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NEPHELIOSYNE A, NEW C_{47} ACETYLENIC ACID FROM THE OKINAWAN MARINE SPONGE *XESTOSPONGIA* SP.

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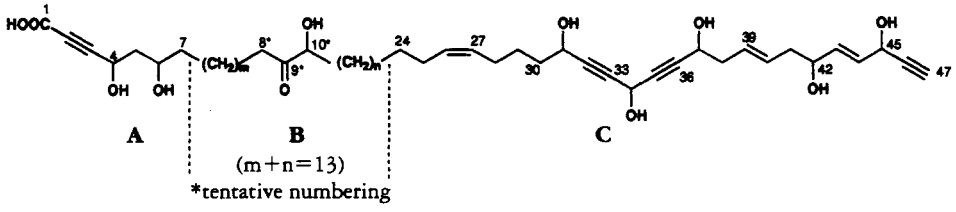
ABSTRACT.—A new C_{47} acetylenic acid, nepheliosyne A [**1**], has been isolated from the Okinawan marine sponge *Xestospongia* sp. and its structure elucidated on the basis of spectroscopic data.

Marine sponges of the genera *Petrosia* or *Xestospongia* (family Nepheliospongiidae) frequently afford polyacetylene metabolites (1) and most of them exhibit various bioactivities such as antifungal, cytotoxic, or antiviral activity. During our studies on bioactive substances from Okinawan marine organisms (2), we recently investigated extracts of the sponge of the genus *Xestospongia* and isolated three new cytotoxic and antimicrobial C_{29} sterols containing a cyclopropane moiety (3). Further examination of the extracts of this sponge resulted in the isolation of a new acetylenic acid metabolite of high molecular weight, nepheliosyne A [**1**], which proved to possess a closely related structure to petrosolic acid, a C_{44} oxooctahydroxytrienetetraynoic carboxylic acid quite recently isolated from a Red Sea sponge *Petrosia* sp. (4). This paper describes the isolation and structure elucidation of **1**.

The sponge *Xestospongia* sp. was collected off Ishigaki Island, Okinawa, and kept frozen until used. The MeOH extract of the sponge was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction was subjected to Si gel cc followed by C_{18} cc to give nepheliosyne A (**1**, 0.01%, wet wt) as a colorless, amorphous solid.

The molecular formula of **1** was deduced as $C_{47}H_{70}O_{11}$ by its hrfabms data [*m/z* 833.4766, $(M+Na)^+$, Δ −4.0 mmu]. The ^{1}H- and ^{13}C-nmr spectra of **1** revealed signals due to a ketone, three disubstituted double bonds, eight oxymethines, and 23 sp^3 methylenes, but no methyl groups. The presence of a carboxylic acid group was suggested by the ir absorption band of **1** at 1700 cm^{-1} as well as the fact that a methyl ester was obtained by treatment of **1** with CH_2N_2. Interpretation of the ^{1}H- and ^{13}C-nmr



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data (Table 1) including ^{1}H-^{1}H COSY, HOHAHA (5), HSQC (6), and HMBC (7) nmr spectra indicated the presence of partial structures **B** and **C** as follows.

The HMBC spectrum showed correlations due to H_2-8*/C-9* and H-10*/C-9*, and the ^{13}C-nmr chemical shift of C-10 (δ_C 78.0) implied that this oxymethine carbon is not at a β-position but rather an α-position to a ketone

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Nepheliosyne A [**1**] in CD_3OD.

Position	^{1}H	J (Hz)		^{13}C
1	—			NA[a]
2	—			NA[a]
3	—			83.0
4	4.60	dd	8.8, 3.8	59.6
5	1.80	m		45.8
	1.75	m		
6	3.80	m		68.6
7	1.55	m		NA[a]
8*	2.55	m		38.7
9*	—			215.5
10*	4.08	dd	7.1, 3.9	78.0
24	2.07 (2H)	m		28.0
25	2.07	m		27.8
26	5.35	m		130.6
27	5.35	m		131.0
28	2.07 (2H)	m		28.0
29	1.45 (2H)	m		25.8
30	1.70	m		38.3
	1.55	m		
31	4.35	br t	6.6	62.63
32	—			83.3
33	—			83.0
34	5.15	t	1.7	52.3
35	—			82.8
36	—			85.9
37	4.35	br t	6.6	62.59
38	2.40 (2H)	m		36.8
39	5.58	m		127.6
40	5.58	m		129.2
41	2.35 (2H)	m		36.3
42	4.15	br q	6.0	72.1
43	5.92	ddd	15.0, 6.0, 1.1	135.7
44	5.75	ddd	15.0, 6.5, 1.1	130.4
45	4.80	br d	6.5	62.5
46	—			84.5
47	2.90	br s		74.9

[a]NA=not assigned.

carbonyl (C-9).[1] The ^{1}H-^{1}H COSY spectrum suggested that both C-8* and C-10* are connected to sp^3 methylene groups. The partial structure **B**[2] was thus deduced.

For the partial structure **C**, the ^{1}H-^{1}H COSY nmr spectrum of **1** revealed the following proton-coupling networks: from H_2-25 to H_2-28, from H_2-30 to H-31, and from H-37 to H-45. The HMBC spectrum of **1** afforded correlations for H-26/C-24, H-26/C-25, H-27/C-28, H-27/C-29, H_2-28/C-29, H_2-28/C-30, and H-31/C-30. The ^{13}C-nmr chemical shifts for C-31 (δ_C 62.63) and C-37 (δ_C 62.59) suggested that these oxymethines are adjacent to acetylenic carbons (8–10), while the oxymethine carbon at C-34 was inferred to be located between two sp car-

[1]In the case of amphidinolide G (11), δ_C values are 77.8 (C-21) and 66.6 (C-18); these carbons correspond to the α and β positions to the ketone carbonyl (C-20), respectively.

