DOI: 10.1002/ejoc.200600907

Bioactive Natural Products from the Endophytic Fungus *Ascochyta* sp. from *Meliotus dentatus* – Configurational Assignment by Solid-State CD and TDDFT Calculations[‡]

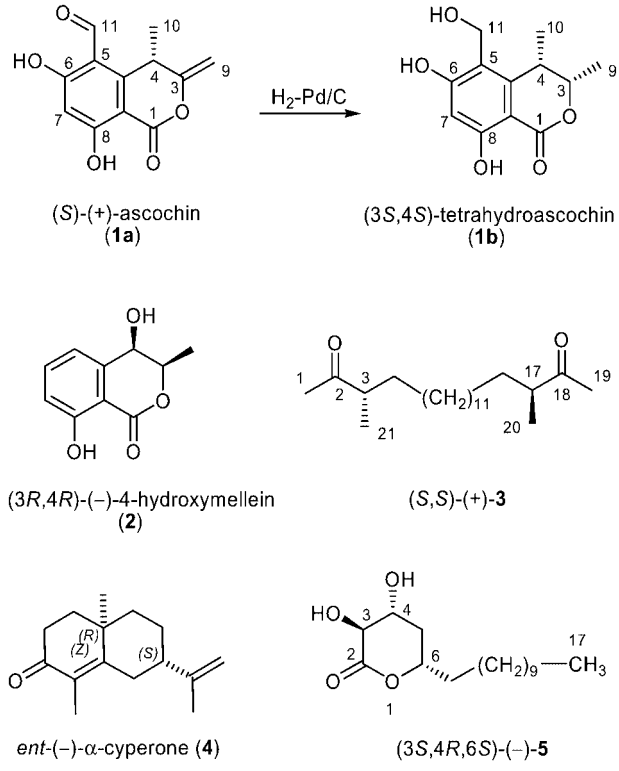
Karsten Krohn,*[a] Ines Kock,[a] Brigitta Elsässer,[a] Ulrich Flörke,[a] Barbara Schulz,[b] Siegfried Draeger,[b] Gennaro Pescitelli,[c] Sándor Antus,[d,e] and Tibor Kurtán[d]

Keywords: Biological activity / Natural products / Isocoumarins / Quantum-mechanical calculation of CD spectra

Two new metabolites, (4*S*)-(+)-ascochin (**1a**) and (*S*,*S*)-(+)-ascodiketone (**3**), together with the known compounds (3*R*,4*R*)-(–)-4-hydroxymellein (**2**), *ent*-α-cyperone (**4**) and (3*S*,4*R*)-(–)-dihydroxy-(6*S*)-undecyl-α-pyranone (**5**) were isolated from cultures of the of the endophytic fungus *Ascochyta* sp. The biologically active isocoumarin derivative, (4*S*)-(+)-ascochin (**1a**), has an unusual substitution pattern which was confirmed by X-ray diffraction. Its absolute configuration was determined by our solid-state TDDFT CD methodology using the X-ray coordinates as input for the calculation. By catalytic hydrogenation, (4*S*)-(+)-ascochin was converted into the corresponding (3*S*,4*S*)-dihydroisocoumarin derivative **1b**. The measured and TDDFT calculated CD spectra enabled studies on the correlation between absolute configuration and n-π* transition Cotton effect.

Introduction

Fungal secondary metabolites are an important source of lead structures for new drugs.[2,3] In our screening program for new biologically active secondary metabolites, we investigated the metabolites of an endophytic fungus, *Ascochyta* sp., isolated from the plant *Meliotus dentatus* near Ahrenshoop on the Baltic Sea coast. Crude extracts of the fungal fermentation were active in the agar diffusion tests against the plant pathogenic fungi *Microbotryum violaceum*, *Phytophtora infestans*, and *Septoria tritici*, as well as against the algae *Chlorella fusca*. The fungus was cultivated for 28 d on biomalt agar; the cultures were subsequently extracted with ethyl acetate. The ethyl acetate extract was subjected to column chromatography and two new metabolites, (4*S*)-(+)-ascochin (**1a**) and (*S*,*S*)-(+)-ascodiketone (**3**), together with the three known compounds (3*R*,4*R*)-(–)-4-hydroxymellein (**2**),[4–6] *ent*-α-cyperone (**4**)[7b] and (3*S*,4*R*,6*S*)-(–)-dihydroxy-6-undecyl-α-pyranone (**5**)[8] were isolated (Scheme 1).



Scheme 1. Structures of compounds **1**–**5** isolated from *Ascochyta* sp. and conversion of **1a** to its *cis*-dihydroisocoumarin derivative **1b**.

[‡] Biologically Active Secondary Metabolites from Fungi, 26. Part 25: Ref.[1]

[a] Department of Chemistry, University of Paderborn, Warburger Straße 100, 33098 Paderborn, Germany
Fax: +49-5251-60-3245
E-mail: k.krohn@upb.de
[b] Institut für Mikrobiologie, Technische Universität Braunschweig, Spielmannstraße 7, 31806 Braunschweig, Germany
[c] Università di Pisa, Dipartimento di Chimica e Chimica Industriale, Via Risorgimento 35, 56126 Pisa, Italy
[d] Department of Organic Chemistry, University of Debrecen, P. O. Box 20, 4010 Debrecen, Hungary
[e] Research Group for Carbohydrates of the Hungarian Academy of Sciences, P. O. Box 55, 4010 Debrecen, Hungary

Results and Discussion

The yellow compound **1a** is crystalline (m.p. 186–187 °C) and optically active ($[\alpha]_D^{20}$ = +288); spots of **1a** on TLC plates turn violet after treatment with anisaldehyde/sulfuric acid and heating. The HRMS spectrum shows a molecular ion at 234.05253, corresponding to the molecular formula $C_{12}H_{10}O_5$, indicating eight sites of unsaturation. The ^1H NMR spectrum reveals the existence of an aldehyde proton at δ = 10.15 ppm and two signals at δ = 11.65 and 12.55 ppm attributable to chelated OH.

