Saldedines A and B, Dibromo Proaporphine Alkaloids from a Madagascan Tunicate

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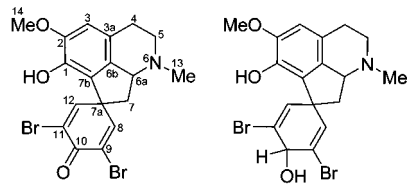
Two new dibromo proaporphine alkaloids, designated saldedines A (**1**) and B (**2**), were isolated from an unidentified tunicate collected at Salary Bay, Madagascar. Saldedines A and B are the first marine proaporphine alkaloids. Their structures were elucidated by extensive spectroscopic means, and the structure of **1** was confirmed by single-crystal X-ray diffraction analysis. Both saldedines A and B were tested for toxicity to brine shrimp and showed moderate activity.

In the search for bioactive substances from marine invertebrates,[1,2] two new dibromo proaporphine alkaloids, designated as saldedines A (**1**) and B (**2**), were isolated from an unidentified Didemnidae tunicate, collected in Salary Bay Madagascar.



1 **2**

3 *N,N*-dimethyl derivative

Plant proaporphine alkaloids, a major isoquinoline group, have been recognized as the biosynthetic precursors of aporphine alkaloids bearing a wide range of oxygenated substitution patterns with mainly a spiro-cyclohexadienone ring system. These alkaloids have, so far, been isolated only from species of Papaveraceae.[3] Several of these alkaloids exhibit interesting biological activities.[4] To the best of our knowledge, the present work is the first report of proaporphine alkaloids from a marine source, and for the first time of bromo proaporphines.

The $CHCl_3$/MeOH (1:1) extract of the frozen tunicate was subjected to solvent partitioning, i.e., aqueous MeOH against hexanes and CH_2Cl_2, and the latter fraction was chromatographed on Sephadex LH-20, eluting with hexanes/MeOH/CH_2Cl_2 (2:1:1), to afford a mixture of two nitrogen-atom-containing compounds. From this mixture we isolated, upon repeated Sephadex LH-20 and silica gel chromatography, two compounds, designated saldedines A (**1**) and B (**2**).

Saldedine A (**1**), colorless crystals, showed a cluster of three (1:2:1) ion peaks at *m*/*z* 454.9553 $[M]^+$ indicative of a dibrominated compound. On the basis of HREIMS data the molecular formula was defined as $C_{18}H_{17}Br_2NO_3$, implying 10 degrees of unsaturation. The ^{13}CNMR and DEPT spectra displayed 18 carbon resonances comprising two methyl groups, one methoxy (δ_C 56.5, δ_H 3.83) and one *N*-methyl (δ_C 43.5, δ_H 2.39), three methylenes (δ_C 27.1, 46.4, 55.0), one sp^3 methine (δ_C 65.5), one sp^3 quaternary carbon (δ_C 55.7), five double bonds (δ_C 110.2, 120.8, 121.4, 121.8, 123.3, 133.8, 140.8, 147.2, 149.5, 153.4), and one carbonyl group (δ_C 174.0). The five double bonds and one carbonyl group accounted for six out of the 10 degrees of unsaturation. The remaining four, therefore, indicated compound **1** was tetracyclic. Determination of the structure of compound **1** was quite straightforward by interpretation of the COSY and HMBC data (Figure 1 and Table 1). The COSY spectrum revealed the presence of two spin systems. Most informative was the HMBC experiment. The various two- and three-bond CH correlations, depicted in Figure 1, established the complete planar structure of **1**. Further confirmation came from the ^{15}NH-HMBC experiment; a $^2J_{NH}$ correlation was observed between Me-13 and a sp^3 nitrogen atom resonating at 48.0 ppm, and $^3J_{NH}$ correlations between methylenes 4 and 7 to the latter nitrogen determined this nitrogen as N-6. The latter ^{15}N chemical shift is in excellent agreement with the value measured for the corresponding atom in mecambrine (δ_N 47.9 ppm), a closely related proaporphine alkaloid, isolated from *Meconopsis cambrica*.[5] Methylation of saldedine A with MeI/K_2CO_3 gave the *N*,*N*-dimethyl ammonium salt of **1** (**3**), leaving, unexpectedly, the C-1 phenol group intact.

