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GRAMINE-DERIVED BROMO-ALKALOIDS FROM THE MARINE BRYOZOAN ZOOBOTRYON VERTICILLATUM

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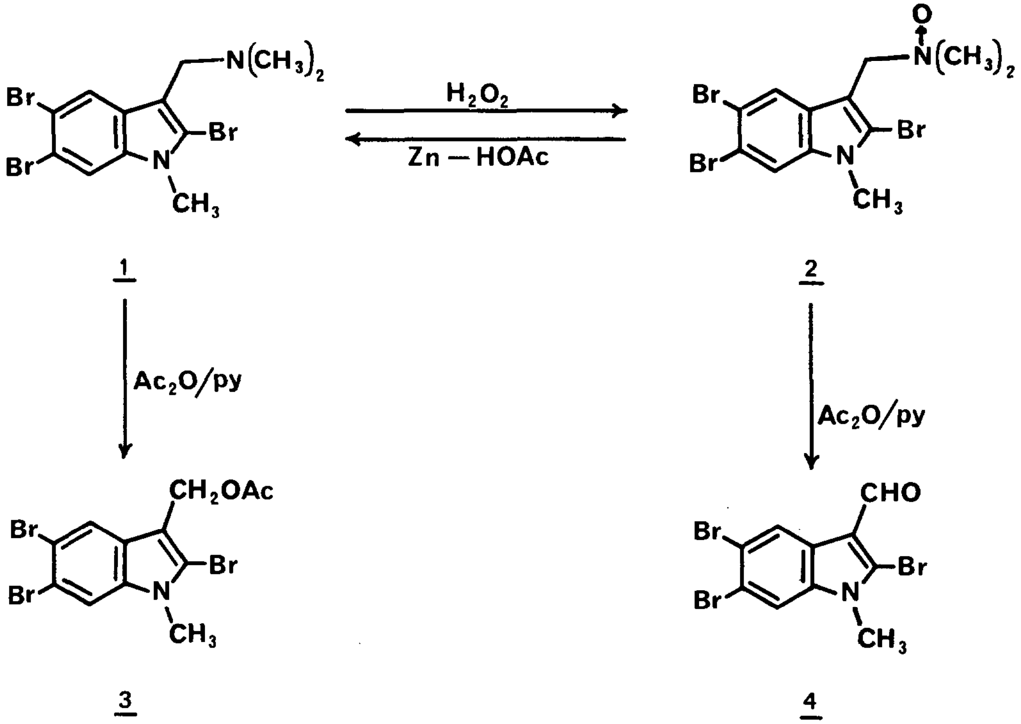
Summary--Two new natural products, 2,5,6-tribromo-N-methylgramine (1) and its corresponding side-chain N-oxide, 2, have been isolated from the subtropical marine bryozoan Zoobotryon verticillatum. The structures of these compounds were illustrated by spectral analyses and by chemical synthesis.

As part of a program to isolate new biologically-active compounds from marine organisms, we have turned our attention to those organisms which are found in abundance in predator-rich, competitive marine habitats. The bryozoan Zoobotryon verticillatum (Delle Chiaja, 1828) (Ectoprocta, Vesiculariidae), a member of the so-called "moss animal" group, is widely-distributed in tropical and subtropical regions, and it is most frequently found fouling wood surfaces in bays and harbors. We wish to report here, that Z. verticillatum, from the San Diego region,[2] contains two bromo-alkaloids, 2,5,6-tribromo-N-methylgramine (1) and its related side-chain N-oxide, 2. While alkaloid N-oxides are commonly isolated along with the free alkaloids from terrestrial sources, this appears to be the first example of an alkaloid N-oxide from marine sources. Several alkaloids bearing the physostigmine and indole nuclei have recently been reported from colder-water representatives of this invertebrate division.[3]

Methanol extraction of freeze-dried Z. verticillatum (400 gm), followed by removal of lipids by solvent partitioning and removal of solvent in vacuo, led to an ethyl acetate-soluble extract (2.6 gm) which contained UV-absorbing, polar metabolites by TLC analysis. Silica gel column chromatography of the extract, followed by medium pressure silica chromatography eluting with various proportions of methanol in chloroform, yielded 1 and 2, (30 mg and 45.5 mg) as 1.15 and 1.73% of the extract, respectively. Alkaloid 1, mp 112-113° (from MeOH), was eluted with 5% methanol in chloroform and alkaloid N-oxide 2, mp 116-120° dec, (aq. MeOH) was eluted with 20% methanol in chloroform.

