Three Diterpenoids (Excoecarins V1—V3) and a Flavanone Glycoside from the Fresh Stem of *Excoecaria agallocha*

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Three new diterpenoids, excoecarins V1—V3 (1—3) and a new flavanone glycoside (7) were isolated from the fresh stem of *Excoecaria agallocha* L. Their structures were elucidated as: 2α,3α,18-trihydroxy-3β,20-epoxybeyer-15-ene (1), *ent*-2,3-secokaur-16-en-2,3-dioic acid (2), *ent*-3,4-*seco*-16α-hydroxyatis-4(19)-en-3-oic acid (3), and 3,5,7,3′,5′-pentahydroxy-2*R*,3*R*-flavanonol 3-*O*-α-L-rhamnopyranoside (7) on the basis of spectroscopic data, chemical evidence, and/or X-ray analysis.

Key words *Excoecaria agallocha*; Euphorbiaceae; diterpenoid; excoecarin V_1; flavanone glycoside

Excoecaria agallocha LINN. (family Euphorbiaceae) is distributed on seashores and edge-mangroves throughout tropical Africa, Asia, and northwest Australia.[1] The *Excoecaria* genus is well known to contain skin irritants, called irritant *Excoecaria* factors. In traditional Thai medicine, the bark and wood of the plant is used to treat flatulence.[2,3] In Sri Lanka, the smoke of the burning wood is used in the treatment of leprosy, and the root, when pounded with ginger, as an embrocation for swollen hands and feet.[4] The milky latex exuded from the bark of *E. agallocha* may cause blindness or blistering of the skin.[2,3] This latex has been used as a poison for fish by adding it to water and to poison arrowheads. The piscicidal constituent excoecariatoxin characterizing the daphnane diterpene ester and some related compounds have been obtained from the twigs, bark, and latex of *Excoecaria agallocha* in Japan and Thailand, respectively.[2,3,5] Daphnane- and tigliane-type diterpene esters are known to be skin irritants and tumor promoters.[2,3]

Previously we reported the isolation and structural elucidation of many diterpenoids from the resinous wood of this plant,[6] as well as their inhibitory effect on Epstein–Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells.[7] In our search for bioactive compounds from plants of the Euphorbiaceae, we isolated three new diterpenoids, excoecarins V1—V3 (**1**—**3**) and a new flavanone glycoside (**7**) with five known compounds, *ent*-atisane-16α-ol (**4**)[8—11] and *ent*-2,3-secobeyer-15-ene-2,3-dioic acid (**5**),[12] *ent*-15,18-dihydroxylabda-8(17),13E-diene (**6**),[13] methyl 3,4,5-trihydroxybezoate (**8**),[14] and 3,4,5-trimethoxyphenol 1-*O*-β-D-(6-galloyl)-glucopyranoside (**9**)[15] from the fresh stem of *E. agallocha* collected in Okinawa. In this paper, we describe the stereochemistry of the new diterpenoids (**1**—**3**) and flavanone glycoside (**7**).

Excoecarin V1 (**1**) was obtained as colorless prisms, mp 177—179 °C and $[\alpha]_D$ −19.3°. The molecular formula of **1** was determined by high-resolution (HR)-EI-MS to be $C_{20}H_{30}O_4$ on the basis of the molecular ion peaks observed at *m*/*z* 334 $[M]^+$. The IR spectrum of **1** showed hydroxyl (3410, 3300 cm^{-1}), ether (1188, 916 cm^{-1}), and disubstituted olefinyl (1600, 746 cm^{-1}) groups. The ^{1}H-NMR spectrum of **1** in DMSO-d_6 (Table 1) showed the presence of two methyl signals at δ 0.90 and 0.97, disubstituted olefin at δ 5.45 (d, J=5.5 Hz) and 5.68 (d, J=5.5 Hz), and three hydroxyl groups at δ 4.41 (dd, J=4.0, 6.0 Hz), 4.42 (d, J=6.5 Hz), and 4.95 (s). The coupling patterns of a methine group at δ 3.81 (ddd, J=2.5, 6.5, 9.7 Hz) and methylene protons [δ 3.16 (dd, J=4.0, 10.5 Hz), 3.25 (dd, J=6.0, 10.5 Hz)] on the carbons bearing the oxygen functions were changed to a double doublet and doublet in $CDCl_3$, respectively. However, the coupling patterns of the methylene protons at δ 3.61 (d, J=8.5 Hz) and 3.67 (dd, J=2.6, 8.5 Hz), which coupled with one of the methylene protons at δ 1.54 (ddd, J=2.6, 9.7, 13.5 Hz), did not change in $CDCl_3$ [δ 3.77 (d, J=9.2 Hz) and 3.87 (dd, J=2.9, 9.2 Hz)]. The ^{13}C-NMR spectrum indicated two methyl carbons at δ 13.7 and 24.5, olefinyl carbons at δ 133.4 and 136.5, three methine carbons at δ 44.8, 45.0 and 66.7, and five quaternary carbons at δ 34.3, 43.3, 43.4, 48.1, and 96.2 (Table 2). Two of the eight methylene carbons showed lower-field chemical shifts at δ 65.1 and 67.3. We therefore concluded that **1** may be a beyerane-type diterpenoid with a ketal group and primary and secondary hydroxyl groups.