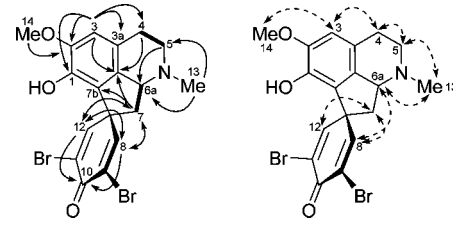


Figure 1. Key COSY (−), HMBC (↷), and NOESY (dashed arrow) correlations of saldedine A (**1**).

Finally, a qualified single crystal was obtained from CH_2Cl_2, enabling X-ray crystallographic analysis (Figure 2) and confirming unequivocally the structure of **1**. Saldedine A crystallized as a racemic mixture in space group *Pbca*, wherein the two enantiomeric species are related by an inversion center.[6]

The spectroscopic data of the second isolated compound, saldedine B (**2**), pointed to high similarity with the structure of **1**, lacking however the cross dienone functionality. The major differences between **1** and **2** in the NMR spectra were the disappearance of the carbonyl group of **1** and the appearance of an oxymethine group resonating at δ_C 73.1 and δ_H 4.56. Furthermore, the two downfield methines [CH-8 (δ_C 135.8, δ_H 6.16) and CH-12 (δ_C 132.6, δ_H 6.28)] are upfield shifted compared to these methines in **1**. The HMBC experiment confirmed the suggested 10-hydroxyl group. Although the differences in the ^{1}H and ^{13}C NMR data pointed toward a different degree of unsaturation, the EI mass spectrum of **2**, surprisingly, exhibited the same molecular peak as **1** at *m*/*z* 455; therefore, the EIMS spectrum represents a pseudomolecular ion peak due to loss of H_2. Although it is possible that **2** could be oxidized to **1** during the isolation process, NMR resonances for both compounds were observed in the crude extract, which suggests that **1** is not an isolation artifact.

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Table 1. NMR Spectroscopic Data (500 MHz, $CDCl_3$) for Saldedine A (**1**)

position	δ_C, mult	δ_H (J in Hz)[a,b]	COSY	HMBC	NOESY
1	140.8, qC				
2	147.2, qC				
3	110.2, CH	6.60, s		1, 2, 3a, 4, 6b	4A, 4B, 14
3a	123.3, qC				
4	27.1, CH_2	2.90 m	4B, 5	3, 5, 3a, 6b	3, 4B, 5A
		2.78, dd (16.7,4.7)	4A, 5	3, 5, 3a, 6b	3, 4A, 5A, 5B
5	55.0, CH_2	3.12, dd (11.8, 6.7)	4, 5B	3a, 4, 6a, 7	4A, 4B, 5B, 13
		2.50, m	4, 5A	3a, 4, 6a, 7	4B, 5A
6a	65.5, CH	3.45, dd (9.5, 6.1)	7	6b	8, 13
6b	133.8, qC				
7	46.4, CH_2	2.50, m	6a, 7B	6a, 6b, 7a, 7b, 8, 12	6a, 7B, 8, 12
		2.28, m	6a, 7A	6a, 6b, 7a, 7b, 8, 12	7A, 8, 12
7a	55.7, qC				
7b	121.8, qC				
8	149.5, CH	7.42, d (2.2)	12	7, 7a, 7b, 9, 10, 12	6a, 7A
9	120.8, qC				
10	174.0, qC				
11	121.4, qC				
12	153.4, CH	7.30, d (2.2)	8	7, 7a, 7b, 8, 10, 11	7B
13	43.5, CH_3	2.39, s		5, 6a	5A, 6a
14	56.5, CH_3	3.83, s		2	3

[a] The CH correlations were assigned by an HSQC spectrum. [b] A and B denote downfield and upfield resonances, respectively, of a geminal pair.

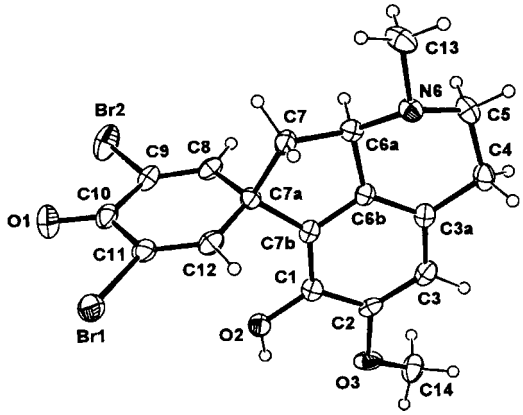

Figure 2. X-ray structure of saldedine A (**1**).

