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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · AUGUST 2000

Impact Factor: 3.8 · DOI: 10.1021/np990408d · Source: PubMed

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Matemone, a New Bioactive Bromine-Containing Oxindole Alkaloid from the Indian Ocean Sponge *Iotrochota purpurea*

Isabelle Carletti,[†,§] Bernard Banaigs,[‡] and Philippe Amade*[,§]

LPTE, Faculté des Sciences, Université de Nice Sophia-Antipolis, 28 Avenue de Valrose, 06108 Nice−Cedex 2, France, and Centre de Phytopharmacie, Université de Perpignan, 52 Avenue de Villeneuve, 66860 Perpignan−Cedex, France

Received August 18, 1999

A new bioactive bromine-containing oxindole alkaloid, matemone (**1**), was isolated from the Indian Ocean sponge *Iotrochota purpurea*, together with the known 6-bromoindole-3-carbaldehyde. The structures were elucidated on the basis of spectral and chemical data. Compound **1** shows mild cytotoxicity against three cancer cell lines and marginal antibacterial activity against *Staphyloccocus aureus*. DNA intercalant screening demonstrated DNA interactions with **1**. Both compounds inhibit division of sea-urchin eggs.

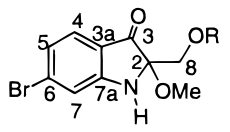
In our continuing studies on bioactive compounds from marine organisms,[1] we have now identified 6-bromoindole alkaloids from the Indian Ocean sponge *Iotrochota purpurea* Bowerbank, 1875 (order Poecilosclerida, family Myxillidae) collected off the coast of Matemo Island, located in the north of the Mozambican Ibo Island (western Indian Ocean). Few investigations of *Iotrochota* species have been reported in the literature.[2−7]

We report here the bioassay-guided fractionation of the crude extract of *I. purpurea*, which resulted in the isolation of two compounds. Structure elucidation led to the identification of known 6-bromoindole-3-carbaldehyde, previously identified from several marine organisms,[8] and a second new bioactive compound that was identified as 6-bromo-2-methoxy-2-hydroxymethane-3-indolinone **(1)** and named matemone. The biological activities of **1** are also reported here.

3-Indolinones have been found in terrestrial sources.[9,10] Their derivatives have been identified as antibiotic compounds produced by bacteria isolated from the toxic mucus of the boxfish[11] and by symbiotic bacteria of crustacean embryos.[12] Dimers of 3-indolinone are responsible for the bright colors of mollusks, such as the Tyrian purple of Muricidae and Thaisidae,[13] the only brominated 3-indolinone described as a natural product. Indolinone derivatives and bromoindole metabolites are often proposed to be repellent or antifeedant substances. Bromoindoles have also been reported to show antimicrobial, antiproliferative, and antifouling effects.[8]

The ethanolic extract of *I. purpurea* showed an inhibitory effect on sea-urchin egg division, with 100% inhibition at 1 mg/mL. This bioassay was used for guiding the fractionation of the extract. The crude ethanolic extract was partitioned between hexane, CH_2Cl_2, MeOH, and H_2O, and the bioactivity was found in the methanolic fraction (100% inhibition at 400 μg/mL), which was subjected to further fractionation. The bioactive metabolites were isolated and identified as 6-bromoindole-3-carbaldehyde and the unstable matemone (**1**).

The structure of 6-bromoindole-3-carbaldehyde was deduced from spectral data (^{1}H and ^{13}C NMR, IR, TLC) and confirmed by comparison with the data of authentic synthetic compound.[8] Matemone (**1**) was obtained as a yellow-green film. The negative electrospray ionization mass spectrum (ESIMS) gave a double peak $[M - H]^+$ at 270/272 (1:1 ratio), suggesting the presence of one bromine atom in the molecule. The UV spectrum was characteristic of an indole nucleus (λ_{max} 245, 273 nm) conjugated with other chromophores (λ_{max} 400 nm).[14] The IR spectrum indicated the presence of hydroxyl or/and amino groups (3334 cm^{-1}), conjugated carbonyl(s) (1709 cm^{-1}) and aromatic double bonds (1451 cm^{-1}). Compound **1** gradually decomposed during NMR experiments carried out in CD_2Cl_2 as solvent, suggesting a solvent-induced polymerization process and the probable presence of a hydroxyl group. Thus **1** was converted to the stable acetate derivative **2**, and all the spectroscopic analyses were carried out with **2**. NMR measurements of **2** were first obtained in CD_2Cl_2, then in acetone-*d*6; those of **1** were obtained in acetone-d_6 (Table 1).



