Chemistry of Natural Compounds, Vol. 48, No. 3, July, 2012 [Russian original No. 3, May-June, 2012]

SECONDARY METABOLITES OF THE MARINE FUNGUS *Aspergillus ustus* KMM 4640

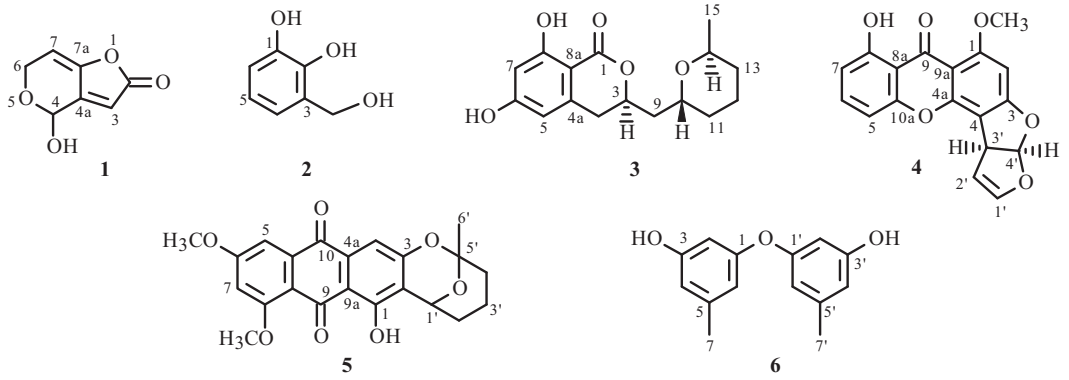
G. K. Oleinikova,* V. A. Denisenko, N. N. Slinkina, and Sh. Sh. Afiyatullov

UDC 577.115.3:582.28

Marine mycelial fungi are known to produce various biologically active compounds with unusual chemical structures [1, 2]. In continuation of research on secondary metabolites of marine fungi-micromycetes, we found that the strain *Aspergillus ustus* (Bainier) Thom & Church KMM 4640 that was isolated from sediment of the Okhotsk Sea shelf of Sakhalin Island (27 m depth) produced compounds with antimicrobial activity.

This strain was cultivated in wort-agar medium with seawater at 22°C for 14 d [3]. Mycelium was extracted with EtOAc. The resulting extracts were concentrated at 10 mm Hg. The dry residue was dissolved in aqueous EtOH (10%) and subsequently extracted with hexane and EtOAc.

Repeated column chromatography over silica gel using hexane:EtOAc (20:1) isolated from the EtOAc extract compounds **1** (5.3 mg), **2** (0.5 mg), **3** (5.3 mg), **4** (1.3 mg), **5** (1.3 mg), and **6** (4.4 mg).



1 **2** **3** **4** **5** **6**

Patulin (1), $C_7H_6O_4$, mp 108°C ($CHCl_3$), racemate. IR spectrum (KBr, ν, cm^{-1}): 2920, 1780, 1740. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 154 (16) $[M]^+$, 136 (18), 126 (26), 110 (55). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.06 (1H, s, H-3), 6.02 (1H, m, H-4), 5.93 (1H, m, H-7), 4.72 (1H, ddd, J = 17.3, 3.0, 1.0, H-6), 4.42 (1H, ddd, J = 17.2, 4.1, 0.8, H-6), 3.37 (1H, br.s, OH-4). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 168.6 (C-2), 149.8 (C-7a), 146.2 (C-4a), 111.2 (C-3), 107.4 (C-7), 88.9 (C-4), 59.6 (C-6).

Spectra data of **1** agreed with the literature for the known mycotoxin patulin (4-hydroxy-4*H*-furo[3,2-*c*]pyran-2(6*H*)-one) [4] produced by fungi of the genera *Aspergillus* and *Penicillium* [5a].

1,2-Dihydroxy-3-hydroxymethylbenzene (2), $C_7H_8O_3$. IR spectrum (DMSO, ν, cm^{-1}): 3456, 1660, 1455. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 140 (44) $[M]^+$, 122 (100), 94(52). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 8.54 (1H, s, OH-1), 8.53 (1H, s, OH-2), 6.73 (1H, d, J = 3.4, H-4), 6.53 (1H, d, J = 8.5, H-6), 6.40 (1H, dd, J = 8.3, 3.5, H-5), 4.87 (1H, t, J = 6.4, OH-3′), 4.39 (2H, d, J = 5.7, H-3′). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 149.2 (C-1), 145.8 (C-2), 129.6 (C-3), 116.3 (C-5), 113.3 (C-6), 111.6 (C-4), 57.8 (C-3′).

