Polyketides and Meroterpenoids from *Neosartorya glabra*

by **Wen-Hong Liu***[a]), **Hong Zhao**[b]), **Rong-Qun Li**[a]), **Hong-Bin Zheng**[a]), and **Qin Yu**[c])

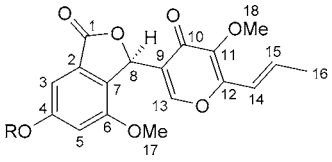
[a]) College of Basic Medical Science, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China (phone/fax: +86-571-86613712; e-mail: lwh687@163.com)

[b]) First Clinical Medical School, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China

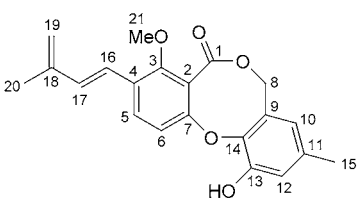
[c]) College of Life Science, Zhejiang Chinese Medical University, Hangzhou 310053, P. R. China

Two new polyketides, neosarphenols A and B (**1** and **2**, resp.), were isolated from *Neosartorya glabra*, together with six known polyketides, **3**–**8**, and two meroterpenoids, **9** and **10**. The structures of the new compounds were elucidated by comprehensive spectroscopic analysis, especially by HR-ESI-MS and NMR experiments. All compounds were evaluated for their cytotoxic activities against MDA-MB-231, MCF-7, and PANC-1 tumor cell lines; **1** and **6** exhibited selective and moderate cytotoxicities against PANC-1 cell line.

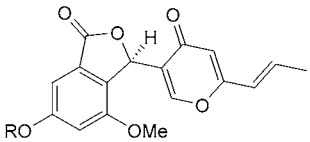
Introduction. – *Neosartorya* species, currently 20–30 in number, are teleomorphs of *Aspergillus* and are like *Aspergillus* [1]. Previous chemical investigations on *Neosartorya glabra* have resulted in the isolation of glabramycins A – C [2] and sartoryglabrins A–C [3], which showed strong antibacterial and selective cytotoxic activities, respectively. During our research on fungi [4–6], the investigation of *N. glabra* afforded two new polyketides, **1** and **2**, and eight known compounds, **3**–**10**.



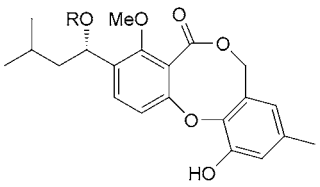
1 R = H
3 R = Me



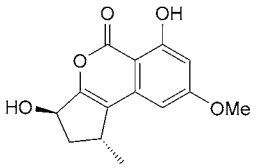
2



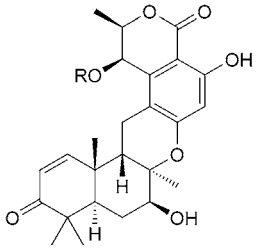
4 R = H
5 R = Me



6 R = H
7 R = Ac



8



9 R = H
10 R = Ac

Their structures were determined by MS and NMR analyses, especially by 2D-NMR techniques (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY). Herein, we report the isolation, structure elucidation, and cytotoxic activities of the metabolites.

Results and Discussion. – Neosarphenol A (**1**) was obtained as white powder. Its molecular formula was determined as $C_{18}H_{16}O_7$ by HR-ESI-MS (*m/z* 345.0969 ($[M+H]^+$), indicating eleven degrees of unsaturation. In the ^{1}H-NMR spectrum (*Table*), signals of a pair of aromatic *meta* H-atoms at δ(H) 6.72 (*d*, *J* = 1.8, H–C(5)[1]) and 6.74 (*d*, *J* = 1.8, H–C(3)), and those of two MeO groups at 3.72 and 3.74 were observed. In addition, its ^{1}H-NMR spectrum also displayed signals of a group of H-atoms (δ(H) 6.54 (*d*, *J* = 15.5, H–C(14)), 6.59 (*d*, *J* = 15.5, 5.5, H–C(15)), and 1.94 (*d*, *J* = 5.5, H–C(16))) assigned to a propenyl group. A low-field signal at δ(H) 10.18 (*s*) was assigned to the phenolic OH group. The ^{13}C-NMR and DEPT spectra of **1** (*Table*) exhibited 18 C-atom signals, including those of nine quaterrary C_q-atoms, and six CH, and three Me groups.