Analysis of the ^{13}C and DEPT NMR spectra allows the identification of one methyl, one CH, one sp^2 CH_2, and one sp^2 CH group, six quaternary sp^2 carbon atoms, plus two carbonyl groups. One carbonyl group is part of a lactone (δ = 165.9 ppm) and the other resonance (δ = 191.6 ppm) is typical of an aldehyde C=O. The chemical shift at δ = 98.3 ppm can be assigned to a CH_2 group linked to an oxygenated double bond (C-3). The signals for olefinic protons at δ = 4.75 and 4.96 ppm show a geminal coupling constant of J = 2.3 Hz, characteristic of methylene protons (C-9). The chemical shift of the sp^2 CH group (C-7) of δ = 103.9 ppm suggests oxygenation at both of its *ortho* positions and the coupling of the methyl group (d, J = 7.2 Hz) implies the presence of a neighboring sp^3 CH group. From this information five fragments can be deduced: an aldehyde, an ester, a CH_3–CH unit, an oxygenated double bond, and an aromatic ring with two hydroxy groups in *meta* position. Thus, the eight unsaturated sites account for six double bonds and two rings. Supported by the HMBC (Figure 1) and COSY spectra, structure **1a** could be assigned to a compound (Scheme 1) that was named ascochin after the producing fungus. Finally, the assumed structure was unambiguously confirmed by X-ray diffraction analysis of its single crystal (Figure 2). X-ray crystal analysis also revealed that the C-4 methyl group is axially oriented, possibly in order to reduce the *peri* interaction with the C-5 formyl group.

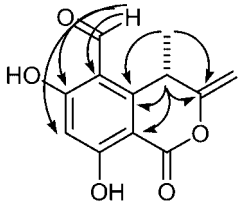


Figure 1. Major HMBC correlations of ascochin (**1a**).

For the configurational assignment of natural products, we recently used quantum-mechanical calculation of their CD spectra, which were then compared with the experimental ones.[9–11,12] Since the X-ray coordinates of **1a** were available, our solid-state TDDFT CD methodology[1] could also be applied in the present case, significantly simplifying the procedure by using the X-ray structure as input for the calculation and the experimental solid-state CD spectrum for comparison. The solution (acetonitrile) and solid-state (KCl disc) CD spectra of **1a** are very similar (Figure 3), implying a substantial structural rigidity. In fact, DFT geometry optimizations resulted in a distinct minimum very close to the crystal structure (RMS deviation for heavy atoms ca. 0.05 Å); this structure is characterized by two intramolecular hydrogen bonds (C1=O→HO-8 and C11=O→HO-6) and an axial disposition of the C-4 methyl group on the heteroring. Other minima were isolated having DFT energy greater than +7.5 kcal/mol compared with the preferred one. The close correspondence between CD spectra shown in Figure 3 is noteworthy because CD intrinsic to the solid-state is seemingly negligible. In principle, intermolecular couplings between electric-dipole allowed π-π* transitions might instead be expected. This is extremely encouraging with respect to the scope and applicability of our methodology, which requires minimal interference from crystal lattice contributions to CD.[1]

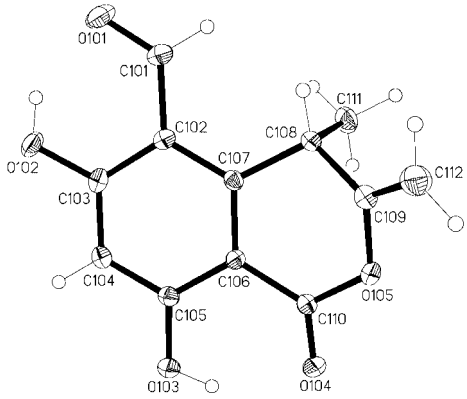


Figure 2. Molecular structure of ascochin (**1a**) in the crystal. Displacement ellipsoids are drawn at the 50% probability level.

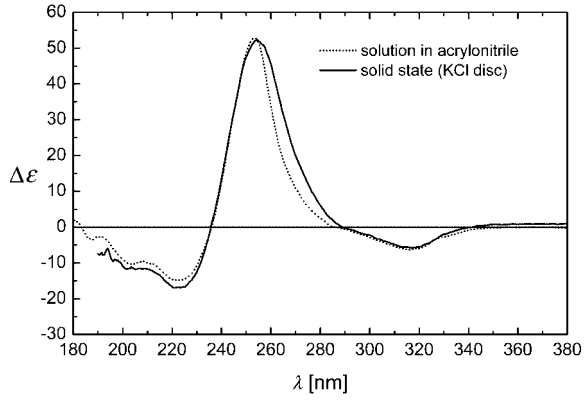


Figure 3. Measured CD spectrum of **1a** in acetonitrile solution (dotted line) and as KCl disc (solid line).

The CD spectra calculated[13] with the TDDFT method[14] using the DFT-computed geometry and the X-ray coordinates for (*S*)-ascochin are also quite similar (Figure 4), except for a small wavelength shift, and reproduce the experimental spectra very well. A slightly better agreement is observed between solid-state experimental and computed spectra. All the 16 computed transitions involve virtual orbitals with negative eigenvalues and have energies well below the estimated ionization potential (7.3 eV).[14] As a consequence, the absolute configuration of compound **1a**

can be unambiguously assigned as (*S*). Although the solution and solid-state methods lead to the same result, compound **1a** represents a useful test for our new solid-state TDDFT CD methodology.