The ^{1}H- and ^{13}C-NMR spectra of **1** were similar to those of excoecarin D (**10**)[16] which was assigned to be 3α,18-dihydroxy-3β,20-epoxybeyer-15-ene, except that the methine proton at C-2 was substituted by a hydroxyl group. This was confirmed by a heteronuclear multiple-bond coherence (HMBC) experiment (Fig. 1). The relative stereochemistry of **1** was assigned on the basis of the nuclear Overhauser effect (NOE) correlations. In the NOE difference NMR experiments, irradiation at δ 3.61 (H_2-20) and 3.67 (H_2-20) enhanced the signal intensities of H-2 and H-15, respectively. NOEs were also observed between H-5 and H-18 and between H_3-17 and H-14, H-16. (Fig. 2) These data suggested that the structure of **1** had the same relative conformation as excoecarin D (**10**). To determine the absolute configuration of C-2 in **1**, a modified Mosher's method[17,18] was applied following the protection of the primary hydroxyl group with trityl chloride in pyridine. Esterifications of 1-tritylate with (*S*)- and (*R*)-MTPA chloride in the presence of pyridine afforded the (*R*)-MTPA ester (**1a**) and (*S*)-MTPA ester (**1b**), respectively. The signals due to protons on C-18 and C-19 in **1b** appeared at higher fields than those of **1a** ($\Delta\delta$: negative),

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Table 1. ^{1}H-NMR Spectral Data for **1**, **2a**, and **3**

No.	**1**[a)]	**1**[b)]	**2a**[c)]	**3**[b)]
1	1.54 (ddd, *J*=2.6, 9.7, 13.5 Hz)	1.75 (ddd, *J*=2.9, 9.7, 14.5 Hz)	2.31 (d, *J*=18.0 Hz)	1.44 (m)
	1.75 (dd, *J*=2.5, 13.5 Hz)	1.93 (dd, *J*=3.0, 14.5 Hz)	2.57 (d, *J*=18.0 Hz)	
2	3.81 (ddd, *J*=2.5, 6.5, 9.7 Hz)	4.05 (dd, *J*=3.0, 9.7 Hz)		2.42 (dd, *J*=9.0, 9.0 Hz)
5	1.16 (m)	1.06 (m)	2.50 (dd, *J*=5.0, 8.0 Hz)	2.00 (dd, *J*=3.0, 11.0 Hz)
6	1.17 (m)	1.08 (m)		1.29 (m)
				1.34 (m)
7	1.15 (m)	1.28 (ddd, *J*=3.6, 13.5, 13.5 Hz)		1.12 (ddd, *J*=3.0, 13.0, 13.0 Hz)
	1.28 (ddd, *J*=4.0, 13.0, 13.0 Hz)	1.56 (m)		1.31 (m)
9	0.99 (m)	0.94 (ddd, *J*=1.3, 3.9, 12.5 Hz)	1.90 (d, *J*=8.0 Hz)	1.32 (m)
11	1.55 (m)	1.67 (ddd, *J*=3.0, 13.4, 13.4 Hz)		1.14 (dd, *J*=9.0, 13.0 Hz)
				1.88 (dd, *J*=13.0, 13.0 Hz)
12	1.51 (m)	1.26 (m)		1.67 (m)
13			2.65 (dd, *J*=4.0, 4.0 Hz)	1.46 (m)
				1.62 (br dd, *J*=12.0, 12.0 Hz)
14	1.00 (d, *J*=10.0 Hz)	0.98 (d, *J*=9.7 Hz)	1.14 (m)	0.84 (ddd, *J*=7.0, 12.0, 12.0 Hz)
			1.86 (dd, *J*=2.5, 11.8 Hz)	1.85 (dd, *J*=12.5, 12.5 Hz)
15	5.45 (d, *J*=5.5 Hz)	5.49 (d, *J*=5.5 Hz)	2.03 (dt, *J*=3.0, 17.0 Hz)	1.22 (m)
			2.13 (ddd, *J*=2.0, 2.0, 17.0 Hz)	1.32 (m)
16	5.68 (d, *J*=5.5 Hz)	5.56 (d, *J*=5.5 Hz)		
17	0.97 (s)	1.01 (s)	4.73 (br s)	1.31 (s)
			4.79 (br s)	
18	3.16 (dd, *J*=4.0, 10.5 Hz)	3.29 (d, *J*=11.5 Hz)	1.25 (s)	1.73 (s)
	3.25 (dd, *J*=6.0, 10.5 Hz)	3.54 (d, *J*=11.5 Hz)		
19	0.90 (s)	1.20 (s)	1.26 (s)	4.65 (br s)
				4.83 (br s)
20	3.61 (d, *J*=8.5 Hz)	3.77 (d, *J*=9.2 Hz)	1.08 (s)	0.94 (s)
	3.67 (dd, *J*=2.6, 8.5 Hz)	3.87 (dd, *J*=2.9, 9.2 Hz)		
OH	4.41 (dd, *J*=4.0, 6.0 Hz)			
OH	4.42 (d, *J*=6.5 Hz)			
OH	4.95 (s)			
OMe			3.61 (s)	
OMe			3.64 (s)	

Measured *a*) in DMSO-d_6 at 400 MHz, *b*) in $CDCl_3$ at 400 MHz, *c*) in $CDCl_3$ at 300 MHz.

