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Marine Products from Bay of Bengal[1]: Constituents of the Sponge Psammaplysilla purpurea

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Abstract : New bromotyrosine derived secondary metabolites have been isolated from the marine sponge Psammaplysilla purpurea, and the structures assigned on the basis of spectroscopic analyses of their acetates **1a'** and **1b'**.

Although several marine organisms have been found to be potential sources of bioactive compounds, sponges received extraordinary attention for the discovery of a variety of structurally novel molecules. Among them, the genera, viz. Aplysina, Ianthella, and Psammaplysilla, belonging to the family Verongidae are known[2-7] to elaborate a broad range of biogenetically related bromotyrosine derived secondary metabolites which are potent antibiotics. Because of our current interest in the chemistry of marine natural products, we undertook the investigation of the sponge Psammaplysilla purpurea collected in the coastal regions of the Bay of Bengal. Considerable work has already been carried out on this organism obtained mostly from the Okinawan coast. The compounds encountered so far include aplysamines[8], bastadin-14[4], (3-bromo-4-hydroxyphenyl)-acetonitrile, 3-bromo-4-hydroxybenzaldehyde[9], 14-debromoprearaplysillin[10], psammaplins A-D[11], psammaplysins A-G[12,13] and purpuramines A-I[14] besides a number of sterols[15]. The present paper deals with the isolation and structure determination of bromotyrosine metabolites **1a-1d.**

The methanol and chloroform-methanol extracts of the sponge on solvent evaporation afforded reddish-yellow residues. These were subjected to a modified Kupchan fractionation procedure[16] and the extracts combined on the basis of TLC analysis.Repeated column chromatography of the combined chloroform extract through

silica gel furnished a light brown substance. Further purification by sephadex LH-20 column chromatography yielded a white amorphous material that appeared homogeneous by TLC. Spectral studies (^{1}H NMR, FAB MS,IR), however, suggested it to be a mixture of closely related compounds containing OH/NH groups. It was therefore acetylated to yield the crude diacetate (s at δ 2.24 and 2.00) which on attempted purification by silica gel chromatography was converted to the monoacetate (s at around δ 2.00). The individual monoacetates could only be separated by reverse-phase HPLC to obtain **1a'-d'**.

The isomeric nature of **1b'** and **1c'** was revealed by the FAB mass spectra having $[M+H]^+$ ion peaks at m/z 662, 664, 666 and 668 (isotopic cluster) which also suggested the presence of three bromine atoms in these molecules. On the other hand, the isotopic distribution pattern of $[M+H]^+$ ions in the mass spectra of **1a'** and **1d'** (vide Experimental) were in good agreement with the presence of two and four bromine atoms respectively.

The ^{1}H NMR spectra (Table 1) of all the four compounds contained exchangeable signals at around δ 10.0(br s), 6.8(m) and 6.5(m), thereby proving the presence of three OH/NH groups. The spectra were virtually identical in the aliphatic region also. Thus, there were signals for one Ar-OMe group (3Hs, δ 3.8),besides an Ar-CH_2(2Ht, 2.7), an ArOCH_2-(2Ht, δ 4.0-4.2) and two >N$COCH_2$- (4Hm, δ 3.3-3.7) groups.

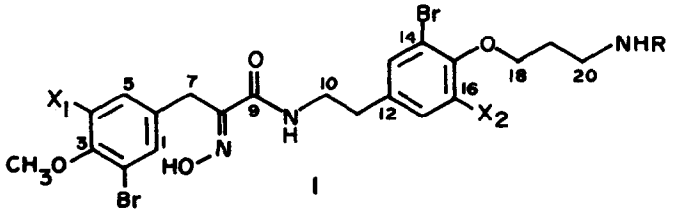
Table 1. ^{1}H NMR Data* of Compounds **1a'-d'**

	1a'	**1b'**	**1c'**	**1d'**
1-H	7.56 d, J=2	7.50 s	7.54 d, J=2	7.52 s
4-H	6.80 d, J=8	-	6.76 d, J=8	-
5-H	7.2-7.5 m	7.50 s	7.3 dd, J=6,2	7.52 s
7-H_2	3.85 s	3.84 s	3.86 brs	3.86 s
10-H_2, 20-H_2	3.4-3.7 m	3.4-3.6 m	3.3-3.7 m	3.4-3.6 m
11-H_2	2.76 t, J=7	2.74 t, J=6	2.70 t, J=7	2.74 t, J=7
13-H	7.2-7.5 m	7.38 d, J=2	7.28 s	7.34 s
16-H	6.80 d, J=8	6.76 d, J=8	-	-
17-H	7.02 dd, J=8,2	7.02 dd, J=8,2	7.28 s	7.34 s
18-H_2	4.20 t, J=6	4.14 t, J=6	4.04 t, J=6	4.08 t, J=6
19-H_2	1.9-2.2 m	1.9-2.2 m	1.9-2.2 m	1.9-2.2 m
2x-NH	6.60 brt, J=6	6.64 t, J=6	6.80 m	6.76 t, J=6
	6.32 m	6.44 m	6.36 m	6.18 m
=NOH	9.80 brs	10.64 brs	10.40 brs	9.92 brs
-$OCOCH_3$	1.94 s	1.94 s	1.98 s	2.00 s
-OCH_3	3.86 s	3.86 s	3.80 s	3.84 s

* Measured in $CDCl_3$; chemical shift in ppm, J in Hz.

