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Ascidiatrienolides A-C, Novel Lactonized Eicosanoids from the Colonial Marine Ascidian *Didemnum candidum*

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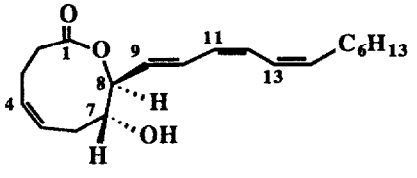
Summary: Three new eicosanoids (**1-3**) possessing a unique 9-membered ring lactone and novel sites of oxidation have been isolated from the colonial marine ascidian *Didemnum candidum* (F. Didemnidae). These compounds further illustrate some intriguing differences in the pathways of eicosanoid production between mammals and several marine invertebrates.

In mammalian tissues, the enzymatic oxidation products of eicosapolyenoic fatty acids (EFAs), especially arachidonic acid, are powerful mediators of diverse physiological events such as tumor growth, inflammation, hypersensitivity reactions, bloodclotting and smooth muscle contraction[1]. Prostaglandins and thromboxanes arise from a cyclooxygenase-derived endoperoxide while various lipoxygenases produce hydroperoxypolyenoic acids (HPETEs) which are converted to leukotrienes and lipoxins. Corey and coworkers[2] recently demonstrated that prostanoids present in marine octocorals are produced via a lipoxygenase type pathway rather than the traditional cyclooxygenase oxidation. This intriguing difference in the pathway of prostanoid production between mammals and some marine invertebrates suggests other unique pathways producing eicosanoids may exist in marine organisms.

In this paper, we wish to report on the chemical investigation of the colonial marine ascidian, *Didemnum candidum*, which contains several new natural products (**1-3**) that appear to have arisen through a novel 7-lipoxygenation of a C_{20} fatty acid. These compounds, isolated as 9-membered ring lactones, further illustrate the uniqueness of eicosanoid production in marine invertebrates.



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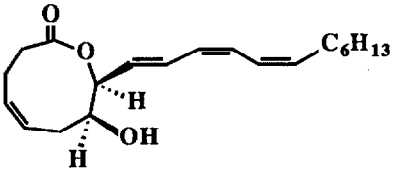

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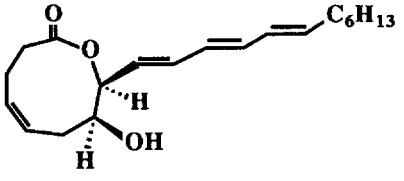

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Table 1. ^{13}C and ^{1}H NMR Assignments for Ascidiatrienolides A-C (1-3)[a].

	ascidiatrienolide A (1)		ascidiatrienolide B (2)		ascidiatrienolide C (3)	
carbon #	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C[b]	^{1}H
1	172.4 C		172.2 C		--	
2	34.8 CH_2	1.73,1H,m	35.3 CH_2	1.36,1H,m	35.7 CH_2	1.35,1H,m
		1.89,1H,m		1.96,1H,m		1.74,1H,m
3	26.4 CH_2	2.25,1H,m	26.9 CH_2	1.80,1H,m	26.9 CH_2	1.51,1H,m
		2.34,1H,m		2.17,1H,m		2.10,1H,m
4	134.2 CH	5.38,1H,ddd(10.6,10.0,6.5)	134.2 CH	5.31,1H,ddd(11.0,10.8,5.2)	134.4 CH	5.24,1H,ddd(10.9,10.9,4.0)
5	125.2 CH	5.61,1H,ddd(10.7,10.6,4.7)	124.6 CH	5.14,1H,ddd(10.8,9.0,4.0)	124.6 CH	5.07,1H,ddd(10.9, 10.0, 4.0)
6	25.4 CH_2	2.15,1H,ddd(13.0,4.7,4.0)	25.7 CH_2	2.12,1H,ddd(13.5,5.5,4.0)	25.8 CH_2	2.00,1H,m
		2.78,1H,ddd(13.0,10.7,2.3)		2.55,1H,ddd(13.5,9.5,9.0)		2.51,1H,ddd(12.4,12.2,10.0)
7	72.1 CH	3.85,1H,m(9.0,4.0,2.3)	71.5 CH	3.49,1H,m(9.5,5.5,2.0)	71.5 CH	3.44,1H,bm
8	76.3 CH	4.95,1H,dd(9.0,6.0)	76.3 CH	5.23,1H,dd(6.7,2.0)	76.3 CH	5.15,1H,dd(6.7,4.0)
9	128.2 CH	5.85,1H,dd(15.2,6.0)	128.5 CH	5.88,1H,dd(15.3,6.7)	128.2 CH	5.72,1H,dd(15.5,6.7)
10	124.0 CH	6.75,1H,dd(15.2,11.4)	124.1 CH	7.05,1H,dd(15.3,11.4)	129.9 CH	6.46,1H,dd(15.5,9.0)
11	131.5 CH	6.01,1H,dd(11.4,11.2)	130.6 CH	6.04,1H,dd(11.4,11.0)	132.5 CH	6.09,1H,d(9.0)
12	129.6 CH	6.30,1H,dd(11.6,11.2)	129.2 CH	6.34,1H,dd(11.6,11.0)	134.8 CH	6.07,1H,d(10.0)
13	126.2 CH	6.48,1H,dd(11.8,11.6)	125.9 CH	6.55,1H,dd(11.6,10.7)	130.9 CH	6.01,1H,m(14.6)
14	131.8 CH	5.55,1H,ddd(11.8,10.5,8.5)	132.6 CH	5.43,1H,ddd(10.7,9.4,9.0)	136.2 CH	5.53,1H,ddd(14.6,7.2,7.2)
15	29.5 CH_2	2.20,2H,m	29.6 CH_2	2.10,2H,m	33.3 CH_2	1.86-1.96,2H,m
16	27.7 CH_2	1.25-1.40,2H,m	27.8 CH_2	1.14-1.33,2H,m	29.5 CH_2	1.10-1.35,2H,m
17	31.7 CH_2	1.25-1.40,2H,m	31.7 CH_2	1.14-1.33,2H,m	31.8 CH_2	1.10-1.35,2H,m
18	33.0 CH_2	1.25-1.40,2H,m	32.7 CH_2	1.14-1.33,2H,m	32.7 CH_2	1.10-1.35,2H,m
19	22.8 CH_2	1.25-1.40,2H,m	22.9 CH_2	1.14-1.33,2H,m	22.8 CH_2	1.10-1.35,2H,m
20	14.1 CH_3	0.88,3H,t(6.7)	14.2 CH_3	0.86,3H,t(6.7)	14.2 CH_3	0.81,3H,t(6.7)

