A new meroterpenoid from the marine fungus *Aspergillus versicolor* (Vuill.) Tirab.

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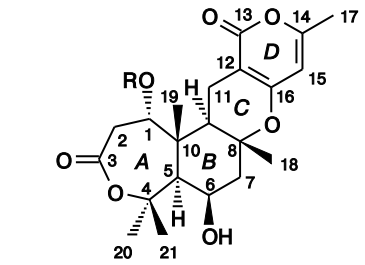
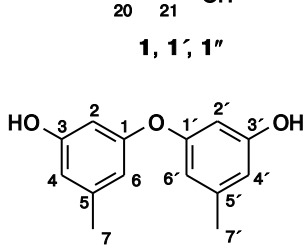
A new meroterpenoid asperdemin (**1**) and two known compounds, *viz.*, diorcinol (**2**) and viridicatol (**3**), were isolated from the marine fungus *Aspergillus versicolor*. The structures of these compounds were established by NMR spectroscopy and high-resolution mass spectrometry. The absolute stereochemistry of **1** was determined by modified Mosher's method. The antimicrobial activity of the total extract from *A. versicolor* was attributed to diorcinol (**2**). Asperdemin exhibits weak cytostatic and membranolytic activities in developing embryos of the sea urchin *Strongylocentrotus nudus*.

Key words: marine fungus *Aspergillus versicolor*, NMR spectroscopy, meroterpenoids, diphenyl ethers, phenylquinolines.

Recent studies have shown that marine fungi are promising sources of both new and known biologically active compounds. Marine and terrestrial forms of the fungus *A. versicolor* can produce compounds characterized by a striking structural diversity. Peptides, terpenoids, anthraquinones, indoles, lactones, nucleosides, *etc*. were found in different extracts of this fungus.[1—8] Metabolites of this fungus exhibit antibacterial, fungicidal, insecticidal, cytotoxic, and some other activities. In continuation of the search for producers of biologically active compounds among marine microscopic fungi, we found that the fungus *Aspergillus versicolor* isolated from marine benthos of the Sakhalin Bay (the Sea of Okhotsk) synthesizes compounds with antibacterial activity. In the present study, we report data on the isolation and identification of a new meroterpenoid asperdemin (**1**) and two known compounds, diorcinol (**2**) and viridicatol (**3**). The fungus was cultured in parallel on two solid media (rice and corn) at 22 °C for 21 days. The biomass grown on each medium was extracted with ethyl acetate. The extracts were concentrated to dryness and successively treated with hexane, chloroform, and butanol. Individual compounds **1—3** were isolated from the chloroform extracts of both media by chromatography on silica gel.



1, 1′, 1″



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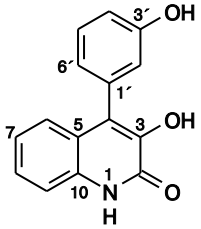

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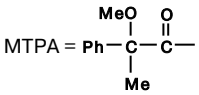
R = H (**1**), (*S*)-MTPA (**1′**), (*R*)-MTPA (**1″**)



The molecular formula of asperdemin (**1**) was determined as $C_{21}H_{28}O_7$ by electrospray mass spectrometry and was then confirmed by the analysis of the ^{13}C NMR spectra.

The ^1H NMR spectrum of compound **1** shows five three-proton singlets at δ 1.2—2.1, which are indicative of the presence of five methyl groups. The DEPT and HSQC spectra of compound **1** confirm the presence of five methyl groups (δ_C 15.4, 19.0, 21.7, 25.9, 33.1) and also provide evidence that there are three methylene groups (δ_C 16.1, 39.2, 46.3) and five methine groups (δ_C 43.2, 49.1, 67.5, 68.0, 99.9). The other eight signals were assigned to carbon atoms bearing no protons (δ_C 171.3, 163.6, 161.9, 159.9, 97.0, 84.8, 80.0, 43.9).

