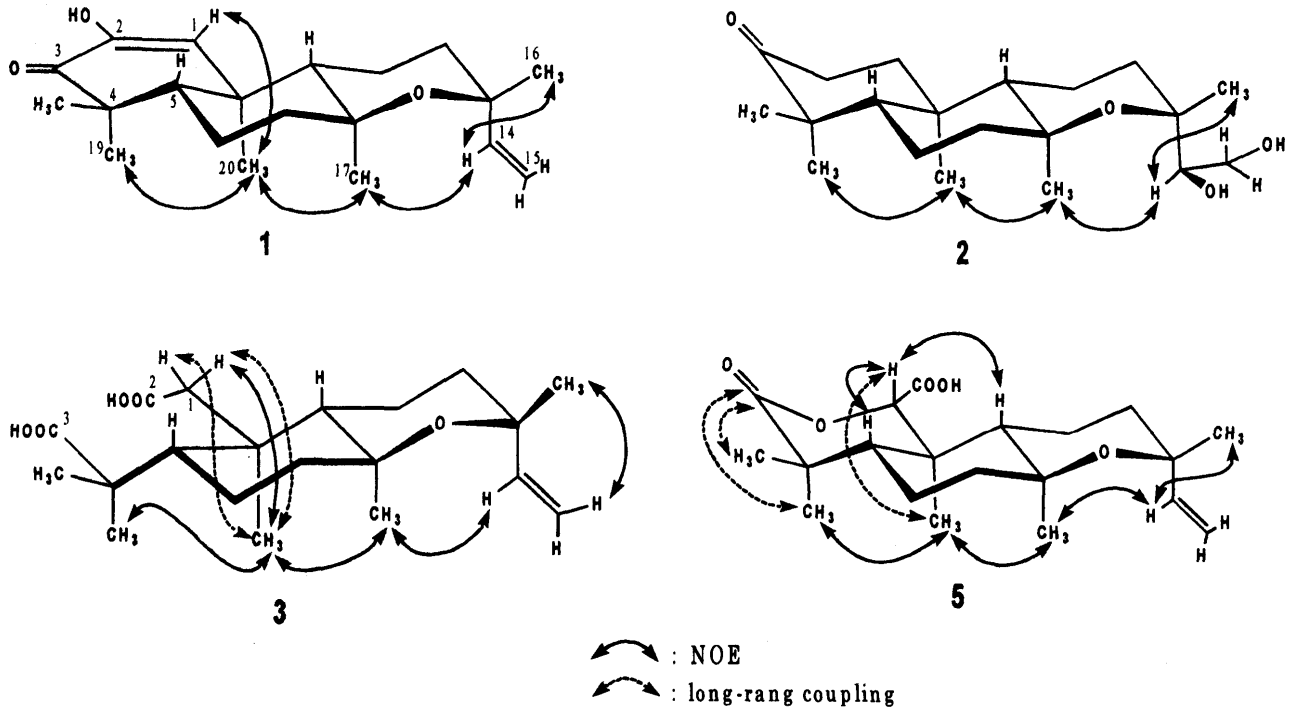

Fig. 1. C-H Long-Range Couplings and NOE Correlations for **1**—**3** and **5**

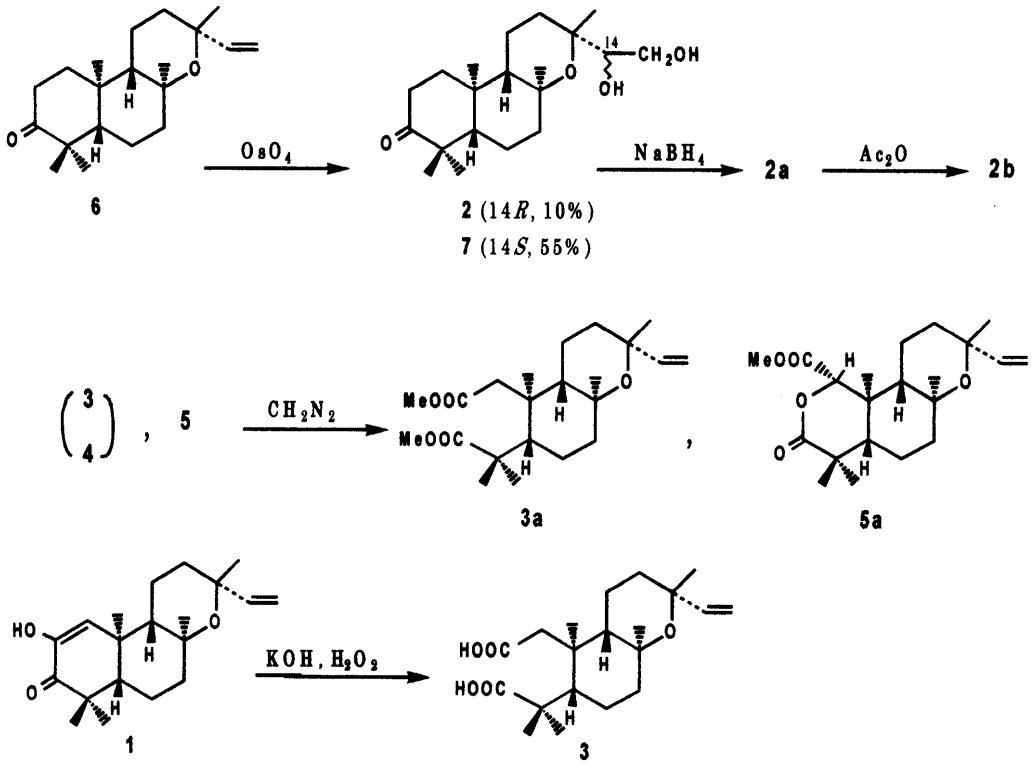

Chart 2

field shift of C-12 (Δ 4.2 ppm, δ 28.8 to 33.0 for acetylated and hydroxylated compounds) and no substantial shift of C-16 (Δ 0.2 ppm, δ 24.6 to 24.8 for both compounds), whereas in the 14*R*-configuration, the same structural variation causes an upfield shift of C-12 (Δ −0.9 ppm, δ 29.5 to 28.6) and a downfield shift of C-16 (Δ 1.1 ppm, δ 26.2 to 26.3).[13] The ^{13}C-NMR spectrum of **2b** showed an upfield shift of C-12 (Δ −1.1 ppm, δ 28.7 to 29.8) and a downfield shift of C-16 (Δ 1.1 ppm, δ 26.3 to 25.2) following acetylation of hydroxyl groups. Thus, the absolute configuration at the C-14 of **2** and **2a** is presumed to be the *R* configuration based on comparison of the ^{13}C chemical shifts at C-12, C-16 for triacetate (**2b**) with the above data.

Finally, **2b** was identified with 3β,14*R*,15-triacetoxy-8,13*R*-epoxylabdan-3-one[13] by comparison of the spectra and physical data for **2b** with those of the reference compound.[13] On the other hand, oxidation of **6** with OsO_4 gave **2** and the 14-isomer of **2** (**7**), in 10% and 55% yield, respectively. Consequently, the absolute structure of **2** has been determined as *ent*-13-*epi*-8,13-epoxy-14*S*,15-dihydroxylabdan-3-one.

The molecular formula of **3** was established by HR-FAB-MS as $C_{20}H_{32}O_5$. This compound was detected by 2,6-dichlorophenol–indophenol reagent.[14] IR spectroscopy revealed the presence of carboxylic acid (3300, 1705, 1697 cm^{-1}), olefinyl (984, 912 cm^{-1}), and ether (1169, 1089 cm^{-1}) groups. Esterification of **3** with diazomethane gave a dimethyl ester (**3a**, $C_{22}H_{36}O_5$), which showed carbonyl absorptions at 1750, 1728 cm^{-1}. The ^{13}C-NMR spectrum of **3** in $CDCl_3$ indicated the presence of two carbonyl groups [δ 177.7 (s), 187.0 (s)], a double bond [δ 109.8 (t), 147.3 (d)], and two oxygenated carbons [δ 75.7 (s), 73,3 (s)]. The ^{1}H-NMR spectrum exhibited five methyl