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Metabolic Products of the Endophytic Fungus *Microsphaeropsis* sp. from *Larix decidua*[‡]

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Dedicated to Professor Hans Paulsen on the occasion of his 85th birthday

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Five new metabolites, palmarumycin M_1 (**1a**) and M_2 (**3**), papyracillic acid C (**6**) and the microsphaeropsins A (**7**) and B (**8**) were isolated from the fungus *Microsphaeropsis* sp. together with the known decaspirone (**2**) and the papyracillic acids A and B (**4** and **5**). Their structures were determined by means of spectroscopic data including HREIMS, ^{1}H NMR, ^{13}C NMR, 2D NMR (HMQC, HMBC, NOESY) and X-ray single crystal analysis. The absolute configuration of palmarumycin M_1 (**1a**) was determined by single-crystal X-ray analysis of the bis(4-bromobenzoate) **1c** and the relationship between **1a**, **2** and **3** established by chemical transformation of **1a** into **2** and of **3** into **2**. The relative configuration of papyracillic acids A–C (**4**–**6**) was revised and their absolute configuration determined by comparison of TDDFT-calculated and experimental solid-state CD spectra.

Introduction

In connection with our ongoing search for biologically active metabolites from fungi,[1] we investigated the constituents of an endophytic fungus, *Microsphaeropsis* sp., internal strain number 7291, isolated from a branch of the tree *Larix decidua*, growing in Hjerting, Denmark. The culture extract of the fungus was found to have good antifungal and moderate antibacterial activities. Extensive column and preparative thin-layer chromatography of the ethyl acetate culture extract afforded five new metabolites belonging to three different structural groups: the palmarumycins M_1 (**1a**) and M_2 (**3**), the papyracillic acid C (**6**), and the microsphaeropsins A (**7**) and B (**8**). The known decaspirone (**2**) and the papyracillic acids A (**4**) and B (**5**) (Figure 1) were also identified. The publication of related spirodioxynaphthalenes[2,3] and the termination of the biological activity screening of our compounds at BASF AG prompted us to disclose the isolation, structural elucidation and herbicidal, antifungal and antibacterial activities of these new compounds.

Results and Discussion

Compound **1a** was obtained as colorless optically active crystals ($[\alpha]_D^{25}$ = +249.4) with the molecular formula $C_{20}H_{20}O_5$ as deduced from HREIMS spectral and ^{13}C NMR spectroscopic data. Its IR spectrum showed strong absorptions for hydroxy groups at 3455 cm^{-1} and the ^{1}H NMR spectrum (Table 1) exhibited the presence of six aromatic and two olefinic methines and three protons bonded to oxygenated C atoms (δ = 3.90, 4.45 and 4.64 ppm, respectively). The ^{13}C NMR spectrum (Table 2) showed signals for 20 carbon atoms, and the DEPT spectrum indicated the presence of two methylenes, thirteen methines and five quaternary carbon atoms.

Analysis of the 2D ^{1}H-^{1}H COSY and HMQC spectra of compound **1a** suggested the presence of the fragments

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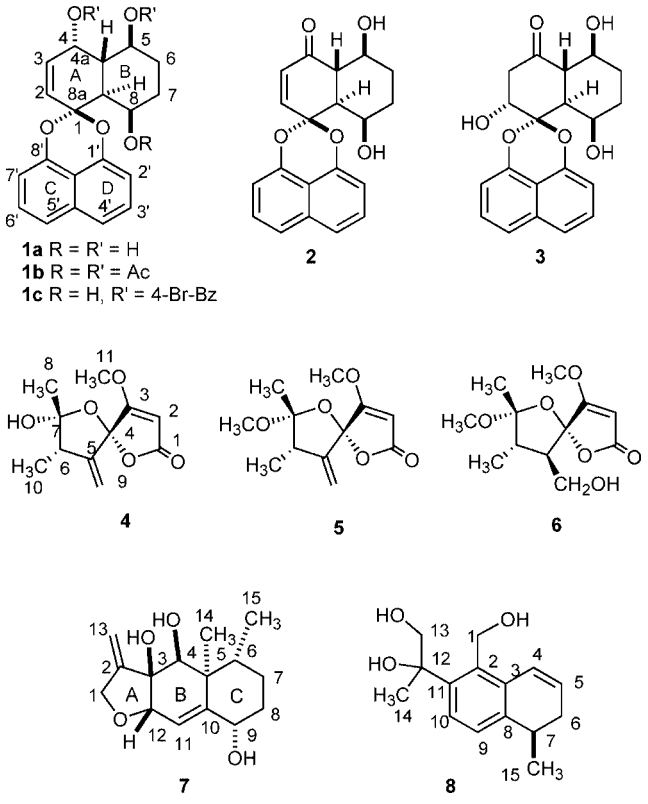

Figure 1. Secondary metabolites isolated from the culture broth of *Microsphaeropsis* sp.

CH(2)–CH(3)–CH(4)–CH(4a)–CH(5)–CH_2(6)-CH_2(7)–CH(8)–CH(8a)-,–CH(2′)–CH(3′)–CH(4′)– and –CH(5′)–CH(6′)–CH(7′)-. The latter aromatic part resembled that of the naphthalene moiety found in the 1,8-dioxynaphthalene part of the palmarumycins isolated from *Coniothyrium palmarum*[4–6] (review[7]). In the HMBC spectrum, ^{13}C-^{1}H long-range correlation signals were found between C-1 and H-2, H-3, H-4a, H-8 and H-8a; between C-1′ and H-2′ and 3′; between C-8′ and H-6′ and H-7′; as well as between C-4a′ and H-4′, H-5′, H-6′ and H-3′. These spectroscopic data did fit for the hydroaromatic upper part of a palmarumycin with gross structure **1a** (Figure 1), named palmarumycin M_1 according to the name of the fungal genus, *Microsphaeropsis*, possessing three hydroxy groups at C-4, C-5, and C-8 and a disubstituted double bond at C-2 and C-3.[5] The relative configuration could be deduced from the coupling constants of relevant protons (see Table 1). Most importantly, the large coupling of J = 13.4 Hz between 4a-H_{ax} and 8a-H_{ax} established the existence of an *anti*-periplanar relationship of these protons and thus the *trans* connection of rings A and B in this octalin system. The equatorial position of 5-OH was deduced from the large coupling of J = 9.9 Hz between 4a-H_{ax} and 5-H_{ax}, while 4-OH

Table 2. ^{13}C NMR spectral data (δ values, ppm) of compounds **1a**–**3** (125 MHz, $CDCl_3$; **1a** in $CD_3OD + CH_3OD$).