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The positions of the signals for two methine carbon atoms at δ 67.5 and 68.0 indicate that these atoms are linked to electronegative susbtituents. The resonance of the tertiary carbon atom of the CH group observed at still lower field (δ_C 99.9) corresponds to the signal for the carbon atom at either the double bond with adjacent electronegative substituents or two oxygen atoms. The signal at δ 171.3 is characteristic of the carbon atom of the ester carbonyl group.

The partial structures **a** and **b** of compound **1** (Fig. 1) were determined from the $^1H—^1H$ COSY, HSQC, and $^1H—^{13}C$ HMBC experiments (Table 1).

The correlations observed in the COSY-45 and HSQC spectra provide evidence for the presence of the isolated spin system —CH(OH)—CH_2— in the structure **a**. These data, as well as the HMBC correlations of the signals for HC(1) (δ_H 3.51) and $H_2C(2)$ (δ_H 2.66 and 3.32) with the signal for the carbonyl carbon atom at δ_C 171.3 and the correlations of OH(1) (δ_H 5.32) with C(1) (δ_C 68.0) and C(10) (δ_C 43.9), indicate that the secondary hydroxy group is bound to the C(1) atom and the lactone carbonyl group is at the C(3) atom.

The position of the methyl group (δ_H 1.26) at C(10) was determined based on the HMBC correlations with the signals for C(10), C(1), and C(5) (δ_C 49.1) and of the

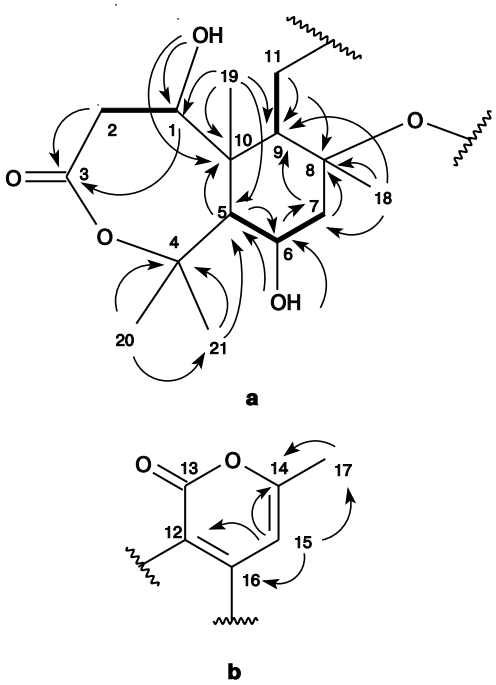

Fig. 1. Structures of the fragments **a** and **b** of compound **1** (HMBC correlations are indicated by arrows; COSY correlations are shown by bold lines).

Table 1. NMR spectroscopic data for compound **1** (500 MHz, DMSO-d_6, δ, *J*/Hz)