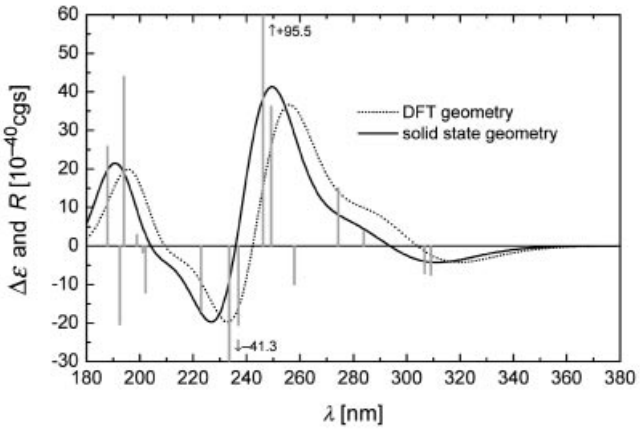


Figure 4. TDDFT-calculated CD spectrum for (*S*)-ascochin using the DFT-computed geometry (dotted line) and the solid-state geometry (solid line; vertical bars represent rotational strengths *R*).

Analysis of Kohn–Sham orbitals allows us to interpret the major features in the CD spectra. The negative band at 300–330 nm is allied to the aldehyde n-π* (transition No. 1 at 309 nm, see vertical bars in Figure 4) and to the aromatic ^1L_b-type π-π* (transition No. 2 at 307 nm); the positive band centered at 255 nm is mainly due to a further aromatic π-π* transition (No. 7 at 246 nm) with a contribution from charge-transfer type transitions from alkene π to aromatic π* (No. 4 and No. 6 at 274 and 249 nm).

For the configurational assignment of dihydroisocoumarins, a semiempirical rule was forwarded which correlates *P*-helicity of the heteroring with a positive n→π* Cotton effect (CE) at around 260 nm.[15] This rule was then used for the determination of configuration in both synthetic[16] and natural derivatives.[6] In order to check the applicability of this rule for 3,4-disubstituted dihydroisocoumarins to the current case, the 3,4-*cis*-dimethyldihydroisocoumarin derivative **1b** was prepared by catalytic (Pd/C) hydrogenation of **1a** (Scheme 1), and its chiroptical data were measured and computed. The hydrogen addition occurs with *cis* diastereoselectivity due to the inherent 4*S* stereogenic center (the formyl group was also reduced during the hydrogenation). The assignment of the *cis* configuration of the C-3 and C-4 methyl groups in **1b** is not only based on chemical considerations, assuming addition of the hydrogen from the less hindered side,[17] but also on the small coupling constant for $J_{3,4}$ = 2.3 Hz for the relevant protons in the ^{1}H NMR spectrum of **1b**. Thus, the absolute configuration of **1b** is 3*S*,4*S*. The CD spectrum shows a negative CE at 307 nm and a positive one at 267 nm (Figure 5). According to the literature,[6] the ester n→π* CD band can be assigned to the latter. Interestingly, the (3*S*)-3-methyldihydroisocoumarin derivative **6**, obtained by the oxidation of the corresponding (3*S*)-3-methylisochroman derivative,[18] has positive CEs at both 300 and 268 nm, and except for the latter transition, its CD curve is nearly a mirror image of that of **1b** (Figure 5). In order to confirm the position of the ester n→π* CD transition and hence the semiempirical rule of dihydroisocoumarins, a TDDFT calculation was carried out on the absolute minimum energy structure computed for **1b**. The conformational analysis of **1b** resulted in a hydrogen-bonded (O-11→HO-7) conformer as the most stable structure (1.7 kcal/mol lower DFT-energy than the second minimum), as shown in Figure 6.

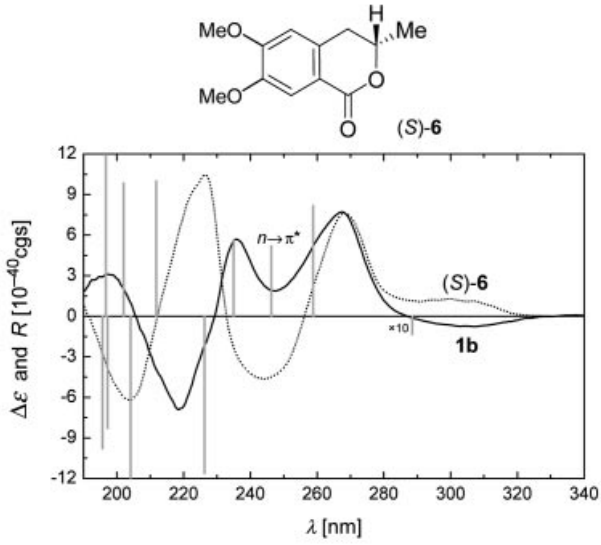


Figure 5. Measured CD spectra of **1b** (solid line) and (*S*)-**6** (dotted line), and TDDFT-computed rotational strengths *R* (vertical bars) for the absolute minimum of (3*S*,4*S*)-**1b** found by DFT (Figure 6).

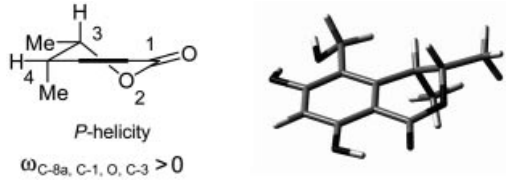


Figure 6. *P*-helicity (left) of (3*S*,4*S*)-6,8-dihydroxy-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-1*H*-isochromen-1-one (**1b**) and DFT-calculated most stable conformation (right).

The TDDFT CD calculation on this conformer demonstrates that the ester n→π* CD transition of **1b** appears with positive CE as the third computed transition from the red at 246 nm, namely as part of the 250–270 nm CD band, overlapped with aromatic π→π* transitions (see Figure 5). In particular, the most red-shifted transition computed at 288 nm is of the ^1L_b-type and it is apparently responsible for the weak CD signal above 280 nm. Since **1b** has (3*S*,4*S*) absolute configuration and *P*-helicity of the heteroring (Figure 6), its computed positive n→π* transition is in accordance with the semiempirical rule. (*S*)-**6** also has *P*-helicity, also resulting in positive n→π* at 268, although all the other corresponding transitions have opposite signs to those of **1b**, due to the different substitution pattern of the

aromatic ring.[18] Since the ester n→π* transition is only one of the contributors to the 267 nm CD band among several π→π* transitions, its application for a safe configurational assignment is endangered in the current case by the overlapping transitions.

