Neobacillamide A, a Novel Thiazole-Containing Alkaloid from the Marine Bacterium *Bacillus vallismortis* C89, Associated with South China Sea Sponge *Dysidea avara*

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A novel thiazole alkaloid, neobacillamide A (**1**), together with a known related one, bacillamide C (**4**), was isolated from the bacterium *Bacillus vallismortis* C89, associated with the South China Sea sponge *Dysidea avara*. The structure of the new compound was elucidated on the basis of its spectroscopic data. A plausible biosynthetic pathway is proposed. Neobacillamide A represents the first example of a thiazole-carboxamide bearing a 2-phenylethylamine moiety.

Introduction. – Marine sponges are known to be precious sources of natural products of great pharmaceutical potential. For instance, the famous Ara-A and Ara-C isolated from sponge *Cryptethia crypta* are widely used as antiviral and antileukemic agents, respectively [1–4]. Among the sponges, the cosmopolitan sponge of genus *Dysidea* (phylum *Porifera*, class *Demospongiae*, order *Dictyoceratida*, family *Dysididea*) is well documented as a rich source for structurally unique and biologically active compounds, including polybrominated phenyl ethers, chlorinated amino acid derivatives, furano-sesquiterpenes, and polyhydroxy steroids [5–8]. However, the challenge is that the *Dysidea* sponges are relatively rare and difficult to collect so that even excellent drug candidates from sponges are not well developed [9]. In 1988, *Stierle et al.* first discovered that a *Micrococcus* sp. isolated from the sponge *Tedania ignis* produced the same diketopiperazines in laboratory cultural media as reported for the exact sponge itself [10]. Recently, increasing evidence has demonstrated that most of the natural products come from sponge-associated microorganisms [11]. We recently isolated the bacterium *Bacillus vallismortis* C89 (GenBank No. DQ091007), with antimicrobial activity from the marine sponge *Dysidea avara* collected off Sanya in the South China Sea [12] and chemically investigated it. Here we describe the isolation and structure elucidation of a novel thiazole alkaloid, neobacillamide A (**1**), produced by the title bacterium. To our knowledge, this is the first report of a thiazole alkaloid bearing a 2-phenylethylamine moiety.

Results and Discussion. – The AcOEt extract of the bacterium was subjected to repeated column chromatography (Silica gel and *Sephadex LH-20*) and semi-preparative HPLC to give the pure compounds **1** and **4** (*Fig. 1*).

