A Novel Cytotoxic Macrolide, Superstolide B, Related to Superstolide A, from the New Caledonian Marine Sponge Neosiphonia superstes

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

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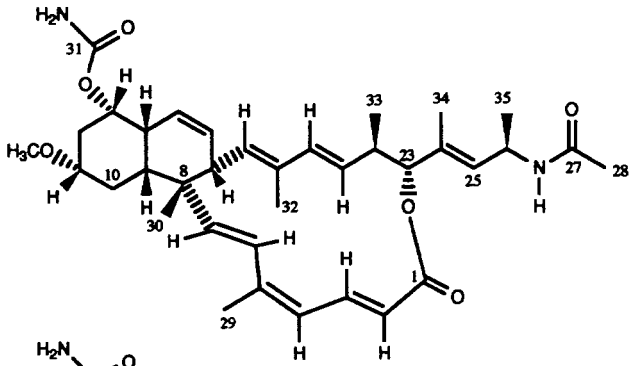
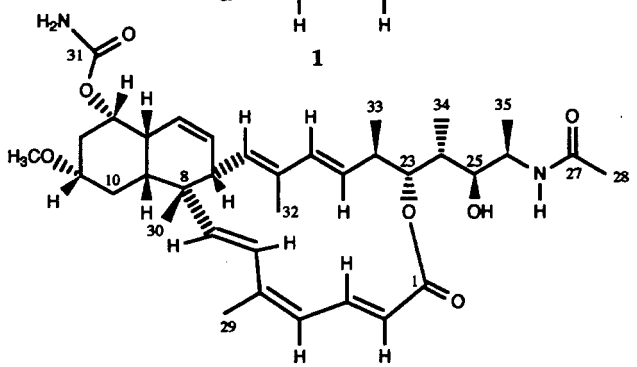
ABSTRACT.—The structure of a new cytotoxic macrolide, superstolide B [**1**], isolated from the deep water sponge *Neosiphonia superstes*, collected off New Caledonia, was elucidated mainly on the basis of nmr data. Compound **1** is closely related to superstolide A [**2**], a major cytotoxic component 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 $(M+H)^+$.

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