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Sphaerolone and dihydrosphaerolone, two bisnaphthyl-pigments from the fungus *Sphaeropsidales* sp. F-24′707

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Abstract

Two new bisnaphthalene compounds, sphaerolone (**1**) and dihydrosphaerolone (**2**), together with 2-hydroxyjuglone (**9**), were isolated from the culture broth of a *Sphaeropsidales* sp. (strain F-24′707) after inhibition of the regular proceeding 1,8-dihydroxynaphthalene (DHN) biosynthesis with tricyclazole. The structures of **1** and **2** were established by detailed spectroscopic analysis and present novel bisnaphthalenes. The biosynthetic origin of **1** and **2** as dimerization products of 1,3,8-trihydroxynaphthalene, an intermediate of the DHN biosynthesis, is discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Fungal metabolite; DHN biosynthesis; *Sphaeropsidales* sp; Sphaerolone; Dihydrosphaerolone

1. Introduction

Spirobisnaphthalenes (e.g. cladospirone bisepoxide (**3**)) are the main polyketide metabolites of the fungus *Sphaeropsidales* sp. (strain F-24′707) (Petersen et al., 1994; Thiergardt et al., 1994, 1995). Variation of the culture conditions resulted in the biosynthesis of at least 15 members of this highly interesting class of compounds (Bode et al., 2000b). In addition a new macrolide, named mutolide, could be detected recently (Bode et al., 2000a). Spirobisnaphthalenes are a growing group of fungal metabolites and exhibit different biological activities. Biosynthetically they are generated via the 1,8-dihydroxynaphthalene (DHN) pathway (Bode et al., 2000c). In order to detect other than spirobisnaphthalene metabolites, we studied the effect of tricyclazole as inhibitor of this pathway (Woloshuk et al., 1980). We isolated two new metabolites, named sphaerolone (**1**) and dihydrospaerolone (**2**) besides the known 2-hydroxyjuglone (**9**) (Stipanovic and Bell, 1977). This paper deals with the isolation and structure elucidation of the new metabolites followed by a discussion of their biosynthesis.

2. Results

The fungus F-24′707 was described previously (Petersen et al., 1994). Cultivation of the strain in shaking flasks with tricyclazole (5 ppm) and extraction with ethyl acetate, as described previously (Bode et al., 2000a), resulted in the yellow crude extract. Column chromatography on silica gel followed by gel permeation chromatography on Sephadex LH-20 afforded pure sphaerolone (**1**, $R_f = 0.81$; $CHCl_3$/MeOH 9:1), dihydrosphaerolone (**2**, $R_f = 0.51$) and 2-hydroxyjuglone (**9**, $R_f = 0.40$) (Stipanovic and Bell, 1977) in yields of 1–3 mg/l.

Sphaerolone (**1**) was obtained as a red amorphous powder that was readily soluble in dichloromethane or chloroform with yellow colour. The molecular formula $C_{20}H_{12}O_6$ results from the HREI mass spectrum ($m/z = 348.0633$) and indicates 15 double bond equivalents. The presence of hydroxyl and carbonyl groups is shown by IR absorbtion bands at ν_{max} 3434,

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Table 1
^{13}C-NMR (A, 125.7 MHz) and ^{1}H-NMR (B, 500 MHz) data of sphaerolone (**1**) and dihydrosphaerolone (**2**) ([D_6]DMSO, δ, multiplicity, J, Hz)

C-atom	**1**		**2**	
	A	B	A	B
1	190.8	–	190.7	–
2	99.6	6.05 (*s*)	98.5	5.95 (*s*)
3	172.5	–	173.2	–
4	121.4	–	118.7	–
4a	128.8	–	129.5	–
5	115.9	7.99 (*d*, 8.0)	115.4	8.00 (*d*, 8.0)
6	134.2	7.52 (*t*, 8.0)	133.7	7.50 (*m*)
7	119.6	7.05 (*d*, 8.0)	118.5[a]	6.98 (*d*, 8.0)
8	161.8	–	161.7	–
8a	114.0	–	114.2	–
1′	200.8	–	63.7	5.17 (*m*)
2′	49.5	3.52 (*dd*, 16.5, 1.0) 3.38 (*d*, 16.5)	42.6	2.96 (*dd*, 14.0, 7.0) 2.01 (*dd*, 14.0, 7.5)
3′	112.3	–	113.8	–
4′	146.6	–	151.5	–
4a′	133.0	–	129.3	–
5′	120.1	7.74 (*d*, 8.0)	118.5[a]	7.50 (*m*)
6′	136.9	7.81 (*t*, 8.0)	128.7	7.36 (*t*, 8.0)
7′	120.9	7.23 (*d*, 8.0)	118.2[a]	7.04 (*d*, 8.0)
8′	161.7	–	156.5	–
8a′	115.5	–	128.0	–
8-OH		13.66 (*s*)		13.84 (*s*)
1′-OH		–		5.45 (*br s*)
3′-OH		8.27 (*d*, 1.0)		7.77 (*s*)
8′-OH		12.18 (*s*)		9.83 (*s*)

[a] Assignments may be interchanged.

