DOI: 10.1002/ejoc.200500471

Novel Highly Substituted Biraryl Ethers, Phomosines D–G, Isolated from the Endophytic Fungus *Phomopsis* sp. from *Adenocarpus foliolosus*[‡]

Jingqiu Dai,[a] Karsten Krohn,*[a] Ulrich Flörke,[a] Dietmar Gehle,[a] Hans-Jürgen Aust,[b] Siegfried Draeger,[b] Barbara Schulz,[b] and Joachim Rheinheimer[c]

In Memoriam Udo Gräfe

Keywords: Fungal secondary metabolites / Phomosines D–G / Biraryl ethers / *Phomopsis* sp. / X-ray single crystal analysis / Structural elucidation / fungal endophyte

Six new metabolites, phomosines D–G (**4**–**7**), 6-hydroxy-6-isopropylcyclohex-1-enecarboxylic acid (**8**) and (1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1a*H*-naphtho[1-*b*]oxirene-4-carboxylic acid (**9**) were isolated together with seven known compounds (**1**–**3**, **10**–**13**) from the endophytic fungus *Phomopsis* sp. The structures of **1**–**13** were determined by spectroscopic methods (mainly extensive 1D and 2D NMR experiments and by mass spectrometric measurements). The antibacterial, fungicidal, and herbicidal properties of the new compounds were evaluated.

Introduction

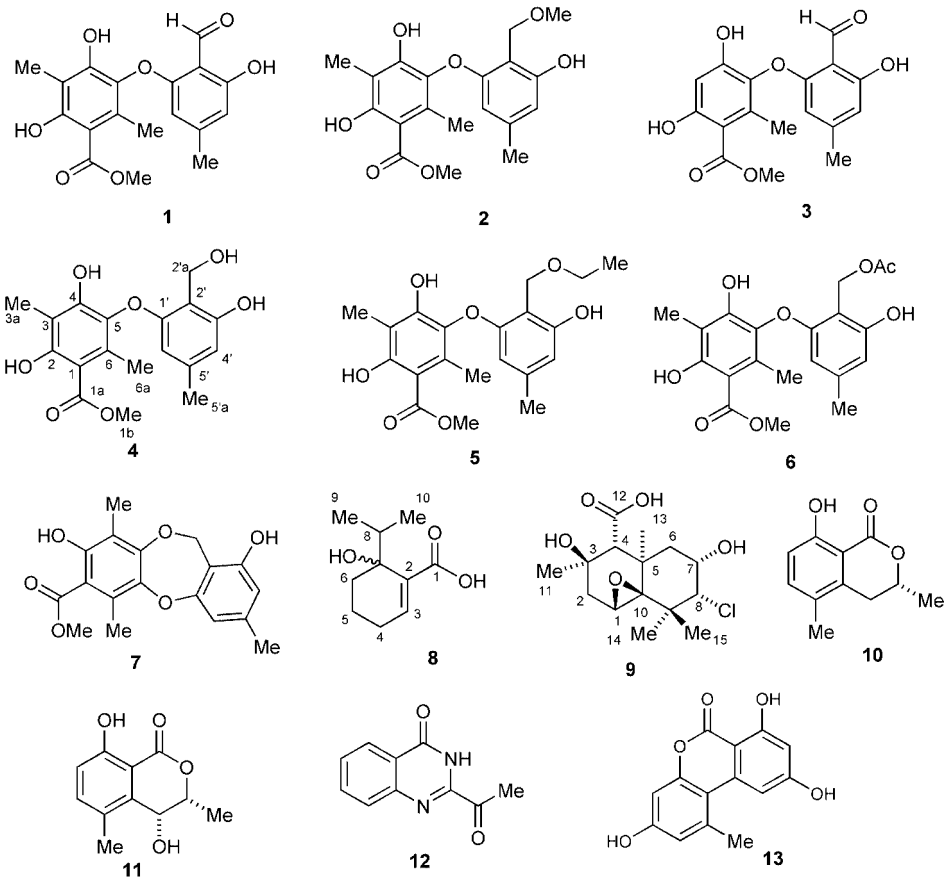
Endophytic fungi have proven to be a rich source of novel organic compounds with interesting biological activities and a high level of biodiversity.[2,3] In our screening program for fungicidal, herbicidal, and antibacterial fungal secondary metabolites, we investigated an endophytic strain of *Phomopsis* sp. (internal strain no. 7111), which was isolated from the halotolerant plant *Adenocarpus foliolosus* from Gomera. From the ethyl acetate culture extract of this fungus six new metabolites, phomosines D–G (**4**–**7**), 6-hydroxy-6-isopropylcyclohex-1-enecarboxylic acid (**8**) and (1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1a*H*-naphtho[1-*b*]oxirene-4-carboxylic acid (**9**) were isolated together with seven known compounds (**1**–**3**, **10**–**13**) (Scheme 1). The structures of the new compounds were determined by spectroscopic methods, mainly extensive 1D and 2D NMR experiments and mass spectrometric measurement. Here we describe the isolation and structural elucidation, as well as the herbicidal, antifungal and antibacterial activities of these new compounds.

