

Notes

Stolonic Acids A and B, New Cytotoxic Cyclic Peroxides from an Indian Ocean Ascidian *Stolonica* Species

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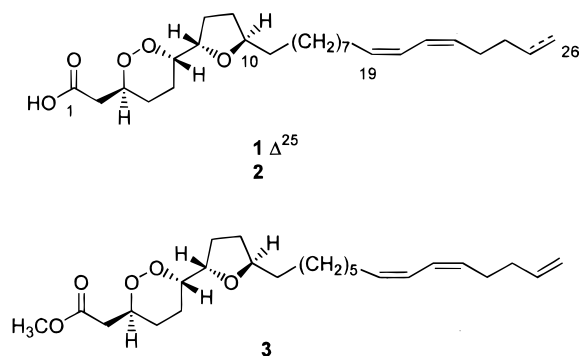
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Two new 3,6-epidioxy-7,10-tetrahydrofurano C₂₆ unsaturated fatty acids, stolonic acids A (**1**) and B (**2**), were isolated from a previously undescribed ascidian species, *Stolonica* sp. collected off the Maldive Islands in the Indian Ocean. The structures and relative stereochemistry of **1** and **2** were determined using conventional spectroscopic methods. Both compounds exhibited antiproliferative activity against selected human melanoma and ovarian tumor cell lines, with IC₅₀ values of approximately 0.05–0.1 μg/mL.

The organic extract of a previously undescribed species of ascidian from the genus *Stolonica*, collected off the Maldive Islands in the northern Indian Ocean, produced a distinctive pattern of differential cytotoxicity in the U.S. National Cancer Institute (NCI)'s 60-cell primary anti-tumor screen.¹ Antiproliferative bioassay-guided fractionation of this extract yielded two new fatty acid-derived cyclic peroxides, stolonic acids A (**1**) and B (**2**). These new metabolites are structural homologues of stolonoxide A, a C₂₄ fatty acid peroxide recently isolated as a methyl ester (**3**) from *S. socialis*.² Prior to the isolation of compounds **1–3**, all peroxy fatty acid derivatives described from marine sources had been confined to sponges of the genera *Chondrilla*, *Plakortis*, and *Xestospongia*.³ It is now clear that *Stolonica* ascidians are an additional source of these aliphatic endoperoxides.

The CH₂Cl₂–MeOH (1:1) extract of *Stolonica* sp. was subjected to a solvent–solvent partitioning protocol⁴ that concentrated the cytotoxic activity primarily into the EtOAc-soluble fraction. Early in our investigation of this material, it was apparent that we were dealing with a mixture of amphipathic carboxylic acids with challenging chromatographic properties. Ultimately, sequential Sephadex LH-20 chromatographic separations employing CH₂Cl₂–hexane–MeOH (5:2:1) and then CH₂Cl₂–MeOH (9:1), followed by repetitive reversed-phase C₁₈ HPLC eluted with MeOH–H₂O (9:1) and CH₃CN–H₂O (85:15) provided purified stolonic acids A (**1**) and B (**2**). The ¹H NMR spectra of compounds **1** and **2**, isolated as pale yellow, optically active oils, suggested these compounds were homologous fatty acid derivatives closely related to stolonoxide A methyl ester (**3**).² A molecular formula of C₂₆H₄₂O₅ was established



for stolonic acid A (**1**) from HRFABMS data, $[M + H]^+$ m/z 435.3089. Of the 26 carbon signals, 24 were clearly resolved in the ¹³C NMR spectrum (DMSO-*d*₆)⁵ of **1** (Table 1), while two resonances (δ 29.0) were overlapped. Data from DEPT and HSQC experiments allowed assignment of a deshielded carbonyl, four oxymethines, five olefinic methines, one olefinic methylene, and 15 aliphatic methylene carbon atoms. The single carbonyl, assigned to a carboxylic acid moiety (IR 3400 and 1709 cm⁻¹), and the six olefinic carbons accounted for four of the six degrees of unsaturation implied by the molecular formula. The remaining two degrees of unsaturation thus required compound **1** to be bicyclic.