singlets (δ 0.82, 1.13, 1.14, 1.22, 1.32) and three olefin protons [δ 4.92 (d, J=11.0 Hz), 4.96 (d, J=18.0 Hz), 5.99 (dd, J=11.0, 18.0 Hz)]. The two-proton doublet signals at δ 2.45 and 2.52 were assigned to CH_2COOH, a grouping which can only result if **3** is a seco-acid derived from ring-A bond fission. These data suggested **3** was a dicyclic diterpene with a labdane or related carbon skeleton. Careful comparison of the NMR data for **3** with those of 1,4-dicarboxy-2,3-dinormanoyl oxide (**8**)[12] clearly showed that **3** is a 2,3-dinormanoyl oxide diterpene. The chemical shifts of the ^{13}C-NMR signals of **3** were nearly identical to those of **8** except for C-9, C-11, C-16, and C-17. This was verified by observation of ^{1}H–^{13}C long-range couplings between the methylene protons at δ 2.45, 2.52 (1-H) and the carbon signal at δ 20.8 (C-20), and by correlations between the 16-H and 12ax-H, 17-H and 11ax-H, 14-H and 15-H_2 behavior of ^{1}H–^{1}H COSY. Accordingly, it was inferred that **3** is the 13-epimer of **8**. The relative stereochemistry of **3** was established by NOE difference spectra measurements (Fig. 1). Thus, irradiation of the methyl group resonance at δ 0.82 [20-H_3] produced NOE enhancements for the signals of 17-H_3 (4.0%), 1-H (3.4%) and 19-H_3 (4.0%), but did not produce any NOE enhancement for the 5-H signal. Furthermore, an NOE was observed between 17-H_3/14-H, which confirmed the *trans*-orientation of the C-16 and C-17 methyl groups. These observations suggested that the relative stereochemistry of **3** was the same as **8**, except for the orientation at C-13. The optical rotation of **3** showed a negative value ([α]$_D$ −14.3°), which confirmed that the structure of **3** was an enantiomer of the 13-epimer of **8**. Compound **8** was obtained by Jones oxidation of 2-oxo-3β-hydroxymanoyl oxide ([α]$_D$ +44°).[12] Oxidation of **1** with 10% hydrogen peroxide–10% potassium hydroxide afforded **3** in 25% yield.[15] Accordingly, **3** was assigned as *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-ene-2,3-dioic acid.