C atom	**1a**	**1b**	**2**	**3**
1	100.6	97.8	98.7	107.1
2	124.4	127.9	141.9	74.8
3	133.2	134.2	130.8	46.3
4	61.2	63.2	203.5	214.8
4a	39.6	36.5	46.3	49.2
5	68.9	70.6	70.6	71.5
6	27.2	24.8	26.4	26.8
7	29.7	28.5	29.7	27.3
8	64.3	64.3	63.7	74.3
8a	42.8	43.3	48.8	43.8
1′	147.2	147.2	146.3	147.2
2′	109.9	109.6	109.9	109.3
3′	127.6	127.7	127.7	127.7
4′	121.3	120.6	121.5	121.2
4a′	134.1	134.2	134.2	134.5
5′	120.6	120.5	121.9	121.7
6′	127.2	127.5	127.4	127.3
7′	109.5	108.5	110.5	109.9
8′	146.3	147.0	145.9	146.9
8a′	113.6	113.3	113.5	114.1

Table 1. ^{1}H NMR spectral data of compounds **1a**–**3** (500 MHz, $CDCl_3$; **1a** in $CD_3OD + CH_3OD$; δ values are given in ppm relative to TMS, J values (Hz) and signal multiplicities are given in parentheses).

H atom	**1a**	**1b**	**1c**	**2**	**3**
2	5.80 (d, 10.2)	6.01 (d, 10.4)	6.05 (d, 10.3)	6.80 (d, 10.3)	4.41 (br.)
3,3α	6.00 (dd, 10.2, 4.8)	5.89 (dd, 10.4, 4.8)	6.13 (dd, 10.3, 4.2)	6.02 (d, 10.3)	2.96 (m, overlap)
3,3β					2.74 (dd, 9.2, 3.0)
4	4.45 (br.)	5.54 (t, 4.8, 4.8)	5.79 (t, 4.3, 4.3)		
4a	2.39 (ddd, 13.4, 9.9, 4.0)	2.94 (ddd, 13.5, 9.4, 4.8)	3.25 (ddd, 13.6, 10.4, 4.3)	3.30 (dd, 13.1, 9.2)	2.96 (m, overlap)
5	3.90 (m)	5.12 (m)	5.40 (m)	4.00 (m)	3.53 (m)
6α	1.82 (m)	1.95 (m)	2.00 (m)	1.91 (m)	1.56 (m)
6β	2.09 (m)	2.09 (m)	2.10 (m)	2.13 (m)	1.80 (m)
7α	1.50 (m)	1.70 (m)	1.75 (m)	1.55 (m)	1.70 (m)
7β	1.98 (m)	2.05 (m)	2.10 (m)	2.10 (m)	2.19 (m)
8	4.64 (br.)	5.87 (br.)	4.85 (br.)	4.75 (m)	4.63 (dd, 5.9, 3.0)
8a	2.26 (d, 13.4)	2.47 (dd, 13.5, 2.2)	2.58 (dd, 13.4, 1.5)	2.49 (dd, 13.1, 1.6)	2.60 (m)
2′	6.90 (d, 7.7)	6.93 (d, 7.5)	7.04 (d, 7.5)	6.99 (d, 8.1)	6.93 (d, 7.5)
3′	7.37 (t, 7.7)	7.37 (t, 7.5)	7.43 (t, 7.5)	7.47 (t, 8.1)	7.45 (t, 7.5)
4′	7.49 (d, 7.7)	7.49 (d, 7.5)	7.53 (d, 7.5)	7.60 (d, 8.1)	7.56 (d, 7.5)
5′	7.47 (d, 7.7)	7.47 (d, 7.5)	7.51 (d, 7.5)	7.60 (d, 8.1)	7.57 (d, 7.5)
6′	7.43 (t, 7.7)	7.43 (t, 7.5)	7.45 (t, 7.5)	7.51 (t, 8.1)	7.48 (t, 7.5)
7′	6.86 (d, 7.7)	6.80 (d, 7.5)	6.98 (d, 7.5)	7.05 (d, 8.1)	7.05 (d, 7.5)
H-phenyl			7.60–7.92 (m)		

adopts an axial position, *anti*-periplanar to 4a-H_{ax}, in accordance with the small gauche coupling of 4-H_{eq} and 4a-H_{ax} (J = 4.0 Hz). The small coupling constant (ca. 2 Hz) between H-8_{eq} and H-$8a_{ax}$ indicates an equatorial orientation of 8-H and thus an axial arrangement of the C-8 hydroxy group.

Structure elucidation was further supported by acetylation of **1a** to afford the triacetate **1b**, confirming the number of hydroxy groups of **1a** and also their location by shifting the respective signals of H-4, H-5 and H-8 by ca. 1.1–1.3 ppm to low field (δ = 4.45 to δ = 5.54, δ = 3.90 to δ = 5.12, and δ = 4.64 to δ = 5.87 ppm). In addition, a further set of coupling constants was provided by **1b** for configurational analysis. In the ^{1}H NMR spectrum of the triacetate **1b**, the coupling of H-8 could be resolved and the small coupling constant of H-8 and H-8a (J = 2.2 Hz; not clearly visible in the spectrum of **1a**) again supported the equatorial position of H-8 and the axial one for 8-OH. This structure and the relative configuration of **1a** were further unambiguously confirmed by X-ray diffraction analysis of a single crystal obtained from its methanol solution (Figure 2).

