Didemniserinolipids A−C, Unprecedented Serinolipids from the Tunicate *Didemnum* sp.

Noemí González, Jaime Rodríguez, and Carlos Jiménez*

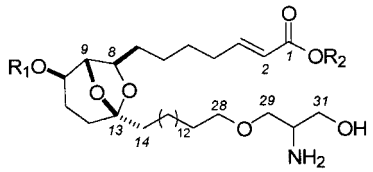
Departamento de Química Fundamental e Industrial, Facultade de Ciencias, Universidade da Coruña, Campus da Zapateira, 15071 A Coruña, Spain

Received March 4, 1999

Marine tunicates belonging to the genus *Didemnum* (Phylum Chordata, class Ascidiacea) have proven to be a particularly rich source of structurally diverse and biologically potent marine metabolites.[1] Most of these metabolites are nitrogen-containing compounds derived from amino acids, which can be classed into two major categories: (1) cyclic and acyclic peptides (2) and aromatic alkaloids. Some representative examples of the first group are the cytotoxic cyclic heptapeptides, such as mollamide[2] and cyclodidemnamide,[3] isolated from *Didemnum molle*, and the first sulfamic acid peptide guanidine derivatives, minalemines D−F, isolated from *Didemnum rodriguesi*.[4] Some recent examples of aromatic alkaloids are the novel predator-deterrent didemnimides A−D,[5] isolated from *Didemnum conchyliatum*, and the β-carbolines, didemnolines A−D.[6] Furthermore, other metabolites with miscellaneous structures have been found, including the HIV-1 protease inhibitor didemnaketals A and B,[7] and a number of enterocin derivatives.[8]

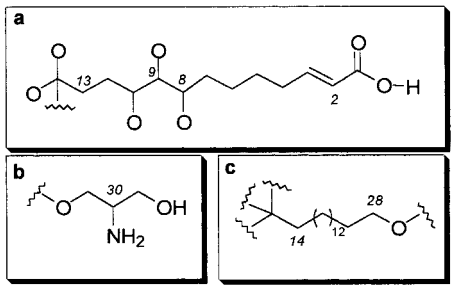
As part of our continuing search for biologically active secondary metabolites from ascidians, particularly those belonging to the *Didemnum* genus,[4] we became interested in the tunicate *Didemnum* sp., which was collected along the coast of Sulawesi Island (Indonesia), when we found a potent cytotoxic activity in its methanolic extract against several tumor cells. Here, we report the isolation and the structural elucidation of the first serinolipids from a marine organism, and these are 2-amino-1,3-propanediols linked to a hydroxylated α,β-unsaturated acid having an unusual 6,8-dioxabicyclo[3.2.1]octane structure (compounds **1**−**3**).

The tunicate samples of *Didemnum* sp. were extracted with methanol, and the extract, after filtration and concentration, was fractionated using our standard partition procedure.[9] The hexane partition was chromatographed on a silica gel flash column and by normal-phase HPLC followed by reversed-phase HPLC to give pheophetin a and pheophetin a′, previously isolated from the tunicate *Trididemnun solidum*.[10] The CH_2Cl_2 partition (150 mg), which exhibited the most potent activity (IC_{50} 0.25 μg/mL against P388, A549, and HT29 tumor cell lines), was chromatographed on a silica gel flash column and by reversed-phase HPLC to give pure compounds **1**−**3**.



1, R_1=Ac, R_2=H
2, R_1=H R_2=Et
3, R_1=R_2=H

The molecular formula of didemniserinolipid A (**1**), $C_{33}H_{59}NO_8$, was determined by positive HRFABMS of its pseudomolecular $[M + H]^+$ ion at *m*/*z* 598.4326 (Δ 0.7 mmu) and indicated the presence of five degrees of unsaturation in the molecule. Extensive NMR analysis (^{1}H NMR, ^{13}C NMR, DEPT, ^{1}H−^{1}H COSY, and HMQC) showed that **1** contained three quaternary carbons, six methine carbons (two olefinic, three attached to oxygen, and the last one linked to nitrogen), an indeterminable number of methylene carbons (three of which are attached to oxygen), and one methyl carbon. The presence of an acetate group in the molecule was easily deduced by the carbon and proton chemical shifts of the methyl group at $\delta_C = 21.3$ and $\delta_H = 2.10$ s and a carbonyl group at $\delta_C = 170.9$. The chemical shift of a quaternary carbon at $\delta = 109.4$ (C-13) indicated the ketal nature of that position. Additionally, interpretation of the spectral data from COSY and TOCSY led to three partial units (**a**−**c**).