[a]Assignments are by analogy to model compounds. ^{1}H (360MHz) and ^{13}C (50MHz) NMR spectra were recorded in benzene-d_6 with TMS as internal standard. Proton coupling constants are given in Hertz. ^{13}C NMR resonance multiplicities were determined from DEPT sequence experiments.

[b]Only DEPT sequence experiments were performed on **3**, hence the carbonyl band was not observed.

As part of our continuing work on the anti-inflammatory properties of metabolites from marine organisms, our interest was directed to *D. candidum* whose crude organic extract showed strong inhibition of phospholipase A_2 in *in vitro* field assays. *D. candidum*, which is devoid of symbiotic algae, was collected while snorkling near Big Pine Key, Florida. The acetone extract of the lyophilized ascidian was initially fractionated by vacuum flash chromatography over TLC grade silica-gel with the final purification by Si HPLC (5% ethyl acetate/isooctane), yielding compounds **1-3**, each as less than 0.02% dry weight of the ascidian. Compounds **1-3** were not active in inducing or inhibiting inflammation in several standard assays[3]. The inhibitory effect of the fresh ascidian crude extract against phospholipase A_2 could have been due to the presence of highly unstable lipoxygenase derived eicosanoids related to compounds **1-3**.

The molecular formula for ascidiatrienolide A (**1**), $C_{20}H_{30}O_3$, was determined from interpretation of the M^+ m/z 318.2186 ion in the HREIMS in conjunction with ^1H and ^{13}C NMR data (Table 1). The molecular formula indicated 6 degrees of unsaturation. Eight olefinic resonances and 1 carbonyl resonance in the ^{13}C NMR spectrum accounted for 5 unsaturations, leaving 1 ring in ascidiatrienolide A. The infrared absorption at 1740 cm^{-1} and the ^{13}C NMR features revealed by DEPT sequence experiments indicated that **1** was a C_{20} polyunsaturated fatty acid derivative which had cyclized to form a lactone moiety. A conjugated triene was also apparent from the characteristic absorptions in the UV spectrum at 282nm (ε27400), 272 (ε35100) and 263 (ε28700). The lactone methine proton (C-8) in the ^1H NMR spectrum proved to be a convenient starting point for determining the positions of all the functionalized carbons in **1**. The C-8 methine proton was coupled (6.0 Hz) to an olefinic proton (C-9) on a **E** double bond. This olefin geometry was indicated by the large 15.2 Hz coupling to the C-10 proton. The C-10 olefinic proton was furthered coupled into a series of 4 other olefinic protons with coupling constants reflecting their **Z** configurations (Table 1). These couplings, visualized by one-dimensional ^1H NMR decoupling and ^1H COSY experiments, positioned the triene side-chain at C-8.