Compound **4**, mp 146—151 °C, [α]$_D$ −35.6°, was isolated

as colorless needles. The IR spectrum showed absorption bands for an ester group (1728 cm^{-1}), and carboxylic acid, double bond and ether groups as observed in **3**. The molecular formula of **4** was established as $C_{21}H_{34}O_5$ on the basis of elemental analysis. The ^{13}C-NMR spectrum indicated the presence of two carbonyl groups [δ 175.7 (s), 180.1 (s)], a double bond [δ 109.7 (t), 147.4 (d)], and two oxygenated carbons [δ 75.7 (s), 73.4 (s)]. The ^1H-NMR spectrum showed four methyl signals at δ 0.83, 1.13, 1.23×2, and 1.26 for five methyl protons, three olefinic protons at δ 4.92 (dd, J=1.0, 11.0 Hz), 4.96 (dd, J=1.0, 18.0 Hz) and 6.00 (dd, J=11.0, 18.0 Hz), and one esterified methyl signal at δ 3.67 (s). Esterification of **4** with diazomethane gave **3a**, which was also obtained from **3** by the same treatment. Careful comparison of the ^{13}C-NMR data of **4** with those of **3** and **3a** clearly identified **4** as the 3-monomethyl ester of **3**. This was verified by ^1H-^{13}C long-range coupling experiments, which showed enhancement of the signal intensity of C-2 (δ 175.7; Wh/2=25 to 10 Hz) by respective irradiation of the signals of 1-H (δ 2.41), and C-3 (δ 180.1; Wh/2=20 to 10 Hz) by 18-H and 19-H (δ 1.23). Accordingly, the structure of **4** was assigned as *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-ene-2,3-dioic acid 3-methyl ester.

Compound **5** was obtained as colorless needles of mp 277—279 °C, $[\alpha]_D$ −6.2°. This compound was detected by 2,6-dichlorophenol–indophenol reagent.[14] This observation suggested the presence of a carboxylic acid group in **5**. Its molecular formula was determined by elemental analysis to be $C_{20}H_{30}O_5$. Its IR spectrum showed the presence of lactone and carboxylic acid (1709, 1620, and 1601 cm^{-1}), olefinic and ether (1163, 1134, 1086, 918, 740 cm^{-1}) groups. The ^1H- and ^{13}C-NMR spectra showed the presence of a monosubstitued olefinic group [δ_C 109.4, δ_H 4.88 (1H, d, J=11.0 Hz, 15-H), 109.4, 4.96 (1H, d, J=18.0 Hz, 15-H), 147.8, 6.00 (1H, dd, J=11.0, 18.0 Hz, 14-H)], a methine proton of a carbon bearing a carboxylic acid group [δ_C 77.2, δ_H 4.51 (1H, br s, 1-H)], and five methyl signals [δ_C 10.4, 22.4, 23.4, 29.7, 32.7, δ_H 0.94, 1.13, 1.18, 1.25, 1.26]. The ^1H- and ^{13}C-NMR spectra of **5** were similar to those of **6**, except for the signal of ring-A. These data suggested that the carboxylic acid and lactone groups were attached to ring-A. The locations of these substitution groups were clarified by measurements of ^1H–^{13}C and ^1H–^1H long-range decoupling spectra and NOE difference spectra for **5**. Enhancement of the signal intensity of C-3 (δ 176.5) was shown by respective irradiation of the signals of 18-H (δ 1.26) and 19-H (δ 1.22) in the ^1H–^{13}C long-range coupling experiments. An ^1H–^1H long-range coupling correlation was observed between 20-H_3 and 1-H. Irradiation of the signal of 1-H produced NOE enhancements for methine protons at δ 1.61 (5-H) and 1.48 (9-H), but no NOE enhancement for the signals of 20-H_3, 19-H_3 and 18-H_3. Thus, the carboxylic acid and carbonyl group of the lactone were located at the C-1 and C-4 positions, respectively. Also the orientation of C-1 was determined as the *R* configuration. The relative stereochemistry of **5** was also established by the NOE experiments. An NOE was detected between 20-H_3 and 17-H_3, 17-H_3 and 14-H, 20-H_3 and 19-H_3. These observations showed that the relative stereochemistry of **5** was the same as **6**. Methylation of **5** with diazomethane provided the monomethyl ester (**5a**), whose ^1H-NMR spectrum showed a signal due to the methoxyl protons at δ 3.76 (3H, s). Comparison of the ^{13}C-NMR data of **5** with those of **5a** showed an esterification shift (7.5 ppm) of the carbon signal at the C-2 position of **5a** following methylation of the carboxylic acid. The CD spectrum of **5a** showed a negative Cotton effect (296 nm) analogous to that of **6**. Consequently, the absolute stereochemistry of **5** was determined to be *ent*-13-*epi*-8,13-epoxy-2-oxa-3-oxolabd-14-ene-1*R*-carboxylic acid.

