Journal of Natural Products
Vol. 57, No. 11, pp. 1595-1597, November 1994

A NOVEL CYTOTOXIC MACROLIDE, SUPERSTOLIDE B, RELATED TO SUPERSTOLIDE A, FROM THE NEW CALEDONIAN MARINE SPONGE *NEOSIPHONIA SUPERSTES*

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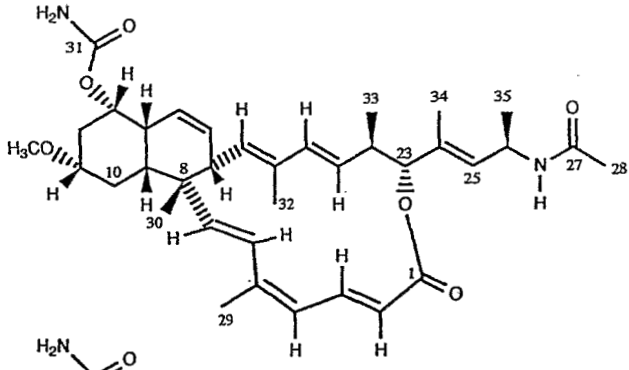
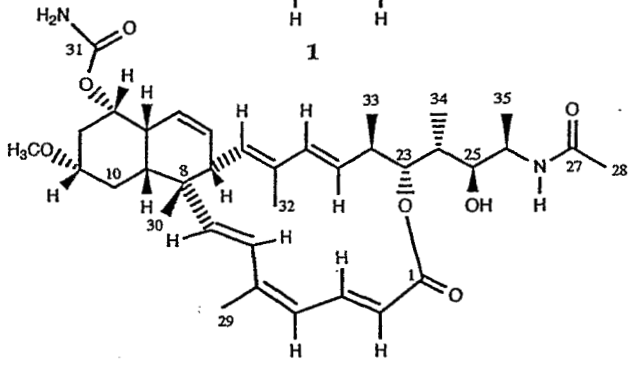
ABSTRACT.—The structure of a new cytotoxic macrolide, superstolide B [**1**], isolated from the deep water sponge *Neosiphonia superstes*, collected off New Caledonia, was elucidated mainly on the basis of nmr data. Compound **1** is closely related to superstolide A [**2**], a major cytotoxic component isolated from that organism, but lacks the 25-hydroxyl group found in **2** and has a C-24 (C-25)-double bond.

The sponge *Neosiphonia superstes* Sollas (Demospongiae, Lithistida, Phymatellidae) has proven to be a rich source of bioactive secondary metabolites possessing novel structural features, namely, the sphinxolides, cytotoxic 26-membered macrolides (1), and the cytotoxic superstolide A [**2**], which is made up of a decalin system fused with a 16-membered macrolide (2). In this paper, we describe the isolation and structural elucidation of a new minor macrolide, superstolide B [**1**], which is closely related to the more abundant superstolide A [**2**]. Superstolide B [**1**] exhibited potent cytotoxicity against KB (IC_{50} 0.005 μg/ml), P-388 (IC_{50} 0.003 μg/ml), and NSCLC-N6-L16 (non-small-cell lung carcinoma, IC_{50} 0.039 μg/ml) cancer cell lines.



1



2

The CH_2Cl_2 extract of the lyophilized specimens (1 kg) of the sponge *N. superstes*, collected off New Caledonia at 500–515 m depth, was fractionated by Si gel flash chromatography. The active fraction (*Artemia salina* assay) eluted with $CHCl_3$-MeOH (199:1) was purified by reversed-phase hplc to give superstolide B (**1**, 3 mg, amorphous solid, $[\alpha]_D$ +47.0°), along with major amounts of superstolide A [**2**].

The fabms of **1** showed a pseudomolecular ion at *m/z* 607 $(M+H)^+$, which is 18 mass units less than that of superstolide A [**2**]. The uv spectrum, λ max (MeOH) 236 (ε 15360) and 303 (ε 5000) nm, which indicated the presence of a conjugated diene and a conjugated triene ester, closely resembled that of **2**. As shown in Table 1, the proton signals in the ^{1}H-nmr spectrum of **1**, which were assigned on the basis of a COSY experi-

TABLE 1. ^{1}H- and ^{13}C-Nmr Data of Superstolides B [**1**] and A [**2**] ($CDCl_3$, 500 MHz).[a]

