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LONGIRABDOLIDE D, A NEW 6,7-SECO-*ENT*-KAURENOID FROM *RABDOSIA LONGITUBA*

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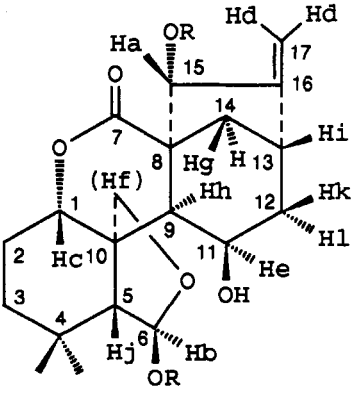
ABSTRACT.—From the aerial parts of *Rabdosia longituba*, a new 6,7-*seco*-*ent*-kaurenoid, longirabdolide D [**1**] was isolated, together with the known compounds, isodocarpin, isolongirabdiol, and jiuhuanin. The structure of the new compound was determined based on its spectroscopic data and a correlation with nodosin [**4**], having known absolute stereochemistry.

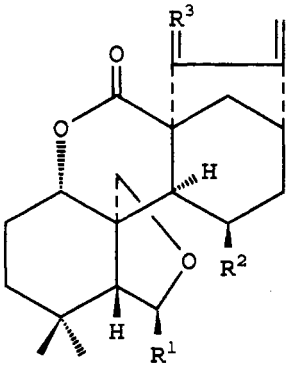
It has been reported that *Rabdosia longituba* (Miq.) Hara (Labiatae) (1) biosynthesizes diterpenoids bearing the *ent*-7β,20-epoxykaurane and 6,7-seco-*ent*-kaurane skeletons (2). Many of these are also known to have biological activities including antibacterial and antitumor activity, and inhibitory activity on respiration of rat mitochondria and insect growth (3). During the course of our studies on the variation in the diterpenoid constituents of this plant collected at different locales, we have examined the diterpenoid constituents of *R. longituba* collected in Toyota County, Hiroshima Prefecture, Japan, and have isolated a new diterpenoid, longirabdolide D [**1**] together with the known compounds, isodocarpin (4), isolongirabdiol (5), and jiuhuanin (6). This paper deals with the structure determination of the new compound.



1 R=COMe
2 R=H

Longirabdolide D [**1**], $[\alpha]_D$ −156.3° ($CHCl_3$), was obtained as colorless needles, mp 257–260°, from the aerial parts of the plant. The molecular formula was determined to be $C_{24}H_{32}O_8$ based on its hreims. Compound **1** did not show any absorption maximum above 220 nm in the uv spectrum and showed absorptions at 3420 (hydroxyl), 1730 (ester), 1710 (δ-lactone), and 1650 (double bond) cm^{-1} in the ir spectrum. The ^1H- and ^{13}C-nmr spectra of **1** showed the presence of two tertiary methyls [δ_H 1.03 and 1.06 (3H each, s), δ_C 20.8 and 33.1 (q)]; two acetoxy groups [δ_H 1.88 and 2.06 (3H each, s), δ_C 23.2 (q), 23.9 (q), 169.6 (s), and 170.0 (s)]; an acetal group [δ_H 6.52 (1H, s) (Hb), δ_C 102.6 (d)]; an *exo*-methylene [δ_H 5.20 (2H, br s) (Hd), δ_C 109.7 (t), and 155.1 (s)]; a δ-lactone [δ_C 173.9 (s)]; a hydroxyl [δ_H 6.70 (1H, d, J=3.2 Hz)]; a methylene bearing an oxygen atom [δ_H 4.19 and 4.23 (each 1H, d, J=8.8 Hz) (Hf), δ_C 74.5 (t)], and three methines having an oxygen functionality [δ_H 4.66 (1H, dd, J=9.6 and 4.2 Hz) (He), 5.96 (1H, dd, J=8 and 8 Hz) (Hc), and 6.81 (1H, t, J=2.8 Hz) (Ha); δ_C 64.8, 77.8, and 80.9 (each d)]. The ^{13}C-nmr spectrum further showed the presence of four

