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Seco-labdane type diterpenes from *Excoecaria agallocha*☆

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Abstract

Labdane-type diterpenes, called excoecarins S, T1, and T2 were isolated from the resinous wood of *Excoecaria agallocha*, along with three known compounds, *ent*-12-oxo-2,3-secobeyer-15-ene-2,3-dioic acid, agallochin H, and *ent*-15-epoxy-beyerane-3α-ol. Their structures were elucidated on the basis of spectroscopic data, chemical evidence, and X-ray analysis.

Keywords: *Excoecaria agallocha*; Euphorbiaceae; Seco-diterpenes; Excoecarin S; Excoecarin T1; Excoecarin T2

1. Introduction

Excoecaria agallocha (family Euphorbiaceae) is found in many tropical countries, including India, Myanmar, Malaysia, Indonesia, Thailand, Philippines and Sri Lanka. The milky latex exuded from the bark of *E. agallocha* may cause blindness or blistering of the skin (Jayaweera, 1980). The milky latex has been used in the past as a poison for fish and as poison applied to arrowheads. In traditional Thai medicine the bark and wood of this plant is used against flatulence (Karalai et al., 1994). In Sri Lanka the smoke of the burning wood has been used in the treatment of leprosy, while the root pounded with ginger has been used as an embrocation for swelling hands and feet (Jayaweera, 1980). Many daphnane and tigliane type diterpene esters which cause skin irritants were isolated from the latex (Karalai et al., 1994). Recently, a new phorbol ester, 12-deoxyphorbol-13-(3*E*,5*E*-decadienoate), with anti HIV properties, has been isolated from the leaves and stems of *E. agallocha* grown in northwestern Australia (Erickson et al., 1995). In the course of our studies on the constituents of *E. agallocha*, we reported the diterpene constituents with an inhibitory effect on Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol 13-acetate (TPA) in Raji cell (Konishi et al., 2000; Konoshima et al., 2001) and new bis-seco-diterpenes (Konishi et al., 2003). Our continuing search led to the isolation of three new seco-diterpenes, excoecarins S, T1 and T2 (**1**–**3**) together with three known compounds, *ent*-12-oxo-2,3-secobeyer-15-ene-2,3-dioic acid (**4**) (Piacenza et al., 1979), agallochin H (**5**) (Anjaneyulu et al., 2002) and *ent*-15,16-epoxy-beyerane-3α-ol (**6**) (Hanson, 1970) from the resinous wood of *Excoecaria agallocha*. This paper deals with the isolation and structure elucidation of the new diterpenes (**1**–**3**).

2. Results and discussion

Excoecarin S (**1**) was obtained as colorless needles, mp 254–256 °C and showed $[\alpha]_D$ −47.2°. The molecular formula for **1** was determined by HR-FABMS as $C_{20}H_{32}O_6$ on the basis of the quasimolecular ion peaks observed at *m*/*z* 759 $[2M+Na]^+$ and 369 $[M+H]^+$ IR spectrum showed the hydroxyl (3400 cm^{-1}), carbonyl (1699 cm^{-1}), and ether (1076 cm^{-1}) groups and mono-substituted olefin (1653, 987, 914 cm^{-1}). The presence of carboxylic acid group revealed by 2,6-dichloro-phenolindophenol sodium salt on TLC. The ^1H NMR spectrum (Table 1) showed the presence of five methyl signals, monosubstituted olefin, at δ 4.90 (*dd*, *J* = 1.5, 11.1 Hz, H-15A), 5.05 (*dd*, *J* = 1.5, 17.8 Hz, H-15B), 5.98 (*dd*, *J* = 11.1, 17.8 Hz, H-14), and a hydroxyl group which disappears upon addition of D_2O at δ 4.43 (*d*, *J* = 5.3 Hz). The ^{13}C NMR and DEPT spectra showed

