Chemistry of Natural Compounds, Vol. 45, No. 2, 2009

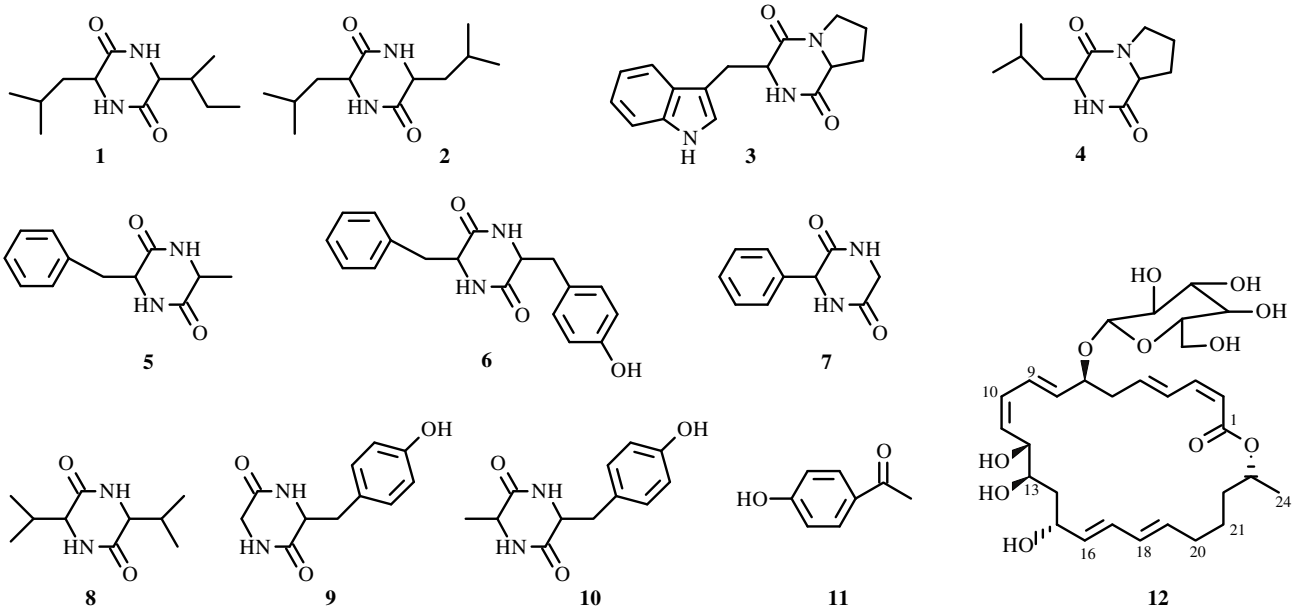
DIKETOPIPERAZINE CONSTITUENTS OF MARINE *Bacillus subtilis*

Xiaoling Lu,[1,2] Yunheng Shen,[3] Yuping Zhu,[1] Qiangzhi Xu,[1] Xiaoyu Liu,[1] Kunyi Ni,[2] Xin Cao,[4] Weidong Zhang,[3*] and Binghua Jiao[1]

UDC 547.9

Marine organisms, especially marine microorganisms, have been proven to be a rich source of diverse arrays of bioactive metabolites with great potential for pharmaceutical and medical applications. Marine *Bacillus subtilis* is a common strain species, which usually produces cyclopeptide, macrolide, indole derivatives, etc. In the course of further studies, 10 diketopiperazines: cyclo(Leu-Ile) (**1**) [1], cyclo(Leu-Leu) (**2**) [2], cyclo(Trp-Pro) (**3**) [3], cyclo(Leu-Pro) (**4**) [4, 5], cyclo(Phe-Ala) (**5**) [6], cyclo(Phe-Tyr) (**6**) [2], cyclo(benzyl-Gly) (**7**) [7], cyclo(Val-Val) (**8**) [8], cyclo(Gla-Tyr) (**9**) [9], cyclo(Ala-Tyr) (**10**) [10], 1-(4-hydroxyphenyl)ethanone (**11**) [11], and macrolactin B (**12**) [12] have been isolated from the marine *Bacillus subtilis*. Among them, compound **7** is a new natural product. All the compounds have not been reported before from this species.