Atom	^{13}C	DEPT	^1H	HMBC	ROESY
1	68.0	CH	3.51 (dd, *J* = 5.0, *J* = 7.1)	19, 2, 10, 5, 3	9, OH(1), 19, 11_α, 11_β, 2_α, 2_β
2	39.2	α-CH_2	2.66 (dd, *J* = 7.2, *J* = 15.8)	10, 1, 3	1
		β-CH_2	3.32 (d, *J* = 15.7)	10, 1, 3	1, OH(1)
3	171.3	C	—	—	—
4	84.8	C	—	—	—
5	49.1	CH	2.00 (d, *J* = 2.2)	19, 20, 21, 10, 6, 4	6, 9, OH(1), 7_α, 20
6	67.5	CH	4.38 (m)	10, 7, 5, 8, 4	5, 20, 7_α, 7_β
7	46.3	α-CH_2	1.78 (dd, *J* = 3.5, *J* = 13.6)	6, 9, 8	6, OH(6)
		β-CH_2	2.14 (m)	6	6
8	80.0	C	—	—	—
9	43.2	CH	2.17 (dd, *J* = 4.4, *J* = 12.4)	7, 19, 18, 10, 5, 12	1, 5, OH(1), 7_β, 11_α
10	43.9	C	—	—	—
11	16.1	α-CH_2	2.33 (dd, *J* = 4.3, *J* = 16.0)	9, 12, 16, 13, 8	1, OH(1)
		β-CH_2	2.06 (dd, *J* = 12.6, *J* = 15.9)	9, 12, 16	1, 18, 19
12	97.0	C	—	—	—
13	163.6	C	—	—	—
14	159.9	C	—	—	—
15	99.9	CH	5.92 (s)	17, 12, 14, 16	17, 18
16	161.9	C	—	—	—
17	19.0	Me	2.12 (s)	15, 14	15
18	21.7	Me	1.38 (s)	9, 7, 8	15, 11_β, 19, OH(6)
19	15.4	Me	1.26 (s)	9, 10, 5, 1	2_β, 1, 11_β, OH(6), 18
20	33.1	Me	1.42 (s)	21, 5, 4	5, 6
21	25.9	Me	1.66 (s)	20, 5, 4	5, 19, OH(6)
OH(1)	—	—	5.32 (d, *J* = 4.8)	10, 1	2_α, 11_α, 5, 9
OH(6)	—	—	4.88 (d, *J* = 4.2)	5, 6	7_β, 18, 19, 21

signal for the methine proton HC(5) (δ_H 2.00, d, J = 2.2 Hz) with the signal for the quaternary carbon atom C(10). The methyl singlets $H_3C(20)$ (δ_C 33.1, δ_H 1.42) and $H_3C(21)$ (δ_C 25.9, δ_H 1.66) were assigned to geminal protons based on the mutual HMBC correlations and the correlations of the signals for the methyl protons with the signal for the carbon atom C(4) (δ_C 84.8) bound to the oxygen atom and with the signal for the C(5) atom.

The structure of the ring *B* was determined based on the analysis of the COSY spectra (see Fig. 1) and the HMBC correlations of HC(5) with C(6) (δ_C 67.5), of HC(6) (δ_H 4.38, m) with C(7) (δ_C 46.3), of the methylene proton $H_2C(7\alpha)$ (δ_H 1.78, dd, J = 3.5 Hz, J = 13.6 Hz) with the carbon atom C(8) (δ_C 80.0) bearing no protons and the methine carbon atom C(9) (δ_C 43.2), and of the proton of the OH(6) group (δ_H 4.88) with C(5) and C(6). The partial structure **a** contains also one angular methyl group at C(8) as evidenced by the HMBC correlation of the signal for the methyl protons $H_3C(18)$ (δ_H 1.38) with C(7), C(8), and C(9).

The signal at δ 163.6 in the ^{13}C NMR spectrum of compound **1** is characteristic of the carbonyl carbon atom of the α,β-unsaturated ester group.[9] The structure of the fragment **b** was assigned based on this fact along with the HMBC correlations (see Fig. 1).

The HMBC correlations of the signal for the methylene proton HC(11α) (δ_H 2.33, dd, J = 4.3 Hz, J = 16.0 Hz) with the signals for C(8), C(9), C(12) (δ_C 97.0), and C(16) (δ_C 161.9) indicate that the C(11) atom is common to the fragments **a** and **b**.

The C(8) and C(16) atoms are bound to the oxygen atom, which is confirmed by the low-field signals for two carbon atoms (δ_C 80.0 and 161.9, respectively). Therefore, the complete primary structure of compound **1** was established.

The relative stereochemistry of compound **1** was determined based on the COSY-45 and ROESY experiments (see Table 1, Fig. 2).