Table. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *of* **1** *and* **2**. δ in ppm, *J* in Hz. Arbitrary atom numbering as indicated in the formulae.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	–	169.8	–	167.0
2	–	128.7	–	121.4
3	6.74 (*d*, *J* = 1.8)	101.7	–	155.1
4	–	160.6	–	130.3
5	6.72 (*d*, *J* = 1.8)	104.9	7.65 (*d*, *J* = 8.5)	130.2
6	–	154.9	6.88 (*d*, *J* = 8.5)	118.0
7	–	125.3	–	151.0
8	6.29 (*s*)	74.2	5.08 (*s*)	69.0
9	–	122.6	–	125.8
10	–	172.9	6.39 (*d*, *J* = 4.0)	121.2
11	–	141.9	–	135.0
12	–	154.3	6.86 (*d*, *J* = 4.0)	117.5
13	8.16 (*s*)	154.5	–	147.2
14	6.54 (*d*, *J* = 15.5)	118.3	–	141.2
15	6.59 (*dq*, *J* = 15.5, 5.5)	135.2	2.25 (*s*)	20.9
16	1.94 (*d*, *J* = 5.5)	18.6	6.89 (*d*, *J* = 17.0)	121.1
17	3.72 (*s*)	55.8	6.74 (*d*, *J* = 17.0)	134.3
18	3.74 (*s*)	60.1	–	141.9
19			5.15 (*s*), 5.17 (*s*)	118.9
20			2.00 (*s*)	18.5
21			4.00 (*s*)	63.1
OH	10.18 (*s*)	–		

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

[1]) Arbitrary atom numbering as indicated in the formulae. For systematic names, see the *Exper. Part.*

The ^{1}H- and ^{13}C-NMR spectra of **1** (*Table*) were highly similar to those of the known compound **3** [7], suggesting that **1** and **3** have the same skeleton. Intensive analysis of the NMR spectrum of **1** indicated that the MeO group at C(4) in **3** was replaced by an OH in **1**. This conclusion was supported by the HMBCs OH/C(5) and OH/C(3), and by the NOEs OH/H–C(5) and OH/H–C(3). Thus, the planar structure of **1** was determined.

The large vicinal coupling constant clearly indicated an (*E*)-configuration of the C(14)=C(15) bond. The absolute configuration of **1** was determined to be the same as that of **3** [7] by comparison of their optical rotations ($[\alpha]_D^{20} = -81.3$ for **1** *vs.* $[\alpha]_D^{20} = -30.0$ for **3**). The structure of **1** was thus established as depicted, and the compound was named neosarphenol A.

Compound **2** was obtained as colorless powder, and on the basis of the $[M+H]^+$ peak at *m*/*z* 353.1385 in its HR-ESI-MS spectrum, it was assigned the molecular formula $C_{21}H_{20}O_5$, indicating twelve degrees of unsaturation. The IR spectrum displayed absorption bands at 3410 and 1739 cm^{-1}, evidencing the presence of OH and C=O groups, respectively. In the ^{1}H-NMR spectrum, signals of a pair of aromatic *meta* H-atoms at δ(H) 6.39 (*d*, *J* = 4.0, H–C(10)) and 6.86 (*d*, *J* = 4.0, H–C(12)) and those of a pair of aromatic *ortho* H-atoms at 6.88 (*d*, *J* = 8.5, H–C(6)) and 7.65 (*d*, *J* = 8.5, H–C(5)) were observed. In addition, the ^{1}H-NMR spectrum displayed two Me signals at δ(H) 2.00 (*s*, Me(20)) and 2.25 (*s*, Me(15)), and one MeO signal at 4.00 (*s*). Four low-field signals at δ(H) 6.89 (*d*, *J* = 17.0, H–C(16)), 6.74 (*d*, *J* = 17.0, H–C(17)), 5.15, and 5.17 (2*s*, CH_2(19)) were assigned to olefinic H-atoms. Twenty-one C-atom signals, attributed to ten quaterrary C-atoms, and six CH, two CH_2, and three Me groups, were evident from the ^{13}C-NMR and DEPT spectra (*Table*), of which one C=O group (δ(C) 167.0), one terminal C=C bond (141.9 and 118.9), a CH_2O (69.0), and three Me groups (18.5, 20.9, and 63.1) were discernable.

The NMR spectra of **2** resembeled those of **6** and **7** [8][9], except for the side chain at C(4). The presence of a Me group and two C=C bonds in the side chain of **2** could be easily deduced from the NMR data. That C(16) was linked to C(17) to form a disubstituted C=C bond could be easily established by analysis of the ^{1}H,^{1}H-COSY spectrum (*Fig.*). The HMBCs CH_2(19)/C(18), Me(20)/C(18), H–C(5)/C(3), CH_2(19)/C(17), and Me(20)/C(17) indicated the linkage of C(17), C(19), and C(20) *via* C(18) (*Fig.*). In the same way, the linkage of C(16) and C(4) was deduced from the HMBCs H–C(17)/C(3) and H–C(16)/C(3). Therefore, the structure of **2** was elucidated as depicted and named neosarphenol B.

