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BROMINATED METABOLITES FROM THE SPONGE *APLYSINA (VERONGIA) THIONA*[1,2]

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ABSTRACT.—In addition to known bromo-compounds **5–11**, the new brominated metabolites aplysinolide [**1**], aplysinimine [**2**] and two mixed ketals, aplysinketal A [**3**] and aplysinketal B [**4**], have been isolated from the sponge *Aplysina (Verongia) thiona*. Their structures were determined by spectroscopic methods. In addition, an X-ray analysis of compound **3** was performed.

Sponges of the order Verongida have yielded a wide variety of brominated compounds with suggested 3,5-dibromotyrosine origin (1). 2,6-Dibromo-4-acetamide-4-hydroxycyclohexadienone [**5**] (2), the hydroxydienoic methyl ester **6** (2), and aeroplysinine 1 [**7**] (3) were responsible for the antimicrobial activity of the MeOH extract of *Aplysina thiona* De Laubenfels, a tropical Eastern Pacific sponge of the family Aplysinidae, order Verongida, collected at Puerto Escondido Bay in Oaxaca, Mexico. Aeroplysinine 2 [**8**] (4), 3,5-dibromo-2-hydroxy-4-methoxyphenylacetamide [**9**] (5), the hydroxydienoic acid **10** (2), and the bis-oxazolidone derivative **11** (6) were other known constituents isolated from this sponge.

The hydroxydienoic methyl ester **6** and the hydroxydienoic acid **10**, previously obtained by synthesis from the dienone **5** and the dimethoxyketal **12**, respectively (2), have now been isolated from this sponge as natural products. Besides the brominated compounds, β-sitosterol was obtained from *A. thiona*.

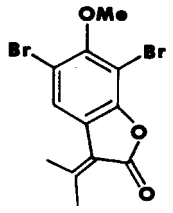
Aplysinolide [**1**] was assigned formula $C_{12}H_{10}O_3Br_2$ based on mass spectral data, which indicated the presence of two bromine atoms (molecular ion cluster at *m/z* 360, 362, 364) as well as fragments corresponding to the successive loss of Me, HCO, and 43 mass units. The ir spectrum exhibited a band for a carbonyl function (1780 cm^{-1}), while the ^1H-nmr spectrum at 400 MHz showed two broad singlets at δ 2.39 and 2.59, indicating the presence of two geminal vinyl methyl groups, a singlet (δ 3.92) for a methoxyl group, and a singlet (δ 7.65) for one aromatic proton in a pentasubstituted benzene ring.

The structure **1** was assigned to this compound based on the chemical shift of the aromatic proton at 7.65 ppm, in accord with the chemical shift (7.69 ppm) of the aromatic proton of the synthetic non-rearranged isomer **14A** of aplysinadiene [**14**] (7). The positive nOe observed between this proton and one of the vinyl methyl group at δ 2.39 is also in agreement with the proposed structure. The ^{13}C-nmr spectrum showed the vinyl methyl group signals at δ 23.69 and 25.21, the methoxyl group at 61.0, one methine at 125.75, and eight fully substituted carbon atoms at 100.74, 111.80, 117.53, 122.35, 150.75, 154.29, 161.97, and 165.23.

The mass spectrum of aplysinimine [**2**] is compatible with the formula $C_9H_7NO_2Br_2$. The molecular ion cluster at *m/z* 319, 321, 323 indicated the presence of one nitrogen atom in the molecule. In addition, it gave significant mass spectral fragments at *m/z* 304, 306, 308 $[M-NH]^+$, 292, 294, 296 $[M-HCN]^+$, and 278, 280,

[1]In memory of Dr. Gerardo Green from the Instituto de Ciencias del Mar y Limnología, UNAM, who studied the ecological aspects of sponges.

[2]Contribution No. 1002 from Instituto de Química, UNAM.



