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APLYSIADIOL, A NEW BROMINATED DITERPENE FROM THE MARINE MOLLUSC *APLYSIA KURODAI*

MAKOTO OJIKA, YOSHIFUMI YOSHIDA, MANABU OKUMURA, SHIGERU IEDA, and KIYOYUKI YAMADA*

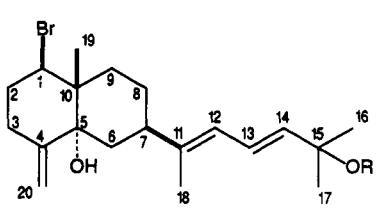
Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

ABSTRACT.—A new brominated diterpene with a prenylated eudesmane skeleton, aplysiadiol [**1**], has been isolated together with the methyl ether derivative **2** from the Japanese marine mollusc *Aplysia kurodai*, and the structures have been determined by spectroscopic data.

Extracts from the marine mollusc *Aplysia kurodai* (Baba) (Aplysiidae) have been shown to contain several novel halogenated terpenoids (1–4). In the course of our search for other terpenoid components of the mollusc, we have isolated a new brominated diterpene named aplysiadiol [**1**] together with the methyl ether derivative **2**. We report herein the isolation and the structure elucidation of these compounds.

The mollusc *A. kurodai* collected in Mie Prefecture, Japan, was extracted with MeOH. The residue from the MeOH extract was subjected to solvent partitioning (EtOAc/H_2O) followed by repeated cc of the EtOAc portion on Si gel, alumina, and C_{18} Si gel. Final purifications were accomplished by C_{18} cc for aplysiadiol [**1**] and by recrystallization for the methyl ether **2**.

Aplysiadiol [**1**] was obtained as a colorless oil, $[\alpha]^{16}D$ −60.7° (c = 0.908, $CHCl_3$). The molecular formula $C_{20}H_{31}BrO_2$ for **1** was determined by hreims (*m/z* 364.1389 $[M-H_2O]^+$, Δ −1.3 mmu). Because the ir spectrum (ν max 3600 and 3430 cm^{-1}) indicated the presence of hydroxyl group(s) in **1**, the ^{13}C-nmr signals at δ 70.6 (s) and 76.5 (s) suggested two tertiary hydroxyl groups. Multiplicities of the ^{13}C signals were determined by DEPT experiments (5). The assignments of the carbons bearing hydrogens were established by the ^1H-^{13}C COSY via one-bond couplings ($^1J_{CH}$) (6) as shown in Table 1. The ^1H- and ^{13}C-nmr spectra of **1** further revealed the presence of three different kinds of the carbon-carbon double bonds: an exomethylene [δ_H 4.52 and 4.60 (s each, H-20), δ_C 109.1 (t, C-20) and 149.8 (s, C-4)], a *trans*-1,2-disubstituted olefin [δ_H 6.60 (dd, J = 15.3 and 10.7 Hz, H-13) and 5.72 (d, J = 15.3 Hz, H-14), δ_C 123.3 (d, C-13) and 140.2 (d, C-14)], and a trisubstituted olefin [δ_H 5.97 (d, J = 10.7 Hz, H-12), δ_C 123.9 (d, C-12) and 141.8 (s, C-11)].



1 R=H
2 R=Me

The analysis of the ^1H-^1H COSY spectrum of **1** revealed the following proton connectivities: H-1/H-2/H-3/H-20, H-6/H-7/H-8/H-9, and H-18/H-12/H-13/H-14. The conjugated diene structure (C-11 to C-14) was supported by uv λ max (cyclohexane) at 239 nm (ε 25,600). Since the information on ^1H-^{13}C long-range couplings ($^2J_{CH}$ and $^3J_{CH}$) was essential to assign five quaternary carbons (C-4, C-5, C-10, C-11, and C-15) and to connect the segments described above through the quaternary carbons, the technique of correlation spectroscopy via long-range coupling (COLOC) (7) was then applied. All long-range couplings detected in the COLOC experiment were tabulated in Table 1. Thus, cross peaks of C-1/H-19, C-4/H-20, C-5/H-6, H-19, and H-20,

