Pergamon

Tetrahedron Letters 41 (2000) 429–432

TETRAHEDRON LETTERS

Structure and absolute stereochemistry of stolonoxide A, a novel cyclic peroxide from the marine tunicate *Stolonica socialis*

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Received 8 October 1999; accepted 2 November 1999

Abstract

Stolonoxide A, a novel peroxide possessing an unprecedented molecular arrangement, has been isolated as its methyl ester from the marine tunicate *Stolonica socialis*. The structure of stolonoxide A has been fully elucidated by spectroscopic methods and its relative stereochemistry secured by chemical conversion. The absolute stereochemistry is suggested on the basis of Mosher's method on the diol derivative obtained by hydrogenation of the natural product.

Keywords: natural products; peroxides; acetogenins.

Linear and cyclic peroxides are quite common in marine organisms belonging mainly to the phylum Porifera.[1,2] Some compounds of this family of natural products show interesting biological activities including inhibition of tumoral cell growth, antimalarial and antimicrobial properties.[2–4] In search of new biologically active metabolites from marine sources, we have investigated the lipid extract of the Mediterranean tunicate *Stolonica socialis* (Hartmeyer Styelidae) collected in Tarifa, Straits of Gibraltar, South Spain, in June 1996. In this paper, we describe the structure elucidation of a novel peroxide compound that we have named stolonoxide A (**1**).

The organic extract of the frozen tunicate (ww 456 g) was fractionated by sequential chromatography on Sephadex and SiO_2. Stolonoxide A, which occurs as the free carboxylic acid (**1**) in the Et_2O extract of the tunicate, was purified as its methyl ester (**1a**) after treatment of the acid-containing fraction with diazomethane. The structure elucidation was achieved by spectroscopic and chemical methods. In particular, the complete NMR assignment of **1a** needed experiments both in $CDCl_3$ and C_6D_6 (Table 1).

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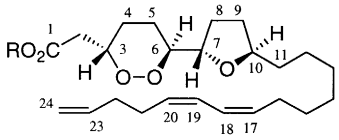
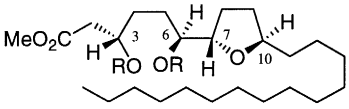
0040-4039/00/$ - see front matter
PII: S0040-4039(99)02077-8

Stolonoxide A methyl ester (**1a**)‡ had the molecular formula $C_{25}H_{40}O_5$ deduced on the basis of both MS [FAB$^+$ molecular ion at *m/z* 421 (M+1); EI molecular ion at *m/z* 420 (M^+)] and ^{13}C NMR spectra. The intense signal at δ 3.68 (-OCH_3) in the ^1H NMR spectrum, the IR band at 1742 cm^{-1}, and the ^{13}C NMR signal at δ 170.0 were all assigned to the methyl ester function. The ^{13}C NMR spectrum also contained six olefin signals and four carbons bearing oxygen. The remaining ^{13}C resonances were all assigned to methylene groups. These data were suggestive of a linear structure with three double bonds and two oxygen-containing cycles. Accordingly, the ^1H NMR spectrum showed four carbinolic protons in the region between 4.60 and 3.80 ppm. These signals were attributed to the 1,2-dioxane and tetrahydrofuran rings on the basis of COSY and HOHAHA experiments (Table 1). In particular, the methylene protons at δ 2.47 and 2.37 (H_2-2), both coupled to the downshifted signal at δ 4.54 (H-3), were diagnostic for a 3-(2-acetyl)-1,2-dioxane residue.[5] Moreover, the COSY cross peak between H-6 (δ 3.94) and H-7 (δ 3.76) in C_6D_6 allowed us to connect the two cycles to give the unusual arrangement of stolonoxide A methyl ester (**1a**). COSY and HOHAHA data in $CDCl_3$ also suggested the depicted alkyl chain, and this was confirmed by the main fragments observed in the EIMS spectrum (Fig. 1).

The all *cis* stereochemistry of the double bonds in **1a** was inferred on the basis of the coupling constants (J_{17-18}=J_{19-20} ≈8.0 Hz) and the chemical shifts of the allylic carbons (C-16, δ 27.7; C-21, δ 26.0). On the other hand, the distinguishing upfield-shift of C-18 (δ 123.5) and C-19 (δ 124.0) in the ^{13}C NMR spectrum suggested the s-*cis* conformation of the diene. Such a conformation was further supported by two very clear NOEs between H-18 and H-21, and between H-19 and H-16. The equatorial orientation of the main substituents of the 1,2-dioxane ring was determined by the analysis of the NOESY spectrum of **1a** in C_6D_6, that showed cross peaks due to 1,3-diaxial interactions between H3 (δ 4.55) and H5 (δ 1.30), as well as between H4 (δ 1.21) and H6 (δ 3.94). On the other hand, the absence of NOEs between H-7 (δ 3.76) and H-10 (δ 3.85) suggested the *trans* geometry of the tetrahydrofuran.



