Stereostructure of Excoecarin H, a Novel *seco*-Labdane-Type Diterpene from *Excoecaria agallocha*

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A novel *seco*-labdane-type diterpene, excoecarin H was isolated from resinous wood of *Excoecaria agallocha* collected from Okinawa prefecture. Stereochemistry of the new diterpene was determined on the basis of chemical and physicochemical evidence.

Key words *Excoecaria agallocha*; Euphorbiaceae; excoecarin H; *seco*-labdane-type diterpene

In the course of our studies on constituents of *Excoecaria agallocha*, which has been used as a fish poison[1] and which is known to be a skin irritant,[2] we reported the diterpene constituents with an inhibitory effect on the Epstein-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in Raji cells.[3] As a continuing study, we isolated nine diterpenes from resinous wood of *E. agallocha* collected in Okinawa prefecture, Japan.[3,4] In this paper, we describe evidence consistent with the stereochemistry of a new compound, excoecarin H (**1**). The ether extract of the resinous wood was purified by repeated ordinary and reverse-phase silica gel column chromatography and, finally, by recycling HPLC to give excoecarin H (**1**, 0.0011%).

1 was obtained as colorless needles, mp 153—156 °C and showed $[\alpha]_D$ −46.0°. Its molecular formula was determined by high-resolution fast atom bombardment MS (HR-FAB-MS) measurement to be $C_{20}H_{34}O_4$ (*m/z* 338), which was 14 mass units less than that of the known *seco*-diterpene (**2**).[5] IR spectrum of **1** showed a hydroxyl group (3400 cm^{-1}), a carbonyl group (1709 cm^{-1}), an ether group (1094, 1068 cm^{-1}), and monosubstituted olefin (1640, 983, 916 cm^{-1}). The positive detection of **1** for 2,6-dichlorophenol-indophenol sodium salt on TLC also revealed the presence of carboxylic acid group.[6]

The ^{1}H-NMR ($CDCl_3$) spectrum of **1** indicated the presence of five *tert*-methyls [δ 0.90 (20-H_3), 1.14 (16-H_3), 1.23 (19-H_3), 1.24 (17-H_3), 1.30 (18-H_3)], six methylenes, and monosubstituted olefin [δ 4.93 (1H, d, J = 11.0 Hz, 15-H), 4.98 (1H, d, J = 18.0 Hz, 15-H), 6.05 (1H, dd, J = 11.0, 18.0 Hz, 14-H)]. The ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra of **1** showed 20 carbons (Table 1). The carbons of B and C rings were similar to those of ribenone (**3**),[4b,7] except for two methyl carbons (δ 27.0, 34.5), three methylene carbons (δ 23.6, 29.0, 33.7), two quaternary carbons (δ 75.9, 179.5), and a methine carbon (δ 50.9). Treatment of **1** with diazomethane in MeOH gave the monomethylester (**1a**). These data and detailed ^{13}C- and ^{1}H-NMR studies of **1** with the aid of ^{13}C–^{1}H correlation spectroscopy (COSY) and the comparison of spectra for **1** and **1a** with those of **2**[5] led us to conclude that **1** and **1a** may be 13-*epi*-*seco*-labdane type diterpenes with cleavage of ring A. The partial structure of ring A was confirmed by the measurements of proton decoupling, ^{13}C–^{1}H long range coupling and nuclear Overhauser effect (NOE) difference spectra for **1a** (Chart 1). On irradiation of methyl group, resonance at δ 0.89 (20-H_3) produced NOE enhancements for the signal of 1-H [δ 2.55 (1H, ddd,

