Three New Prenylated Diketopiperazines from *Neosartorya fischeri*

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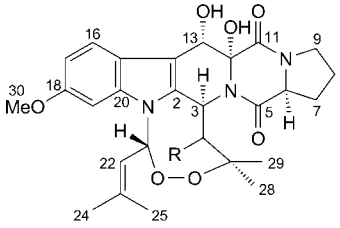
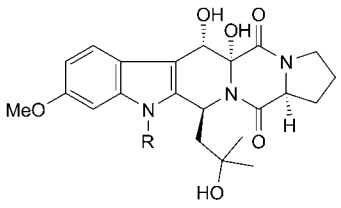
Three new prenylated 2,5-diketopiperazines, namely neofipiperazines A–C (**1**–**3**, resp.), were isolated from the culture of *Neosartorya fischeri* CGMCC 3.5378, together with six known ones. The structures of the new compounds were elucidated by comprehensive spectroscopic analysis, especially HR-ESI-MS and NMR experiments. All the prenylated diketopiperazines, except **7**, were isolated for the first time from the species.

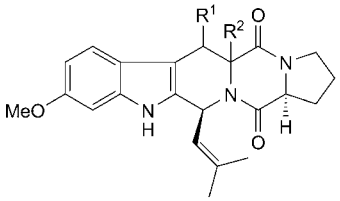
Introduction. – *Neosartorya fischeri*, one of the most frequently reported heat-resistant moulds [1], is a common environmental fungus, which could cause human endocarditis [2], virulent peritonitis in mice [3], and spoilage in fruit products [4]. Previous chemical investigations indicated that the fungus *N. fischeri* produce an amazing variety of metabolites, including polyketides [5–8], cylcopeptides [9], and alkaloids [3][10]. These metabolites possess a variety of biological features, including antifungal [5], antiviral [8], and cytotoxic [8][11] activities. Herein, we describe the isolation and identification of nine prenylated diketopiperazines from the culture of the fungus fermented in solid medium, including three new ones, namely neofipiperzines A–C (**1**–**3**, resp.), and six known analogs, **4**–**9**. The structures of the new compounds



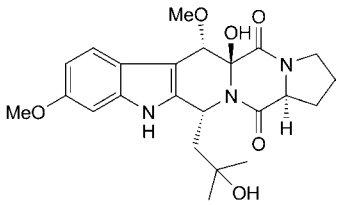
1 R = α-OH
2 R = β-OH
4 R = H



3 R = $Me_2C{=}CHCH_2$
5 R = H



6 R^1 = α-OH, R^2 = α-OH
7 R^1 = H, R^2 = α-H
8 R^1 = α-MeO, R^2 = β-OH



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were determined by NMR-spectroscopic, especially 2D-NMR techniques (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY), and mass spectrometric analyses.

Results and Discussion. – Neofipiperzine A (**1**) was obtained as white amorphous powder. The molecular formula of **1** was established as $C_{27}H_{33}N_3O_8$ by HR-ESI-MS (*m*/*z* 528.2336 ($[M+H]^+$; calc. 528.2346)), indicating 13 degrees of unsaturation. The IR spectrum of **1** showed a strong absorption band at 3412 cm^{-1} for OH groups. The ^{1}H-NMR spectrum (*Table*) of **1** exhibited signals of four tertiary Me groups (δ(H) 1.10 (Me(28)), 1.64 (Me(29)), 1.77 (Me(24)), and 2.01 (Me(25))), and one MeO group (δ(H) 3.83 (Me(30))). In addition, one olefinic H-atom signal (δ(H) 5.12 (*dd*, *J* = 1.0, 8.0, H–C(22))) and five low-field CH signals (δ(H) 6.61 (*d*, *J* = 8.0, H–C(21)), 6.29 (*d*, *J* = 9.5, H–C(3)), 5.69 (*s*, H–C(13)), 4.53 (*dd*, *J* = 7.5, 10.0, H–C(6)), and 3.15 (*d*, *J* = 9.0, H–C(26))). Three signals at δ(H) 7.90 (*d*, *J* = 9.0), 6.81 (*dd*, *J* = 2.0, 8.5), and 6.57 (*d*, *J* = 2.0) were ascribed to a 1,3,4-trisubstituted phenyl group. The ^{13}C-NMR and DEPT spectra of **1** (*Table*) displayed 27 C-atom signals, including those of ten quaternary C-atoms, and of nine CH, three CH_2, and five Me groups. Two low-field quaternary C-atom signals at δ(C) 166.0 (C(11)) and 174.5 (C(5)) indicated that **1** contained two C=O groups. The signals at δ(C) 85.7 (C(21)), 85.2 (C(27)), 82.9 (C(12)), 82.5 (C(26)), 68.8 (C(13)), and 55.7 (MeO) were attributed to the O-bearing C-atoms. Interpretation of NMR data of **1** indicated the presence of an indole group, which was confirmed by the UV data. The data mentioned above evidenced that **1** was a prenylated diketopiperazine alkaloid containing an indole group.