Unit a. COSY experiments gave straightforward connectivities from H-2 to H-5 and from H-6 to H-12. TOCSY correlations from the olefinic proton H-3 to the methine H-8 allowed us to link the former spin systems. The *E* geometry of the Δ^2 double bond assigned to an α,β-unsaturated acid was derived from the coupling constant of 14.9 Hz between H-2 and H-3. The carbon and proton chemical shifts at C-10 ($\delta_C = 68.4$, $\delta_H = 4.68$ m) indicated that this carbon must be linked to the acetoxy group.

Unit b. The presence of a monosubstituted serinol moiety in the molecule was deduced from the ^{1}H−^{1}H

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1998**, *15*, 113−158. Davidson, B. S. *Chem. Rev.* **1993**, *93*, 1771−1791.
(2) Carroll, A. R.; Bowden, B. F.; Coll, J. C.; Hockless, D. C. R.; Skelton, B. W.; White, A. H. *Aust. J. Chem.* **1994**, *47*, 61−69.
(3) Toske, S. G.; Fenical, W. *Tetrahedron Lett.* **1995**, *36*, 8355−8358.
(4) Expósito, M. A.; López, B.; Fernández, R.; Vázquez, M. J.; Debitus, C.; Iglesias, T.; Jiménez, C.; Quiñoá, E.; Riguera, R. *Tetrahedron* **1998**, *54*, 7539−7550.
(5) Vervoort, H. C.; Richards-Gross, S. E.; Fenical, W.; Lee, A. Y.; Clardy, J. *J. Org. Chem.* **1997**, *62*, 1486-1490.
(6) Schumacher, R. W.; Davidson, B. S. *Tetrahedron* **1995**, *51*, 10125−10130.
(7) Potts, B. C.; Faulkner, D. J.; Chan, J. A.; Simolike, G. C.; Offen, P.; Hemling, M. E.; Francis, T. A. *J. Am. Chem. Soc.* **1991**, *113*, 6321−6322.
(8) Kang, H.; Jensen, P. R.; Fenical, W. *J. Org. Chem.* **1996**, *61*, 1543−1546.
(9) Rodríguez, J.; Nieto, R.; Jiménez, C. *J. Nat. Prod.* **1998**, *61*, 313−317.
(10) Rinehart, K. L.; Kishore, V.; Bible, K. C.; Sakai, R.; Sullins, D. W.; Li, K. *J. Nat. Prod.* **1988**, *51*, 1−21.

10.1021/jo9903914 CCC: $18.00
Published on Web 06/30/1999

Table 1. NMR Data for Didemniserinols (1−3) in Cl_3CD

	1		2		3	
C no.	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H
1	169.0 s		166.7 s			
2	121.4 d	5.77 d, 14.9	121.4 d	5.80 d, 15.2		5.85 d, 15.3
3	150.5 d	6.98 dt, 7.3; 14.9	149.1 d	6.95 dt, 7.4; 15.2		6.89 dt, 7.2; 15.3
4	32.2 t	2.17 m	32.0 t	2.20 c, 7.0	33.0 t	2.17 c, 7.2
5	27.9 t	1.43 m	27.9 t	1.47 m	27.1 t	1.50 m
6	25.2 t	1.27 m	25.3 t	1.26 m	25.9 t	
7	35.1 t	1.41/1.54 m	35.1 t	1.43/1.54 m	36.4 t	1.47/1.54 m
8	77.8 d	3.91 m	77.8 d	3.87 m	79.0 d	3.95 m
9	79.8 d	4.15 br s	82.4 d	4.06 br s	83.8 d	4.06 br s
10	68.4 d	4.68 br s	66.2 d	4.61 br s	66.9 d	3.65 br s
11	37.1 t	1.69/2.06 m	37.5 t	1.69/1.96 m	38.5 t	1.63/2.04 m
12	30.8 t	1.53 m/1.78 dt, 5.6; 12.6	30.1 t	1.53 m/1.79 dt, 5.4; 13.1	31.4 t	1.46 m/1.89 dt, 5.5; 12.9
13	109.4 s		109.5 s		110.6 s	
14	22.5 t	1.40/1.66 m	23.0 t	1.41/1.66 m	23.9 t	1.3−1.6 m
15−25	25.0−29.7 t	1.21/1.36 m	25.0−29.8 t	1.2−1.3	26.4−30.8 t	1.3−1.6 m
26	25.0−29.7 t	1.19 m	25.0−29.8 t	1.2−1.3	26.4−30.8 t	1.3−1.6 m
27	25.0−29.7 t	1.50 m	25.0−29.8 t	1.60 m	26.4−30.8 t	1.63 m
28	71.9 t	3.42 m	72.0 t	3.47 m	72.9 t	3.56 m
29	65.3 t	4.23/4.30 m	65.2 t	4.26/4.34 m	66.2 t	4.17 dd, 6.2, 11.5/4.25 dd, 3.4, 11.5
30	51.2 d	3.82 m	51.6 d	3.81 m	52.3 d	3.67 m
31	67.0 t	3.64 m	66.8 t	3.65 m	68.6 t	3.65/3.70 m
O**C**OCH$_3$	170.9 s					
OCO*CH*$_3$	21.3 c	2.10 s				
O*CH*$_2$CH$_3$			60.1 t	4.18 c, 7.1		
OCH$_2$*CH*$_3$			14.3 c	1.27 t		