The ROESY correlations of the signal for $H_3C(19)$ (δ_H 1.26) with the signals for $H_2C(2\beta)$ (δ_H 3.32) and $H_3C(21)$ (δ_H 1.66) and of the signal for $H_3C(20)$ (δ_H 1.42) with H(5) (δ_H 2.00) show that the rings *A* and *B* are *trans*-fused. This is confirmed by the long-range COSY correlations of H(5) with $H_3C(19)$ and $H_3C(21)$.

The cross-peaks $H_3C(19)$/OH(6) (δ_H 4.88) and $H_3C(19)/H_3C(18)$ (δ_H 1.38) indicate that the groups under consideration are on the same side of the molecule. Based on these data, as well as on the correlations of HC(5) with HC(9) (δ_H 2.17) and the long-range COSY correlations of HC(9) with $H_3C(18)$ and $H_3C(19)$, the rings *B* and *C* were found to be *trans*-fused.

The absolute configuration of compound **1** was determined by modified Mosher's method.[10] The mass spectra of the resulting MTPA esters show that only one hydroxy group underwent esterification ($[M]^+$, m/z 608). The downfield shift of the proton at the C(1) atom in the ester compared to its position in the spectrum of compound **1** indicates that the hydroxy group at the C(1) atom is involved in the formation of the ester (Table 2). Based on the difference in the chemical shifts for *S*- and *R*-MTPA esters, the *S* configuration can be assigned to the C(1) atom.

Taking into account the above facts and the results of ROESY experiments, the configurations of the other asymmetric centers were determined as 5(*S*), 6(*R*), 8(*R*), 9(*R*), 10(*R*). Therefore, the absolute configuration of the new metabolite of the facultative marine fungus *A. versicolor*, which we called asperdemin, was established.

It should be noted that asperdemin is structurally similar to several already known compounds, such as tropo-

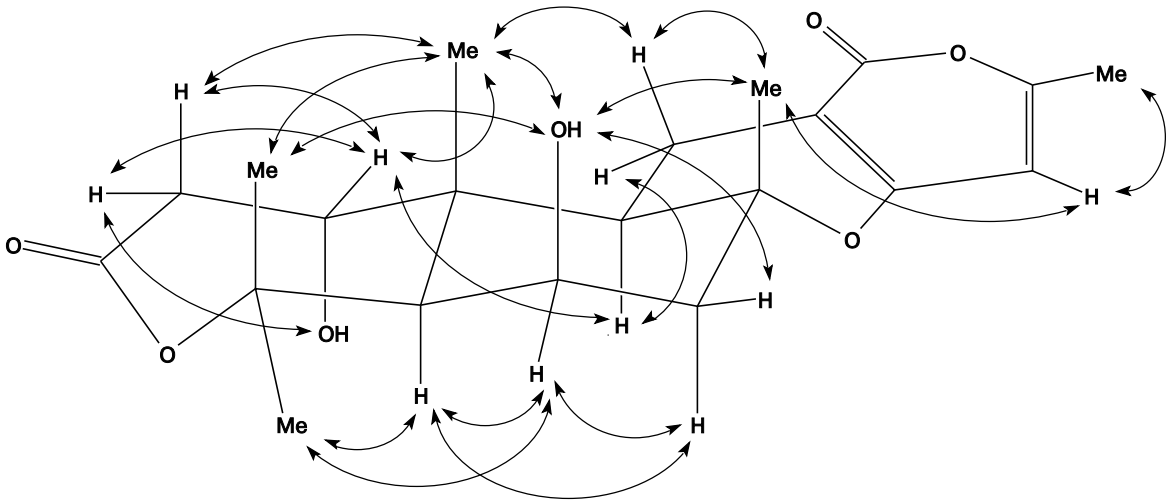

Fig. 2. Main ROESY correlations found in compound **1**.