Results and Discussion

The endophytic fungus *Phomopsis* sp. (internal strain no. 7111), was isolated from *Adenocarpus foliolosus* from Gomera and was cultivated at room temperature on biomalt solid agar media for 21 d. The 12 L of culture medium was then extracted with ethyl acetate to afford 6.5 g of a crude extract. The culture extract was both antibacterial and antifungal, in particular against *Septoria tritici* and *Pyricularia oryzae*, even at 8 ppm. The extract was separated into three fractions by column chromatography (CC) on silica gel. The less polar fraction 1 (2.3 g) contained mainly fatty acids and lipids. The remaining two fractions were each further purified by CC on silica gel and Sephadex (LH-20). The second fraction again gave two subfractions A and B. Column chromatography of fraction A gave crude products **1**, **2**, **3**, **5** and **6** and fraction B **7**, **8**, **10**, **11**, and **12**. The crude compounds were further purified by preparative TLC to afford the pure compounds **1** (40 mg), **2** (20 mg), **3** (20 mg), **5** (5 mg), **6** (10 mg), **8** (15 mg), **10** (20 mg), **11** (5 mg), and **12** (7 mg). Chromatography of the third polar fraction afforded crystals of compounds **4**, **9**, and **13**, which were further purified by crystallization from MeOH to yield the pure compounds **4** (60 mg), **9** (10 mg), and **13** (40 mg).

The structures of compounds **1**–**3** were identified as phomosines A–C, previously isolated by our group from another *Phomopsis* sp.;[4] structures **10**–**13** were identified as 5-methylmellein,[5] 4-hydroxy-5-methylmellein,[6] 2-quinazolin-4(3*H*)-one,[7,8] and the mycotoxin alternariol,[9] by

[‡] Biologically Active Metabolites from Fungi, 23. Part 22: Ref.[1]
[a] Department of Chemistry, University of Paderborn, Warburger Straβe 100, 33098 Paderborn, Germany
Fax: +49-5251-60-3245
E-mail: karsten.krohn@uni-paderborn.de
[b] Institut für Mikrobiologie, Technische Universität Braunschweig, Spielmannstraße 7, 31806 Braunschweig, Germany
[c] BASF AG, GVA/G-A030, 67056 Ludwigshafen



Scheme 1. Structures of compounds isolated from *Phomopsis* sp.

comparing their NMR spectroscopic data with those reported in literature.

A new compound **4** was obtained as colorless crystals with the empirical formula $C_{18}H_{20}O_7$, as deduced from HREIMS and ^{13}C NMR data. Its IR spectra shows strong absorptions for hydroxy groups ($\tilde{\nu}$ = 3413 cm^{-1}), while the ^1H NMR spectrum (Table 1) exhibits signals of four methyl groups at δ = 2.07, 2.10, 2.44, and 3.97 ppm, three phenolic OH groups at δ = 11.89, 9.77, and 8.69 ppm, and two aromatic protons at δ = 5.93 and 6.44 ppm. The ^{13}C NMR spectrum of compound **4** (Table 2) shows signals for 18 carbon atoms, and the DEPT spectrum indicates the presence of four methyl groups, one methylene group, two methine groups and eleven quaternary carbon atoms. Comparison of the ^{13}C NMR spectroscopic data with those of compound **1**, another biaryl ether isolated previously from a *Phomopsis* sp. by our group,[4] show the analogy of the chemical shifts, with the exception of that for C-2′a. In the HMBC spectrum of **4**, ^{13}C-^1H long-range correlation signals are seen between 2′a-H and C-2′, C-1′ and C-3′. Therefore, the structure of biaryl ether **4** is deduced as methyl 2,4-dihydroxy-5-[3-hydroxy-2-(hydroxymethyl)-5-methylphenoxy]-3,6-dimethylbenzoate and assigned the trivial name phomosine D. The structure of **4** was further unambiguously confirmed by X-ray diffraction analysis of a single crystal obtained from methanol (Figure 1).

