

Polyketides and Meroterpenoids from *Neosartorya glabra*

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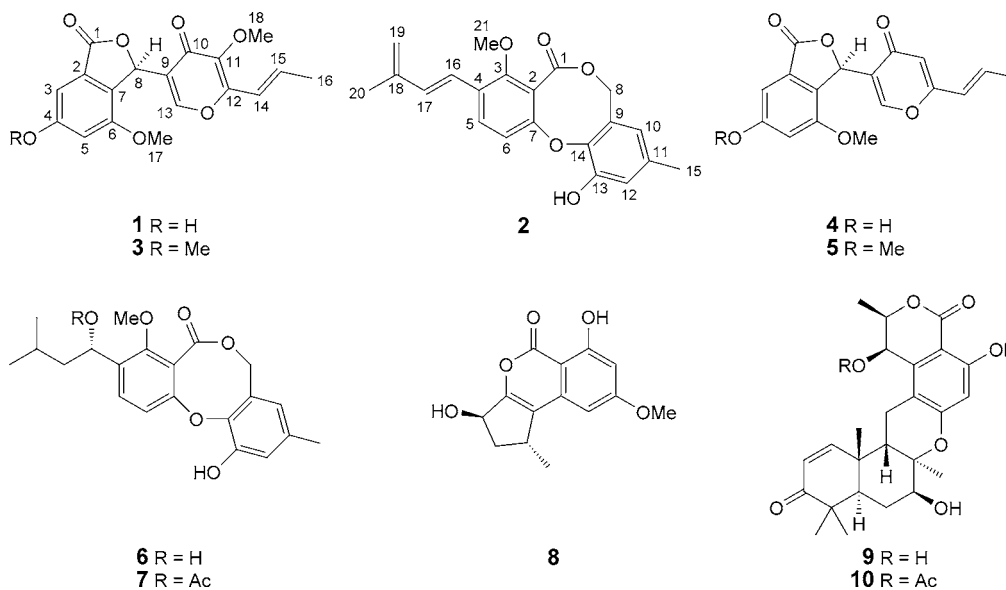
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Two new polyketides, neosarphenols A and B (**1** and **2**, resp.), were isolated from *Neosartorya glabra*, together with six known polyketides, **3–8**, and two meroterpenoids, **9** and **10**. The structures of the new compounds were elucidated 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 sartoryglabrans 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**.



Their structures were determined by MS and NMR analyses, especially by 2D-NMR techniques (^1H , ^1H -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 $\text{C}_{18}\text{H}_{16}\text{O}_7$ by HR-ESI-MS (m/z 345.0969 ($[M + \text{H}]^+$), indicating eleven degrees of unsaturation. In the ^1H -NMR spectrum (Table), signals of a pair of aromatic *meta* H-atoms at $\delta(\text{H})$ 6.72 ($d, J = 1.8, \text{H}-\text{C}(5)^1$) and 6.74 ($d, J = 1.8, \text{H}-\text{C}(3)$), and those of two MeO groups at 3.72 and 3.74 were observed. In addition, its ^1H -NMR spectrum also displayed signals of a group of H-atoms ($\delta(\text{H})$ 6.54 ($d, J = 15.5, \text{H}-\text{C}(14)$), 6.59 ($d, J = 15.5, 5.5, \text{H}-\text{C}(15)$), and 1.94 ($d, J = 5.5, \text{H}-\text{C}(16)$)) assigned to a propenyl group. A low-field signal at $\delta(\text{H})$ 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 quaternary C_q -atoms, and six CH, and three Me groups.

Table. ^1H - 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)}	
	$\delta(\text{H})$	$\delta(\text{C})$	$\delta(\text{H})$	$\delta(\text{C})$
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 ^1H - 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 ($[\alpha]_{\text{D}}^{20} = -81.3$ for **1** vs. $[\alpha]_{\text{D}}^{20} = -30.0$ 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 $\text{C}_{21}\text{H}_{20}\text{O}_5$, 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 ^1H -NMR spectrum, signals of a pair of aromatic *meta* H-atoms at $\delta(\text{H})$ 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 ^1H -NMR spectrum displayed two Me signals at $\delta(\text{H})$ 2.00 (*s*, Me(20)) and 2.25 (*s*, Me(15)), and one MeO signal at 4.00 (*s*). Four low-field signals at $\delta(\text{H})$ 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*, $\text{CH}_2(19)$) were assigned to olefinic H-atoms. Twenty-one C-atom signals, attributed to ten quaternary 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 ($\delta(\text{C})$ 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** resembled 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 $^1\text{H}, ^1\text{H}$ -COSY spectrum (*Fig.*). The HMBCs $\text{CH}_2(19)/\text{C}(18)$, Me(20)/C(18), H–C(5)/C(3), $\text{CH}_2(19)/\text{C}(17)$, 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]

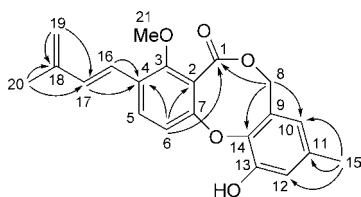


Figure. Key HMBCs (H → C) of **2**

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.

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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} . ^1H - 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 \times 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 \times 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 \times 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 \rightarrow 1:1 \rightarrow 0:1) to give two fractions, *Fr. 1* and *2*. *Fr. 1* (3.0 g) was further purified by CC (*MCI CHP20P* gel; MeOH/ H_2O 60:40 \rightarrow 80:20) to give three subfractions, *Fr. 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 separated 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 \rightarrow 85:15) to give two fractions, *Fr. 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 \rightarrow 6:1) to give **7** (27.2 mg) and **3** (13.2 mg).

Neosarphenol A (= (3R)-6-Hydroxy-4-methoxy-3-[5-methoxy-4-oxo-6-[(1E)-prop-1-en-1-yl]-4H-pyran-3-yl]-2-benzofuran-1(3H)-one; **1**). White powder. $[\alpha]_{\text{D}}^{20} = -81.3$ ($c = 0.32$, $\text{CHCl}_3/\text{MeOH}$ 1:1). UV (CHCl_3): 302 (3.97). IR: 3214, 2921, 1768, 1639, 1597, 1460, 1429, 1346, 1286, 1115, 1038, 971, 854. ^1H - and ^{13}C -NMR: see the *Table*. ESI-MS (pos.): 345 ($[M + \text{H}]^+$). HR-ESI-MS (pos.): 345.0969 ($[M + \text{H}]^+$, $\text{C}_{18}\text{H}_{17}\text{O}_7$; calc. 345.0969).

Neosarphenol B (= 11-Hydroxy-4-methoxy-9-methyl-3-[(1E)-3-methylbuta-1,3-dien-1-yl]-5H,7H-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. ^1H - and ^{13}C -NMR: see the *Table*. ESI-MS (pos.): 353 ($[M + \text{H}]^+$). HR-ESI-MS (pos.): 353.1385 ($[M + \text{H}]^+$, $\text{C}_{21}\text{H}_{21}\text{O}_8$; 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-2H-tetrazolium bromide (MTT) assay according to a standard protocol [14] with paclitaxel as positive control.

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