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Stereostructures of New Labdane-Type Diterpenes, Excoecarins F, G1, and G2 from the Wood of *Excoecaria agallocha*

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Three new labdane-type diterpenes, excoecarins F, G1, and G2, were isolated from resinous wood of *Excoecaria agallocha* LINN collected from Okinawa prefecture. Stereochemistries of the new diterpenes were determined on the basis of chemical and physicochemical evidence. Excoecarin F was found to possess a chloride element in its structure.

Key words *Excoecaria agallocha*; excoecarin F; excoecarin G1; excoecarin G2; labdane-type diterpene; chlorinated diterpene

Excoecaria agallocha LINN (Euphorbiaceae) is distributed on seashores and edge-mangroves throughout tropical Africa, Asia, and northwest Australia.[1,2] The *Excoecaria* genus is well known to contain skin irritants, generally called irritant *Excoecaria* factors. The bark and wood of this tree have been used in traditional medicines for flatulence in Thailand.[2] The resinous wood including the latex of *E. agallocha* has been used as a substitute for agarwood (Jinko)[3] incense in Okinawa, Japan. The piscicidal constituent excoecariatoxin[4] characterizing the daphnane diterpene ester and some related compounds has been obtained from the twigs, bark, and latex of *E. agallocha* in Japan[4] and Thailand,[2] respectively. Daphnane- and tigliane-type diterpene esters are known to be skin irritants and tumor promoters.[2] A novel phorbol ester acting as an anti-HIV agent was isolated from the leaves and stems of *E. agallocha* collected in northwest Australia.[5] We reported the isolation and structure elucidation of some diterpenes from the resinous wood of this plant,[6] and also reported their inhibitory effect on Epsetin-Barr virus early antigen (EBV-EA) activation induced by 12-*O*-tetra-decanoyl phorbol-13-acetate (TPA) in Raji cells.[7] As a continuing study, we isolated three new diterpenes, excoecarin F (**1**), excoecarin G1 (**2**), and G2 (**3**) from the resinous wood of *E. agallocha*. In this paper, we describe the stereochemistry of the new compounds. The ether extract of the resinous wood was purified by repeated ordinary and reversed-phase silica-gel column chromatography and finally preparative HPLC to give excoecarins F (**1**, 0.0011%), G1 (**2**, 0.0008%), and G2 (**3**, 0.0007%).

The positive detection of excoecarin F (**1**), $[\alpha]_D$ $-20.8°$ ($c=1.0$, MeOH), for 2,6-dichlorophenol-indophenol sodium salt on TLC revealed the presence of a carboxylic acid group. The molecular formula of colorless syrup dimethyl ester **1a**, $[\alpha]_D$ $-21.7°$ ($c=1.0$, $CHCl_3$), was determined by HR-FAB-MS measurement to be $C_{22}H_{37}O_6Cl$. The IR spectrum of **1a** showed hydroxyl (3358 cm^{-1}), carbonyl (1740, 1726 cm^{-1}), ether (1109, 1074, 987 cm^{-1}), and alkyl halide (1259, 740 cm^{-1}) groups. The ^1H-NMR spectrum of **1a** showed signals for methine and methylene protons on each carbon bearing a hydroxyl group and/or chloride group (δ 3.44, 3.60, 3.71) (Table 1). The proton and carbon signals in the ^1H- and ^{13}C-NMR spectra of **1a** were very similar to those of *ent*-13-*epi*-8,13-epoxy-2,3-secolabd-14-en-2,3-oic acid dimethyl ester (**4**),[6] except for some signals due to the side chain [**1a**: δ_C 77.4, δ_H 3.71 (1H, dd, $J=2.3$, 9.0 Hz, 14-H), 46.4, 3.44 (1H, dd, $J=9.0$, 11.0 Hz, 15-H), 46.4, 3.60 (1H, dd, $J=2.3$, 11.0 Hz, 15-H)] (Tables 1 and 2). These data and detailed ^{13}C- and

Table 1. ^1H-NMR Spectra Data for Compounds **1a**, **2**, and **3** in $CDCl_3$[a)]