Table 1. ^1H NMR spectral data of compounds **4**–**7** [500 MHz, $(CD_3)_2CO$, **7** in $CDCl_3$].

Proton	4	5	6	7
1b	3.97 s	3.98 s	3.95 s	3.94 s
3a	2.07 s	2.06 s	2.05 s	2.21 s
6a	2.44 s	2.45 s	2.30 s	2.23 s
2′a	5.00 br. s	4.82 s	5.34 s	5.01 s
2′b		3.70 m		
2′c		1.26 t (J = 7.1 Hz)	2.07 s	
4′	6.44 s	6.47 s	6.46 s	6.39 s
5′a	2.10 s	2.08 s	2.06 s	2.16 s
6′	5.93 s	5.94 s	5.81 s	5.81

Compound **5** has an empirical formula of $C_{20}H_{24}O_7$, as deduced from HREIMS and ^{13}C NMR data. The 1D (^1H, ^{13}C/DEPT) (Tables 1 and 2) and 2D (COSY, HMQC, HMBC) NMR spectra of **5** are similar to those of **4**, except that the hydroxy group on C-2′a is replaced by an ethoxy group. Therefore, compound **5** is deduced as methyl 3-[2-(ethoxymethyl)-3-hydroxy-5-methylphenoxy]-4,6-dihydroxy-2,5-dimethylbenzoate and assigned the trivial name phomosine E.

Compound **6** has the empirical formula $C_{20}H_{22}O_8$, as seen from the HREIMS and ^{13}C NMR data. The 1D (^1H, ^{13}C/DEPT) (Tables 1 and 2) and 2D (COSY, HMQC, HMBC) NMR spectra of **6** are similar to those of **4**. However, the hydroxy group at C-2′a is replaced by an acetyl group. Therefore, the biaryl ether **6** is assigned as methyl 3-

Table 2. ^{13}C NMR spectral data of compounds **4**–**7** [125 MHz, $(CD_3)_2CO$, **7** in $CDCl_3$].

Carbon	4	5	6	7
1	103.3	103.4	104.2	105.1
2	160.1	160.2	160.0	160.3
3	110.2	110.2	110.1	110.3
4	154.2	153.8	153.5	153.2
5	134.8	134.7	133.3	132.9
6	131.4	131.5	131.3	130.7
1′	157.6	157.6	157.6	152.2
2′	112.9	110.5	108.2	99.4
3′	155.9	156.3	157.4	152.3
4′	110.8	110.7	110.5	111.9
5′	139.2	139.9	140.7	138.9
6′	106.2	106.2	104.3	104.9
1a	172.6	172.5	172.4	172.4
1b	51.4	51.5	51.5	51.9
3a	7.5	7.5	7.5	8.2
6a	14.9	14.9	14.4	15.1
2′a	53.9	61.8	56.2	57.9
2′b		65.9	171.3	
2′c		14.3	20.0	
5′a	20.6	20.7	20.7	21.5

[2-(acetoxymethyl)-3-hydroxy-5-methylphenoxy]-4,6-dihydroxy-2,5-dimethylbenzoate and named phomosine F.

Compound **7** has the empirical formula $C_{18}H_{18}O_6$, as deduced from HREIMS and ^{13}C NMR data. The 1D (^1H, ^{13}C/DEPT) (Tables 1 and 2) and 2D (COSY, HMQC, HMBC) NMR spectra of **7** are similar to those of **4** except for the absence of the hydroxys group on C-4 and C-2′a. The HMBC spectrum shows correlation of C-4 with 3a-H, 2′a-H and of C-2′ with 2′-H. Therefore, the phenolic hydroxy group at C-4 and the benzylic hydroxy group at C-2′ in biaryl ether **4** must form a bridge to generate the dibenzo-1,4-dioxepin structure of **7**, which is named phomosine G.