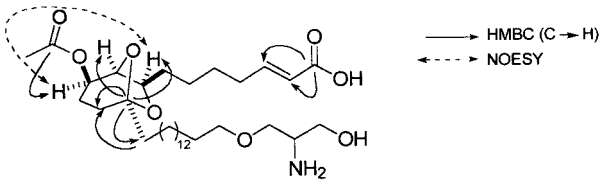


Figure 1. HMBC experiments and stereostructure of **1** deduced by NOESY.

COSY spectrum and proton and carbon chemical shifts at positions C-29 to C-31. Thus, the methine proton H-30 ($\delta_H = 3.82$ m), linked to a nitrogen-bearing carbon ($\delta_C = 51.2$), is coupled to two methylene protons linked to an oxygen-bearing carbon: H_2-29 ($\delta_H = 4.23/4.30$ m, $\delta_C = 65.3$) and H_2-31 ($\delta_H = 3.64$ m, $\delta_C = 67.0$).

Unit c. A long saturated chain with an indeterminable number of methylene carbons was deduced by the broad peak at $\delta_H = 1.0-1.3$ and $\delta_C = 22.9-29.8$ in the ^{1}H- and ^{13}C NMR spectra, respectively. HMQC and COSY experiments suggested this long saturated chain to be connected to oxygen at one end and to a quaternary carbon at the other end. Subtraction of the atoms of the former fragments (units **a** and **b** and the acetyl group) from the molecular formula of **1** indicated that this part of the molecule had to account for 15 methylene groups.

HMBC techniques revealed that these fragments were linked through cross-peaks due to $^2J_{CH}$ and $^3J_{CH}$ long-range coupling (Figure 1). Thus, long-range couplings between the carbonyl C-1 and the olefinic protons H-2 and H-3 were consistent with the presence of an α,β-unsaturated acid, which was also supported by the UV absorption at 215 nm and the IR band at 1730 cm^{-1}. The HMBC NMR correlations between protons and carbons from H-3 to H-12 (see Figure 1) allowed us to confirm the proposed structure for unit **a**. The existence of the 6,8-dioxabicyclo[3.2.1]octane system in **1** was deduced from the HMBC correlations between the quaternary ketal carbon assigned as C-13 ($\delta_C = 109.4$) to protons H-8, H-9, H_2-12, and H_2-14. The long-range correlation between the acetate carbonyl carbon at 170.9 ppm and H-10 (δ_H 4.68 br s) confirmed the location of this group at that position. Furthermore, HMBC cross-peaks between methylene protons H_2-12 and C-14 connected the long saturated chain (unit **c**) to the bicyclic system (unit **a**). At this point, the link between the monosubstituted serinol (unit **b**) and the long saturated chain through oxygen established the structure of the molecule as **1**.

^{1}H NMR coupling constants and NOESY data allowed us to determine the relative stereochemistry around the [3.2.1]bicyclic system. An intense NOESY cross-peak between H-8 and H-10 revealed their cis relationship. The almost zero coupling constants between H-9/H-8 and H-9/H-10 agree with the relative stereochemistry shown in Figure 1.