The strain 201721 has been derived from sea sediment of East Sea of China. It was isolated on F1 medium [Glucose (6 g), peptone (5 g), and yeast extract (1 g) were dissolved in artifical seawater (1L)] with incubation for 4 days at 28°C. Due to its chemical and morphological features as well as the 16S rDNA (GenBank accession number EF417872), the strain can be identified as the genus *Bacillus* sp.



1) Department of Biochemistry and Molecular Biology, College of Basic Medical Sciences, Second Military Medical University, No. 800, Xiangyin Rd., Shanghai 200433, People's Republic of China, e-mail: jiaobh@uninet.com.cn; 2) Department of Analytical Chemistry, China Pharmaceutical University, Nanjing, 210009, P. R. China; 3) College of Pharmacy, Second Military Medical University, Shanghai, 200433, P. R. China, e-mail: wdzhangY@hotmail.com; 4) Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing, 210009, P. R. China. Published in Khimiya Prirodnykh Soedinenii, No. 2, pp. 244–246, March–April, 2009. Original article submitted September 3, 2007.

 0009-3130/09/4502-0290

The entire culture broth was centrifugated at 12000 rpm for 15 min, yielding the supernatant. The supernatant was extracted with ethyl acetate 3 times. The combined extracts were evaporated in vacuo at 30°C to yield 5 g of the crude extract, which was subjected to Sephadex LH-20 column and eluted with $CHCl_3$–CH_3OH (4:1) to get five fractions, one of which was rechromatographed on middle-pressure LC with the gradient CH_3OH–H_2O. The fractions obtained were chromatographyed on a C_8 semi-preparative LC (Zorbax Columns, R_X-C_8, 9.4 mm × 25 cm, 2 mL/min, 218 nm) using isocratic elution of CH_3CN–H_2O, yielding compounds **1** (2 mg), 2 (2 mg), **3** (3 mg), **4** (2 mg), **5** (1 mg), **6** (3 mg), **7** (2 mg), **8** (1 mg), **9** (5 mg), **10** (3 mg), **11** (5 mg), and **12** (3 mg).

Cyclo(Leu-Ile) (1): white powder, $C_{12}H_{22}N_2O_2$. ESI-MS: *m/z* 249.5 $[M+Na]^+$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, δ): 4.0 (1H, m, Leu_α), 3.8 (1H, m, Ile_α), 1.93 (1H, m, Ile_β), 1.74 (1H, m, Leu_β), 1.72 (1H, m, Leu_β), 1.71 (1H, m, Ile_γ), 1.54 (1H, m, Ile_γ), 1.41 (1H, m, Leu_γ), 1.2 (3H, d, $Ile_{\beta\text{-}CH3}$), 1.19 (3H, m, $Leu_{\gamma\text{-}CH3}$), 0.88 (3H, d, $Leu_{\gamma\text{-}CH3}$), 0.80 (3H, d, Ile_δ); ^{13}C NMR (150 MHz, CD_3OD, CD_3Cl, δ): 169.58 (s, Leu_{CO}), 168.08 (s, Ile_{CO}), 60.07 (d, Ile_α), 53.26 (d, Leu_α), 44.49 (t, Leu_β), 38.98 (d, Ile_β), 24.44 (t, Ile_γ), 24.33 (d, Leu_γ), 23.29 (q, $Ile_{\beta\text{-}CH3}$), 21.26 (q, Ile_δ), 15.29 (q, $Leu_{\gamma\text{-}CH3}$), 11.72 (q, $Leu_{\gamma\text{-}CH3}$).