High similarity was observed between the NMR data of **1** and those of **4** [12], implying a close structural resemblance of the two compounds. The major difference was the change of an aliphatic CH_2 C-atom signal at δ(C) 45.3 in **4** to a low-field CH signal (δ(C) 82.5; δ(H) 3.15) in **1**, suggesting the presence of a OH group at C(26) in **1**, which was confirmed by HMBCs H–C(26)/C(2), H–C(26)/C(27), H–C(3)/C(26), Me(29)/C(26), and Me(28)/C(26) (*Fig. 1*). The single-crystal X-ray diffraction analysis (*Fig. 2*) of **1** indicated the configuration of OH at C(26) was α-face. Accordingly, the relative configuration of **1** was identified as α-orientation for H–C(3), H–C(6), HO–C(12), HO–C((13) and Me(28), and β-orientation for H–C(21), H–C(26) and Me(29). The structure of **1** was thereby established as neofipiperzine A.

Neofipiperzine B (**2**) had the molecular formula $C_{27}H_{33}N_3O_8$, which was identical with that of **1**, based on by HR-ESI-MS analysis (*m*/*z* 528.2350). The NMR spectra of **2** were very similar to those of **1**. The extensive analysis of 2D-NMR spectra (^{1}H,^{1}H-COSY, HMQC, and HMBC) revealed that **2** had the same constitution as **1**. The major difference between the two compounds was the chemical shift of C(26) (δ(C) 82.5) in **1** and δ(C) 78.0 in **2**, which indicated that **2** was a 26-epimer of **1**. The relative configuration of **2** was established by single-crystal X-ray diffraction analysis (*Fig. 3*), which confirmed that the structure of **2** was 26-epineofipiperzine A, and was named neofipiperzine B.

Compound **3** had the molecular formula $C_{27}H_{35}N_3O_6$ as deduced from HR-ESI-MS (*m*/*z* 498.2608 ($[M+H]^+$; calc. 498.2604)). The NMR data of **3** were identical to those of **5** [13], except for the signals of an additional prenyl unit in **3** compared with **5**. The downfield CH_2(21) chemical shift indicated that the prenyl unit was located at N(1) in **3** by comparison with those of **5**, which was confirmed by HMBCs H–C(21)/C(22),

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of* **1**–**3**. δ in ppm, J in Hz. Arbitrary atom numbering as indicated in the *Formulae*.