Zoobotryon alkaloid 1, was assigned a molecular formula of $C_{12}H_{13}Br_3N_2$ by interpretation of its ^{13}C NMR and high-resolution mass spectral features. A complex quartet of parent ions was observed at 421.8628 (calc. 421.8623) reflecting a $^{79}Br_3$ composition, and bands at 423.8608 (calc. 423.8602), 425.8587 (calc. 425.8565) and 427.8567 (calc. 427.8565) illustrated compositions of $^{79}Br_2{}^{81}Br$, $^{79}Br{}^{81}Br_2$ and $^{81}Br_3$, respectively. In addition, an intense Br_3-containing base

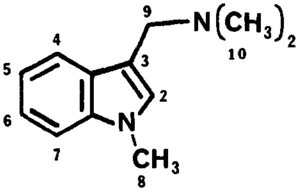


fragment at m/z - 377.8131 (calc. 377.8128) reflected an efficient loss of $-N(CH_3)_2$ from the molecule. Infrared absorptions ($CHCl_3$) were observed at 1455, 1420, 1340, 1305, 860 and 835 cm^{-1}, and ultraviolet absorptions (MeOH) were recorded for this metabolite at 232 (ε 46,800), 298 (ε 9,900) and 308 nm (ε 9,100).

Interpretation of these latter spectral features, and in particular the correlation of the ^{13}C NMR features of **1** (Table 1) with those of gramine,[4] ultimately allowed the formulation of the structure **1** for this compound. The ^{1}H NMR spectrum (Table 1) consisted of 5 singlets assigned to two 1 H aromatic protons, the C-9 methylene group, the indole N-methyl group and the equivalent methyl substituents of the dimethylamino functionality. That the aromatic protons were singlet resonances indicated their positions at C-4 and C-7, respectively.[5]

The more polar metabolite isolated, **2**, showed almost identical high resolution mass spectral features as **1**. The largest mass fragment ion observed, now recognized as $M^{+}-O$, was indicative of a formula of $C_{12}H_{13}Br_3N_2$, isomeric with that of **1**. UV and IR data for **2**[6] were also in close correlation with alkaloid **1**. The ^{1}H and ^{13}C features for **2** also showed strong similarities. However, both the proton and carbon resonances associated with the side-chain in **2** (C-9 and C-10) showed strong deshieldings indicative of a cationic nitrogen. While **2** was initially considered to be the quaternary ammonium salt of **1**, treatment of **2** with numerous bases failed to generate **1**. The assignment as the N-oxide derivative was then considered and confirmed by interconversion of **1** and **2**. Oxidation of **1** (30% H_2O_2/MeOH) yielded **2** in quantitative yield, and reduction of **2** (Zn-HOAc) yielded the free amine **1**.

Table 1. ^{1}H and ^{13}C NMR features of alkaloids 1 and 2.



C#	^{1}H (360 MHz, CDCl$_3$) 1	^{1}H (360 MHz, CDCl$_3$) 2	^{13}C (50 MHz, CDCl$_3$) 1	^{13}C (50 MHz, CDCl$_3$) 2
2			106.6 (s)	105.5 (s)
3			111.6 (s)	120.9 (s)
3a			128.0 (s)	128.3 (s)
4	7.94 (1H, s)	8.10 (1H, s)	123.3 (d)	124.0 (d)
5			117.2 (s)	118.4 (s)
6			115.0 (s)	117.1 (s)
7	7.58 (1H, s)	7.63 (1H, s)	114.0 (d)	114.5 (d)
7a			136.2 (s)	136.5 (s)
8	3.71 (3H, s)	3.79 (3H, s)	31.7 (q)	32.4 (q)
9	3.51 (2H, s)	4.55 (2H, s)	54.3 (t)	67.3 (t)
10	2.25 (6H, s)	3.21 (6H, s)	45.4 (2xq)	58.3 (2xq)

To confirm the structures of 1 and 2, both compounds were synthesized from the commercially available gramine (3-dimethylaminomethyl indole). Indole N-methylation of gramine (NaH/DMSO-DMF and CH_3I) proceeded in 75% yield. Bromination of N-methylgramine, as described by DaSettino et al.,[7] proceeded in low yield (∿15%), but generated the 2,5,6-tribromo derivative (1) as the major product. Synthetic 1, and the natural product, were found to be identical spectrally, and no depression was observed on mixed melting point analysis.

