VOL. 56 NO. 11, NOV. 2003 THE JOURNAL OF ANTIBIOTICS pp. 970 — 972

A New Bicyclic Guanidine Alkaloid, Sch 575948, from a Marine Sponge, *Ptilocaulis spiculifer*

SHU-WEI YANG,* TZE-MING CHAN, SHIRLEY A. POMPONI,†
GUODONG CHEN, AMY E. WRIGHT,† MAHESH PATEL,
VINCENT GULLO, BIRENDRA PRAMANIK and MIN CHU

Schering-Plough Research Institute,
2015 Galloping Hill Road, Kenilworth, NJ 07033, USA
† Harbor Branch Oceanographic Institution,
5600, U.S. 1 North, Fort Pierce, FL 34946, USA

(Received for publication August 18, 2003)

In the course of our continuing search for novel antimicrobial agents,[1,2] we have isolated a new antibacterial compound Sch 575948 (**1**) along with a known alkaloid ptilomycalin A (**2**) from a marine sponge *Ptilocaulis spiculifer* (or *Crambe crambe*). Sch 575948 was identified as a bicyclic bis-guanidine type of alkaloid, a homolog of crambescin A (or crambine A (**3**)), identified as a major component of the homolog complex.[3,4]

Several compounds with biological activity have been discovered from extract of *P. spiculifer* and related sponges.[3,5] The biological activities were found to be derived from a guanidine family of alkaloids. Many of these alkaloids possess unique structures. These polycyclic-guanidine type alkaloids, isolated from related sponges, could be represented by the following compounds: ptilomycalin A, ptilocaulin,[6] crambescidins, bazelladines,[7] and crambescins A, B,[4] C1 and C2.[8] Among them, ptilomycalin A, a pentacyclic guanidine alkaloid, possesses potent antitumor, antiviral, and antifungal activities.[9] Its analog, crambescidin 816, has been discovered as a potent calcium channel blocker.[5] These guanidine-type alkaloids with a wide range of biological activity, including anti-HIV properties, have drawn a lot of research interest in the biological and synthetic areas.[10]

Crude sponge sample (~20 g) was ground and extracted with ethanol.[11] The dried organic extract (710 mg) was fractionated on a CG161 column (~200 ml) to generate 100 fractions by eluting with an aqueous methanol step-wise gradient solution (10%, 20%, 30%, 40%, 60%, 80%, and 100%), followed by elution with methanol-EtOAc. Two pure compounds, **1** (0.9 mg) and **2** (2.6 mg), were obtained in fractions 14 and 81, respectively.

Mass spectral data and the detailed NMR analysis identified **2** as ptilomycalin A, which had been isolated previously from the same species.[9]

The structure of **1** was determined based on extensive NMR and HRMS analyses.[12] From the high-resolution ESI-MS, the molecular formula of **1** was established as $C_{18}H_{32}N_6O_2$ (found *m/z* 365.2665; calcd. *m/z* 365.2659 for $[M+H]^+$). The high number of nitrogen atoms and the history of this marine sponge, *P. spiculifer*, indicated the existence of guanidine functionalities. Proton signals in ^1H NMR can be interpreted and assigned to their attached carbons by HSQC analysis. The connectivities of protonated fragments were determined primarily by HSQC-TOCSY data (Table 1). Thus three fragments A, B, and C were established (Figure 1). In the fragment A, a nitrogen and an oxygen atoms were attached to C-2 (δ 40.2) and C-5 (δ 63.5), respectively, based on their carbon chemical shifts. In the HMBC spectrum, H_2-5 (δ 4.11) and C-6 (δ 164.3) long-range correlation indicated an ester group adjacent to C-5. Since the rest of the hetero-atoms were all nitrogen atoms, C-2 was assigned adjacent to a guanidine group on the basis of a long-range correlation of H_2-2 (δ 3.13) to C-1 (δ 156.9), which had no correlations with any other protons.

In the fragment B, C-11 was connected to a nitrogen atom due to the downfield carbon chemical shift (δ 47.8). Long-range correlations of H_2-9 (δ 2.88 and 3.15) and H-13 (δ 4.28) to C-7 (δ 100.8) and C-8 (δ 151.5) allowed us to assign the double bond position between C-9 (δ 30.6) and C-13 (δ 49.0), which was adjacent to a nitrogen atom and connected to the fragment C. The downfield chemical shift of olefin C-8 (δ 151.5) indicated the attachment of a nitrogen atom. At this point, the only unassigned carbon C-12 (δ 151.1) could only be identified as a guanidine group connecting N-8 and N-13, cyclizing a 6-member ring. Hence, the fragment A with an ester group can only be located to quaternary olefin C-7 (δ 100.8). With an additional degree of unsaturation, fragment B should be cyclized to form a 5-member ring with *N*-8 nitrogen. This was supported by the evidence of the uneqivalence of α and β methylene proton resonances of H_2-9, H_2-10, and H_2-11 observed in the NMR spectrum due to a rigid formation of a five-membered ring fused with a six-membered ring carbon skeleton.

