Journal of Natural Products
Vol. 58, No. 1, pp. 121-123, January 1995

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

M. VALERIA D'AURIA, LUIGI GOMEZ PALOMA, LUIGI MINALE,* ANGELA ZAMPELLA,

Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli "Federico II", Via Domenico Montesano 41, 80131 Napoli, Italy

CÉCILE DEBITUS,

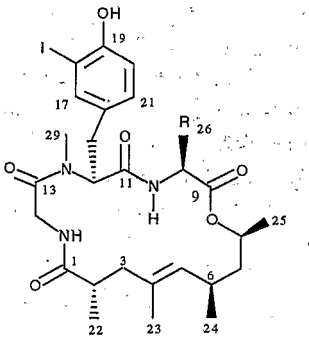
Centre ORSTOM, B.P. A5, Nouméa, New Caledonia

and JOSEPH PEREZ

Centre de Recherche de Vitry-Alfortville 13, Quai Jules Guesde-BP 14, 94403 Vitry Sur Seine Cedex, France

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, [α]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.

ACKNOWLEDGMENTS

This contribution is part of the project SMIB "Substances Marines d'Intérêt Biologique," ORSTOM-CNRS, Nouméa, New Caledonia. This work was partly supported by C.N.R., Rome "Contributo 93.03093.CT 03" and partly by MURST, Rome. The antifungal tests were performed by Rhône-Poulenc Rorer, Centre de Recherche de Vitry-Alfortville, and we thank Drs. J. Provost and J. Perez. We are also grateful to the staff of the Servizio di Spettrometria di Massa del CNR dell'Università di Napoli for mass spectra.

LITERATURE CITED

1. C.M. Ireland, T.F. Molinski, D.M. Roll, T.M. Zabriskie, T.C. McKee, J.C. Swersey, and M.P. Foster, in: "Biorganic Marine Chemistry." Ed. by P.J. Scheuer, Springer-Verlag, Berlin, 1989, Vol. 3, pp. 1–46.
2. N. Fusetani and S. Matsunaga, *Chem. Rev.*, **93**, 1793 (1993).
3. T.M. Zabriskie, J.A. Klocke, C.M. Ireland, A.H. Marcus, T.F. Molinski, D.J. Faulkner, C. Xu, and J.C. Clardy, *J. Am. Chem. Soc.*, **108**, 3123 (1986).
4. P. Crews, L.V. Manes, and M. Boehler, *Tetrahedron Lett.*, **27**, 2797 (1986).
5. W.R. Chan, W.F. Tinto, P.S. Manchand, and L.J. Todaro, *J. Org. Chem.*, **52**, 3091 (1987).
6. E.D. de Silva, R.J. Andersen, and T.M. Allen, *Tetrahedron Lett.*, **31**, 489 (1990).
7. M.V. D'Auria, L. Gomez Paloma, L. Minale, A. Zampella, J.F. Verbist, C. Roussakis, and C. Debitus, *Tetrahedron*, **49**, 8657 (1993).
8. P. Marfey, *Carlsberg Res. Commun.*, **49**, 591 (1984).

Received 18 May 1994