This is the first example of the isolation of *ent*-2,3-secomanoyl oxide derivatives (**3**, **4**), and lactone (**5**) from *E. agallocha*. Until now, this type of diterpene had been isolated from *Dacrydium colensoi*,[16] but not from *E. agallocha*. Examination of the constituents of the resinous wood of this plant resulted in the isolation of five new diterpenes. These compounds were derivatives of highly oxygenated diterpenes.

Experimental

General Methods Optical rotations and CD were determined on a Horiba digital polarimeter and JASCO J-500C spectrometer, respectively. IR spectra were measured with a Shimadzu FT-IR-8100 spectrometer. NMR spectra were recorded on a Varian XL-300 spectrometer in $CDCl_3$ solution using tetramethylsilane (TMS) as an internal standard. The FAB-MS and HR positive FAB-MS (70 eV, 3-nitrobenzyl alcohol as the matrix) were measured on a JEOL JMS-SX 102AQQ mass spectrometer and electron impact (EI)-MS was carried out on a JEOL MS-BU20 with a direct probe. Column chromatography was carried out on Kiesel gel 60 (120—230 μm, Merck), Lichroprep Rp-18 (ODS, 4—63 μm, Merck), Lichroprep Si 60 (40—60 μm, Merck), and Sephadex LH-20 (Pharmacia).

Isolation The more polar fraction (fr. 8, 4.65 g) of the ether extract obtained in the previous paper[1] was fractionated by silica-gel column chromatography with a binary solvent system (*n*-hexane–AcOEt gradient) into six fractions: fr. 1 (300 mg), fr. 2 (544.8 mg), fr. 3 (550.2 mg), fr. 4 (1359.6 mg), fr. 5 (840.5 mg), fr. 6 (1067.6 mg). Rechromatography on fr. 3 on silica-gel (hexane : AcOEt=4 : 1) gave the crude fraction (113 mg) containing compound **1**. The crude fraction was chromatographed repeatedly on silica-gel (hexane : AcOEt=3 : 1) and octadecyl silica (ODS) (35% MeOH) to afford pure compound **1** (25.4 mg). Fraction 4 was chromatographed on silica-gel ($CHCl_3$: MeOH : H_2O=4 : 1 : 0.1) to give fr. 4-1 (203 mg) containing compounds **4** and **5**, and fr. 4-2 (848.6 mg) containing compound **3**. Furthermore, fr. 4-1 was repeatedly chromatographed on silica-gel ($CHCl_3$: MeOH : H_2O=4 : 1 : 0.1) and ODS (58% MeOH) to give compounds **4** (198 mg) and **5** (7.7 mg). Rechromatography of fr. 4-2 on silica-gel using $CHCl_3$: MeOH : H_2O=4 : 1 : 0.1 solvent system and Sephadex LH-20 (MeOH) gave compound **3** (518 mg). Fraction 5 was chromatographed on silica-gel (*n*-hexane : AcOEt=1 : 3) and Lichroprep Si 60 ($CHCl_3$: MeOH=10 : 1) to give compound **2** (52.6 mg).