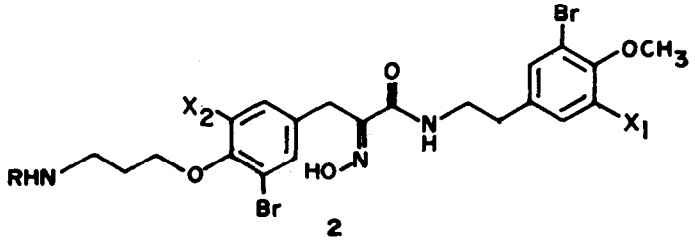
Furthermore, a two-proton singlet near δ 3.9 was consistent with the presence of an Ar-CH_2-C(=X)- moiety. There was however significant difference in the signals for the aromatic protons which established the substitution patterns of the aromatic rings and clearly distinguished the individual compounds.

The above evidences would be compatible with any one of the structures **1**, **2** and **3**; similar compounds have been encountered earlier in this family. However, structure **3** could be eliminated on the basis of decoupling experiments in the ^{1}H NMR spectrum of the original mixture where all the methylene protons showed distinct signals against the overlapping signals of the two amidomethylene groups in the acetates. The spectrum contained a 3H multiplet around δ 8 ppm for NH protons which changed to a 1H t for the slow exchanging amide proton on addition of D_2O. Warming the solution to complete the exchange process changed the δ 3.4 multiplet to a broad triplet, identifying it to be the amidomethylene signal. This multiplet was however unaffected on irradiation of the peak (δ 2.1 m) for the central methylene of the propanolamine unit which transformed only the δ 3.1 and 4.1 multiplets. Thus, the propanolamine moiety could not have been involved in amide formation.



1

1a. R = X_1 = X_2 = H	**1a'**. R = Ac, X_1 = X_2 = H
b. R = X_2 = H, X_1 = Br	**b'**. R = Ac, X_1 = Br, X_2 = H
c. R = X_1 = H, X_2 = Br	**c'**. R = Ac, X_1 = H, X_2 = Br
d. R = H, X_1 = X_2 = Br	**d'**. R = Ac, X_1 = X_2 = Br



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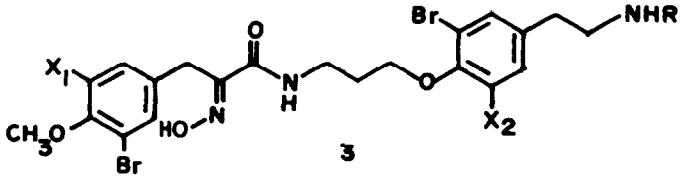
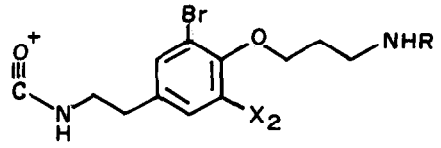
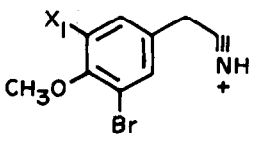


3

Evidence in favour of structure **1** came from a study of the mass fragmentation of **1a'** - **1d'**. All the FAB mass spectra showed prominent fragment peak clusters assignable to the acylium ion (**i**; R=$COCH_3$) and weaker peaks for the protonated nitrile (**ii**) conceivably arising out of the cleavage of the C_8-C_9 bond. Peaks derived by the loss of 42 mass units ($COCH_2$) from the acylium ion (**i**; R=H) were also observed. The formation of these ion peaks cannot be explained on the basis of structure **2**.



i



ii

^{13}C NMR spectra of **1b'** and **1c'** are in general agreement with the proposed structure. Assignments (vide Experimental) are mainly based on chemical shift and multiplicity considerations, taking into account the fact that substitution by two bulky groups at the o-positions forces an aromatic alkoxy group out of plane, deshielding the alkyl group as also the o and p carbons significantly[17]. The geometry of the oxime grouping is suggested to be E by comparing the chemical shift of C_7 with the reported values for geometrical isomers of 2-butanone oxime[18]. The stereochemistry would thus be identical with those of analogous sponge metabolites.

Since the completion of this work, aplysamine-3 (Purpuramine H) and aplysamine-4, assigned structures **1c** and **1d** respectively have been reported[8,14] from the same source. The published ^{13}C NMR values for purpuramine H[14] is in reasonable agreement with that of **1c'**, taking into account the effects of N-acylation and solvent difference.

EXPERIMENTAL :

General Procedure : Infrared spectra were recorded as KBr disks on a JASCO IR-700 spectrophotometer. ^1H and ^{13}C NMR spectra were measured in a JEOL FX-100, Bruker AM-300L or a Bruker WH-270 spectrometer; chemical shifts are reported in ppm relative to TMS. Mass spectra were run on a JEOL AX-500 instrument. Column chromatography was performed on Si-gel (60-120 mesh) or on sephadex LH-20. TLC analyses were carried out using Si-gel plates. HPLC was done on a Waters-440 instrument. Solvents used were either spectral grade or distilled.