1 R = H
2 R = Ac

The molecular formula of **2** was determined as $C_{12}H_{12}NO_4Br$ by HREIMS, as expected for the monoacetate derivative of **1**. Its ^{1}H NMR spectrum shows the presence of a trisubstituted aromatic ring (ortho, meta, meta) [δ 7.40 (1H, d, J = 7.6 Hz), 7.10 (1H, d, J = 1.2 Hz), and 6.99 ppm (1H, dd, J = 8.4, 1.6 Hz)], two aliphatic protons [δ 4.44 (1H, d, J = 11.2 Hz), δ 4.07 (1H, d, J = 11.2 Hz)], a methoxy group at δ 3.16 (3H, s), the acetate methyl group at δ 1.92 (3H, s), and a broad one-proton signal at δ 5.07 ppm (Table 1). The ^{13}C NMR spectrum of **2** showed signals for six quaternary carbons (δ 197.5, 170.9, 160.7, 133.9, 119.5, 90.9), three sp^2 carbons (δ 125.8, 123.5, 115.5), one sp^3 carbon (δ 65.3), and two methyl groups (δ 51.3, 20.7).

The NMR data of **2** supported the presence of an aromatic trisubstituted ring, a ketone, and a methylene group with two unequivalent protons, shifted downfield by the acetate (1 ppm relative to **1**), a methoxy group, and one amino proton.

The connectivities observed in the ^{1}H−^{1}H COSY and HMQC spectra supported placement of the bromine atom

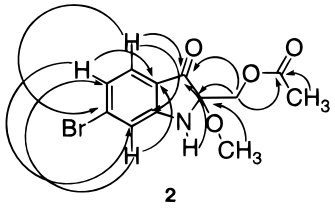
* To whom correspondence should be addressed. Tel.: +33 04 92 07 65 84. Fax: +33 04 92 07 65 63. E-mail: amade@unice.fr.
† This work is part of the Thèse de Doctorat.
§ Nice University.
‡ Perpignan University.

10.1021/np990408d CCC: $19.00
Published on Web 06/29/2000

Table 1. ^{1}H and ^{13}C NMR Assignments for Compounds **1** and **2**[a]

	1[b]		**2**[b]	**2**[c]	
carbon	δ^{13}C	δ^1H[d]	δ^1H[d]	δ^{13}C	δ^1H[d]
1 − NH		5.05 (1H, br)	4.95 (1H, br)		5.07 (1H,br)
2	93.7			90.9	
3	197.0			197.5	
1	133.1			133.9	
4	125.7	7.31 (1H, d, 8.1)	7.41 (d, 8.2)	125.8	7.40 (d, 7.6)
5	122.4	6.89 (1H, dd, 8.1, 1.1)	7.00 (dd, 8.2, 1.6)	123.5	6.99 (dd, 8.4, 1.6)
6	120.8			119.5	
7	116.0	7.15 (1H, d, 1.1)	7.21 (d, 1.6)	115.5	7.10 (d, 1.2)
7a	163.0			160.7	
8	65.3	3.65 (1H, s)	4.33 (1H, d, 11.1)	65.3	4.44 (1H, d, 11.2)
		3.28 (1H, s)			4.07 (1H, d, 11.2)
-OMe	50.8	3.08 (3H, s)	3.15 (3H, s)	51.3	3.16 (3H, s)
-CO*Me*			1.87 (3H, s)	20.7	1.92 (3H, s)
-*C*OMe				170.9	

[a] Assignments are based on extensive ^{1}H and 2D measurements (COSY, HMQC, HMBC). [b] Recorded in acetone-d_6 (200 MHz). [c] Recorded in CD_2Cl_2 (400 MHz). [d] δ values are in ppm (integration, multiplicity, J in Hz).



2

Figure 1. C/H long-range correlations obtained from the HMBC spectra of **2**.

on either C-5 or C-6 of the indole skeleton. Also, the LREIMS of **2** showed the molecular ion peak at *m*/*z* 313/315 (1:1) and the base peak at *m*/*z* 240/242 (1:1) corresponding to the loss of $-CH_2OCOMe$. An ion at *m*/*z* 208/210, with molecular formula C_8H_5ONBr, was assigned to the brominated indole fragment, consistent with previous reports for indole subunits.[15] The oxo-indole subunit was confirmed by an observed HMBC connectivity, between H-4 (δ 7.40) and C-3 (δ 197.5). This HMBC connectivity together with the ortho and meta proton coupling for H-5, revealed the location of the bromine atom at C-6. This substitution pattern was confirmed by comparison of the calculated and observed chemical shifts for the C-5 and C-6 substituted isomers. The calculated shifts were based on data obtained for peronatin[9] by using the substituent effects.[15] Selective HMQC (Table 1) and HMBC connectivities (Figure 1) allowed the unambiguous assignments of all proton and carbon resonances in **2**. On the basis of HMBC measurements (long-distance H−C correlations), the quaternary carbon at δ 90.9 bearing the methoxy and CH_2OCOMe groups was assigned as C-2. Finally, the quaternary carbon at δ 160.7 ascribed to C-7a showed an unusual high-field chemical shift due to the carbonyl effect, in accordance with the spectral data of peronatin[9] and 2,2-di(3-indolyl)-3-indolinone,[11] whose C-7a resonances appear at 160.5 and 161.6 ppm, respectively.