Compound **2** was isolated as an optically active amorphous solid that gave a pseudomolecular $[M + H]^+$ ion at *m/z* 584.4501 (Δ 2.5 mmu) in the positive HRFABMS, corresponding to the molecular formula $C_{33}H_{59}NO_8$. The ^{1}H and ^{13}C NMR spectra of **2** had many features in common with those of **1**, and indeed, ^{1}H NMR, COSY, HMQC, and HMBC experiments confirmed many of the same partial structures as determined for **1**. Distinctively different spectral chemical shifts allowed us to determine the structure of **2**. Indeed, the absence of the signals in the NMR spectra of **2** corresponding to the acetate group, which were present in **1**, suggested that the hydroxyl group at C-10 must be free. This was confirmed by the upfield chemical shift of C-10 (−2.2 ppm) and the downfield chemical shifts of C-9 (+2.6 ppm) and C-11 (+0.4 ppm) in **2** in relation to **1**. On the other hand, the presence of carbon and proton chemical shifts of an additional ethyl group in the NMR spectra of **2** suggested that the carboxylic acid in **1** must be esterified as an ethyl carboxylate in **2**. The presence of an α,β-unsaturated ester was confirmed by the carbon chemical shift of the carbonyl at $\delta_C = 166.7$ in the ^{13}C NMR spectrum and the infrared band at 1720 cm^{-1}. Furthermore, the HMBC

cross-peak between this carbonyl carbon and the methylene protons at $\delta_H = 4.18$, corresponding to the additional ethyl group, confirmed the presence of an ethyl carboxylate moiety in **2**. These data indicated that **2** is the ethyl carboxylate 10-deacetyl derivative of **1**. ROESY experiments on **2** revealed the same relative stereochemistry as in **1**.

Compound **3** was isolated in a small amount in comparison to **1** and **2**. Comparison of the chemical shifts of the ^{1}H and ^{13}C NMR, DEPT, and ^{1}H−^{1}H COSY spectra of **3** with those of **1** and **2** showed the presence of the same framework as in **1** and **2**, but with a free hydroxyl group at C-10, as in **2**, and the nonesterified carboxylic acid at C-1, as in **1**. This was confirmed by its FABMS, which showed $[M + H]^+$ and $[M + Na]^+$ pseudomolecular ions at *m/z* 556 and 578, respectively, which agree with the molecular formula $C_{31}H_{57}NO_7$.

Compounds **1**−**3** have structures without any precedent as they incorporate a serinol unit joined to a hydroxylated α,β-unsaturated acid having an unusual 6,8-dioxabicyclo[3.2.1]octane structure. Although the glycerolipids are very common in marine organisms, this is the first example of a monosubstituted serinol (2-aminoglycerol) derivative isolated from a marine source. One example of an aminoglycerol reported previously is rhapsamine, a 1,3-diaminoglycerol linked to a linear C28 polyene, which has been previously isolated from the Antarctic sponge *Leucetta leptorhapsis*.[11] On the other hand, the unusual 6,8-dioxabicyclo[3.2.1]octane substructure is present in very potent toxins such as palytoxins[12] from the soft coral *Palythoa toxica*, pinatoxins from the bivalve *Pinna muricata*,[13] and rubrobramide, which is a cytotoxic and phytotoxic metabolite from *Cladobotryum rubrobrunnescens*.[14]

Although the CH_2Cl_2 fraction was very active, compounds **1**−**3** showed no cytotoxicity activity against P388, A549, and HT29 tumor cell lines ($IC_{50} > 20$ μg/mL).