[2]We were not able to define the values of m and n, and the numbering for C-8* to C-10* is tentative. The positions of the carbonyl (C-9*) and oxymethine (C-10*) groups may be reversed. The total of m+n=13 was deduced by a process of elimination from the numbers of sp^3 methylenes contained in partial structures **A** and **C**.

bons from its high-field ^{13}C-nmr resonance (δ_C 52.3) (8,9). The signal for H-34 was observed as a triplet (J=1.7 Hz) and the ^{1}H-^{1}H COSY spectrum showed cross-peaks due to H-31/H-34 and H-34/H-37; these observations were ascribable to long-range coupling through a triple bond. Thus, the two oxymethines of C-31 and C-37 were revealed to be connected via the C-32–C-36 moiety. The ^{13}C-nmr chemical shift for C-45 (δ_C 62.5) also implied that C-45 is connected to an acetylene carbon, and the ^{1}H-^{1}H COSY spectrum showed a cross-peak between H-45 and H-47 (δ_H 2.90). The latter is assignable to the terminal acetylene proton, one end of the molecule of **1**. The allylic methylenes of C-25 and C-28 resonated at δ_C 27.8 and 28.0, respectively, implying the geometry of the $\Delta^{26,27}$-double bond to be *Z* (12), while the ^{13}C-nmr chemical shifts of C-38 and C-41 (δ_C 36.8 and 36.3, respectively) suggested a 39*E*-configuration (12). The $\Delta^{43,44}$-double bond was deduced as having the *E*-configuration from the coupling constant ($J_{43,44}$=15.0 Hz). From these observations the structure of C-24 to C-47 (**C**) was reasonably elucidated.

From the COSY and HOHAHA nmr spectra of **1** proton connectivities from H-4 to H_2-7 were clearly revealed. Because **1** has no sp^3 methines or trisubstituted olefins, no branched substituents are contained except hydroxyl groups. The remaining acetylenic bond at C-2/C-3, therefore, must be connected to the carboxylic acid group (C-1), which is another terminal of the molecule. The low-field ^{13}C-nmr chemical shift of C-4 (δ_C 59.6) suggested that this oxymethine carbon is adjacent to an acetylenic carbon (8–10). C-4 was therefore shown to be connected to the C-3 sp carbon, thus leading to the partial structure **A**.

Because compound **1** contains no branched substituents as described above, the partial structure **A–C** must be connected linearly to construct the whole structure of nepheliosyne A as **1**. It was revealed that this structure of **1** corresponded to a homologue of petrosolic acid (4), which contains three fewer CH_2 units than **1**. Spectral data of petrosolic acid (4) were consistent with those of nepheliosyne A [**1**].

Nepheliosyne A [**1**] is a polyacetylene carboxylic acid with the highest molecular weight among those obtained hitherto from extracts of marine sponges. Although C_{46} polyacetylene metabolites were reported previously (8,9), nepheliosyne A [**1**] is the first example of a C_{47} polyacetylene natural product. The cytotoxicity of compound **1** proved to be weak; it showed only 40% and 14% inhibition at 20 μg/cm^3 against lymphoma L-1210 and human epidermoid carcinoma KB cells in vitro, respectively.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Optical rotations were recorded on a Jasco DIP-370 digital polarimeter. Uv and ir spectra were taken on a Jasco Ubest-35 and a Jasco Report-100 infrared spectrometer, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers. Fabms were obtained on a JEOL HX-110 spectrometer.

EXTRACTION AND ISOLATION.—The sponge material *Xestospongia* sp. (SS-217) was previously characterized in detail (3). The sponge (1.4 kg) was extracted with MeOH (1 liter×2) and the MeOH extract was partitioned between EtOAc (500 ml×3) and H_2O (500 ml). A portion (1.1 g) of the EtOAc-soluble material (5.8 g) was subjected to Si gel cc (Wako gel C-300, Wako Pure Chemical, 2.3×45 cm) with MeOH-$CHCl_3$ (0:100, 4:96, 8:92, 16:92, 50:50, and 100:0, each 260 ml). The fraction eluted with 50% MeOH in $CHCl_3$ was further purified by the second Si gel column (1.0×24 cm) with MeOH-$CHCl_3$ (35:65). The fraction eluted from 12 to 60 ml was finally purified by a C_{18} Sep-Pak cartridge (Waters) with 70% MeOH to give nepheliosyne A (**1**, 0.01% wet wt).

Nepheliosyne A [**1**].—Colorless amorphous solid: $[\alpha]^{22}$D +7.0° (c=0.26, MeOH); uv (MeOH) end absorption; ir (film) ν max 3600–3000, 1700, 1580, 1300, and 1050 cm^{-1}; ^{1}H and ^{13}C nmr, see Table 1; fabms (positive, *m*-nitrobenzyl alcohol matrix) *m/z* 833 (M+Na)$^+$; fabms (negative, glycerol matrix) *m/z* 809 (M−H)$^-$; hrfabms *m/z* 833.4766 (M+Na; calcd for $C_{47}H_{70}O_{11}Na$, 833.4726).

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