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ISOLONGIRABDIOL, A NEW DITERPENOID FROM *RABDOSIA LONGITUBA*

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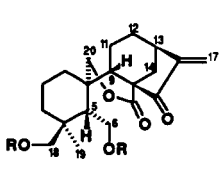
ABSTRACT.—A new *ent*-spirosecokaurene diterpenoid, named isolongirabdiol [**1**], was isolated from the aerial parts of *Rabdosia longituba* together with the known compounds oridonin [**4**], lasiokaurin [**5**], and nodosin [**6**]. The structure of the new compound was elucidated on the basis of spectroscopic and chemical evidence.

Rabdosia longituba (Miq.) Hara (Labiatae) is a perennial herb distributed in the western part of Japan (1). From the aerial parts of this plant, many diterpenoids biosynthesized from *ent*-kaurene have been found and characterized (2–5). Some of these showed antibacterial and cytotoxic activities, while other showed inhibitory activity against respiration of rat liver mitochondria (2). In our continuing search for biologically active substances of *R. longituba*, we examined the diterpenoid constituents of the plant collected in Hiroshima Prefecture, Japan and isolated a new diterpenoid, named isolongirabdiol [**1**], together with the known compounds oridonin [**4**], lasiokaurin [**5**], and nodosin [**6**] (2). This paper describes the structure elucidation of the new compound.

Isolongirabdiol [**1**], $[\alpha]^{26}D$ +37.7° (c = 0.69, MeOH) was isolated as an amorphous powder from the dried aerial parts of the plant by the method described in the Experimental section. The molecular formula was assigned as $C_{20}H_{28}O_5$ based on its hreims spectrum. The spectral data of **1** showed the presence of a five-membered ring containing a ketone group conjugated with an exomethylene group [uv λ max (MeOH) 233 nm (ε 6840); ir ν max ($CHCl_3$) 1740 and 1640 cm^{-1}; ^1H nmr see Table 1; (C_5D_5N) δ 5.31 and 5.95 (each 1H, br s); ^{13}C nmr (C_5D_5N) δ 117.4 (t), 151.3 (s), 203.2 (s)], a δ-lactone (ir ν max 1710 cm^{-1}; ^{13}C nmr δ 171.3), a methylene group having an acyloxy group [^1H nmr δ 4.87 and 4.94 (each 1H, d, J = 11.5 Hz), ^{13}C nmr δ 71.1 (t)], two hydroxymethyl groups [ir ν max 3440 cm^{-1}; ^1H nmr δ 3.39 and 3.84 (each 1H, d, J = 11 Hz), 4.01 (2H); ^{13}C nmr δ 58.0 and 71.7 (each t)], and a tertiary methyl group [^1H nmr δ 0.97 (3H, s); ^{13}C nmr δ 19.9 (q)] as partial structures. Acetylation of isolongirabdiol [**1**] gave the diacetate **2** [^1H nmr δ 1.97 and 2.06 (each 3H, s)]. In addition to the above-mentioned signals, the ^{13}C-nmr spectrum (see Table 2) showed signals due to six methylene groups, three methine groups, and three quaternary carbon atoms. Thus, isolongirabdiol [**1**] is tetracyclic. When the spectral data of **1** were compared with those of closely related diterpenoids, the data suggested that



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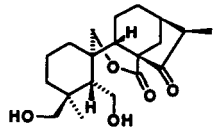

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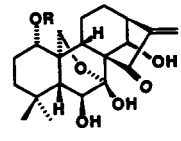

4 R=H
5 R=Ac

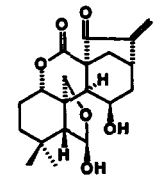

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TABLE 1. ^{1}H-nmr Data of Isolongirabdiol [**1**].

Proton	C_5D_5N (400 MHz)	$CDCl_3$ (200 Hz)
H_2-1	1.29–1.38	
H_2-2	1.29–1.38, 1.48 (1H, m)	
H_2-3	1.29–1.38	
	1.88 (1H, dt, J = 13.5 and 4 Hz)	
H-5	2.41 (t, J = 4 Hz)	1.89 (dd, J = 5.5 and 3.5 Hz)
H_2-6	4.01 (2H, m)	3.62 (1H, dd, J = 11.5 and 5.5 Hz)
		3.72 (1H, dd, J = 11.5 and 3 Hz)
H-9	3.16 (dd, J = 12.5 and 5 Hz)	2.50 (1H, br t, J = 8.5 Hz)
H_2-11	1.60, 1.78 (each 1H, m)	
H_2-12	1.29–1.38, 2.04 (each 1H, m)	
H-13	2.91 (br dd, J = 9 and 5 Hz)	3.12 (m)
H_2-14	2.22 (1H, d, J = 12.5 Hz)	2.15 (1H, d, J = 12.5 Hz)
	2.57 (1H, dd, J = 12.5 and 4.5 Hz)	2.40 (1H, dd, J = 12.5 and 4.5 Hz)
H_2-17	5.31, 5.95 (each 1H, br s)	5.50, 6.00 (each 1H, br s)
H_2-18	3.39, 3.84 (each 1H, d, J = 11 Hz)	3.12, 3.48 (each 1H, J = 11.5 Hz)
H_3-19	0.97 (s)	0.88 (s)
H_2-20	4.87, 4.94 (each 1H, d, J = 11.5 Hz)	4.10, 4.57 (each 1H, d, J = 11.5 Hz)
OH	6.34 (1H, br)	

