FUMITREMORGINS FROM THE MARINE ISOLATE OF THE FUNGUS *Aspergillus fumigatus*

Sh. Sh. Afiyatullov, A. I. Kalinovskii, M. V. Pivkin, P. S. Dmitrenok, and T. A. Kuznetsova

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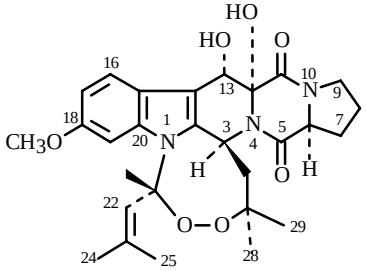
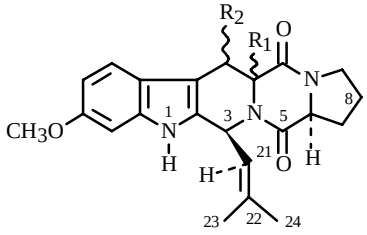
Marine fungi are a rich source of biologically active compounds of various nature [1, 2]. In continuation of research on secondary metabolites from marine-fungus isolates, we investigated the strain *Aspergillus fumigatus* KMM 4631, which was isolated from a soft coral *Simularia* sp. (Kunashir island, Kuril islands, 52 m deep). It has been demonstrated that this strain produces during cultivation on modified rice medium [3] for 21 days compounds that inhibit the growth of gram-positive bacteria and exhibit cytotoxic activity toward Erlich carcinoma tumor cells.

The cultivation was carried out at 22°C in 20 Ehrlenmeyer flasks (500 mL) containing rice (20 g), yeast extract (20 mg), KH_2PO_4 (10 mg), and natural seawater (40 mL).

The mycelium together with the medium was ground and extracted three times with $CHCl_3$:alcohol (2:1). The extract was evaporated. The solid was chromatographed over a column of silica gel L (40/100 μm) with elution successively by $CHCl_3$ and $CHCl_3$:alcohol (10:1 and 5:1). Fractions eluted by $CHCl_3$:alcohol (10:1) were combined, concentrated in vacuum to the minimal volume, and separated by HPLC over Silasorb Si (ethylacetate:hexane, 4:1) and Diaspher-110-C18 (55% MeOH) columns to afford **1** (48 mg), **2** (42 mg), **3** (36 mg), **4** (56 mg), and **5** (40 mg).



1



2 - 5

2: R_1= β-OH, R_2 = α-OH; **3:** R_1 = β-OH, R_2 = α-OCH_3
4: R_1= α-OH, R_2 = α- OH; **5:** R_1 = α- H, R_2 = H

Verruculogen (1), $C_{27}H_{33}N_3O_7$, mp 232-234°C (MeOH), $[\alpha]_D^{26}$ -22.2° (*c* 1.0, $CHCl_3$), R_f 0.65 (ethylacetate:hexane, 6:1).

Mass spectrum (EI, 70 eV) *m/z*: 511 (12) $[M]^+$, 479 (4), 386 (6), 371 (8), 346 (10), 330 (8), 315 (14), 300 (14), 285 (24), 239 (28), 199 (16), 149 (36), 84 (100).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 1.01 (3H, s, CH_3-28), 1.72 (3H, s, CH_3-29), 1.74 (3H, d, J = 1.2, CH_3-24), 2.00 (3H, d, J = 1.2, CH_3-25), 1.65-2.55 (6H, m, H-7, H-8, H-26), 3.63 (2H, t, H-9), 3.82 (3H, s, OCH_3), 4.10 (1H, br.s, OH-12), 4.48 (1H, m, H-6), 4.78 (1H, br.s, OH-13), 5.05 (1H, dm, J = 8.0, H-22), 5.65 (1H, br.s, H-13), 6.05 (1H, d, J = 10.0, H-3), 6.59 (1H, d, J = 2.2, H-19), 6.64 (1H, d, J = 8.0, H-21), 6.83 (1H, dd, J = 2.2, 8.7, H-17), 7.90 (1H, d, J = 8.7, H-16).

Pacific Institute of Bioorganic Chemistry, Far-East Division, Russian Academy of Sciences, 690022, Vladivostok, pr. 100-letiya Vladivostoka, 159, fax 7-(4232) 31 40 50, e-mail: kuzta@piboc.dvo.ru. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 506-507, November-December, 2004. Original article submitted September 10, 2004.