Compound **8** was obtained as colorless crystals with the empirical formula $C_{10}H_{16}O_3$, in agreement with HREIMS and ^{13}C NMR data. Its IR spectrum shows strong absorptions for hydroxy groups ($\tilde{\nu}$ = 3415 cm^{-1}) and a lactone moiety ($\tilde{\nu}$ = 1687 cm^{-1}), while the ^1H NMR spectrum (Table 3) exhibits the signals of two methyl groups (δ = 0.88 and 0.90 ppm) and one alkene proton at δ = 6.96 (br. s) ppm. The ^{13}C NMR spectrum of **8** (Table 3) shows signals for 10 carbon atoms, and the DEPT spectrum indicates the presence of two methyl groups, three methylene groups, two methine groups, and three quaternary carbon atoms. Analysis of the ^1H-^1H COSY and HMQC spectra of **8** enables deduction of the fragment –CH_2–CH_2–CH_2–CH–. In the HMBC spectrum of **8**, ^{13}C-^1H long-range correlation signals are seen between C-7 and 6-H, 4-H and 9-H, between C-8 and 9-H, 5-H, and 6-H, and between C-10 and 9-H and 6-H. These data prove the structure of compound **8** as 6-hydroxy-6-isopropylcyclohex-1-enecarboxylic acid. The absolute configuration of the α-hydroxy acid **8** has not yet been elucidated.

Compound **9** was obtained as colorless crystals with the empirical formula $C_{15}H_{23}ClO_5$ in accordance with MS and ^{13}C NMR data. Its IR spectrum shows strong absorptions for hydroxy groups ($\tilde{\nu}$ = 3487 cm^{-1}), while the ^1H NMR spectrum (Table 4) exhibits the signals of four methyl

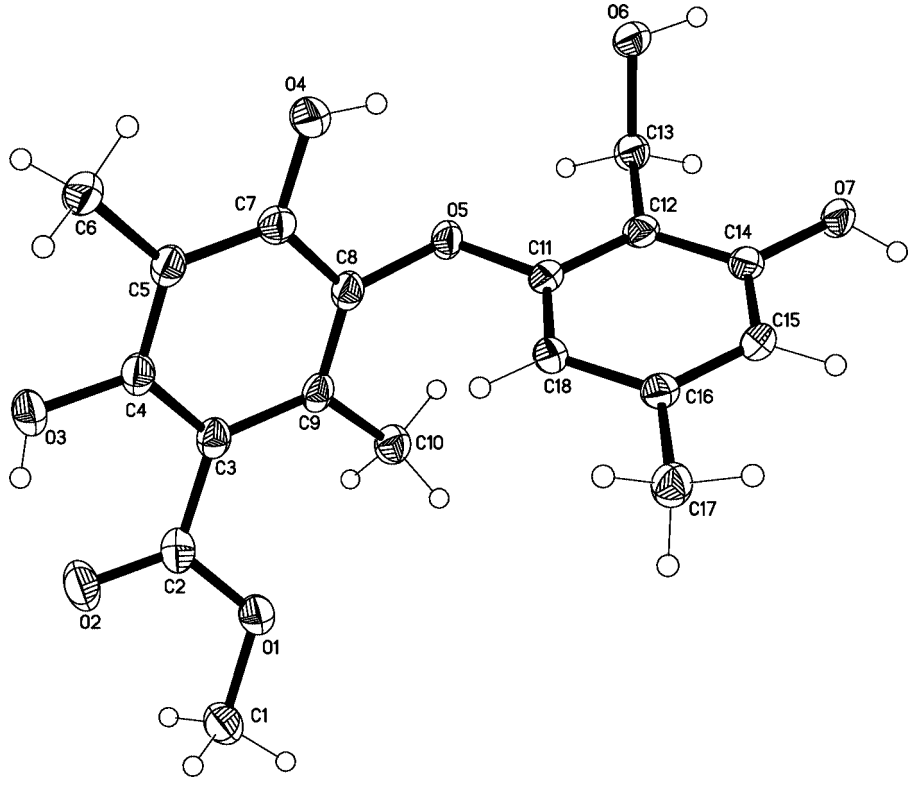


Figure 1. Molecular structure of phomosine D (**4**) in the crystal.

Table 3. NMR data for compound **8** (125 and 500 MHz, $CDCl_3$).