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20 carbons (Table 2). The ^{13}C NMR spectrum indicated two carbonyl carbons and olefinic carbons, three methine carbons, and four quaternary carbons. These data and detailed ^{13}C and ^{1}H NMR studies with the aid of ^{13}C–^{1}H COSY led us to conclude that excoecarin S (**1**) may be a seco-labdane type diterpene with a cleaved ring A and a secondary hydroxyl group. The ^{1}H NMR spectrum was similar to that of **7** (Konishi et al., 1998) except for the presence of an extra signal for a methine proton, δ 3.84. Comparison of the ^{13}C NMR spectra (Table 2) of **1** and **7** suggested that the hydroxyl group was located at C-12, based on the substitution effects at C-9, C-11, C-12 and C-16. The relative stereochemistry of excoecarin S (**1**) was established by NOE difference spectra measurements. On irradiation of methyl proton at δ 0.79, H_3-20 produced NOE enhancement for the signals of H_3-17 and irradiation of olefin proton at δ 5.98, H-14 produced NOE enhancement for H_3-17, furthermore, the NOEs were detected between the signals of H_3-16 and H-12, H-15 and H-12, and H-9 and 12-OH (Fig. 1). Thus the relative stereostructure was deduced as 13-*epi*-12-hydroxy-2,3-secolabda-14-ene. The *ent*-labdane skeleton was confirmed by the optical rotation showing the same negative sign ($[\alpha]_D^{26}$ −47.2°) as that of compound **7**.

The absolute configuration of the hydroxyl group was determined by the application of a modification of Mosher's method (Kusumi, 1993; Ohtani et al., 1991) to excoecarin S (**1**). Treatment of **1** with (*R*)- and (*S*)-MTPA chloride in the presence of pyridine afforded the (*S*)-MTPA ester (**1a**) and (*R*)-MTPA ester (**1b**), respectively. The signals due to protons on C-12, 14, 15 and 16 in **1a** appeared at higher fields than those of **1b** ($\Delta\delta$: negative), while the signals due to protons attached to C-7, 9, 11, 17, and 20 of **1a** were observed at lower fields compared with those of **1b** ($\Delta\delta$: positive). Consequently, the configuration at C-12 in excoecarin S (**1**) has been elucidated to be *R*. Finally, the structure of excoecarin S (**1**) was confirmed by X-ray structure analysis showing the β-orientation of 12-OH (Fig. 2).

Excoecarin T1 (**2**) and T2 (**3**) were also treated with diazomethane and isolated as dimethyl esters (**2a** and **3a**), respectively. IR spectra of **2a** and **3a** showed very similar absorption bands ascribable to ester carbonyl groups (**2a**, 1749, 1732 cm^{-1}; **3a**, 1750, 1728 cm^{-1}) and ether groups (**2a**, 1146 cm^{-1}; **3a**, 1152 cm^{-1}). Both compounds were found to have the same molecular formula, $C_{22}H_{36}O_6$, on the basis of HR-FABMS. The ^{1}H NMR spectra (Table 1) of both compounds showed the presence of five tertiary methyls and the presence of the methylene (δ 2.77, 2.80 for **2a** and δ 2.50, 2.79 for **3a**) and methine protons (δ 2.90 for **2a** and δ 3.09 for **3a**) bearing an oxygen atom. The ^{13}C NMR spectra (Table 2) displayed 22 carbon signals including two methoxyl carbons (δ 51.0 and 51.8 for **2a** and **3a**) and a methine carbon (δ 58.9 for **2a** and δ 57.6 for **3a**) bearing an oxygen atom. Analyses of the ^{13}C NMR and HSQC spectra revealed that excoecarin T1 dimethyl ester (**2a**) and T2 dimethyl ester (**3a**) are 2,3-seco-labdane-type diterpenes with 14,15-epoxy ring as a side chain. Previous workers reported the assignment of the stereochemistry at C-14 in 14,15-epoxy-13-*epi*-manoyl oxides resolved using only NMR spectroscopic data (Fraga et al., 2001). The stereochemistry at C-14 was determined in the same way as reference for **2a** and **3a**. The chemical shifts of H_3-17, one of H_2-15 and H-14 signals appeared at δ 1.26 (*s*), 2.77 (*dd*, J = 3.0, 4.5 Hz) and 2.80 (*dd*, J = 4.0, 4.5 Hz) for **2a**, whilst in **3a**, the chemical shifts of those protons appeared at δ 1.41, 2.50 (*dd*, J = 3.0, 4.5 Hz) and 2.79 (*dd*, J = 4.5, 4.5 Hz), respec-