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^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 131.7 (C-2), 49.0 (C-3), 166.3 (C-5), 58.8 (C-6), 29.1 (C-7), 22.7 (C-8), 51.3 (C-9), 170.8 (C-11), 82.6 (C-12), 68.7 (C-13), 105.6 (C-14), 121.1 (C-15), 121.7 (C-16), 109.4 (C-17), 156.5 (C-18), 94.0 (C-19), 136.3 (C-20), 85.9 (C-21), 118.6 (C-22), 143.2 (C-23), 18.8 (C-24), 24.3 (C-25), 45.4 (C-26), 82.2 (C-27), 27.2 (C-28), 25.7 (C-29), 55.8 (CH_3O-18).

The spectral data of **1** correspond with those in the literature for verruculogen [4, 5].

Cyclotryprostatin A (2), $C_{22}H_{25}N_3O_5$, mp 178-182°C ($CHCl_3$), $[\alpha]_D^{26}$ +83.0° (*c* 0.4, $CHCl_3$), R_f 0.24.

Mass spectrum (EI, 70 eV) *m/z*: 411 (72) $[M]^+$, 243 (66), 200 (100).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.82 (1H, br.s, NH), 6.65 (1H, d, J = 9.7, H-3), 4.42 (1H, dd, J = 6.0, J = 10.5, H-6), 1.99, 2.49 (2H, m, H-7), 1.99, 2.08 (2H, m, H-8), 3.64-3.80 (2H, m, H-9), 5.10 (1H, s, H-13), 7.45 (1H, d, J = 8.5, H-16), 6.81 (1H, dd, J = 2.2, J = 8.5, H-17), 6.86 (1H, d, J = 2.2, H-19), 5.60 (1H, dm, J = 9.6, H-21), 1.79 (3H, s, CH_3-23), 2.04 (3H, s, CH_3-24), 4.43 (1H, br.s, OH-12), 2.20 (1H, br.s, OH-13), 3.82 (3H, s, OCH_3).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 133.4 (C-2), 48.9 (C-3), 165.6 (C-5), 60.5 (C-6), 29.9 (C-7), 22.1 (C-8), 45.9 (C-9), 166.9 (C-11), 85.6 (C-12), 68.9 (C-13), 107.5 (C-14), 120.7 (C-15), 118.4 (C-16), 110.0 (C-17), 156.8 (C-18), 95.4 (C-19), 136.8 (C-20), 123.5 (C-21), 138.0 (C-22), 26.0 (C-23), 18.3 (C-24), 55.8 (CH_3O-18).

Cyclotryprostatin B (3), $C_{23}H_{27}N_3O_5$, mp 155-160°C ($CHCl_3$), $[\alpha]_D^{26}$ +108.0° (*c* 0.2, $CHCl_3$), R_f 0.56.

Mass spectrum (EI, 70 eV) *m/z* (I_{rel}, %): 425 (59) $[M]^+$, 257 (100), 226 (56).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.90 (1H, br.s, NH), 6.65 (1H, d, J = 9.8, H-3), 4.42 (1H, dd, J = 6.2, J = 10.8, H-6), 2.00, 2.49 (2H, m, H-7), 2.00, 2.10 (2H, m, H-8), 3.70, 3.75 (2H, m, H-9), 4.73 (1H, s, H-13), 7.44 (1H, d, J = 8.5, H-16), 6.82 (1H, dd, J = 2.2, J = 8.5, H-17), 6.88 (1H, d, J = 2.2, H-19), 5.55 (1H, dm, J = 9.7, H-21), 1.79 (3H, d, J = 0.9, CH_3-23), 2.04 (3H, d, J = 1.3, CH_3-24), 4.40 (1H, br.s, OH-12), 3.36 (3H, s, CH_3O-13), 3.82 (3H, s, CH_3O-18).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 133.8 (C-2), 49.2 (C-3), 167.1 (C-5), 60.0 (C-6), 29.8 (C-7), 22.2 (C-8), 45.9 (C-9), 166.0 (C-11), 84.8 (C-12), 76.6 (C-13), 105.4 (C-14), 122.7 (C-15), 118.7 (C-16), 110.1 (C-17), 156.5 (C-18), 95.3 (C-19), 136.7 (C-20), 123.7 (C-21), 137.9 (C-22), 26.1 (C-23), 18.3 (C-24), 56.7 (CH_3O-13), 55.8 (CH_3O-18).

