Aplyzanzine A, a New Dibromotyrosine Derivative from a Verongida Sponge

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Aplyzanzine A (**1**), a novel bisdibromotyrosine derivative, has been isolated from the Indo-Pacific sponge *Aplysina* sp. Its structure was elucidated mainly on the basis of 1D and 2D NMR and MS spectroscopic data.

In connection with our long-standing interest in the chemistry of marine sponges, we have investigated Indo-Pacific sponges that were collected near the coast of Zanzibar. From one of the sponges, an *Aplysina* sp., we have isolated a new dibromotyrosine derivative (**1**) designated aplyzanzine A.

The genus *Aplysina* belonging to the Verongida sponges (order Verongida, family Aplysinidae) is well known for containing dibromotyrosine metabolites.[1−8] The freshly collected sponge was frozen on site and kept frozen until needed. Aplyzanzine A (**1**) was obtained from the ethyl acetate extract, after chromatography on Sephadex LH-20, as a pale orange oil.

Aplyzanzine A (**1**),[10] obtained as pale orange oil, analyzed for $C_{25}H_{33}Br_4N_3O_3$, from the CIMS and NMR data; the CIMS showed a cluster of peaks at *m/z* 740/742/744/746/748, in a ratio of 1:4:6:4:1, characteristic for a tetrabrominated compound. The EIMS showed a similar cluster of peaks at *m/z* 739/741/743/745/747, while the main peak (i.e., *m/z* 743) had an intensity of only 4%. The IR spectrum revealed bands at 1036, 1678, 3222, and 2968 cm^{-1}, suggesting an ethereal C−O, an amide, and an aryl CH group, respectively. The presence of an amide group was confirmed by the δ_C 170.8 s and δ_H 8.67 br t resonances. Furthermore, the multiplicity of the NH signal suggested a CH_2NHCO group. Additional functionalities were two NMe_2 groups (δ_H 2.26 and 2.67 s, 6H each), one aromatic methoxy group (δ_C 60.4 q, δ_H 3.74 s, 3H), and two 1,3,4,5-tetrasubstituted aromatic rings (Table 1), accounting, together with the amide, for the nine degrees of unsaturation of **1**. From the multiplicity (DEPT experiment) and δ_C values it was clear that each ring is tetrasubstituted, bearing an ethereal oxygen (δ_C 152.3 and 150.9 s, for C-4 and 15, respectively). The chemical shifts of the other ring carbon atoms and especially the three- and two-bond CH correlations, observed in a HMBC experiment (Table 1), determined the alkyldibromophenolic structure of the two rings. A 1D INAPT experiment[11] assisted with the distinction between the close aromatic carbon chemical shifts. All chemical shifts of the aromatic rings are in good agreement with literature values.[1] Three additional spin systems were established by a COSY experiment (Table 1), that is, one CH_2CH, one CH_2CH_2, and one $OCH_2CH_2CH_2N$ system (C-7, 8; C-10, 11; and C-18−20, respectively). All the above functional groups accounted for all the molecule's atoms and the nine degrees of unsaturation. Assembly of the various moieties of aplyzanzine A (**1**) was principally achieved from the HMBC CH correlations (Table 1) and partially also confirmed by NOE measurements (Table 1).

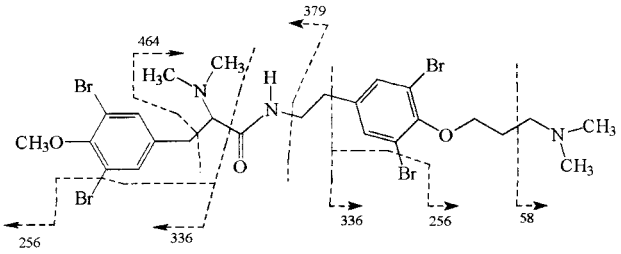
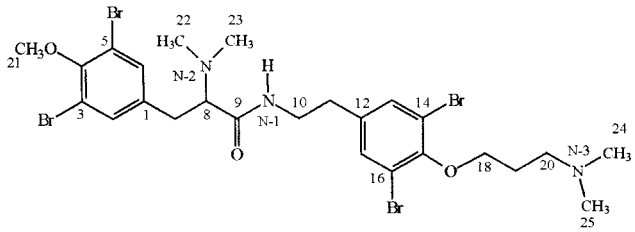


Figure 1. EIMS fragmentation of **1**.