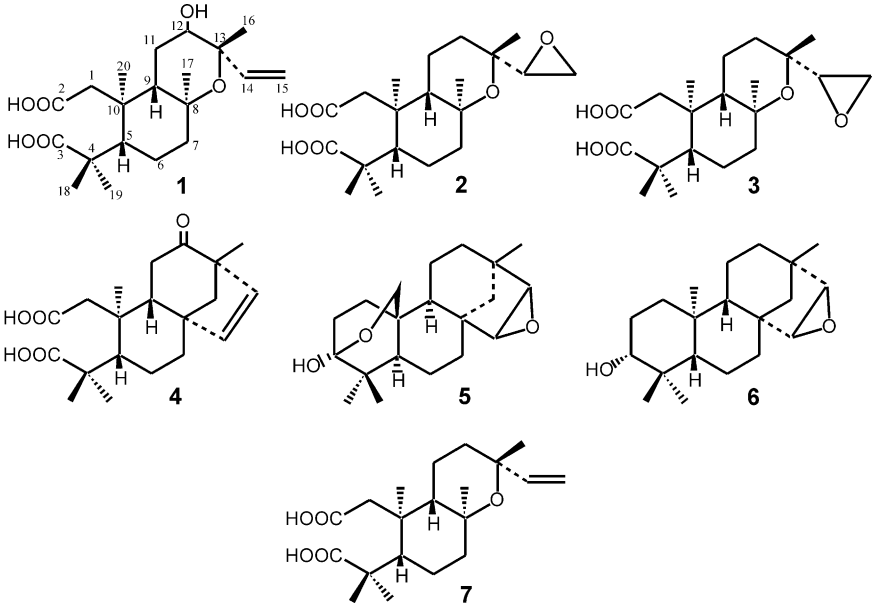

Table 1
^{1}H NMR spectral data of compounds **1**, **2a**, **3a** and **4a**[a]

Position	**1**[b]	**2a**[c]	**3a**[c]	**4a**[d]
1	2.24 (*d*, 18.3)	2.35 (*d*, 18.0)	2.38 (*d*, 18.0)	2.15 (*d*, 19.0)
	2.45 (*d*, 18.3)	2.40 (*d*, 18.0)	2.41 (*d*, 18.0)	2.28 (*d*, 19.0)
5	2.54 (*dd*, 5.5, 10.0)	2.51 (*dd*, 3.0, 12.0)	2.53 (*d*, 3.5, 12.0)	2.51 (*dd*, 3.5, 11.0)
6	1.45 (*m*)	1.46 (*m*)	1.52 (*m*)	1.66 (*m*)
		1.64 (*ddd*, 3.0, 6.0, 13.0)	1.64 (*ddd*, 3.0, 5.5, 13.0)	
7	1.29 (*ddd*, 5.0, 11.0, 11.7)	1.51 (*m*)	1.53 (*m*)	1.56 (*ddd*, 5.5, 12.5, 12.5)
	1.61 (*ddd*, 3.0, 3.0, 11.7)	1.75 (*ddd*, 3.0, 3.5, 12.0)	1.76 (*ddd*, 3.0, 3.5, 12.0)	1.84 (*ddd*, 3.0, 3.0, 12.5)
9	2.67 (*dd*, 5.5, 12.0)	2.12 (*dd*, 3.0, 12.0)	2.05 (*dd*, 3.0, 12.5)	2.78 (*dd*, 7.0, 10.5)
11	1.45 (*m*)	1.34 (*m*)	1.41 (*m*)	2.19 (*dd*, 7.0, 16.5)
	1.70 (*ddd*, 5.0, 12.5, 12.5)	1.48 (*m*)	1.60 (*m*)	2.33 (*dd*, 10.5, 16.5)
12	3.84 (*ddd*, 5.0, 5.3, 5.5)	1.30 (*m*)	1.26 (*ddd*, 5.0, 13.0, 13.0)	
		1.58 (*ddd*, 3.0, 6.5, 13.0)	1.50 (*ddd*, 3.0, 3.0, 13.0)	
14	5.98 (*dd*, 11.1, 17.8)	2.90 (*dd*, 3.0, 4.0)	3.09 (*dd*, 3.0, 4.5)	1.69 (*d*, 11.0)
				1.90 (*d*, 11.0)
15	4.90 (*dd*, 1.5, 11.1)	2.77 (*dd*, 3.0, 4.5)	2.50 (*dd*, 3.0, 4.5)	6.05 (*d*, 5.5)
	5.05 (*dd*, 1.5, 17.8)	2.80 (*dd*, 4.0, 4.5)	2.79 (*dd*, 4.5, 4.5)	
16	1.04 (*s*)	1.19 (*s*)	1.10 (*s*)	5.60 (*d*, 5.5)
17	1.12 (*s*)	1.26 (*s*)	1.41 (*s*)	1.09 (*s*)
18	1.16 (*s*)	1.24 (*s*)	1.25 (*s*)	1.24 (s)
19	1.12 (*s*)	1.21 (*s*)	1.23 (*s*)	1.25 (*s*)
20	0.79 (*s*)	0.83 (*s*)	0.86 (*s*)	0.83 (*s*)
2-OMe		3.65 (*s*)	3.66 (*s*)	3.63 (*s*)
3-OMe		3.62 (*s*)	3.63 (*s*)	3.62 (*s*)
OH	4.43 (*d*, 5.3)			