Position	Compound			
	1		**2**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	—	166.8	—	167.0
2	5.64 d (15.3)	121.0	5.70 d (15.3)	121.3
3	7.29 dd (15.3, 11.2)	138.6	7.21 dd (15.3, 11.2)	139.2
4	5.93 d (11.2)	125.4	5.92 d (11.2)	125.5
5	—	142.3	—	142.5
6	6.90 d (16.6)	126.1	6.88 d (16.3)	125.8
7	5.62 d (16.6)	142.5	5.60 d (16.3)	142.7
8	—	40.9	—	40.4
9	1.55 m	41.2	1.48 m	41.3
10	1.55 m, 1.90 m	31.3	1.45 m, 1.80 m	30.7
11	3.12 m	77.4	3.10 m	77.0
12	1.30 m, 2.23 br d (10.2)	33.7	1.31 m, 2.24 br d (10.5)	33.7
13	4.75 overlapped	72.9	4.76 br t (9.8)	72.6
14	2.88 br s ($W_{1/2}$ 11.8)	36.1	2.88 br s ($W_{1/2}$ 10.5)	36.0
15	5.54 dt (9.8, 3.7)	121.1	5.52 dt (9.8, 3.4)	120.3
16	5.65 d (9.8)	130.3	5.68 d (9.8)	130.3
17	3.11 br d (3.0)	42.7	3.10 br d overlapped	42.9
18	5.83 d (9.8)	132.6	5.78 d (10.8)	132.9
19	—	132.2	—	132.4
20	6.32 d (15.3)	137.3	6.29 d (15.3)	137.1
21	5.32 dd (15.3, 9.5)	129.3	5.32 dd (15.3, 9.8)	129.4
22	2.53 m	41.9	2.71 m	40.7
23	4.41 d (10.5)	83.2	4.79 dd (10.5, 2.0)	77.0
24	—	135.1	1.82 m	37.5
25	5.44 d (8.8)	131.5	3.16 dd (10.5, 2.7)	73.1
26	4.77 m	43.3	4.18 m	45.4
27	—	169.0	—	169.7
28	1.94 s	23.5	1.96 s	23.5
29	1.93 s	20.9	1.92 s	20.7
30	1.15 s	30.7	1.15 s	29.7
31	—	156.0	—	156.0
32	1.81 s	11.9	1.77 s	12.0
33	0.93 d (6.9)	16.9	1.07 d (6.9)	18.0
34	1.65 s	21.8	0.90 d (6.9)	8.8
35	1.21 d (6.9)	11.7	1.05 d (6.9)	12.7
NH	5.90 overlapped	—	6.22 d (8.8)	—
OCH_3	3.34 s	56.1	3.35 s	56.1
$COONH_2$	4.66 br s	—	4.66 br s	—

[a]Chemical shifts are expressed as δ values, with multiplicities indicated as *J* values in Hz in parentheses.

ment (3), also closely resembled those of superstolide A [2] except in the side-chain C-24 through C-28 region, where the structural difference occurred. Above all, the hydroxymethine signal at δ 3.16 in **2** (H-25) was replaced in **1** by an olefinic doublet at δ 5.44 and the Me-34 resonance was observed as a singlet shifted downfield to δ 1.65 ppm. Consequently, superstolide B [**1**] has been elucidated as a 25-dehydrated analogue of **2**, with the double bond placed at the C-24 (C-25)-position. Intense nOes between H-25 and H-23 and between Me-34 and H-26 revealed the trans- stereochemistry of the newly formed double bond. The close similarity in the ^{1}H- and ^{13}C-nmr shifts observed for the macrolides **1** and **2** (Table 1) implied that the chiral centers in the decalin and in the macrolide fragments have the same configurations in both molecules. The configuration at C-26 in **1** was assumed to be *R* by analogy with **2**.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr measurements were performed on a Brucker AMX-500 instrument interfaced with a Brucker X-32 computer. The superstolide B [**1**] sample was prepared by dissolving 3.0 mg in 0.4 ml of $CDCl_3$. The optical rotation was measured on a Perkin-Elmer 141 polarimeter using a sodium lamp operating at 589 nm. Fabms were recorded in a glycerol-thioglycerol matrix in the positive-ion mode on a VG ZAB instrument (argon atoms of energy 2–6 kV). Uv spectra were performed on a DU 70 Beckmann spectrophotometer.

ANIMAL MATERIAL.—*Neosiphonia superstes* was collected during the dredging campaigns (1987, 1989) of the ORSTOM-CNRS, Programme Substances Marines d'Intérêt Biologique (SMIB) in the South of New Caledonia (Banc Eponge region) at a depth of 500–515 m. The taxonomic identification was performed by Lévi and Lévi of the Museum Nationale d' Histoire Naturelle, Paris, France; reference specimens are on file at ORSTOM Centre de Nouméa (reference 1408).

EXTRACTION AND ISOLATION.—Preliminary assays for cytotoxic (KB cells and P-388 leukemia cells) and antifungal activities (*Fusarium oxysporum, Phythophthora hevea,* and *Penicillium digitatum*) showed marked activities associated with the CH_2Cl_2 extract. The organisms were freeze dried and the lyophilized material (1 kg) was extracted with *n*-hexane and CH_2Cl_2 in a soxhlet apparatus, then with CH_2Cl_2-MeOH, 8:2 (3×1 liter) and finally with MeOH (3×1 liter) at room temperature. The extract was filtered and concentrated under reduced pressure to give 2 g of a yellow cytotoxic oil. The crude CH_2Cl_2 extract was chromatographed by mplc on a SiO_2 column (50 g) using a solvent gradient system from $CHCl_3$ to $CHCl_3$-MeOH, 98:2. Fractions eluted with $CHCl_3$-MeOH, 199:1 (74 mg) were further purified by hplc on a Waters C-18 μ-Bondapak column (7.8 mm i.d.×30 cm) with MeOH-H_2O (73:27) as eluent (flow rate 5 ml/min) to give 31.2 mg of superstolide A [**1**] (R_t=10.4 min) and 3.0 mg of superstolide B [**2**] (R_t=16.0 min). Superstolide B [**1**] was obtained as a colorless amorphous solid, $[\alpha]_D$ +47.0°, uv (MeOH) λ max 236 (ε=15360), 303 (ε=5000); ^{1}H and ^{13}C nmr, see Table 1; fabms *m/z* 607 $(M+H)^+$.

ACKNOWLEDGMENTS

This contribution is part of the project SMIB "Substances Marines d'Intérêt Biologique" ORSTOM-CNRS, Nouméa, New Caledonia. We thank Professor J.F. Verbist of the Faculty of Pharmacy of the University of Nantes, France, for cytotoxicity tests. We are also grateful to the staff of the Servizio di Spettrometria di Massa del CNR dell'Università di Napoli for mass spectra. This work was partly supported by both C.N.R., Rome "P.F. Chimica Fine 2" and MURST, Rome.

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Received 18 May 1994