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J. Nat. Prod., **1993**, 56 (4), 633-636• DOI:
10.1021/np50094a031 • Publication Date (Web): 01 July 2004

Journal of Natural Products
Vol. 56, No. 4, pp. 633-636, April 1993

A NEW BROMINATED INDOLE-3-CARBALDEHYDE FROM THE MARINE BRYOZOAN *ZOOBOTRYON VERTICILLATUM*

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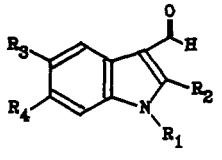
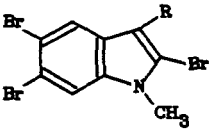
ABSTRACT.—In addition to the metabolites previously reported, a new natural product 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**] has been isolated from the marine bryozoan *Zoobotryon verticillatum* collected in two different locations of the Spanish coast. Its structure was elucidated by interpretations of spectral data and chemical interconversions. Compound **1** delays the metamorphosis in fertilized sea urchin eggs at low concentrations.

Although marine bryozoans ("moss animals") emerged as a source of new natural compounds with promising biological activities, the studies have been limited by the inherent difficulties in collecting sufficient material (1). This is not the case with *Zoobotryon verticillatum* Delle Chiaje 1828 (Vesicularcidae), a widely distributed bryozoan frequently found fouling surfaces in harbors and fish-farms. Despite being considered a tropical species, *Z. verticillatum* is very abundant on the southern Atlantic coast of Spain.

In 1983 Sato and Fenical (2) reported the isolation and characterization of two cytotoxic brominated alkaloids, 2,5,6-tribromo-*N*-methylgramine [**2**] and its related side-chain *N*-oxide **3**, from colonies of *Z. verticillatum* collected in San Diego, California. The study of pharmacologically active metabolites of two different collections of *Z. verticillatum* from Cádiz and Huelva has led to the isolation of a new natural product, 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**], together with the known brominated gramine alkaloids **2** and **3** previously described. The related indole-3-carbaldehyde **4** and 6-bromo-indole-3-carbaldehyde **5** had been described from an unidentified yellow marine bacterium species of the genus *Pseudomonas* (3).



1 R_1=Me, R_2=R_3=R_4=Br
4 R_1=R_2=R_3=R_4=H
5 R_1=R_2=R_3=H, R_4=Br



3 R=Me_2NCH_2-
2 R=Me_2NCH_2-
6 R=(Me)(Ac)NCH_2-

Two collections of *Z. verticillatum* from two different locations of the Atlantic coast of Spain were treated separately, leading to similar results. In both cases the MeOH extract was chromatographed on Si gel to obtain fractions that were separated by hplc on Partisil to obtain 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**] (0.010% dry wt for both specimens) as a white crystalline solid, mp 228.5–229.5°, 2,5,6-tribromo-*N*-methylgramine [**2**] (0.088% and 0.100% dry wt), and its related side chain *N*-oxide **3** (0.058% and 0.020% dry wt). Compounds **2** and **3** were identified by comparison of their spectral data with those already reported (2).

The hrms of 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**] contained the correct cluster of peaks for the molecular formula $C_{10}H_6Br_3NO$. The presence of an aldehyde group was indicated by the ir absorption at 1652 cm^{-1}, the ^{1}H-nmr signal at δ 9.96 (s, 1H), and

the ^{13}C-nmr doublet at δ 183.5. A singlet on the ^{1}H-nmr spectrum at δ 3.80 and a quartet on the ^{13}C-nmr spectrum at δ 32.5 indicated the presence of a methyl group attached to nitrogen. These latter spectral features together with the correlations of the ^{13}C-nmr features of **1** (see Experimental section) with those of **2** and **3** (2) strongly support the proposed structure, 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde. Furthermore, two singlets on the ^{1}H-nmr spectrum at δ 8.58 (s, 1H) and 7.62 (s, 1H) were assigned to H-4 and H-7, respectively. The uv absorption at 223 nm (ε 18100) was in agreement with the presence of the indole nucleus (4).

2,5,6-Tribromo-*N*-methylindole-3-carbaldehyde [**1**] had been reported to form when compound **3** was treated with Ac_2O in pyridine (2). In order to confirm the proposed structure for **1**, compound **3** was dissolved in pyridine and treated with Ac_2O to give **1** as the major product (62% yield), together with 2,5,6-tribromo-3-(*N'*-acetyl-*N'*-methylaminomethyl)-*N*-methylindole [**6**] (15% yield). *O*-Acetylation products of amine *N*-oxides readily undergo the Polonovski reaction (5) affording acetoxy derivatives α to nitrogen. As neither the 9-acetoxy **7** nor the 10-acetoxy **8** gramine derivatives were obtained in our reaction, a subsequent rearrangement (6) must occur. The nitrogen acetoxy derivatives **7** and **8** would then lead to the formation of **1** and **6**, respectively (Scheme 1). The different acidity between the side chain methylene and methyl protons as well as the higher stability of the indole-3-carbaldehyde moiety might be responsible for the ratio in which **1** and **6** are obtained.