1643, 1606 and 1586 cm^{-1}. The ^{1}H-NMR spectrum exhibits 12 proton signals in [D_6]DMSO, three of them (δ_H 8.27, 12.18, 13.66) are readily exchangeable with D_2O. In addition, seven aromatic/quinoid protons at δ_H 6.05, 7.05, 7.23, 7.52, 7.74, 7.81 and 7.99 and a methylene group at δ_H 3.52/3.38 are seen. The ^{13}C-NMR spectrum exhibits 20 carbon signals (Table 1). Besides the proton attached carbon atoms, the signals of 12 quaternary carbon atoms are observable. Two carbon atoms at δ_C 190.8 and 200.8 confirm the presence of carbonyl groups. A pair of signals at δ_C 99.6 and 172.5 reveals the presence of a strongly polarized double bond. Proton and carbon signal assignments result from a ^{1}H–^{13}C correlation spectrum (HSQC). Proton connectivities arose from a ^{1}H–^{1}H COSY experiment and led to two fragments that can be connected with the quaternary carbon atoms and the hydroxy groups by $^{n}J_{C,H}$ couplings observed in a HMBC experiment to give two naphthalene fragments, as depicted in Fig. 1. Correlations obtained from a NOESY experiment between both *peri* aromatic protons at δ_H 7.99 (5-H) and 7.74 (5′-H) confirmed the connection of the two halves as depicted in **1**. No CD spectrum could be obtained from **1** indicating a racemic mixture of the possible enantiomers, which results from the center of chirality (C-3′) of the hemiketal (δ_C 112.3).

Dihydrosphaerolone (**2**), isolated as optically active yellow amorphous powder, exhibits two hydrogens more than **1**, resulting in the molecular formula $C_{20}H_{14}O_6$ (HR-EIMS $m/z = 350.0790$, M^+). In the ^{1}H-NMR spectrum of **2** close analogies to **1** are observable, e.g. the signals for protons of both aromatic rings (δ_H 6.98/7.50/8.00 and 7.04/7.36/7.50) and the

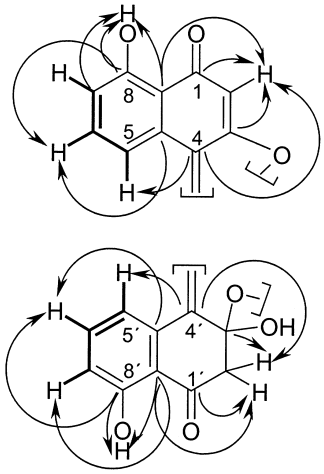


Fig. 1. Substructures of sphaerolone (**1**) derived from a ^{1}H–^{1}H COSY (bold lines) and a HMBC experiment (arrows).

singlet of the enolic proton at δ_H 5.95. The main difference is the lack of one chelated hydroxy group, the presence of a methine proton at δ_H 5.17 and an additional exchangeable proton at δ_H 5.45 pointing to a secondary hydroxy group. In the ^{13}C-NMR spectrum the signals of all 20 carbon atoms of **2** are observable. As expected, instead of two carbonyl groups as in **1**, only one carbonyl group (δ_C 190.7) and a methine group at δ_C 63.7 are observable. A ^{1}H–^{1}H COSY experiment confirmed the presence of the methine group next to the methylene group. HMBC and NOESY correlations and the close similarity to **1** led to the structure as depicted in **2**. The CD spectrum and the optical rotation of $[\alpha]_D^{20} = +57$ indicate the optical activity of **2** with the enantiomeric purity and stereochemistry remains to be clarified.