Position	δ_C	δ_H
1	171.9	
2	129.3	
3	139.7	6.96 br. s
4	35.3	2.13 m, 2.30 m
5	20.9	2.36 m
6	30.4	1.50 m, 1.69m
7	71.7	
8	37.0	1.68 m
9	16.6	0.88 d (J = 6.9 Hz)
10	16.7	0.90 d (J = 6.9 Hz)

groups, which are singlets (δ = 0.94, 1.30, 1.46, and 1.75 ppm). The ^{13}C NMR spectrum of **9** (Table 4) shows signals for 15 carbon atoms, and the DEPT spectrum indicates the presence of four methyl groups, two methylene groups, four methine groups, and five quaternary carbon atoms. Analysis of the 2D ^1H-^1H COSY and HMQC spectra of compound **9** suggests the presence of the fragments –CH_2(6)–CH(7)–CH(8)– and –CH(1)–CH_2(2)–. In the HMBC spectrum (Figure 2), ^{13}C-^1H long-range correlation signals are seen between C-3 and 1-H, 2-H, 4-H, and 11-H; between C-5 and 4-H, 6-H, 7-H, and 13-H; between C-9 and 1-H, 7-H, 8-H, 14-H, and 15-H; between C-10 and 1-H, 2-H, 13-H, 14-H, and 15-H; as well as between C-12 and 4-H. These spectroscopic data suggest that **9** is a sesquiterpene acid with two hydroxy groups at C-3 and C-7. The absolute configuration could be confirmed by X-ray analysis (Figure 3) because there is a chlorine atom at C-8 in this molecule giving rise to an anisotropy effect. The absolute configuration of **9** was thus assigned as (1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1a*H*-naphtho[1-*b*]oxirene-4-carboxylic acid.

Table 4. NMR data for compound **9** (125 and 500 MHz, CD_3OD).

Position	δ_C	δ_H
1	53.9	3.28 dd (J = 3.4, 1.2 Hz)
2	38.9	2.32 d (J = 3.4 Hz), 2.26 d, (J = 1.2 Hz)
3	68.7	
4	59.5	3.10 s
5	36.3	
6	40.4	1.70 dd (J = 14.5, 3.8 Hz), 2.22 dd (J = 14.5, 3.1 Hz)
7	70.9	4.22 m
8	71.2	4.09 d (J = 3.2 Hz)
9	40.7	
10	66.8	
11	28.4	1.30 s
12	174.2	
13	19.0	1.75 s
14	22.3	1.46 s
15	23.1	0.94 s

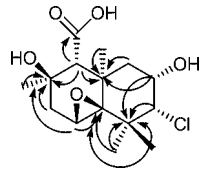


Figure 2. HMBC (H→C) correlations of compound **9**.

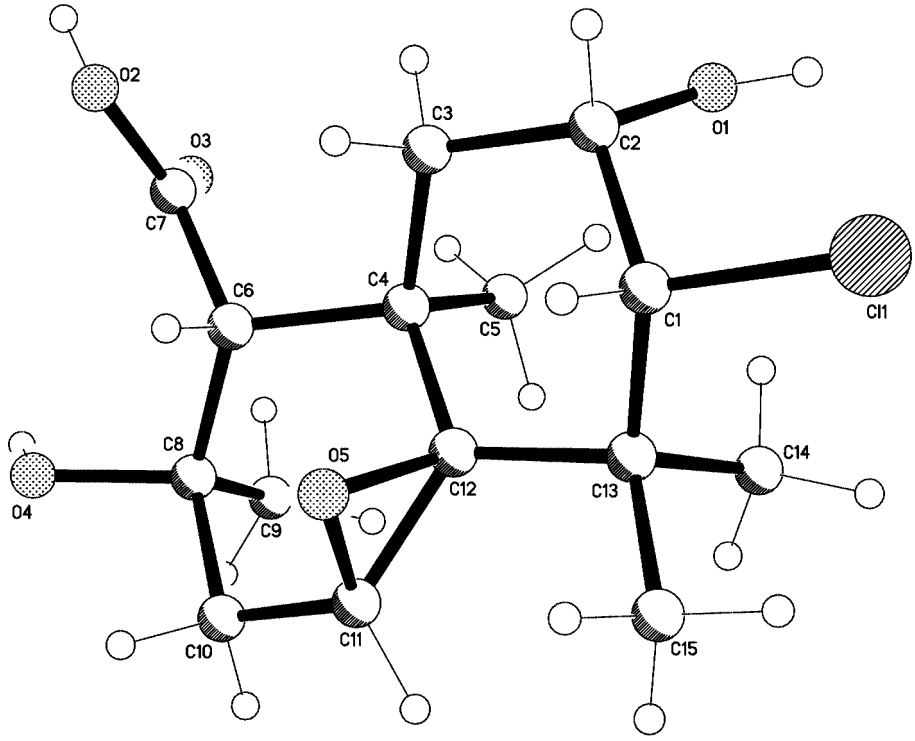


Figure 3. Molecular structure of compound **9** showing the correct absolute configuration.

