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LONGIRABDOLIDE C, A NEW DITERPENOID FROM *RABDOSIA LONGITUBA*

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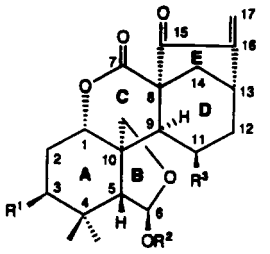
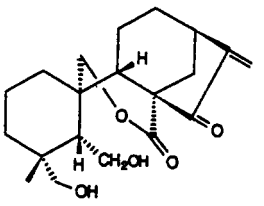
ABSTRACT.—A new *ent*-6,7-secokaurene diterpenoid, longirabdolide C [**1**], was isolated from the aerial parts of *Rabdosia longituba* together with the known compounds nodosin [**4**], enmein [**5**], macrocalyxoformin D [**6**] and longirabdacetal [**7**]. The structure of the new compound was elucidated based on spectroscopic and chemical evidence.

As diterpenoid constituents of *Rabdosia longituba* (Miq.) Hara (1) (Labiatae), compounds such as oridonin, nodosin, and isolongirabdiol, that are biosynthesized from *ent*-kaurene, have been isolated (2,3). These substances have shown various biological activities such as antibacterial and cytotoxic effects. During the course of our systematic search for biologically active substances from plants of the genus *Rabdosia*, we examined the diterpenoid constituents of *R. longituba* collected in Hiroshima Prefecture and isolated a new diterpenoid, named longirabdolide C [**1**], together with the known compounds nodosin [**4**] (4), enmein [**5**] (5), macrocalyxoformin D [**6**] (6) and longirabdacetal [**7**] (7). This paper deals with the structure elucidation of the new compound **1**.

Longirabdolide C [**1**] was isolated as colorless needles, mp 204–206°, $[\alpha]_D$ −120.6° (MeOH), from the MeOH extract of the dried aerial parts of *R. longituba*, by the methods described in the Experimental section. Based on its hreims, the molecular formula was determined as $C_{20}H_{26}O_7$. Spectral data of this compound indicated that it contained a five-membered-ring carbonyl group conjugated with an *exo*-methylene group [uv λ max nm (ε) 229 (6088) nm; ir ν max 1750 and 1640 cm^{-1}; ^1H nmr δ 5.31 and 5.98 (each 1H, br s) (Table 1); ^{13}C nmr δ 117.8 (t), 151.0 (s), and 200.9 (s) (Table 2)], δ-lactone [ν max 1710 cm^{-1}; δ_C 172.1 (s)], a hemiacetal group [δ_H 5.95 (1H, d, J=2.2 Hz, changed to a singlet on addition of D_2O) and δ_C 102.5 (d)], a methylene attached to an oxygen atom [δ_H 4.44 and 4.66 (each 1H, ABd, J=9.2 Hz)], two methine hydrogens geminal to a hydroxyl group and an oxygen atom of a lactone group [δ_H 3.82 (1H, m) and 6.20 (1H, dd, J=12.5 and 6.6 Hz); δ_C 75.4 and 75.8 (each d)], three hydroxyl groups, [δ_H 6.16 (1H, d, J=3.4 Hz), 6.77 (1H, d, J=5.6 Hz) and 8.46 (1H, d, J=2.2 Hz)], and two tertiary methyl groups [δ_H 1.08 and 1.31 (each 3H, s); δ_C 23.2 and 28.4 (each q)]. The ^{13}C-nmr spectrum of **1** (see Table 2) showed the presence of three methylene groups, three methine groups, and three quaternary carbon atoms in addition to the above-mentioned signals. These spectral data, together with those reported for other diterpenoids isolated from the genus *Rabdosia* (2), suggested that longirabdolide C [**1**] has a structure in which two hydroxyl groups are introduced to the structure of isodocarpin [**3**] (8). Dihydrolongirabdolide C [**8**] obtained by catalytic hydrogenation showed a negative Cotton effect in the cd spectrum, proving the reported absolute stereochemistry (9,10). Two hydroxyl groups were located at C-3 and C-11 by comparing the ^{13}C-nmr data of longirabdolide C [**1**] with those of nodosin [**4**], enmein [**5**], and carpalasionin [**9**] (11) (Table 2). In particular, the ^{13}C-nmr chemical shifts of carbons in rings D and E in **1** were very