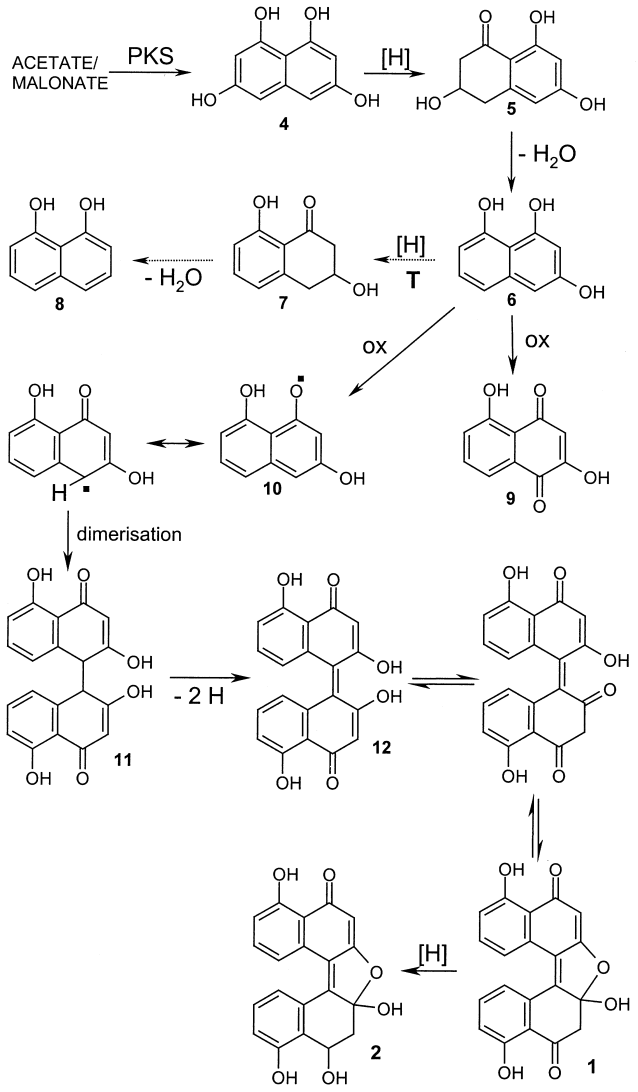


Fig. 2. Proposed biosynthesis of **1** and **2**. PKS = polyketide synthase; [H] = reduction reaction; ox = oxidation reaction; T = site of tricyclazole inhibition.

3. Discussion

Two novel bisnaphthalenes, named sphaerolone (**1**) and dihydrosphaerolone (**2**), were detected as metabolites of the fungus *Sphaeropsidales* sp. (strain F-24′707) after inhibition of the 1,8-dihydroxynaphthalene (**8**, DHN) biosynthesis with tricyclazole. DHN is the precursor of DHN melanin in various fungi and of the spirobisnaphthalenes [e.g. cladospirone bisepoxide (**3**)] in strain F-24′707 (Bode et al., 2000c). Biosynthetically, all these compounds are the product of a fungal polyketide synthase (Bell and Wheeler, 1986) leading to 1,3,6,8-tetrahydroxynaphthalene (**4**) that is further modified in post-PKS reactions to **8** via scytalone (**5**), 1,3,8-trihydroxynaphthalene (**6**) and vermelone (**7**) as intermediates (Wheeler and Bell, 1988; Kubo and Furusawa, 1991). Tricyclazole inhibits the reduction of **6** to **7** at low concentrations (Woloshuk et al., 1980), leading to an accumulation of **6**. Under aerobic conditions, **6** can be easily oxidized to 2-hydroxyjuglone (**9**) or to radical **10**. Dimerization would result in the formation of **11** that can be oxidized to **12**. The transformation from **12** to **1** is a thermodynamically controlled equilibrium and perhaps needs no enzyme. Keto-enol tautomerism and non-stereospecific formation of the hemiketal would lead to both enantiomers of **1**. Stereoselective reduction of **1** and thermodynamically controlled acid catalyzed opening and closure of the hemiketal would result in **2**, as depicted in Fig. 2. Several bisnaphthalenes from fungi are described in the literature (Bode et al., 2000b; Krohn et al., 1994, 1999; Arnone et al., 1986; Singh et al., 1994; McDonald et al., 1999) and all of them can be characterized as dimerization products of intermediates of the DHN pathway via C–C [stemphytriol (**13**)], C–O–C [3 or preussomerin G (**14**)] or C–C/C–O–C bond connections [spiroxin A (**15**)]. **16** is the only compound similar to **1** and **2** described in the literature, it is patented as an aldose reductase inhibitor (Yoshida et al., 1993). One could postulate an identical pathway for its biosynthesis starting from 1,3,6,8-tetrahydroxynaphthalene (**4**), as described for the biosynthesis of **1** starting from **6**.