[a] δ in ppm and J (in parentheses) in Hz.
[b] Measured in DMSO-d_6 at 400 MHz.
[c] Measured in $CDCl_3$ at 500 MHz.
[d] Measured in $CDCl_3$ at 400 MHz.

Table 2
^{13}C NMR spectral data of compounds **1**, **2a**–**4a** and **7**

	1[a]	**2a**[b]	**3a**[c]	**4a**[c]	**7**[d]
1	40.6 *d*	41.1 *t*	40.9 *t*	39.4 *d*	40.5
2	172.3 *s*	171.6 *s*	171.4 *s*	171.3 *s*	177.7
3	180.0 *s*	179.6 *s*	179.5 *s*	179.5 *s*	187.0
4	45.6 *s*	46.2 *s*	46.1 *s*	45.8 *s*	45.2
5	46.8 *d*	48.0 *d*	48.0 *d*	46.7 *d*	48.6
6	22.0 *t*	22.0 *t*	22.0 *t*	21.9 *t*	20.5
7	41.8 *t*	41.7 *t*	41.5 *t*	35.4 *t*	42.3
8	75.5 *s*	74.9 *s*	74.6 *s*	49.4 *s*	75.7
9	42.6 *d*	49.7 *d*	49.4 *d*	48.2 *d*	49.6
10	40.3 *s*	41.5 *s*	41.5 *s*	41.0 *s*	41.0
11	24.9 *t*	17.2 *t*	17.0 *t*	36.3 *t*	16.5
12	67.5 *t*	31.8 *t*	30.8 *t*	211.5 *s*	34.4
13	74.2 *s*	71.5 *s*	71.0 *s*	57.0 *s*	73.3
14	147.3 *d*	58.9 *d*	57.6 *d*	57.6 *t*	147.3
15	110.4 *t*	45.9 *t*	47.2 *t*	139.1 *t*	109.8
16	24.6 *q*	28.4 *q*	28.7 *q*	36.3 *d*	32.3
17	24.4 *q*	22.0 *q*	23.3 *q*	17.0 *q*	23.8
18	24.6 *q*	27.6 *q*	27.6 *q*	28.3 *q*	30.1
19	25.8 *q*	23.9 *q*	23.8 *q*	23.5 *q*	32.3
20	18.6 *q*	19.5 *q*	19.3 *q*	17.9 *q*	20.8
2-OMe		51.0 *q*	51.0 *q*	51.0 *q*	
3-OMe		51.8 *q*	51.8 *q*	51.9 *q*	

[a] Measured in DMSO-d_6 at 400 MHz.
[b] Measured in $CDCl_3$ at 400 MHz.
[c] Measured in $CDCl_3$ at 500 MHz.
[d] Measured in $CDCl_3$ at 300 MHz.