The second new compound was obtained as a colorless, optically active oil ($[\alpha]_D^{20}$ = +20). From the ^1H and ^{13}C NMR spectra, two methyl and six methylene signals, plus one keto and one methine signal were identified. The coupling pattern of the methyl signal (d, J = 6.9 Hz) at δ = 1.05 ppm is indicative of its connection to a methine group, while the downfield-shifted signal of another methyl group and a methine group at δ = 2.11 and 2.47 ppm, respectively, verify their proximity to a carbonyl group. The coupling pattern of the CH signal (tq, J = 6.9 Hz) points to an additional neighboring CH_2 group. This information shows that the methylene groups form a linear chain CH_3–CO–$CHCH_3$–CH_2–CH_2–CH_2–CH_2–CH_2–CH_2 fragment. However, the molecular ion of 324 in the mass spectrum together with the single set of NMR signals suggests a symmetric structure in which the two linear fragments are connected by a central methylene group. The HRMS spectrum confirms the molecular formula $C_{21}H_{40}O_2$ for 3,17-dimethylnonadecan-2,18-dione, named ascodiketone (**3**) (Scheme 1). Since ascodiketone (**3**) is optically active ($[\alpha]_D^{20}$ = +19.8), the (*S*,*R*) *meso* absolute configuration can be excluded; **3** must have either the absolute configuration (*S*,*S*) or (*R*,*R*); differentiation was possible on the basis of its CD spectrum. As the two identical remote chromophores have the same configuration and equivalent contributions to the CD spectra, the CD of **3** can be compared with those of synthetic compounds (*R*)-**7**[19] and (*R*)-**8**[20] (Figure 7). They contain the ketone chromophore within a similar chiral pattern $CH_3COCH(Me)R$, where R is a long alkyl chain. Both (*R*)-**7** and (*R*)-**8** exhibit a negative CE around 280 nm for the n-π* transition; since ascodiketone **3** has a positive CE with maximum at 286 nm, its absolute configuration is assigned as (*S*,*S*).

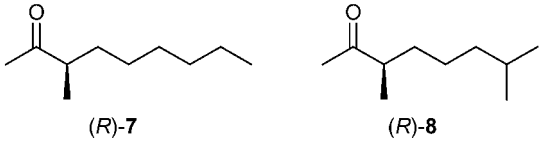


Figure 7. Structures of synthetic α-methyl ketones (*R*)-**7** and (*R*)-**8**.

The optically active ($[\alpha]_D^{20}$ = –37) compound **5** was identified as (3*S*,4*R*)-dihydroxy-(6*S*)-undecyl-α-pyranone, recently published by Li et al.[8] The compound exhibits biological activity against *Microbotryum violaceum*, *Bacillus megaterium* and *Septoria tritici* (Table 1). The relative configuration of the substituents on the tetrahydropyranone ring was deduced from the coupling constants of the relevant protons as shown in Figure 8. The coupling constant of $J_{3,4}$ = 9.7 Hz indicates an axial orientation between H-3 and H-4, and thus a bisequatorial position of the two hydroxy groups at C-3 and C-4. In addition, the axial–equatorial relationship follows from the couplings $J_{4a,5e} = J_{5e,6a}$ = 3.6 Hz. Thus, the C_{11} side chain also adopts an equatorial position. On the basis of the optical rotation and CD data, compound **5** has the same absolute configuration as (3*S*,4*R*)-dihydroxy-(6*S*)-undecyl-α-pyranone reported by Li et al.[8]

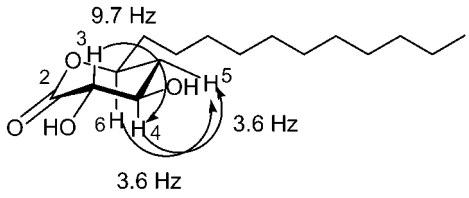


Figure 8. The relative configuration of (3*S*,4*R*)-dihydroxy-(6*S*)-undecyl-α-pyranone (**5**) as deduced from the coupling constants.

The known compound **4** was identified as *ent*-α-cyperone: the ^{13}C NMR spectroscopic data match those of α-cyperone[21] and the negative optical rotation is in contrast to the positive value of α-cyperone.[7a] The second isocoumarin isolated from *Ascochyta* sp. is identical with (3*R*,4*R*)-4-hydroxymellein (**2**) (Scheme 1) on the basis of the published data.[4,5]

Bioactivity

The crude culture extract shows antifungal activity against *Microbotryum violaceum*, *Septoria tritici* and *Phytophtora infestans* as well as antibacterial activity against *Bacillus megaterium* and algicidal activity against *Chlorella fusca*. The pure compounds were tested against the Gram-positive bacterium (*Bacillus megaterium*), the fungus *Microbotryum violaceum*, and the algae *Chlorella fusca* (Table 1). As shown in Table 1, hydroxymellein (**2**) was particularly antibacterially active. Höller et al.[5] previously reported on the activity of **2** against the fungus *Eurotium repens*. (3*S*,4*R*)-Dihydroxy-(6*S*)-undecyl-α-pyranone (**5**) shows the highest activity against the fungus *Microbotryum violaceum*. Toshima et al.[22] reported on the abscisic effects of the C_9 analogue of **5** on the leaves of *Chamaecyparis obtuse*.

Table 1. Activity of compounds **1a**, **2** and **5** against the Gram-positive bacterium *Bacillus megaterium*, the fungus *Microbotryum violaceum*, and the algae *Chlorella fusca*.[a]

Compound	*Bacillus megaterium*	*Microbotryum violaceum*	*Chlorella fusca*
1a	7 gi*	10**	10
2	25	12	12
5	7	13	5 gi

[a] Application of 0.5 mg (50 μL of 10 mg/mL) in an agar diffusion test. *gi = indicates that some growth occurred in the zone of inhibition. **Radius of zone of inhibition in mm.