Table 2. ^{13}C-NMR Spectral Data for **1**, **2a**, **3**, **4**, and **5a**

	1[a)]	**2a**[b)]	**3**[c)]	**4**[c)]	**5a**[b)]
1	43.3 t	41.8 t	33.0 t	39.3 t	40.3
2	66.7 d	172.0 s	27.6 t	18.7 t	177.9
3	96.2 s	179.9 s	177.7 s	42.2 t	171.8
4	43.3 s	46.4 s	147.5 s	33.1 s	46.2
5	44.8 d	47.6 d	50.4 d	56.4 d	44.6
6	20.4 t	18.9 t	24.6 t	18.2 t	20.6
7	35.2 t	39.2 t	38.1 t	39.7 t	36.4
8	48.1 s	44.2 s	33.5 s	33.8 s	49.0
9	45.0 d	48.2 d	41.9 d	51.3 d	49.2
10	34.3 s	43.3 s	39.3 s	37.1 s	41.4
11	21.8 t	22.9 t	23.3 t	24.1 t	22.1
12	32.0 t	33.3 t	37.5 d	37.9 d	33.2
13	43.4 s	43.6 d	23.8 t	23.2 t	43.4
14	59.8 t	39.7 t	26.8v	27.3 t	60.7
15	133.4 d	48.6 t	56.1 t	57.7 t	136.9
16	136.5 d	155.5 s	73.3 s	72.2 s	134.3
17	24.5 q	103.1 t	30.1 q	30.4 q	24.7
18	65.1 t	27.4 q	23.7 q	33.4 q	23.9
19	13.7 q	23.9 q	113.2 t	21.7 q	23.9
20	67.3 t	21.1 q	17.9 q	13.9 q	18.7
2-OMe		50.8 q			50.8
3-OMe		51.7 q			51.7

Measured *a*) in DMSO-d_6 at 100 MHz, *b*) in $CDCl_3$ at 75 MHz, *c*) in $CDCl_3$ at 100 MHz.

while the signals due to protons attached to C-1, C-5, C-9, and C-11 of **1b** appeared at lower fields compared with those of **1a** ($\Delta\delta$: positive). Thus the configuration at C-2 in **1** was elucidated to be *R* (Fig. 3). The optical rotation of **1** showed a negative sign, similar to that of excoecarin D.[16] Consequently, the structure of **1** was assigned to be 2α,3α,18-trihydroxy-3β,20-epoxybeyer-15-ene.

Excoecarin V2 (**2**) was isolated as a methyl ester (**2a**) when treated with diazomethane. The molecular formula of **2a** was determined to be $C_{22}H_{34}O_4$ from the HR-EI-MS data (*m/z* 362.2452, $[M]^+$). The IR spectrum of **2a** showed the absorption bands of ester carbonyl and olefinyl groups. The ^{1}H-NMR spectrum of **2a** ($CDCl_3$) showed three methyls at δ 1.08, 1.25, and 1.26, two methoxyl signals at δ 3.61 and 3.64, exomethylene protons at δ 4.73 (br s) and 4.79 (br s), and two methylene protons at δ 2.31 and 2.57 (each d, *J*=18.0 Hz). The ^{13}C-NMR spectrum indicated two ester carbonyl carbons at δ 172.0 and 179.9, three methine carbons at δ 43.6, 47.6, and 48.2, three quaternary carbons at δ 43.3, 44.2, and 46.4, and an olefin group at δ 103.1 and 155.5. These spectral data was similar to those of **5a**[12] except for the chemical shifts of the C-ring moiety. This evidence suggested that excoecarin V2 (**2**) could be a ring A 2,3-*seco*-16-kaurenoid. The relative stereochemistry of **2a** was established by the NOE difference spectra measurements shown in Fig. 2. Irradiation of the methyl protons at δ 1.08, H_3-20 produced NOE enhancement of the signal of H-14, and irradiation of the exomethylene protons at δ 4.73 and 4.79, H_2-17 produced NOE enhancements of H_2-15 and H-13, respectively. Furthermore, NOEs were detected between the signals of H-5 and H-9. The *enantiomer*-type skeleton of **2a** was