The biosynthesis of proaporphine alkaloids derives in nature from oxidative phenolic coupling of the benzylisoquinoline alkaloide.[7] The benzylisoquinoline precursor was previously obtained only from the starfish *Dermasterias imbricata*.[8]

Saldedines A (**1**) and B (**2**) were both tested for toxicity to brine shrimp (*Artemia salina*)[9] and were found moderately active. Saldedine A (**1**) shows a greater potency with a LD_{50} value of 4.4 μM, while saldedine B (**2**) has a LD_{50} value of 10.9 μM.

Experimental Section

General Experimental Procedures. Optical rotations were obtained with a Jasco P-1010 polarimeter. UV spectra were recorded on an Agilent model 8453 UV−visible spectrometer. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. NMR spectra were acquired on a Bruker Avance-500 spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C using the residual solvent signals as an internal reference ($CDCl_3$ δ_H 7.26 ppm, δ_C 77.0). The ^{15}NH-HMBC experiment was optimized for a delay of 65 ms, and the ^{15}N chemical shift is reported with respect to liquid NH_3 as the reference standard. High-resolution mass spectrometric data were obtained on a Fisons, Autospec Q instrument.

Biological Material. The Didemnidae tunicate was collected at Salary Bay, ca. 100 km north of Tulear, Madagascar (22°, 32.935′ S; 43°, 13.049′ E), in January 2007. Two samples of the Didemnidae tunicate were sent to Dr. F. Monniot (Museum d'Histoire Naturelle de Paris, France) for characterization. Since neither of them had gonads, the genus could not be determined. Furthermore spicules and consistency were indicative of Lissoclinum, but very long appendices fixative pointed out rather Didemnum or Polysyncraton. A color image of the Didemnidae tunicate is attached in the Supporting Information. A voucher specimen is deposited at Museum d'Histoire Naturelle de Paris with Dr. F. Monniot (voucher no. AMSA-69 and -70).

Extraction and Isolation. The frozen wet sample (95 g) of the tunicate was homogenized and exhaustively extracted with $CHCl_3$/MeOH (1:1). The organic extract was concentrated to yield a crude extract (457 mg). Partitioning using a modified Kupchan procedure[10] yielded 150 mg of the CH_2Cl_2 fraction. The latter CH_2Cl_2 fraction was chromatographed on Sephadex LH-20, eluting with hexanes/MeOH/CH_2Cl_2 (2:1:1), to afford a mixture containing two compounds, **1** and **2**. This mixture was further purified by Sephadex LH-20, as above, and Si gel flash column chromatography eluting with a gradient of hexanes/EtOAc, which led to the isolation of saldedines A (**1**, 4 mg) and B (**2**, 2 mg).

Saldedine A (1): colorless oil; $[\alpha]^{20}_D$ 0 (*c* 0.40, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (4.24), 296 (3.75), 379 (2.98) nm; IR (CH_2Cl_2) ν_{max} 3450, 2925, 1660, 1380, 1260 cm^{-1}; see Table 1 for tabulated NMR spectroscopic data; EIMS *m*/*z* 455 $[M]^+$; HREIMS *m*/*z* 454.9553 (calcd for $C_{18}H_{17}Br_2NO_3$, 454.9555).

Saldedine B (2): colorless oil; $[\alpha]^{20}_D$ −50 (*c* 0.20, MeOH); UV (MeOH) λ_{max} (log ϵ) 283 (3.85), 399 (3.31) nm; IR (CH_2Cl_2) ν_{max} 3520, 3450, 2900, 1385, 1259 cm^{-1}; ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.65 (1H, s, H-3), 3.00 (1H, m, 4A), 2.76 (1H, dd, J = 16.8, 4.9 Hz, 4B), 3.21 (1H, m, H-5A), 2.52 (1H, m, H-5B), 3.48 (1H, m, H-6a), 2.46 (1H, m, H-7A), 2.09 (1H, m, H-7B), 6.28 (1H, s, H-8), 4.56 (1H, H-10), 6.16 (1H, s, H-12), 2.44 (3H, s, NMe-14), 3.82 (3H, m, OMe-13); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 140.1 (C, C-1), 147.3 (C, C-2), 109.7 (CH, C-3), 122.8 (C, C-3a), 26.6 (CH_2, C-4), 54.9 (CH_2, C-5), 65.0 (CH, C-6a), 132.6 (C, C-6b), 46.4 (CH_2, C-7), 53.2 (C, C-7a), 124.4 (C, C-7b), 132.6 (CH, C-8), 121.1 (C, C-9), 73.1 (CH, C-10), 121.4 (C, C-11), 135.8 (CH, C-12), 43.1 (CH_3, OMe-13), 56.6 (CH_3, NMe-14), C-9 and C-11 signals are interchangeable; EIMS *m*/*z* 455 $[M - H_2]^+$; HREIMS *m*/*z* 454.9553 (calcd for $C_{18}H_{17}Br_2NO_3$, 454.9555).