Cyclo(Leu-Leu) (2): white powder, $C_{12}H_{22}N_2O_2$. ESI-MS: *m/z* 249.3 $[M+Na]^+$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, δ): 3.9 (1H, m, Leu_α), 1.8 (2H, m, Leu_β), 1.7 (1H, m, Leu_γ), 1.5 (3H, d, $Leu_{\gamma\text{-}CH3}$), 1.2 (3H, d, $Leu_{\gamma\text{-}CH3}$); ^{13}C NMR (150 MHz, CD_3OD, CD_3Cl, δ): 169.12 (s, Leu_{CO}), 53.23 (d, Leu_α), 43.82 (t, Leu_β), 24.09 (d, Leu_γ), 23.06 (q, $Leu_{\gamma\text{-}CH3}$), 21.00 (q, $Leu_{\gamma\text{-}CH3}$).

Cyclo(Trp-Pro) (3): white powder, $C_{16}H_{17}N_3O_2$.ESI-MS: *m/z* 306.4 $[M+Na]^+$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, δ, J/Hz): 7.53 (1H, m, J = 7.8, $Trp_{2'}$), 7.50 (1H, m, J = 7.2, $Trp_{4'}$), 7.34 (1H, dd, J = 7.2, $Trp_{7'}$), 7.12 (2H, m, J = 7.2, $Trp_{6'}$), 7.03 (1H, m, J = 7.2, $Trp_{5'}$), 4.36 (1H, m, Trp_α), 4.00 (1H, m, Pro_α), 3.52 (2H, m, Pro_δ), 3.4 (1H, m, Trp_β), 3.1 (1H, m, Trp_β), 2.1 (1H, m, Pro_β), 1.7 (2H, m, Pro_β, Pro_γ), 1.4 (1H, m, Pro_γ); ^{13}C NMR (150 MHz, CD_3OD, CD_3Cl, δ): 170.22 (s, Pro_{CO}), 166.68 (s, Trp_{CO}), 137.36 (s, $Trp_{9'}$), 127.48 (s, $Trp_{4'}$), 124.72 (d, $Trp_{9'}$), 122.43 (d, $Trp_{6'}$), 119.69 (d, $Trp_{7'}$), 118.83 (d, $Trp_{5'}$), 112.08 (d, $Trp_{8'}$), 108.92 (s, $Trp_{3'}$), 59.66 (d, Pro_α), 55.84 (d, Trp_α), 45.71 (t, Pro_δ), 28.57 (t, Pro_β), 27.90 (t, Trp_β), 22.55 (t, Pro_δ).

Cyclo(Leu-Pro) (4): white powder, $C_{11}H_{18}N_2O_2$. ESI-MS: *m/z* 233.1 $[M+Na]^+$ 211.1 $[M+H]^+$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, δ): 4.1 (1H, m, Leu_α), 4.0 (1H, m, Pro_α), 3.5 (2H, m, Pro_δ), 2.6 (2H, m, Pro_β), 1.9 (2H, m, Pro_γ), 1.8 (2H, m, Leu_β), 1.71 (1H, m, Leu_γ), 1.1 (3H, d, $Leu_{\gamma\text{-}CH3}$), 0.9 (3H, d, $Leu_{\gamma\text{-}CH3}$); ^{13}C NMR (150 MHz, CD_3OD, CD_3Cl, δ): 170.48 (s, Leu_{CO}), 166.26 (s, Pro_{CO}), 58.83 (d, Pro_α), 53.4 (d, Leu_α), 45.41 (t, Pro_δ), 38.49 (t, Leu_β), 24.54 (t, Pro_β), 23.16 (d, Leu_γ), 22.67 (q, $Leu_{\gamma\text{-}CH3}$), 21.55 (t, Pro_γ), 21.24 (q, $Leu_{\gamma\text{-}CH3}$).

Cyclo(Phe-Ala) (5): white needle, $C_{12}H_{14}N_2O_2$. ESI-MS: *m/z* 241.2 $[M+Na]^+$ *m/z* 217.6 $[M-H]^-$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, J/Hz, δ): 7.20–7.35 (5H, m, Phe_{Ar}), 4.27 (1H, m, J = 4.8, Phe_α), 3.8 (1H, m, J = 7.2, Ala_α), 3.19 (1H, m, Phe_β), 3.09 (1H, m, Phe_β), 0.8 (3H, d, Ala_β); ^{13}C NMR (CD_3OD, CD_3Cl, 150 MHz): δ 167.00 (s, Phe_{CO}), 166.26 (s, Ala_{CO}), 134.85 (s, $Phe_{1'}$), 130.00 (d, $Phe_{3'}$), 128.60 (d, $Phe_{2'}$), 127.30 (d, $Phe_{4'}$), 56.06 (d, Phe_α), 50.47 (d, Ala_α), 39.62 (t, Phe_β), 19.69 (q, Ala_β).

