Three New Phenyl Ether Derivatives from *Aspergillus carneus* HQ889708

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Three new phenyl ether derivatives, 3-hydroxy-5-(3-hydroxy-5-methylphenoxy)benzoic acid (**1**), 3,4-dihydroxy-5-(3-hydroxy-5-methylphenoxy)benzoic acid (**2**), 3-[3-hydroxy-5-(hydroxymethyl)phenoxy]-5-methylphenol (**3**), and three known compounds **4**–**6** were obtained from the fermentation broth of *Aspergillus carneus* HQ889708, which was isolated from sea water from South China Sea. The structures of compounds **1**–**3** were established on the basis of spectroscopic methods including ESI-MS and NMR. Compounds **4**–**6** were reported before as synthesized products, herein, they are reported from nature for the first time.

Introduction. – *Aspergillus* is a large genus of the fungus kingdom, which is distributed all over the world. *A. carneus* has been source of bioactive secondary metabolites with diverse structures such as dihydrocinereain [1], carneamides A–C [2], aspergillicins A–E [3], which exhibited activities in insecticide [4][5], herbicide [6], mutagen [7][8], and others [9]. In recent years, people have made new progress on the study of benzene ethers [10]. In our recent study of *A. carneus* HQ889708, three new compounds were obtained (*Fig. 1*). In addition, three known secondary metabolites, dehydrocyclopeptine (**4**), viridicatin (**5**), and viridicatol (**6**) were obtained.

Results and Discussion. – Compound **1** was obtained as white amorphous solid. The molecular formula was deduced as $C_{14}H_{12}O_5$ from the ESI-MS data (*m*/*z* 259 ($[M-H]^-$), 519 ($[2M-H]^-$)), HR-ESI-MS (259.2135 ($[M-H]^-$, $C_{14}H_{11}O_5^-$; calc. 259.0606)), and ^{13}C-NMR. The ^{13}C-NMR spectrum displayed 14 C-atom signals (*Table*). Analysis of the ^{1}H- and ^{13}C-NMR data revealed that compound **1** contains two Ph rings and one Me group (δ(H) 2.26 (*s*, 3 H) and δ(C) 20.2 (*q*)). According to the coupling constants (7.09 (*t*, *J* = 1.7, 1 H), 6.64 (*t*, *J* = 2.3, 1 H), 7.20 (*t*, *J* = 1.7, 1 H), 6.27 (*t*, *J* = 2.1), 6.34 (br. *s*, 1 H), 6.44 (br. *s*, 1 H)), those two Ph rings were *meta*-substituted. The HMBC spectrum exhibited the correlations (*Fig. 2*): H–C(2′) (δ(H) 6.27 (*t*, *J* = 2.1))/C(1′) (δ(C) 157.5) and C(3′) (δ(C) 158.7); H–C(4′) (δ(H) 6.34 (br. *s*))/C(2′) (δ(C) 103.3), C(3′) (δ(C) 158.7) and C(6′) (δ(C) 111.4); H–C(6′) (δ(H) 6.44 (br. *s*))/C(1′) (δ(C) 157.5) and C(2′) (δ(C) 103.3); Me(7′)/C(4′) (δ(C) 110.8), C(5′) (δ(C) 140.6), C(6′) (δ(C) 111.4), indicating a Me-substituted benzene ring. Correlations H–C(2) ((7.09 (*t*, *J* = 1.7))/C(1) (δ(C) 132.9), C(3) (158.3), C(4) (109.7), C(6) (110.9) and C(7) (168.2); H–C(4) (6.64 (*t*, *J* = 2.3))/C(3) (δ(C) 158.3) and C(5) (158.6); H–C(6) (7.20 (*t*, *J* = 1.7))/C(1) (δ(C) 132.9), C(5) (158.6), and C(7) (168.2) indicated

