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NEOSIPHONIAMOLIDE A, A NOVEL CYCLODEPSIPEPTIDE, WITH ANTIFUNGAL ACTIVITY FROM THE MARINE SPONGE *NEOSIPHONIA SUPERSTES*

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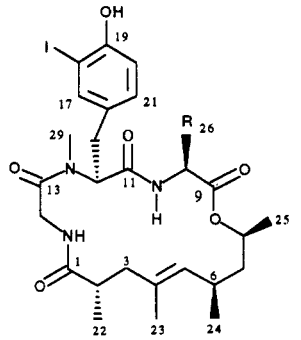
ABSTRACT.—A novel cyclodepsipeptide, neosiphoniamolide A [**1**], has been isolated from the sponge *Neosiphonia superstes*. The structure of **1**, which contains a 12-carbon hydroxy acid, glycine, valine, and a halogenated tyrosine residue in an 18-membered ring, is related to jaspamide and the geodiamolides, previously isolated from sponges. The structure was solved by spectroscopic analysis.

Marine sponges are a well-established source of unique and biologically active peptides (1,2). Jaspamide from *Jaspis* sp. (order Choristida) (3,4) and the geodiamolides from *Geodia* sp. (order Choristida) (5) and also from *Pseudoaxinissa* sp. (6), belonging to the order Axinellida (which is taxonomically distant from *Geodia*), are recent examples. They are four-residue cyclic depsipeptides, which contain a common 12-carbon polypropionate residue and three amino acid residues found in the tripeptide portion of the 18-membered macrocycle. All these metabolites were reported to exhibit potent antimicrobial and cytotoxic activities (3,6); jaspamide was also reported to be insecticidal (3). As a part of an ongoing study of biologically active metabolites from New Caledonian marine invertebrates, we have been working on the bioactive extracts of the sponge *Neosiphonia superstes*, from which we have isolated sphinxolides, potent cytotoxic 26-membered macrolides (7). We now report the isolation and structure determination of a new cyclodepsipeptide, neosiphoniamolide A [**1**], which is related to the previously known jaspamide and the geodiamolides.



1 R=$CH(CH_3)_2$
2 R=CH_3

The CH_2Cl_2-MeOH (8:2) extract of the sponge was chromatographed by Si gel mplc (MeOH: $CHCl_3$, 2:98) followed by reversed-phase C-18 μ-Bondapak hplc with 73% aqueous MeOH to give neosiphoniamolide A (**1**, 2 mg, colorless glass) and major amounts of the previously isolated sphinxolides. The fabms of **1** gave a pseudomolecular ion at *m/z* 656 $[M+H]^+$. Resonances in the ^1H- and ^{13}C-nmr spectra could be assigned to a 12-carbon polypropionate unit (C-1 to C-8 and attached methyls) identical to that found in jaspamide (3) and the geodiamolides (5,6). Additional ^1H- and ^{13}C-nmr resonances could be assigned to an *N*-methyl-3-iodotyrosine residue and

one glycine residue by comparison of their chemical shifts and coupling constants to those reported for the resonances assigned to the same residues in geodiamolide D [**2**] (6). Uv absorptions at λ max 219, 284, and 292 (ε13270, 4000, 3000) supported the presence of a 3-iodotyrosine residue (5). The remaining resonances in the ^{1}H-nmr spectrum of neosiphoniamolide A [**1**] could be readily assigned to a valine residue [δ 0.75 and 0.79 (3H each, d, J=6.5 Hz); 1.99 m; 4.33 (dd, J=7.0 and 8.8 Hz)] supported by decoupling experiments, which allowed the doublet at δ 6.42 (J=8.8 Hz) to be assigned to NH-Val and by the ^{13}C-nmr shifts at 18.1 (×2), 31.5, and 58.4 ppm. The above fragments identified by the nmr data accounted for the mol wt of neosiphoniamolide A [**1**]. It was apparent, therefore, that **1** was a valine analogue of geodiamolide D [**2**] (6) in which the alanyl residue is replaced in **1** by a valine residue, and what remained to be determined was the sequence. An intense nOe between the glycine NH proton resonating at δ 6.46 t and H-2 at 2.47 m revealed that the glycine was attached via an amide linkage to the polypropionate fragment. In addition, an intense nOe between the valine NH resonating at δ 6.42 d and the iodotyrosine methine signal H-12 at δ 5.13 dd demonstrated that the valine residue was connected via a peptidic linkage to the *N*-methyl-iodotyrosine residue as shown in **1**. The similarity in ^{1}H- and ^{13}C-nmr shifts observed for neosiphoniamolide A [**1**] and the geodiamolides (e.g., **2**) implied that the stereogenic centers in the iodotyrosine and polypropionate fragments had the same relative configurations in all molecules. The absolute configuration of geodiamolides A and B was determined by X-ray crystallographic analysis, which revealed the unusual *R* configuration for 3-iodotyrosine (3). Hydrolysis of **1** with 6 N HCl followed by derivatization with Marfey's reagent and hplc analysis (8) showed that valine had the L configuration and confirmed that 3-iodotyrosine had the unusual D configuration.