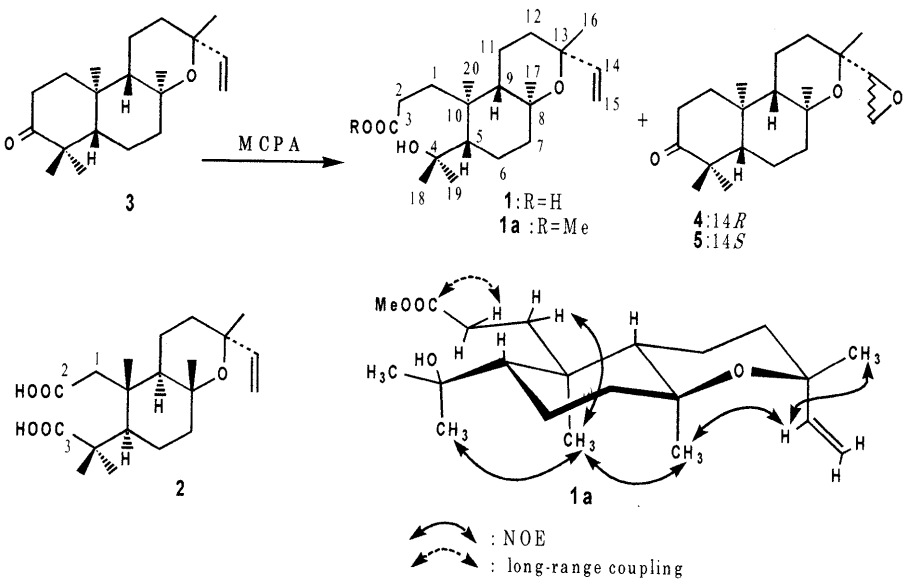

Chart 1

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Table 1. ^{13}C-NMR Spectral Data of Compounds **1**, **1a**, **2** and **3**[a]

Carbon	1	1a	2	3
1	33.7	33.6	40.1	38.2
2	29.0	28.7	177.5	33.9
3	179.5	175.5	187.6	17.4
4	75.9	75.5	45.0	47.3
5	50.9	50.8	46.1	54.6
6	23.6	23.7	20.3	20.8
7	42.2	42.2	42.4	42.2
8	76.2	76.0	74.7	75.5
9	51.0	51.1	48.5	57.7
10	40.8	40.8	41.0	36.4
11	15.6	15.7	15.8	16.4
12	34.8	34.9	34.8	34.8
13	73.3	73.3	73.3	73.6
14	147.4	147.4	147.0	147.4
15	109.8	109.8	110.7	109.8
16	32.5	32.6	25.4	32.7
17	23.2	23.2	28.6	23.4
18	34.5	34.2	30.5	26.7
19	27.0	27.3	25.4	20.9
20	20.3	20.3	20.3	15.5
OMe		51.7		

a) Those of ^{13}C-signals were determined by DEPT and ^{13}C–^{1}H COSY experiments.

J=5.0, 10.5, 15.0 Hz)] which coupled with 2-H [δ 2.16 (1H, ddd, J=4.5, 10.5, 16.0 Hz), 2.47 (1H, ddd, J=5.0, 11.0, 16.0 Hz)]. In the ^{13}C–^{1}H long range coupling spectrum, irradiation of H-2 proton (δ 2.16) produced the enhancement of carbon signal at δ 175.5 (C-3).

These data confirmed that ring A possessed the structure with cleavage between C-3 and C-4. The relative stereochemistry of **1** was established by NOE difference spectra measurements. On irradiation of methyl protons at δ 0.90, 20-H_3 produced NOE enhancements for the signal of 19-H_3, furthermore, the NOE were detected between the signals of 20-H_3 and 17-H_3, 17-H_3 and 14-H and between the signal of 14-H and 16-H_3. Thus the relative stereochemistry of this compound was shown to be **1**. The stereochemistry of **1** was confirmed by chemical evidence. To determine the total stereochemistry of **1** including its absolute configuration, we carried out chemical correlation of **1** and **3**. Oxidation of **3** with *m*-chloroperbenzoic acid (MCPA) gave the compound (**1**, yield 9.6%) together with *R,S*-epoxides (**4**, **5**[4a] yield 20, 25%, respectively) (Chart 1). The structure of this compound was identified from the measurement of the ^{1}H-NMR, ^{13}C-NMR and NOE difference spectra. Consequently, the absolute stereochemistry of excoecarin H (**1**) was determined as shown in Chart 1. This compound is the first example of a 3,4-*seco*-labdane type of diterpene.

Experimental

The instrument used for obtaining physical data and the conditions for chromatography were as described in the preceding paper, Silica gel (Merck), Sephadex LH-20 (Pharmacia), Jai-gel GS-310 (Nihon Bunseki Kogyo), and Lichroprep Rp-18 (Merck) were used for column chromatography. Preparative recycling HPLC was carried out on an LC-09 instrument (Nihon Bunseki Kogyo).