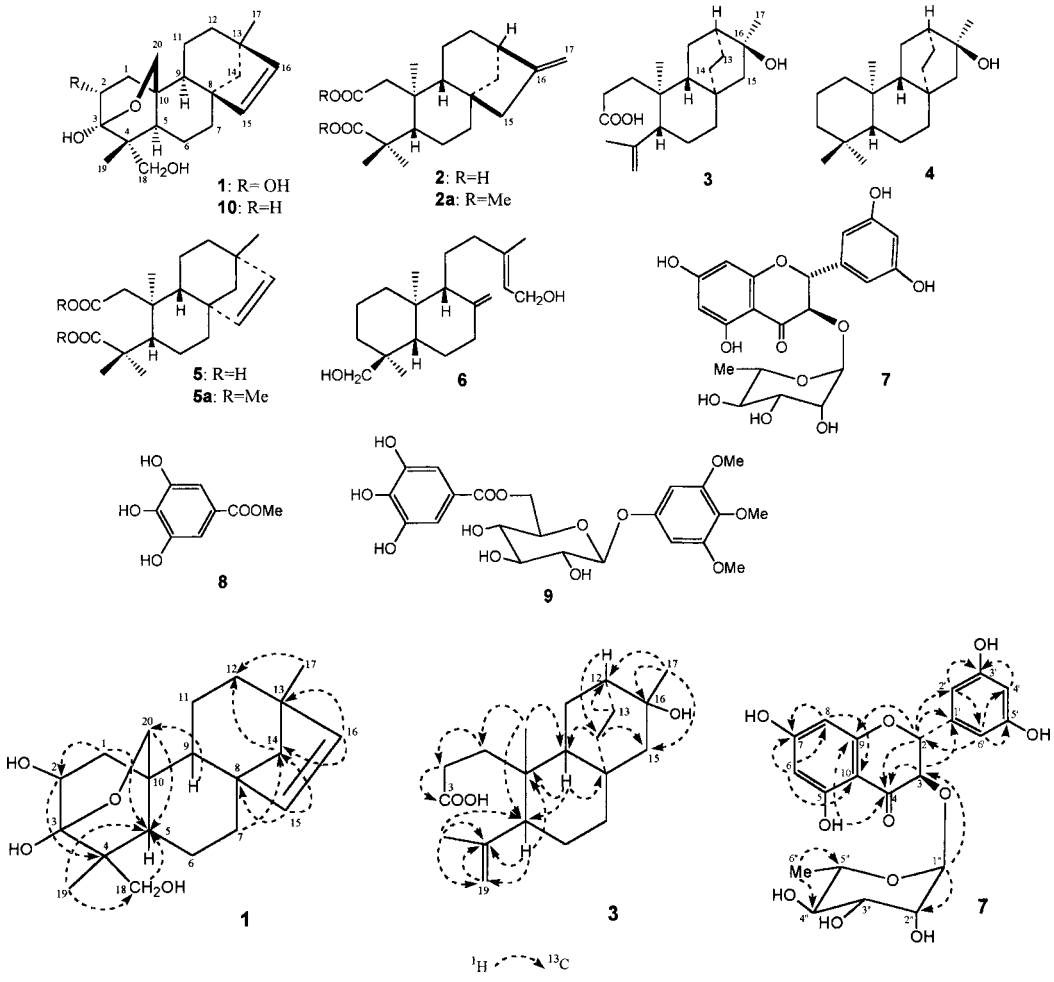

Fig. 1. HMBC Correlations of **1**, **3**, and **7**

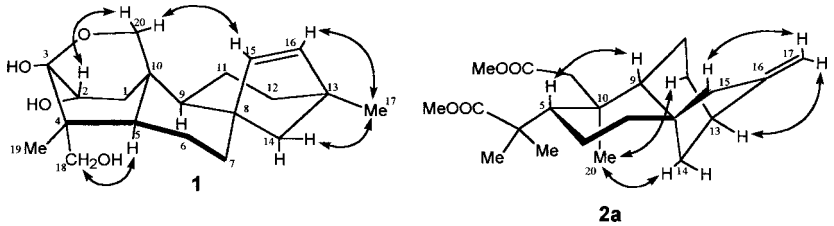

Fig. 2. NOE Correlations of **1** and **2a**

confirmed by the optical rotation ($[\alpha]_D$ −27.7°) showing the same negative sign as that of *ent*-kaurene.[19] Consequently, the structure of **2** was determined to be *ent*-2,3-secokaur-16-en-2,3-dioic acid.

Excoecarin V3 (**3**) was isolated as colorless plates, mp 101—102 °C and $[\alpha]_D$ −53.7°, and its molecular formula was established to be $C_{20}H_{32}O_3$ from HR-EI-MS showing $[M]^+$ ion at *m/z* 320.2396. The IR spectrum of **3** showed a hydroxyl group (3560 cm^{-1}), a carbonyl group (1713 cm^{-1}), and disubstituted olefin (1637, 758 cm^{-1}). The positive detection of **3** for 2,6-dichlorophenol-indophenol sodium salt on TLC also revealed the presence of a carboxylic acid group.[20,21] The presence of carboxyl and isopropenyl groups was evident from the ^{1}H- and ^{13}C-NMR spectral data (Tables 1 and 2).