Metabolites **1**, **3**, **7** and **10** exhibited moderate inhibitory activity against the test organisms used in these studies as shown in Table 5. Metabolite **1** had good antibacterial activities, whereas **10** had the best fungicidal properties.

Table 5. Antimicrobial activities of compounds **1**–**10** and **12**; agar diffusion test with radius of zone of inhibition in mm. GI = growth inhibition.

Compound	Chl[a] 5 mg/mL	Chl 10 mg/mL	Mv[a] 5 mg/mL	Mv 10 mg/mL	Bm[a] 5 mg/mL	Bm 10 mg/mL
1	0	5	0	7GI	8	11
2		0		0		0
3		0		0		7
4	0	0	0	0	0	0
5		0		0		0
6		0		0		0
7		7GI		0		6GI
8		0		0		0
9		0		0		0
10	5	6	6GI	9	0	0
12		0		0		0

[a] *Chlorella fusca* (Chl), *Bacillus megaterium* (Bm), and *Microbotryum violaceum* (Mv).

Conclusion

In summary, the investigated Phomopsis sp. Nr. 7111 shows remarkable biodiversity in the production of bioactive secondary metabolites in two ways. The first is exemplified in the functional group transformation of one basic skeleton ranging from changements in oxidation state, alkylation or acylation degree to chain and ring-closed forms. The second way is the diversity expressed in the production of products of different ring systems (carbocyclic: benzene, decahydronaphthalene; or heterocyclic: isochromene, quinazoline, benzo[*c*]chromene) generated by different biosynthetic pathways (polyketide, terpene, amino acid incorporation).

Experimental Section

General Experimental Procedures: For general methods and instrumentation see ref.[1] and for microbiological methods and conditions of culture see refs.[10,11] Melting points were determined with a Gallenkamp micro-melting point apparatus and are uncorrected. NMR spectra were run with a Bruker Avance-500 NMR spectrometer with TMS as internal standard. EIMS data were obtained with an MAT 8200 mass spectrometer.

Extraction and Isolation: The endophytic fungus *Phomopsis* sp., internal strain No. 7111, was isolated from *Adenocarpus foliolosus* from Gomera and was cultivated on 12 L of 5% w/v biomalt solid agar media at room temperature for 21 d.[11] The culture media were then extracted with ethyl acetate to afford 6.5 g of a residue after removal of the solvent under reduced pressure. The extract was separated into three fractions by column chromatography (CC) on silica gel (200 g), using gradients of dichloromethane/ethyl acetate (85:15, 50:50, 0:100). The less polar fraction 1 (2.3 g) contained mainly fatty acids and lipids. The remaining two fractions were each further purified by silica gel column chromatography (CC), preparative TLC and Sephadex (LH-20). The next polar fraction (1.8 g) was separated by CC on 200 g of silica gel with hexane/ethyl acetate (10:1, 1000 mL; 5:1, 1000 mL) to give two subfractions A and B. Fraction A (500 mg) was separated by CC on silica gel (10 g) with hexane/ethyl acetate (7:1, 550 mL) to give crude compounds **1**, **2**, **3**, **5** and **6**. Fraction B (450 mg) was separated by CC on silica gel (10 g) with hexane/ethyl acetate (4:1, 450 mL) to give crude compounds **7**, **8**, **10**, **11** and **12**. Subsequently, each crude fraction was further purified by preparative TLC on silica gel (1 mm, Macherey–Nagel) and Sephadex (LH-20) to give compounds **1** (40 mg), **2** (20 mg), **3** (20 mg), **5** (5 mg), **6** (10 mg), **8** (15 mg), **10** (20 mg), **11** (5 mg), and **12** (7 mg). The more polar fractions (1.1 g) were separated by silica gel column chromatography eluting with dichloromethane/ethyl acetate (6:1, 980 mL) to give crude compounds **4**, **9** and **13** as crystals. The samples were then recrystallized from MeOH to give the natural products **4** (60 mg), **9** (10 mg), and **13** (40 mg).