1 $R^1=R^3=OH$; $R^2=H$
2 $R^1=OAc$; $R^2=Ac$; $R^3=OH$
3 $R^1=R^2=R^3=H$
4 $R^1=R^2=H$; $R^3=OH$
5 $R^1=OH$; $R^2=R^3=H$



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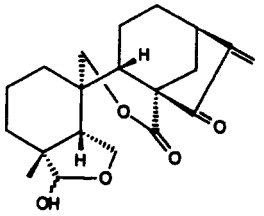

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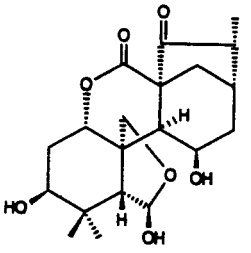

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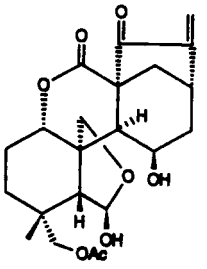

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similar to those of **4** and **9**, and the chemical shifts of carbons in rings A and B were very similar to those of **5**. Acetylation of **1** with a mixture of Ac_2O and pyridine gave the diacetate [**2**] [^{1}H nmr δ 1.96 and 2.08 (each 3H, s); 4.95 (1H, dd, J=4.5 and 2 Hz) and 6.25 (1H, s, 6-H)]. A hydroxyl group which was presumed to be located at C-11 was not acetylated under these conditions. The location of the OH group was finally determined by analyzing the ^{1}H-COSY nmr spectrum. By following the cross-peaks from δ 3.11 (H-9) to δ 5.11 (H-11), 1.87 (H-12α), 2.51 (H-12β), and 3.10 (H-13), successively, the hydroxyl group was positioned at C-11. The location of another hydroxyl group at C-3 was also determined by following cross-peaks from δ 3.82 (H-3) to 2.31 (H_2-2) and 6.20 (H-1). The stereochemistry of the hydroxyl groups at both C-3 and C-11 was determined as β based on the results of ^{1}H-NOESY nmr experiments. The signal at δ 3.82 (H-3) showed cross-peaks with the signals at δ 1.08 (H_3-19) and 1.31 (H_3-18) and the resonance at δ 5.11 (H-11) showed cross-peaks with the signal at δ 2.91 (H-5). Thus, the structure of longirabdolide C was elucidated as **1**, and was further supported by

TABLE 1. ^{1}H-Nmr Data of Longirabdolide C [1].[a]

Proton	Compound 1
1β-H	6.20 (dd, 6.6, 12.5)
2-H_2	2.31 (m)
3α-H	3.82 (m)
5-H	2.91 (s)
6-H	5.95 (d, 2.2)[b]
9-H	3.11 (d, 3.7)
11α-H	5.11 (m)[c]
12α-H	1.87 (dd, 15.0, 5.3)
12β-H	2.51 (dd, 15.0, 9.2)
13-H	3.10 (m)
14α-H	2.18 (dd, 12.0, 5.3)
14β-H	3.65 (d, 12.0)
17-H_2	5.31, 5.98 (br s)
18-H_3	1.31 (s)
19-H_3	1.08 (s)
20-H_2	4.44, 4.66 (d, 9.2)
3-OH	6.77 (d, 5.6)
6-OH	8.46 (d, 2.2)
11-OH	6.16 (d, 3.4)

[a]Measured at 200 MHz in C_5D_5N. The splitting pattern and the coupling constants are shown in parentheses.