Cyclo(Phe-Tyr) (6): white needle, $C_{18}H_{18}N_2O_3$. ESI-MS: *m/z* 333.4 $[M+Na]^+$. ^1H NMR (600 MHz, DMSO-d_6, δ, J/Hz): 9.234 (1H, s, Tyr_{OH}), 7.836 (2H, s, Tyr_{NH}, Phe_{NH}), 7.287 (2H, d, J = 7.2, $Phe_{2'}$), 7.203 (1H, d, J = 7.2, $Phe_{4'}$), 7.043 (2H, d, J = 7.8, $Phe_{3'}$), 6.841 (2H, d, J = 8.4, $Tyr_{2'}$), 6.679 (2H, d, J = 8.4, $Tyr_{3'}$), 3.942 (1H, s, Tyr_α), 3.886 (1H, d, Phe_α), 2.58 (1H, m, Tyr_β), 2.50 (1H, m, Tyr_β), 2.18 (2H, m, Phe_β); ^{13}C NMR (125 MHz, DMSO-d_6, δ): 166.13 (s, Tyr_{CO}), 166.12 (s, Phe_{CO}), 156.07 (s, $Tyr_{4'}$), 136.62 (s, $Phe_{1'}$), 130.75 (s, $Tyr_{1'}$), 129.69 (d, $Tyr_{2'}$), 128.13 (d, $Phe_{3'}$), 127.90 (d, $Phe_{2'}$), 126.38 (d, $Phe_{4'}$), 114.98 (d, $Tyr_{3'}$), 55.68 (d, Tyr_α), 55.36 (d, Phe_α), 39.08 (t, Tyr_β), 38.48 (t, Phe_β).

Cyclo(benzyl-Gly) (7): white powder, $C_{10}H_{10}N_2O_2$. ESI-MS: *m/z* 213.2 $[M+Na]^+$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, δ, J/Hz): 7.216–7.337 (5H, m, $benzyl_{Ar'}$), 4.28 (1H, dd, J = 6, $benzyl_\alpha$), 3.38 (1H, m, Gly_α), 2.89 (1H, m, Gly_α); ^{13}C NMR (150 MHz, CD_3OD, CD_3Cl, δ): 174.81 (s, $benzyl_{CO}$), 157.60 (s, Gly_{co}), 135.31 (s, $Ar_{1'}$), 129.15 (d, $Ar_{2'}$), 128.63 (d, $Ar_{3'}$), 127.17 (d, $Ar_{4'}$), 59.70 (d, $benzyl_\alpha$), 37.61 (t, Gly_α).

Cyclo(Val-Val) (8): white powder, $C_{10}H_{18}N_2O_2$. ESI-MS: *m/z* 222 $[M+Na+H]^+$. ^1H NMR (600 MHz, CD_3Cl, δ): 5.923 (1H, s, NH), 4.116 (1H, d, Val_α), 2.5 (1H, m, Val_β), 1.25 (3H, d, Val_γ), 0.9 (3H, d, Val_γ); ^{13}C NMR (125 MHz, CD_3Cl, δ): 167.42 (s, CO), 59.83 (d, Val_α), 30.91 (d, Val_β), 18.26 (q, Val_γ), 16.27 (q, Val_γ).