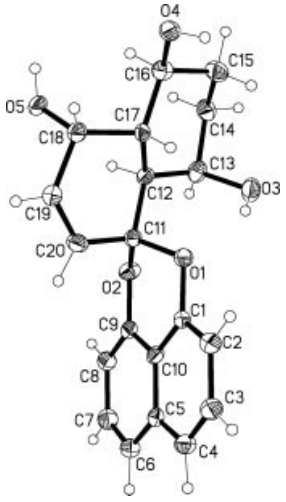

Figure 2. The molecular structure of compound palmarumycin M_1 (**1a**) in the crystalline state.[8]

Whereas the X-ray analysis of triol **1a** did not allow for the determination of the absolute configuration, we were able to obtain suitable crystals of the 4,5-bis(4-bromobenzoate) **1c**. Interestingly, the hydroxy group at C-8 was less reactive in the benzoylation reaction, most probably due to weak chelation to the two neighboring ketal oxygens as can be seen in Figure 2. X-ray analysis of the single crystal of **1c** allowed the determination of the absolute configuration to be 4*S*,4a*S*,5*S*,8*R*,8a*S*, as shown in Figures 1 and 3.

The absolute configuration of the three related diepoxines was also independently determined by employing the exciton chirality CD method.[9] The CD spectrum of **1c** is one order of magnitude more intense than that of parent **1a** (Figure 4), due to the exciton coupling between the strong dipole-allowed transitions of the 4-bromobenzoate chromophores. In particular, in the region corresponding to the 4-

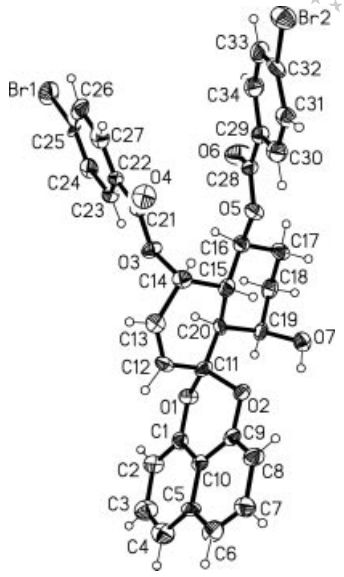

Figure 3. The molecule of 4,5-bis(4-bromobenzoate) **1c** of palmarumycin M_1 (**1a**) in the crystal.[8]

bromobenzoate ^1L_a (or charge-transfer) transition,[9] a moderate positive CD couplet appears [251 nm (+48.17), 240 (0.0), 227 (–19.03)] which is related to a positive chirality (*P* helicity) defined by the two electric transition moments (Figure 4, top), polarized along the long axes of the two 4-bromobenzoates and then parallel to the C–O ester bonds. The so-called non-degenerate couplings between each benzoate and the very strong 1,8-dihydroxynaphthalene chromophore are much weaker than the benzoate–benzoate coupling, because of a larger distance and a less favorable arrangement.[9] Therefore, they affect the CD spectrum of **1c** to a small extent (vide infra). Since the relative configurations of the C-4 and C-5 ester groups were known from either the ^{1}H NMR coupling constants or the X-ray data of **1a** and **1c**, the measured positive exciton couplet unambiguously defines the absolute configuration of **1a**–**c** as 4*S*,5*S*. The X-ray data of **1c** clearly proved that, in the solid state, the ^1L_a electric transition moments of the two 4-bromobenzoates define a positive chirality, namely, the projection angle O-4, C-4, C-5, O-5 was +98.5° (Figure 4, top). In addition to the solution CD of **1c**, its solid-state CD spectrum was also recorded by using a KCl disc, which allowed direct comparison with the X-ray structure. The solid-state CD spectrum was very similar to the one in solution (Figure 4), which indicated that the same conformer dominant in solution is found in the single crystal and, incidentally, that there are no significant intermolecular exciton interactions in the crystal lattice.[1,10] Thus, the X-ray structure of **1c** was employed as input for exciton-CD calculations with DeVoe's method.[11] They proved that the so-called degenerate coupling between the two benzoates is fully responsible for the observed CD of **1c**, while non-degenerate benzoate–naphthalene couplings give much weaker effects (see Supporting Information).

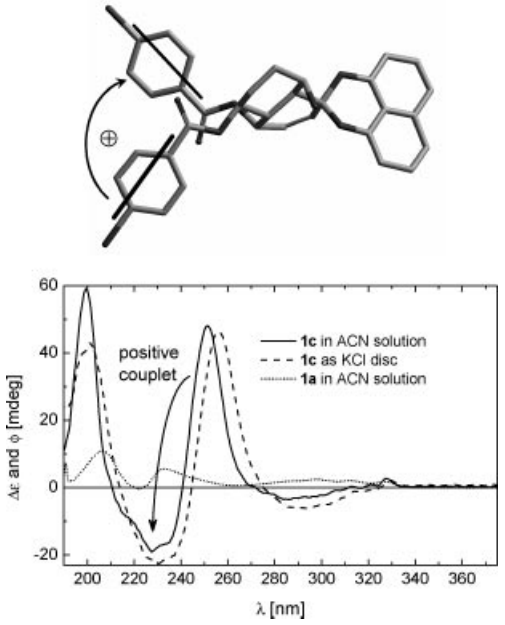

Figure 4. Bottom: CD spectra of **1c** in solution (acetonitrile, solid line) and in the solid state (KCl, dashed line), compared to the CD spectrum of **1a** in solution (acetonitrile, dotted line). The first two exhibit positive exciton CD couplets due to a positive chirality defined by the long axes of the two 4-bromobenzoate chromophores (top).