[b]The signal changed to a singlet on addition of D_2O.

[c]The signal changed to a doublet of doublets (J=5.3 and 3.7 Hz) on addition of D_2O.

the results of a ^{1}H-^{13}C long range COSY nmr spectrum.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanagimoto micro melting point apparatus. Uv spectra were recorded on a Hitachi 330 spectrophotometer. Ir spectra were recorded on a Hitachi 215 spectrometer. Optical rotations were determined with a Union Giken PM 201 digital polarimeter. Cd spectra were measured on a Jasco J-600 spectropolarimeter. Mass spectra were obtained on a JEOL D-300 mass spectrometer using an electron-impact ion source (70 eV). Nmr spectra were measured with either a JEOL FX-200 or a JEOL GSX-400 nmr spectrometer. Kieselgel 60 F_{254} precoated plates (0.25 mm or 0.5 mm, Merck) were used for tlc.

PLANT MATERIAL.—*Rabdosia longituba* was collected and identified by one of the authors (H.O.) from Kohda Town, Hiroshima Prefecture, Japan, in late September 1987. A voucher specimen has been deposited in the laboratory of H.O.

ISOLATION OF DITERPENOIDS.—The dried aerial parts (275 g) of *R. longituba* were extracted with MeOH (5 liters) for one month at room temperature. The MeOH extract was evaporated *in vacuo*. The residue was dissolved in 90% MeOH (330 ml) and the solution was washed with *n*-

TABLE 2. ^{13}C-Nmr Data of Longirabdolide C [1] and Related Compounds (Measured in C_5D_5N).

Carbon No.	Compound			
	1	4	5	9[a]
1	75.4	78.5	75.1	78.2
2	30.4	24.1	30.9	23.5
3	75.8	37.5	74.1	31.1
4	35.9	31.6	35.9	35.3
5	52.5	55.8	51.1	56.5
6	102.5	102.1	102.4	100.5
7	172.1	171.8	172.2	171.7
8	56.8	56.5	57.0	56.5
9	48.9	48.7	46.5	49.0
10	49.3	50.0	51.1	49.9
11	66.7	66.1	19.9	66.1
12	40.3	41.4	29.6	41.2
13	35.4	35.6	35.3	35.5
14	34.1	34.2	32.8	34.2
15	200.9	201.0	200.9	201.0
16	151.0	151.3	151.5	151.1
17	117.8	117.3	117.5	117.5
18	28.4	33.1	28.4	27.2
19	23.2	23.4	23.3	66.5
20	74.7	74.0	74.4	74.2
CH_3CO				20.7
CH_3CO				170.6

[a]Taken from Takeda *et al.* (11).

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 (7.2 g). The residue was chromatographed over Si gel (250 g) with $CHCl_3$/Me_2CO as eluent using next for elution Me_2CO/$CHCl_3$ (1 liter), $CHCl_3$-Me_2CO (19:1, 1.5 liters), $CHCl_3$-Me_2CO (9:1, 1.5 liters), $CHCl_3$-Me_2CO (17:3, 1.5 liters), and $CHCl_3$-Me_2CO (4:1, 1.5 liters) (75 ml fractions collected).

Fractions 47–52 gave a residue (373 mg) which was recrystallized from MeOH to give nodosin [**4**] (197 mg).

Fractions 60–64 gave a residue (1.218 g) which was recrystallized from MeOH to give enmein [**5**] (384 mg). The mother liquor gave a residue (498 mg) on concentration *in vacuo* which was then subjected to cc over Si gel (50 g) with $CHCl_3$/Me_2CO mixtures as eluent, viz., $CHCl_3$ (200 ml) and $CHCl_3$-Me_2CO (93:7, 1 liter) being used for elution successively, with 8 ml fractions collected. Fractions 89–113 gave a residue (166 mg) which was separated by hplc [column: M&S pack C-18 B, 20×250 mm; solvent: CH_3CN-H_2O 3:7, 7 ml/min; detection 230 nm] to give macrocalyxoformin D [**6**] (4.4 mg) and longirabdacetal [**7**] (6.8 mg).