Experimental Section

General Experimental Procedures: For microbiological methods and culture conditions see ref.[23] and for instrumentation see ref.[24] Melting points were determined in open capillaries on a Büchi melting point apparatus and are uncorrected. Optical rotations

were measured on a Perkin–Elmer 241 MC polarimeter and IR spectra on a Perkin–Elmer 16PC FT-IR instrument. Silica gel (230–400 mesh) was used for column chromatography (CC). The X-ray data were generated on a Bruker Smart 1000 CCD system diffractometer. The CD spectra were recorded on a J-810 spectropolarimeter and the concentrations are given in mol/dm^3. For the solid-state CD protocol, see ref.[1] ESI-TOF MS measurement was performed on a MicroTOF-Q instrument (Bruker Daltonik GmbH, Bremen, Germany). Spots on TLC were detected under UV and by heating after spraying with 0.5 mL anisaldehyde (0.5 mL) in HOAc (50 mL)/H_2SO_4 (1 mL).

Extraction und Isolation: The endophytic fungus, *Ascochyta* sp., internal strain number 6651, was isolated following surface sterilization from the plant *Meliotus dentatus* from the shores of the Baltic Sea, near Ahrenshoop, Germany. The fungus was cultured for 28 d at room temperature on 12 L of biomalt (5% w/v) solid agar medium on 480 petri dishes. The cultures were frozen for 3 d, warmed to room temperature and the liquid and solid phases separated by filtration. Liquid and solid phases were extracted separately with ethyl acetate. The TLCs of both extracts were identical and therefore combined to afford 29.00 g of crude extract after removal of solvent under reduced pressure. The extract was then separated into 7 fractions by column chromatography on silica gel, using gradients of dichloromethane/methanol (0–10% methanol in 1%-steps of 500 mL each). Fraction 1 gave ascochin (**1a**, 1.600 g) after crystallization from dichloromethane. Fractions 2 and 3 each contained a mixture of fatty acids as well as ascodiketone (**3**, 89 mg) and *ent*-α-cyperone (**4**, 9 mg), which were purified by column chromatography on Sephadex LH 20 with dichloromethane/methanol (3:2) and preparative TLC on silica gel with dichloromethane. Fraction 4 contained (3*R*,4*R*)-4-hydroxymellein which was further purified by column chromatography on Sephadex LH 20 with dichloromethane/methanol (3:2) to yield 527 mg of (**2**). Fraction 5 gave (3*S*,4*R*)-dihydroxy-(6*S*)-undecyl-α-pyranone (**5**) (413 mg) after crystallization from diethyl ether.

(3*R*,4*R*)-6,8-Dihydroxy-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-1*H*-isochromen-1-one (Ascochin, 1a): Colorless crystals (CH_2Cl_2), m.p. 186–187 °C. $[\alpha]_D^{20}$ = +287.9 (c = 0.28, CH_2Cl_2). UV: λ_{max}, nm (log ε, CH_3CN) = 186 (4.46), 253 (4.64), 277 (4.12), 318 (3.62). CD (CH_3CN, c = 6.0·10^{-4}) λ ($\Delta\varepsilon$) = 188 (–3.45), 204 (–10.38), 211 (–10.21), 222 (–14.87), 253 (52.70), 317 (–5.83). CD (KCl, c = 7.16·10^{-4}) λ ($\Delta\varepsilon$) = 201 (–11.65), 221 (–16.94), 254 (52.37), 317 (–5.79). IR (KBr): $\tilde{\nu}_{max}$ = 3126, 2981, 2931, 1703, 1664, 1626, 1597, 1452, 1419, 1365, 1338, 1288, 1271, 1227, 1107, 1063, 1024 (cm^{-1}). ^{1}H NMR (200 MHz, $CDCl_3$): δ = 1.63 (d, $J_{10,4}$ = 7.2 Hz, 3 H, 10-H), 4.36 (q, $J_{4,10}$ = 7.2 Hz, 1 H, 4-H), 4.75 (d, J_{gem} = 2.3 Hz, 1 H, 9-H^a), 4.96 (d, J_{gem} = 2.3 Hz, 1 H, 9-H^b), 6.44 (s, 1 H, 7-H), 10.15 (s, 1 H, 11-H), 11.65 (s, 1 H, 6-OH), 12.55 (s, 1 H, 8-OH). ^{13}C NMR (50 MHz, $CDCl_3$): δ = 25.4 (CH_3, C-10), 33.2 (CH, C-4), 98.3 (CH_2, C-9), 100.7 (C_q, C-8a), 103.9 (CH, C-7), 109.9 (C_q, C-5), 152.1 (C_q, C-4a), 155.0 (C_q, C-3), 165.9 (C_q, C-1), 168.8 (C_q, C-8), 170.3 (C_q, C-6), 191.6 (CH, C-11). EIMS (70 eV, 175 °C): *m*/*z* (%) = 234 [M$^+$] (100), 217 (13.8), 191 (9.1), 174 (31.9), 164 (39.7), 69 (10.1). HRMS (EI): calcd. for $C_{12}H_{10}O_5$ 234.05282; found 234.05253.

Crystal Structure Determination of Ascochin (1a):[25] $C_{12}H_{10}O_5$, Mr = 234.2, monoclinic, space group $P2_1$, a = 6.4068(4), b = 25.7674(15), c = 12.8166(8) Å, β = 91.563(1)°, V = 2115.1(2) Å^3, Z = 8, D_x = 1.471 g/cm^3, F(000) = 976, T = 120(2) K. Bruker-AXS SMART APEX,[26] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.116 mm^{-1}, colorless crystal, size 0.50 × 0.24 × 0.16 mm^3, 26761 intensities collected 1.6 < θ < 28.3°, –8 < h < 8, –34 < k < 34, –17 < l < 16. Structure solved by direct methods,[26] full-matrix least-squares refinement[26] based on F^2 and 625 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with U = 1.5 U_{iso}(methyl-C and OH) or 1.2 U_{iso}(C). There are four independent but geometrically identical molecules A, B, C and D per asymmetric unit with numbering schemes 1xx, 2xx, 3xx and 4xx, respectively. **1a** crystallizes in the non-centrosymmetric space group $P2_1$; however, in the absence of significant anomalous scattering effects, the Flack[27] parameter is essentially meaningless. Accordingly, Friedel pairs were merged. Refinement converged at $R_1[F > 4\sigma(F)]$ = 0.040, $wR_2(F^2$, all data) = 0.103, S = 1.064, max(δ/σ) < 0.001, min./max. height in the final ΔF map: –0.21/0.37 eÅ^{-3}. Figure 2 shows the molecular structure.