1 R = H
1a R = Me



2 R = H
3a R = (*S*) - MTPA
3b R = (*R*) - MTPA

This was further confirmed by comparison of the NMR data of the diol derivative **2** with those of similar compounds reported in the literature.[6–8] Compound **2** was easily obtained by hydrogenation of **1a** with 5% Pd/C in EtOH. ^1H and ^{13}C NMR data of the derivative **2**§ were also in agreement with the *threo* relative configuration of the substituents at C-6 and C-7 (Fig. 2). In fact, the NMR values of both carbons and hydrogens at positions 6, 7 and 10 in the derivative **2** (δ 3.42 and 74.0, H-6 and C-6; δ 3.78 and 81.7, H-7 and C-7; δ 3.89 and 79.4, H-10 and C-10) were very similar to those reported in the

‡ Stolonoxide A methyl ester (**1a**): colorless oil, $[\alpha]_D$ −33.3 (*c* 0.1, $CHCl_3$); IR (film) 2927, 2859, 1742, 1171 cm^{-1}; UV λ_{max} (EtOH) 237 nm; NMR data, see Table 1; EIMS *m/z* 420 (20), 261 (15), 229 (10), 159 (55), 135 (40), 121 (70), 67 (100); FAB+MS *m/z* 421 $(M+1)^+$; HREIMS *m/z* 420.2871 (required 420.2876 for $C_{25}H_{40}O_5$).

§ Compound **2**: colorless oil, $[\alpha]_D$ −2.5 (*c* 0.2, $CHCl_3$); IR (film) 3444, 2926, 2872, 1745 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz): δ 4.08 (1H, *m*, H-3), 3.89 (1H, *m*, H-10), 3.78 (1H, *dt*, *J*=15.4 and 6.9 Hz, H-7), 3.71 (3H, *s*, OCH_3), 3.42 (1H, *m*, H-6), 2.49 (2H, *d*, *J*=2.9 Hz), 1.99 (1H, *m*, H-9a), 1.94 (1H, *m*, H-8a), 1.80 (1H, *m*, H-8b), 1.75 (2H, *m*, H-4a and H-5a), 1.58 (1H, *m*, H-11a), 1.50 (1H, *m*, H-4b and H-5b), 1.45 (1H, *m*, H-9b), 1.35 (1H, *m*, H-11b), 1.30–1.22 (22H, *bs*), 0.89 (3H, *t*, *J*=6.4 Hz, H_3-24); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.4 (s, C-1), 81.7 (d, C-7), 79.4 (d, C-10), 74.0 (d, C-6), 67.9 (d, C-3), 51.7 (q, OMe), 41.4 (t, C-2), 35.6 (t, C-11), 32.9 (t, C-4), 32.4 (t, C-9), 32.2 (t, C-5), 31.9 (t, C-22), 29.6–29.3 (t, alkyl chain), 28.3 (t, C-8), 22.7 (t, C-23), 14.1 (q, C-24).

Table 1
NMR data (500 MHz) for stonoloxide A methyl ester (**1a**) in $CDCl_3$ and C_6D_6

pos.	$CDCl_3$		C_6D_6
	^1H δ (*m*, Hz)	^{13}C δ, m	^1H δ (*m* Hz)
1	--	170.0, s	--
2	2.47 (*dd*, 15.6, 7.5)	38.4, t	2.28 (*dd*, 15.5, 7.6)
	2.37 (*dd*, 15.6, 5.4)		2.03 (*dd*, 15.5, 5.7)
3	4.54 (*m*)	77.6, d	4.55(*dddd*, 11.1, 7.6, 5.7, 2.0)
4	1.91 (*m*)	29.1, t	1.50 (*m*)
	1.57 (*m*)		1.21 (*m*)
5	1.78(*m*)	25.2, t	1.75 (*m*)
			1.30 (*m*)
6	4.05 (*m*)	83.8, d	3.94(*ddd*,11.5, 4.4, 2.1)
7	3.87 (*m*)	78.6, d	3.76 (*dt*, 7.4, 4.4)
8	1.91 (*m*)	27.7, t	1.68 (*m*)
	1.77 (*m*)		1.59 (*m*)
9	1.97 (*m*)	31.7, t	1.75 *m*
	1.43 (*m*)		1.21 *m*
10	3.87 (*m*)	79.9, d	3.85 *m*
11	1.57 (*m*)	35.6, t	1.57 *m*
	1.36 (*m*)		1.33 *m*
12	1.29 (*m*)	29.6, t	1.39 *m*
13	1.29 (*m*)	29.6, t	1.27 *m*
14	1.29 (*m*)	29.6, t	1.27 *m*
15	1.36 (*m*)	29.6, t	1.40 *m*
	1.29 (*m*)		
16	2.15 (*m*)	27.7, t	2.14 *m*
17	5.45 (*m*)	132.4, d	5.48 *m*
18	6.23 (*bt*, 7.5)[a]	123.5, d	6.37 (*bt*, 9.0)
19	6.24 (*bt*, 7.5)[a]	124.0, d	6.37 (*bt*, 9.0)
20	5.44 (*m*)	132.3, d	5.48 *m*
21	2.27 (*m*)	26.0, t	2.21 *m*
22	2.15 (*m*)	27.7, t	2.14 *m*
23	5.82 (*m*)	138.2, d	5.76 *m*
24	5.03 (*dd*, 17.9, < 1.0)	114.7, t	5.01(*dd*, 18.8, < 1.0)
	4.97 (*dd*, 10.0, < 1.0)		4.98 (*dd*, 10.0, < 1.0)
OMe	3.68 *s*	51.9, q	3.28 *s*