TABLE 1. ^{1}H- and ^{13}C-nmr Data for Aplysiadiol [1] and the Methyl Ether Derivative 2 (C_6D_6).[a]

Position	Compound				
	1			2	
	δ_H	δ_C	COLOC (^{1}H)[b]	δ_H	δ_C
1	4.81 dd (9.4, 8.0)	63.9 d	H-3	4.81 dd (8.7, 8.7)	63.8 d
2	2.08 m	34.5 t		2.07 m	34.5 t
3	1.76 ddd (13.0, 4.0, 4.0) 2.42 m	32.7 t	H-20	1.75 ddd (13.5, 3.5, 3.5) 2.44 m	32.7 t
4		149.8 s	H-3, 20		149.8 s
5		76.5 s	H-3, 6, 19, 20		76.5 s
6	1.28 ddd (13.0, 4.0, 1.3) 1.70 dd (13.0, 13.0)	36.4 t	H-8	1.21 ddd (13.0, 4.1, 1.4) 1.68 dd (13.0, 13.0)	36.4 t
7	2.40 dddd (13.0, 13.0, 4.0, 4.0)	41.8 d	H-9, 18	2.38 dddd (13.0, 13.0, 4.1, 4.1)	41.8 d
8	1.38 dddd (13.0, 13.0, 13.0, 5.3) 1.48 br d (13.0)	26.1 t	H-6, 9	1.36 dddd (13.0, 13.0, 13.0, 5.4) 1.49 m	26.1 t
9	1.83 ddd (13.0, 13.0, 4.3) 1.88 ddd (13.0, 5.3, 2.7)	33.1 t	H-19	1.87 m	33.1 t
10		43.4 s	H-6, 8, 9, 19		43.4 s
11		141.8 s	H-18		142.0 s
12	5.97 d (10.7)	123.9 d	H-18	6.00 d (10.7)	123.9 d
13	6.60 dd (15.3, 10.7)	123.3 d		6.58 dd (15.7, 10.7)	126.0 d
14	5.72 d (15.3)	140.2 d	H-16, 17	5.73 d (15.7)	138.0 d
15		70.6 s	H-14, 16, 17		75.0 s
16	1.22 s	30.2 q	H-14	1.32 s	26.3 q
17	1.23 s	30.2 q	H-14	1.32 s	26.3 q
18	1.65 s	15.1 q		1.64 s	15.1 q
19	0.94 s	15.1 q		0.95 s	15.0 q
20	4.52 s 4.60 s	109.1 t		4.49 s 4.58 s	109.0 t
OH	0.80 s, 1.36 br s			0.60 s	
OMe				3.14 s	50.2 q

[a]J values (in Hz) are given in parentheses. ^{1}H and ^{13}C nmr spectra were measured at 500 MHz and 67.8 MHz, respectively.

[b]Protons to which a long-range connectivity ($^2J_{CH}$ and $^3J_{CH}$) was observed in the COLOC experiment. The delays were optimized for $J_{CH} = 11$ Hz.

C-7/H-18, C-9/H-19, C-10/H-19, C-11/H-18, C-14/H-16 and H-17, and C-15/H-16 and H-17 clarified all carbon connectivities and established the gross structure of **1** (no stereochemistry implied). The relative stereochemistry of **1** was determined by the ^{1}H-^{1}H coupling information of the decalin skeleton (see Table 1) and nOe experiments of the methyl ether derivative **2** as described below.