***N*,*N*-Dimethylsaldedine A (3).** Saldedine A (3 mg) was dissolved in a mixture of acetone (2 mL) and methyl iodide (0.5 mL) in the presence of anhydrous K_2CO_3 (5 mg), and the mixture was heated to 70 °C for 24 h in a sealed reaction vial. After cooling, the mixture was filtered and the solvent was evaporated. ^{1}H NMR (DMSO-d_6, 400 MHz) 9.19 (1H, s, OH), 7.82 (1H, d, J = 2.3 Hz, H-12), 7.61 (1H, d, J = 2.3 Hz, H-8) 6.92 (1H, s, H-3) 5.33 (1H, dd, J = 9.7, 6.9 Hz, H-6a), 3.89 (1H, dd, J = 9.7, 6.9 Hz, H-5A), 3.77 (3H, s, OMe-13), 3.63 (1H, m, H-5B), 3.19 (3H, s, NMe), 3.07 (2H, m, 2H-4), 2.80 (3H, s, NMe-14), 2.70 (1H, m, H-7A), 2.60 (1H, m, H-7B).

Crystal Structure of Saldedine A (1). A single crystal suitable for X-ray was obtained by crystallization from CH_2Cl_2, and the structure of saldedine A was confirmed by diffraction analysis. The measurements were carried out on a Nonius KappaCCD diffractometer at low temperature (ca. 110 K) in order to optimize the precision of the crystallographic determination, with Mo Kα radiation. Crystal data:

$C_{18}H_{17}Br_2NO_3$, $M = 455.15$, orthorhombic, space group *Pbca*, $a = 14.5123(3)$ Å, $b = 10.7256(2)$ Å, $c = 21.7980(5)$ Å, $V = 3392.9(1)$ Å^3, $Z = 8$, $T = 110(2)$ K, $D_c = 1.782$ g/cm^3, μ(Mo Kα) = 4.80 mm^{-1}, 4004 unique reflections to $2\theta_{max} = 55.7°$, 220 refined parameters, $R_1 = 0.055$ for 2541 observations with $I > 2\sigma(I)$, $R_1 = 0.097$ ($wR_2 = 0.168$) for all unique data.

The molecular geometry of saldedine A reveals common bond lengths and bond angles. The saturated *N*-methyl piperidine fragment, which is fused to the aromatic ring, exhibits a pseudoenvelope conformation with the N-atom lying above the mean plane of the five C-atoms. The crystal structure is centrosymmetric, wherein neighboring molecules related to one another by the b-glide symmetry are hydrogen bonded to one another. The hydrogen bonding involves the OH group as proton donor and the N-site as proton acceptor at an O···N distance of 2.670(5) Å, linking each molecule to two adjacent species. The crystal packing between the hydrogen-bonded chains is stabilized by dispersion forces, exhibiting intermolecular van der Waals contacts within the normal range. The relatively high *R*-factor of 5.5% can be attributed to the wide-amplitude riding motion of the terminal heavy Br atoms. The somewhat sizable *R* for the averaging of equivalent reflections of 7.2% can be explained by the fact that only an approximate empirical (but not the more rigorous analytical) correction for absorption has been applied to the diffraction data. The original material subjected to crystallization was probably racemic as well (or it could racemize easily); oherwise it would be difficult to explain why the crystalline compound is racemic.

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Supporting Information Available: Color image of the Didemnidae tunicate and ^{1}H and ^{13}CNMR spectra for **1** and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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