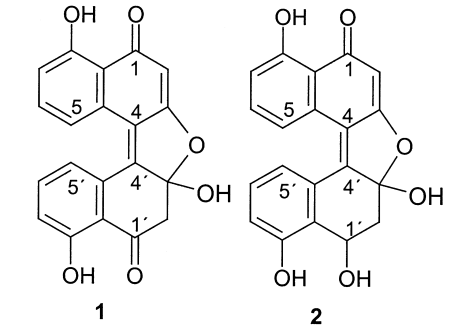
4. Experimental section

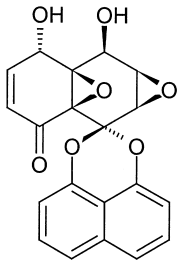
4.1. General

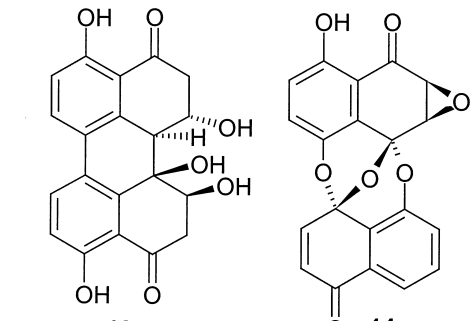
The strain was described previously (Petersen et al., 1994). For general methods and instrumentation see Bode et al. (2000a).

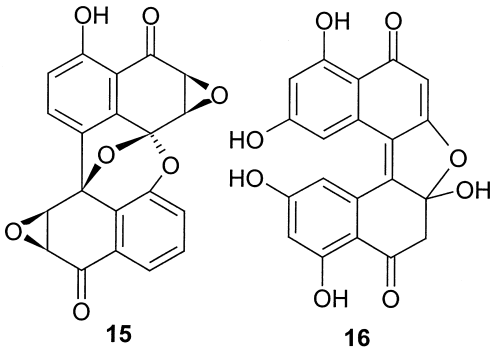
4.2. Fermentation and isolation

The fungus *Spahaeropsidales* sp. (strain F-24′707)









was cultivated for 72 h in 300 ml Erlenmeyer flasks with three intrusions using 100 ml of medium A (2% glucose, 2% soybean meal, 2% oatmeal) with 5 ppm of tricyclazole (250 rpm, 28°C). An equal amount of ethyl acetate was added and the culture broth was homogenized with a blender. After centrifugation to remove insoluble material, the phases were separated and the water layer was extracted twice with ethyl acetate. The combined organic layers were dried over anhydrous sodium sulfate and evaporated to give the crude extract. Chromatography on silica gel ($CHCl_3$/MeOH, 9:1) followed by gel permeation chromatography (Sephadex LH-20, acetone) of the yellow fractions yielded pure sphaerolone (**1**) (1 mg/l), dihydrosphaerolone (**2**) (3 mg/l) and 2-hydroxyjuglone (**9**) (2 mg/l).

4.3. Sphaerolone (*1*) $C_{20}H_{12}O_6$

Red amourphous powder; mp 210°C (decomp.). HREIMS: calcd. for $C_{20}H_{12}O_6$, m/z 348.0633 $[M]^+$, found: 348.0633. $R_f = 0.81$ ($CHCl_3$/MeOH [9:1]). IR (KBr): ν_{max} cm^{-1}: 3434, 1643, 1606, 1586, 1475, 1400, 1236, 1209, 1169, 1117, 1017, 948. EIMS (70 eV) m/z (%) = 348 (100) $[M]^+$. UV (MeOH): λ_{max} nm (logε) 254 (3.93), 286 (3.92), 327 (4.07), 423 (3.84). −MeOH/HCl: 254 (3.90), 286 (3.87), 327 (4.05), 424 (3.82). −MeOH/NaOH: 236 (4.41), 350 (3.96), 587 (2.15). ^{1}H- and ^{13}C- NMR data: see Table 1.

4.4. Dihydrosphaerolone (*2*) $C_{20}H_{14}$

Orange amorphous solid; mp 139°C (decomp.). $[\alpha]_D^{20} = +57$ (c 0.94 MeOH). HREIMS: calcd. for $C_{20}H_{14}O_6$, m/z 350.0790 $[M]^+$, found 350.0790. R_f = 0.51 ($CHCl_3$/MeOH [9:1]). IR (KBr) ν_{max} cm^{-1} 3417, 2926, 2854, 1640, 1584, 1463, 1402, 1346, 1294, 1238, 1209, 1169, 1127, 1083, 1018, 930, 753. −EIMS (70 eV) m/z (%) = 350 (12) $[M]^+$, 332 (20) $[M - H_2O]^+$, 43 (100). UV (MeOH): λ_{max} nm (logε) 201 (4.49), 264 (3.72), 329 (4.19), 419 (3.92). MeOH/HCl: 200 (4.51), 264 (3.76), 329 (4.20), 419 (3.92). MeOH/NaOH: 206 (4.44), 284 (3.84), 338 (4.07), 417 (4.00). CD (MeOH): $\lambda_{extr.}$ nm ($[\Phi]^{22}$) 204 (−4300), 216 (25,900), 233 (−67,800), 259 (−15,800), 275 (−22,400), 345 (22,100). ^{1}H- and ^{13}C-NMR data: see Table 1.

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