In two recent papers Jiao et al.[2] and Hu et al.[3] reported on the isolation of related spirodioxynaphthalenes from the aquatic fungus *Descaisnelle thyridoides* and the saprophytic fungus *Helicoma virids*, respectively. Palmarumycin M_1 (**1a**) is the 6,7-dihydro derivative of decaspirone A.[2] The data for this dihydro compound **1a** are in agreement with the relative configuration, notably the *trans* connection of the rings A and B, and also the absolute configuration, assigned by the Mosher method on decaspirone A[2] or by X-ray analysis of 5-(4′-bromobenzoyl)-decaspirone F.[3]

Compound **2** was isolated as colorless optically active gum ($[\alpha]_D^{25}$ = +173, *c* = 0.06, CH_3OH). The similarity of the spectroscopic data of compounds **1a** and **2** confirmed their membership in the palmarumycin family of natural products.[7] The ^1H, ^{13}C and DEPT NMR spectra of compound **2** were particularly close to those of **1a** (see Tables 1 and 2). In the ^{13}C NMR spectrum of **2**, the signal for C-4 was shifted downfield (δ = 203.5 ppm) compared to that of **1a** (δ = 61.2 ppm). These data suggest structure **2** for the ketone in which the hydroxy group at C-4 is oxidized to a carbonyl group. The oxidation of this allylic hydroxy group at C-4 was easily performed chemically by treatment of palmarumycin M_1 (**1a**) with active manganese dioxide to afford ketone **2**, identical in all respects including the sign of optical rotation with the isolated natural product **2**. This chemical conversion also unambiguously established the absolute configuration of ketone **2** to be (4a*S*,5*S*,8*R*,8a*S*). The data, except a deviation in the optical rotation perhaps due to a different concentration (ref.[2] $[\alpha]_D^{25}$ = +89, *c* = 0.58, CH_3OH), were in good agreement with those published for decaspirone C.[2]

Compound **3** was also a colorless optically active gum with a strong positive rotation of $[\alpha]_D^{25}$ = +355.7 (*c* = 0.007, CH_2Cl_2). Comparison of the NMR spectra of **3** (Tables 1 and 2) with those of **2** revealed that **3** had a similar structure. This was confirmed by the fragment for the bottom part aromatic protons–CH(2′)–CH(3′)–CH(4′)– and –CH(5′)–CH(6′)–CH(7′)– as deduced from the COSY and HMQC spectra. However, the signals for the olefinic carbons at C-2 and C-3 (δ = 124.4 and 133.2) and the respective protons (δ = 5.80 and 6.00) were missing. Instead, signals for methylene protons appearing at δ = 2.96 (3α-H) and δ = 2.74 (3β-H) and a lower field signal at δ = 4.41 (2-H) indicated the presence of a hydroxy group on C-2. This assumption was confirmed by analysis of the COSY and HMQC spectra showing the fragment –CH(2)–CH_2(3)–CH(4a)–CH(5)–CH_2(6)–CH_2(7)–CH(8)–CH(8a)– for the hydroaromatic part of this spiroacetal. The ^{13}C-^1H long-range correlations found in the HMBC spectrum between C-4 and H-3, H-4a, H-2, and H-8a, and as well as between C-1 and H-3, H-4a, H-2, and H-8a, were also in agreement with this analysis. The equatorial position of 2-H (and consequently the axial position of 2-OH) could be deduced from the couplings of *J* = 9.2 and 3.0 Hz observed in the signal for 3β-H (axial). This stereochemical assignment was supported by the NOESY spectrum, exhibiting clear correlations between H-2 and H-3β, and between H-3β and H-4a, showing that these protons are on the same side of the molecule. Finally, a chemical correlation to decaspirone C (**2**) could be established by base treatment of **3**, affording the α,β unsaturated olefin **2** by β-elimination of the C-2 hydroxy group (identified by TLC). Therefore, the compound was assigned structure **3**, as shown in Figure 1, and named palmarumycin M_2.

The new palmarumycins are noteworthy for their near (**1a** and **2**) or complete hydrogenation of the two top rings of the spiroketals (see also ref.[7]) and their *trans* junction of the hydrogenated rings A and B. They further increase the number of related spirodioxynaphthalenes[2,3] and are examples of the remarkable capability of the fungi to create chemical diversity by plain functional group transformations, starting the biosynthesis from simple 1,8-dihydroxynaphthalene.[12,13]

The ^1H NMR spectrum of compound **4**, isolated as a crystalline material, m.p. 115–117 °C, showed a mixture of four isomers in the ratio of ca. 1:1:2:4, according to the integrals of the ^1H NMR spectrum in chloroform. Analysis of the signals for the major isomer (Table 3) showed characteristic signals for three methyl groups, of which two were singlets (δ = 1.59, 3.88) and one was a doublet (δ = 1.20, d, *J* = 6.9 Hz), for an olefinic proton (δ = 5.18, s) and for an exomethylene group (δ = 5.27, m). The ^{13}C and DEPT NMR spectra (Table 4) of compound **4** showed signals for 11 carbon atoms, including three for methyl groups (δ = 10.9, 24.5, 59.8), two for oxygenated quaternary C atoms (δ = 107.3, 107.4), two for double-bond C atoms (δ = 88.5, 178.0, C-2/C-3; δ = 111.4, 148.3, C-5/C-9) and for a lactone group (δ = 170.1, C-1). These data, in conjunction with those of the ^1H NMR spectrum, were in agreement with