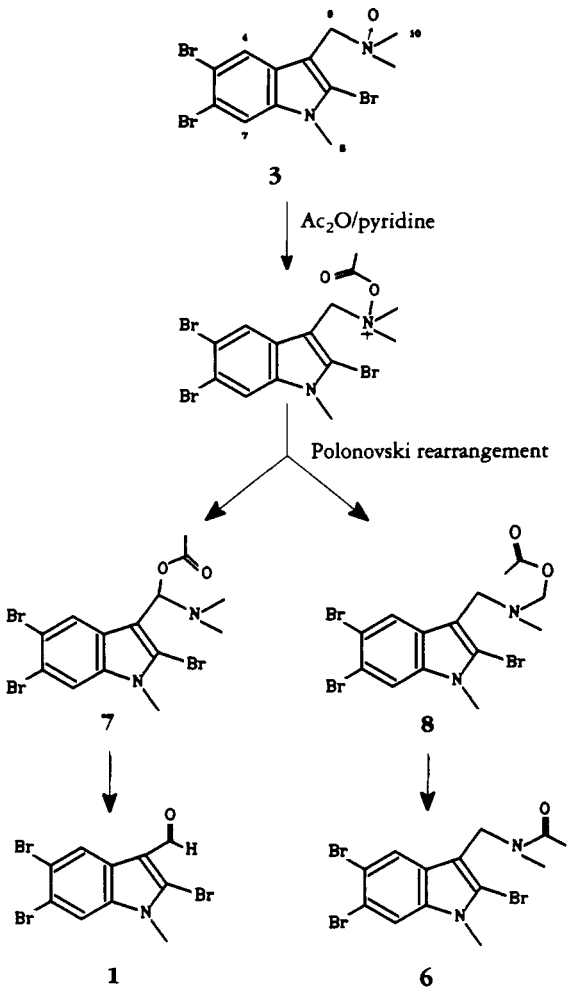
2,5,6-Tribromo-*N*-methylgramine [**2**] and its related N-oxide **3** exhibited significant inhibition of cell division of sea urchin eggs (5 μg/ml and 20 μg/ml, respectively). These bioassays for 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**] showed that cell division appears to proceed normally but metamorphosis is significantly delayed at concentrations of 5 μg/ml. Furthermore, concentrations of 10 μg/ml promote a decrease of mobility in the pluteus larvae. Complete biomedical evaluation of **1** is in progress.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were recorded on a Perkin-Elmer 257 spectrophotometer. Uv spectra were measured in MeOH solution using a Phillips PU 8710 spectrophotometer. ^{1}H-nmr and ^{13}C-nmr spectra were recorded on a Varian Gemini-200 instrument at 200 MHz and 50 MHz, respectively. Mass spectra were measured on a VG 12250 or on Kratos MS 80RFA spectrometers. Thioglycerol + NaI was used as matrix for fabms. Melting points (uncorrected) were determined on a Reichert-Jung apparatus. All solvents were distilled from glass prior to use.

COLLECTION, EXTRACTION, AND ISOLATION PROCEDURES.—The specimen of *Z. verticillatum* from Huelva (57 g dry wt) was collected by hand and stored in MeOH for 6 months at 3°. A voucher specimen (No. 25.03/196) is deposited in Museo Nacional de Ciencias Naturales, Madrid, Spain. The specimen of *Z. verticillatum* from San Fernando, Cádiz (100 g dry wt) was collected by hand and stored in MeOH for 2 weeks at 3°. A voucher specimen (No. 25.03/195) is deposited in Museo Nacional de Ciencias Naturales, Madrid, Spain. The extraction and isolation procedures were quite similar for both specimens (unless otherwise indicated). The MeOH was carefully decanted, and the bryozoans were washed with fresh MeOH at room temperature over a period of 4 h. The respective MeOH extracts were evaporated to obtain aqueous suspensions that were diluted with distilled H_2O and extracted several times with CH_2Cl_2 (500 ml). The extracts were dried over anhydrous Na_2SO_4 and the solvent evaporated. The organic extracts were chromatographed on Si gel under a positive pressure of N_2 using solvents of increasing polarity from hexane to Et_2O, and subsequently $CHCl_3$ to MeOH. The fraction eluted with hexane-Et_2O (1:9) showed on tlc spots active both to uv light and to Dragendorff's reagent, as well as aromatic signals in its ^{1}H-nmr spectrum. Separation by hplc on Partisil afforded 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**] (6 mg, 0.010% dry wt from the Huelva specimen; 10 mg, 0.010% dry wt from the San Fernando specimen); 2,5,6-tribromo-*N*-methylgramine [**2**] (50 mg, 0.088% dry wt; 100 mg, 0.100% dry wt) and 2,5,6-tribromo-*N*-methylgramine oxide [**3**] (35 mg, 0.058% dry wt; 20 mg, 0.020% dry wt).