The positions of the carboxyl group at C-3 and an iso-

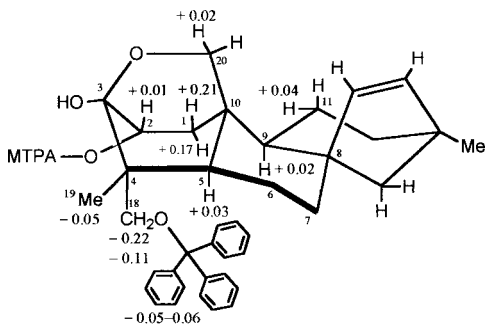

Fig. 3. Results Obtained with the Modified Mosher's Method for **1**
The $\Delta\delta$ values are in Hz ($\delta S - \delta R$, 400 MHz).

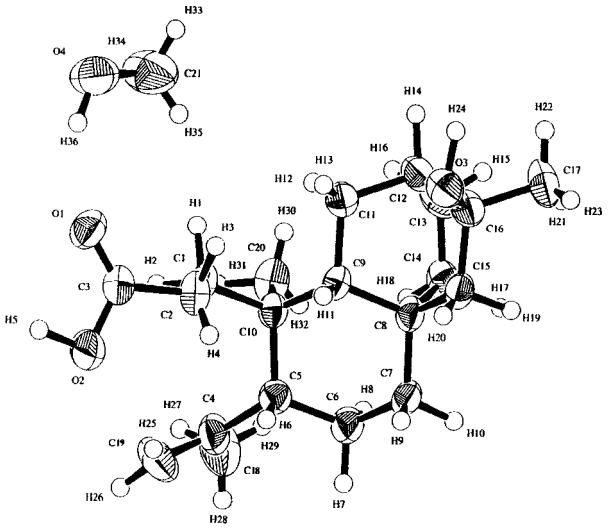

Fig. 4. ORTEP Drawing of **3**

propenyl group at C-5 were confirmed by the HMBC correlations between the carbonyl carbon at δ 177.7 and H_2-2, between a quaternary carbon at δ 147.5 (C-4) and H_3-18, H-5, and H_2-19, between C-5 (δ 50.4) and H-9, H_3-18, and H_3-20, and between a methine carbon at δ 37.5 (C-12) and H_3-17, H_2-13. In addition, other correlations for the quaternary carbons (C-8, C-10, and C-16) were also observed in this HMBC spectrum (Fig. 1). The above-mentioned evidence suggests that **3** has a planar structure of a secoatisane-type diterpenoid with a carboxylic acid at C-3 and an olefinyl group at C-19. This proposition is supported even by the ^{13}C chemical shift values of **3**, which agreed very well with respect to the carbons of ring A with agallochin O.[22] The relative structure was established on the basis of the X-ray crystal structure analysis (Fig. 4). A methanol, as the crystal solvent, was contained in the crystal lattice. The methyl groups at C-17 and C-20 and methylene groups of C-13 and C-14 were *cis* oriented to each other. The hydroxyl group at 16-OH and the methine protons at C-5 and C-9 were also *cis* oriented. The absolute stereostructure of **3** was determined by the comparison of the optical rotation ($[\alpha]_D$ −53.7°) with that of **4**.[8—11] Consequently, the structure of **3** was established as *ent*-3,4-*seco*-16α-hydroxyatis-4(19)-en-3-oic acid.

Compound **7** was obtained as pale yellow needles from MeOH. The IR spectrum of **7** showed the presence of hydroxyl (3300 cm^{-1}), carbonyl (1649 cm^{-1}), and aromatic ring (1605, 1525, 1390 cm^{-1}) groups. The fast atom bombardment (FAB)-MS of **7** showed a quasimolecular ion peak at *m/z* 451 $[M+H]^+$ together with a fragment ion peak at *m/z* 303 $[C_{15}H_{11}O_7]^+$. The molecular formula $C_{21}H_{22}O_{11}$ of **7** was determined from the quasimolecular ion peak and high-resolution MS measurement. The ^1H- and ^{13}C-NMR (DMSO-d_6) spectra of **7** showed signals assignable to a ring B in the flavanonol structure by a characteristic coupling pattern at δ 4.66 (d, J=10.0 Hz, H-2) and 5.24 (d, J=10.0 Hz, H-3), five aromatic protons at δ 5.89 (d, J=2.1 Hz, H-8), 5.91 (d, J=2.1 Hz, H-6), 6.74 (2H, s, H-2′, 6′), 6.89 (s, H-4′), an anomeric proton at δ 4.03 (br s, H-1″), and seven hydroxyl protons at δ 4.52 (br d, J=4.5 Hz), 4.54 (br d, J=5.0 Hz), 4.75 (br d, J=4.5 Hz), 9.07 (s), 9.11 (s), 10.85 (br s), and 11.80 (s, OH-5). The sugar was assigned to be an L-rhamnose by comparison with the ^{13}C chemical shift values of the reference.[23] In the HMBC experiment for **7**, the H–C long-range correlation was observed between an anomeric proton at δ 4.03 and a methine carbon at δ 75.7 (C-3) (Fig. 1). Thus the rhamnose moiety was connected with C-3 in ring B. The absolute structure of **7** was determined on the basis of the circular dichromic (CD) spectroscopic method. The CD spectrum of **7** showed positive Cotton effects at 252 nm ($\Delta\varepsilon$+1.85) and 332 nm ($\Delta\varepsilon$+5.71) and a negative Cotton effect at 297 nm ($\Delta\varepsilon$−10.0), which indicated the configurations of the 2- and 3-positions to be *R* and *R*, respectively.[24—26] Thus the absolute stereostructure of **7** was confirmed to be 3,5,7,3′,5′-pentahydroxy-2*R*,3*R*-flavanonol 3-*O*-α-L-rhamnopyranoside.