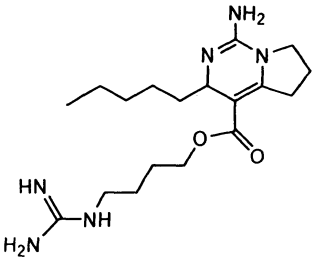
The identified structure **1** is a new member of crambescin A (**3**) class of compounds with a shorter alkyl

* Corresponding author: shu-wei.yang@spcorp.com

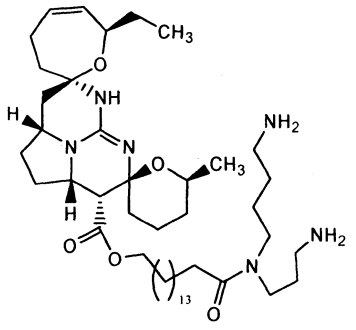
Table 1. NMR spectral data for compound Sch 575948 (**1**) in DMSO-d_6.

C/H no.	^{1}H (δ)	^{13}C (δ)	Some HSQC-TOCSY (2 bond)
1		156.9 s	
2	3.13, m	40.2 t	C-3
3	1.51, quintet, J = 7.0	25.2 t	C-2
4	1.63, quintet, J = 7.0	25.4 t	C-5
5	4.11, m	63.5 t	C-4
6		164.3 s	
7		100.8 s	
8		151.5 s	
9	2.88, ddd, J = 19, 9.5, 9.5	30.6 t	C-10
	3.15, m		
10	2.11, m; 1.97, m	21.4 t	C-9, C-11
11	3.62, ddd, J = 9.5, 9.5, 9.5	47.8 t	C-10
	3.72, ddd, J = 9.5, 9.5, 2.5		
12		151.1 s	
13	4.28, t, J = 6.5	49.0 d	C-14
14	1.45, m	35.9 t	C-13, C-15
15	1.23, m; 1.35 m	23.2 t	C-14
16	1.23, m	30.7 t	
17	1.23, m	21.9 t	C-18
18	0.85, t, J = 7.0	13.8 q	C-17
NH	7.81, t, J = 5.5		C-2
NH_2	8.35, brs		
NH	9.20, brs		

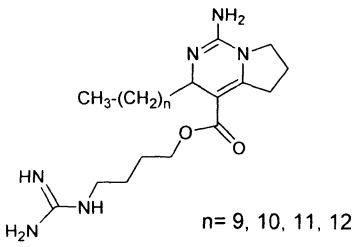
δ in ppm; J in Hz



Sch575948 (**1**)



Ptilomycarin (**2**)



n= 9, 10, 11, 12

Crambescin A or crambine A (**3**)
major homolog, n = 10

side chain. Crambescin A (**3**) and its homologs, n=9~12, have been identified previously but were not isolated in pure form based on the literature.[3)]

The stereochemistry at C-13 position could not be defined by NMR methods. Determination of the chiral center at C-13 position was not pursued due to the limited amount of compound available.

Compound **1** exhibited antibacterial activity against a super sensitive strain of *Staphylococcus aureus*. This super sensitive strain is a genetically engineered *S. aureus* (RN4220) with the knockout gene of the NorA efflux pump which is a member of the major facilitator superfamily. The resistance markers were inserted to this strain for MLS (macrolides, lincosamides, streptogramins), aminoglycosides, tetracyclines, chloramphenicol, and β-lactams. Therefore, the microorganisms only producing these known classes of antibiotics would be excluded due to the inserted resistance genes during the primary screening. The inhibition zones of 13, 15, and 18 mm in diameter, were observed at 25, 50, and 100 μg of **1** impregnated on the paper disc (8 mm), respectively, in an agar diffusion assay. The standard disc with gentamicin (10 μg) showed a 16 mm inhibition zone. Compound **1** did not show antifungal

Fig. 1. Fragments A, B, and C determined by HSQC-TOCSY.

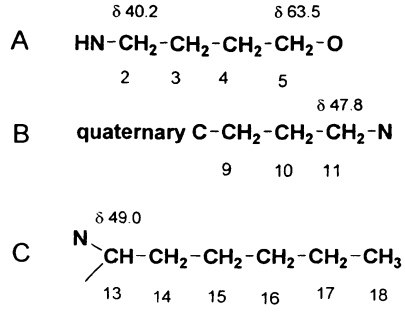
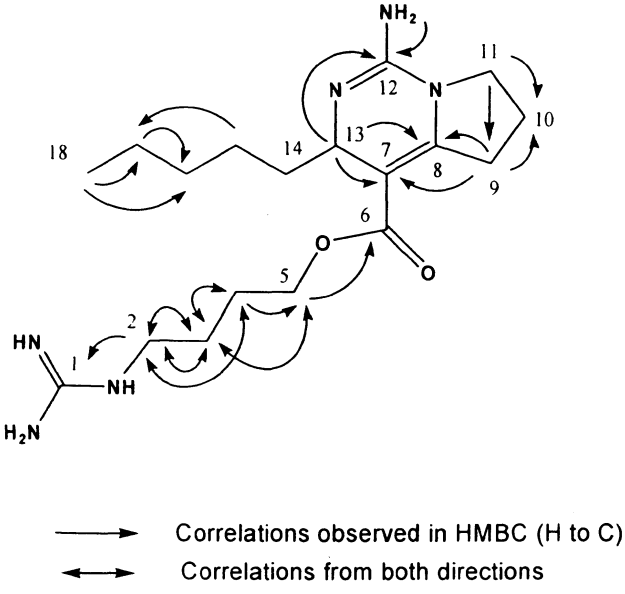