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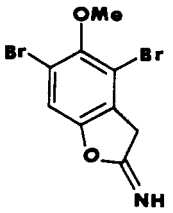
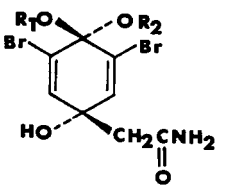
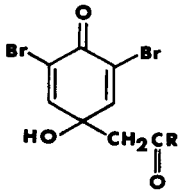


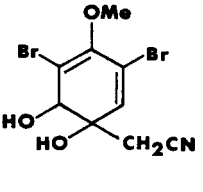
2A



3 R_1=Me, R_2=butyl
4 R_1=Me, R_2=pentyl
12 R_1=Me, R_2=Me
15 R_1=Me, R_2=Et



5 R=NH_2
6 R=OMe
10 R=OH



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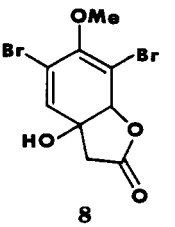

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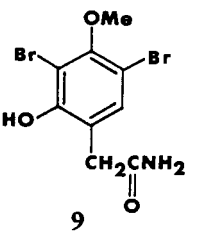

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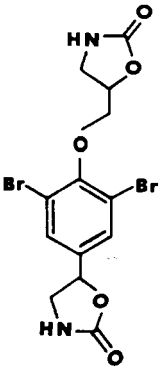

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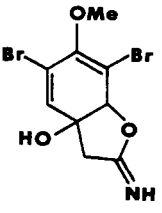

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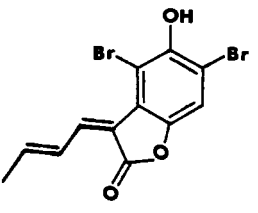

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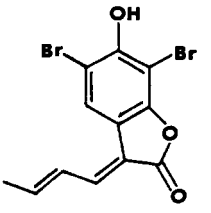

14A

282 $[M - CH_2HCN]^+$. The ir spectrum showed an absorption band at 3500 cm^{-1} typical for a nitrogen-hydrogen bond of an imine group. The ^{1}H-nmr spectrum revealed singlets assigned to an isolated methylene group in the α position to two double bonds (δ 3.67), a methoxyl group (δ 3.85), a D_2O exchangeable proton (δ 5.82) for the N-H imine, and an aromatic proton (δ 7.48). During the synthetic work on aplysinadiene [**14**], two isomeric lactones closely related to aplysinimine [**2**] were synthesized (8). Based on the chemical shift of the aromatic protons of these compounds (7.52 and 7.34), we propose that aplysinimine is very likely the non-rearranged dibromotyrosine derivative **2** rather than the rearranged one **2A**.

The third new compound isolated from *A. thiona* was a mixed ketal that we named aplysinketal A [**3**]. The mass spectrum showed no molecular ion but contained peaks corresponding to the loss of methoxy $[M - 31]^+$ and butoxy $[M - 73]^+$ groups or loss of both groups $[M - 105]^+$. The ir spectrum indicated the presence of hydroxyl (3410 cm^{-1}), amide-NH_2 (3220 cm^{-1}), and carbonyl (1660 cm^{-1}) functions. The ^{1}H-nmr spectrum at 400 MHz exhibited signals for methoxy (δ 3.07) and butoxy (δ 0.89, 1.41, 1.55, and 3.55) protons, together with two olefinic proton singlets (δ 6.77), a methylene group (δ 2.58), and three downfield D_2O-exchangeable broad singlets (δ

5.88, 6.68, and 7.20) due to hydroxyl and amide protons. The ^{13}C-nmr showed a methyl group signal at δ 14.10, a methoxyl group at 51.04, four methylene groups at 20.04, 32.34, 45.03, and 63.98, one methine signal at 142.11, and three fully substituted carbon signals at 71.92, 123.55, and 172.99. These spectral assignments have been confirmed by X-ray crystallography, which established structure **3** for aplysinketal A. A stereo-drawing of the skeleton of **3**, as determined from the X-ray study, is shown in Figure 1, and the atomic coordinates are listed in Table 1.

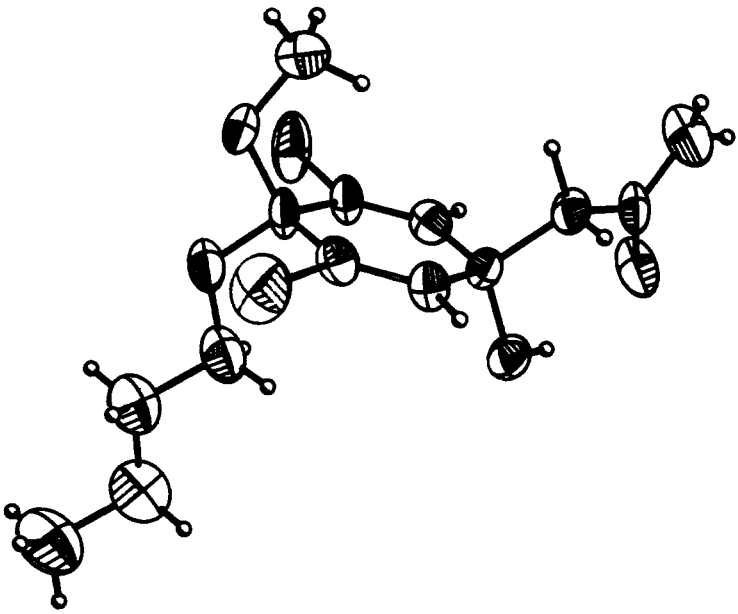

FIGURE 1. Perspective view of aplysinketal A [**3**].