Collection, Extraction and Purification : The sponge was collected 8km from Gopalpur Light House (District Ganjam, Orissa) in the coastal area of the Bay of Bengal in the month of December,1991. The sponge (800 wet wt) was homogenised in MeOH, filtered, extracted (3 times each) with MeOH followed by $CHCl_3$:MeOH (2:1) and filtered again.The filtrates were evaporated in a rotary evaporator to a reddish

yellow mass to which aq.methanol was added and successively extracted with pet.ether, CCl_4, $CHCl_3$ and n-BuOH under a modified Kupchan procedure. Each extract was concentrated under vaccum. TLC analyses were carried out using solvent systems of varying polarity ($CHCl_3$:MeOH=19:1,9:1 or 17:3). Extracts having identical TLC pattern were mixed together. The total $CHCl_3$ extractive (4 g) was chromatographed several times through silica-gel columns to obtain a brown substance (1.75 g; eluent - $CHCl_3$: MeOH = 19:1) which was further purified over sephadex LH-20 to afford a white amorphous solid (Rf 0.3 in $CHCl_3$:MeOH=9:1), IR ν_{max}:3844, 3750, 1661, 1530, 1495, 1458, 1254, 1209, 1153, 990cm^{-1}.

Acetylation of the solid : A part (270 mg) of the white solid was dissolved in 0.5 ml of pyridine to which 2 ml of acetic anhydride was added. The mixture was heated on a steam-bath for 2 hr. and then left at room temperature overnight. Usual work-up yielded the crude diacetate (340 mg; Rf 0.5 and 0.6 in $CHCl_3$:MeOH=9:1); IR ν_{max} 3304, 2934, 1780, 1664, 1531, 1495, 1455, 1398, 1256, 1191, 1053, 999, 956 cm^{-1}.

Column chromatography of the crude diacetate through silica gel afforded a white compound (Rf 0.5 in $CHCl_3$:MeOH 9:1). Four individual acetates were separated by reverse-phase HPLC of the sample using a μBondapak C_{18} column and methanol-water (7:3) solvent at the flow rate of 1.2 ml/min. The peaks were identified by UV detector (280 nm) at the sensitivity of 0.5 AUFS. The compounds **1a'**, **1c'**, **1b'** and **1d'** were eluted from the column after 6, 7.5, 10.5 and 13 minutes at the ratio of 2:16:14:3 respectively.

1a' : FABMS-588(4), 586(7), 584(4)$[M+H]^+$, 572(5), 570(8), 568(5), 492(4), 490(4), 343(11), 341(10), 301(9), 299(9); 228(19), 226(21), 201(47), 199(57%).

1c' : ^{13}CNMR - 171.3(-NH$\underline{C}$OCH$_3$), 162.8(C-9), 153.1(C-3), 152.5(C-8), 151.2(C-15), 135.3(C-12), 133.5(C-1,C-5), 132.6(C-6), 129.2(C-17), 117.8(C-2,C-4), 113.0(C-16), 111.9(C-14), 68.4(C-18), 60.6(-OCH$_3$), 39.9(C-10), 38.6(C-20), 34.0(C-11), 28.1(C-19), 27.9(C-7), 23.1(-CO$\underline{C}$H$_3$); FABMS - 668(2), 666(4), 664(4), 662(2), $[M+H]^+$, 652(2), 650(4), 648(4), 646(2), 586(2), 584(1), 572(3), 570(4), 568(3), 492(1), 490(1), 343(8), 341(7), 308(2), 306(2), 304(3), 281(8), 279(13), 277(7%).

1b' : ^{13}CNMR - 170.6(-NH$\underline{C}$OCH$_3$), 163.2(C-9), 152.6(C-8), 151.5(C-15), 137.9(C-12), 134.1(C-1), 133.0(C-13,C-17), 130.2(C-6), 129.5(C-5), 118.2(C-14,C-16), 113.3(C-2), 112.0(C-4), 71.9(C-18), 56.3(-OCH$_3$), 40.2(C-10), 37.7(C-20), 34.3(C-11), 29.6(C-19), 28.0(C-7), 23.3(-CO$\underline{C}$H$_3$), FABMS - 668(4), 666(10), 664(10), 662(4)$[M+H]^+$, 652(4), 650(9), 648(9), 646(4), 588(2), 586(4), 584(2), 572(3), 570(5), 568(3), 423(12), 421(14), 419(11), 381(2), 379(4), 377(2), 343(13), 341(13), 228(12), 226(14), 201(29), 199(36%).

1d' : FABMS - 748(3), 746(10), 744(14), 742(10), 740(4)$[M+H]^+$, 732(2), 730(4), 728(6), 726(5), 724(4), 423(7), 421(10), 419(7), 381(7), 379(11), 377(7), 343(6), 341(5), 308(8), 306(15), 304(10), 281(20), 279(40), 277(25%).

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