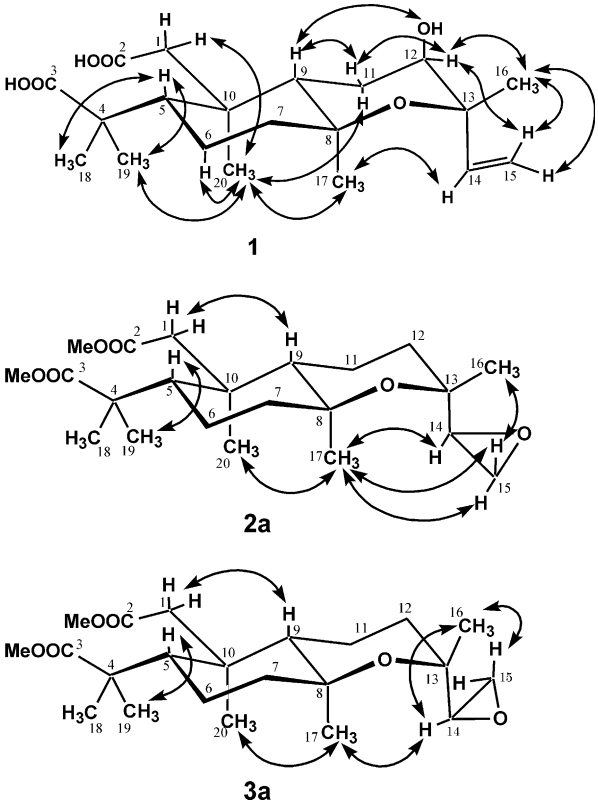

Fig. 1. Some important NOE correlations of **1**, **2a** and **3a**.

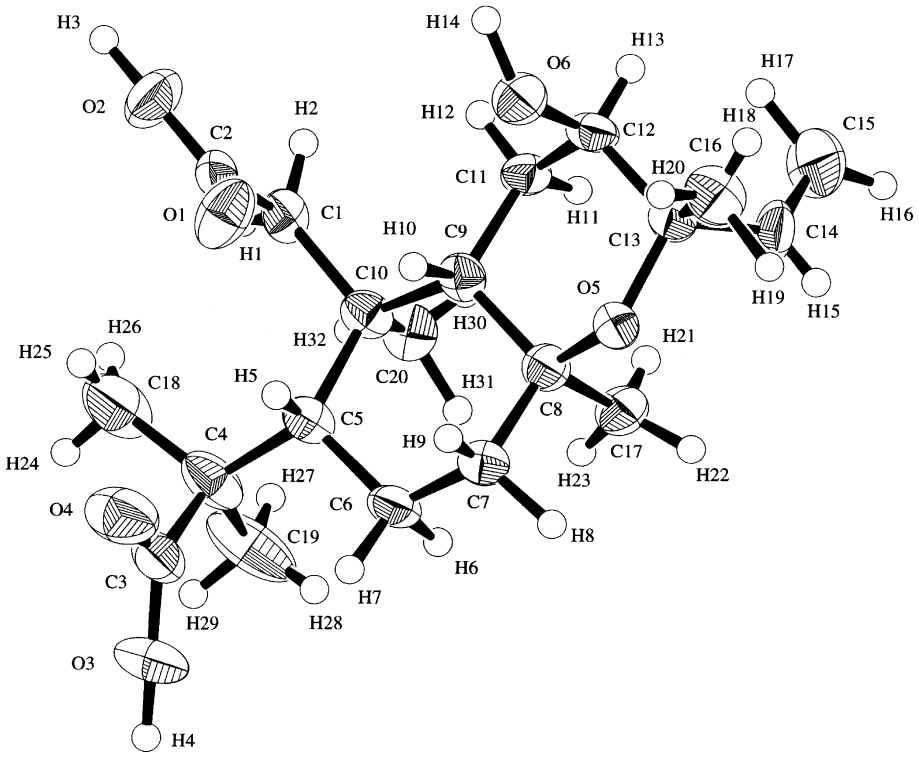

Fig. 2. X-ray crystal structure of compound **1**.