TABLE 1. Atomic Coordinates (×10^4) of Aplysinketal A [**3**].

Atom	*x*	*y*	*z*
Br-1	−4288 (1)	2879(1)	2905(1)
Br-2	1960 (1)	977(1)	2584(1)
O-1	−1933 (7)	2073(4)	1905(3)
O-2	−1828 (7)	979(5)	2901(4)
O-3	390 (7)	3262(5)	4972(3)
O-4	3206 (8)	4272(5)	5006(4)
N-1	3664 (9)	5454(6)	4015(5)
C-1	−1409(10)	3498(7)	3799(5)
C-2	−2039 (9)	2828(7)	3224(5)
C-3	−1127(10)	1984(6)	2783(5)
C-4	647 (9)	2014(6)	3093(5)
C-5	1306(10)	2666(7)	3656(5)
C-6	341(10)	3503(7)	4088(5)
C-7	1077(10)	4585(6)	3943(5)
C-8	2740(10)	4744(6)	4367(5)
C-9	−803(13)	3041(7)	1560(6)
C-10	−1836(12)	593(8)	3747(6)
C-11	−3083(12)	−281(7)	3740(6)
C-12	−3139(14)	−820(8)	4568(7)
C-13	−4454(14)	−1694(9)	4542(8)

Compound **4**, which was named aplysinketal B, showed spectral properties similar to aplysinketal A [**3**]. The mass spectrum did not give a molecular ion but contained peaks due to the loss of both methoxy and pentoxy groups at $[M-31]^+$ and $[M-87]^+$. The ir and ^{1}H-nmr spectra were very similar to those of **3**, and its structure and stereochemistry are assigned as **4** by analogy with the structure of **3**.

The sponge yielded brominated metabolites similar to those obtained from other *Aplysina* (=*Verongia*) species (9). The isolation of aplysinimine [**2**] is remarkable because this compound could be considered as a possible precursor of other bromo-compounds obtained from *Aplysina* sponges. In fact, substance **2** could be derived from the hypothetical precursor imine-ether **13** proposed by Andersen and Faulkner (10) by dehydration of the tertiary alcohol to the aromatic compound. Aplysinolide [**1**] is somewhat similar to the known aeroplysinine 2 [**8**], but compound **1** has an unusual α,β-unsaturated side chain linked to the lactone ring. The presence of such an unusual side chain suggested that compound **1** could be an artifact formed by reaction of the imine **2** with Me_2CO during the purification process; however, this possibility has not yet been confirmed. To our knowledge, the only known bromo-compound containing an α,β-unsaturated side chain is the rearranged dibromotyrosine derivative aplysinadiene [**14**], recently isolated from *Aplysina aerophoba* (7). Two ketals have been obtained from *Verongia* sponges, the dimethoxy ketal **12** from *Verongia fistularis* (2) and the methoxy-ethoxy ketal **15** from *Verongia* sp. (10), both ketals showing antibacterial activity. Two new mixed ketals have now been isolated; one proved to be the methoxy-butoxy ketal **3** and the second one the methoxy-pentoxy ketal **4**. Ketals **12** and **15** have been considered as artifacts generated during the extraction process because of the ^{1}H-nmr spectrum of **15**, which showed two methoxy signals at 220 MHz, indicating the presence of two diastereoisomers (10). The ^{1}H-nmr spectrum of aplysinketal A [**3**], with a bulkier butoxy group, showed only one methoxy signal at 200 and 400 MHz, indicating the presence of only one diastereoisomer. Furthermore, the mixed ketals **3** and **4** would not be expected to be formed without simultaneous formation of the dimethoxy ketal **12**, which was never detected. According to these results, aplysinketals A [**3**] and B [**4**] are very likely to be natural products. However, we acknowledge that they might be reaction products of an unknown metabolite in the sponge (10). Furthermore, compounds containing C_4 and C_5 chains, such as aerothionine and homoaerothionine, have been isolated from *A. thiona* and other *Aplysina* species (11). It was suggested that the C_4 and C_5 chains may be derived from ornithine and lysine, respectively (12).