Compound **1** showed marginal antimicrobial activity against the bacterium *Staphylococcus aureus* at 50, 100, and 200 μg/disk, affording inhibitory zones of 7, 9, and 11 mm, respectively. No activity was detected against the yeast *Candida albicans* at 200 μg/disk. Matemone (**1**) inhibits sea-urchin egg division[16] (IC_{50}, 35 μg/mL). Potential DNA intercalant activity was evaluated using the HPLC method,[17] by which **1** showed significant binding ability (40−50%; ethydium bromide, 100%). Compound **1** also displayed mild cytotoxicity against the growth of the NSCLC−N6 L16 strain[18] (lung cancer), Mia PaCa-2 cell line (pancreas cancer), and DU145 cell line (prostate cancer), with IC_{50} values of 30, 24, and 27 μg/mL, respectively.

Experimental Section

General Experimental Procedures. Melting points were determined with a Büchi 510 apparatus and are uncorrected. IR spectra were recorded with a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer and the UV spectra with a Uvikon 930 Kontron spectrophotometer. Optical rotation was measured with an Optical Activity Polarimeter (Type AA-5). NMR spectra were acquired using a Bruker 200 and a JEOL EX400 spectrometer operating at frequencies of 400 and 100 MHz for ^{1}H and ^{13}C nuclei, respectively, in Me_2CO-d_6 or CD_2Cl_2 solutions. The chemical shifts are given in parts per million (ppm) and are referenced to the solvent signal. ESI was run on a LCQ Finnigan spectrometer and LREIMS on a Finnigan Mat 500 spectrometer at 70 eV. HREIMS was performed at the Service Central d'Analyses du CNRS, Solaize, France. HPLC analyses were carried out on a Waters HPLC System: 600 pump, 996 diode array detector, 717+ autosampler. Final HPLC purifications were carried out using Intersphere Si gel (250 mm × 10 mm, 10 μm, Interchrom) and Magnum RP_{18} (250 mm × 10 mm, 10 μm, Whatman) columns.

Animal Material. The sponge *I. purpurea* was collected by scuba at a depth of 12 m at Matemo Island, located north of the Mozambican Ibo Island (western Indian Ocean) in November 1995. Taxonomic identification was provided by Dr. Jean Vacelet from the Station Marine d'Endoume, Marseille, France. A voucher specimen (MHNM1999Im545) has been deposited at the Museum d'Histoire Naturelle (Marseille, France).

Extraction and Isolation. The freshly collected sponge was stored in ethanol prior to its extraction. The crude ethanolic extract (20 L) was concentrated under reduced pressure to yield a brown viscous residue (191 g). This extract was then partitioned between hexane and water, and the aqueous layer was further extracted with CH_2Cl_2. The two organic layers were mixed, dried over Na_2SO_4, and evaporated. The residue was extracted successively with hexane, CH_2Cl_2, MeOH, and H_2O. A portion (25 g) of the MeOH solubles (54 g) was subjected to C_{18} reversed-phase vacuum flash chromatography, using as eluents increasing amounts of MeOH in H_2O, then using EtOAc−MeOH (1:1), and finally rinsing with EtOAc. The 100% MeOH fraction (1.2 g) was purified on Si gel with hexane−EtOAc and then EtOAc−MeOH mixtures of increasing polarity. The hexane−EtOAc (60:40) fraction was further subjected to semipreparative HPLC on a Si gel column with hexane−EtOAc (80:20) followed by C_{18} column with H_2O−MeOH (60:40) to yield the yellow-green compound **1** (19 mg, 0.0099% based on the crude ethanolic extract). The known colorless 6-bromoindole-3-carbaldehyde (6 mg, 0.0031%) was obtained from the EtOAc−MeOH (80:20) fraction purified on

a semipreparative HPLC Si gel column with hexane−EtOAc (60:40) and was further crystallized from CH_2Cl_2.