tively. The NOESY spectrum of **2a** showed NOE correlations between H-15 (δ 2.77 and 2.80) and H-17 (δ 1.41), whilst in **3a** this effect was not observed (Fig. 1). These data for **2a** and **3a** resemble those of the 14*S*,15- or 14*R*,15-epoxy-13-*epi*-manoyl oxide derivatives (Konishi et al., 1996). Consequently, the C-14 stereochemistry was determined to be a *S* configuration for **2a** and an *R* for **3a**. To determine the absolute configurations for **2a** and **3a**, we carried out chemical correlation of these compounds and **7** (Konishi et al., 1998). Oxidation of **7** with *m*-chloroperbenzoic acid (MCPA) gave the compounds **2a** ($[\alpha]_D^{28}$ −7.8°) (yield 22%) and **3a** ($[\alpha]_D^{28}$ −14.9°) (31%), respectively. These compounds were identified from the measurements of the ^{1}H, ^{13}C NMR and NOE spectra.

Consequently, the absolute stereostructures of excoecarin T1 (**2**) and T2 (**3**) were determined as *ent*-14*S*,15- and *ent*-14*R*,15-epoxy-2,3-seco-13-*epi*-labdane-2,3-dioic acid, respectively.

Compound **4** was isolated as a dimethyl ester (**4a**) by using diazomethane. **4a,** colorless prisms showed mp 171–172 °C and $[\alpha]_D^{25}$ −275.4°. From the spectral data, optical rotation and x-ray analysis and alkali hydrolysis of **4a**, compound **4** was identified as an *ent*-12-oxo-2,3-secobeyer-15-ene-2,3-dioic acid which was synthesized by oxidation of a diosphenol, *ent*-2-hydroxy-beyer-1,15-dien-3,20-dione (Piacenza et al., 1979). The isolation of this diacid thus forms its first report from nature.

3. Experimental

3.1. General experimental procedures

Melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded using a Shimadzu FTIR-8100A. Optical rotations were recorded in $CHCl_3$ or MeOH using a Jasco DIP-370 digital polarimeter. ^{1}H NMR (300, 400 and 500 MHz) and ^{13}C NMR (75, 100 and 125 MHz) spectra were recorded on a Varian XL-300, a Varian INOVA-400 and a Varian INOVA-500 spectrometers in $CDCl_3$ with tetramethylsilane (TMS) as internal standard, respectively. Coupling constants (*J*) are given in Hz. MS were obtained with a JEOL MS-BU 20 and a JEOL LMS-SX-120A QQ mass spectrometer. Chromatographic separations were achieved by column chromatography (CC) using silica gel 60 (70–230 mesh, Merck) or Lichroprep Rp-18 (40–63 μm 200 mm×10 mm, i.d., Merck) or Sephadex LH-20 (Pharmacia) and by high performance liquid chromatography (HPLC). HPLC was run using a Shimadzu LC-10AS Micro pump with a Shimadzu RID-2A RI-Detector and preparative recycling HPLC was carried out on an LC-09 instrument (Nihon Bunseki Kogyo). For HPLC, Nova-Pak Cartridge C_{18} (100 mm×5 mm i.d., Millipore Co. Ltd.) and GS-310 (20 mm×500 mm, Nihon Bunseki Kogyo) were used. Analytical TLC was performed on precoated silica gel 60 F254 plates (Merck) and

precoated octadecyl-functionalized silica gel F254s plates (Merck 15389) and detection was achieved by spraying with anisaldehyde reagent, followed by heating.

3.2. Plant material

The resinous wood of *Excoecaria agallocha* L. was collected in August 1996, on Okinawa Island, Japan. A voucher specimen (KPU 001950) has been deposited in the Herbarium of the Department of Pharmaceutical Sciences of Natural Resources, Kyoto Pharmaceutical University, Japan.

