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3β-Methoxyolean-18-ene (miliacin) from the marine fungus *Chaetomium olivaceum*

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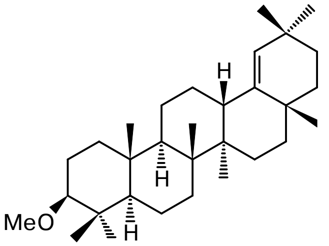
A pentacyclic tritepenoid, 3β-methoxyolean-18-ene (miliacin), was isolated for the first time from the marine fungus *Chaetomium olivaceum*. Its structure was determined on the basis of 2D NMR spectroscopy and X-ray diffraction data.

Key words: pentacyclic triterpenoid, 3β-methoxyolean-18-ene, miliacin, marine facultative fungus, *Chaetomium olivaceum*.

Recently, it was found[1—3] that microscopic fungi isolated from marine objects are sources of biologically active compounds. In a search for biologically active compounds in the isolates of marine facultative and obligate microscopic fungi, we found that the fungus *Chaetomium olivaceum* produces antibacterial and cytotoxic compounds. The chloroform—ethanolic extract of mycelium of the fungus was separated by column chromatography on silica gel, and the fraction exhibiting the highest cytotoxic activity towards mouse erythrocytes was rechromatographed. Compound **1** was the major component of this fraction.



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The ^{1}H NMR spectrum of compound **1** contained singlets due to the protons of eight angular methyl groups at δ 0.73 (3 H), 0.75 (3 H), 0.88 (3 H), 0.94 (6 H), 0.95 (3 H), 1.02 (3 H), and 1.07 (3 H), a signal for the methoxy-group protons at δ 3.36, and a broad singlet for the proton at the double bond, δ 4.85 (Table 1). The molecular mass of compound **1** found by GLC/MS was 440 Da. Comparison of the NMR spectroscopic data of **1** and 3β-hydroxyolean-18-ene (germanicol)[4,5] showed that compound **1** is a 3-*O*-methyl derivative of germanicol. The signals in the spectra of 3β-methoxyolean-18-ene were assigned on the basis of the results of DEPT and HMQC experiments (see Table 1).

Table 1. ^{1}H and ^{13}C NMR data for compound **1** (CDCl$_3$)

Atom	δ (*J*/Hz) [lit. data[8]]	
	^{1}H	^{13}C
C(1)		39.0
C(2)		22.4
C(3)	2.65 (dd, 1 H, J_1 = 4.3; J_2 = 11.8) [2.66]	88.8
C(4)		39.0
C(5)		56.3
C(6)		18.3
C(7)		34.8
C(8)		41.0
C(9)		51.4
C(10)		37.4
C(11)		21.3
C(12)		26.4
C(13)	2.26 (d, 1 H, *J* = 11.9)	38.6
C(14)		43.5
C(15)		27.7
C(16)		37.9
C(17)		34.5
C(18)		142.9
C(19)	4.86 (br.s, 1 H) [4.86]	129.8
C(20)		32.5
C(21)		33.5
C(22)		37.5
C(23)	0.75 (s, 3 H)* [0.73]	16.3
C(24)	0.95 (s, 3 H) [0.93]	28.1
C(25)	0.88 (s, 3 H) [0.87]	16.8
C(26)	1.07 (s, 3 H)* [1.06]	16.3
C(27)	0.73 (s, 3 H) [0.73]	14.6
C(28)	1.02 (s, 3 H) [1.02]	25.4
C(29)	0.94 (s, 3 H) [0.93]	29.4
C(30)	0.94 (s, 3 H) [0.93]	31.4
C(31)	3.36 (s, 3 H, OMe) [3.36]	57.6

* The assignment is ambiguous.

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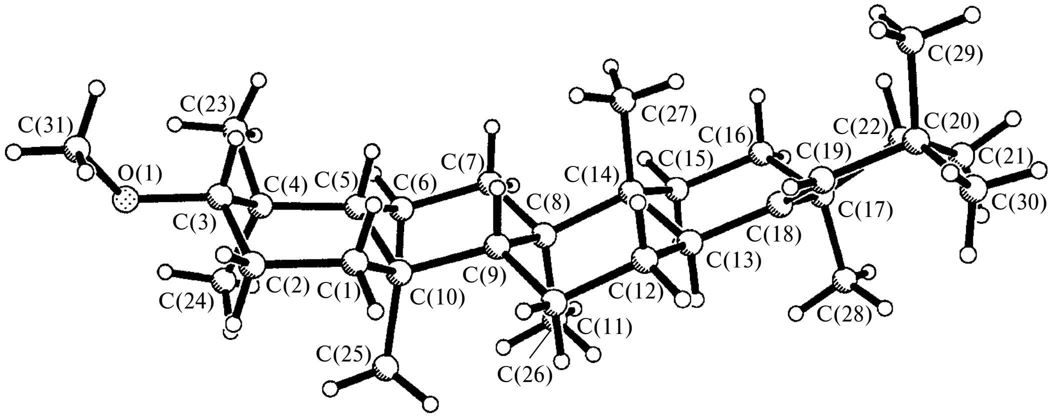

Fig. 1. Structure of compound **1**.

The structure of the isolated triterpenoid was confirmed by X-ray diffraction analysis (Fig. 1, Table 2).