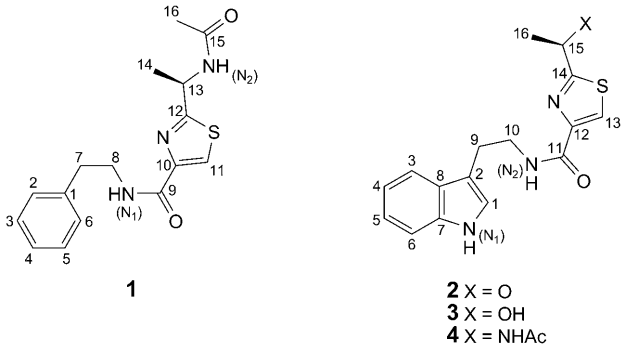


Fig. 1. *Structures of compounds* **1**–**4**

Neobacillamide A (**1**) was obtained as an optically active yellow oil with $[\alpha]_D^{24} = -16.0$. The ESI-MS spectrum displayed a *pseudo*-molecular ion peak at *m*/*z* 340.1 ($[M+Na]^+$). The HR-ESI-MS experiment established the molecular formula as $C_{16}H_{19}N_3O_2S$ (*m*/*z* 340.1081 ($[M+Na]^+$), calc. for $C_{16}H_{19}N_3NaO_2S^+$, 340.1096), indicating nine degrees of unsaturation. The IR spectrum of compound **1** showed absorption bands for an amide group (ν_{max} 3423 and 1655 cm^{-1}). In the ^{1}H-NMR spectrum (*Table*), five signals for aromatic H-atoms between δ(H) 7.23 and 7.35 indicated the presence of one mono-substituted benzene ring. Furthermore, one aromatic H-atom at δ(H) 8.00 (*s*, 1 H), one CH group at δ(H) 5.38 (*q*, *J* = 6.7, 1 H), two CH_2 groups at δ(H) 2.93 (*t*, *J* = 7.2, 2 H) and 3.70 (*dt*, *J* = 7.2, 6.6, 2 H), and two Me groups at 2.04 (*s*, 3 H) and 1.57 (*d*, *J* = 6.7, 3 H) were observed. The ^{13}C-NMR and DEPT spectra exhibited 16 C-atom signals (2 Me, 2 CH_2, 7 CH, 5 quaternary C), whose chemical-shift values and multiplicities confirmed the presence of a mono-substituted benzene ring (δ(C) 138.8 (*s*), 128.8 (*d*), 128.8 (*d*), 128.6 (*d*), 128.6 (*d*), 126.5 (*d*)), two CH groups (δ(C) 123.1 (*d*), 47.0 (*d*)), two CH_2 groups (δ(C) 35.8 (*t*), 40.5 (*t*)), and two Me groups (δ(C) 23.3 (*q*), 21.5 (*q*)). Analysis of the ^{1}H,^{1}H-COSY spectrum led to the identification of two spin systems: **a** (CH_2(7) to CH_2(8)), and **b** (H–C(13) to Me(14)) (*Fig. 2*). Long range HMBC correlations of CH_2(7) to C(1), C(2) and C(6), and CH_2(8) to C(1) suggested that C(7) was connected to the mono-substituted benzene ring. In addition, CH_2(8) showed a strong HMBC cross-peak to the CO C-atom at δ(C) 160.8 (*s*, C(9)), together with the ^{1}H,^{1}H-COSY correlation of CH_2(8) to the NH signal δ(H) 7.31–7.35, implied that C(8) was connected to C(9) by an amide linkage. Both H–C(13) and Me(16) showed HMBC correlations to the CO C-atom at δ(C) 169.5 (*s*, C(15)), and H–C(13) also showed a ^{1}H,^{1}H-COSY correlation with the NH signal at δ(H) 5.97–6.01, suggesting that an Ac unit is connected to C(13) through an amide linkage. The other significant HMBC cross-peaks were observed from H–C(11), H–C(13), and Me(14) to the quaternary C-atom at δ(C) 172.3, and from H–C(11) to another quaternary C-atom at δ(C) 149.7. Considering the molecular formula, the presence of a disubstituted thiazole ring was suspected. Comparison of the NMR data of the thiazole ring with those reported [13][14] confirmed the above assignments. The structure of compound **1** showed strong similarities to that of the co-occurring alkaloid **4**. In fact, comparison of the NMR data of the two compounds revealed that the only

difference between them was that the indole ring in **4** was replaced by a benzene ring in **1**. Thus, the constitution of compound **1** was determined to be 2-[(1*R*)-1-(acetylamino)ethyl]-*N*-(2-phenylethyl)-1,3-thiazole-4-carboxamide. The absolute configuration at C(13) was tentatively determined to be the same as that in compound **4** by comparison of their optical rotation data: $[\alpha]_D^{24} = -16.0$ ($c = 0.10$, MeOH) for compound **1** and $[\alpha]_D^{24} = -15.2$ ($c = 0.10$, MeOH) for compound **4**.

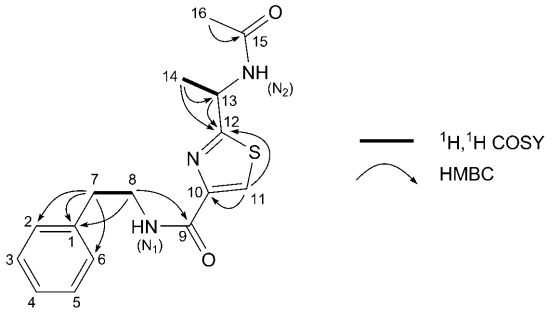


Fig. 2. *Selected ^{1}H,^{1}H-COSY and HMBC correlations for compound* **1**

Table. *NMR Data of Compounds* **1** *and* **4** (in $CDCl_3$)