The methyl ether **2** was obtained as colorless plates from hexane, mp 102–103°, $[\alpha]^{11}D$ −62.5° (c=0.848, $CHCl_3$). The ^{1}H- and ^{13}C-nmr (Table 1), uv, and eims data of **2** suggested that **2** was a monomethyl ether of **1**. This was confirmed by a ready transformation of **1** to **2** under acidic conditions (Cl_3CCOOH/MeOH). The location of the methyl ether group in **2** was easily determined by the downfield shift (α effect) of the signal due to C-15 (δ 70.6↦75.0) and the upfield shift (β effect) of the signal due to C-16 and C-17 (δ 30.2↦26.3). This was also supported by a cross peak observed between C-15 (δ 75.0) and methoxy protons (δ 3.14) in the COLOC spectrum. The hydroxyl proton at C-5 (δ 0.60 in C_6D_6 and δ 1.18 in $CDCl_3$) was useful to determine the relative stereochemistry of **2** by nOe experiments. The results of the nOe experiments in C_6D_6 and in $CDCl_3$ are summarized in Figure 1, demonstrating that **1** possesses a *trans*-decalin skeleton with an equatorial diene substituent and that the stereochemistry of the trisubstituted olefin at C-11 is *E*. It should be noted that whereas the vicinal coupling constants for H-1 in Table 1 (9.4 and 8.0 Hz in **1**, 8.7 and 8.7 Hz in **2**) suggest that the A ring is somewhat flattened in the C_6D_6 solution, the A ring is shown to be in the chair conformation illustrated in Figure 1 on the basis of the coupling constants for H-1 (12.0 and 5.0 Hz in **1** and **2**) in the $CDCl_3$ solution. Thus, the structure of aplysiadiol was determined to be **1**.

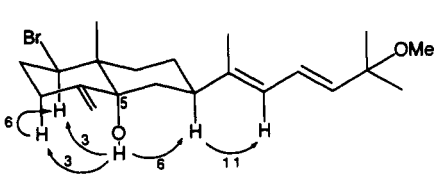


FIGURE 1. Relative stereochemistry of **2** demonstrated by nOe (% enhancement).

Because **2** was easily obtained on treatment of **1** with acid as described above, the methyl ether **2** may be an artifact produced from **1** during MeOH extraction. Aplysiadiol [**1**] is biogenetically regarded as an "extended sesquiterpene" (8) and is an example of the rare prenylated eudesmane type. A few compounds of this type have been isolated from a brown alga (9) and soft corals (10–12): one of the food sources of *A. kurodai* is known to be brown algae, from which **1** may originate.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp is uncorrected. Optical rotations were measured on a JASCO DIP-181 polarimeter. Uv spectra were recorded on a JASCO UVIDEC-510 spectrophotometer and ir spectra on a JASCO IR-810 spectrophotometer. ^{1}H-nmr spectra were recorded on a JEOL GX-500 (500 MHz) using TMS (δ_H 0.00) as an internal standard. ^{13}C nmr and 2D nmr spectra were recorded on a JEOL JNM-C675 spectrometer (270 MHz for ^{1}H nmr and 67.8 MHz for ^{13}C nmr) in C_6D_6 (δ_C 128.0 as a standard) solution. Mass spectra (eims) were obtained with a JEOL JMS LG-2000 spectrometer at 70 eV. Fuji-Davison Si gel BW-820 MH and Merck neutral alumina (Aluminiumoxid 90, Activity II-III) were used for cc.

EXTRACTION AND SEPARATION.—*A. kurodai* (10.7 kg wet wt) was collected in Mie Prefecture, Japan, in May 1985. A voucher specimen (no. MO-N-35) is deposited in the Laboratory of Organic Chemistry, Faculty of Science, Nagoya University. The MeOH (40 liters) extract was suspended in H_2O (2 liters), and the mixture was extracted with EtOAc (4 × 2 liters). The EtOAc-soluble material (60.7 g) was subjected to a Si gel (1 kg) column eluted with C_6H_6, C_6H_6-EtOAc (4:1, 1:1), EtOAc, and MeOH, successively. The fraction (2.5 g) eluted with C_6H_6-EtOAc (1:1) was suspended in MeOH, and the MeOH-soluble material (1.55 g) was passed through an alumina (30 g) column using EtOAc as an eluent. The resulting oily material (1.22 g) was chromatographed on Si gel (30 g) using hexane-EtOAc