Experimental

General Methods 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. ^1H-NMR (300, 400 MHz) and ^{13}C-NMR (75, 100 MHz) spectra were recorded on a Varian XL-300 and a Varian INOVA-400 spectrometer in $CDCl_3$ or DMSO-d_6 with TMS as an internal standard, respectively. Coupling constants (J) are given in Hz. MS were obtained with JEOL MS-BU 20 and JEOL LMS-SX-120A QQ mass spectrometers. Silica gel 60 (70—230 mesh, Merck), reverse-phase silica gel (Cosmosil 75 C_{18}-OPN, Nacalai tesque), Avicel (Funakoshi), and Sephadex LH-20 (Pharmacia) were used for column chromatography. Silica gel 60 F_{254} (0.25 mm, Merck) and Rp-18 F_{254}s (0.25 mm, Merck) were used for analytical TLC. HPLC was performed 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 column chromatography, Nova-Pak Cartridge C_{18} (Millipore Co. Ltd., 100 mm×5 mm i.d.) was used.

Plant Material The fresh stem of *E. agallocha* L. was collected in August 2001, on Okinawa Island, Japan. A voucher specimen (KPU 001951) has been deposited in the Herbarium of the Department of Pharmaceutical Sciences of Natural Resources, Kyoto Pharmaceutical University, Japan.

Extraction and Isolation The chopped fresh stem of *E. agallocha* (6.5 kg) was extracted twice with MeOH at room temperature. Removal of the solvent from the combined methanol extracts yielded a brown viscous mass (351 g). The extract was suspended in H_2O and partitioned with *n*-hexane, $CHCl_3$, AcOEt, and *n*-BuOH. The *n*-hexane layer (49.9 g) was chromatographed on silica gel with $CHCl_3$–MeOH (10 : 1) to obtain 10 fractions, 1—10. Fraction 2 (6.9 g) was subjected to reverse-phase silica gel [MeOH–H_2O (3 : 1) and MeOH] to yield fractions 2-1—2-5. Fraction 2-2 (443 mg) was subjected to silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1) and MeOH] to give a crude fraction (70.8 mg) containing excoecarin V2 (**2**) and *ent*-2,3-secobeyer-15-ene-2,3-dioic acid[12] (**5**). The crude fraction was extracted with MeOH and treated with diazomethane. The reaction mixture was concentrated under reduced pressure. The residue was analyzed using HPLC [MeOH–H_2O (7 : 3)] to produce **2**-methyl ester (**2a**, 11 mg) and **5**-methyl ester (**5a**, 18.8 mg). Fraction 10 (238 mg) was chromatographed on silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1)] and recycle HPLC (MeOH) was used to provide *ent*-atisane-16α-ol[8—11] (**4**, 34.8 mg). Fraction 5 (11.5 g) was subjected to silica gel [$CHCl_3$–MeOH–H_2O (4 : 1 : 0.1) and MeOH] and reverse-phase silica gel [MeOH–H_2O (10 : 1)], and recycle HPLC (MeOH) to give excoecarin V3 (**3**, 77.5 mg) and *ent*-15,18-dihydroxy-labda-8(17),3*E*-diene[13] (**6**, 63.2 mg). Fraction 6 was subjected to cellulose [$CHCl_3$–MeOH–H_2O (4 : 1 : 1, lower phase)], reverse-phase silica gel [MeOH–H_2O (8 : 1)], and silica gel [$CHCl_3$–MeOH–H_2O (9 : 1 : 0.1)] to give excoecarin V1 (**1**, 5.5 mg). The AcOEt layer (10.0 g) was chromatographed on cellulose with $CHCl_3$–MeOH–H_2O (10 : 1 : 1, lower layer) to give six fractions, 1—6. Fraction 2 was subjected to reverse-phase silica gel [MeOH–H_2O (3 : 7)] and Sephadex LH-20 (MeOH) to produce methyl 3,4,5-trihydroxybenzoate[14] (**8**, 171.5 mg). Fraction 4 was chromatographed on reverse-phase silica gel [MeOH–H_2O (1 : 4)] and Sephadex LH-20 (MeOH) to give 3,4,5-trimethoxyphenol 1-*O*-β-D-(6-galloyl)-glucopyranoside[15] (**9**, 174.5 mg). Fraction 5 was subjected to silica gel [hexane–AcOEt (1 : 1)], reverse-phase silica gel [MeOH–H_2O (1 : 5)], and Sephadex LH-20 (MeOH) to provide **7** (62.2 mg).