2,5,6-Tribromo-N-methylindole-3-carbaldehyde [**1**].—Eluted from Partisil with 10% hexane in



SCHEME 1

$CHCl_3$; white solid, mp 228.5–229.5°; ν max (film) cm^{-1} 1652, 1486, 1426, 862; uv λ max (MeOH) nm 223 (ε 18100), 256 (ε 13700), 309 (ε 6900); ^1H nmr ($CDCl_3$) δ ppm 3.80 (s, 3H), 7.62 (s, 1H), 8.58 (s, 1H), 9.96 (s, 1H); ^{13}C nmr ($CDCl_3$) δ ppm 183.5 (d, -CHO), 136.6 (s, C-7a), 127.0 (s, C-3a), 124.9 (d, C-4), 119.2 (s, C-3), 118.8 (s, C-5), 115.0 (s, C-6), 114.1 (d, C-7), 110.4 (s, C-2), 32.5 (q, $-NCH_3$); hrms *m/z* 396.7961 ($C_{10}H_6{}^{79}Br^{81}Br_2NO$ requires 396.7962), 394.7974 ($C_{10}H_6{}^{79}Br_2{}^{81}BrNO$ requires 394.7981); eims *m/z* (intensity, %) 398.6, 396.6, 394.6, 392.6 (29.4:97.5:100:29.7), 316.7, 314.7, 312.7 (1.4:2.4:1.2), 235.8, 233.8 (9.4:9.4).

REACTION OF **3** WITH Ac_2O IN PYRIDINE.—An excess of Ac_2O was added to a solution of 15 mg of **3** in pyridine. After 24 h at room temperature, the pyridine and the excess of Ac_2O were removed by distillation under reduced pressure. The crude reaction mixture (14 mg) was chromatographed on a Si gel column using $CHCl_3$ as eluent to obtain 2,5,6-tribromo-*N*-methylindole-3-carbaldehyde [**1**] (8 mg) and 2,5,6-tribromo-3-(*N'*-acetyl-*N'*-methylaminomethyl)-*N*-methylindole [**6**] (2 mg).

*2,5,6-Tribromo-3-(*N'*-acetyl-*N'*-methylaminomethyl)-*N*-methylindole* [**6**].—Amorphous powder: ν max (film) cm^{-1} 1645, 1420, 1415, 875; uv λ max (MeOH) nm 204 (ε 26400), 234 (ε 36700), 298 (ε 7700); ^1H nmr ($CDCl_3$) δ ppm 2.11 (s, 3H), 2.84 (s, 3H), 3.72 (s, 3H), 4.71 (s, 2H), 7.57 (s, 1H), 7.98 (s, 1H); eims *m/z* (intensity, %) $[M-C_3H_6NO]^+$ 383.9, 381.9, 379.8, 377.9 (1.4:4.2:4.9:1.6); $[M-Br]^+$ 375.0, 373.0, 371.0 (4.3:8.8:4.8); fabms (intensity, %) $[M+Na]^+$ 479, 477, 475, 473 (26.9:79.2:100:57.7).

ACKNOWLEDGMENTS

The bryozoan from Huelva was collected and identified by Dr. Carlos M. López de la Cuadra, Laboratorio de Biología Marina, Universidad de Sevilla, and the specimen from San Fernando, Cádiz, was collected and identified by Eloisa Millán, CUPIMAR S.A. The biological tests were performed by Dra. M.J. Uríz, Centre d'Estudis Avançats de Blanes, Girona. Fabms and hrms were measured at Servicio de Espectrometría de Masas, Universidad de Sevilla by Dra. María Angeles Pradera.

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Received 10 August 1992

ERRATUM

For the paper by Gribble entitled "Naturally Occurring Organohalogen Compounds-A Survey," *J. Nat. Prod.*, **55**, 1353 (1992), a quotation was attributed to the Great Lakes Science Advisory Board of the International Joint Commission that was not of the Board's making. The quotation, which was taken from a secondary source, linked phrases from three separate sentences used in three different contexts, thereby distorting the meaning. Therefore, the sentence appearing on page 1353: "Not only are these statements incorrect, but, coming from a "Science Advisory Board," they reflect a disturbing ignorance of the chemical composition of our environment and of biochemical processes therein," should be deleted. The author apologizes for this error.