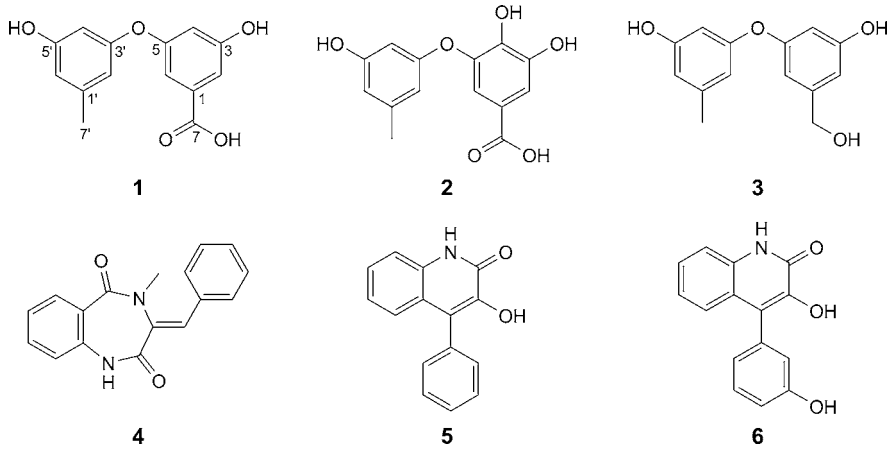

Fig. 1. *Structures of compounds* **1**–**6**[1])

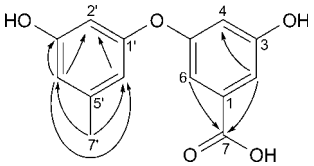

Fig. 2. *Key HMBCs* (H → C) *of* **1**[1])

Table. *^{1}H- and ^{13}C-NMR Data* (600 and 150 MHz, resp., in CD_3OD) *of* **1**–**3**[1]). δ in ppm, J in Hz.

Position	**1**		**2**		**3**	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
1		132.9		121.1		144.2
2	7.09 (*t*, *J* = 1.7)	110.3	7.12 (*d*, *J* = 1.4)	113.4	6.48 (*d*, *J* = 0.5)	107.9
3		158.3		143.9		158.4
4	6.64 (*t*, *J* = 2.3)	109.7		141.9	6.33 (*t*, *J* = 2.2)	104.4
5		158.6		146.1		158.5
6	7.20 (*t*, *J* = 1.7)	110.9	7.33 (*d*, *J* = 1.4)	112.3	6.56 (*d*, *J* = 0.6)	108.2
7		168.2		168.5	4.51 (*s*)	63.5
1′		157.5		158.5		158.2
2′	6.27 (*t*, *J* = 2.1)	103.3	6.23 (br. *s*)	101.7	6.25 (*t*, *J* = 2.0)	103.0
3′		158.7		158.1		158.1
4′	6.34 (br. *s*)	110.8	6.38 (br. *s*)	110.5	6.31 (br. *s*)	110.6
5′		140.6		140.3		140.4
6′	6.44 (br. *s*)	111.4	6.31 (br. *s*)	109.3	6.39 (br. *s*)	110.8
7′	2.26 (*s*)	20.2	2.24 (*s*)	20.2	2.23 (*s*)	20.2

that there is a benzoic acid structure (*Fig. 2*). Based on the evidences, the structure of compound **1** was identified as 3-hydroxy-5-(3-hydroxy-5-methylphenoxy)benzoic acid, as depicted in *Fig. 1*.

[1]) Arbitrary atom numbering used for the NMR interpretation.