6-Bromoindole-3-carbaldehyde: colorless needles; mp 199−201 °C (CH_2Cl_2); ESIMS *m/z* 222 and 224 [$(M - H)^+$, 1:1]. For data, see Olguin-Uribe et al.[8]

Matemone (1): yellow-green film; $[\alpha]^{25}_D$ + 8.9° (*c* 0.45, MeOH); UV (MeOH); λ_{max} (log ϵ) 228 (4.34), 245 (4.33), 273 (4.01), 399 (3.50); IR (KBr) 3334, 2951, 2920, 2851, 1702, 1608, 1573, 1450, 1311, 1083, 1088 cm^{-1}; ^1H NMR (200 MHz, acetone-d_6) δ 7.31 (1H, d, J = 8.1 Hz, H-4), 7.15 (1H, d, J = 1.1 Hz, H-7), 6.89 (1H, dd, J = 8.1, 1.1 Hz, H-5), 5.07 (NH, H-1), 3.65 (1H, s, H-8b), 3.28 (1H, s, H-8a), 3.06 (3H, s, OCH_3); ^{13}C NMR (75 MHz, acetone-d_6) δ 197.0 (C-3), 163.0 (C-7a), 133.1 (C-1), 125.7 (C-4), 122.4 (C-5), 120.8 (C-6), 116.0 (C-7), 93.7 (C-2), 65.3 (C-8), 50.8 (OCH_3); ESIMS *m/z* 270 and 272-[$(M - H)^+$, 1:1].

Acetate Derivative 2. Compound **1** (10 mg) was dissolved in anhydrous Ac_2O (0.6 mL) and pyridine (0.6 mL) and kept at room temperature overnight. After addition of H_2O, the mixture was extracted three times with CH_2Cl_2. The combined organic layers were dried over anhydrous $CaCl_2$ and concentrated. Purification on TLC Si gel afforded the monoacetate **2** (6 mg): UV (MeOH) λ_{max} (log ϵ) 228 (4.19), 245 (4.16), 274 (3.88), 395 (3.40); ^1H NMR (400 MHz, CD_2Cl_2) δ 7.40 (1H, d, J = 7.6 Hz, H-4), 7.10 (1H, d, J = 1.2 Hz, H-7), 6.99 (1H, dd, J = 8.4, 1.6 Hz, H-5), 5.07 (NH, H-1), 4.44 (1H, d, J = 11.2 Hz, H-8b), 4.07 (1H, d, J = 11.2 Hz, H-8a), 3.16 (3H, s, OCH_3), 1.92 (3H, s, $COCH_3$); ^{13}C NMR (400 MHz, CD_2Cl_2) δ 197.5 (C-3), 170.9 ($COCH_3$), 160.7 (C-7a), 133.9 (C-1), 125.8 (C-4), 123.5 (C-5), 119.5 (C-6), 115.5 (C-7), 90.9 (C-2), 65.3 (C-8), 51.3 (OCH_3), 20.7 (CH_3CO); EIMS *m/z* 313/315 [M^+] (22), 240/242 (100), 208/210 (3), 197 (20), 185 (19), 43 (39); HREIMS *m/z* 312.9955 [M^+] (calcd for $C_{12}H_{12}NO_4Br$, 312.9949).

Antimicrobial Assay. The antimicrobial activity of **1** was tested against *S. aureus* (Pasteur Institute Production, IPC 53146) and *C. albicans* (IPC 1283) using the paper-disk agar diffusion method (standard disk diameter, 6 mm). Growth-inhibition diameter, including disk diameter, was measured in millimeters after 18 h of incubation at 37 °C.

Toxicity Evaluation. Cytoxicity was estimated on the sea-urchin egg division assay (*Paracentrotus lividus*, Lamarck, 1816) according to the procedure previously described.[16] The percentage of divided eggs was determined in controls 30 min after the beginning of cell division.

DNA Binding. The potential DNA intercalant activity was evaluated by HPLC. A μ-Bondapak C_{18} column (250 × 4 mm, 10 μm, Waters) was equilibrated with H_2O−MeOH, 80:20. Co-injection of equal volumes of calf thymus DNA (10 μL at 0.1 mg/mL; ref. D4522 Sigma-Aldrich) and **1** (10 μL at 1 μg/μL) without incubation was used. The flow rate was maintained at 1 mL/min, and the free DNA was eluted from the column in approximately 1.5 min. DNA binding was expressed as a percentage of the DNA peak exclusion. Ethidium bromide was used as a typical intercaling agent with major binding (100%).

Acknowledgment. We thank the Ardoukoba Association for help in collecting the Mozambican sponge samples. We are grateful to Dr. Jean Vacelet for identification of the sponge and to Dr. Danielle Pesando (LPTE, Université de Nice, France) for assistance during the biological assays. Thanks are also extended to Dr. Christos Roussakis (Faculté de Pharmacie de Nantes, France) and to L'Institut Henri Beaufour (particularly Mrs. Marie Christine Brezac, IHB, Les Ulis, France) for cytotoxicity assays. Financial support for this research was provided by INSERM and Le Conseil Régional Provence-Alpes Côte d'Azur.

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NP990408D