Table 2. ^{1}H NMR spectroscopic data for MTPA esters of asperdemin (**1**) (500 MHz, DMSO-d_6)

Atom	δ, *J*/Hz		$\Delta^{\delta S-\delta R}$/Hz
	1′	**1″**	
1	5.01 (d, *J* = 7.3)	4.96 (d, *J* = 7.1)	+28.4
2_α	3.07 (d, *J* = 7.3, *J* = 16.8)	3.07 (dd, *J* = 7.1, *J* = 17.1)	+0.9
2_β	3.72 (d, *J* = 16.8)	3.72 (d, *J* = 17.0)	–2.8
3	—	—	—
4	—	—	—
5	1.97 (d, *J* = 1.9)	1.84 (d, *J* = 1.8)	+38.8
6	4.41 (m)	4.36 (m)	+31.5
7_α	1.68 (dd, *J* = 3.7, *J* = 13.7)	1.51 (dd, *J* = 3.8, *J* = 13.2)	+86.4
7_β	—	—	—
8	—	—	—
9	1.78 (dd, *J* = 4.6, *J* = 12.9)	1.50 (dd, *J* = 4.0, *J* = 13.0)	+136
10	—	—	—
11_α	2.01 (dd, *J* = 4.5, *J* = 16.7)	1.83 (dd, *J* = 4.9, *J* = 16.7)	+92.4
11_β	2.24 (dd, *J* = 13.0, *J* = 16.5)	—	—
12	—	—	—
13	—	—	—
14	—	—	—
15	5.96 (s)	5.84 (s)	+55.8
16	—	—	—
17	2.18 (s)	2.19 (s)	–4
18	1.41 (s)	1.32 (s)	+44.5
19	1.44 (s)	1.39 (s)	+26.5
20	1.23 (s)	1.23 (s)	+0.5
21	1.73 (s)	1.72 (s)	+7.3
OH(1)	—	—	—
OH(6)	5.1 (d, *J* = 4.3)	5.06 (d, *J* = 4.4)	+28.7

lactone D isolated from the marine fungus *Aspergillus sp.* (strain CNK-371)[11] and isoterreulactone A isolated from *A. terreus*.[12]

In addition to asperdemin **1**, compounds **2** and **3** were also isolated from the fungus *A. versicolor*. These compounds were identified as diorcinol and viridicatol, respectively, based on the mass spectra and the comparison of their ^{1}H and ^{13}C NMR spectra with the data published in the literature.[2,13]

Biological activity assays. Asperdemin at a concentration of 6.38 mmol L^{-1} showed a weak cytostatic effect (an increase in the number of eggs in the four-cell stage blastomere by 2.4%) and weak membranolytic activity causing an increase in the number of lysed cells compared to the reference sample by 9.3%. In addition, asperdemin induced hemolysis of human erythrocytes (the effective concentration EC_{50} = 1.15 mmol L^{-1}).

Diorcinol showed the antimicrobial activity against the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis* (the minimum inhibitory concentration MIC = 4.35 mmol L^{-1}), as well as against the yeast fungi *Candida albicans* (MIC = 3.45 mmol L^{-1}). Like asperdemin, diorcinol exhibited the hemolytic activity (EC = 1.96 mmol L^{-1}). Diorcinol was found to have the inhibitory effect (the inhibitory concentration IC_{50} = = 0.078 mmol L^{-1}) on the sperm fertilizing ability. The test using fertilized eggs showed that diorcinol (at a concentration of 10.87 mmol L^{-1}) has the pronounced cytostatic effect and increases the number of fertilized but undivided cells by 74%. The cytotoxic activity of diorcinol was determined in mouse splenic cells at a minimum inhibiting concentration of 0.11 mmol L^{-1}.

Viridicatol inhibited the sperm fertilizing ability (IC_{50} = 11.86 mmol L^{-1}), as well as (at a concentration of 9.88 mmol L^{-1}) showed weak cytostatic and membranolytic effects.