As part of the early chemical analyses of 1 and 2, each were treated with acetic anhydride in pyridine to determine if acylatable -OH or -NH functionalities were present. Unexpected and unusual reactivities were observed. Acetylation of 1 resulted in displacement of the side-chain dimethylamino group to yield the primary acetate 3.[8] Similarly, acetylation of 2 yielded the corresponding 3-carboxaldehyde derivative 4.[9] This latter reaction may possibly be explained by the intermediacy of the corresponding N to O rearrangement product which could then eliminate dimethylamine.

Although 1 had been earlier synthesized,[7] this is the first report of this compound, and its N-oxide 2, as natural products. Initial biotests indicated 1 to inhibit cell division of the fertilized sea urchin egg ($ED_{50} \cong 16$ μg/ml). Complete pharmacological evaluations of 1 and 2 are in progress.

Acknowledgements

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References and Notes

1. Permanent address: Fermentation Research Laboratories, Sankyo Co. Ltd., 2-58, 1-Chome Hiromachi, Singawa-ku, Tokyo 104, Japan.
2. *Z. verticillatum* was found fouling pier pilings in the southern part of San Diego Harbor (September, 1981). San Diego represents the northernmost distribution of this tropical cosmopolitan species. We thank Mr. James Lance for the collection and identification of this organism.
3. J.S. Carlé and C. Christophersen, *J. Am. Chem. Soc.*, **101**(14), 4012 (1979); *J. Org. Chem.*, **45**, 1586 (1980); *J. Org. Chem.*, **46**, 3440 (1981); P. Wulff, J.C. Carlé and C. Christophersen, *J. Chem. Soc.*, PT1, **1981**, 2895; *Comp. Biochem. Physiol.*, **71B**, 523 (1982); *Comp. Biochem. Physiol.*, **71B**, 525 (1982).
4. M. Shamma and D.M. Hindenlang, *Carbon-13 NMR Shift Assignments of Amines and Alkaloids*, Plenum Press Inc., 1979, p. 205.
5. Substitution by bromine at C-5 and C-6 in the indole nucleus has been reported in several marine natural produts, see: G.E. Van Lear, G.O. Morton, and W. Fulmor, *Tetrahedron Lett.* 299 (1973); G.T Carter, K.L. Rinehart, Jr., L.H. Li, S.L. Kuentzel and J.L. Connors, *Tetrahedron Lett.*, 4479 (1978).
6. Additional spectral features for **2** are: IR (nujol): 3150, 1525, 1455, 1340, 1310, 1290, 1250, 1090, 1030, 960, 890, 870, 860, 825, 815 cm^{-1}; UV (MeOH): 230 (ε 38,800), 294 (ε 11,800), 306 (ε 10,700) nm.
7. A. DaSettino, V. Santerini, G. Primofioro and C. Veneziano, *Chim. Ind.* (Milan) **59**, 454 (1977), CA**87**:201 244C (1977).
8. For compound **3**: mp = 133-136°; IR ($CHCl_3$): 1730, 1460 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz): δ 7.89 (1 H, s), 7.57 (1 H, s), 5.22 (2 H, s), 3.72 (3 H, s), 2.07 (3 H, s).
9. For compound **4**: mp = 225-226°; IR ($CHCl_3$): 1650, 1485, 1450, 885 cm^{-1}; ^1H NMR ($CDCl_3$, 360 MHz): δ 9.96 (1 H, s), 8.59 (1 H, s), 7.63 (1 H, s), 3.81 (3 H, s), UV (MeOH): 222 (ε 24,000), 255 (ε 15,500), 277 (ε 8,300), and 309 nm (ε 8,300); HRMS: M^+ m/z = 377.8119, 379.8101, 381.8081, 383.8067 for the expected bromine isotope composition for $C_{10}H_6NOBr_3$.

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