ent-13-*epi*-8,13-Epoxy-2-hydroxylabda-1,14-dien-3-one (**1**): A white powder (mp 79—80 °C), $[\alpha]_D^{30}$ −79.2° (c=0.475, $CHCl_3$). IR (KBr, cm^{-1}): 3400 (OH), 1650 (C=O), 1638, 1145, 1089, 985, 914, 843. CD (c=1.18×10^{-4}, $CHCl_3$) $\Delta\varepsilon$=0 (367), −2.86 (278), 0 (240 nm). ^1H-NMR ($CDCl_3$) δ: 1.04 (3H, s, 20-H), 1.10 (3H, s, 18-H), 1.15 (3H, s, 16-H), 1.22 (3H, s, 19-H), 1.29 (1H, s, 17-H), 2.31 (1H, ddd, J=3.0, 3.0, 13.0 Hz, 5-H or 12-H), 4.95 (1H, d, J=11.0 Hz, 15-H), 5.02 (1H, d, J=18.0 Hz, 15-H), 6.20 (1H, dd, J=11.0, 18.0 Hz, 14-H), 6.46 (1H, s, 1-H), 5.97 (1H, br, OH). ^{13}C-NMR ($CDCl_3$): see Table 1. EI-MS: m/z 318 $(M)^+$. FAB-MS m/z: 319 $(M+H)^+$. HR-FAB-MS m/z: Calcd for $C_{20}H_{30}O_3$: 318.2195. Found: 318.2176 $(M)^+$.

ent-13-*epi*-8,13-Epoxy-14*S*,15-dihydroxylabdan-3-one (**2**): Colorless needles, mp 98—99 °C, $[\alpha]_D^{26}$ −34.0° (c=2.48, $CHCl_3$). IR (KBr, cm^{-1}): 3457 (OH), 1699 (C=O), 1454, 1383, 1076, 989, 958, 877. ^1H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 20-H), 1.03 (3H, s, 19-H), 1.10 (3H, s, 18-H), 1.12 (3H, s, 16-H), 1.34 (3H, s, 17-H), 1.84 (1H, dd, J=3.5, 9.0 Hz, 7eq-H), 1.86 (1H, ddd, J=4.0, 7.5, 13.5 Hz, 1eq-H), 2.15 (1H, dd, J=7.0, 13.5 Hz, 11ax-H), 2.42 (1H, ddd, J=4.0, 7.0, 16.0 Hz, 2ax-H), 2.56 (1H, ddd, J=7.5, 11.0, 16.0 Hz, 2eq-H), 2.92 (1H, br s, OH), 3.58 (1H, dd, J=3.0, 6.0 Hz, 14-H), 3.64 (1H, dd, J=3.0, 11.0 Hz, 15-H), 3.72 (1H, dd, J=6.0, 11.0 Hz, 15-H). ^{13}C-NMR ($CDCl_3$): see Table 1. Positive-FAB-MS m/z: 361 $(M+Na)^+$, 339 $(M+H)^+$, 321 $(M+H-H_2O)^+$. HR-FAB-MS m/z: Calcd for $C_{20}H_{35}O_4$: 339.2535. Found: 339.2550 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{34}O_4$: C, 70.97; H, 10.13. Found: C, 70.73; H, 10.15.

Osmylation of 6 To a solution of **6** (200 mg) in 6 ml acetone were added

2 ml absolute ether and a reaction mixture composed of 2 ml 0.5% osmium tetroxide catalyst solution and 2 ml 30% hydrogen peroxide. The reaction mixture became brown and the mixture was stirred for 16 h at room temperature. The reaction mixture was concentrated under reduced pressure, and water added followed by extraction with chloroform three times. The chloroform solutions were combined and evaporated under reduced pressure. The residue was purified by column chromatography on silica-gel to give **2** (10%) and the 14-isomer of **2** (**7**, 55%). **7**: Colorless needles, mp 112—114 °C, $[\alpha]_D^{28}$ −35.7° (*c*=1.8, $CHCl_3$). IR (KBr, cm^{-1}): 3338 (OH), 1707 (C=O), 1089, 1078, 1057, 1010, 966, 916. ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s, 20-H), 1.03 (3H, s, 19-H), 1.10 (3H, s, 18-H), 1.19 (3H, s, 16-H), 1.28 (3H, s, 17-H), 1.83 (1H, ddd, *J*=3.0, 3.5, 9.0 Hz, 7eq-H), 1.86 (1H, ddd, *J*=4.0, 7.5, 13.0 Hz, 1eq-H), 2.12 (1H, m, 12-H), 2.41 (1H, ddd, *J*=4.0, 7.0, 16.0 Hz, 2ax-H), 2.56 (1H, ddd, *J*=7.5, 11.0, 16.0 Hz, 2eq-H), 2.61 (1H, br s, OH), 3.59 (2H, m, 14-, 15-H), 3.78 (1H, dd, *J*=7.8, 14.0 Hz, 15-H). ^{13}C-NMR ($CDCl_3$): see Table 1. Positive-FAB-MS *m/z*: 361 $(M+Na)^+$, 339 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{20}H_{35}O_4$: 339.2535. Found: 339.2545 $(M+H)^+$.