^2J and ^3J CH correlations from 2H-7, H-8, and Me's 22, 23 to C-1, 2 (and 6), 8, and 9; and between 2H-11, H-13 (and 17), and 2H-18 and the second aromatic ring C atoms; and, similarly, between 2H-10 and 11, 2H-18, 19, 20, and Me's 24, 25 and their adjacent C atoms established the full structure of **1**. The suggested structure was further confirmed by the NOE measurements (Table 1) and several MS fragments shown in Figure 1. All fragmentations agree well with known cleavages α to heteroatoms.



1

The structure of aplyzanzine A (**1**) points clearly to a bisdibromotyrosine derivative. Parts of **1** are well known from other Verongida sponge metabolites (e.g., moloka'inamine).[2] Closest in structure is purealidin C, reported by Kobayashi, from *Psammaplysilla purea*.[1] Both **1** and the latter compound have in common the dibromotyrosine−dibromotyramine skeleton; however they differ in substitution. To the best of our knowledge, the structure of a *N,N*-dimethyltyrosine is without precedent as a marine natural product.

Several recently reported additional dibromotyrosine derivatives are ceretinamine,[3] ceratinamides A and B,[4] 7-hydroxyceratinamine,[5] and other metabolites reported by Fattorusso.[6]

The biogenesis of **1** can best be described by amidation of the *O,N,N*-trimethyldibromotyrosine with the appropriate *O*-3-dimethylaminopropyldibromotyrosine.

Experimental Section

General Experimental Procedures. IR spectra were obtained with a Bruker FTIR Vector 22 spectrophotometer. ^1H NMR, ^{13}C NMR, and 2D NMR spectra were recorded on a

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Table 1. ^{1}H and ^{13}C NMR Data for **1**[a]

no.	δ_C (mult)	δ_H[a] (mult, *J* in Hz)	COSY	HMBC (H to C) correlations	INAPT[11] (H to C)	1D NOE
1	137.66 s					
2, 6	133.21 d	7.31 (s, 2H)		C2/C6, C3/C5, C4, C7	C2/C6, C3/C5, C4	H7a, H7b
3, 5	117.58 s					
4	152.34 s					
7a	31.57 t	2.71 (dd, 1H, 4.5, 13.8)	H7b, H8	C2/C6, C8, C9	C1, C2/C6,	H22/H23
7b		2.94 (dd, 1H, 8.8, 13.5)	H7a, H8	C2/C6, C8, C9	C22/C23	
8	69.84 d	3.14 (dd, 1H,4.5, 8.8)	H7a, H7b	C1, C9, C22/C23		
9	170.82 s					
10	39.81 t	3.29 (dt, 2H, 2.8, 7.0)	H11a, H11b	C9, C11, C12		
11a	34.20 t	2.54 (m, 1H)	H10	C10, C13/C17		
11b		2.57 (m, 1H)		C10, C13/C17		
12	137.94 s					
13, 17	132.78 d	7.23 (s, 2H)		C11, C13/C17, C14/C16, C15	C13/C17, C14/C16, C15	H11a, H11b
14, 16	117.74 s					
15	150.87 s					
18	69.71 t	3.96 (t, 2H,5.5)	H19	C15, C19, C20		
19	25.38 t	2.18 (m, 2H)	H18, H20	C18, C20		
20	55.41 t	3.16 (m, 2H)	H19	C18, C19, C24/C25		
21	60.39 q	3.74 (s, 3H)		C3/C5, C4		
22, 23	41.51 q	2.26 (s, 6H)		C8, C22/C23	C8	H7a, H7b
24, 25	42.92 q	2.67 (s, 6H)		C20, C24/C25	C20	H20
N-1[a]		8.67 (br t, 1H)	H10			

[a] Data recorded in $CDCl_3$+CD_3OD (10:1) at 500 MHz (^{1}H) and 125 MHz (^{13}C) at 27 °C. [b] CH assignments are based on the HMQC spectrum. [c]Obtained from a spectrum taken in $CDCl_3$ only.