methylenes, three methines, and three quaternary carbon atoms. The above-mentioned spectral data, together with a consideration of the structures of diterpenoids isolated so far from the genus *Rabdosia* (7), suggested that longirabdolide D [**1**] has a structure in which a hydroxyl group and two acetoxyl groups are present in a 6,7-seco-*ent*-kaurene nucleus [**3**]. The location of one acetoxy group was determined as being on the C-6 position by consideration of the chemical shift of Hb. The location of two other oxygen functional groups, a hydroxyl and the other acetoxyl, was made by interpretation of the ^{1}H-^{1}H-COSY spectrum of **1**. Starting with the H-9 resonance (Hh, δ_H 3.01, d, J=4.0 Hz) and following correlations revealed by the cross-peaks, the following assignments were made: H-9 (Hh)→H-11 (He)→H-12α (Hl, δ_H 1.95, dd, J=14.4 and 6.0 Hz) and H-12β (Hk, δ_H 2.53, m)→H-13 (Hi, δ_H 2.94, dd, J=8.4 and 5.2 Hz). The hydroxyl proton showed both a vicinal coupling with H-11 and a long-range coupling with H-9. The latter observation allowed us to assign the hydroxyl as being in the β-configuration. The location of the remaining acetoxyl group was similarly deduced by starting with the H-17 exocyclic methylene resonance (Hd) and following the allylic coupling to H-13 (Hi) and H-15 (Ha). A NOESY spectrum which revealed the spatial proximity of H-15 and H-14α (δ_H 1.84, m) indicated that the acetoxyl was α-oriented (H-15β). Based on these findings, the structure of longirabdolide D was elucidated as **1**.



3 R^1=R^2=H; R^3=H_2
4 R^1=R^2=OH; R^3=O

The structure of **1** corresponds to the 6,15-diacetate of dihydronodosin [**2**] in which the carbonyl group in nodosin [**4**] (8) is reduced to an allylic alcohol. To confirm the above-mentioned assignment, nodosin [**4**], having known absolute stereochemistry, was chemically converted into longirabdolide D [**1**]. Nodosin [**4**] was reduced with $NaBH_4$ in the presence of $CeCl_3 \cdot 7H_2O$ (9) to give an allylic alcohol [**2**]. The stereochemistry of the newly generated hydroxyl group takes an α-orientation as the result of a hydride attack from the less hindered β-side. Acetylation of **2** under the usual conditions gave a diacetate which was identical with natural longirabdolide D [**1**]. Thus, the structure of longirabdolide D was unequivocally established as **1**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Ir spectra were recorded with a Shimadzu IR 400 or Perkin-Elmer 1720 infrared spectrophotometer. Optical rotations were determined with a Jasco DIP 360 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 either a JEOL-FX 200 or a JEOL GSX-400 spectrometer. Kieselgel 60 F_{254} precoated plates (0.25 mm, Merck) were used for tlc.

PLANT MATERIAL.—*R. longituba* was collected from Toyota County, Hiroshima Prefecture, Japan, in early September 1988, and identified by one of the authors (H.O.). A voucher specimen [88-RL-Toyota (12)] has been deposited in the laboratory of H.O.

EXTRACTION AND ISOLATION.—The dried aerial parts (1 kg) of *R. longituba* were extracted with MeOH (25 liters) for 1 month at room temperature. The MeOH extract was evaporated *in vacuo*. The residue was dissolved in 90% MeOH (440 ml) and the solution was washed with *n*-hexane (400 ml×3). The aqueous MeOH solution was concentrated *in vacuo*. The residue was suspended in H_2O (400 ml) and partitioned with EtOAc (400 ml×3). After being washed with

H_2O, the EtOAc extract was dried and evaporated *in vacuo* to give a residue (23 g). The residue was chromatographed over Si gel (700 g) with $CHCl_3$/Me_2CO mixtures as eluents, with increasing Me_2CO content. Thus, $CHCl_3$ (1 liter), $CHCl_3$-Me_2CO (19:1, 10 liters), $CHCl_3$-Me_2CO (9:1, 16 liters), $CHCl_3$-Me_2CO (17:3, 5.7 liters), $CHCl_3$-Me_2CO (4:1, 3 liters), and Me_2CO (1.5 liters) were successively used for elution, collecting 250-ml fractions.

The residue (2.1 g) from fractions 11–14 was treated with active charcoal in MeOH to give a residue (165 mg) that was chromatographed over Si gel (8 g) with *n*-hexane-Et_2O (3:7) as eluent, collecting 5-ml fractions. Fractions 6–8 gave jiuhuanin A (6) (105 mg) on evaporation. Fractions 11–13 gave a residue (7.9 mg) that was recrystallized from MeOH to give longirabdolide D [**1**] (5.6 mg). Fractions 15–35 gave isodocarpin (4) (34.9 mg) on evaporation.

Fractions 84–101 gave a residue (0.6 g) on evaporation, which was chromatographed over Si gel (30 g) with Et_2O as eluent, collecting 10-ml fractions. Fractions 25–50 gave isolongirabdiol (5) (71.4 mg) on evaporation.

The known compounds isolated were identified by comparing spectral data with those reported.