Reduction of 2 with $NaBH_4$ A methanol solution (2 ml) of compound **2** (10 mg) was treated with $NaBH_4$ (10 mg) at room temperature for 1 h. After removal of methanol, the residue was recrystallized from aqueous methanol to give **2a** (7.5 mg). **2a**: Colorless needles, mp 142—145 °C, $[\alpha]_D^{28}$ −14.0° (*c*=0.75, $CHCl_3$). IR (KBr, cm^{-1}): 3385 (OH), 1088, 1037, 987. 1H-NMR ($CDCl_3$) δ: 0.77 (3H, s, 20-H), 0.81 (3H, s, 19-H), 0.98 (3H, s, 18-H), 1.10 (3H, s, 16-H), 1.30 (3H, s, 17-H), 1.83 (1H, ddd, *J*=3.0, 3.5, 9.0 Hz, 7eq-H), 2.05 (1H, m, 12-H), 3.20 (1H, dd, *J*=6.0, 10.0 Hz, H-3), 3.60 (2H, m, 14-, 15-H), 3.70 (1H, dd, *J*=2.5, 12.0 Hz, 15-H). ^{13}C-NMR ($CDCl_3$–CD_3OD) δ: 14.4 (C-11), 15.0 (C-20), 15.1 (C-19), 19.5 (C-6), 24.6 (C-16), 24.7 (C-17), 26.3 (C-2), 27.7 (C-18), 29.8 (C-12), 36.7 (C-10), 37.1(C-1), 38.6 (C-4), 43.4 (C-7), 53.9 (C-9), 55.2 (C-5), 63.1 (C-15), 75.1 (C-13), 75.5 (C-8), 76.2 (C-14), 78.1 (C-3). Positive-FAB-MS: *m/z* 363 $(M+H)^+$, 341 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{20}H_{37}O_4$: 341.2692. Found: 341.2709 $(M+H)^+$.

Acetylation of 2a A mixture of **2a** (7.7 mg) and Ac_2O–pyridine (1 : 1) (2 ml) was left to stand overnight. The reaction solution was evaporated under reduced pressure and the residue was crystallized with aqueous methanol to give **2b** as colorless needles (4.0 mg). **2b**: mp 208—209 °C, $[\alpha]_D^{30}$ +14.1° (*c*=0.5, $CHCl_3$). IR (KBr) cm^{-1}: 1753, 1740, 1738, 1240, 1223, 1088, 1047, 997, 945, 868. ^{1}H-NMR ($CDCl_3$): δ 0.82 (3H, s, 20-H), 0.84 (3H, s, 16-H), 0.86 (3H, s, 18- or 19-H), 1.22 (3H, s, 17-H), 1.60 (3H, s, 19- or 18-H), 2.02, 2.05, 2.11 (each 3H, s, $COCH_3$), 4.07 (1H, dd, *J*=9.0, 12.0 Hz, 15-H), 4.44 (1H, dd, *J*=2.5, 12.0 Hz, 15-H), 4.80 (1H, dd, *J*=5.0, 11.0 Hz, 3-H), 5.04 (1H, dd, *J*=2.5, 9.0 Hz, 14-H). ^{13}C-NMR ($CDCl_3$) δ: 14.5 (C-11), 15.1 (C-20), 16.5 (C-19), 19.6 (C-6), 26.3 (C-16), 25.3 (C-17), 23.4 (C-2), 28.0 (C-18), 28.7 (C-12), 36.8 (C-10), 36.8(C-1), 37.7 (C-4), 43.5 (C-7), 53.1 (C-9), 55.1 (C-5), 63.6 (C-15), 73.1 (C-13), 74.6 (C-8), 76.6 (C-14), 80.7 (C-3), 20.9, 21.2, 21.3 (CH_3), 170.7, 171.0, 171.1 (C=O). FAB-MS *m/z*: 467 $[M+H]^+$. HR-FAB-MS *m/z*: Calcd for $C_{26}H_{43}O_7$: 467.3009. Found: 467.3001$(M+H)^+$.