Neosiphoniamolide A [**1**] inhibited the growth of the fungi *Piricularia oryzae* and *Helminthosporium gramineum* with IC_{90} values of 5 ppm, but exhibited weaker activity against a panel of fungi used by Rhône-Poulenc in their in vitro primary screening search for antifungal compounds. More potent activities were exhibited by the co-occurring macrolides, the sphinxolides (see Experimental).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr measurements were performed on a Bruker AMX-500 instrument interfaced with a Bruker X-32 computer. The neosiphoniamolide A [**1**] samples were prepared by dissolving 2 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 obtained on a Beckmann DU 70 spectrophotometer.

ANIMAL MATERIAL.—*Neosiphonia superstes* Sollas (Demospongiae, Lithistida, Phymatellidae) was collected during the dredging campaigns (1987, 1989) of the ORSTOM-CNRS Programme "Substances Marines d'Intéret Biologique (SMIB)," in the South of New Caledonia (Banc Eponge region) at a depth of 500–515 m. 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 activity by an initial $CHCl_3$ extract. To follow up on this observation, 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 CH_2Cl_2/MeOH extract was filtered and concentrated under reduced pressure to give 20 g of a yellow cytotoxic oil. The crude CH_2Cl_2/MeOH extract was chromatographed by mplc on a SiO_2 column (400 g) using a solvent gradient system from $CHCl_3$ to $CHCl_3$-MeOH (98:2). Fractions eluted with $CHCl_3$-MeOH (995:5) (400 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 2 mg of neosiphoniamolide A [**1**], 10.5 mg of sphinxolide (R_t=12.0 min), 13.7 mg of sphinxolide C (R_t=19.8 min), and 9.8 mg of sphinxolide D (R_t=21.6 min) (7).