	1a[b)]	**2**[c)]	**3**[c)]
1	2.32 d (18)	1.50 m	1.49 m
	2.38 d (18)	2.07 ddd (3.5, 6.5, 13.0)	2.08 ddd (3.5, 6.5, 13.0)
2		2.40 ddd (3.5, 5.5, 15.0)	2.40 ddd (3.5, 5.5, 15.0)
		2.65 ddd (6.5, 13.0, 15.0)	2.64 ddd (6.5, 13.0, 15.0)
5	2.48 dd (3.5, 6.5, 6.5)		
6	1.43 ddd (3.3, 12.3, 13.5)		
	1.64 ddd (3.5, 6.8, 13.5)		
7	1.75 dt (3.2, 12.3)	2.00 ddd (4.5, 13.0, 13.0)	2.01 ddd (4.5, 13.0, 13.0)
	1.50 m	2.45 ddd (2.5, 4.0, 13.0)	2.45 ddd (2.5, 4.0, 13.0)
9	2.47 dd (5.5, 11.0)	1.68 m	1.69 m
11	1.49 m		
	1.56 ddd (5.5, 5.5, 19.0)		
12	1.51 m		
	1.98 ddd (6.5, 11.0, 17.0)		
14	3.71 dd (2.3, 9.0)	3.48 t (4.5)	3.47 t (4.5)
15	3.44 dd (9.0, 11.0)	3.76 d (4.5)	3.76 d (4.5)
	3.60 dd (2.3, 11.0)		
16	1.10 s	1.25 s	1.25 s
17	1.22 s	4.68 br s	4.55 br s
		4.92 br s	4.91 br s
18	1.24 s	1.10 s	1.10 s
19	1.28 s	1.03 s	1.03 s
20	0.88 s	0.89 s	0.88 s

a) Values in parentheses are coupling constants in Hz. *b*) Spectrum was measured at 600 MHz. *c*) Spectra were measured at 300 MHz.

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Table 2. ^{13}C-NMR Spectra Data for Compounds **1**—**5** and **1a** in $CDCl_3$[a)]

Carbon	1[b)]	1a	2	3	4	5
1	77.0	40.6	37.7	37.6	41.2	37.6
2	176.2	171.5	34.7	34.7	171.5	34.7
3	176.5	179.5	216.8	216.9	179.6	216.9
4	40.3	45.9	47.8	47.8	46.1	47.8
5	52.6	46.1	55.2	55.2	47.9	55.2
6	20.5	21.9	25.0	25.1	21.9	25.1
7	42.0	42.2	37.9	37.9	41.7	38.2
8	75.4	75.4	146.9	147.5	75.6	147.2
9	50.5	48.3	56.2	56.5	50.6	55.2
10	41.5	41.5	39.4	39.5	41.6	39.2
11	16.0	15.6	17.7	17.6	16.7	22.3
12	30.4	30.2	36.7	36.7	34.6	37.8
13	74.4	74.5	74.7	74.7	73.1	139.8
14	77.4	77.4	76.2	76.6	147.4	123.4
15	46.4	46.4	63.3	63.2	109.6	59.3
16	24.1	24.2	23.3	23.3	32.3	16.4
17	24.3	24.3	107.9	107.4	22.9	107.5
18	27.8	27.9	26.0	26.0	27.5	26.0
19	23.4	23.6	21.7	21.7	23.8	21.7
20	19.3	19.1	14.0	14.0	19.6	14.1
OMe		51.0			51.0	
		51.9			51.4	

a) Those of ^{13}C signals were determined by DEPT and ^{13}C–^{1}H COSY experiments. *b*) Spectrum was measured in DMSO-d_6.