the molecular formula $C_{11}H_{14}O_5$, determined by MS. Accordingly, the major isomer of compound **4** could be identified as a spiro compound containing two double bonds and a lactone group. The UV band at 224 nm ($\varepsilon \approx 10^4$) is in agreement with a α,β-unsaturated lactone. In the HMBC spectrum of **4**, the proton signal of H-2 was correlated to the carbon signals of the quaternary carbons (C-1, 3 and 4) and the proton signal of H-6 was correlated to the C signals of the quaternary carbon atoms (C-5 and 7), indicating that methyl and methine are located at C-6 and C-5, respectively. The ^1H and ^{13}C NMR spectroscopic data of the major isomer of compound **4** were in agreement with those published for papyracillic acid (see Tables 3 and 4), isolated from the ascomycete *Lachnum papyraceum*.[14] The published spectra also displayed an approximate 1:1:2:4 mixture. In this mixture, ring opening at the acetal carbon C-7 also induced equilibration at C-4. The NMR spectroscopic data did not allow a definite assignment of the four sets of signals to a specific stereoisomer.[14] Fortunately, our crystalline material permitted X-ray diffraction analysis of **4** and the molecular structure in the crystal is shown in Figure 5. Comparison of the NMR spectroscopic data of the major isomer with those of the methyl acetal **5** (see Tables 3 and 4) then allowed assignment of the major isomer in solution to structure **4**, as shown in Figure 1.

Table 3. ^1H NMR spectral data of compounds **4**–**6** and the published data for structure assumed to be **9**[14] (500 MHz, $CDCl_3$; δ values are given in ppm relative to TMS, *J* values (Hz) and signal multiplicities are given in parentheses).

H atom	**4**[a]	**5**	**9**	**6**
2	5.18 (s)	5.07 (s)	5.01 (s)	5.10 (s)
5				2.63 (td, 12.6, 5.1, 5.1)
6	2.70 (m)	2.70 (m)	2.62 (ddq)	2.28 (dq, 12.6, 6.8)
8	1.59 (s)	1.51 (s)	1.43 (s)	1.59 (s)
9	5.27 (m)	5.20 (m)	5.12, 5.10	3.75 (d, 5.1)
10	1.20 (d, 6.9)	1.16 (d, 6.8)	1.08 (d, 6.8)	1.14 (d, 6.8)
11-OCH_3	3.88 (s)	3.87 (s)	3.81 (s)	3.95 (s)
6-OCH_3		3.31 (s)	3.23 (s)	

[a] Data of the major isomer.

Table 4. ^{13}C NMR spectral data of compounds **4**–**6** and the published data for structure assumed to be **9**[14].

C atom	**4**	**5**	**9**	**6**
1	170.1	170.2	170.1	169.3
2	88.5	88.4	88.1	89.7
3	178.0	178.2	178.0	177.4
4	107.3	107.3	106.8	108.9
5	148.3	148.8	148.5	50.2
6	47.4	48.8	48.5	42.3
7	107.4	109.3	109.1	107.5
8	24.5	18.9	18.7	25.9
9	111.4	109.8	109.1	59.2
10	10.9	10.6	10.3	11.7
11	59.8	59.8	59.6	59.8
6-OCH_3		49.5	49.2	

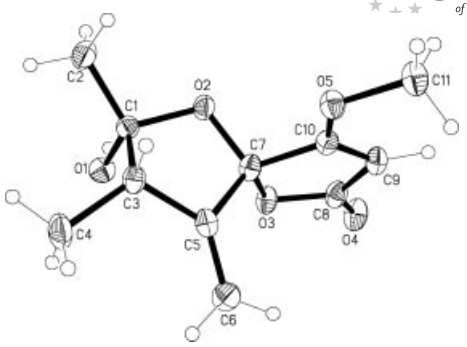

Figure 5. The molecular structure of compound papyracillic acid A (**4**) in the crystal.

The absolute configurations of papyracillic acid A (**4**) and its methyl acetal (**5**) were determined through the comparison of quantum-mechanical calculated and experimental CD spectra.[15–17] In the case of compound **4**, whose X-ray structure was available, we applied our recently developed solid-state TDDFT/CD approach.[1,18–21] In this method, the experimental solid-state CD spectrum is compared with that computed by the TDDFT method,[22,23] using the X-ray coordinates as input structure. Since the calculation and experiment are based on the same conformation of the compound in question, a very good agreement is usually observed. This way, the many steps required for the analogous procedure in solution can be skipped, e.g. conformational analysis, geometry optimizations and excited-state calculation on each conformer, as well as Boltzmann weighting of different spectra.[17] This latter procedure was employed for assigning the absolute configuration of compound **5**, whose X-ray structure was not available. In the case of **4**, the solid-state procedure allowed an unambiguous assignment in spite of different structures equilibrating in solution, which would probably hamper any configurational assignment based on the solution procedure.

The CD spectrum of papyracillic acid A (**4**) was calculated with TDB3LYP/TZVP[24] using X-ray coordinates with (4*S*,6*S*,7*R*) absolute configuration as input structure, upon re-optimization of all bond lengths. The calculated CD spectrum matches well the shape of the experimental solid-state one (Figure 6, a); the absolute configuration may be then safely assigned as above. The couplet-like feature appearing at 195–250 nm in the CD spectrum of **4** is due to four transitions (see no. 2–5 in Figure 6, a) with rotational strengths of alternating signs involving the four highest occupied and the two lowest virtual orbitals (both with negative eigenvalues[25]), mostly confined to the enone and the alkene chromophores. Similar to an analogous case involving a compound with a compact polycyclic structure,[20] we re-optimized all bond lengths found in the solid-state geometry to explore the impact of possible libration effects introducing unnatural bond shortening in the X-ray structure.[26] This correction was in fact beneficial for obtaining a better agreement between experimental and computed spectra, but not decisive for assigning the absolute configuration;