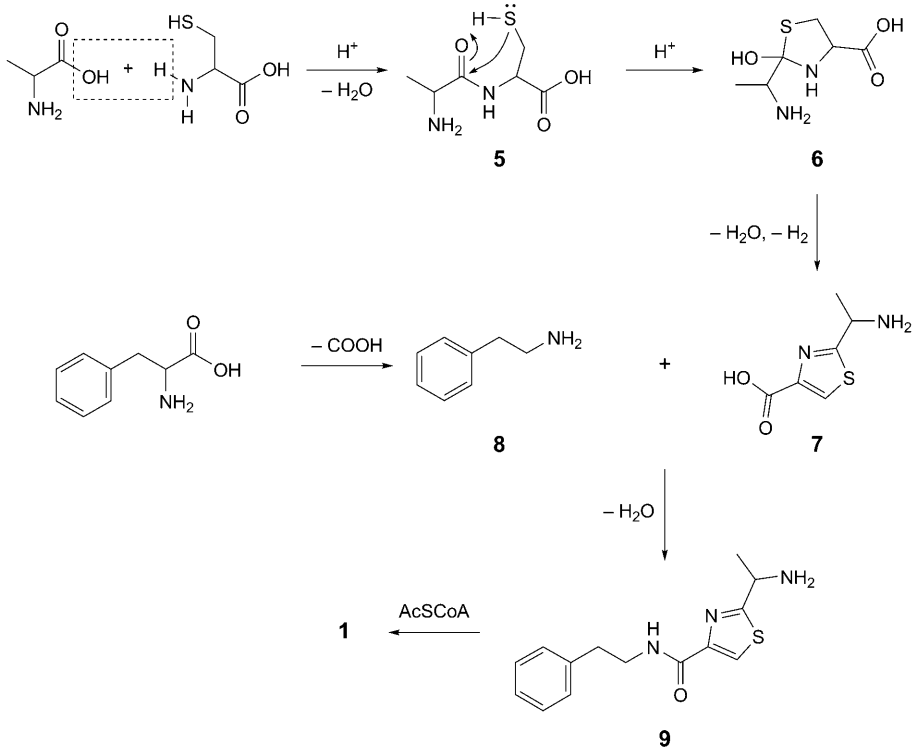
	1		**4**
	δ(H)[a])	δ(C)[b])	δ(C)[b])
C(1) or H–C(1)	–	138.8 (*s*)	122.3 (*d*)
H–C(2) or C(2)	7.24–7.28 (*m*)	128.8 (*d*)	113.3 (*s*)
H–C(3)	7.30–7.34 (*m*)	128.6 (*d*)	119.0 (*d*)
H–C(4)	7.23–7.26 (*m*)	126.5 (*d*)	119.6 (*d*)
H–C(5)	7.31–7.35 (*m*)	128.6 (*d*)	122.3 (*d*)
H–C(6)	7.24–7.27 (*m*)	128.8 (*d*)	111.4 (*d*)
CH_2(7) or C(7)	2.93 (*t*, $J = 7.2$)	35.8 (*t*)	136.6 (*s*)
CH_2(8) or C(8)	3.70 (*dt*, $J = 7.2, 6.6$)	40.5 (*t*)	127.6 (*s*)
C(9) or CH_2(9)	–	160.8 (*s*)	25.6 (*t*)
C(10) or CH_2(10)	–	149.7 (*s*)	39.9 (*t*)
H–C(11) or C(11)	8.00 (*s*)	123.1 (*d*)	161.0 (*s*)
C(12)	–	172.3 (*s*)	150.1 (*s*)
H–C(13)	5.38 (*q*, $J = 6.7$)	47.0 (*d*)	123.1 (*d*)
Me(14) or C(14)	1.57 (*d*, $J = 6.7$)	21.5 (*q*)	172.4 (*s*)
C(15) or H–C(15)	–	169.5 (*s*)	47.2 (*d*)
Me(16)	2.04 (*s*)	23.3 (*q*)	21.7 (*q*)
C(17)	–		169.5 (*s*)
Me(18)	–		23.4 (*q*)
NH-1	7.31–7.35 (*m*)		
NH-2	5.97–6.01 (*m*)		

[a]) Measured at 400 MHz, in $CDCl_3$, referred to the residual $CHCl_3$ (δ(H) 7.26 ppm). [b]) Measured at 100 MHz, in $CDCl_3$, referred to the residual $CDCl_3$ (δ(C) 77.0 ppm).

The known compound **4** was identified as bacillamide C by analysis of its NMR spectral data and optical rotation, as well as by comparison with the reported data [13][14].

A plausible biosynthetic pathway for compound **1** is proposed as shown in *Scheme 1*. Biogenetically, compound **1** could be derived from the amino acids alanine, cysteine, and phenylalanine [15][16]. The condensation of alanine and cysteine will afford 2-(1-aminoethyl)thiazole-4-carboxylic acid (**7**) *via* the two intermediates **5** and **6**. On the other hand, decarboxylation of phenylalanine will give 2-phenylethylamine (**8**). Compound **7** reacts with **8** (amidation) by the formation of an amide bond to yield **1** (*Scheme*).

Scheme. *Plausible Biogenetic Pathway to Compound* **1**



Neobacillamide A is structurally related to bacillamides A – C (**2** – **4**), all of which were previously isolated from the bacteria of the genus *Bacillus* [14][17]. It is interesting to note that bacillamides A – C all contain a common tryptamine moiety in their molecules while in **1** the amine portion is replaced by a phenethylamine. Neobacillamide A is the first member of *Bacillus* thiazole alkaloids containing a phenethylamine moiety. In addition, bacillamide C (**4**) was isolated from the sponge-associated bacterium for the first time.