Fractions 77–84 gave a residue (110 mg) which was purified sequentially by prep. tlc [solvent: $CHCl_3$-Me_2CO (3:1), developed twice] and hplc [conditions were the same as before except for the detection; 215 nm], to afford longirabdolide C [**1**] (25.0 mg).

Compounds **4**, **5**, **6** and **7** were identified by direct comparison with authentic samples.

Longirabdolide C [**1**].—Colorless needles from MeOH, mp 204–206° $[\alpha]^{22}D$ −120.6° (c=0.80, MeOH); uv λ max (MeOH) (ε) 229 (6088) nm; ir ν max (KBr) 3450 (br), 1750, 1710, 1640, 1550, 1455, 1355, 1305, 1250, 1185, 1075, 1025 and 970 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; hreims *m/z* $[M]^+$ 378.1637 ($C_{20}H_{26}O_7$ requires 378.1678).

Dihydrolongirabdolide C [**8**].—To a solution of longirabdolide C [**1**] (6.0 mg) in MeOH (5 ml), 5% Pd/C (9.0 mg) was added and the mixture was stirred for 1 h under an atmosphere of hydrogen. After the catalyst was filtered off, the filtrate was concentrated *in vacuo* to give **8** (5.5 mg). Mp 201–203° (from MeOH); ir ν max (KBr) 3400 (br), 1760, 1705, 1250 cm^{-1}; ^1H nmr (C_5D_5N) δ 1.02 (3H, d, J=6.8 Hz, H_3-17), 1.07 and 1.30 (each 3H, s, 4-Me_2), 2.87 (1H, d, J=3.7 Hz, H-9), 2.93 (1H, s, H-5), 3.78 (1H, d, J=11.0 Hz, H-14β), 3.81 (1H, m, H-3), 4.43 and 4.67 (each 1H, d, J=9.0 Hz, H_2-20), 4.97 (1H, m, H-11), 5.95 (1H, s, H-6) and 6.18, 6.59 and 8.39 (each 1H, OH×3); cd (MeOH; 3.737 mM) $\Delta\epsilon_{308.8}$=−0.21 and $\Delta\epsilon_{317.4}$=−0.20; hreims *m/z* $[M]^+$ 308.1871, calcd for $C_{20}H_{28}O_7$, 380.1835.

Longirabdolide C Diacetate [**2**].—Longirabdolide C [**1**] (6.0 mg) was acetylated with a mixture of Ac_2O (0.5 ml) and pyridine (0.5 ml) for 24 h at room temperature. After addition of excess MeOH, the solution was concentrated *in vacuo* to give a residue which was purified by tlc [$CHCl_3$-Me_2CO (4:1)] to give **2** (2.0 mg). Mp 292–293° (from MeOH); ir ν max ($CHCl_3$) 1750, 1720, 1645, 1240–1220 cm^{-1}; ^1H nmr δ ($CDCl_3$) 1.10 and 1.13 (each 3H, s, 4-Me_2), 1.96 and 2.08 (each 3H, s, 2×OAc), 2.34 (1H, s, H-5), 3.26 (1H, m, H-13), 3.26 (1H, d, J=11.0 Hz, H-14β), 4.12 (2H, br s, H_2-20), 4.47 (1H, m, H-11), 4.95 (1H, dd, J=4.5 and 2 Hz, H-3), 5.39 (1H, dd, J=11.9 and 6.2 Hz, H-1), 5.56 and 6.13 (each 1H, br s, H_2-17) and 6.25 (1H, s, H-6); hreims *m/z* $[M]^+$ 462.1926, calcd for $C_{24}H_{30}O_9$, 462.1890.

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