METABOLITES OF THE MARINE FUNGUS *Asperigillus varians* KMM 4630

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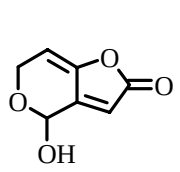
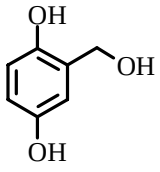
Microscopic marine fungi are rich sources of various biologically active compounds [1-3]. In searching for these compounds in fungi isolated frm various marine habitats, we observed that the ethylacetate extract of *Aspergillus varians* (Wehner) KMM 4630 culture medium contained compounds with antimicrobial activity. The fungus was isolated from the bottom of Aniva Bay (Sakhalin Island, 12 m).

The evaporated extract was dissolved in ethanol (70%) and extracted with hexane. The aqueous alcohol part was evaporated to an aqueous solution that was extracted with butanol. The butanol extract (820 mg) was placed on polychrom-1. The aqueous effluent (550 mg) was evaporated. Then, the active fraction was chromatographed several times over silica gel using hexane:hexane-ethylacetate (15%).

Thus, we isolated **1**-**3**, which exhibited antimicrobial activity toward gram-positive and gram-negative bacteria.



1



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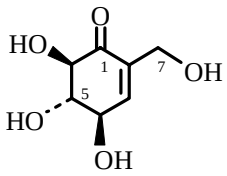

3

Compound **1**, $C_7H_6O_4$, mp 108°C, racemate, IR spectrum (KBr, cm^{-1}): 2920, 1780, 1740. Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 154 (15) $[M]^+$, 136 (17), 126 (27), 110 (54).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = TMS): 6.06 (1H, d, J = 3, H-1), 6.03 (1H, br.d, J = 1.0, H-4), 5.96 (1H, m, H-7), 4.73 (1H, ddd, J = 17.3, 3.1, 1.0, H-5), 4.42 (1H, ddd, J = 17.3, 4.2, H-5), 3.6 (OH-1).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$, δ, ppm, 0 = TMS): 168.7 (C-8), 150 (C-2), 146 (C-3), 111 (C-4), 107 (C-7), 88 (C-1), 60 (C-5).

Spectral data for **1** agree with those reported [4] for patulin [4-hydroxy-4H-furo[3,2-*c*]pyran-2(6H)-one], a known mycotoxin produced by terrestrial fungi of the genera *Aspergillus* and *Penicillium* [4, 5]. Based on two-dimensional (2D) NMR spectroscopy, we assigned fully the signals for protons and C atoms in **1**.

Compound **2**, $C_7H_8O_3$, mp 107-108°C ($CHCl_3$), $[\alpha]_D^{20}$ +4.92° (*c* 0.51, EtOH). Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 140 (42) $[M]^+$, 122 (100), 110 (4), 94 (77), 82 (13), 66 (29), 55 (15).

PMR and ^{13}C NMR spectra and 2D NMR experiments enabled signals for **2** to be assigned fully: δ ^1H 6.75 (1H, d, J = 3, H-2)—δ ^{13}C 116.2 (C-2); δ ^1H 6.61 (1H, d, J = 8, H-5)—δ ^{13}C 117.0 (C-5); δ ^1H 6.53 (1H, dd, J = 8.5, 3, H-4)—δ ^{13}C 115.6 (C-4); δ ^1H 4.61—δ ^{13}C 60.66 (C-7); 150.8 (C-3); 148.7 (C-6), 130.1 (C-1). Spectral data of **2** correspond with those for gentisyl alcohol (2-hydroxymethyl-1,4-benzenediol) [6, 7].

Compound **3**, $C_7H_{10}O_5$, wax, $[\alpha]_D^{30}$ +76.0° (*c* 0.25, EtOH). Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 174 (11) $[M]^+$, 156 (24), 145 (12), 139 (32), 128 (12), 114 (83), 112 (14), 111 (18), 96 (99), 68 (100). The compound is a mixture of two inseparable components with the same molecular weight.

PMR spectrum [300 MHz, $(CD_3)_2CO$, δ, ppm, J/Hz] of the main component: 4.57 (1H, d, J = 11.4, H-6), 3.68 (1H, dd, J = 8.3, 11.4, H-5), 4.43 (1H, dq, J = 8.3, 2.2, H-4), 6.86 (1H, q, J = 1.7, H-3), 4.13 (2H, m, H_2-7).

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^{13}C NMR spectrum [75.4 MHz, $(CD_3)_2CO$, δ, ppm, 0 = TMS]: 67.2 (C-6), 78.0 (C-5), 71.7 (C-4), 147.1 (C-3), 137.3 (C-2), 192.1 (C-1), 58.4 (C-7). Data from the mass spectrum, COSY-45, HSQC, and HMBC experiments showed that **3** is a new alcohol, 2-hydroxymethyl-4β,5α,6β-trihydroxycyclohex-2-en-1-one, which is the 4-*epi*-isomer of a known compound [8, 9]. The large spin—spin coupling constants between H-6, H-5, and H-4 indicate that the hydroxyls on C-6, C-5, and C-4 are equatorial. The minor component has PMR spectrum [300 MHz, $(CD_3)_2CO$, δ, ppm, J/Hz]: 4.61 (1H, m, J = 3.2, H-6), 4.10 (1H, dd, J = 3.2, 6.4, H-5), 4.46 (1H, m, H-4), 6.82 (1H, dt, J = 3.2, 1.6, H-3), 4.12 (2H, m, H-7).

^{13}C NMR spectrum [75.4 MHz, $(CD_3)_2CO$, δ, ppm, 0 = TMS]: 62.9 (C-6), 74.2 (C-5), 68.4 (C-4), 145.2 (C-3), 136.8 (C-2), 58.5 (C-7).

GC—MS analysis of the active fraction showed that it contained chlorogentisyl alcohol (**4**) and its amudol isomer (**5**), which made up 7 and 12% of the total fraction weight, respectively. These compounds were previously observed in terrestrial fungi [6, 7, 10, 11].

It was demonstrated that **1**-**3** exhibit high cytotoxic activity toward embryos of the sea urchin *Strongylocentrotus intermedius* at concentrations of 5.0, 5.0, and 10.0 μg/mL, respectively. The preparation of gametes, fertilization of ova, incubation of embryos, and determination of normal growth stages were performed by the previously described method [12]. Compounds **1**-**3** at concentrations of 15.0, 10.0, and 10.0 μg/mL, respectively, immobilize sea-urchin sperm, i.e., are spermatotoxic. A biotest based on estimating the fertilizing ability of sea-urchin spermatozoids (FAS test) [13] incubated beforehand with the studied compounds showed that the percent fertilized ova were 45, 0, and 0% for **1**, **2**, and **3**, respectively.

Thus, we determined that the marine isolate of the fungus *Aspergillus varians* KMM 4630 produces cytotoxins, among which one new compound was identified. New data were given for the biological activity of the isolated compounds that enable **1**-**3** to be considered potential contraceptive preparations. Based on data of 2D NMR spectroscopy of **1**-**3**, we assigned completely the signals for protons and C atoms.

ACKNOWLEDGMENT

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