(3*R*,4R)-6,8-Dihydroxy-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-1*H*-isochromen-1-one (Tetrahydroascochin, 1b): A solution of ascochin (**1a**) (40 mg, 0.17 mmol) in dry THF (10 mL) was treated with 10 mg of 5%Pd/C and the suspension was stirred under an atmosphere of hydrogen for 2 h. The progress of the reaction was monitored by TLC. The reaction mixture was filtered over celite and the solvent was removed under reduced pressure. The resulting crude product was purified by preparative TLC with CH_2Cl_2/methanol (95:5) to yield (3*R*,4*R*)-6,8-dihydroxy-5-(hydroxymethyl)-3,4-dimethyl-3,4-dihydro-1*H*-isochromen-1-one (**1b**, 32 mg, 0.13 mmol, 80%) as a white, amorphous powder, m.p. 200 °C (decomp.). UV: λ_{max} (log ε, CH_3CN) = 218 nm (4.45), 231 sh (4.16), 265 (4.03), 305 (3.70). CD (CH_3CN, c = 6.0·10^{-4}): λ ($\Delta\varepsilon$) = 197 (3.09), 218 (–6.9), 236 (6.0), 247 (1.8), 267 (7.71), 307 (–0.75). IR (KBr): $\tilde{\nu}_{max}$ = 3442, 2980, 1738, 1650, 1470, 1454, 1380, 1260, 1180, 1154, 1114, 1012. ^{1}H NMR (200 MHz, CD_3OH): δ = 1.19 (d, $J_{10,4}$ = 7.1 Hz, 3 H, 10-H), 1.49 (d, $J_{9,3}$ = 6.5 Hz, 3 H, 9-H), 3.26 (dq, $J_{4,10}$ = 7.1 Hz, $J_{4,3}$ = 2.5 Hz, 1 H, 4-H), 4.61 (d, J_{gem} = 11.8 Hz, 1 H, 11-H^a), 4.70 (m, 1 H, 3-H), 4.69 (d, J_{gem} = 11.8 Hz, 1 H, 11-H^b), 6.32 (s, 1 H, 7-H). ^{13}C NMR (50 MHz, CD_3OH): δ = 12.7 (CH_3, C-10), 16.9 (CH_3, C-9), 33.6 (CH, C-4), 54.3 (CH_2, C-11), 77.9 (CH, C-3), 99.3 (C_q, C-8a), 101.0 (CH, C-7), 116.2 (C_q, C-5), 148.8 (C_q, C-4a), 164.2 (C_q, C-8)*, 164.3 (C_q, C-6)*, 171.1 (C_q, C-1). For signals marked with an asterisk * the assignments are interchangeable. ESI-TOF MS: Calcd. mass for $C_{12}H_{14}O_5$ [M + Na]$^+$ 261.0733, found 261.0739, [2M + Na]$^+$ 499.1575, found 499.1543.

3,17-Dimethylnonadecan-2,18-dione (Ascodiketone, 3): Colorless oil. $[\alpha]_D^{20}$ = +19.8 (c = 0.44, CH_2Cl_2). UV: λ_{max} (log ε, CH_3CN) = 216 nm (2.98), 256 (2.58), 314 (2.03). CD (CH_3CN, c = 7.2·10^{-4}): λ ($\Delta\varepsilon$) = 213 (–0.14), 236 sh (0.11), 286 (0.56), 305 sh (0.19), 321 (–0.02). IR (film): $\tilde{\nu}_{max}$ = 3460, 2925, 2854, 1712, 1460, 1356 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.05 (d, $J_{20,3}$ = 6.9 Hz, 6 H, 20-H, 21-H), 1.23 (s, 22 H, 5-H, 6-H, 7-H, 8-H, 9-H, 10-H, 11-H, 12-H, 13-H, 14-H, 15-H), 1.32 (m, 2 H, 4-H^a, 16-H^a), 1.62 (m, 2 H, 4-H^b,16-H^b), 2.11 (s, 6 H, 1-H, 19-H), 2.47 (tq, $J_{3,20}$ = $J_{3,4}$ = 6.9, 2 H, 3-H, 17-H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 16.1 (CH_3, 2 C, C-20, C-21′), 27.2 (CH_2, 2 C, C-5, C-15), 27.9 (CH_3, 2 C, C-1, C-19), 29.4 (CH_2, 2 C, C-7, C-13)*, 29.5 (CH_2, 3 C, C-9, C-10, C-11)*, 29.5 (CH_2, 2 C, C-8, C-12)*, 29.6 (CH_2, 2 C, C-6, C-14)*, 32.9 (CH_2, 2 C, C-4, C-16), 47.2 (CH, 2 C, C-3, C-17), 212.7 (C_q, 2 C, C-2, C-18). For signals marked with an asterisk * the assignments are interchangeable. EIMS (70 eV, 175 °C): *m*/*z* (%) = 324 [M$^+$] (7), 253 (21), 85 (14), 72 (100), 43 (56). HRMS (EI): calcd. for $C_{21}H_{40}O_2$ 324.30283; found 324.30268.