Position	**1**[b])		**2**[b])		**3**[a])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
2	–	128.3	–	125.0	–	132.7
3	6.29 (*d*, *J* = 9.5)	51.9	6.28(*s*)	52.7	5.73 (*dd*, *J* = 3.0, 9.0)	46.3
5	–	174.5	–	171.1	–	173.0
6	4.53 (*dd*, *J* = 7.5, 10.0)	59.0	4.47 (*dd*, *J* = 7.0, 9.0)	58.4	4.53 (*dd*, *J* = 7.0, 9.5)	59.4
7	2.14–2.16 (*m*), 2.45–2.48 (*m*)	29.1	1.94–1.96 (*m*), 2.34–2.36 (*m*)	29.0	2.08–2.11 (*m*), 2.48–2.50 (*m*)	28.9
8	1.95–1.97 (*m*), 2.05–2.07 (*m*)	22.8	1.92–1.94 (*m*)	22.1	1.99–2.01 (*m*), 2.09–2.11 (*m*)	22.9
9	3.61–3.64 (*m*)	45.2	3.46–3.49 (*m*)	45.0	3.61–3.63 (*m*)	45.1
11	–	166.0	–	166.0	–	166.0
12	–	82.9	–	82.9	–	84.0
13	5.69 (*s*)	68.8	5.55 (*s*)	68.0	5.80 (*s*)	68.9
14	–	106.8	–	111.7	–	105.3
15	–	120.8	–	120.8	–	120.5
16	7.90 (*d*, *J* = 9.0)	121.8	7.74 (*d*, *J* = 9.5)	121.1	7.85 (*d*, *J* = 8.5)	121.4
17	6.81 (*dd*, *J* = 8.5, 2.0)	109.4	6.67 (*dd*, *J* = 9.5, 2.5)	108.4	6.81 (*dd*, *J* = 8.5, 2.0)	109.6
18	–	156.4	–	155.3	–	156.3
19	6.57 (*d*, *J* = 2.0)	93.7	6.69 (*d*, *J* = 2.5)	93.5	6.77 (*d*, *J* = 2.5)	94.0
20	–	135.9	–	135.6	–	137.7
21	6.61 (*d*, *J* = 8.0)	85.7	6.75 (*d*, *J* = 8.0)	84.9	4.68 (*d*, *J* = 6.0)	41.9
22	5.12 (*dd*, *J* = 8.0, 1.0)	118.3	4.98 (*dd*, *J* = 8.0, 1.0)	118.4	5.16 (*t*, *J* = 6.0)	119.9
23	–	143.7	–	142.4	–	135.6
24	1.77 (*s*)	22.7	1.70 (*s*)	25.2	1.72 (*s*)	25.6
25	2.01 (*s*)	18.8	1.98 (*s*)	18.5	1.87 (*s*)	18.3
26	3.15 (*d*, *J* = 9.0)	82.5	3.40 (*d*, *J* = 5.0)	78.0	1.70 (*m*)	50.7
27	–	85.2	–	84.9	–	68.9
28	1.10 (*s*)	25.4	1.56 (*s*)	23.8	1.09 (*s*)	29.6
29	1.64 (*s*)	18.1	0.91 (*s*)	22.8	1.24 (*s*)	30.4
30	3.83 (*s*)	55.7	3.76 (*s*)	55.2	3.86 (*s*)	55.8

[a]) Recorded in $CDCl_3$. [b]) Recorded in (D_6)DMSO.