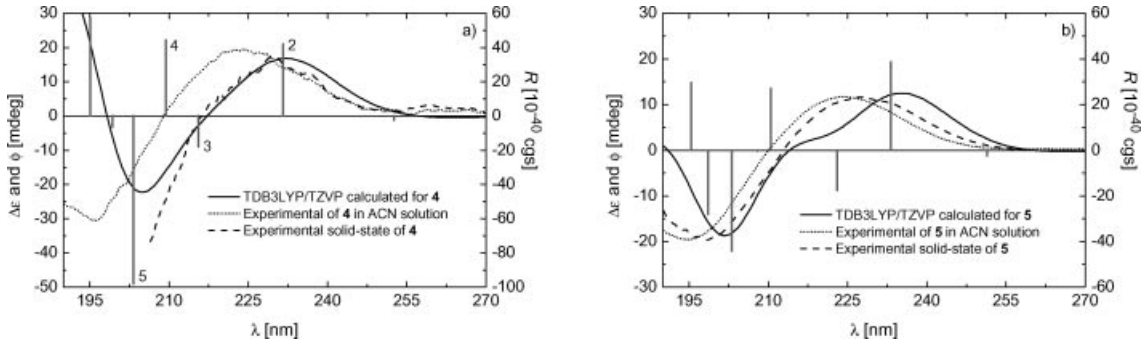

Figure 6. CD spectra of (a) (4*S*,6*S*,7*R*)-papyracillic acid A (**4**) and (b) its methyl acetal (**5**) measured in solution (acetonitrile, dotted line) and in the solid state (KCl disc, dashed line), and TDB3LYP/TZVP calculated CD spectra (solid line) using either X-ray coordinates (a) or DFT-computed structures (b). Vertical bars represent rotational strengths *R*.

the CD spectrum computed on the original X-ray structure still showed the same trend as in Figure 6 (a), only with a weaker short-wavelength negative band. It must be stressed that, in the current case, re-optimized bond lengths did not increase systematically with respect to the X-ray structure,[20] but rather increased/decreased within ±2% in a random way.

Compound **5** was obtained as a colorless optically active ($[\alpha]_D^{25}$ = –42.1) resin with the molecular formula $C_{12}H_{16}O_5$, as deduced from mass spectral and NMR spectroscopic data. Comparison of the NMR spectra of **5** (Tables 3 and 4) with those of the major solution isomer of **4** revealed that **5** had a nearly identical structure. The only difference was the replacement of the hydroxy group at C-7 by a methoxy group. The signal for C-7 in the ^{13}C NMR spectrum for **5** (δ = 109.3 ppm) was shifted slightly but characteristically downfield compared to that of **4** (δ = 107.4 ppm). The near identity of the signals for C-6, C-10 as well as 6-H and 10-H in the NMR spectra also indicated the same configuration at the anomeric center at C-7 and the structure of papyracillic acid methyl acetal **5** was therefore assigned as shown in Figure 1. Since no methanol was used during the extraction process or in the course of the chromatography, the methyl acetal **5**, isolated from the culture broth, is not an artefact.

The absolute configuration of compound **5** was presumably the same as the parent papyracillic acid (**4**), also based on the similar appearance of the CD spectra of the two compounds (Figure 6). This was further confirmed through TDDFT calculations of the CD spectrum of **5**. A molecular-mechanics conformational analysis followed by geometry optimizations with DFT at B3LYP/6-31G(d) level resulted in only two conformations populated at room temperature, differing in the orientation of the C7-attached methoxy group. The relative rigidity of compound **5** is additionally proven by the close resemblance between the CD spectra measured in solution and in the solid state (Figure 6, b). CD spectra were calculated for the low energy structures with the TDB3LYP/TZVP method and weighted with relative Boltzmann factors; the average spectrum computed for (4*S*,6*S*,7*R*) absolute configuration matches well the experimental one, thus confirming the configurational assignment. The origin of the observed CD bands is the same as for compound **4** (see above).

Since the X-ray analysis of papyracillic acid (**4**) showed a different relative configuration than that assumed by Sterner et al.[14] for the corresponding methyl acetal, this question had to be addressed. In their paper, Sterner et al. prepared a methyl acetal from papyracillic acid (**4**) and assigned structure **9** (Figure 7) to the major product produced in the acid catalysed acetalization.[14] Comparison of the ^1H and ^{13}C NMR spectroscopic data with only minor deviations in chemical shifts[27] and the same coupling constant of 6.8 Hz for the designative $J_{6,10}$ coupling (see Tables 3 and 4) showed that our compound **5** was in fact identical with the one prepared semi-synthetically from **4**. The optical rotation of –41°, measured for both samples, showed their identical absolute configuration. Therefore, structure **9**, published for the methyl acetal,[14] has to be revised to structure **5** as shown in Figure 7. The relative configuration of the centers at C-6 and C-7 were assigned correctly by the authors, considering that the absolute configuration of their methyl acetal was unknown and arbitrarily selected in structure **9**.

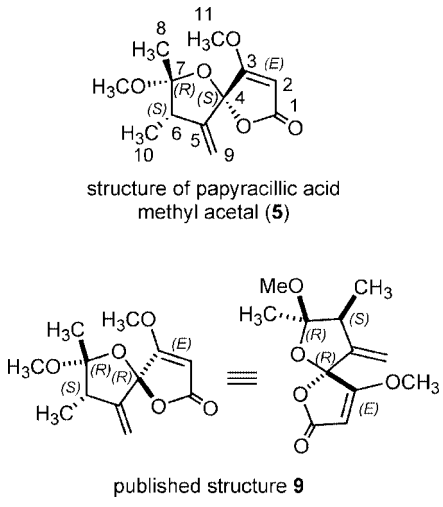

Figure 7. Revised (**5**) and published[14] structure **9** of papyracillic acid A methyl acetal.

Compound **6** was obtained as colorless crystals with the molecular formula $C_{11}H_{16}O_6$, as deduced from HREIMS spectroscopic data. The ^1H, ^{13}C and DEPT NMR spectra of compound **6** (Tables 3 and 4) were related to those of **5**. However, the exomethylene group at C-5 was replaced by oxygenated methylene group in **6**. The NOE spectrum exhibited correlation between 10-CH_3 and H-5, and the coupling pattern of H-5 and H-6 (J = 12.6 Hz) indicated that these two protons are in antiperiplanar position and therefore the methyl and the hydroxymethyl group had to be on different sides of the molecule. Based on synthesis by the same fungus, an identical absolute configuration can be assumed for compounds **4**–**6** and compound **6** was named papyracillic acid C.