Compounds **1** and **4** were evaluated for their inhibitory activity against HL60 human leukemia cells and A549 human lung cancer cells. Unfortunately, the results indicated that both compounds **1** and **4** were inactive. Other bioassay studies for other bioactivities are currently underway.

Further study should be conducted to understand the real role of these metabolites in the relations between sponge and its associated bacteria as well as to conduct fermentation of the titled bacterium on a large scale and to explore the potential pharmaceutical values of compounds **1** and **4**.

Experimental Part

General. Column chromatography (CC): commercial silica gel (SiO_2; *Qing Dao Hai Yang Chemical Group Co.*; 200–300 mesh), and *Sephadex LH-20* (*Amersham Biosciences*). TLC: precoated silica gel plates (*Yan Tai Zi Fu Chemical Group Co.*; *G60*, *F-254*). Reversed-phase HPLC: *Agilent 1100* series liquid chromatograph with a *VWD G1314A* detector at 210 nm using a semi-prep. *ODS-HG-5* (5 μm, 10 mm (i.d.) × 25 cm) column. Optical rotations: *Perkin-Elmer 341* polarimeter. UV Spectra: *UNICO UV-2102 PCS* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Nicolet Magna FT-IR 750* spectrophotometer; ν_{max} in cm^{-1}. ^1H and ^{13}C-NMR spectra: *Varian Mercury 400* (400 MHz for ^1H and 100 MHz for ^{13}C) spectrometer; chemical shifts δ in ppm, with residual $CDCl_3$ (δ(H) 7.26, δ(C) 77.0) as internal standard, coupling constant J in Hz. ^1H- and ^{13}C-NMR assignments were supported by ^1H,^1H-COSY, HMQC, HMBC experiments. ESI-MS and HR-ESI-MS: *Q-TOF Micro* LC-MS-MS spectrometer in m/z.

Sponge and Bacterium Material. The sponge *Dysidea avara* was collected by SCUBA diving at depth of about 20 m around Sanya Island in the South China Sea in November 2002 and identified by Professor *J.-H. Li* of Institute of Oceanology, Chinese Academy of Sciences [12]. *Bacillus vallismortis* C89 was isolated from *Dysidea avara* and identified as *Bacillus vallismortis* by 16S rDNA sequencing (GenBank No. DQ091007), showing significant activity against *A. Niger* and *P. Variotii* [12].

Extraction and Isolation. The bacterium *B. vallismortis* was incubated on solid plates at 28° using as a medium 5 g of beef extract, 10 g of peptone, 20 g of agar in every 1000 ml of artificial seawater with pH 7.0–7.2. After 5–6 d of incubation, bacterial clones were razed and the solid medium was extracted four times with AcOEt. Evaporation of AcOEt extracts gave an oil (6.4 g) which was subjected to CC (SiO_2; $CHCl_3$/MeOH). Consequently, 5 fractions (*Fr. 1–5*) were obtained according to the increasing proportion of MeOH ($CHCl_3$/MeOH (100 : 1, 80 : 1, 40 : 1, 30 : 1, 10 : 1)). *Fr. 2* (188.9 mg), *Fr. 3* (83.9 mg), and *Fr. 4* (595.0 mg) were further chromatographed over *Sephadex LH-20* columns with $CHCl_3$/MeOH 1 : 1, resp. The subfractions (*Fr. 2.1*, *3.1*, *4.1*) which showed positive reactions with *Dragendorff*'s reagent were further purified on semi-prep. HPLC at a flow rate of 2 ml/min with different ratios of MeOH/H_2O and monitored at 210 nm. *Fr. 3.1* (16.7 mg) was subjected to MeOH/H_2O (75 : 25) to yield **1** (1.8 mg) with a t_R value of 12.4 min. *Fr. 4.1* (30.6 mg) was eluted with MeOH/H_2O (70 : 30) to deliver compound **4** (4.0 mg) with a t_R value of 11.3 min.

Neobacillamide A (=*2-[(1*R*)-1-(Acetylamino)ethyl]*-N-*(2-phenylethyl)-1,3-thiazole-4-carboxamide*; **1**). Yellow oil. $[\alpha]_D^{24} = -16$ ($c = 0.10$, MeOH). UV (MeOH): 209 (3.76), 228 (3.63), 235 (3.61), 328 (3.22). IR (KBr): 3423.1, 2923.6, 2852.2, 1654.7, 1546.7, 1496.5, 1454.1, 1375.0, 1253.5, 1062.6, 750.2, 700.0. ^1H- and ^{13}C-NMR: *Table.* ESI-MS: 340.1 ($[M+Na]^+$). HR-ESI-MS: 340.1081 ($[M+Na]^+$; calc. 340.1096).

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