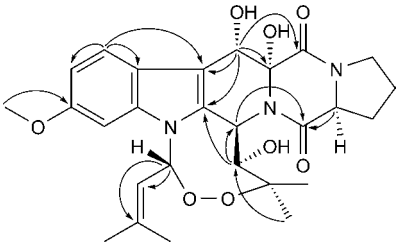

Fig. 1. *Key HMBCs* (H → C) *of* **1**

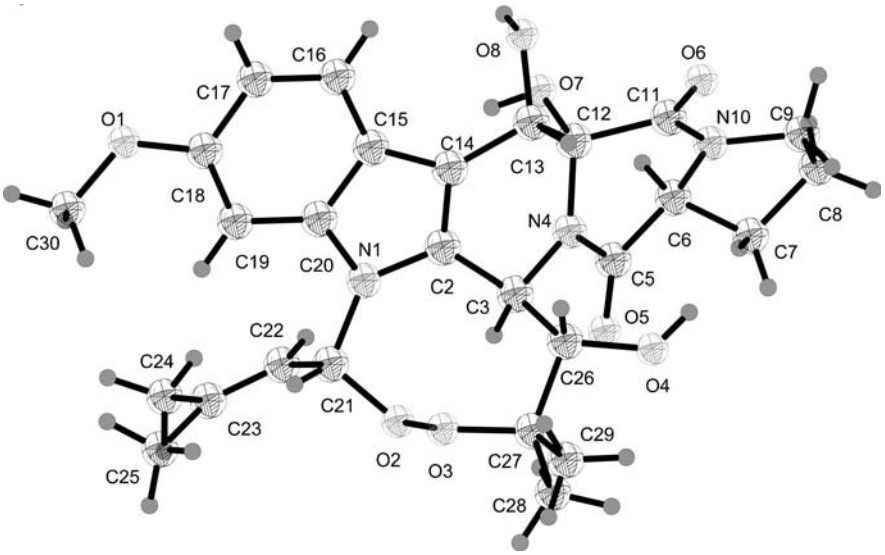

Fig. 2. *Single-crystal X-ray structure of* **1**

H–C(21)/C(23), H–C(21)/C(20) and H–C(21)/C(2). Thus, the structure of **3** was elucidated as depicted and named neofipiperzine C.

The structures of six known compounds were identified as verruculogen (**4**) [12], verruculogen TR-2 (**5**) [13], 12α,13α-dihydroxyfumitremorgin C (**6**) [14], fumitremorgin C (**7**) [12], cyclotryprostatin B (**8**) [15] and *rel*-(8S)-19,20-dihydro-9,20-dihydroxy-8-methoxy-9,18-diepifumitremorgin C (**9**) [16] by comparison of their spectroscopic data with those reported in the literature.

Diketopiperazines are an important class of fungal metabolites not only for their privileged structures, but also for their promising biological properties [17], such as antifungal [18], cytotoxic [13][14][16][19], and anti-inflamatory [20] activities. In this study, all isolated compounds belong to prenylated diketopiperazines biosynthesized from the condensation of proline and tryptophan. Among them, compounds **6** and **7** were reported to display potent and specific inhibition of the breast cancer resistance protein [11], while **8** was reported to be an inhibitor of the mammalian cell cycle [15].