^{1}H-NMR studies with the aid of ^{13}C–^{1}H COSY, HMBC spectra led us to conclude that **1** and **1a** may be 2,3-secolabdane-type diterpenes possessing side chains with polar groups. The connectivities of a hydroxyl group at C-14 and a chloride group at C-15 were determined by the effect of substituents on ^{13}C chemical shifts in the side chain and comparison of the ^{13}C-NMR spectrum for **1a** with that of **4**, as shown in Table 2. The presence of **1** and **1a** was detected from the ether extract and its methyl ester products using diazomethane by TLC, respectively. Thus the possibility of an artifact of **1** in the process of separating was eliminated. The absolute configuration of the hydroxyl group was determined by the application of a modification of Mosher's method[8)] to **1a**. Treatment of **1a** with (*R*)- and (*S*)-MTPA chloride in the presence of pyridine afforded the (*S*)-MTPA ester (**1b**) and (*R*)-MTPA ester (**1c**), respectively. The signals due to protons on C-12, -14, -16, -17, and -20 in **1b** appeared at higher fields than those of **1c** ($\Delta\delta$: negative), while the signals due to protons attached to C-15 of **1b** were observed at lower fields compared with those of **1c** ($\Delta\delta$: positive). Consequently, the configuration at C-14 in **1a** has been elucidated to be *R*. The absolute stereostructure of **1a** was clarified by the chemical evidence. The reaction of **4** with calcium hypochloride in the presence of acetic acid gave the chlorohydrin **1a** ($[\alpha]_D$ $-20.1°$, 10.0%) and 14-isomer of **1a** ($[\alpha]_D$ $-22.4°$, 12.0%). Consequently, the stereostructure of **1** was determined as shown.

Excoecarin G1 (**2**), mp 130—132 °C, $[\alpha]_D$ $-14.8°$ (c=0.5, $CHCl_3$) and G2 (**3**), mp 118—120 °C, $[\alpha]_D$ $-9.4°$ (c=0.5, $CHCl_3$), were found to have the same molecular formula, $C_{20}H_{34}O_4$, on the basis of HR-FAB-MS. The IR spectra of **2** and **3** showed absorption bands ascribable to the carbonyl (**2**, **3** 1707 cm^{-1}), and olefinyl (**2**, **3** 1645, 892 cm^{-1}) groups. The ^{1}H- and ^{13}C-NMR spectra of **2** and **3** indicated the presence of four *tert*-methyls (δ 0.89, 1.03, 1.10, 1.25 in **2**, 0.88, 1.03, 1.10, 1.25 in **3**), six methylenes, exomethylenes (δ 4.68, 4.92

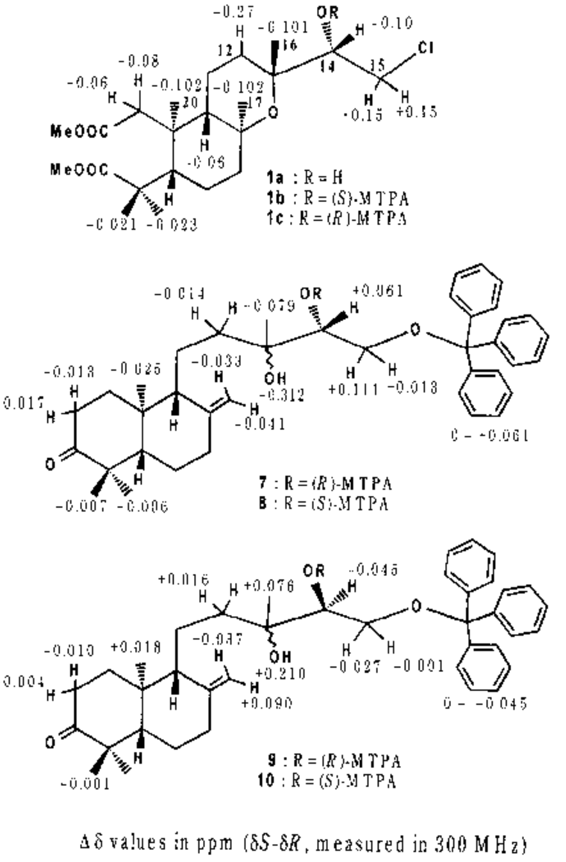

Fig. 1. Chemical Shift Differences for the (*R*)-MTPA Esters (**1c**, **7**, **9**) and (*S*)-MTPA Esters (**1b**, **8**, **10**) in ppm at 300 MHz