ent-13-*epi*-8,13-Epoxy-2,3-secolabd-14-ene-2,3-dioic Acid (**3**): mp 202—204 °C, $[\alpha]_D^{30}$ −14.3° (*c*=0.77, $CHCl_3$). IR (KBr) cm^{-1}: 3300, 1705, 1697, 1640, 1169, 1089, 984, 912. ^{1}H-NMR ($CDCl_3$): δ 0.82 (3H, s, 20-H), 1.13 (3H, s, 16-H), 1.14 (3H, s, 18- or 19-H), 1.22 (3H, s, 17-H), 1.32 (3H, s, 19- or 18-H), 1.79 (1H, dd, *J*=3.0, 9.0 Hz, 6-H), 1.83 (1H, dd, *J*=3.0, 9.0 Hz, 7-H), 2.15 (1H, dd, *J*=7.5, 11.0 Hz, 9-H), 2.45 (1H, d, *J*=19.0 Hz, 1-H), 2.52 (1H, d, *J*=19.0 Hz, 1-H), 2.60 (1H, dd, *J*=2.0, 12.0 Hz, 5-H), 2.40 (1H, m, 12-H), 4.92 (1H, d, *J*=11.0 Hz, 15-H), 4.96 (1H, d, *J*=18.0 Hz, 15-H), 5.99 (1H, dd, *J*=11.0, 18.0 Hz, 14-H). ^{13}C-NMR ($CDCl_3$): see Table 1. FAB-MS *m/z*: 353 $[M+H]^+$. HR-FAB-MS *m/z*: Calcd for $C_{20}H_{33}O_5$: 353.2328. Found: 353.2315 $(M+H)^+$. *Anal.* Calcd for $C_{20}H_{32}O_5 \cdot 0.2H_2O$: C, 67.46; H, 9.17. Found: C, 67.45; H, 9.28.

Oxidation of 1 by Alkaline Peroxide 10% KOH (10 ml) was added to an ice-cooled solution of **1** (20 mg) in methanol (20 ml), then a solution of 30% hydrogen peroxide (5 ml) was added with stirring at 0—5 °C. After this addition, stirring was continued at room temperature for 24 h. The reaction mixture was acidified with 10% HCl and extracted with $CHCl_3$. The organic phase was dried with Na_2SO_4, filtered and evaporated under reduced pressure. The residue was chromatographed on silica-gel ($CHCl_3$: MeOH : H_2O=10 : 1 : 0.1) to give **3** (5 mg), which was identified on the basis of IR, NMR spectra comparisons with authentic sample.

ent-13-*epi*-8,13-Epoxy-2,3-secolabd-14-ene-2,3-dioic Acid 3-Methyl Ester (**4**): mp 146—151 °C; $[\alpha]_D^{30}$ −35.6° (*c*=1.0, $CHCl_3$). IR (KBr) cm^{-1}: 3300, 1728, 1713, 1253, 1228, 1145, 1092, 1068, 984, 918. ^{1}H-NMR ($CDCl_3$) δ 0.83 (3H, s, 20-H), 1.13 (3H, s, 16-H), 1.23 (6H, s, 18-, 19-H), 1.26 (3H, s, 17-H), 1.65 (1H, dd, *J*=3.0, 9.0 Hz, 6-H), 1.74 (1H, dd, *J*=3.0, 9.0 Hz, 7-H), 2.06 (1H, dd, *J*=7.5, 11.0 Hz, 9-H), 2.21 (1H, m, 12-H), 2.41 (1H, d, *J*=17.5 Hz, 1-H), 2.49 (1H, d, *J*=17.5 Hz, 1-H), 2.52 (1H, ddd, *J*=3.5, 6.5, 6.5 Hz, 5-H), 3.67 (3H, s, OCH_3), 4.92 (1H, dd, *J*=1.0, 11.0 Hz, 15-H), 4.96 (1H, dd, *J*=1.0, 18.0 Hz, 15-H), 6.00 (1H, dd, *J*=11.0, 18.0 Hz, 14-H). ^{13}C-NMR ($CDCl_3$): see Table 1. FAB-MS *m/z* 367 $[M+H]^+$. *Anal.* Calcd for $C_{21}H_{34}O_5 \cdot 0.33H_2O$: C, 67.72; H, 9.38. Found: C, 67.82; H, 9.35.

Methylation of 3 and 4 Compound **3** (180 mg) was dissolved in MeOH and a solution of CH_2N_2 (3.0% w/w) in diethyl ether (10 ml) was added. The reaction mixture was allow to stand for 3 h and the solvent was then evaporated under reduced pressure to give a residue. This residue was purified by column chromatography to give the dimethyl ester (**3a**, 130 mg). Compound **4** (77 mg) gave 64 mg dimethyl ester **3a** by the same treatment.