In the other direction, the lactone methine proton was coupled (9.0 Hz) to the proton at δ 3.85, thus placing the free hydroxyl group at C-7. The C-7 proton was also coupled to allylic methylene protons at C-6 which were coupled to the olefinic proton at C-5. The 10.6 Hz coupling constant between the C-5 and C-4 protons characterized the **Z** geometry of this isolated double bond. The C-4 olefinic proton was coupled to the C-3 allylic protons which were in turn coupled to the methylene protons at C-2 adjacent to the carbonyl. These data established the lactone as a rare 9-membered ring, the position of the free hydroxyl at C-7 and the double bonds as 4**Z**, 9**E**, 11**Z** and 13**Z**.

The last structural feature to be addressed was the stereochemistry at C-7 and C-8, but the small quantity of compounds isolated, combined with their relative instabilities, left us with no material with which to pursue the absolute stereochemistry by chemical and spectroscopic means. However, a molecular model of the **RS** or **SR** enantiomers of **1** revealed a likely dihedral angle of 170° between the C-7 and C-8 protons after minimizing gauche and eclipsed interactions of substituents on adjacent carbons and cross-ring interactions when the side-chain assumed a pseudoequatorial position. This ring conformation was also predicted by computer analysis using the Alchemy conformational analysis program[5] as ascidiatrienolide A reached its energy minimum.

Ascidiatrienolide B (**2**) was structurally similar to **1**, possessing the 9-membered ring lactone and the identical number of double bonds, positions and geometries (Table 1 and ref. 4). The coupling constant between the C-7 (δ 3.49) and C-8 (δ 5.23) protons was 2.0 Hz in **2** indicating a change in their stereochemistry to either **SS** or **RR**. A molecular model of the **SS** or **RR** enantiomers, using those conformational considerations applied above, produced a structure with a likely dihedral angle of 75° between the C-7 and C-8 protons consistent with their reduced coupling constant.

Ascidiatrienolide C (**3**) also possessed the 9-membered ring lactone and either the **SS** or **RR** absolute stereochemistries at C-7 and C-8, as indicated by the small 4.0 Hz coupling between the relevant protons. From the ^1H

NMR spectrum of **3**, the coupling constants of the olefinic protons established the olefin geometries as **4Z**, **9E**, and **13E**. Since the C-11 and C-12 protons overlaped in the ^{1}H NMR spectra, the magnitude of their coupling could not be determined. But, the relative downfield shift in the triene ^{13}C NMR bands, when compared to several model compounds[6], lead us to assign the **E** configuration to the C-11 - C-12 double bond.

The ascidiatrienolides are unique among marine natural products and of special interest as members of an important class of compounds, most of which exhibit physiologically potent activities at the cellular level. They also add the structural complexity of a 9-membered ring lactone never seen before in the eicosanoids. The role of the ascidiatrienolides in *D. candidum* is unknown, but oxidized metabolites of EFAs are important in the reproduction of some marine invertebrates[1a].

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References and Notes

1. (a) Stanley-Samuelson, D. W. *Biol. Bull.*, 1987, **173**, 92-109. (b) Lefer, A. *Biochem. Pharm.* 1986, **35**, 123-127. (c) Borgeat, M. Nadeau, H. Salari, P. Poubelle and B. Fruteau de laclos. *Adv. Lipid Res.*, 1985, **21**, 47-77.

2. Corey, E. J., S. P. T. Matsuda, R. Nagata and M. B. Cleaver. *Tetrahedron Lett.*, 1988, **29**, 2555-2558., and references cited therein.

3. Inflammatory assays were performed topically using the standard mouse ear assay. Anti-inflammatory assays were performed using the PMA-induced mouse ear edema assay applying ascidiatrienolide topically at doses of 20 microgram/ear.

4. Additional spectral data: For **1**, HREIMS obs. 318.2190, $C_{20}H_{30}O_3$, calc. 318.2196; IR(film) 3550-3350, 1740, 1713, 1220, 1142, 995 cm^{-1}; UV (MeOH) 282nm (ε27400), 272 (ε35100), 263 (ε28700); $[\alpha]_D$ -14.8° (c 4.5, $CHCl_3$): For **2**, HREIMS obs. 318.2186, $C_{20}H_{30}O_3$, calc. 318.2196; IR (film) 3550-3350, 1740, 1715, 1450, 1262, 1219, 1185, 1145, 1090, 1000 cm^{-1}; UV (MeOH) 280nm (ε19200), 271 (ε24500), 263 (ε20100); $[\alpha]_D$ -4.1° (c 3.4, $CHCl_3$): For **3**, HREIMS obs. 318.2195, $C_{20}H_{30}O_3$, calc. 318.2196; IR (film) 3550-3350, 1735, 1450, 1260, 1220, 1190, 1090, 990 cm^{-1}; UV (MeOH) 280nm (ε41300), 269 (ε52400), 260 (ε39600); $[\alpha]_D$ -10.6° (c 11.3, $CHCl_3$).

5. *Alchemy*, copyright 1987, a program available from Tripos Associates.

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