COSY NMR data, supported by TOCSY and HSQC–TOCSY experiments, established a contiguous proton coupling sequence from the diastereotopic, deshielded methylene protons at C-2 (δ 2.21 and 2.40) through to the C-12 methylene protons (2H, δ 1.22). The 2D NMR data unequivocally placed the four oxymethine protons (δ 4.29, 3.87, 3.67, and 3.75) at C-3, C-6, C-7, and C-10, respectively, and thus confirmed that stolonic acid A (**1**) possessed the same novel 3,6-epidioxy-7,10-tetrahydrofurano structural motif found in stolonoxide A. A prominent C₂₀H₃₃O fragment ion in the HRFABMS spectrum of **1** (m/z 289.2535), arising from cleavage of the C-6,C-7 bond,

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Table 1. ^{13}C and ^1H NMR Data^a for Compounds **1** and **3**²

C/H	1 ^b		1 ^c		3 ^b	
	^{13}C	^1H (J/Hz)	^{13}C	^1H (J/Hz)	^{13}C	^1H (J/Hz)
1	173.9 (s)		171.2 (s)		170.0 (s)	
2	37.8 (t)	2.40 dd (5.6, 15.6) 2.48 dd (7.5, 15.6)	38.2 (t)	2.21 dd (4.8, 16.1) 2.40 dd (8.7, 16.1)	38.4 (t)	2.37 dd (5.4, 15.6) 2.47 dd (7.5, 15.6)
3	77.3 (d)	4.52 m	77.6 (d)	4.29 m	77.6 (d)	4.54 m
4	28.8 (t)	1.56 m 1.93 m	28.3 (t)	1.47 m 1.82 m	29.1 (t)	1.57 m 1.91 m
5	25.1 (t)	1.72 m 1.77 m	24.7 (t)	1.50 m 1.63 m	25.2 (t)	1.78 m
6	83.8 (d)	4.06 m	83.4 (d)	3.87 m	83.8 (d)	4.05 m
7	78.5 (d)	3.85 q	78.0 (d)	3.67 q	78.6 (d)	3.87 q
8	27.7 (t)	1.76 m 1.93 m	27.5 (t)	1.61 m 1.88 m	27.7 (t)	1.77 m 1.91 m
9	31.5 (t)	1.41 m 1.97 m	31.3 (t)	1.33 m 1.93 m	31.7 (t)	1.43 m 1.97 m
10	79.9 (d)	3.85 m	78.7 (d)	3.75 m	79.9 (d)	3.87 m
11	35.4 (t)	1.35 m 1.56 m	35.2 (t)	1.31 m 1.43 m	35.6 (t)	1.36 m 1.57 m
12	29.9 (t)	1.25 m	25.6 (t)	1.22 m	29.6 (t)	1.29 m
13	29.9 (t)	1.25 m	28.9 (t)	1.22 m	29.6 (t)	1.29 m
14	29.9 (t)	1.25 m	29.0 (t)	1.22 m	29.6 (t)	1.29 m
15	29.9 (t)	1.25 m	29.0 (t)	1.22 m	29.6 (t)	1.29 m
16	29.9 (t)	1.25 m	28.5 (t)	1.22 m	27.7 (t)	1.36 m
17	25.9 (t)	1.35 m	28.7 (t)	1.32 m	132.4 (d)	2.15 m
18	27.4 (t)	2.14 m	26.7 (t)	2.11 m	123.5 (d)	5.45 m
19	132.6 (d)	5.43 m	132.0 (d)	5.44 m	124.0 (d)	6.23 bot (7.5)
20	123.6 (d)	6.21 m	123.5 (d)	6.23 m	132.3 (d)	6.24 bot (7.5)
21	124.1 (d)	6.25 m	123.8 (d)	6.23 m	26.0 (t)	5.44 m
22	130.8 (d)	5.42 m	130.7 (d)	5.44 m	27.7 (t)	2.27 m
23	26.7 (t)	2.25 m	26.2 (t)	2.22 m	138.2 (t)	2.15 m
24	33.5 (t)	2.13 m	33.1 (t)	2.08 m	114.7 (t)	5.82 m
25	138.3 (d)	5.80 m	137.9 (d)	5.78 (m)		4.97 dd (17.9)
26	114.9 (t)	4.95 dd (17.5) 5.01 dd (10.1)	115.1 (t)	4.94 dd (17.1) 5.02 dd (10.2)		5.03 dd (10.0)
OMe					51.9 (q)	3.68 s

^a Spectra were acquired at 500 MHz for ^1H and 125 MHz for ^{13}C , ^{13}C multiplicities inferred from the DEPT pulse sequence. ^b CDCl_3 . ^c $\text{DMSO}-d_6$.

further supported this structural assignment. Close similarity between the ^1H and ^{13}C NMR data of **1** (Table 1), and those reported for the methyl ester **3**,² suggested that **1** was a higher homologue of stolonoxide A that differed only by the addition of two methylene groups in the acyclic portion of the molecule.