Thus, we demonstrated for the first time that the isolate from the marine fungus *C. olivaceum* produces 3β-methoxyolean-18-ene. This compound was first isolated from the seeds of switch-grass *Panicum miliaceum*.[6] Later, it was found that this product is frequently encountered in higher plants.[7,8] In our experiments, the highest production of miliacin (20 mg L^{-1} of the culture medium) was noted when the fungus was cultivated in a medium prepared on the basis of L. rice *Oryza sativa*. The control study of the medium showed the absence of miliacin, which is consistent with the data of Japanese researchers who have found that rice contains other pentacyclic triterpenoids, namely, arundoin, cylindrin, and isoarborinol.[9] Miliacin is known to possess hepatoprotector properties.[10] We found that miliacin induces hemolysis of erythrocytes at pH = 7.0 (HC_{50} = $2 \cdot 10^{-4}$ mol L^{-1}).

Table 2. Crystallographic data and data collection and structure refinement parameters for **1**

Parameter	Value
Molecular formula	$C_{31}H_{52}O$
Molecular mass	440.75
Temperature/K	296(2)
Radiation	Mo-Kα
Wavelength/Å	0.71073
System	Orthorhombic
Space group	$P2_12_12_1$
a/Å	7.743(1)
b/Å	10.966(1)
c/Å	31.720(2)
V/Å^3	2693.2(5)
Z	4
d_{calc}/g cm^{-3}	1.114
μ/mm^{-1}	0.064
F(000)	1004
Crystal shape	Prism
Crystal size/mm	0.30×0.30×0.25
Data collection range over θ/deg	1.96—31.43
Ranges of reflection indices	$-10 \le h \le 10$, $-14 \le k \le 15$, $-46 \le l \le 41$
The number of measured reflections	22141
The number of independent reflections	8207
R_{int}	0.0727
The number of reflections with $I > 2\sigma(I)$	3167
The number of refinement parameters	299
GOOF	0.791
R-Factors over $F^2 > 2\sigma(F^2)$	
R_1	0.0488
wR_2	0.0987
R-Factors over all reflections	
R_1	0.1493
wR_2	0.1221
Extinction coefficient	0.0017(3)
Residual electron density, ($\rho_{min}/e \cdot Å^{-3}$)/($\rho_{max}/e \cdot Å^{-3}$)	−0.155/0.146

Experimental

X-ray diffraction analysis was performed using a faceted single crystal on a SMART 1000 CCD diffractometer (Mo-Kα radiation, graphite monochromator). The data were collected in groups of 606, 435, and 230 frames each for the angles φ = 0, 90, and 180°, respectively. The ω-scanning was performed with a step of 0.3° and an exposure of 10 s per frame; the crystal—detector distance was 45 mm. The structure was solved by the direct method and refined by the least-squares method in the anisotropic approximation for nonhydrogen atoms. The positions of the H atoms were calculated geometrically and included in the refinement in the riding model.

The data collection and edition and the refinement of the unit cell parameters were carried out by the SMART and SAINT-Plus program packages.[11] All calculations on the structure solution and refinement were carried out by the SHELXTL/PC [12] program packages. The main crystallographic data and the results of structure refinement are presented in Table 2.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM-250 using Me_4Si as the internal standard. The optical rotation was measured on a Perkin—Elmer 141 polarimeter. Mass spectrum was measured on an LKB 9000s instrument (70 eV, direct injection). GLC/MS analysis was done on a Hewlett Packard 6890 GC System instrument with an HP 5973 mass detector and an HP-5MS capillary column

(5% Me Siloxane, 30 m × 250 μm × 0.25 μm) at 270 °C (helium, 1 mL min^{-1}, 70 eV). Melting point was measured on a Leica Galen III instrument.

Column chromatography was carried out using silica gel L (40/100 μm, Chemapol, Czechoslovakia). Thin layer chromatography was performed on glass plates (4.5×6.0 cm) with a fixed layer of silica gel Sorbfil (5—17 μm, Russia).

Fungus cultivation. The fungus *C. olivaceum* was isolated from marine bottom sediments collected at a depth of 44 m near the Paramushir island (Kuriles). The fungus was cultivated for 3 weeks at 22 °C in ten 0.5 L flasks, each containing 60 g of the nutrient medium of the following composition (g): sodium tartrate, 0.01; yeast extract, 0.02; rice, 20; KH_2PO_4, 0.01; sea water, 40.

Isolation of compound 1. The fungus mycelium with the medium was homogenized for 3 min using a MRM-324 type homogenizer. Then it was extracted three times with a $CHCl_3$—EtOH mixture (2 : 1) and the extract was concentrated. The residue (1 g) was chromatographed on a column (50×3 cm) with silica gel in the $CHCl_3$—EtOH system (99 : 1 → 50 : 50). The second purification was carried out on a column with silica gel in the hexane—ethyl acetate system (99 : 1) to give 12 mg of compound **1**, $C_{31}H_{52}O$, m.p. 286 °C (from a 1 : 1 benzene—acetone mixture) (see Ref. 7: m.p. 285—286 °C), $[\alpha]_D$ +19 (*c* 0.2, $CHCl_3$) (see Ref. 7: $[\alpha]_D$ +16 (*c* 1, $CHCl_3$)). MS (EI), *m/z* (I_{rel} (%)): 440 $[M]^+$ (30), 425 $[M - Me]^+$ (27), 393 $[M - Me - MeOH]^+$ (5), 204 (87), 189 (100), 177 (73) (see Ref. 4: 440 $[M]^+$ (44), 425 $[M - Me]^+$ (23), 393 (5), 204 (100), 189 (75), 177 (63)).

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