Experimental

The melting points were determined on a Leica VMTG instrument (Germany). The optical rotation was measured on a Perkin—Elmer 343 polarimeter (Germany). The UV spectra were recorded on a Shimadzu UV-1601 PC spectrophotemeter (Japan) in methanol. The IR spectra were measured on a Bruker OPUS Vector-22 spectrophotemeter in chloroform. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer (500 and 125.6 MHz, respectively) with Me_4Si as the internal standard. The high-resolution mass spectra were obtained on an AMD 604 S spectrometer (Germany). Column chromatography was carried out on silica gel L (40/100 μm, Chemapol, Czechoslovakia). The TLC analysis was performed on silica gel plates (10.0×5.0 cm) (5—17 μm, Sorbfil, Russia).

Culturing of the fungus. The fungus *A. versicolor* was isolated from benthos of the Sakhalin Bay (the Sea of Okhotsk, the depth was 26.5 m). The culturing of the fungus was carried out at 22 °C in forty 1-L flasks: 20 flasks contained the rice medium I (sodium tartrate, 0.005 g; yeast extract, 0.01 g; rice, 10 g; KH_2PO_4, 0.005 g; seawater, 20 mL) and 20 flasks contained the corn medium II (sodium tartrate, 0.001 g; corn grains, 10 g; KH_2PO_4, 0.001 g; $MgSO_4 \cdot 7H_2O$, 0.001 g; $FeSO_4 \cdot 7H_2O$, 0.0001 g; $ZnSO_4 \cdot 7H_2O$, 0.0001 g; seawater, 20 mL).

Extraction and isolation of compounds 1—3. The extraction and isolation from both media were carried out in parallel according to similar schemes. The fungal mycelia grown on the media (I and II) were extracted with ethyl acetate (2×2.25 L). The extracts were concentrated, and the dry residues (2.5 and 2.2 g, respectively) were dissolved in an ethanol—water system (1 : 4). The resulting solutions were extracted with hexane (3×100 mL), chloroform (3×100 mL), and butanol (3×100 mL). The chloroform extracts were concentrated to dryness and the residues from the media I and II (1.5 and 1.0 g, respectively) were repeatedly chromatographed on columns (2×8 cm) with silica gel (hexane—ethyl acetate, stepwise gradient, 1 : 0 → 2 : 1). Compounds **1** and **2** were isolated (3.0 and 64.9 mg, respectively) from the extract of the rice-grown fungus; compounds **1** and **3** were isolated (10.0 and 6.5 mg, respectively) from the extract of the mycelium grown on the corn medium.

Asperdemin (1). White needle-like crystals, m.p. 209—210 °C (hexane—ethyl acetate, 9 : 1), $[\alpha]^{20}_D$ + 87.2 (*c* 0.28, EtOH), $C_{21}H_{28}O_7$. UV (EtOH), λ_{max}/nm (logε): 285 (3.72); 206 (4.33). Electrospray mass spectrometry (ES-MS), *m/z* (I_{rel} (%)): 393 $[M + H]^+$ (100), 375 (6), 333 (38), 315 (8), 273 (12), 219 (8), 159 (8), 139 (57). High-resolution ES-MS. Found: *m/z* 393.1922 $[M + H]^+$. $C_{21}H_{29}O_7$. Calculated: 393.1915. The NMR spectroscopic data for compound **1** are given in Table 1.

Synthesis of (*S*)- and (*R*)-MTPA esters of asperdemin (1′ and 1″). Several crystals of 4-dimethylaminopyridine and (*R*)-MTPA-Cl (15 μL) were added to a solution of asperdemin (**1**) (1.5 mg) in pyridine. The reaction mixture was stirred at ~20 °C for 72 h. The solvent was evaporated, and the residue was purified on silica gel in ethyl acetate—hexane (3 : 7). (*S*)-MTPA ester **1′** was obtained in a yield of 2.3 mg. (*R*)-MTPA ester **1″** (2.3 mg) was synthesized according to the same procedure starting from (*S*)-MTPA-Cl.