Bruker ARX-500 spectrometer with TMS as internal standard. MS data were recorded on a Fisons Autospec-Q spectrometer. $[\alpha]_D$ spectra were obtained with a Jasco P-1010 polarimeter.

Animal Material. *Aplysina* sp. (order Verongida, family Aplysinidae) was collected by diving (−4 m) in a coral reef at Changuu, Zanzibar, on March 14th, 1999. The sponge has a globular to massive morphology with firm, yet compressible texture. Its surface bears small conules. The sponge's color in life was yellow, which turned deep purple upon collection and preservation. It was covered and encrusted by several other species of sponges and therefore collected as an assemblage of species, in which the major component was the *Aplysina* sp. The sponge mesohyl contains a relatively large number of small broken mollusk shells as well as foreign sponge spicules. Fibers have a thick pith component. No fibers with foreign detritus incorporated were noticed, and the choanosomal skeleton is not organized into one plane. The species does not belong to the genus *Verongula*, since it is devoid of the typical honeycromb-like appearance to the surface. All these characteristics assign the species to the genus *Aplysina*. A voucher sample of *Aplysina* sp. (#M0032) is deposited in the Department of Zoology, Tel-Aviv University.

Extraction and Isolation. The fresh sponge was immediately frozen and kept at −25° C until investigated chemically. The freeze-dried sponge (70 g) was extracted at room temperature in EtOAc (3 × 0.3 L) and in EtOAc−MeOH, (1:1, 3 × 0.3 L). The EtOAc extract gave 0.4 g of a brown gum after evaporation, which was subsequently partitioned between aqueous methanol and CCl_4, $CHCl_3$, and *n*-butanol.[9] The $CHCl_3$ phase was then subjected to chromatography on Sephadex LH-20 (eluting with CH_2Cl_2−MeOH, 1:1) to afford aplyzanzine A (**1**, 15 mg, 0.02%).

Aplyzanzine A (1): pale orange oil; $[\alpha]_D$ 0° (*c* 5.0, $CHCl_3$); IR ($CHCl_3$) ν_{max} 1036, 1211, 1221, 1259, 1473, 1545, 1678, 2450, 2969, 3023, 3222 cm^{-1}; ^{1}H NMR and ^{13}C data see Table 1; CIMS *m/z* (relative intensity) 740(22)/742(70)/744(100)/746(64)/748(20) [MH^+], 696(1)/698(5)/700(7)/702(4)/704(1) [$MH^+ - NMe_2$], 662(4)/664(10)/666(8)/668(2) [$MH^+ - Br$], 582(3)/584-(6)/586(3) [$MH^+ - Br_2$], 462(3)/464(12)/466(12)/468(3), 334(15)/336(26)/338(15) [$C_{11}H_{14}Br_2NO^+$], 309(12)/311(21)/313(8), 118-(22); EIMS *m/z* (relative intensity) 696(1)/698(3)/700(7)/702(3)/704(1) [$MH^+ - NMe_2$], 462(4)/464 (9)/466(7) [$C_{17}H_{26}Br_2N_3O_2^+$], 377(5)/379(6)/381(3) [$C_{12}H_{15}Br_2N_2O_2^+$], 334(52)/336(99)/338(50) [$C_{11}H_{14}Br_2NO^+$], 256(20)/258(15) [$C_{11}H_{14}BrNO^+$], 84(17), 58(100) [$CH_2NMe_2^+$].

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