3.3. Extraction and isolation

The powdered resinous wood (1.2 kg) was extracted three times with diethyl ether at room temperature. Removal of the solvent from the combined diethyl ether extracts gave a brown syrup (240 g). A portion (100 g) of the brown syrup was subjected to CC over silica gel using solvents of increasing polarity from hexane through EtOAc to give 10 fractions. Fraction 9 (15.4 g) was applied to silica gel with $CHCl_3$–MeOH–H_2O (10:1:0.1 and 9:1:0.1) and MeOH to yield fractions 11–16. Fraction 13 (553 mg) was subjected to CC using silica gel with $CHCl_3$–MeO–H_2O, 9:1:0.1 and using Lichroprep Rp-18 with MeOH–H_2O, 3:2 to give **5** (5.5 mg) and **6** (4.8 mg). Fraction 15 (805 mg) was subjected to CC using silica gel and eluting with $CHCl_3$–MeOH–H_2O, 4:1:0.1 to give fractions 17–23. Fraction 17 (108 mg) was further purified by silica gel with $CHCl_3$–MeOH–H_2O, 9:1:0.1, by Sephadex LH-20 with MeOH, by Lichroprep Rp-18 with MeOH–H_2O, 55:45, and by HPLC (MeOH) recycling to give excoecarin S (**1**, 25 mg). Fraction 18 (314 mg) was subjected to silica gel CC with $CHCl_3$–MeOH–H_2O (8:2:0.1) to give fractions 24–26. Fraction 26 (58 mg) was treated with diazomethane and the reaction mixture was performed on Lichroprep Rp-18 with MeOH–H_2O, 7:3 to give a dimethyl ester (**4a**, 25.5 mg). Fraction 22 (228 mg) was treated with diazomethane and then the reaction mixture was subjected to CC over silica gel with hexane–EtoAc (2:1) and HPLC with MeOH–H_2O (77:23) to give excoecarin T1 dimethyl ester (**2a**, 7.4 mg) and T2 dimethyl ester (**3a**, 9.4 mg). The compounds **4–6** were identified by comparison of their physical and spectroscopic data with those reported in the literature (Anjaneyulu et al., 2002; Hanson, 1970; Piacenza et al., 1979).

3.3.1. Excoecarin S (*1*)

Colorless needles from MeOH, mp 254–256 °C, $[\alpha]_D^{26}$ −47.2° (*c* 0.9, MeOH). IR (KBr) ν_{max} 3400, 1699, 1653, 1076, 987, 914, 837 cm^{-1}. FABMS *m/z*: 759 $[2M+Na]^+$, 737 $[2M+H]^+$, 369 $[M+H]^+$, 351 $[M-OH]^+$, 333 $[351-H_2O]^+$. HR-FABMS *m/z*: 369.2292 $[M+H]^+$ (calc. for $C_{20}H_{33}O_6$, 369.2278).

3.3.2. Excoecarin T1 dimethyl ester (*2a*)

Colorless needles from aqueous MeOH, mp 102–103 °C, $[\alpha]_D^{30}$ −8.1° (*c* 1.0, $CHCl_3$). IR (KBr) ν_{max} 1749, 1732, 1264, 1146, 1102, 932, 866 cm^{-1}. FABMS *m/z*: 397 $[M+H]^+$. HR-FABMS *m/z*: 397.2593 $[M+H]^+$(calcd for $C_{22}H_{37}O_6$, 397.2590).

3.3.3. Excoecarin T2 dimethyl ester (*3a*)

Colorless needles from aqueous MeOH, mp 130–133 °C, $[\alpha]_D^{30}$ −14.7° (*c* 0.5, $CHCl_3$). IR (KBr) ν_{max} 1750, 1728, 1258, 1152, 1098, 905, 866 cm^{-1}. FABMS *m/z*: 397 $[M+H]^+$. HR-FABMS *m/z*: 397.2598 $[M+H]^+$(calcd for $C_{22}H_{37}O_6$, 397.2590).