Resonances in PMR and ^{13}C NMR spectra were assigned based on HSQC and HMBC data. The structure 1,2-dihydroxy-3-hydroxymethylbenzene was proposed for **2** [6].

Cladosporin (3), $C_{16}H_{20}O_5$, mp 182–184°C (EtOH). IR spectrum ($CHCl_3$, ν, cm^{-1}): 3600, 2930, 1665, 1628, 1598, 1378, 1251. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 292 (17) $[M]^+$, 179 (12), 149 (13), 99 (98). PMR spectrum (500 MHz,

Pacific Institute of Bioorganic Chemistry, Far-East Branch, Russian Academy of Sciences, 690022, Vladivostok, Prosp. 100-Letiya Vladivostoka, 159, Russia, fax: (4232) 31 40 50, e-mail: oleingk@mail.ru. Translated from *Khimiya Prirodnykh Soedinenii*, No. 3, May–June, 2012, pp. 421–422. Original article submitted December 22, 2011.

0009-3130/12/4803-0467

$CDCl_3$, δ, ppm, J/Hz): 11.11 (1H, s, OH-8), 6.60 (1H, br.s, OH-6), 6.29 (1H, s, H-7), 6.17 (1H, s, H-5), 4.70 (1H, m, H-3), 4.10 (1H, m, H-10), 3.99 (1H, m, H-14), 2.84 (2H, m, H-9), 1.94 (1H, m, H-4), 1.84 (1H, m, H-4), 1.72 (4H, m, H-11, 2H-12, H-13), 1.36 (2H, m, H-11,13), 1.23 (3H, d, J = 6.8, CH_3-15). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 169.8 (C-1), 164.4 (C-8), 162.7 (C-6), 141.8 (C-4a), 106.6 (C-5), 101.9 (C-7), 100.4 (C-8a), 76.4 (C-3), 67.8 (C-14), 66.5 (C-10), 39.4 (C-4), 33.7 (C-9), 30.92 (2C-11,13), 18.9 (C-15), 18.2 (C-12).

Spectral data of **3** agreed with those published earlier in the literature for cladosporin (3,4-dihydro-6,8-dihydroxy-3[(tetrahydro-6-methyl-2*H*-pyran-2-yl)methyl]-1*H*-2-benzopyran-1-one) [7], a known antibiotic produced by fungi of the genera *Cladosporium* and *Aspergillus* [5b].

Sterigmatocystin (4), $C_{18}H_{12}O_6$, mp 245–246°C (MeOH), $[\alpha]_D^{23}$ –205.8° (*c* 0.18, MeOH). IR spectrum (acetone, ν, cm^{-1}): 3445, 3002, 1662, 1549. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 324 (100) $[M]^+$, 306 (28), 295 (81), 265 (21). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.21 (1H, s, OH-8), 7.50 (1H, t, J = 8.3, H-6), 6.84 (2H, m, H-5,4′), 6.76 (1H, dd, J = 1.15, 8.55, H-7), 6.50 (1H, dd, J = 1.6, 2.8, H-3′), 6.44 (1H, s, H-2), 5.45 (1H, t, J = 2.6, H-2′), 4.81 (1H, dt, J = 2.2, 7.1, H-1′), 4.00 (3H, s, CH_3-1). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 181.4 (C-9), 164.6 (C-3), 163.4 (C-1), 162.4 (C-8), 155.0 (C-10a), 154.1 (C-4a), 145.4 (C-3′), 135.7 (C-6), 113.3 (C-4′), 111.3 (C-7), 109.0 (C-8a), 106.5 (C-4), 106.0 (C-9a), 105.9 (C-5), 102.5 (C-2′), 90.5 (C-2), 56.8 (C-11), 48.1 (C-1′).

Spectral data of **4** agreed with those for sterigmatocystin (2a,12c-dihydro-8-hydroxy-6-methoxy-7*H*-furo[3′,2′:4,5]furo[2,3-*c*]xanthen-7-one) [8], a known mycotoxin isolated earlier from terrestrial producers of the genus *Aspergillus* [5c].