Longirabdolide D [**1**].—Colorless needles, mp 257–260°, $[\alpha]^{22}D$ −156.3° (*c*=0.45, $CHCl_3$); uv, no absorption maxima above 220 nm; ir ν max ($CHCl_3$) 3420, 1730, 1710, 1650, 1360, 1230–1200, 1140, and 1050 cm^{-1}; ^1H nmr δ (C_5D_5N) 1.03 and 1.06 (3H each, s, 2×*tert.*-Me), 1.84 (1H, m, H-14α), 1.88 and 2.06 (3H each, s, 2×OAc), 1.95 (1H, dd, *J*=14.4 and 6.0 Hz, H-12α), 2.53 (1H, m, H-14β), 2.75 (1H, s, H-5), 2.94 (1H, dd, *J*=8.4 and 5.2 Hz, H-13), 3.01 (1H, d, *J*=4.0 Hz, H-9), 3.22 (1H, d, *J*=11.6 Hz, H-14β), 4.19 and 4.23 (1H each, d, *J*=8.8 Hz, H_2-20), 4.66 (1H, dd, *J*=9.6 and 4.2 Hz, H-11), 5.20 (2H, br s, H_2-17), 5.96 (1H, dd, *J*=8.8 and 8.8 Hz, H-1), 6.52 (1H, s, H-6), 6.70 (1H, d, *J*=3.2 Hz, OH), 6.81 (1H, t, *J*=2.8 Hz, H-15); ^{13}C-nmr data, see Table 1; hreims *m/z* $[M]^+$ 448.2067 ($C_{24}H_{32}O_8$ requires 448.2097).

TABLE 1. ^{13}C-Nmr Data of Longirabdolide D [**1**] (measured in C_5D_5N).

Carbon	δ	Carbon	δ
C-1	77.8	C-12	45.3
C-2	23.9	C-13	38.1
C-3	37.3	C-14	33.9
C-4	31.8	C-15	80.9
C-5	54.4	C-16	155.1
C-6	102.6	C-17	109.7
C-7	173.9	C-18	33.1
C-8	51.4	C-19	23.2
C-9	43.1	C-20	74.5
C-10	49.2	CH_3CO-	20.8, 21.2
C-11	64.8	CH_3CO-	169.6, 169.9

SODIUM BOROHYDRIDE REDUCTION OF NODOSIN [**4**].—Nodosin [**4**] (72.1 mg) was dissolved in MeOH (4 ml) and a solution of $CeCl_3 \cdot 7H_2O$ (74 mg) dissolved in 1 ml of MeOH was added. To the stirring solution, $NaBH_4$ (8 mg) was added portionwise. After stirring for 5 min at room temperature, the reaction mixture was neutralized with AcOH and concentrated *in vacuo*. The residue was partitioned between H_2O (30 ml) and *n*-BuOH (30 ml×3). The *n*-BuOH layer was concentrated *in vacuo* to give a residue (80.2 mg) that was purified by cc (solvent $CHCl_3$-Me_2CO with increasing amounts of Me_2CO) to give an allylic alcohol [**2**] (30.0 mg) as an amorphous powder, $[\alpha]^{20}D$ −136.2° (*c*=1.44, $CHCl_3$); ir ν max ($CHCl_3$) 3400, 1750, 1710, 1650, and 1220 cm^{-1}; ^1H nmr (C_5D_5N) δ 1.05 and 1.07 (3H each, s), 2.90 (1H, s), 3.29 (1H, d, *J*=10.8 Hz), 4.27 and 4.90 (1H, each, d, *J*=8.2 Hz), 5.15 and 5.48 (1H each, br s), 5.78 (1H, br s), 5.85 (1H, s), 6.10 (1H, t, *J*=2.8 Hz), 6.34 (1H, br s, OH); hreims *m/z* $[M]^+$ 364.1903 ($C_{20}H_{28}O_6$ requires 364.1887).

ACETYLATION OF THE ALLYLIC ALCOHOL [**2**].—The allylic alcohol [**2**] (15.0 mg) was dissolved in a mixture of pyridine (1 ml) and Ac_2O (1 ml) and the solution was left overnight at room temperature. Excess MeOH was added to the mixture and the solution was concentrated *in vacuo* to give an allylic alcohol diacetate [**1**] (16.7 mg) that was recrystallized from MeOH to give colorless needles, mp 248–250°, $[\alpha]^{22}D$ −155.7° (*c*=0.84, $CHCl_3$); hreims *m/z* $[M]^+$ 448.2094 ($C_{24}H_{32}O_8$ requires 448.2097). This compound was identical to natural longirabdolide D [**1**] based on the mixed melting point determination and a comparison of their ir and ^1H-nmr spectra.

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