Cyclo(Gla-Tyr) (9): white powder, $C_{11}H_{12}N_2O_3$. ESI-MS: *m/z* 243.7 $[M+Na]^+$ 219.8 $[M-H]^-$. ^1H NMR (600 MHz, DMSO-d_6, δ, J/Hz): 9.85(1H, s, OH), 8.059 (1H, d, Tyr_{NH}), 7.817 (1H, s, Gly_{NH}), 6.915 (2H, d, J = 8.4, $Tyr_{2'}$), 6.636 (2H, d, J = 8.4, $Tyr_{3'}$), 3.954 (1H, d, Tyr_α), 3.28 (1H, m, Gly_α), 2.97 (1H, m, Gly_α), 2.74 (1H, m, Tyr_β), 2.68 (1H, m, Tyr_β); ^{13}C NMR (DMSO-d_6, 150 MHz): δ 167.38 (s, Tyr_{CO}), 165.73 (s, Gly_{CO}), 156.27 (s, $Tyr_{4'}$), 131.02 (d, $Tyr_{2'}$), 125.73 (s, $Tyr_{1'}$), 114.97 (d, $Tyr_{3'}$), 55.76 (d, Tyr_α), 43.66 (t, Gly_α), 38.17 (t, Tyr_β).

Cyclo(Ala-Tyr) (10): white powder, $C_{12}H_{14}N_2O_3$. ESI-MS: *m/z* 257.8 $[M+Na]^+$. ^1H NMR (600 MHz, CD_3OD, δ, J/Hz): 6.994 (2H, d, J = 8.4, $Tyr_{2'}$), 6.704 (2H, d, J = 9, $Tyr_{3'}$), 4.22 (1H, m, Tyr_α), 3.76 (1H, m, Ala_α), 3.15 (1H, dd, J = 3.6, Tyr_β), 2.8 (1H, dd, J = 4.8, Tyr_β), 0.6 (3H, d, Ala_β); ^{13}C NMR (125 MHz, CD_3OD, δ): 170.71 (s, Tyr_{CO}), 170.63 (s, Ala_{CO}), 157.95 (s, $Tyr_{4'}$), 132.75 (d, $Tyr_{2'}$), 127.1 (s, $Tyr_{1'}$), 116.32 (d, $Tyr_{3'}$), 57.6 (d, Tyr_α), 51.75 (d, Ala_α), 39.55 (t, Tyr_β), 20.43 (q, Ala_β).

1-(4-Hydroxyphenyl)ethanone (11): a light green oil, $C_8H_8O_2$. ESI-MS: *m/z* 159.0 $[M+Na]^+$, *m/z* 135.1 $[M-H]^-$. ^1H NMR (600 MHz, CD_3OD, CD_3Cl, δ, J/Hz): 7.8 (2H, dd, J = 4.8, Ar-2,2′), 6.8 (2H, dd, J = 4.8, Ar-3,3′), 2.543 (3H, s, CH_3); ^{13}C NMR (125 MHz, CD_3OD, CD_3Cl, δ): 197.87 (s, CO), 161.9 (s, $Ar_{4'}$), 130.82 (d, $Ar_{2'}$), 129.00 (s, $Ar_{1'}$), 115.13 (d, $Ar_{3'}$), 25.95 (t, CH_3).