(*S*)-MTPA ester 1′. The ^{1}H NMR spectroscopic data are given in Table 2. ES-MS (70 eV), *m/z*: 608 $[M]^+$.

(*R*)-MTPA ester 1″. The ^{1}H NMR spectroscopic data are given in Table 2. ES-MS (70 eV), *m/z*: 608 $[M]^+$.

Diorcinol (2). Pale-yellow oil, $C_{14}H_{14}O_3$. UV (EtOH), λ_{max}/nm (logε): 215 (4.59), 275.4 (3.64); 282.0 (3.62) (see Ref. 13). IR ($CHCl_3$), ν/cm^{-1}: 3596 (Ar—OH), 3036 (C_{Ar}—H), 3008 (C_{Ar}—H), 1600 (C_{Ar}=C_{Ar}), 1591 (C_{Ar}=C_{Ar}), 1487 (C_{Ar}=C_{Ar}), 1466 (C_{Ar}=C_{Ar}), 1147 (Ar—O—Ar). ES-MS (70 eV), *m/z* (I_{rel} (%)): 230 $[M]^+$ (100), 214 (7), 202 $[M - CO]^+$ (3), 187 (5), 159 (14), 145 (6), 108 (6), 77 $[C_6H_5]^+$ (6). High-resolution EI-MS. Found: *m/z* 230.0932 $[M]^+$. $C_{14}H_{14}O_3$. Calculated: 230.0943. ^{13}C NMR (DMSO-d_6), δ: 21.1, 102.9, 110.0, 111.1, 140.0, 157.6, 158.4 (see Ref. 2). ^{1}H NMR (DMSO-d_6), δ: 2.17 (s); 3.32 (s); 6.14 (br.s); 6.23 (br.s; 6.32 (br.s); 9.42 (s).

Viridicatol (3). White crystals, m.p. 265—267 °C (hexane—ethyl acetate, 2 : 1), $C_{15}H_{11}O_3N$. UV (EtOH), λ_{max}/nm (logε): 223 (4.63), 286 (3.93), 307 (3.93), 317 (4.01), 330 (3.88). ES-MS (70 eV), *m/z* (I_{rel} (%)): 253 $[M]^+$ (100), 236 (17), 224 (5), 207 (11), 198 (19), 184 (11), 152 (9). High-resolution EI-MS. Found: *m/z* 253.0732 $[M]^+$. $C_{14}H_{14}O_3$. Calculated: 253.07389. ^{13}C NMR (DMSO-d_6), δ: 114.6, 115.2, 116.7, 120.4, 120.9, 122.1, 124.1, 124.5, 126.4, 129.3, 133.1, 134.9, 142.2, 157.3, 158.3 (see Ref. 2). ^{1}H NMR (DMSO-d_6), δ: 6.70 (m); 6.71 (m); 6.81 (m); 7.06 (ddd, *J* = 8.1 Hz, *J* = 6.0 Hz, *J* = 1.9 Hz); 7.09 (m); 7.29 (t, *J* = 8.0 Hz); 7.30 (m); 7.33 (m); 9.06 (s); 9.47 (s); 12.16 (s).

Biological activity assays. The antimicrobial activity was determined against the Gram-positive bacteria *Bacillus subtilis* (KMM 430) and *Staphylococcus aureus* (ATCC 21027), the Gram-negative bacteria *Pseudomonas aeruginosa* (KMM 433) and *Escherichia coli* (ATCC 15034), and the yeast fungi *Candida albicans* (KMM 455) according to a procedure described previously.[14]

The cytotoxic activity was assayed for mouse splenic lymphocytes according to a known procedure.[15]

The hemolytic activity was tested using human blood erythrocytes according to a procedure described previously.[16]

The tests for the inhibition of the sperm fertilizing ability and the assays in fertilized eggs were carried out using gametes of the sea urchin *Strongylocentrotus nudus* according to a standard procedure.[17]

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