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ARTICLE *in* JOURNAL OF NATURAL PRODUCTS · NOVEMBER 2000

Impact Factor: 3.8 · DOI: 10.1021/np000158x · Source: PubMed

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

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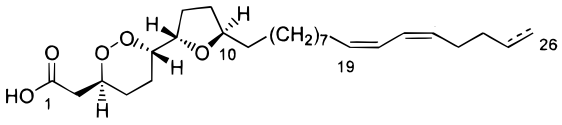
Received April 10, 2000

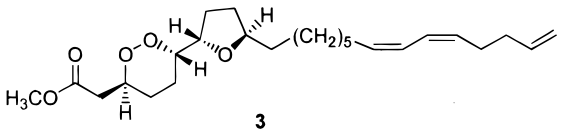
Two new 3,6-epidioxy-7,10-tetrahydrofurano C_{26} unsaturated fatty acids, stolonic acids A (**1**) and B (**2**), were isolated from a previously undescribed ascidian species, *Stolonica* sp. collected off the Maldive Islands in the Indian Ocean. The structures and relative stereochemistry of **1** and **2** were determined using conventional spectroscopic methods. Both compounds exhibited antiproliferative activity against selected human melanoma and ovarian tumor cell lines, with IC_{50} 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 antitumor 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 C_{24} 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 CH_2Cl_2−MeOH (1:1) extract of *Stolonica* sp. was subjected to a solvent−solvent partitioning protocol[4] 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 CH_2Cl_2−hexane−MeOH (5:2:1) and then CH_2Cl_2−MeOH (9:1), followed by repetitive reversed-phase C_{18} HPLC eluted with MeOH−H_2O (9:1) and CH_3CN−H_2O (85:15) provided purified stolonic acids A (**1**) and B (**2**). The ^{1}H 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 $C_{26}H_{42}O_5$ was established



1 Δ^{25}
2



3

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 ^{13}C 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 $C_{20}H_{33}O$ 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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10.1021/np000158x CCC: $19.00
Published on Web 08/09/2000

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

	1[b]		**1**[c]		**3**[b]	
C/H	^{13}C	^{1}H (*J* Hz)	^{13}C	^{1}H (*J* Hz)	^{13}C	^{1}H (*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 1.36 m
16	29.9 (t)	1.25 m	28.5 (t)	1.22 m	27.7 (t)	2.15 m
17	25.9 (t)	1.35 m	28.7 (t)	1.32 m	132.4 (d)	5.45 m
18	27.4 (t)	2.14 m	26.7 (t)	2.11 m	123.5 (d)	6.23 bot (7.5)
19	132.6 (d)	5.43 m	132.0 (d)	5.44 m	124.0 (d)	6.24 bot (7.5)
20	123.6 (d)	6.21 m	123.5 (d)	6.23 m	132.3 (d)	5.44 m
21	124.1 (d)	6.25 m	123.8 (d)	6.23 m	26.0 (t)	2.27 m
22	130.8 (d)	5.42 m	130.7 (d)	5.44 m	27.7 (t)	2.15 m
23	26.7 (t)	2.25 m	26.2 (t)	2.22 m	138.2 (t)	5.82 m
24	33.5 (t)	2.13 m	33.1 (t)	2.08 m	114.7 (t)	4.97 dd (17.9) 5.03 dd (10.0)
25	138.3 (d)	5.80 m	137.9 (d)	5.78 (m)		
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)		
OMe					51.9 (q)	3.68 s

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

further supported this structural assignment. Close similarity between the ^{1}H 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-epidioxy 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 stolonic acid A (**1**) was made by comparison of the ^{1}H 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°, *c* 0.43, $CHCl_3$) was in close agreement with the rotation reported for **3** ($[\alpha]_D$ −33.3°, *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 stolonic acid 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 $C_{26}H_{44}O_5$, established for stolonic acid 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 ^{1}H 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 stolonic acid B (**2**) and stolonic acid A (**1**) share the same relative configuration at each of their four chiral centers.

Stolonic acids 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 μg/mL with OVCAR-3, and 0.05 to 0.09 μg/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 photodiode 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 Maldive Islands 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 # 0CDN5257) 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 H_2O. The H_2O was removed by centrifugation, and the remaining solids were lyophilized and then sequentially extracted with CH_2Cl_2−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 CH_2Cl_2−hexane−MeOH (5:2:1) and then LH-20 eluted with CH_2Cl_2−MeOH (9:1). Repeated C_{18} HPLC purification using MeOH−H_2O (9:1) and CH_3CN−H_2O (85:15) solvent systems provided 10 mg of stolonic acid A (**1**) and 7 mg of stolonic acid B (**2**).

Stolonic acid A (1): pale yellow oil; $[\alpha]_D$ −30.5° (*c* 0.43, $CHCl_3$); UV (MeOH−$CHCl_3$; 1:1) λ_{max} 239 (ϵ 4855) nm; IR ν_{max} (KBr) 3400 (br), 2921, 2854, 1709, 1444, 1191 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; FABMS $[M + H]^+$ *m*/*z* 435.3089 (calcd for $C_{26}H_{43}O_5$, 435.3110).

Stolonic acid B (2): pale yellow oil; $[\alpha]_D$ −18.4° (*c* 0.42, $CHCl_3$); UV (MeOH−$CHCl_3$; 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); ^{13}C 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 $C_{26}H_{45}O_5$, 437.3267)

Bioassay. DMSO solutions of the chromatography fractions and purified stolonic 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]

Acknowledgment. The authors thank the Coral Reef Research Foundation for the collections, D. J. Newman for coordinating collections, T. McCloud for extractions, D. Scuderio and A. Monks for antitumor screening, and T. Johnson for antiproliferative evaluations of the chromatography fractions and pure compounds. MTD-C acknowledges sabbatical support from the South African National Research Foundation, Rhodes University and an NIH Visiting Scientist Scholarship.

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

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NP000158X