Isolation of Compound 1 The ether extract (30 g) of *E. agallocha* was subjected to column chromatography on silica gel with a binary solvent system (hexane+AcOEt gradient and $CHCl_3$+MeOH gradient) to obtain ten fractions, Frs. 1—10.[4] Fraction 7 (2.3 g) was chromatographed on silica gel ($CHCl_3$: MeOH = 10 : 1), Lichroprep Rp-18 (MeOH : H_2O = 6 : 4), and recycling HPLC (MeOH) to afford compound **1** (25.4 mg).

Excoecarin H (**1**): Colorless needles, mp 153—156 °C, $[\alpha]_D^{23}$ −46.0° (c=0.77, $CHCl_3$), 2,6-dichlrophenol-indophenol reagent: positive. IR (KBr, cm^{-1}): 3400, 1709, 1640, 1094, 1068, 983, 916. ^{1}H-NMR ($CDCl_3$, δ): 0.90 (3H, s, 20-H_3), 1.14 (3H, s, 16-H_3), 1.23 (3H, s, 19-H_3), 1.24 (3H, s, 17-H_3), 1.30 (3H, s, 18-H_3), 1.73 (1H, m, 7-H), 1.74 (1H, m, 1a-H), 2.15 (1H, m, 2a-H), 2.22 (1H, m, 12-H), 2.49 (1H, m, 2b-H), 2.52 (1H, br d, J=15.0 Hz, 1b-H), 4.93 (1H, d, J=11.0 Hz, 15-H), 4.98 (1H, d, J=18.0 Hz, 15-H), 6.05 (1H, dd, J=11.0, 18.0 Hz, 14-H). EI-MS m/z: 337 $(M-H)^+$, 323 $(M-15)^+$, 306 $(M-H_2O)^+$. HR-FAB-MS (m/z): Calcd for $C_{20}H_{34}O_4$ $(M)^+$: 338.4722. Found: 338.4701.

Methylation of Excoecarin H (1) with Diazomethane A solution of **1** (20 mg) in MeOH (5 ml) was treated with 3.0% CH_2N_2-ether solution, and the reaction mixture was left standing at room temperature for 10 min. The reaction solution evaporated to dryness under reduced pressure to give **1a** (18 mg).

1a: A white powder. $[\alpha]_D^{23}$ −42.4° (c=0.40, $CHCl_3$). IR (KBr, cm^{-1}): 3260, 1728, 1194, 1091, 1070, 981, 918. ^{1}H-NMR ($CDCl_3$, δ): 0.89 (3H, s, 20-H_3), 1.14 (3H, s, 16-H_3), 1.21 (3H, s, 19-H_3), 1.24 (3H, s, 17-H_3), 1.28 (3H, s, 18-H_3), 1.60 (1H, m, 7-H), 1.72 (1H, m, 1a-H), 2.16 (1H, ddd, J=4.5, 10.5, 16.0 Hz, 2a-H), 2.23 (1H, m, 12-H), 2.47 (1H, ddd, J=5.0, 11.0, 16.0 Hz, 2b-H), 2.55 (1H, ddd, J=5.0, 10.5, 15.0 Hz, 1b-H), 3.68 (3H, s, OCH_3), 4.93 (1H, d, J=11.0 Hz, 15-H), 4.97 (1H, d, J=18.0 Hz, 15-H), 6.00 (1H, dd, J=11.0, 18.0 Hz, 14-H). HR-FAB-MS (m/z) : Calcd for $C_{21}H_{37}O_4$ $(M+H)^+$: 353.2691. Found: 353.2697.

Oxidation of 3 with *m*-Chloroperbenzoic Acid A solution of **3** (100 mg) and *m*-chloroperbenzoic acid (110 mg) in $CHCl_3$ (15 ml) was stirred at room temperature for 38 h. The reaction mixture was diluted with $CHCl_3$ (35 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 **1** (9.6 mg) as colorless needles which was identified as excoecarin H by direct comparison with authentic sample, together with **4** (20.0 mg) and **5** (25.0 mg).

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