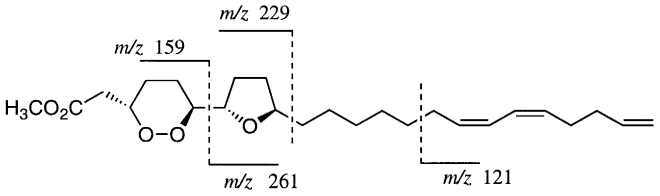


Fig. 1. Mass fragmentation of stolonoxide A methyl ester (**1a**) under EI conditions.

literature for annonaceus acetogenins with *threo* configuration, whereas they differed significantly from the chemical shifts of compounds with *erythro* geometry.[6,7]

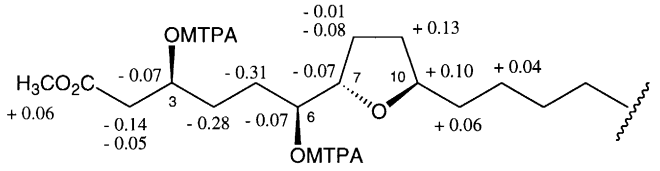


Fig. 2. Chemical shift differences ($\Delta\delta$) between the MTPA derivatives of stolonoxide A (**1**) [500 MHz, $CDCl_3$].

The absolute stereochemistry of stolonoxide A methyl ester (**1a**) was inferred by application of Mosher's method to the diol **2**.¶ Chemical shift differences between the esters **3a** (*S*-ester) and **3b** (*R*-ester) (Fig. 2) gave apparently conflicting results due to the double esterification of the hydroxy functions at C-3 and C-6. After a more careful examination, however, these data could be explained by separately considering the influence of each MTPA group on the adjacent hydrogens. In particular, the effect of the two MTPA groups is *on phase* (the diamagnetic shift has the same direction) upon C-1, C-4, C-5, C-9 and C-10, whereas it is *anti phase* (the diamagnetic shift has the opposite direction) upon C-2, C-7 and C-8. Accordingly, the final effect is additive for the first set of centers, whereas it gives ambiguous results for the second group of atoms (Fig. 2). On this basis, the absolute stereochemistry of stolonoxide A (**1**) is suggested to be *3S,6S,7S,10R*. This hypothesis has been confirmed by Prof. R. Riguera through studies on model compounds (personal communication; manuscript in preparation).

In conclusion, cyclic peroxides are quite common in organic extracts from marine organisms.[1,2] The molecular arrangement of stolonoxide A is new in nature, although it resembles that of trunculins, polycyclic metabolites isolated from Australian sponges of the genus *Latrunculia*.[8,9] The tunicate *S. socialis* contained other minor metabolites, the investigation of which is still in progress.

Acknowledgements

Thanks are due to Dr. E. Mollo and Dr. G. Villani for collecting the tunicate, to Mr. F. Castelluccio and Dr. A. De Napoli for the isolation work and to Mr. G. Scognamiglio for spectrophotometric measurements. The authors are grateful to Prof. R. Riguera (University of Santiago de Compostela, Spain) for valuable discussions. Thanks are also due to Dr. Valentin Buencuerpo (Complutense University of Madrid) for the taxonomic identification of the tunicate. NMR and MS spectra were recorded at 'Servizio di Risonanza Magnetica Nucleare dell' ICMIB' and at 'Servizio di Spettrometria di Massa'. Staff of both services are acknowledged. We wish to thank Conselleria d'Educaciò i Ciències, Generalitata Valenciana, for the post-doctoral grant to M.C.G. This work is part of a Spanish–Italian bilateral project between CNR (ICMIB) and CSIC (Museo Nacional de Ciencias Naturales).

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¶ *S*- and *R*-MTPA esters were prepared by reaction of **1a** with (*R*-MTPA Cl) or (*S*-MTPA Cl) in dry pyridine at room temperature.