Confirmation of the relative stereochemistry of the 3,6-epidioxo ring in **1** was well supported by 1D and 2D NOESY data. NOE correlations between H-3 and both the equatorial H-4e (δ 1.82) proton and the axial H-5a (δ 1.50) proton established H-3 as axial. In a similar manner, the axial orientation of H-6 was defined by an NOE interaction with the axial H-4a (δ 1.47). Thus, the alkyl substituents at C-3 and C-6 in compound **1** were both equatorial. The trans geometry of the tetrahydrofuran ring was tentatively suggested by an absence of NOEs between H-7 (δ 3.72) and H-10 (δ 3.75). Assignment of a threo relative configuration for the substituents at C-6 and C-7 in stolonoxide A (**1**) was made by comparison of the ^1H and ^{13}C chemical shifts at positions 6, 7, and 10 in **1** with the analogous positions in stolonoxide A methyl ester (**3**). The optical rotation of **1** ($[\alpha]_D -30.5^\circ$, c 0.43, CHCl_3) was in close agreement with the rotation reported for **3** ($[\alpha]_D -33.3^\circ$, c 0.1, CHCl_3),² thus we propose that **1** and **3** share the same stereochemistry at their four chiral centers.

The positions of the acyclic diene and terminal olefin group in **1** were confirmed by a combination of TOCSY and HMBC data, while the *Z* geometries of the Δ^{19} and Δ^{21} olefins followed from NOEs observed between the olefinic protons H-20 and H-21 and the allylic protons on C-23 and

C-18, respectively. The upfield chemical shifts of the allylic carbons C-18 (δ 26.7) and C-23 (δ 26.2) further supported the *Z,Z* configuration assigned to the diene in **1** (represented here in the compound's more energetically favored *S-trans* conformation). The configuration of the Δ^{19} , Δ^{21} diene in **1** is in accordance with the analogous vinyl moieties in **3**,² and therefore stolonoxide A (**1**) differs from stolonoxide A only by having two additional methylenes inserted between the tetrahydrofuran ring and the conjugated diene in the side chain.

A molecular formula of $\text{C}_{26}\text{H}_{44}\text{O}_5$, established for stolonoxide B (**2**) from HRFABMS data, together with the absence of the terminal olefinic proton resonances and the presence of a methyl signal (δ 0.85, 3H, t, $J = 3.7$ Hz) in the ^1H NMR spectrum of **2**, suggested that **2** was the dihydro analogue of **1**. NOESY and selective 1D NOE experiments were used to define the stereochemistry of **2**. The NOE data of **2** were consistent with those of **1**, which indicated that stolonoxide B (**2**) and stolonoxide A (**1**) share the same relative configuration at each of their four chiral centers.

Stolonoxide A (**1**) and B (**2**) exhibited potent cytotoxic activity against LOX (melanoma) and OVCAR-3 (ovarian) human tumor cell lines. In a two-day in vitro assay, experimental details of which have been described previously,⁶ compounds **1** and **2** provided IC_{50} values of approximately 0.1 $\mu\text{g}/\text{mL}$ with OVCAR-3, and 0.05 to 0.09 $\mu\text{g}/\text{mL}$ with LOX.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Beckman DU-640 spectrophotometer, and IR spectra were obtained on a Perkin-Elmer Spectrum 2000 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. HRMS were obtained on a JEOL SX102 spectrometer. NMR data were recorded on a Varian INOVA-500 spectrometer, and HPLC separations were performed on a Waters 600E system using a Waters 990 photo-diode array detector.

Animal Material. Colonies of the small orange ascidian *Stolonica* sp. were collected in September 1997, from a depth of 18 m off the North Male Atoll of the Maldives in the northern Indian Ocean. The ascidian has been identified as a new species by C. Monniot (personal communication), and a photograph is available in the Supporting Information section. A voucher specimen (voucher # OCDN5257) for this collection is maintained at the Smithsonian Institute.

Extraction and Isolation. Frozen ascidian samples (212 g, wet wt) were ground to a fine powder and extracted with H₂O. The H₂O was removed by centrifugation, and the remaining solids were lyophilized and then sequentially extracted with CH₂Cl₂-MeOH (1:1) followed by 100% MeOH. Solvent was removed from the combined organic extracts in vacuo to yield 3.1 g of material. A 1.0 g aliquot of the organic extract was separated by a solvent-solvent partitioning protocol,⁴ which concentrated the antiproliferative activity into the EtOAc-soluble fraction. The active material (130 mg) was further fractionated on Sephadex LH-20 eluted with CH₂Cl₂-hexane-MeOH (5:2:1) and then LH-20 eluted with CH₂Cl₂-MeOH (9:1). Repeated C₁₈ HPLC purification using MeOH-H₂O (9:1) and CH₃CN-H₂O (85:15) solvent systems provided 10 mg of stolononic acid A (**1**) and 7 mg of stolononic acid B (**2**).