Compound **7** was obtained as a colorless gum with the molecular formula $C_{15}H_{22}O_4$ as deduced from HREIMS spectral and ^{13}C NMR spectroscopic data. Its IR spectrum showed strong absorptions for hydroxy groups (3500, 3420 cm^{-1}), while the ^1H NMR spectrum (Table 5) exhibited the presence of two methyl groups, one of which was a singlet (δ = 1.20 ppm) and one was a doublet (δ = 1.03, d, J = 6.6 Hz), and five protons on oxygenated carbons at δ = 4.18 (d, J = 12.6), 4.30 (d, J = 12.6), 3.11 (s), 4.30 (m, overlap), 4.64 (d, J = 2.3 Hz), respectively. In the low-field area, signals for an olefinic proton (δ = 5.48, d, J = 2.3) and an exomethylene group (δ = 5.32, d, J = 20) could be detected. The ^{13}C NMR spectrum of **7** showed signals for 15 carbon atoms, and the DEPT spectra indicated the presence of two methyl, four methylene, five methine groups and four quaternary carbon atoms.

Table 5. NMR data for compound **7** (125 and 500 MHz, $CDCl_3$).

Position	δ_C	δ_H
1α	63.8	4.18 (d, 12.6)
1β		4.30 (d, 12.6)
2	145.8	
3	64.9	
4	68.5	3.11 (s)
5	38.7	
6	36.3	1.75 (m)
7α	24.8	1.40 (m)
7β		1.83 (m)
8α	32.9	1.62 (m)
8β		1.89 (m)
9	73.8	4.30 (m, overlap)
10	142.7	
11	124.2	5.48 (d, 2.3)
12	66.8	4.64 (d, 2.3)
13	115.7	5.32 (d, 20.0)
14	18.4	1.20 (s)
15	15.8	1.03 (d, 6.6)

Analysis of the COSY and HMQC spectra of **7** enabled the deduction of the fragment –CH_3(15)–CH(6)–CH_2(7)–CH_2(8)–CH(9)–. In the HMBC spectrum of **7**, ^{13}C-^1H long range correlations were found between C-2 and H-1, H-13; C-3 and H-1, H-4, H-11, H-12, H-13; C-5 and H-4, H-9, H-11, H-14, H-15; C-10 and H-4, H-9, H-11, H-14. The NOESY spectrum exhibited clear correlation between H-14 and H-4, H-15; H-15 and H-7α as well as between H-9 and H-7β, showing that these protons are on the same side of the molecule. The OH group on C-3 should be on the same side as H-12 due to the rigidity of the five-membered exomethylene furane ring A, attached to ring B. Therefore, the compound was assigned structure **7** as shown in Figure 1 and named microsphaeropsin A because of its synthesis by a *Microsphaeropsis* sp. The absolute configuration has not yet been determined.

Compound **8** was also obtained as a colorless gum. The HREIMS revealed a molecular ion peak at 248.14125. The ^{13}C NMR and DEPT spectra of **8** exhibited 15 carbon signals (2 × CH_3, 3 × CH_2, 5 × CH, 5 × C), and the molecular composition of **8** was deduced to be $C_{15}H_{20}O_3$, confirmed by HR-EIMS (m/z 248.14125, calcd. 248.14125). Its IR spectrum showed absorption for hydroxy groups and double bonds (3234 and 1656 cm^{-1}, respectively). The ^1H NMR spectrum demonstrated the presence of two methyl groups, one of which was a singlet (δ = 2.51, s) and one a doublet (δ = 1.10, d, J = 7.0 Hz), four olefinic protons at δ = 5.93 (m), 6.43 (dd, J = 9.5, 3.2 Hz), 6.91 (d, J = 8.0 Hz), 7.23 (d, J = 8.0 Hz), respectively, and two hydroxymethylene protons (δ = 4.05, overlap). Elucidation of the COSY and HMQC spectra of **8** enabled deduction of the fragments –CH(4)–CH(5)–CH_2(6)–CH(7)–CH_3(15)– and –CH(9)–CH(10)–. In the HMBC spectrum of **8**, ^{13}C-^1H long-range correlation signals were found between C-2 and H-1, H-4, and H-10; between C-3 and H-1, H-4, H-5 and H-9; between C-8 and H-6, H-7, H-9, H-10 and H-15; between C-11 and H-1, H-9, H-10, H-13, and H-14; as well as between C-12 and H-10, H-13 and H-14 (Table 6). The coupling pattern of H-7 and H-6β (J = 6.9 Hz) indicated the pseudo diaxial relationship of these protons and thus an equatorial position of the methyl group. The compound was thus assigned structure **8** and named microsphaeropsin B. The relative configuration at C-12 in the side chain with respect to C-7 and the absolute configuration have not yet been assigned.

Table 6. NMR data for compound **8** (125 and 500 MHz, $CDCl_3$).

Position	δ_C	δ_H
1	67.4	4.05 (overlap)
2	133.4	
3	132.5	
4	127.2	6.43 (dd, 9.5, 3.2)
5	126.3	5.93 (m)
6α	30.9	2.24 (ddd, 17.3, 6.4, 1.3)
6β		2.55 (ddt, 17.3, 6.9, 2.7)
7	27.7	3.25 (q)
8	140.9	
9	124.3	6.91 (d, 8.0)
10	124.5	7.23 (d, 8.0)
11	137.5	
12	77.2	
13	67.5	4.05 (overlap)
14	16.2	2.51 (s)
15	18.4	1.10 (d, 7.0)

Biological Activity

The crude culture extract of *Microsphaeropsis* sp. was very active against the Oomycete *Phytophthora infestans*

Table 7. Biological activity the crude culture extract of *Microsphaeropsis* sp. (7291) against microbial test organisms in an agar diffusion test and against the grass, *Agrostis stolonifer*. Radius of zone of inhibition in mm.