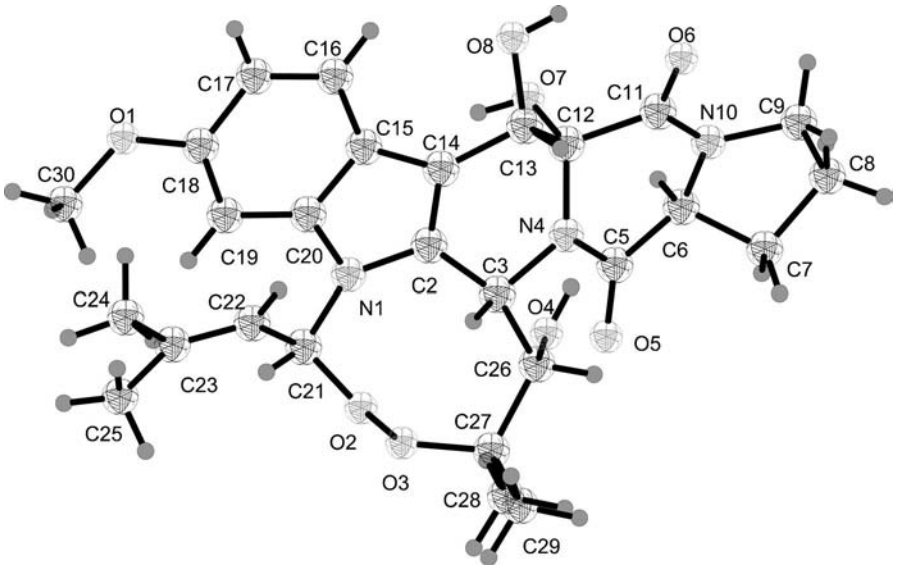

Fig. 3. *Single-crystal X-ray structure of* **2**

Experimental Part

General. All solvents used were of anal. grade (*Hangzhou Gaojin Fine Chemical Plan Chemical Plant*). TLC: Precoated silica-gel GF_{254} plates (*Qingdao Haiyang Chemical Plant*). Column chromatography (CC): silica gel (SiO_2; 230–400 mesh), *MCI CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries Ltd.*), *Lichroprep RP-18* gel (40–63 μm, *YMC ODS-A*), and *Toyopearl-HW-40C* gel (*Tosoh corporation*). HPLC: *Waters DELTA-600*. M.p.: *X-4 Digital Dispaly Microscopic Instrument* (*Beijing Tektronix Instrument Ltd.*). Optical rotations: *Rudolph-Autopol-IV* polarimeter. UV Spectra: *Shimadzu UV-2450* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Thermo-Nicolet-6700* spectra-photometer; $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker AM-500* apparatus; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Agilent-6210-Lc/Tof* mass spectrometer; in *m*/*z*. X-Ray single-crystal diffraction: *Agilent Gemini Altas Ultra*.

Microbial Material and Fermentation. The working fungus *Neosartorya Fischeri* CGMCC 3.5378 was obtained from the Chinese Academy of Science. To resuscitate strains, the powdered fungal spores were grown on the *Czapek*'s agar culture plates composed of sucrose (30 g/l), $NaNO_3$ (30 g/l), $MgSO_4 \cdot 7\ H_2O$ (0.5 g/l), KCl (0.5 g/l), $FeSO_4 \cdot 7\ H_2O$ (0.012 g/l), K_2HPO_4 (1.0 g/l), agar (15.0 g/l), and dist. H_2O (1 l) at pH 6.0–6.5, 28° for 5 d. The producing strains were prepared on potato dextrose agar slants and stored at 4°.

The strains were grown under static conditions at 28° for 28 d. *Erlenmeyer* flasks (500 ml × 113), each containing 90 g of solid medium composed of 56 g of dried wheat bran and 34 ml of dist. H_2O.

Extraction and Isolation. The culture was extracted with EtOH at r.t. (5 × 20 l). The combined extracts were evaporated to dryness under reduced pressure to afford a residue (1.5 kg). The residue was dissolved in H_2O (3.0 l) to form a suspension, which was then extracted with AcOEt (5 × 1.0 l). The org. solvent was evaporated to dryness under reduced pressure to give a crude extract (300 g).

The crude extract was separated into *Frs. A* and *B* by CC (*MCI-CHP20P* gel; MeOH/H_2O, 1:4 → 9:1). *Fr. A* (4.6 g) was further purified by CC (SiO_2; $CHCl_3$/MeOH 30:1) to give *Frs. A-1* and *A-2*. Compound **9** (3.0 mg) was obtained from *Fr. A-1* (140.0 mg) by semiprep. HPLC (25% MeCN/H_2O, 20.0 ml/min). *Fr. A-2* was subjected to CC (*Toyopearl HW-40C*; MeOH) to yield **5** (14.7 mg). *Fr. B* (11.6 g) was submitted to CC (SiO_2; $CHCl_3$/MeOH, 40:1 → 20:1 → 10:1) to afford three major

subfractions, *Frs. B-1 – B-3*. Compound **4** (42.4 mg) was recrystallized in MeOH/$CHCl_3$ from *Fr. B-1* (2.2 g). *Fr. B-2* (1.8 g) was further separated by CC (*RP-18* CC; MeOH/H_2O 5:5 → 6:4) to afford **1** (32.0 mg), **2** (26.4 mg), **3** (8.3 mg), and **6** (2.4 mg). Compound **7** (3.0 mg) was obtained from *Fr. B-3* (116.3 mg) by CC (SiO_2; $CHCl_3$/MeOH 100:1).

Single crystals of **1** and **2** were recrystallized from MeOH mounted in inert oil and transferred to the cold gas stream of the diffractometer.

Neofipiperzine A (=(*1*R,*5*R,*10*S,*10a*R,*14a*S,*15b*R)*-1,10,10a,14,14a,15b-Hexahydro-1,10,10a-trihydroxy-7-methoxy-2,2-dimethyl-5-(2-methylprop-1-en-1-yl)-12*H*-3,4-dioxa-5a,11a,15a-triazacyclooct a[1,2,3-*lm*]indeno[5,6-*b*]fluorene-11,15(2*H*,13*H*)-dione*; **1**). White amorphous powder. M.p. 173–175°. $[\alpha]_D^{20} = -69.3$ ($c = 0.31$, MeOH/$CHCl_3$ 1:1). UV (MeOH): 277 (3.31), 256 (3.19), 204 (4.37). IR (KBr): 3412, 2950, 2838, 1652, 1450, 1417, 1112, 1021. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 528 ($[M+H]^+$). HR-ESI-MS: 528.2336 ($[M+H]^+$, $C_{27}H_{34}N_3O_8^+$; calc. 528.2346).