Compound **2** was obtained as white amorphous solid. The molecular formula was deduced as $C_{14}H_{12}O_6$ from the ESI-MS data (*m/z* 275 ($[M-H]^-$)), HR-ESI-MS (275.2225 ($[M-H]^-$, $C_{14}H_{11}O_6^-$; calc. 275.0556)), and ^{13}C-NMR. The ^{1}H- and ^{13}C-NMR data of compound **2** were similar to those of compound **1**, Therefore, it was assumed that they have the same skeleton, except for the signal of the C(4) (δ(C) 141.9; *Table*). The HMBCs H–C(2) (δ(C) 7.12 (*t*, *J* = 1.4))/C(3) (δ(C) 143.9), C(6) (112.3), and C(7) (168.5); H–C(6) (δ(C) 7.33 (*t*, *J* = 1.4))/C(1) (δ(C) 121.1), C(5) (146.1), and C(7) (168.5) indicated that there is a benzoic acid moiety (*Fig. 3*). C(4) (δ(C) 141.9) is a quaternary atom. Therefore, compound **2** has one more OH group than **1** has, this OH connected to C(4) (δ(C) 141.9). The HMBC also exhibited the following correlations: H–C(2′) (δ(H) 6.23 (br. *s*))/C(3′) (δ(C) 158.1); H–C(4′) (δ(H) 6.38 (br. *s*))/C(2′) (δ(C) 101.7), C(3′) (158.1), and C(6′) (109.3); H–C(6′) (6.31 (br. *s*))/C(1′) (δ(C) 158.5) and C(2′) (101.7); Me(7′)/C(4′) (δ(C) 110.5), C(5′) (140.3), and C(6′) (109.3), indicating a Me-substituted benzene ring. Based on the results, the structure of compound **2** was identified as 3,4-dihydroxy-5-(3-hydroxy-5-methylphenoxy)benzoic acid, as depicted in *Fig. 1*.

Compound **3** was obtained as colorless oil. The molecular formula was deduced as $C_{14}H_{14}O_4$ from the ESI-MS data (*m/z* 245 ($[M-H]^-$)), HR-ESI-MS (245.2494 ($[M-H]^-$, $C_{14}H_{13}O_4^-$; calc. 245.0814), and ^{13}C-NMR. ^{1}H-NMR data displayed signals at δ(H) 6.48, 6.33, 6.56, 4.51, 6.25, 6.31, 6.39, and 2.23, and the ^{13}C-NMR displayed 14 signals at δ(C) 144.2, 107.9, 158.4, 104.4, 158.5, 108.2, 63.5, 158.2, 103.0, 158.1, 110.6, 140.4, 110.8, 20.2. The ^{1}H- and ^{13}C-NMR data of compound **3** were similar to those of the former two compounds. Thus, it was assumed that these compounds have similar skeletons. The C=O group of C(7) (168.2) in compound **1** was replaced by a CH_2OH group (63.5) in compound **3**. The HMBCs H–C(2) (6.48 (*d*, *J* = 0.5))/C(3) (δ(C) 158.4); H–C(4) (6.33 (*d*, *J* = 2.2))/C(3) (δ(C) 158.4), C(5) (158.5); H–C(6) (6.56 (*d*, *J* = 0.6))/C(2) (δ(C) 107.9), C(4) (104.4), C(5) (158.5); CH_2(7) (4.51 (*s*))/C(1) (δ(C) 144.2), C(2) (107.9), C(6) (108.2) defining the structure of the benzyl alcohol moiety (*Fig. 4*). Based on the evidences, the structure of compound **3** was identified as 3-[3-hydroxy-5-(hydroxymethyl)phenoxy]-5-methylphenol, as depicted in *Fig. 1*.

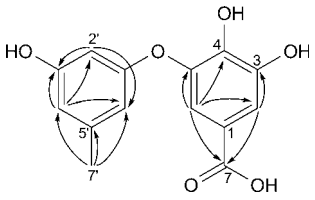

Fig. 3. *Key HMBCs* (H → C) *of* **2**[1])

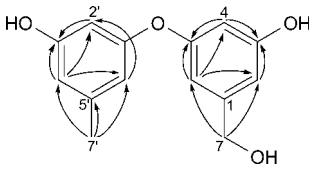

Fig. 4. *Key HMBCs* (H → C) *of* **3**[1])

In addition, three known compounds, dehydrocyclopeptine (**4**) [11], viridicatin (**5**) [12], and viridicatol (**6**) [13] were obtained (*Fig. 1*). Their structures were established on the basis of NMR spectroscopic methods.

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Experimental Part

General. Column chromatography (CC): SiO_2 (200–300 mesh), *ODS* (40–63 mm, *YMC Co.*, Japan), or *Sephadex LH-20* (*Pharmacia, Co.*). HPLC: *Shimadzu* apparatus (*C18* column, 5 μm, 19 × 250 mm). 1D- and 2D-NMR spectra: *Bruker Avance III* 600 MHz NMR instrument; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS: *Bruker Apex-Ultra 7.0 T* mass instrument (neg.); in *m*/*z*.