The known compounds were identified by comparison of their physical

and spectral data with the values given in the literature.

Excoecarin V1 (**1**): Colorless prisms (MeOH), mp 177—179 °C. $[\alpha]_D^{26}$ −19.3° (c=0.5, MeOH). IR (KBr) cm^{-1}: 3410, 3300, 1600, 1188, 916, 746. EI-MS m/z: 334 $[M]^+$, 316 $[M-18]^+$, 298 $[316-18]^+$. HR-EI-MS m/z: 334.2150 $[M]^+$ (Calcd for $C_{20}H_{30}O_4$: 334.2144). ^{1}H- and ^{13}C-NMR data are shown in Tables 1 and 2.

Excoecarin V2 methyl ester (**2a**): Colorless syrup, $[\alpha]_D^{26}$ −27.7° (c=1.1, $CHCl_3$). IR (KBr) cm^{-1}: 1742, 1728, 1655, 873. EI-MS m/z (rel. int.): 362 $[M]^+$ (1.5), 347 $[M-15]^+$ (1.0), 331 $[M-31]^+$ (1.5), 303 $[M-CH_3OCO]^+$ (5.3), 289 $[303-14]^+$ (35), 187 (100). HR-EI-MS m/z: 362.2452 $[M]^+$ (Calcd for $C_{22}H_{34}O_4$: 362.2457). ^{1}H- and ^{13}C-NMR data are shown in Tables 1 and 2.

Excoecarin V3 (**3**): Colorless plates (MeOH), mp 101—102 °C. $[\alpha]_D^{28}$ −53.7° (c=1.0, $CHCl_3$). IR (KBr) cm^{-1}: 3560, 3000, 1713, 1637, 1219, 758, 667. EI-MS m/z (rel. int.): 320 $[M]^+$ (6.0), 302 $[M-18]^+$ (100), 287 $[302-15]^+$ (13.7). HR-EI-MS m/z: 320.2396 $[M]^+$ (Calcd for $C_{20}H_{32}O_3$: 320.2398). ^{1}H- and ^{13}C-NMR data are shown in Tables 1 and 2.

3,5,7,3′,5′-Pentahydroxy-2*R*,3*R*-flavanonol 3-*O*-α-L-rhamnopyranoside (**7**): Pale yellow needles (MeOH), mp 191—193 °C. $[\alpha]_D^{34}$ −3.0° (c=1.06, MeOH). IR (KBr) cm^{-1}: 3300, 1649, 1605, 1525, 1471, 1390, 1294, 1262, 1160, 1116, 1039, 976, 825, 779, 735. UV λ_{max} (MeOH) nm (log ε): 204 (4.75), 230 (4.35), 290 (4.37), 335 sh (3.62). FAB-MS m/z: 451 $[M+H]^+$, 303 $[C_{15}H_{11}O_7]^+$. HR-FAB-MS m/z: 451.1237 $[M+H]^+$ (Calcd for $C_{21}H_{23}O_{11}$: 451.1240). CD (c=1.06×10^{-4}, MeOH) $\Delta\varepsilon^{24}$ (nm): +5.71 (332), −10.0 (297), +1.85 (252). ^{1}H-NMR (400 MHz in DMSO-d_6) δ: 1.07 (1H, d, J=6.5 Hz, H-6″), 3.13 (1H, dt, J=3.5, 9.5 Hz, H-4″), 3.39 (2H, m, H-2″, 3″), 3.89 (1H, dq, J=6.5, 9.0 Hz, H-5″), 4.03 (1H, br s, H-1″), 4.52 (1H, br d, J=4.5 Hz, OH), 4.54 (1H, br d, J=5.0 Hz, OH), 4.66 (1H, d, J=10.0 Hz, H-2), 4.75 (1H, br d, J=4.5 Hz, OH), 5.24 (1H, d, J=10.0 Hz, H-3), 5.89 (1H, d, J=2.1 Hz, H-8), 5.91 (1H, d, J=2.1 Hz, H-6), 6.74 (2H, s, H-2′, 6′), 6.89 (1H, s, H-4′), 9.07 (1H, s, OH), 9.11 (1H, s, OH), 10.85 (1H, br s, OH), 11.80 (1H, s, OH); (+D_2O) δ: 1.06 (1H, d, J=6.5 Hz, H-6″), 3.13 (1H, dd, J=9.5, 9.5 Hz, H-4″), 3.34 (1H, dd, J=1.5, 3.5 Hz, H-2″), 3.42 (1H, dd, J=3.5, 9.5 Hz, H-3″), 3.89 (1H, dq, J=6.5, 9.5 Hz, H-5″), 4.04 (1H, d, J=1.5 Hz, H-1″), 4.66 (1H, d, J=10.0 Hz, H-2), 5.25 (1H, d, J=10.0 Hz, H-3), 5.91 (1H, d, J=2.1 Hz, H-8), 5.93 (1H, d, J=2.1 Hz, H-6), 6.76 (2H, s, H-2′, 6′), 6.91 (1H, s, H-4′). ^{13}C-NMR (100 MHz in DMSO-d_6) δ: 17.8 (C-6″), 69.0 (C-5″), 70.1 (C-3″), 70.4 (C-2″), 71.7 (C-4″), 75.7 (C-3), 81.6 (C-2), 95.1 (C-8), 96.1 (C-6), 100.1 (C-1″), 101.0 (C-10), 114.8 (C-6′), 115.4 (C-2′), 118.9 (C-4′), 127.0 (C-1′), 145.2 (C-3′), 145.9 (C-5′), 162.2 (C-9), 163.5 (C-5), 167.0 (C-7), 194.6 (C-4).