Phomosine D (4): Colorless crystals (acetone), m.p. 225–226 °C. IR (KBr, film): $\tilde{\nu}$ = 3425, 1636, 787 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. MS (EI, 70 eV): *m*/*z* (%) = 348 (50) [M$^+$], 330 (100), 298 (55), 270 (35), 199 (20), 148 (30), 83 (20), 42 (15). HREIMS (EI, 70 eV, $C_{18}H_{20}O_7$): calcd. 348.1209, found 348.1208.

Crystal Structure Determination of Phomosine D (4):[12] $C_{18}H_{20}O_7$, M = 348.3, monoclinic, space group $P2_1/c$, a = 8.129(3), b = 10.367(3), c = 19.591(6) Å, β = 101.97(1)°, V = 1615.0(9) Å^3, Z = 4, D_X = 1.433 g/cm^3, F(000) = 636, T = 120(2) K. Bruker-AXS SMART APEX,[13] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.11 mm^{-1}, colorless prismatic crystal, size 0.45 × 0.38 × 0.36 mm, 13708 intensities collected 2.1° < θ < 27.9°, –10 < h < 10, –11 < k < 13, –25 < l < 25. Structure solved by direct methods,[13] full-matrix least-squares refinement[13] based on F^2 and 234 parameters, all but H atoms refined anisotropically, H atoms refined with a riding model on idealized positions with U = 1.5 × U_{iso}(methyl-C and OH) or 1.2 × U_{iso}(C). Refinement converged at $R_1(F)$ = 0.052, $wR^2(F^2$, all data) = 0.153, S = 1.182, max(δ/σ) < 0.001, min/max height in final ΔF map –0.34/0.53 e/Å^3. Figure 1 shows the molecular structure.

Phomosine E (5): Amorphous powder. IR (KBr, film): $\tilde{\nu}$ = 3374, 2360, 2339, 1636, 658 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. MS (EI, 70 eV): *m*/*z* (%) = 376 (75) [M$^+$], 330 (100), 298 (50), 270 (30), 167 (20), 149 (70), 57 (70), 29 (10). HREIMS (EI, 70 eV, $C_{20}H_{24}O_7$): calcd. 376.15219, found 376.15231.

Phomosine F (6): Amorphous powder. IR (KBr, film): $\tilde{\nu}$ = 3420, 1646, 1418, 1217, 596 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. MS (EI, 70 eV): *m*/*z* (%) = 390 (20) [M$^+$], 330 (60), 298 (100), 270 (20), 180 (10), 149 (30), 43 (90), 28 (10). HREIMS (EI, 70 eV, $C_{20}H_{22}O_8$): calcd. 390.13147, found 390.13164.

Phomosine G (7): Amorphous powder. IR (KBr, film): $\tilde{\nu}$ = 3431, 1636 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data, see Tables 1 and 2. MS (EI, 70 eV): *m*/*z* (%) = 330 (100) [M$^+$], 167 (50), 149 (95), 57 (70). HREIMS (EI, 70 eV, $C_{18}H_{18}O_6$): calcd. 330.1103, found 330.1097.

6-Hydroxy-6-isopropylcyclohex-1-enecarboxylic Acid (8): Colorless crystals (MeOH), m.p. 135–136 °C. IR (KBr, film): $\tilde{\nu}$ = 3487, 2950, 2370, 1687, 1418, 1248 cm^{-1}. $[\alpha]_D^{25}$ = –192.8 (c = 0.07, CH_2Cl_2). For ^{1}H and ^{13}C NMR spectroscopic data, see Table 3. MS (EI, 70 eV): *m*/*z* (%) = 184 (40) [M$^+$], 141 (20), 95 (100), 55 (95), 28 (40).

(1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-Chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1a*H*-naphtho[1-*b*]oxirene-4-carboxylic Acid (9):

Colorless crystals (MeOH), m.p. 185–186 °C. IR (KBr, film): $\tilde{\nu}$ = 3487, 1636 cm^{-1}. $[\alpha]_D^{25}$ = –58.3 (c = 0.24, CH_3OH). For ^1H and ^{13}C NMR spectroscopic data, see Table 4. MS (ESI, 70 eV): m/z (%) = 317 (90) $[M - 1]^+$, 263 (100), 227 (25), 191 (15), 81 (10).