6,8-Di-*O*-methylaverufin (5), $C_{22}H_{20}O_7$, mp 211–212°C (EtOH). Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 396 (68) $[M]^+$, 353 (63), 338 (99), 325 (78). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 13.55 (1H, s, OH-1), 7.46 (1H, d, J = 2.5, H-5), 7.21 (1H, s, H-4), 6.78 (1H, d, J = 2.5, H-7), 5.38 (1H, d, J = 3.0, H-1′), 4.02 (3H, s, OCH_3-8), 3.97 (3H, s, OCH_3-6), 2.07 (1H, m, H-2′), 2.04 (1H, m, H-4′), 1.89 (1H, m, H-2′), 1.83 (1H, m, H-4′), 1.66 (1H, m, H-3′), 1.59 (CH_3, s, C-6′), 1.57 (1H, m, H-3′). ^{13}C NMR spectrum (125 MHz, $CDCl_3$, δ, ppm): 186.8 (C-9), 182.6 (C-10), 164.9 (C-6), 162.8 (C-8), 159.6 (C-1), 159.6 (C-3), 137.6 (C-10a), 132.5 (C-4a), 116.8 (C-2), 115.3 (C-8a), 110.0 (C-9a), 107.0 (C-4), 104.9 (C-7), 104.0 (C-5), 100.9 (C-5′), 67.1 (C-1′), 56.6 (OMe, C-8), 56.0 (OMe, C-6), 35.9 (C-4′), 27.9 (C-6′), 27.5 (C-2′), 16.0 (C-3′).

Spectral data of **5** agreed with those for the natural anthraquinone-type compound 6,8-di-*O*-methylaverufin (7,9-dimethoxy-11-hydroxy-2-methyl-3,4,5,6-tetrahydro-2*H*-2,6-epoxyanthra[2,3-*b*]oxocin-8,13-dione), which was isolated from mycelium of an unidentified fungus-endophyte of the mangrove tree *Acanthus ilicifolius* [9].

Diorcinol (6), $C_{14}H_{14}O_3$, light-yellow oil. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 230 (100) $[M]^+$, 214 (14), 186 (10), 158 (25). PMR spectrum (500 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.40 (4H, d, J = 7.6, H-4,6,4′,6′), 6.29 (2H, br.s, H-2,2′), 5.13 (2H, br.s, OH-3,3′), 2.25 (6H, s, CH_3-7,7′).

Spectral data of **6** corresponded exactly to those given for diorcinol (3,3′-dihydroxy-5,5′-dimethylphenylether), which was isolated earlier from extract of mycelium of *Emericella falconensis* and showed antibacterial activity [10] and also from the marine isolate of the fungus *A. versicolor* [11].

We did not find data on the production of the isolated compounds by marine isolates of the fungus *A. ustus*.

ACKNOWLEDGMENT

The work was supported by grants from the RFBR No. 11-04-00772-a and No. 11-04-98544-r_vostok_a and the RAS Presidium Program “Molecular and Cellular Biology.”

REFERENCES

1. T. S. Bugni and C. M. Ireland, *Nat. Prod. Rep.*, **21**, 143 (2004).
2. H. B. Liu, R. Edrada-Ebel, Y. Wang, B. Schulz, S. Draeger, W. Muller, V. Wray, W. H. Lin, and P. Proksh, *J. Nat. Prod.*, **72**, 1585 (2009).
3. V. I. Bilai (ed.), *Methods of Experimental Mycology* [in Russian], Naukova Dumka, Kiev, 1982.
4. M. Tada, K. Ohtsu, and K. Chiba, *Chem. Pharm. Bull.*, **42**, 2167 (1994).

5. B. F. Bycroft (ed.), *Dictionary of Antibiotics and Related Substances*, Chapman and Hall, London, New York, 1988, a) p. 551; b) p. 229; c) p. 661.
6. F. Richter (ed.), *Beilsteins Handbuch der Organischen Chemie*, **6**, Springer-Verlag, Berlin, 1949.
7. J. P. Springer, H. G. Culter, F. G. Crumler, R. H. Cox, E. E. Davis, and J. E. Thean, *J. Agric. Food Chem.*, **29**, 853 (1981).
8. Y. M. Lee, H. Li, J. Hong, H. Y. Cho, K. S. Bae, M. A. Kim, D. K. Kim, and J. H. Jung, *Arch. Pharm. Res.*, **33**, 231 (2010).
9. C. Shao, C. Wang, M. Wei, S. Li, Z. She, Y. Gu, and Y. Lin, *Magn. Reson. Chem.*, **46**, 886 (2008).
10. T. Itabashi, K. Nozawa, S. Nakajima, and K.-I. Kawai, *Chem. Pharm. Bull.*, **41**, 2040 (1993).
11. A. I. Yurchenko, O. F. Smetanina, A. I. Kalinovskii, M. V. Pivkin, P. S. Dmitrenok, and T. A. Kuznetsova, *Izv. Akad. Nauk, Ser. Khim.*, 834 (2010).