Dimethyl Ester (**3a**): A colorless syrup, $[\alpha]_D^{30}$ −30.0° (*c*=2.25, $CHCl_3$). IR (KBr) cm^{-1}: 1750, 1728, 1718, 1252, 1145, 1090, 1010, 983, 925. ^{1}H-NMR ($CDCl_3$): δ 0.81 (3H, s, 20-H), 1.13 (3H, s, 16-H), 1.22 (3H, s, 17-H), 1.236 (3H, s, 18-H), 1.244 (3H, s, 19-H), 1.63 (1H, dd, *J*=3.0, 9.0 Hz, 6-H), 1.72 (1H, dd, *J*=3.0, 8.0 Hz, 7-H), 2.06 (1H, dd, *J*=7.5, 11.0 Hz, 9-H), 2.20 (1H, m, 12-H), 2.41 (2H, s, 1-H), 2.53 (1H, ddd, *J*=3.5, 6.5, 6.5 Hz, 5-H), 3.62 (3H, s, OCH_3), 3.67 (3H, s, OCH_3), 4.92 (1H, dd, *J*=1.0, 11.0 Hz, 15-H), 4.96 (1H, d, *J*=18.0 Hz, 15-H), 5.99 (1H, dd, *J*=11.0, 18.0 Hz, 14-H). ^{13}C-NMR ($CDCl_3$) δ: 16.7 (C-11), 19.6 (C-20), 21.9 (C-6), 22.9 (C-17), 23.8 (C-19), 27.5 (C-18), 32.3 (C-16), 34.6 (C-12), 41.2 (C-1), 41.6 (C-10), 41.7 (C-7), 46.1 (C-4), 47.9 (C-5), 50.6 (C-9), 73.1 (C-13), 75.6 (C-8), 109.6 (C-15), 147.4 (C-14), 171.5 (C-2), 179.6 (C-3), 51.0, 51.4 (OMe). FAB-MS *m/z*: 381 $(M+H)^+$. HR-FAB-MS *m/z*: Calcd for $C_{22}H_{37}O_5$: 381.2641. Found: 381.2648 $(M+H)^+$.

ent-13-*epi*-8,13-Epoxy-2-oxa-3-oxolabd-14-ene-1*R*-carboxylic Acid (**5**): Colorless needles, mp 277—279 °C, $[\alpha]_D^{30}$ −6.2° (*c*=2.0, $CHCl_3$). IR (KBr) cm^{-1}: 3400, 1709, 1620, 1601, 1388, 1163, 1134, 1086, 918, 740. ^{1}H-NMR ($CDCl_3$): δ 0.94 (3H, s, 20-H), 1.13 (3H, s, 16-H), 1.18 (3H, s, 19-H), 1.25 (3H, s, 17-H), 1.26 (3H, s, 18-H), 4.51 (1H, br s, 1-H), 4.88 (1H, d, *J*=11.0 Hz, 15-H), 4.96 (1H, d, *J*=18.0 Hz, 15-H), 6.00 (1H, dd, *J*=11.0, 18.0 Hz, 14-H). ^{13}C-NMR ($CDCl_3$): see Table 1. FAB-MS *m/z*: 350 $(M)^+$. *Anal.* Calcd for $C_{20}H_{30}O_5$: C, 68.54; H, 8.63. Found: C, 68.50; H, 8.52.

Methylation of 5 with Diazomethane **5** was treated by the same procedure for the methylation of **3**, to afford **5a**. **5a**: $[\alpha]_D^{27}$ +4.57° (*c*=2.16, MeOH). IR (KBr, cm^{-1}): 2361, 1760, 1740, 1387, 1163, 1130, 918, 740. CD (*c*=1.3×10^{-4}, MeOH); $\Delta\varepsilon$=−0.61 (296), 0 (330), 0 (250 nm). ^{1}H-NMR ($CDCl_3$) δ: 1.04 (3H, s, 20-H), 1.14 (3H, s, 16-H), 1.22 (3H, s, 19-H), 1.28 (3H, s, 17-H), 1.32 (3H, s, 18-H), 1.84 (1H, ddd, *J*=3.0, 3.0, 7.0 Hz, 7ax-H), 2.20 (1H, ddd, *J*=3.0, 3.0, 13.0 Hz, 12ax-H), 3.76 (3H, s, OCH_3), 4.54 (1H, s, 1ax-H), 4.95 (1H, d, *J*=11.0 Hz, 15-H), 4.97 (1H, d, *J*=18.0 Hz, 15-H), 5.99 (1H, dd, *J*=11.0, 18.0 Hz, 14-H). ^{13}C-NMR ($CDCl_3$) δ: 10.2 (C-20), 18.0 (C-11), 20.1 (C-6), 22.9 (C-19), 23.4 (C-17), 29.7 (C-18), 32.5 (C-16), 34.3 (C-12), 40.6 (C-4), 41.0 (C-10), 41.3(C-7), 51.3 (C-9), 52.4 (OMe), 52.9 (C-5), 73.6 (C-13), 75.1 (C-8), 87.6 (C-1), 110.0 (C-15), 147.0 (C-14), 168.7 (C-2), 175.6 (C-3). EI-MS : *m/z* 365 $(M+H)^+$, 349 $(M-15)^+$, 289 $(365-HCOOCH_3)$. HR-FAB-MS *m/z*: Calcd for $C_{21}H_{33}O_5$: 365.2328. Found: 365.2344 $(M+H)^+$.

Acknowledgements We are thankful to Dr. M. Takasaki for NMR spectra and to Ms K. Oda for MS spectra measurements.

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