Macrolactin B (12): a white solid, $C_{30}H_{44}O_{10}$. ESI-MS: *m/z* 587.76 $[M+Na]^+$, *m/z* 600.20 $[M+Cl]^-$. ^1H NMR (600 MHz, CD_3OD, δ, J/Hz): 7.19 (1H, dd, J = 15, H-4), 6.71 (1H, dd, J = 15, H-9), 6.68 (1H, dd, J = 11.4, H-3), 6.23 (1H, dd, J = 15, H-5), 6.16 (1H, dd, J = 11.2, H-17; 1H, dd, J = 15.2, H-10), 6.03 (1H, dd, J = 15, H-18), 5.62 (1H, dd, J = 15, H-8), 5.59 (1H, dd, J = 11.2, H-11), 5.58 (1H, dd, J = 15, H-19), 5.56 (1H, dd, J = 11.4, H-2; 1H, dd, J = 15.2, H-16), 5.0 (1H, m, H-23), 4.49 (1H, dd, H-7), 4.36 (1H, dd, H-15), 4.3 (1H, m, H-1′), 4.1 (1H, m, H-2′), 3.9 (1H, dd, H-13), 3.87 (1H, m, H-6′), 3.34 (1H, m, H-4′), 3.28 (1H, m, H-3′), 3.22 (1H, m, H-5′), 2.4, 2.5 (2H, m, H-12), 2.4, 2.5 (2H, m, H-6), 2.1, 2.2 (2H, m, H-20), 1.6 (2H, m, H-14), 1.6 (2H, m, H-22), 1.5 (2H, m, H-21), 1.2 (3H, d, H-24); ^{13}C NMR (125 MHz, CD_3OD, δ): 167.95 (s, C-1), 145.30 (d, C-3), 142.2 (d, C-5), 135.46 (d, C-16), 134.85 (d, C-19), 133.96 (d, C-8), 131.78 (d, C-18), 131.37 (d, C-17), 130.93 (d, C-4), 129.87 (d, C-10), 129.42 (d, C-9), 129.09 (d, C-11), 117.78 (d, C-2), 101.12 (d, C-1′), 78.26 (d, C-4′), 77.99 (d, C-7), 75.18 (d, C-3′) 72.41 (d, C-5′), 71.88 (d, C-2′), 71.84 (d, C-23), 69.73 (d, C-15), 69.10 (d, C-13), 62.9 (t, C-6′), 43.66 (t, C-14), 41.4 (t, C-6), 36.54 (t, C-12), 35.99 (t, C-22), 32.83 (t, C-20), 25.54 (t, C-21), 20.13 (q, C-24).

ACKNOWLEDGMENT

The work was supported by the Shanghai Outstanding Field Leaders Program (05XD1423) and the National Hi-tech R&D Program (2007AA091501) to Dr. B. Jiao, Dr. X. Liu (2006 AA09Z416) and Dr. Q. Xu (2006 AA 09Z425).

REFERENCES

1. Hu Yong-mei, Wang Hao, Ye Wen-cai, and Liu Ge, *Zhongguo Yaoke Daxue Xuebao*, **6**, 523 (2005).
2. Cheng Zhi-hong, Wu Tao, and Yu Boyang, *Tianran Chanwu Yanjiu Yu Kaifa.,* **17** (1), 1 (2005).
3. V. Ivanova, U. Graefe, R. Schlegel, B. Schlegel, A. Gusterova, M. Kolarova, and K. Aleksieva, *Biotechnol. Biotechnol. Equip.,* **17** (2), 128 (2003).
4. F. J. Schmitz, D. J. Vanderah, K. H. Hollenbeak, C. E. L. Enwall, V. Gopichand, P. K. Sen Gupta, M. B. Hossain, and D. V. Helm, *J. Org. Chem.,* **48**, 3941 (1983).
5. J. Kang and R. Y. Chen, *Chin. J. Chin. Mater. Med.,* **28** (11), 1038 (2003).
6. H. J. Li, Y. C. Lin, and X. H. Liu, *Acta Sci. Nat. Univ. Suny,* **41** (1), 110 (2002).
7. Sato Nobuhiro and Kato Yukihiro, *J. Heter. Chem.*, **23**, 1677 (1986).
8. Ogura Haruo, Furuhata Kimio, and Furuhata Katsuko, *Chem. Pharm. Bull*, **23**, 2474 (1975).
9. K. Suzuki, Y. Sasaki, N. Endo, and Y. Mhara, *Chem. Pharm. Bull*, **29**, 233 (1981).
10. Guangying Chen, Yongcheng Lin, L. L. P. Vrijmoed, and Wang-Fun Fong, *Chem. Nat. Comp.,* **42**, 138 (2006).
11. X. W. Yang, J. S. Wang, Y. H. Wang, H. T. Xiao, X. J. Hu, Sh. Zh. Mu, Y. L. Ma, H. Lin, H. P. He, L. Li, and X. J. Hao, *Planta Med.,* **73**, 496 (2007).
12. K. Gustafson, M. Roman, and William Fenical, *J. Am. Chem. Soc.,* **111**, 75199 (1989).