Neosiphoniamolide A [**1**].—Colorless amorphous solid, $[\alpha]_D$ +5.2°; uv (MeOH) λ max 219 (ε 13270), 284 (ε 4000), 292 (ε 3000) nm; fabms *m/z* 656 $(M+H)^+$; ^{1}H nmr ($CDCl_3$, 500 MHz) δ 7.53 (1H, d, *J*=2.0 Hz, H-17), 7.10 (1H, dd, *J*=8.5 and 2.0 Hz, H-21), 6.90 (1H, d, *J*=8.5 Hz, H-20), 6.46 (1H, t, *J*=3.4 Hz, NH-gly), 6.42 (1H, d, *J*=8.8 Hz, NH-val), 5.13 (1H, dd, *J*=9.8 and 6.4 Hz, H-12), 4.99 (1H, d, *J*=8.5 Hz, H-5), 4.84 (1H, sextet, *J*=6.4 Hz, H-8), 4.33 (1H, dd, *J*=8.8 and 7.0 Hz, H-10), 4.15 (1H, dd, *J*=18.3 and 4.1 Hz, H-14), 3.79 (1H, dd, *J*=18.3 and 3.4 Hz, H-14), 3.25 (1H, dd, *J*=13.2 and 9.0 Hz, H-15), 2.96 (3H, s, H-29), 2.80 (1H, dd, *J*=13.2 and 6.1 Hz, H-15), 2.47 (1H, m, H-2), 2.21 (1H, m, H-6), 2.10 (1H, m, H-3), 1.99 (1H, m, H-26), 1.68 (1H, m, H-7), 1.56 (3H, s, H-23), 1.40 (1H, m, H-7), 1.25 (3H, d, *J*=6.4 Hz, H-25), 1.17 (3H, d, *J*=6.8 Hz, H-22), 0.90 (3H, d, *J*=6.8 Hz, H-24), 0.79 (3H, d, *J*=6.5 Hz, H-27), 0.75 (3H, d, *J*=6.5 Hz, H-28); ^{13}C nmr ($CDCl_3$, 125 MHz) δ 175.9 (s, C-11), 169.7 (s, C-1), 169.5 (s, C-13), 168.5 (s, C-9), 154.0 (s, C-19), 138.6 (d, C-17), 133.2 (s, C-16), 131.6 (d, C-5), 130.9 (d, C-21), 130.5 (s, C-4), 115.2 (d, C-20), 85.5 (s, C-18), 71.5 (d, C-8), 58.4 (d, C-10), 57.8 (d, C-12), 43.3 (s, C-3), 43.3 (t, C-7), 42.0 (d, C-2), 41.9 (t, C-14), 32.7 (t, C-15), 31.5 (d, C-26), 29.7 (q, C-29), 29.1 (d, C-6), 20.8 (q, C-25), 20.5 (q, C-24), 18.8 (q, C-22), 18.1 (q, C-27), 18.1 (q, C-28), 17.8 (q, C-23).

ANTIFUNGAL ACTIVITY.—Neosiphoniamolide A [**1**] and the co-occurring sphinxolides (7) were subjected to in vitro primary screening for antifungal activity by Rhône-Poulenc Rorer and were tested at 5 and 2 ppm. The fungi used were: *Phythothora citrophthora, Phythothora citnnamomi, Pythium rostatum, Pythium vexans, Botrytis cinerea, Pericularia oryzae, Fusarium roseum, Alternaria alternata, Rhizoctonia solani, Pseudocercosporella herpotrichoides, Septoria nodorum, Helminthosporium gramineum*, and *Ustilago nuda*.

Sphinxolide and sphinxolides B and C were very active for all microorganisms used showing IC_{90} values on mycelium growth of <2 ppm. Neosiphoniamolide A [**1**] inhibited the growth of *Pericularia oryzae* and *Helminthosporium gramineum* with IC_{90} values of 5 ppm, while the IC_{90} values against the remaining fungi tested were >5 ppm.

HYDROLYSIS OF NEOSIPHONIAMOLIDE A [**1**].—1 mg of neosiphoniamolide A [**1**] in 0.2 ml of 6 N HCl was heated at 110° with stirring for 24 h in a Pyrex tube sealed with a Teflon screw cap. The cooled reaction mixture was evaporated to dryness under N_2.

DERIVATIZATION OF AMINO ACIDS WITH MARFEY'S REAGENT AND HPLC ANALYSIS.—To a 0.5-ml vial containing 1 mg of pure amino acid standard in 100 μl of H_2O, was added 2 mg of FDAA in 200 μl Me_2CO followed by 40 μl 1 N $NaHCO_3$. The mixture was heated for 1 h at 40°. After cooling to room temperature, 40 μl of 2 N HCl was added and the resulting solution was taken to dryness and then dissolved in 500 μl of DMSO.

The neosiphoniamolide A [**1**] residual hydrolysate in 100 μl of H_2O was reacted with 2 mg of FDAA in 200 μl of Me_2CO as described above. A 5-μl aliquot of the FDAA derivatives was analyzed by reversed-phase hplc. A linear gradient from triethylammonium phosphate (50 mM, pH 3.0)/MeCN 90% to triethylammonium phosphate (50 mM, pH 3.0)/MeCN 50% over 45 min (flow rate 2 ml/min) was used to separate the FDAA derivatives which were detected by uv at 340 nm. L-Valine and L-3-iodotyrosine derivatized with Marfey's reagent were used as references.

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