Experimental Section

Collection, Extraction, and Isolation Procedures. Ascidian was collected using scuba (16 M) along the coast of Sulawesi (0° 58.113′ N, 126° 09.417′ E), Indonesia, in 1996 and kept frozen until used. A voucher specimen (UDC no. 96040) is held in the Dpto. de Química Fundamental e Industrial, Universidade da Coruña, A Coruña, Spain. The samples (1.5 kg) were extracted three times with methanol and the combined methanol extracts partitioned between CH_2Cl_2 and H_2O (1:1). The fraction soluble in CH_2Cl_2 was evaporated under reduced pressure and partitioned between 10% aqueous MeOH (400 mL) and hexane (2 × 400 mL). Water was added to the polar fraction until the mixture became 50% aqueous MeOH, and this was then extracted with CH_2Cl_2 (3 × 400 mL). After evaporation, the combined organic layers yielded 3.4 g of product from the hexane fraction and 150 mg of product from the CH_2Cl_2 fraction. A 1.6 g portion of the product from the hexane fraction was purified by flash column chromatography (silica gel 230−400 mesh, eluting with hexane/EtOAc mixtures of increasing polarity) followed by normal-phase (hexane/EtOAc 20:80) and reversed-phase (acetonitrile/THF 95:5) HPLC to give pheophetin a (16 mg) and pheophetin a′ (4 mg). The product obtained from the methylene chloride fraction (150 mg) was separated by flash column chromatography (silica gel 230−400 mesh, eluting with CH_2Cl_2/MeOH mixtures of increasing polarity) to give several fractions. Purification of the individual fractions was achieved by C18 reversed-phase HPLC using H_2O/MeOH (2:8) to give compounds **1** (7 mg, 0.46×10^{-3}% wet weight), **2** (4 mg, 0.26×10^{-3}% wet weight), and **3** (1.4 mg, 0.09×10^{-3}% wet weight).

Didemniserinolipid A (1): colorless solid; $[\alpha]^{24}_D$ +12.5° (*c* = 0.085, MeOH); IR ν_{max} (film) 3485, 2990, 1730 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 201 (4.39), 215 (3.96) nm; ^{1}H and ^{13}C NMR, see Table 1; (+) LRFABMS (thioglycerol) *m/z* (rel intensity) 598 ($[M + H]^+$; 40), 215 (100); (+) HRFABMS *m/z* 598.4326 $[M + H]^+$ (calcd for $C_{33}H_{60}NO_8$, 598.4319).

Didemniserinolipid B (2): colorless solid; $[\alpha]^{24}_D$ +10.3° (*c* = 0.225, $CHCl_3$); IR ν_{max} (film) 3485, 2990, 1720 cm^{-1}; UV (MeOH) λ_{max} (log ϵ) 206 (4.10) nm; ^{1}H and ^{13}C NMR, see Table 1; (+) LRFABMS *m/z* (rel intensity) 584 ($[M + H]^+$; 100); (+) HRFABMS *m/z* 584.4501 $[M + H]^+$ (calcd for $C_{33}H_{62}NO_7$, 584.4526).

Didemniserinolipid C (3): colorless solid; $[\alpha]^{24}_D$ +32.3° (*c* = 0.075, MeOH); ^{1}H and ^{13}C NMR, see Table 1; (+) LRFABMS *m/z* (rel intensity) 556 ($[M + H]^+$; 100), 578 ($[M + Na]^+$; 30).

Acknowledgment. Taxonomic information was generously provided by Dr. Elsa Vazquez (Departamento de Ecología y Biología Animal, Facultad de Ciencias, Universidade de Vigo, 36200 Vigo, Spain). We appreciate the assistance and support in the collection of *Didemnum* sp. of Prof. Phillip Crews (University of Santa Cruz, CA). We would like to thank CACTI (Universidad de Vigo, Spain) for recording HRFAB mass spectra. Xunta de Galicia (XUGA-10301A97) provided financial support. We are grateful to BIOMAR S.A. for the pharmacological assays. N.G. acknowledges a fellowship from Xunta de Galicia.

Supporting Information Available: ^{1}H, ^{13}C, DEPT, and 2D NMR spectra for compounds **1**−**3**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO9903914

(11) Jayatilake, G. S.; Baker, B. J.; MacClintock, J. B. *Tetrahedron Lett.* **1997**, *38*, 7507−7510.

(12) Armstrong, R. W.; Beau, J. M.; Cheon, S. H.; Christ, W. J.; Fujioka, H.; Ham, W. H.; Hawking, L. D.; Jin, H.; Kang, S. H.; Kishi, Y.; Martinelli, M. J.; McWhorter W. W.; Mizuno, M.; Nakata, M.; Stutz, A. E.; Talamas, F. X.; Taniguchi, M.; Tino, J. A.; Ueda, K.; Uenishi, J.; White, J. B.; Yonaga, M. *J. Am. Chem. Soc.* **1989**, *111*, 7530−7533.

(13) McCauley, J. A.; Nagasawa, K.; Lander, P. A.; Mischke, S. G.; Semones, M. A.; Kishi, Y. *J. Am. Chem. Soc.* **1998**, *120*, 7647−7648.

(14) Wagner, C.; Anke, H.; Sterner, O. *J. Nat. Prod.* **1998**, *61*, 501−502.