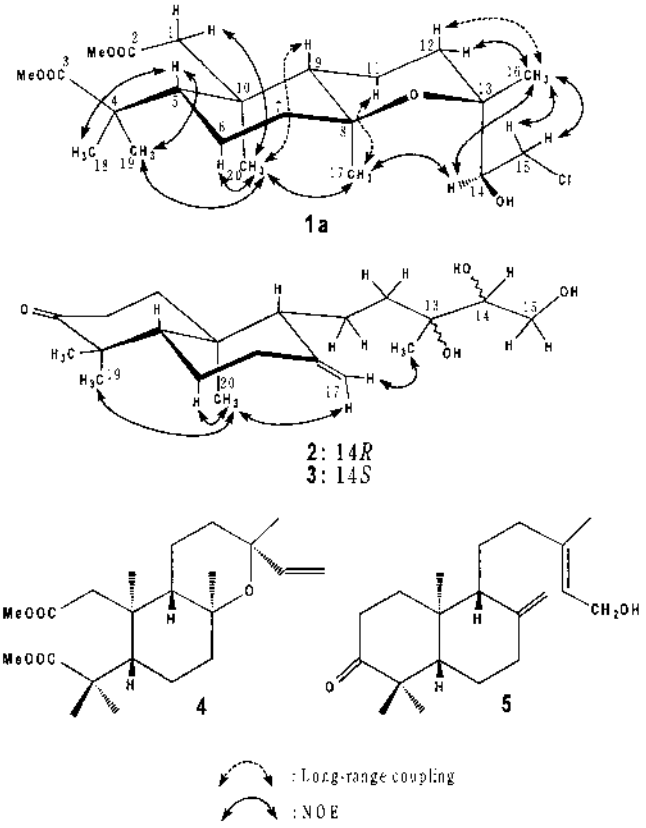

Fig. 2. Structures of **1a**, **2**—**5**, and C–H Long-Range Coupling and NOE Correlations for **1a**, **2**, and **3**

in **2**, 4.55, 4.91 in **3**), and methine and methylene protons (δ 3.48, 3.76 in **2**, 3.47, 3.76 in **3**) on the carbons bearing the hydroxyl groups (Tables 1 and 2). The mass spectra measurements and ^{13}C chemical shift data led us to presume that **2** and **3** were labdane-type diterpenes analogous to those occurring along with *ent*-15-hydroxylabda-8(17),13*E*-dien-3-one (**5**).$^{9)}$ The *sp*^{3} carbon signals at δ 74.7 and 76.2 in **2**, analogous to the signals at δ 74.7 and 76.6 in **3**, were observed instead of the *sp*^{2} carbon signals of C-13 and -14 in **5**. Thus the hydroxyl groups were present at C-13, -14, and -15 in **2** and **3**. The configuration of the secondary hydroxyl group at C-14 was determined by a modification of Mosher's method following the protection of the primary hydroxyl group in **2** and **3** with trityl chloride in pyridine, respectively. In the MTPA esters prepared from **2**, the signals due to protons on C-12, -16, -17, and -20 and hydroxyl proton in (*R*)-MTPA ester (**7**) appeared at lower fields than those of (*S*)-MTPA ester (**8**) ($\Delta\delta$: negative), while the signals due to protons attached to C-15 and the protons of the trityl group of (*R*)-MTPA ester were observed at higher fields at compared with those of (*S*)-MTPA ester ($\Delta\delta$: positive). In the MTPA esters (**9**, **10**) derived from **3**, $\Delta\delta$ showed opposite signs to the MTPA esters (**7**, **8**). These observations suggest that the configurations of C-14 in **2** and **3** were *R* and *S*, respectively. However, the configurations at C-13 of **2** and **3** were not determined. The CD spectra of **2** and **3** showed negative Cotton effect (315 m) analogues to that of **5** (315 nm). Consequently, the absolute stereostructures of **2** and **3** except for the configuration of C-13 were determined as shown.

More labdane-type diterpenes possessing halide groups were found in *Aplysia kurodai*,$^{10)}$ *Laurencia venusta*,$^{11)}$ and *Chondria tenuissima*,$^{12)}$ a marine animal and plants, respectively. Excoecarin F is the first example of the isolation of a labdane-type diterpene with a chloride group from the shore plant *Excoecaria agallocha*, however.

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