***ent*-α-Cyperone (4):** Colorless oil. $[\alpha]_D^{20}$ = –36.6 (c = 0.9, CH_2Cl_2), ref.[7a] –92.2 (c = 2.04). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.25 (s, 3 H, 9-H), 1.46 (m, 1 H, 5-H^a), 1.65 (m, 1 H, 6-H^a), 1.73 (m, 2 H, 6-H^b, 5-H^b), 1.78 (m, 2 H, 4-H), 1.80 (s, 3 H, 10-H), 1.81 (d, $J_{13,12}$

= 1.1 Hz, 3 H, 13-H), 2.07 (m, 2 H, 8-H^a, 7-H), 2.42 (ddd, J_{gem} = 16.9 Hz, $J_{3a,4a}$ = 4.5 Hz, $J_{3a,4b}$ = 3.5 Hz, 1 H, 3-H^a), 2.54 (ddd, J_{gem} = 16.9 Hz, $J_{3b,4a}$ = 13.8 Hz, $J_{3b,4b}$ = 5.8 Hz, 1 H, 3-H^b), 2.76 (m, 1 H, 8-H^b), 4.80 (d, $J_{12,13}$ = 1.1 Hz, 2 H, 12-H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 10.9 (CH_3, C-10), 20.7 (CH_3, C-13), 22.5 (CH_3, C-9), 26.9 (CH_2, C-6), 32.9 (CH_2, C-8), 33.8 (CH_2, C-3), 35.8 (C_q, C-4a), 37.5 (CH_2, C-4), 41.9 (CH_2, C-5), 45.9 (CH, C-7), 109.2 (CH_2, C-12), 128.8 (C_q, C-1), 149.2 (C_q, C-11), 162.1 (C_q, C-8a), 199.1 (C_q, C-2).

(3*R*,4*R*)-4-Hydroxymellein (2): Orange crystals, m.p. 108–110 °C (ref.[4] 112–117 °C). $[\alpha]_D^{20}$ = –39.2 (*c* = 0.25, MeOH), ref.[28] = –31 (MeOH). ^{1}H NMR ($CDCl_3$, 200 MHz): δ = 1.58 (d, $J_{9,3}$ = 6.6 Hz, 3 H, 9-H), 4.57 (d, $J_{4,3}$ = 2.1 Hz, 1 H, 4-H), 4.70 (dq, $J_{3,9}$ = 6.6 Hz, $J_{3,4}$ = 2.1 Hz, 1 H, 3-H), 6.93 (d, $J_{7,6}$ = 7.4 Hz, 1 H, 7-H)*, 6.99 (dd, $J_{5,6}$ = 8.4 Hz, $J_{5,7}$ = 0.9 Hz, 1 H, 5-H)*, 7.52 (dd, $J_{6,5}$ = 8.4 Hz, $J_{6,7}$ = 7.4 Hz, 1 H, 6-H), 10.93 (br. s, 1 H, 8-OH). ^{13}C NMR ($CDCl_3$, 50 MHz): δ = 16.4 (CH_3, C-9), 67.5 (CH, C-3), 78.8 (CH, C-4), 107.2 (C_q, C-8a), 118.7 (C_q, C-7)*, 118.9 (C_q, C-5)*, 137.2 (CH, C-6), 141.0 (C_q, C-4a), 162.3 (C_q, C-8), 169.7 (C_q, C-1). For signals marked with an asterisk * the assignments are interchangeable.

(3*S*,4*R*,6*S*)-3,4-Dihydroxy-6-undecyl-α-pyranone (5): Amorphous powder, m.p. 101–102 °C. ref.[8] 105–107 °C. $[\alpha]_D^{20}$ = –37 (*c* = 0.8, CH_2Cl_2). ref.[8] –42 (*c* = 0.13, MeOH). UV: λ_{max}, nm (logε, CH_3CN) = 224 (1.99). CD (CH_3CN, *c* = 9.0·10^{-4}): λ ($\Delta\varepsilon$) = 224 (1.73), negative below 199 nm. ref.[8] positive maximum at 222 nm. IR (KBr): $\tilde{\nu}_{max}$ = 3440, 2954, 2920, 2850, 1755, 1722, 1469, 1385, 1227, 1122, 1092, 1070 (cm^{-1}). ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.90 (t, $J_{17,16}$ = 6.9 Hz, 3 H, 17-H), 1.28 (s, 16 H, 8× CH_2), 1.39 (m, 1 H, 8-H^a), 1.48 (m, 1 H, 8-H^b), 1.64 (m, 1 H, 7-H^a), 1.77 (m, 1 H, 7-H^b), 1.83 (dt, J_{gem} = 13.6 Hz, $J_{5a,4}$ = $J_{5a,6}$ = 11.7 Hz, 1 H, 5-H^a), 2.28 (dt, J_{gem} = 13.6 Hz, $J_{5b,4}$ = $J_{5b,6}$ = 3.6 Hz, 1 H, 5-H^b), 3.27 (br. s, 1 H, OH), 3.88 (br. s, 1 H, OH), 4.01 (d, $J_{3,4}$ = 9.7 Hz, 1 H, 3-H), 4.06 (m, 1 H, 4-H), 4.34 (m, 1 H, 6-H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ = 14.1 (CH_3, C-17), 22.7 (CH_2, C-16), 24.8 (CH_2, C-8), 29.3 (CH_2), 29.3 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 29.7 (CH_2), 31.9 (CH_2, C-15), 35.7 (CH_2, C-7)*, 35.9 (CH_2, C-5)*, 69.1 (C_q, C-4), 74.4 (C_q, C-3), 78.6 (C_q, C-6), 173.2 (C_q, C-2). For signals marked with an asterisk * the assignments are interchangeable. EIMS (70 eV, 200 °C): *m*/*z* (%) = 286 (3), 223 (7), 211 (9), 193 (5), 180 (4), 166 (4), 137 (5), 123 (7), 111 (9), 95 (17), 69 (17), 60 (100), 43 (23). HRMS (EI): calcd. for $C_{16}H_{30}O_4$ 286.21442; found 286.21405.