X-Ray Crystal-Structure Analysis of **1**[1]). Colorless and transparent crystals of **1** were obtained by recrystallization in MeOH. The crystal belongs to the monoclinic system, with the formula $C_{27}H_{33}N_3O_8$; space group, *I2*; $a = 19.7614(13)$, $b = 9.7968(6)$, $c = 15.8269(12)$ Å, $\alpha = \gamma = 90.00°$, $\beta = 99.850(7)°$, $V = 3018.9(4)$ Å^3, $Z = 4$, and $\rho_{calc} = 1.161$ mg/mm^3. The intensity data were collected within the range of 6.12– to 52° using graphite-monochromated MoK_α radiation ($\lambda = 0.71073$ Å). The final R_1 was 0.0450 and wR_2 was 0.1102 ($I >= 2\sigma(I)$).

Neofipiperzine B (=(*1*S,*5*R,*10*S,*10a*R,*14a*S,*15b*R)*-1,10,10a,14,14a,15b-Hexahydro-1,10,10a-trihydroxy-7-methoxy-2,2-dimethyl-5-(2-methylprop-1-en-1-yl)-12*H*-3,4-dioxa-5a,11a,15a-triazacyclooct a[1,2,3-*lm*]indeno[5,6-*b*]fluorene-11,15(2*H*,13*H*)-dione*; **2**). White amorphous powder. M.p. 210–212. $[\alpha]_D^{20} = -11.7$ ($c = 0.24$, $CHCl_3$). UV (MeOH): 276 (3.67), 255 (3.52), 207 (4.97). IR (KBr): 3422, 2256, 2129, 1659, 1049, 1025, 1002, 826, 764. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 528 ($[M+H]^+$). HR-ESI-MS: 528.2350 ($[M+H]^+$, $C_{27}H_{34}N_3O_8^+$; calc. 528.2346).

X-Ray Crystal-Structure Analysis of **2**[1]). Colorless and transparent crystals of **2** were obtained by recrystallization in MeOH. The crystal belongs to the orthorhombic system, with the formula $C_{27}H_{33}N_3O_8$; space group $P2_12_12_1$; $a = 10.8093(5)$, $b = 13.7261(7)$, $c = 17.434(9)$ Å, $\alpha = \beta = \gamma = 90.0°$, $V = 2586.7(20)$ Å^3, $Z = 4$, and $\rho_{calc} = 1.355$ mg/mm^3. The intensity data were collected within the range of 6–52° using graphite-monochromated MoK_α radiation (λ 0.71073 Å). The final R_1 was 0.0422, and wR_2 was 0.1013 ($I >= 2\sigma(I)$).

Neofipiperzine C (=(*5a*R,*6*S,*12*S,*14a*S)*-1,2,3,5a,6,11,12,14a-Octahydro-5a,6-dihydroxy-12-(2-hydroxy-2-methylpropyl)-9-methoxy-11-(3-methylbut-2-en-1-yl)-5*H,*14*H*-pyrrolo[1'',2'':4',5']pyrazino-[1',2':1,6]pyrido[3,4-*b*]indole-5,14-dione*; **3**). White amorphous powder. $[\alpha]_D^{20} = -38.4$ ($c = 0.07$, $CHCl_3$). UV (MeOH): 259 (3.20), 210 (4.84). IR (KBr): 3356, 2945, 2833, 2522, 2044, 1656, 1450, 1114, 1030. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 498 ($[M+H]^+$). HR-ESI-MS: 498.2608 ($[M+H]^+$, $C_{27}H_{36}N_3O_6^+$; calc. 498.2604).

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