The spectral data for **2** and **3** correspond with those in the literature for cyclotryprostatins A and B [6].

12,13-Dihydroxyfumitremorgin C (4), $C_{22}H_{25}N_3O_5$, mp 196-199°C (MeOH), $[\alpha]_D^{26}$ +9.0° (*c* 0.4, $CHCl_3$), R_f 0.60.

Mass spectrum (EI, 70 eV) *m/z* (I_{rel}, %): 411 (56) $[M]^+$, 394 (13), 371 (4), 330 (4), 315 (8), 257 (44), 243 (100), 226 (36), 214 (30), 200 (86), 187 (42), 159 (16).

PMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.67 (1H, br.s, NH), 5.87 (1H, dd, J = 1.2, J = 9.5, H-3), 4.42 (1H, dd, J = 6.6, J = 9.1, H-6), 1.99, 2.49 (2H, m, H-7), 1.99, 2.08 (2H, m, H-8), 3.65 (2H, m, H-9), 5.75 (1H, dd, J = 1.3, J = 2.8, H-13), 7.80 (1H, d, J = 8.5, H-16), 6.81 (1H, dd, J = 2.2, J = 8.5, H-17), 6.84 (1H, d, J = 2.2, H-19), 4.79 (1H, dm, J = 9.5, H-21), 1.66 (3H, s, CH_3-23), 2.01 (3H, s, CH_3-24), 4.10 (1H, s, OH-12), 4.67 (1H, d, J = 2.8, OH-13), 3.82 (3H, s, CH_3O-18).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 130.2 (C-2), 50.2 (C-3), 166.2 (C-5), 58.7 (C-6), 29.2 (C-7), 22.6 (C-8), 45.3 (C-9), 171.0 (C-11), 83.0 (C-12), 68.7 (C-13), 105.5 (C-14), 120.8 (C-15), 121.3 (C-16), 109.9 (C-17), 156.8 (C-18), 95.1 (C-19), 137.6 (C-20), 124.0 (C-21), 134.6 (C-22), 25.7 (C-23), 18.3 (C-24), 55.8 (CH_3O-18).

The spectral data for **4** correspond with those in the literature for 12,13-dihydroxyfumitremorgin C [7, 8].

Fumitremorgin C (5), $C_{22}H_{25}N_3O_3$, mp 128-132°C (MeOH), $[\alpha]_D^{26}$ -27.0° (*c* 0.2, $CHCl_3$), R_f 0.40.

Mass spectrum (EI, 70 eV) *m/z* (I_{rel}, %): 379 (97) $[M]^+$, 364 (20), 324 (30), 315 (25), 281 (100), 212 (40).

^{13}C NMR spectrum (75.4 MHz, $CDCl_3$): 132.2 (C-2), 56.9 (C-3), 165.8 (C-5), 59.3 (C-6), 28.7 (C-7), 23.1 (C-8), 45.5 (C-9), 169.6 (C-11), 51.1 (C-12), 45.5 (C-13), 106.5 (C-14), 120.9 (C-15), 119.0 (C-16), 109.7 (C-17), 156.7 (C-18), 95.4 (C-19), 137.1 (C-20), 124.3 (C-21), 134.1 (C-22), 18.2 (C-23), 25.7 (C-24), 55.9 (CH_3O-18).

The spectral data for **5** correspond with those in the literature for fumitremorgin C [4].

It has been found that **1-5** at concentrations of 10.0-50.0 μg/mL stop division of fertilized egg cells of the sea urchin *Strongylocentrotus intermedius* at the stage of 1-4 blastomers. The most active alkaloid was unhydroxylated fumitremorgin C (IC_{50} = 10 μg/mL). Compounds **1-5** exhibit cytotoxic activity toward Erlich carcinoma tumor cells *in vitro* (IC_{50} = 20-50 μg/mL).

Thus, the marine isolate of *Aspergillus fumigatus* KMM 4631 that was isolated from a soft coral *Sinularia* sp. is a good producer of fumitremorgins, potential antitumor preparations [9]. The yields of cyclotryprostatins A and B was three orders of magnitude greater than the previously reported yields of these compounds [7].

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