Tritylation of Excoecarin V1 (1) A solution of **1** (2.6 mg) in pyridine (1 ml) was treated with trityl chloride (5 mg) and the mixture was left to stand at room temperature for 36 h. The reaction mixture was poured into ice-water and the precipitate was filtered. The residue (5.9 mg) was dried and purified by silica gel column chromatography with hexane–AcOEt (2 : 1) to give 1-tritylate (2.2 mg).

Preparation of (*R*)- and (*S*)-MTPA Esters (1a, 1b) from 1-Tritylate A solution of 1-tritylate (1.0 mg) in 1 ml of pyridine was reacted with (*S*)-(+)-MTPA chloride (10 μl) in pyridine (20 μl), and the mixture was left to stand at room temperature for 10 h. After the ice-water (3 ml) was added, the water solution was passed through a Sep-Pak C_{18} cartridge, washed with 5 ml of MeOH–H_2O (2 : 1), and then eluted with MeOH. The MeOH solution was removed under reduced pressure, and the residue subjected to silica gel column chromatography by elution with hexane–AcOEt, 1 : 2, to obtain the (*R*)-(+)-MTPA ester (**1a**) (1.0 mg). Through a similar procedure, (*S*)-(−)-MTPA ester (**1b**) (1.1 mg) was prepared from 1-tritylate (1.5 mg) using (*R*)-(−)-MTPA chloride.

1a: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.92 (1H, ddd, J=1.3, 3.9, 12.5 Hz, H-9), 0.98 (1H, d, J=9.7 Hz, H-14), 1.03 (3H, s, H-17), 1.01 (1H, m, H-5), 1.08 (1H, m, H-6), 1.21 (3H, s, H-19), 1.65 (1H, dd, J=13.4, 14.5 Hz, H-11), 1.78 (1H, ddd, J=3.0, 9.6, 14.5 Hz, H-1), 1.95 (1H, dd, J=3.0, 14.5 Hz, H-1), 3.54 (1H, d, J=11.4 Hz, H-18), 3.74 (1H, d. J=11.4 Hz, H-18), 3.77 (1H, d, J=9.0, H-20), 3.89 (1H, d, J=9.0 Hz, H-20), 5.05 (dd, J=3.0, 9.6 Hz, H-2), 5.49 (1H, d, J=5.5 Hz, H-15), 5.56 (1H, d, J=5.5 Hz, H-16), 6.0—7.5 (aromatic-H).

1b: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 0.94 (1H, ddd, J=1.3, 3.9, 12.5 Hz, H-9), 0.98 (1H, d, J=9.7 Hz, H-14), 1.03 (3H, s, H-17), 1.04 (1H, m, H-5), 1.08 (1H, m, H-6), 1.16 (3H, s, H-19), 1.69 (1H, dd, J=13.4, 14.5 Hz, H-11), 1.99 (1H, ddd, J=3.0, 9.6, 14.5 Hz, H-1), 2.12 (1H, dd, J=3.0, 14.5 Hz, H-1), 3.32 (1H, d, J=11.5 Hz, H-18), 3.63 (1H, d. J=11.5 Hz, H-18), 3.79 (1H, d, J=9.0, H-20), 3.89 (1H, d, J=9.0 Hz, H-20), 5.06 (dd, J=3.0, 9.7 Hz, H-2), 5.49 (1H, d, J=5.5 Hz, H-15), 5.56 (1H, d, J=5.5 Hz, H-16), 6.2—7.8 (aromatic-H).

X-Ray Diffraction Structure Determination of 3 A single crystal of **3** was obtained by recrystallization from MeOH. Crystal data of **3**: $C_{21}H_{36}O_4$; Mr=352.51, colorless plate, space group $P2_1$ (#4), a=8.582(1) Å, b=8.422(1) Å, c=13.816(8) Å, β=92.31(1) Å, V=997.9(2) Å^3, Z=2, D_{calc}=1.173 g/cm^3, μ(Cu$K\alpha$)=6.28 cm^{-1}. The R (R_w) value of **3** was 0.102 (0.145). The data were collected on a Rigaku AFC7R diffractometer at 23±1 °C using graphite-monochromated Cu$K\alpha$ (λ=1.54178 Å) radiation. The structure was solved by direct methods (MITHRIL84[27]). The nonhydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. Neutral atom scattering factors were taken from Cromer and Waber.[28] All calculations were performed using the teXsan[29] crystallographic software package of Molecular Structure Corporation.

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