Fig. 2. Key HMBC correlations of **1**.



activity at 60 μg in a disc agar assay against *Saccharomyces cerevisiae*. IC_{50} values were not obtained due to the limited amount of isolated material. Ptilomycalin A (**2**) showed antifungal activity with inhibition zones, 10, 11, 12, and 13 mm, in a disc agar diffusion assay against *S. cerevisiae* at 10, 20, 40, and 80 μg, respectively.

Acknowledgement

The authors wish to acknowledge Mr. LEWIS B. FAN for sample processing, and Ms. ELEANOR SHARGORODSKAYA for database search.

References

1) YANG, S.-W.; T.-M. CHAN, S. A. POMPONI, G. CHEN, D. LOEBENBERG, A. WRIGHT, M. PATEL, V. GULLO, B. PRAMANIK & M. CHU: Structure elucidation of a new antifungal sterol sulfate, Sch 575867, from a deep-water marine sponge (Family: Astroscleridae). J. Antibiotics 56: 186~189, 2003
2) YANG, S.-W.; A. BUEVICH, T.-M. CHAN, J. TERRACCIANO, G. CHEN, D. LOEBENBERG, M. PATEL, E. BOEHM, V. GULLO, B. PRAMANIK & M. CHU: A New Antifungal Sterol Sulfate, Sch 601324, from *Chrysosporium* sp. J. Antibiotics 56: 186~189, 2003
3) BERLINCK, R. G. S.; J. C. BRAEKMAN, D. DALOZE, K. HALLENGA, R. OTTINGER, I. BRUNO & R. RICCIO: Two new guanidine alkaloids from the Mediterranean *Crambe crambe*. Tetrahedron Lerrers 31: 6531~6534, 1990
4) JARES-ERIJMAN, E. A.; A. A. INGRUM, F. SUN & K. L. RINEHART: On the structures of crambescins B and C1. J. Nat. Prod. 56: 2186~2188, 1993
5) BERLINCK, R. G. S.; J. C. BRAEKMAN, D. DALOZE, I. BRUNO, R. RICCIO, S. FERRI, S. SPAMPINATO & E. SPERONI: Polycyclic guanidine alkaloids from the marine sponge *Crambe crambe* and Ca^{++} channel blocker activity of crambescidin 816. J. Nat. Prod. 56: 1007~1015, 1993
6) HARBOUR, G. C.; A. A. TYMIAK, K. L. RINEHART Jr., P. D. SHAW, R. G. HUGHES Jr., S. A. MIZSAK, J. H. COATS, G. E. ZURENKO, L. H. LI & S. L. KUENTZEL: Ptilocaulin and isoptilocaulin, antimicrobial and cytotoxic cyclic guanidines from the Caribbean sponge *Ptilocaulis* aff. *P. spiculifer* (Lamarck, 1814). J. Am. Chem. Soc. 103: 5604~5606, 1981
7) PATIL, A. D.; A. J. FREYER, P. OFFEN, M. F. BEAN & R. K. JOHNSON: Three new tricyclic guanidine alkaloids from the sponge *Batzella* sp. J. Nat. Prod. 60: 704~707, 1997
8) BERLINCK, R. G. S.: Natural guanidine derivatives. Nat. Prod. Rep. 16: 339~365, 1999
9) KASHMAN, Y.; S. HIRSH, O. J. MCCONNELL, I. OHTANI, T. KUSUMI & H. KAKISAWA: Ptilomycalin A: a novel polycyclic guanidine alkaloid of marine origin. J. Am. Chem. Soc. 111: 8925~8926, 1989
10) HEYS, L.; C. G. MOORE & P. J. MURPHY: The guanidine metabolites of *Ptilocaulis spiculifer* and related compounds; isolation and synthesis. Chem. Soc. Rev. 29: 57~67, 2000
11) YANG, S.-W.; A. BUEVICH, T.-M. CHAN, M. SMITH, J. LACHOWICZ, S. A. POMPONI, A. E. WRIGHT, R. MIERZWA, M. PATEL, V. GULLO & M. CHU: A new sterol sulfate, Sch 572423, from a marine sponge, *Halichondrida* sp. Bioorg. Med. Chem. Lett. 13: 1791~1794, 2003
12) General Experimental Procedures: NMR spectra were recorded in DMSO-d_6 on a Varian Unity 500 NMR instrument at 500 MHz for ^1H and 125 MHz for ^{13}C, using standard Varian pulse sequence programs (VNMR Version 6.1 Software). High resolution electrospray ionization (ESI)-MS experiments were performed on PE Sciex QSTAR mass spectrometer. Two internal standards CsI and verapamil were used as internal standards for positive ion accurate mass measurements.