3.4. Preparation of (*S*)-(−)- and (*R*)-(+)-MTPA ester derivatives of *1*

Excoecarin S(**1**) (2.0 mg) was treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (20 μl) in chloroform (10 μl) and pyridine (20 μl) at room temperature overnight, and then ice water (3 ml) was added. The water solution was passed through a Sep-Pak C_{18} cartridge, washed with 4 ml of MeOH–H_2O (7:3), and eluted with MeOH. The MeOH solution was removed in vacuo, the residue was subjected to silica gel CC eluted with hexane–EtOAc (3:1) to obtain the *S*-(−)-MTPA ester, **1a**: ^1H NMR (300 MHz, DMSO-d_6) δ: 0.86 (3H, *s*, H-20), 1.01 (3H, *s*, H-16), 1.04 (3H, *s*, H-19), 1.20 (3H, *s*, H-17), 1.23 (3H, *s*, H-18), 1.30 (1H, *ddd*, *J* = 5.0, 5.0, 11.5 Hz, H-7ax), 1.35 (1H, *m*, H-11), 1.61 (2H, *m*, H-7, H-11), 1.89 (1H, *m*, H-6), 2.03 (1H, *m*, H-9), 2.05 (1H, *m*, H-5), 4.94 (1H, *d*, *J* = 11.2 Hz, H-15), 5.07 (1H, *d*, *J* = 17.5 Hz, H-15), 5.29 (1H, *m*, H-12), 5.61 (1H, *dd*, *J* = 11.2, 17.5 Hz, H-14). HR-FABMS *m/z* 584.2590 $[M+H]^+$ (calc. for $C_{30}H_{39}O_8F_3$ 584.2597). Treatment of 1.0 mg of **1** with (*S*)-(+)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride, as described above, afforded the *R*-(+)-MTPA ester, **1b**: ^1H NMR (300 MHz, DMSO-d_6) δ: 0.85 (3H, *s*, H-20), 1.03 (3H, *s*, H-19), 1.08 (3H, *s*, H-16), 1.12 (3H, *s*, H-17), 1.22 (3H, *s*, H-18), 1.31 (1H, *ddd*, *J* = 5.0, 5.0, 11.5 Hz, H-7ax), 1.41 (1H, *m*, H-11), 1.56 (1H, *m*, H-7), 1.68 (1H, *ddd*, *J* = 5.0, 12.5, 12.5 Hz, H-11ax), 1.89 (1H, *m*, H-6), 1.98 (1H, *m*, H-9), 2.06 (1H, *m*, H-5), 5.07 (1H, *d*, *J* = 11.0 Hz, H-15), 5.17 (1H, *d*, *J* = 17.5 Hz, H-15), 5.37 (1H, *m*, H-12), 5.89 (1H, *dd*, *J* = 11.0, 17.5 Hz, H-14). HR-FABMS *m/z* 584.2587 $[M+H]^+$ (calc. for $C_{30}H_{39}O_8F_3$ 584.2597).

3.5. X-ray crystallographic analysis of *1*

Refraction data were measured with a Rigaku AFC7R diffractmeter using Cu-K_α (λ = 1.54178 Å) radiation for **1**. Monoclinic $P2_1$. *a* = 6.8130(8) Å, *b* = 11.3867(7) Å, *c* = 12.8622(8) Å, β = 96.003(7) Å, *V* = 992.3(1) $Å^3$, *Z* = 2, D_{calc} = 1.233 g/cm^3. Final *R*-factor and weighted *R*-factor were 0.043 and 0.026.

3.6. Epoxidation of ent-13-epi-2,3-seco-labda-14-ene-2,3-dioic acid (7)

A solution of **7** (50 mg) and *m*-chloroperbenzoic acid (60 mg) in $CHCl_3$ (10 ml) was stirred at room temperature for 38 h. The reaction mixture was diluted with $CHCl_3$ (25 ml), washed with aqueous Na_2CO_3, dried with Na_2SO_4 and evaporated under reduced pressure. The residue was treated with diazomethane. The reaction mixture was purified by HPLC to obtain excoecarin T1 dimethyl ester (**2a**) (11.0 mg), $[\alpha]_D^{28}$ -7.8° (*c* 1.0, $CHCl_3$) and T2 dimethyl ester (**3a**) (15.6 mg) $[\alpha]_D^{28}$ -14.9° (*c* 1.3, $CHCl_3$) as colorless needles, which were identified by comparison of their physical data (^1H, ^{13}C NMR and NOE) with those of authentic samples, respectively.

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