The known compounds were identified as methoxyvermistatin (**3**) [7], 6-demethylvermistatin (**4**) [10], vermistatin (**5**) [11], penicillide (**6**) [8], purpactin A (**7**) [9], phialophoriol (**8**) [12], chrodrimanin A (**9**) [13], and chrodrimanin B (**10**) [13]

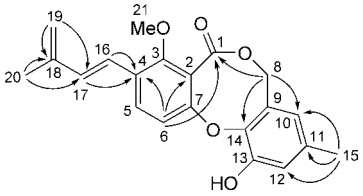


Figure. *Key HMBCs* (H → C) *of* **2**

by comparison of their spectroscopic data with those in the literature. All known compounds were isolated from the fungus for the first time.

Three tumor cell lines, MCF-7, MDA-MB-231, and PANC-1, were used to evaluate the cytotoxic activities of all isolates; paclitaxel was used as positive control. Compounds **1** and **6** showed selective and moderate cytotoxicities against the PANC-1 cell line with IC_{50} values of 14.38 and 10.93 μM (IC_{50} 0.45 μM for paclitaxel), respectively. The other compounds were all inactive against the three tested cell lines.

Financial support by the *National Natural Science Foundation* (31270051), *Key Project of Zhejiang Province Education Department* (Z201121522), and *Science and Technology Department of Zhejiang Province* (2011C21034) is gratefully acknowledged.

Experimental Part

General. All solvents used were of anal. grade (*Hangzhou Gaojin Fine Chemical Co., Ltd.*). TLC: Precoated silica gel GF_{254} plates (SiO_2; *Qingdao Haiyang Chemical Co., Ltd.*). Column chromatography (CC): SiO_2 (230–400 mesh), *MCI CHP20P* gel (75–150 μm; *Mitsubishi Chemical Industries, Ltd.*), *LiChroprep RP-18* gel (*YMC ODS-A*; 40–63 μm; *Merck Millipore*), and *Toyopearl-HW-40C* gel (50–100 μm; *Tosoh Corporation*). UV Spectra: *Shimadzu UV-2450* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Thermo-Nicolet-6700* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Bruker AM-500* apparatus; δ in ppm rel. to Me_4Si as internal standard, J in Hz. ESI-MS: *Agilent 6210 TOF LC/MS* mass spectrometer; in *m/z*. HR-ESI-MS: *Agilent 6210* TOF LC/MS; in *m/z*.

Fungus and Culture Conditions. The working fungus *N. glabra* CGMCC 32286 was obtained from the Chinese Academy of Sciences. To resuscitate strains, the powdered fungal spores were grown on potato dextrose agar (PDA) plates at 28° for 4 d. The producing strains were prepared on PDA slants and stored at 4°.

The strains were grown under static conditions at 28° for 28 d in *Erlenmeyer* flasks (88 × 500 ml), 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. (3 × 20 l). Evaporation of the combined EtOH phase under reduced pressure provided an EtOH extract (147 g). The extract was suspended in 2.0 l of H_2O and extracted with AcOEt (4 × 0.5 l). The org. phase was evaporated to dryness under reduced pressure to give a crude extract (80 g).

The crude extract was separated by CC (SiO_2; petroleum ether (PE)/acetone 1:0 → 1:1 → 0:1) to give two fractions, *Frs. 1* and *2*. *Fr. 1* (3.0 g) was further purified by CC (*MCI CHP20P* gel; MeOH/H_2O 60:40 → 80:20) to give three subfractions, *Frs. 1.1–1.3*. *Fr. 1.1* was subjected to CC (SiO_2; PE/acetone 2.5:1) to yield **9** (7.5 mg) and **4** (8.9 mg). *Fr. 1.2* was submitted to CC (SiO_2; PE/acetone 3:1) to give **1** (19.7 mg). *Fr. 1.3* was seperated by CC (SiO_2; PE/acetone 3.5:1) to yield **2** (6.2 mg), **8** (10.6 mg), and **6** (4.3 mg). *Fr. 2* (3.8 g) was subjected to CC (*MCI CHP20P* gel; MeOH/H_2O 60:40 → 85:15) to give two fractions, *Frs. 2.1* and *2.2*. *Fr. 2.1* was purified by CC (*ODS C-18*; MeOH/H_2O 50:50) to afford **10** (4.3 mg) and **5** (14.5 mg). *Fr. 2.2* was subjected to CC (SiO_2; PE/acetone 3.5:1 → 6:1) to give **7** (27.2 mg) and **3** (13.2 mg).