Stolononic acid A (1): pale yellow oil; [α]_D -30.5° (c 0.43, CHCl₃); UV (MeOH-CHCl₃; 1:1) λ_{\max} 239 (ϵ 4855) nm; IR ν_{\max} (KBr) 3400 (br), 2921, 2854, 1709, 1444, 1191 cm⁻¹; ¹H and ¹³C NMR, see Table 1; FABMS [M + H]⁺ *m/z* 435.3089 (calcd for C₂₆H₄₃O₅, 435.3110).

Stolononic acid B (2): pale yellow oil; [α]_D -18.4° (c 0.42, CHCl₃); UV (MeOH-CHCl₃; 1:1) λ_{\max} 239 (ϵ 8200) nm; IR ν_{\max} (KBr) 3400 (br), 2922, 2857, 1698, 1458, 1192 cm⁻¹; ¹H NMR (500 MHz, DMSO-*d*₆) δ 6.21 (1H, m, H-20), 6.21 (1H, m, H-21), 5.42 (1H, m, H-19), 5.42 (1H, m, H-22), 4.29 (1H, m, H-3), 3.87 (1H, m, H-6), 3.74 (1H, m, H-10), 3.68 (1H, m, H-7), 2.39 (1H, dd, *J* = 16.1, 8.7 Hz, H-2), 2.20 (1H, dd, *J* = 16.1, 4.8 Hz, H-2),

2.12 (2H, m, 2H-23), 2.10 (2H, m, 2H-18), 1.92 (1H, m, H-9), 1.88 (1H, m, H-8), 1.82 (1H, m, H-4), 1.63 (1H, m, H-5), 1.61 (1H, m, H-8), 1.50 (1H, m, H-5), 1.47 (1H, m, H-4), 1.43 (1H, m, H-11), 1.33 (1H, m, H-9), 1.32 (2H, m, 2H-17), 1.31 (1H, m, H-11), 1.30 (2H, m, 2H-24), 1.28 (2H, m, 2H-25), 1.24 (10H, m, 2H-12 to 2H-16), 0.85 (3H, t, *J* = 3.7, 3H-26); ¹³C NMR (125 MHz, DMSO-*d*₆) δ 171.2 (C-1), 131.7 (C-22), 131.6 (C-19), 123.6 (C-20), 123.6 (C-21), 83.4 (C-6), 78.8 (C-10), 78.0 (C-7), 77.6 (C-3), 38.1 (C-2), 35.2 (C-11) C-4, 33.3 (C-24), 31.1 (C-9), 29.0 (C-14), 29.0 (C-15), 28.9 (C-13), 28.7 (C-17), 28.5 (C-16), 28.3 (C-4), 27.5 (C-8), 26.7 (C-18), 26.5 (C-23), 25.6 (C-12), 24.7 (C-5), 21.6 (C-25), 13.7 (C-26); FABMS [M + H]⁺ *m/z* 437.3250 (calcd for C₂₆H₄₅O₅, 437.3267)

Bioassay. DMSO solutions of the chromatography fractions and purified stolononic acids were evaluated for antiproliferative properties using LOX (melanoma) and OVCAR-3 (ovarian) human tumor cell lines. Experimental details of the 2-day, in vitro assay have been previously described.⁶

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Supporting Information Available: This material is available free of charge via the Internet at <http://pubs.acs.org>.

References and Notes

- Boyd, M. R. In *Cancer Drug Discovery and Development; Vol. 2, Drug Development: Preclinical Screening, Clinical Trial and Approval*; Teicher, B. A., Ed. Humana: Totowa, NJ, 1997; pp 23-42.
- Fontana, A.; Gonzalez, M. C.; Gavagnin, M.; Templado, J.; Cimino, G. *Tetrahedron Lett.* **2000**, *41*, 429-432.
- Casteel, D. A. *Nat. Prod. Rep.* **1999**, *16*, 55-73 and Casteel, D. A. *Nat. Prod. Rep.* **1992**, *9*, 289-312.
- Van Wagenen, B. C.; Cardellina, J. H., II; Lidert, Z. C.; Swithenbank, C. *J. Org. Chem.* **1993**, *58*, 335-337.
- Although it is possible to acquire NMR data for the stolononic acids in either CDCl₃ or DMSO-*d*₆, the latter solvent gave enhanced ¹³C signal dispersion and better solubility.
- Bokesch, H. R.; Blunt, J. W.; Westergaard, C. K.; Cardellina, J. H., II; Johnson, T. R.; Michael, J. A.; Hollingshead, M. G.; Boyd, M. R. *J. Nat. Prod.* **1999**, *62*, 633-635.

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