All natural products isolated from *A. thiona* were tested for their antibacterial activity against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Salmonella typhi*, and *Shigella sonnei*. The new bromo-compounds were inactive in all tests.

EXPERIMENTAL

SPONGE COLLECTION AND TAXONOMY.—*A. thiona* was collected by scuba diving at Puerto Escondido Bay, Oaxaca, Mexico, during November 1984. The sponge is preserved in the invertebrate collection of the Laboratory of Farmacología Marina of the Instituto de Ciencias del Mar y Limnología, UNAM. *A. thiona* is a massive encrusting sponge, cavernous, ochre-yellow in life but turning to dark purple in alcohol, and spongy in consistency. The surface is a polygonal reticulation of reddish fibers that are stratified and pithed; the fiber diameters measure 31–124 μm.

GENERAL REMARKS.—Ir spectra in $CHCl_3$ and KBr were recorded on a Perkin-Elmer IR 283-B or Nicolet IR FT-5SX. Uv spectra of MeOH solutions were recorded on a Perkin-Elmer UV 552. Nmr spectra of $CDCl_3$ or $(CD_3)_2CO$ solutions, with TMS as internal standard, were recorded on a Varian FT-80 instrument. Mass spectra were recorded on a Hewlett-Packard MS 5985 instrument. X-ray crystal structure determination was carried out on a Nicolet R3m Automatic Diffractometer. Both cc and tlc were carried out on Merck Si gel 60 F_{254} 10–40 μm. Antibiosis tests were done by the Petri-disc zonal inhibition technique at doses of 100 and 500 μg of test compound per disc.

EXTRACTION AND ISOLATION OF BROMO-COMPOUNDS.—A frozen sample of the sponge (4 kg) was cut into small pieces at room temperature, and the H_2O was separated under pressure and lyophilized (140 g). The resulting solid residue was packed on a column and eluted using hexane-EtOAc mixtures (95:5, 90:10, 80:20, 50:50). Preparative tlc [CH_2Cl_2-Me_2CO (1:1)] of fractions eluted with hexane-EtOAc (1:1) yielded **8** (5 mg). The wet and chopped sponge was blended with MeOH (Waring blender) (4 × 1000 ml) and filtered to remove solids. The combined extracts were concentrated (81 g) in vacuo; the residue was poured into H_2O (200 ml) and extracted with EtOAc (5 × 1000 ml). The organic layer was dried and the solvent removed to give a reddish-brown oil (22 g). This last residue was treated with CH_2Cl_2. The CH_2Cl_2-soluble material was chromatographed on a Si gel column (200 g) and eluted with hexane containing increasing proportions of EtOAc and then with EtOAc to afford 180–250 ml fractions. The first bromo-compound **1** isolated from the organic extract eluted with hexane-EtOAc (95:5). Fractions eluted with hexane-EtOAc (90:10) were combined and rechromatographed on a column of Si gel (30 g); compound **2** (10 mg) was obtained from fractions eluted with hexane-EtOAc (90:10); preparative tlc (CH_2Cl_2) of the final fractions eluted with hexane-EtOAc (87.5:12.5) yielded **6** (20 mg) and **10** (10 mg). Preparative tlc [CH_2Cl_2-Me_2CO (95:5)] of fractions eluted with hexane-EtOAc (80:20) and (70:30) yielded **7** (12 mg). Fractions eluted with hexane-EtOAc (50:50) yielded **9** (30 mg), **5** (40 mg), and **3** (15 mg) after column rechromatography and preparative tlc [CH_2Cl_2-Me_2CO (90:10)]. Fractions eluted with hexane-EtOAc (40:60) were purified on a column of Si gel (30 g), yielding **4** (10 mg) [hexane-EtOAc (70:30)]. Fractions eluted with 100% EtOAc were rechromatographed on a column of Si gel (40 g) to give compound **11** (20 mg) as colorless crystals from fractions eluted with hexane-EtOAc (70:30). β-Sitosterol (3.65 g) was isolated from the lyophilized solid residue after extraction with MeOH.