Neosarphenol A (=*(3*R*)-6-Hydroxy-4-methoxy-3-[5-methoxy-4-oxo-6-[(1*E*)-prop-1-en-1-yl]-4*H*-pyran-3-yl]-2-benzofuran-1(3*H*)-one*; **1**). White powder. $[\alpha]_D^{20} = -81.3$ ($c = 0.32$, $CHCl_3$/MeOH 1:1). UV ($CHCl_3$): 302 (3.97). IR: 3214, 2921, 1768, 1639, 1597, 1460, 1429, 1346, 1286, 1115, 1038, 971, 854. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 345 ($[M+H]^+$). HR-ESI-MS (pos.): 345.0969 ($[M+H]^+$, $C_{18}H_{17}O_7^+$; calc. 345.0969).

Neosarphenol B (=*11-Hydroxy-4-methoxy-9-methyl-3-[(1*E*)-3-methylbuta-1,3-dien-1-yl]-5*H*,7*H*-dibenzo[*b*,*g*][1,5]dioxocin-5-one*; **2**). Colorless powder. UV ($CHCl_3$): 293 (4.01). IR: 3410, 2923, 2852, 1739, 1593, 1492, 1469, 1292, 1234, 1208, 1051, 1026. ^{1}H- and ^{13}C-NMR: see the *Table*. ESI-MS (pos.): 353 ($[M+H]^+$). HR-ESI-MS (pos.): 353.1385 ($[M+H]^+$, $C_{21}H_{21}O_5^+$; calc. 353.1384).

Cytotoxicity Assays. Compounds **1**–**10** were evaluated for their cytotoxic activities against MCF-7 (human breast cancer cell), MDA-MB-231 (human breast cancer cell), and PANC-1 (human pancreatic

cancer cell) by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assay according to a standard protocol [14] with paclitaxel as positive control.

REFERENCES

[1] R. A. Samson, S. Hong, S. W. Peterson, J. C. Frisvad, J. Varga, *Stud. Mycol.* **2007**, *59*, 147.
[2] H. Jayasuriya, D. Zink, A. Basilio, F. Vicente, J. Collado, G. Bills, M. L. Goldman, M. Motyl, J. Huber, G. Dezeny, K. Byrne, S. B. Singh, *J. Antibiot.* **2009**, *62*, 265.
[3] A. Kijjoa, S. Santos, T. Dethoup, L. Manoch, A. P. Almeida, M. H. Vasconcelos, A. Silva, L. Gales, W. Herz, *Nat. Prod. Commun.* **2011**, *6*, 807.
[4] Y.-M. Ying, Z.-Z. Zheng, L.-W. Zhang, W.-G. Shan, J.-W. Wang, Z.-J. Zhan, *Helv. Chim. Acta* **2014**, *97*, 95.
[5] Y.-M. Ying, W.-G. Shan, L.-W. Zhang, Z.-J. Zhan, *Phytochemistry* **2013**, *95*, 360.
[6] Y.-M. Ying, W.-G. Shan, L.-W. Zhang, Y. Chen, Z.-J. Zhan, *Helv. Chim. Acta* **2013**, *96*, 2092.
[7] X.-K. Xia, H.-R. Huang, Z.-G. She, J.-W. Cai, L. Lan, J.-Y. Zhang, L.-W. Fu, L. L. P. Vrijmoed, Y.-C. Lin, *Helv. Chim. Acta* **2007**, *90*, 1925.
[8] K. Suzuki, K. Nozawa, S.-I. Udagawa, S. Nakajima, K.-I. Kawai, *Phytochemistry* **1991**, *30*, 2096.
[9] S.-I. Komai, T. Hosoe, T. Itabashi, K. Nozawa, T. Yaguchi, K. Fukushima, K.-I. Kawai, *J. Nat. Med.* **2006**, *60*, 185.
[10] X.-K. Xia, F. Liu, Z.-G. She, L.-G. Yang, M.-F. Li, L. L. P. Vrijmoed, Y.-C. Lin, *Magn. Reson. Chem.* **2008**, *46*, 693.
[11] J. Fuska, D. Uhrín, B. Proksa, Z. Votický, J. Ruppeldt, *J. Antibiot.* **1986**, *39*, 1605.
[12] F. Ye, G.-D. Chen, J.-W. He, X.-X. Li, X. Sun, L.-D. Guo, Y. Li, H. Gao, *Tetrahedron Lett.* **2013**, *54*, 4551.
[13] M. Nakajima, H. Takahashi, K. Furuya, S. Sato, K. Itoi, T. Kagasaki, M. Hara, JP 90 – 221 – 227, 1990 (*Chem. Abstr.* **1990**, *114*, 99993h).
[14] D. A. Scudiero, R. H. Shoemaker, K. D. Paull, A. Monks, S. Tierney, T. H. Nofziger, M. J. Currens, D. Seniff, M. R. Boyd, *Cancer Res.* **1988**, *48*, 4827.

Received July 11, 2014