Crystal Structure Determination of (1a*S*,3*R*,4*R*,4a*R*,6*S*,7*R*,8a*S*)-7-Chloro-3,6-dihydroxy-3,4a,8,8-tetramethyl-octahydro-1a*H*-naphtho-[1-*b*]oxirene-4-carboxylic Acid (9):[12] $C_{15}H_{23}ClO_5$, M = 318.8, monoclinic, space group $C2$, a = 18.312(2), b = 6.0007(8), c = 14.0839(18) Å, β = 102.157(3)°, V = 1512.9(3) $Å^3$, Z = 4, D_X = 1.400 g/cm^3, $F(000)$ = 680, T = 120(2) K. Bruker-AXS SMART APEX[13] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.27 mm^{-1}, colorless prismatic crystal, size 0.40 × 0.10 × 0.05 mm, 7452 intensities collected $1.5° < \theta < 27.5°$, $-22 < h < 23$, $-7 < k < 7$, $-18 < l < 18$. Structure solved by direct methods,[13] full-matrix least-squares refinement[13] based on F^2 and 197 parameters, all but H atoms refined anisotropically, H atoms refined with a riding model on idealized positions with $U = 1.5 \times U_{iso}$(methyl-C and OH) or $1.2 \times U_{iso}$(C). The title compound crystallizes in the non-centrosymmetric space group $C2$; the correct absolute configuration was unambiguously assigned from refinement of the Flack[14] parameter. Refinement converged at $R_1(F)$ = 0.052, $wR^2(F^2$, all data) = 0.117, S = 0.968, max(δ/σ) < 0.001, min/max height in final ΔF map –0.33/0.34 e/$Å^3$. Figure 3 shows the molecular structure.

Acknowledgments

We thank the BASF AG and the BMBF (Bundesministerium für Bildung und Forschung, project no. 0310722 B1) for financial support, and Qunxiu Hu for excellent technical assistance. We are grateful to Prof. Kuck and Dr. Matthias C. Letzel for ESIMS measurements.

[1] J. Dai, K. Krohn, D. Gehle, I. Kock, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, J. Rheinheimer, *Eur. J. Org. Chem.* **2005**, 4009–4016.
[2] B. Schulz, C. Boyle, S. Draeger, H.-J. Aust, A.-K. Römmert, K. Krohn, *Mycolog. Res.* **2002**, 996–1004.
[3] B. Schulz, C. Boyle, *Mycolog. Res.* **2005**, *109*, 661–686.
[4] K. Krohn, A. Michel, E. Roemer, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, V. Wray, *Nat. Prod. Lett.* **1995**, *9*, 309–314.
[5] J. R. Anderson, R. L. Edwards, *J. Chem. Soc., Perkin Trans. 1* **1983**, 2185–2192.
[6] T. Kokubun, N. C. Veitch, P. D. Bridge, M. S. J. Simmonds, *Phytochemistry* **2003**, *62*, 779–782.
[7] J. P. Michael, *Nat. Prod. Rep.* **1994**, *11*, 163–172.
[8] D. Niederer, C. Tamm, W. Zürcher, *Tetrahedron Lett.* **1992**, *33*, 3997–4000.
[9] E. E. Stinson, W. B. Wise, R. A. Moreau, A. J. Jurewicz, P. E. Pfeffer, *Can. J. Chem.* **1986**, *64*, 1590–1594.
[10] K. Krohn, U. Flörke, M. S. Rao, K. Steingröver, H.-J. Aust, S. Draeger, B. Schulz, *Nat. Prod. Lett.* **2001**, *15*, 353–361.
[11] B. Schulz, J. Sucker, H.-J. Aust, K. Krohn, K. Ludewig, P. G. Jones, D. Doering, *Mycol. Res.* **1995**, 1007–1015.
[12] CCDC-270159 (**4**) and -269676 (**9**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
[13] SMART (Ver. 5.62), SAINT (Ver. 6.02), SHELXTL (Ver. 6.10) and SADABS (Version 2.03), Bruker AXS Inc., Madison, Wisconsin, USA, **2002**.
[14] H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.

Received: June 27, 2005
Published Online: October 13, 2005