APLYSINOLIDE [**1**].—Yellow crystals: mp 142–144°; uv λ max (MeOH) 207 nm (ε 6717), 327 nm (ε 3732); ir ν max ($CHCl_3$) 1780 cm^{-1} (CO); ^{1}H-nmr ($CDCl_3$, 400 MHz) δ 2.39 (s, Me), 2.59 (s, Me), 3.92 (s, MeO), 7.65 (s, Ar-H); ms *m/z* [M^+, M^{+2}, M^{+4}] 360, 362, 364 (49, 100, 48%), 345, 347, 349 [M − Me]$^+$ (15, 25, 14%), 331, 333, 335 [M − 29]$^+$, (5, 9, 4%), 317, 319, 321 [M − 43]$^+$ (36, 70, 35%).

APLYSINIMINE [**2**].—Amorphous solid: mp 146°; uv λ max (MeOH) 206 nm (ε 30173), 289 nm (ε 1742); ir ν max ($CHCl_3$) 3500 cm^{-1} (N-H); ^{1}H-nmr ($CDCl_3$, 80 MHz) δ 3.67 (s, CH_2), 3.85 (s, MeO), 5.82 (s, N-H), 7.48 (s, Ar-H); ms *m/z* [M^+, M^{+2}, M^{+4}] 319, 321, 323 (30, 61, 31%), 304, 306, 308 [M − NH]$^+$ (52, 100, 46%), 292, 294, 296 [M − HCN]$^+$ (8, 15, 8%), 278, 280, 282 [M − CH_2HCN]$^+$ (2, 4, 2%).

APLYSINKETAL A [**3**].—Colorless crystals: mp 194–195°, uv λ max (MeOH) 204 nm (ε 9912); ir ν max (KBr) 3410 (OH) 3200 (amide), 1660 cm^{-1} (CO); ^{1}H-nmr [$(CD_3)_2CO$, 400 MHz] δ 0.98 (t, Me), 1.41, 1.55 (m, CH_2CH_2), 2.58 (s, CH_2CO), 3.07 (s, MeO), 3.25 (t, CH_2O), 5.88 (s, OH), 6.68 (s, H), 6.77 (s, vinylic-H), 7.20 (s, H); ms *m/z* 380, 382, 384 [M − MeO]$^+$ (8, 14, 8%), 338, 340, 342 [M − BuO]$^+$ (40, 78, 39%), 306, 308, 310 [M − MeOH − BuO]$^+$ (14, 27, 18%).

CRYSTAL DATA[3].—$C_{13}H_{19}NO_4Br_2$, MW = 413.11, monoclinic space group $P2_1/C$; cell dimensions: a = 8.235 (4), b = 12.649 (6), c = 15.896 (8) Å, β = 93.60 (4)°, V = 1652.43 (1.21) Å^3, Z = 4, D_c = 1.659 g·cm^{-3}, μ = 48.68 cm^{-1}. Intensity data of colorless crystal (0.38 × 0.38 × 0.40 mm) were collected (2155) on a Nicolet R3m four circle diffractometer using graphite monochromated MoK_α radiation (λ = 0.71069 Å); ω scan mode in the range of $3<2\theta<45°$ (a quarter of the full sphere) of which 1501 unique reflections were considered as observed [$F \geq 3\sigma(F)$], Lp corrections but not absorption correction. The structure was solved by direct methods using SHELXTL Rev. 4.1 (13) and refined by blocked-cascade least squares procedure using anisotropic thermal parameters for non-hydrogen atoms and fixed isotropic thermal parameter (U = 0.06 Å^2) for hydrogens. Hydrogens were located on difference-density maps and forced to ride on carbon atoms to converge to an R = 0.059 (Rw = 0.55).

APLYSINKETAL B [**4**].—Colorless crystals: mp 166–168°, uv λ max (MeOH) 203 nm (ε 12134); ir ν max (KBr) 3390 (OH) 3185 (amide), 1668 cm^{-1} (CO); ^{1}H-nmr [$(CD_3)_2CO$, 80 MHz] δ 0.98 (t, Me), 1.50 (m, $CH_2CH_2CH_2$), 2.57 (s, CH_2CO), 3.10 (s, MeO), 3.22 (t, CH_2O), 5.85 (s, OH), 6.65 (s, H), 6.76 (s, vinylic-H), 7.14 (s, H); ms *m/z* 394, 396, 398 [M − MeO]$^+$ (1, 1.5, 1%), 338, 340, 342 [M − PenO]$^+$ (21, 40, 20%), 306, 308, 310 [M − MeOH − PenO]$^+$ (12.5, 25, 13%).

[3]Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained on request from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.

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