(10:1, 5:1) and EtOAc, successively. A portion (242 mg) of the fraction eluted with hexane-EtOAc (5:1) was separated three times by medium pressure cc [(1) Micro Bead Si gel B-(30-70) μ (64.5 g) (Fuji-Davison), flow rate 20 ml/min, hexane-Me_2CO (6:1→4.5:1) gradient elution; (2) Micro Bead Si gel B-(30-70) μ (64.5 g) (Fuji-Davison), flow rate 10 ml/min, CH_2Cl_2-Et_2O (15:1); (3) Develosil ODS 30/60 (60 g) (Nomura Chemical), flow rate 10 ml/min, MeCN-H_2O (70:30)] to afford aplysiadiol [**1**] (77.4 mg, 7.2×10^{-4}% wet wt) as a colorless oil. On the other hand, a part (140 mg) of the fraction eluted with hexane-EtOAc (10:1) was separated three times by medium pressure cc [(1) Micro Bead Si gel B-(30-70) μ (64.5 g) (Fuji-Davison), flow rate 10 ml/min, hexane-Me_2CO (15:1→5:1) gradient elution; (2) Micro Bead Si gel B-(30-70) μ (64.5 g) (Fuji-Davison), flow rate 10 ml/min, C_6H_6-EtOAc (20:1); (3) Micro Bead Si gel B-(30-70) μ (64.5 g) (Fuji-Davison), flow rate 10 ml/min, C_6H_6-EtOAc (40:1)] to afford aplysiadiol methyl ether [**2**] (53.8 mg, 5.0×10^{-4}%), which was further purified by recrystallization from hexane.

APLYSIADIOL [**1**].—A colorless oil, $[\alpha]^{16}D$ −60.9° ($c = 0.908$, $CHCl_3$); ir ν max ($CHCl_3$) 3600, 3430, 3080, 1650 cm^{-1}; uv λ max (cyclohexane) 239 nm (ε 25,600); eims *m/z* (rel. int.) $[M]^+$ 384 (1) and 382 (1), 366 (25) and 364 (25), 348 (25) and 346 (25), 267 (65), 107 (100); hreims found *m/z* 364.1389, calcd for $C_{20}H_{29}{}^{79}BrO$ $[M - H_2O]^+$ 364.1402.

APLYSIADIOL METHYL ETHER [**2**].—Colorless plates; mp 102–103° (hexane); $[\alpha]^{11}D$ −62.5° ($c = 0.848$, $CHCl_3$); ir ν max ($CHCl_3$) 3590, 3420, 3080, 1650, 1065 cm^{-1}; uv λ max (cyclohexane) 240 nm (ε 26,800); eims *m/z* (rel. int.) $[M]^+$ 398 (0.9) and 396 (0.7), 380 (2) and 378 (2), 348 (25) and 346 (25), 267 (60), 107 (100); hreims found *m/z* 364.1411, calcd for $C_{20}H_{29}{}^{79}BrO$ $[M - MeOH]^+$ 364.1402.

TRANSFORMATION OF **1** TO **2**.—A solution of aplysiadiol [**1**] (8.0 mg) and trichloroacetic acid (0.8 mg) in MeOH (0.5 ml) was stirred at room temperature for 15 min. After addition of saturated $NaHCO_3$ (3 ml), the reaction mixture was extracted with hexane (3 × 6 ml), and the combined hexane extracts were dried on Na_2SO_4 and concentrated. Purification of the resulting solid material by Si gel (1 g) cc, eluting with hexane-EtOAc (40:1), afforded methyl ether **2** (4.5 mg) as colorless crystals. The spectral and physical properties of synthetic **2** were identical with those of natural **2**.

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