isolongirabdiol [**1**] has an *ent*-15-oxo-spirosecokaurene skeleton. The structure around rings C and D was confirmed by the ^{1}H-COSY spectrum of isolongirabdiol [**1**], which is shown in Figure 1. The connectivities for H_2-17↦H-13↦H-12β↦H-11↦H-9 were demonstrated by following the cross peaks through the line A. The connectivities for H-13↦H-14β↦H-14α were also demonstrated by following the cross peaks through the line B. The cross peaks between H-20 and H-9, due to long range coupling via W letter interaction, were also observed. The location of two primary hydroxyl groups was determined on the basis of the ^{1}H-COSY and ^{1}H-NOESY. The signal at δ 4.01 showed a cross peak with a methine signal at δ 2.41 (1H, t, J = 4 Hz), which was assigned to H-5 in the ^{1}H-COSY spectrum. Thus, a hydroxyl group is located at C-6. Isolongirabdiol [**1**] showed only one tertiary methyl group in the ^{1}H- and ^{13}C-nmr spectra, suggesting that one of the methyl groups at C-4 was oxidized to a hydroxymethyl group. On the basis of the results from the ^{1}H-NOESY spectrum summarized in Figure 2, another hydroxyl group is located at C-18. Namely, cross peaks between the tertiary methyl group and protons at C-20 were observed. The tertiary methyl group showed cross peaks to H_2-18 and H_2-6, and cross peaks between H-9 and H-6

TABLE 2. ^{13}C-nmr Data of Isolongirabdiol [**1**] (measured in C_5D_5N).

Carbon		Carbon	
C-1	27.9	C-11	17.5
C-2	17.6	C-12	29.9
C-3	36.7	C-13	35.4
C-4	38.9	C-14	29.8
C-5	44.8	C-15	203.2
C-6	58.0	C-16	151.3
C-7	171.3	C-17	117.4
C-8	58.2	C-18	71.7
C-9	58.2	C-19	19.9
C-10	42.1	C-20	71.1