The fungus, which was isolated from sea water of South China Sea, has been indentified as *A. carneus* HQ889708 (*Beijing Sunbiotech Co., Ltd.*).

Fermentation, Extraction, and Isolation. The fungus *A. carneus* HQ889708 was maintained on potato dextrose agar (PDA) at 28°. After incubation on PDA liquid medium at 28° for 5 d on a rotary shaker (200 rpm), each primary culture was transferred into a 1 l *Erlenmeyer* flask containing 0.5 l of the rice solid culture, and incubated at 28° for 40 d. After the solid fermentation, the 150 bottles of carrier were poured in a large container, and extracted with 25 l AcOEt for three times. The combined AcOEt soln. was concentrated under reduced pressure to afford a brown gum (600 g). The gum was subjected to CC (SiO_2; CH_2Cl_2/MeOH (50:1 to 1:1), step gradient elution) to afford eleven fractions, *Frs. 1–11. Fr. 6* (3 g) was subjected to CC (SiO_2; CH_2Cl_2/MeOH (30:1 to 10:1), step gradient elution) to afford compound **5** (20 mg). Compound **4** (12 mg) was obtained from *Fr. 9* (0.9 g) using the same method. *Fr. 10* (6 g) was further fractionated and subjected to *ODS* (eluted with MeOH/H_2O 80:20) and *Sephadex LH-20* (eluted with MeOH) to afford compound **1** (122 mg), compound **2** (16 mg) and compound **3** (57 mg). *Fr. 11* (1 g) was further purified by semi-prep. HPLC over a reversed *C18* column (19 × 250 mm) using MeOH/H_2O (70:30) as eluting solvent (flow rate 2.0 ml/min, detector wave length 254 nm) to afford compound **6** (12 mg).

REFERENCES

[1] O. I. Zhuravleva, S. S. Afiyatullov, E. A. Yurchenko, V. A. Denisenko, N. N. Kirichuk, P. S. Dmitrenok, *Nat. Prod. Commun.* **2013**, *8*, 1071.
[2] O. I. Zhuravleva, S. S. Afiyatullov, V. A. Denisenko, S. P. Ermakova, N. N. Slinkina P. S. Dmitrenok, N. Y. Kim, *Phytochemistry* **2012**, *80*, 123.
[3] R. J. Capon, C. Skene, M. Stewart, J. Ford, R. A. J. O'Hair, L. Williams, E. Lacey, J. H. Gill, K. Heiland, T. Friedel, *Org. Biomol. Chem.* **2003**, *1*, 1856.
[4] J. J. Fourie, L. J. Fourie, I. G. Horak, M. G. Snyman, *J. S. Afr. Vet. Assoc.* **2012**, *81*, 33.
[5] P. K. Mittal, U. Sreehari, R. K. Razdan, A. P. Dash, *J. Vector Borne Dis.* **2009**, *46*, 241.
[6] S. C. Kim, I. B. Im, *Weed Biol. Manage.* **2002**, *2*, 65.
[7] T. Matsushita, Y. Matsui, Y. Matsui, T. Inoue, *J. Environ. Sci. Health Part* **2005**, *40*, 851.
[8] S. Kitamori, Y. Tanaka, Y. Ishiguro, H. Kondo, *Sangyo Eiseigaku Zasshi* **1995**, *37*, 143.
[9] B. Erkmen, M. Caliskan, S. Yerli, *Vet. Hum. Toxicol.* **2000**, *42*, 5.
[10] J. L. Wu, *J. Tech.* **2012**, *26*, 41.
[11] N. Zelenkova, N. Vinokurova, M. Arinbasarov, *Appl. Biochem. Microbiol.* **2003**, *39*, 44.
[12] Y. Kobayashi, T. Harayama, *Org. Lett.* **2009**, *11*, 1603.
[13] M.-Y. Wei, R.-Y. Yang, C.-L. Shao, *Chem. Nat. Compd.* **2011**, *47*, 322.

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