Computations: DFT and TDDFT calculations were run with the Gaussian'03 program (Gaussian, Inc., Pittsburgh PA, 2003). Geometry optimization was executed with the DFT method at B3LYP/6-31G(d) level starting from MM structures obtained through conformational searches. For the solid-state geometry, the positions of the hydrogen atoms were re-optimized with the DFT method. TDDFT calculations were executed with the hybrid functional B3LYP and Ahlrich's TZVP basis set. (See Gaussian 03 documentation at http://www.gaussian.com/g_ur/g03mantop.htm for details on basis sets and DFT functionals.) CD spectra were generated by using dipole length-computed rotational strengths to which a Gaussian band-shape was applied with 4,000 cm^{-1} half-height width (corresponding to 24.2 nm at 245 nm). Rotational strenghts calculated with the dipole-velocity formulation differed from dipole-length ones by less than 10% for most computed transitions.

Acknowledgments

K. K., I. K., B. S. and S. D. thank BASF AG and the BMBF (Bundesministerium für Bildung und Forschung, project no. 03F0360A), and S. A. and T. K. thank the Hungarian Scientific Research Fund (OTKA, T-049436, F-043536, NI-61336) for financial support.

[1] H. Hussain, K. Krohn, U. Flörke, B. Schulz, S. Draeger, G. Pescitelli, S. Antus, T. Kurtán, *Eur. J. Org. Chem.* **2007**, 292–295.
[2] M. M. Chapela, I. H. Dreyfuss, *Potential of fungi in the discovery of novel low-molecular weight pharmaceuticals* in *The discovery of natural products with therapeutic potential* (Ed.: V. P. Gullo), vol. 3, Butterworth-Heinemann, Boston, **1994**, pp. 49–80.
[3] M. Corrado, K. F. Rodrigues, *J. Basic Microbiol.* **2004**, *44*, 157–160.
[4] R. J. Cole, J. H. Moore, N. D. Davis, J. W. Kirksey, U. L. Diener, *J. Agric. Food Chem.* **1971**, *19*, 909–911.
[5] U. Höller, G. M. König, A. D. Wright, *J. Nat. Prod.* **1999**, *62*, 114–118.
[6] K. Krohn, R. Bahramsari, U. Flörke, K. Ludewig, C. Kliche-Spory, A. Michel, H.-J. Aust, S. Draeger, B. Schulz, S. Antus, *Phytochemistry* **1997**, *45*, 313–320.
[7] a) Optical rotation: V. N. Zhabinskii, A. J. Minnaard, J. B. P. A. Wijnberg, A. de Groot, *J. Org. Chem.* **1996**, *61*, 4022–4027; b) *ent*-α-cyperone: Y. Asakawa, M. Tori, T. Masuya, J. P. Frahm, *Phytochemistry* **1990**, *29*, 1577–1584.
[8] H.-J. Li, Y.-C. Lin, J.-H. Yao, L. L. P. Vrijmoed, E. B. Gareth, *J. Asian Nat. Prod. Res.* **2004**, *6*, 185–191.
[9] J. Dai, K. Krohn, U. Flörke, H.-J. Aust, S. Draeger, B. Schulz, A.-K. Szikszai, S. Antus, T. Kurtán, T. van Ree, *Eur. J. Org. Chem.* **2006**, 3498–3506.
[10] G. Bringmann, S. Busemann, K. Krohn, K. Beckmann, *Tetrahedron* **1997**, *53*, 1655–1664.
[11] X. Wu, X. Liu, Y. Lin, J. Luo, Z. She, L. Houjin, W. W. L. Chan, S. Antus, T. Kurtan, B. Elsässer, K. Krohn, *Eur. J. Org. Chem.* **2005**, 4061–4064.
[12] G. Bringmann, S. Busemann, *The Quantummechanical Calculation of CD Spectra: the Absolute Configuration of Chiral Compounds from Natural or Synthetic Origin*, in: *Natural Product Analysis, Chromatography, Spectroscopy, Biological Testing* (Eds.: P. Schreier, M. Herderich, H.-U. Humpf, W. Schwab), Vieweg, Braunschweig, Wiesbaden, **1998**, pp. 195–211.
[13] C. Diedrich, S. Grimme, *J. Phys. Chem. A* **2003**, *107*, 2524–2539.
[14] C. J. Cramer, *Essentials of Computational Chemistry: Theories and Models*, Wiley, Chichester, **2002**.
[15] S. Antus, G. Snatzke, I. Steinke, *Liebigs Ann. Chem.* **1983**, 2247–2261.
[16] P. Salvadori, S. Superchi, F. Minutolo, *J. Org. Chem.* **1996**, *61*, 4190–4191.
[17] F. Zymalkowski, *Katalytische Hydrierungen*, Enke, Stuttgart, **1965**.
[18] G. Kerti, T. Kurtán, T.-Z. Illyés, K. E. Kövér, S. Sólyom, G. Pescitelli, N. Fujioka, N. Berova, S. Antus, *Eur. J. Org. Chem.* **2007**, 296–305.
[19] E. P. Siqueira Filho, J. A. R. Rodrigues, P. J. S. J. S. Moran, *Tetrahedron: Asymmetry* **2001**, *12*, 847–852.
[20] H. Ageta, Y. Arai, *Chem. Lett.* **1982**, 881–884.
[21] J. W. Huffman, W. E. Swain, J. Jacobus, A. T. McPhail, *J. Org. Chem.* **1980**, *45*, 3088–3096.
[22] H. Toshima, A. Watanabe, H. Sato, A. Ichihara, *Tetrahedron Lett.* **1998**, *39*, 9223–9226.
[23] U. Höller, A. D. Wright, G. F. Matthée, G. M. König, S. Draeger, H.-J. Aust, B. Schulz, *Mycological Res.* **2000**, *104*, 1354–1365.
[24] 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.
[25] CCDC-617319 contains 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.
[26] Bruker (**2002**). SMART (Ver. 5.62), SAINT (Ver. 6.02), SHELXTL (Ver. 6.10) and SADABS (Version 2.03). Bruker AXS Inc., Madison, Wisconsin, USA.
[27] H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, *39*, 876–881.
[28] G. Assante, R. Locci, L. Camarda, L. Merlini, G. Nasini, *Phytochemistry* **1977**, *16*, 243–247.

Received: October 18, 2006
Published Online: January 8, 2007