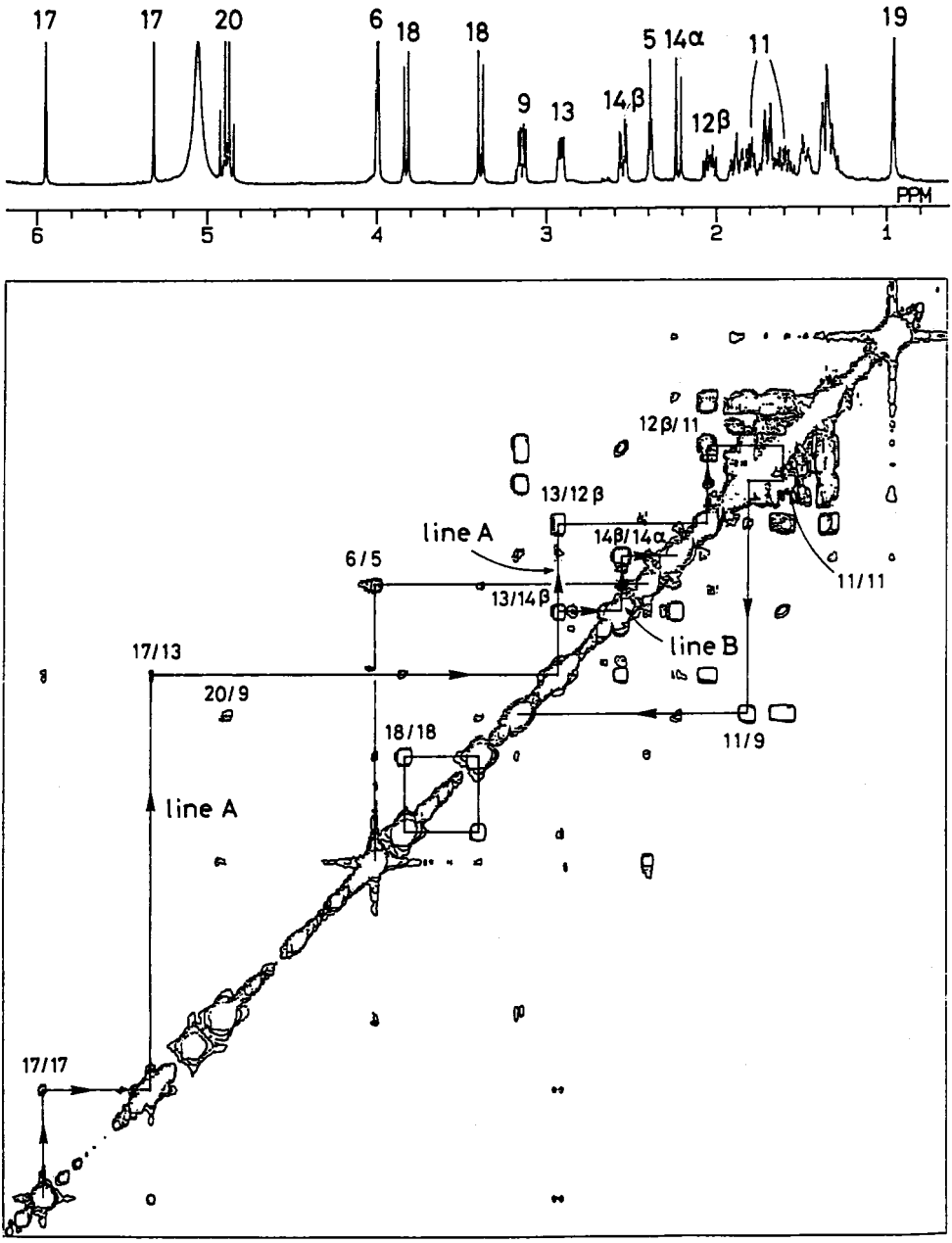

FIGURE 1. ^{1}H-COSY spectrum of isolongirabdiol [**1**].

were also observed. Thus, the relative stereostructure of isolongirabdiol was elucidated as **1**. The absolute stereochemistry was established from the negative Cotton effect [$\Delta\epsilon_{305}$ −0.99] in the cd spectrum of the dihydro compound **3** obtained by catalytic hydrogenation of **1** (6). It is known that there are two possible chair conformations for ring A in the *ent*-spirosecokaurane diterpenoid series (7). One has a C-9 equatorial and C-20 axial configuration, the other has a C-9 axial and C-20 equatorial configuration. The above-mentioned results from the ^{1}H-NOESY spectrum suggested that ring A of isolongirabdiol is in the former chair conformation.

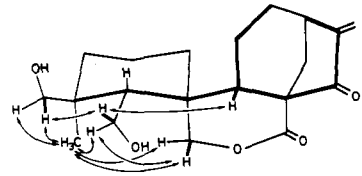

FIGURE 2. Summary of the results of ^{1}H-NOESY spectrum for isolongirabdiol [**1**].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Shimadzu UV-160 spectrophotometer. Ir spectra were recorded with a Hitachi 215 spectrophotometer. Optical rotations were determined with a Union Giken PM-201 digital polarimeter. Mass spectra were obtained with a JEOL D-300 mass spectrometer using a 70 eV electron impact ion source. Nmr spectra were measured with a JEOL-FX 200 or JEOL GSX-400 spectrometer. Kieselgel 60 (0.040–0.063 mm; Merck) was used for cc, and Kieselgel 60 F_{254} precoated plates (0.25 mm or 0.5 mm, Merck) were used for tlc and layer chromatography.

PLANT MATERIAL.—*R. longituba* was collected by one of the authors (H.O.) from Aki-ku, Hiroshima Prefecture, Japan, in early October 1987. It was identified by Professor T. Seki of Miyajima Natural Botanical Garden, Faculty of Sciences, Hiroshima University. A voucher specimen is deposited in the herbarium of Faculty of Pharmaceutical Sciences, The University of Tokushima.

ISOLATION OF DITERPENOIDS.—Dried aerial parts (505 g) of *R. longituba* were extracted with MeOH (10 liters) for 2 weeks at room temperature. The plant was extracted again with the same volume of MeOH for 1 month. The combined MeOH extract was evaporated in vacuo. The residue was dissolved in 90% MeOH (330 ml), and the solution was washed with *n*-hexane (300 ml × 3). The aqueous MeOH solution was concentrated in vacuo. The residue was suspended in H_2O (300 ml) and partitioned with EtOAc (300 ml × 3). After being washed with H_2O, the EtOAc extract was dried and evaporated in vacuo to give a residue (15.2 g). The residue was chromatographed over Si gel (450 g) with $CHCl_3$/Me_2CO as eluent with increasing Me_2CO content. $CHCl_3$ (1 liter), $CHCl_3$-Me_2CO (19:1, 2.5 liters), $CHCl_3$-Me_2CO (9:1, 2.5 liters), $CHCl_3$-Me_2CO (17:3, 2.5 liters), $CHCl_3$-Me_2CO (4:1, 2 liters), $CHCl_3$-Me_2CO (7:3, 2 liters) were eluted successively, collecting 150-ml fractions. Fractions 46–55 gave a residue (2.36 g) that was recrystallized from MeOH to give lasiokaurin [**5**] (422 mg). The mother liquor gave a residue (1.24 g) on concentration in vacuo. An aliquot (133 mg) was separated by layer chromatography [$CHCl_3$-Me_2CO (4:1), developed twice] to give nodosin [**6**] (46 mg). Fractions 61–64 gave a residue (186 mg) on evaporation, which was purified by layer chromatography [$CHCl_3$-Me_2CO (3:1), developed twice] to give isolongirabdiol [**1**] (67 mg). Fractions 70–79 gave a residue (822 mg), an aliquot (132 mg) of which was purified by layer chromatography [$CHCl_3$-Me_2CO (3:1) developed three times] to give oridonin [**4**]. Compounds **4**, **5**, and **6** were identified with authentic samples by mixed mp and comparisons of spectral data.

ISOLONGIRABDIOL [**1**].—An amorphous powder, $[\alpha]^{26}D$ +37.7° (c = 0.69, MeOH); uv λ max (MeOH) nm (ε) 233 (6840); ir ν max ($CHCl_3$) 3440, 1740, 1710, 1640, 1260–1190, 1120, 1040 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; eims *m/z* $[M]^+$ 348.1938 ($C_{20}H_{28}O_5$ requires 348.1937).

ISOLONGIRABDIOL DIACETATE [**2**].—Isolongirabdiol [**1**] (24.6 mg) was acetylated with a mixture of Ac_2O (0.5 ml) and pyridine (0.5 ml) for 20 h at room temperature. Excess MeOH was added to the mixture, and the solution was concentrated in vacuo to give a residue which was purified by layer chromatography [$CHCl_3$-Me_2CO (9:1), developed twice] to give **2** (15.7 mg) as an amorphous powder: ir ν max ($CHCl_3$) 1740, 1715, 1640, 1380, 1365, 1260–1200, 1120, 1035 cm^{-1}; ^{1}H nmr δ ($CDCl_3$) 1.00 (3H, s, H_3-19), 1.97 and 2.06 (each 3H, s, 2 × OAc), 2.14 (1H, d, J = 12.5 Hz, H-14α), 3.11 (1H, br dd, J = 9 and 4.5 Hz, H-13), 3.68 and 3.97 (each 1H, d, J = 11 Hz, H_2-18), 4.12 (2H, m, H_2-6), 4.27 and 4.63 (each 1H, d, J = 11.5 Hz, H_2-20), and 5.51 and 6.03 (each 1H, br s, H_2-17); eims *m/z* $[M]^+$ 432.2136, calcd for $C_{24}H_{32}O_7$, 432.2139.

DIHYDROISOLONGIRABDIOL [**3**].—Isolongirabdiol [**1**] (19.8 mg) was dissolved in MeOH (5 ml), and 5% Pd/C (30 mg) was added to the solution. The mixture was stirred for 3 h in an atmosphere of H_2. After the catalyst was filtered off, the filtrate was concentrated in vacuo. The residue was purified by layer chromatography [$CHCl_3$-Me_2CO (4:1), developed twice] to give **3** (9.5 mg) as an amorphous powder: ir ν max ($CHCl_3$) 3600, 3420, 1740, 1710, 1460, 1400, 1260–1190, 1100, 1035, 1020 cm^{-1}; ^{1}H nmr δ

(C_5D_5N) 0.97 (3H, s, H_3-19), 1.03 (3H, d, J = 7 Hz, H_3-17), 2.24 (1H, d, J = 13 Hz, H-14α), 2.36 (1H, t, J = 4.5 Hz, H-5), 2.96 (1H, dd, J = 11 and 6 Hz, H-9), 3.40 and 3.83 (each 1H, d, J = 11 Hz, H_2-18), 4.01 (2H, m, H_2-6), 4.87 (2H, s, H_2-20), 6.30 (1H, s, OH); cd $\Delta\epsilon_{305} = -0.99$ (MeOH, 4.565 mM); eims *m/z* $[M]^+$ 350.2082, calcd for $C_{20}H_{30}O_5$, 340.2094.

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