Bacillus megaterium	*Microbotryum violaceum*	*Chlorella fusca*	*Septoria tritici*	*Botrytis cinerea*	*Phytophthora infestans*	*Agrostis stolonifer*
12[a], g.i.	7, g.i.	0	8	10, g.i.	15	0

[a] g.i. = growth inhibition: there was some microbial growth within the zone of inhibition; 0 = no inhibition.

and moderately antibacterial (*Bacillus megaterium*) and antifungal against the three fungal test organisms (Table 7). These activities were also found with the individual pure compounds **1a**, **2**, **3**, and **7** as shown in Table 8. The other compounds were not active in these tests.

Table 8. Inhibition by compounds **1a**, **2**, **3**, and **7** in an agar diffusion test of the bacterium *Bacillus megaterium* (Bm), the fungus *Microbotryum violaceum* (Mv), and the alga *Chlorella fusca* (Cf).[a]

Compound	Bm	Mv	Cf
1a	6	0	6
2	6	18	13
3	7	0	0
7	0	0	7

[a] Radius of inhibition in mm. 50 µL of the test substance (1 mg/mL, 5 mg/mL for *Microbotryum violaceum*) were applied to a filter disc (see Experimental Section).

Summary

In conclusion, five new metabolites of three different compound classes were isolated from culture extracts of the endophytic fungus *Microsphaeropsis* sp., demonstrating the rich metabolic diversity of this fungus. In addition, the configurations of papyracillic acids were revised. The absolute configurations of the new palmarumycins were established by X-ray of the bis(4-bromobenzoate) **1c**, chemical correlation, and the exciton chirality approach. The solid-state CD/TDDFT method was employed to establish the absolute configuration of papyracillic acid A **4**.

Experimental Section

General Experimental Procedures: For general methods and instrumentation see ref.[28] and for microbiological methods and culture conditions see ref.[29,30] Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 MC polarimeter. The IR spectra were taken on a NICOLET-510P spectrometer. NMR spectra were run on a Bruker Avance 500 NMR spectrometer with TMS as internal standard. EIMS were obtained on a MAT 8200 mass spectrometer. Silica gel (70–230 mesh) was used for CC. Spots were detected on TLC under UV or by heating after spraying with 0.5 mL of anisaldehyde in 50 mL of HOAc and 1 mL of H_2SO_4. For the solid-state CD measurement of **1a**, the disc was prepared by mixing about 241 mg of KCl (optical grade, heated at 100 °C) and 38 µg **1a** with a Perkin–Elmer vibrating mill for 5 min. Then the mixture was pressed at ten tons with a Perkin–Elmer press under vacuum to get a transparent disc for CD measurement. In order to check the microscopic anisotropy of the KCl disc, four spectra were recorded by rotating the disc with 90° intervals, which were slightly different. Solid-state CD spectra are shown as ϕ [mdeg].

Extraction and Isolation: The endophytic fungus *Microsphaeropsis* sp., internal strain no. 7291, was isolated following surface sterilization from a branch of the tree *Larix decidua* from Hjerting, Denmark, and was cultivated at room temperature for 28 d on 5% w/v biomalt solid agar media. 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 subjected to chromatography over a silica gel column, eluted with dichloromethane/ethyl acetate (85:15, 50:50, 0:100), to give three fractions. 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 fraction (1.2 g) was separated by CC over 200 g of silica gel with hexane/ethyl acetate (10:1, 500 mL, 5:1, 500 mL) to give two subfractions A and B. Fraction A (300 mg) was separated by CC over 5 g of silica gel with hexane/ethyl acetate (7:1, 550 mL) to give crude **2**, **3** and **5**. Fraction B (450 mg) was separated by CC over 5 g of silica gel with hexane/ethyl acetate (4:1, 450 mL) to give crude **4**, **7** and **8**. Subsequently, each crude fraction was further purified by preparative TLC chromatography on silica gel (1 mm, Macherey & Nagel) and Sephadex (LH-20) to give compounds **2** (5 mg), **3** (6 mg), **4** (60 mg), **5** (8 mg), **7** (7 mg) and **8** (6 mg). The more polar fractions (2.0 g) were separated by silica gel column chromatography eluted with dichloromethane/ethyl acetate (5:1, 480 mL) to give crude crystals of **1a** and **6**, successively. The samples were then recrystallized from MeOH to give **1a** (70 mg) and **6** (10 mg).

Biological Testing: 2 mg of the culture extract was applied to a filter disc on appropriate growth media and sprayed with a suspension of the respective test organism. The culture extract was found to be biologically active against the fungal test organisms *Microbotryum violaceum*, *Septoria tritici* and *Botrytis cinera*, antibacterial against *Bacillus megaterium*, herbicidal against *Agrostis stolonifer*, algicidal against *Chlorella fusca*, but also inhibitory of the Oomycete, *Phytophthora infestans*.

For tests of the pure substances, 0.05 mg of the test substance (0.25 mg for *Microbotryum violaceum*) were applied to a filter disc and sprayed with a suspension of the respective test organism.

Palmarumycin M_1 (1a): Colorless crystals (MeOH), m.p. 230–231 °C. $[\alpha]_D^{25}$ = +249 (c = 0.03, CH_3OH). CD (MeOH, λ [nm] ($\Delta\varepsilon$), $c = 3.64 \times 10^{-4}$): 327 sh (2.24), 312 sh (2.15), 297 (2.51), 285 sh (1.99), 246 sh (2.75) 233 (5.68), 222 (–0.34), 206 (10.99). CD (KCl, λ [nm] ϕ [mdeg]), 38 µg and 241 mg of KCl: 233 sh (2.72), 330 (3.06), 316 (2.22), 300 (2.59), 288 (2.97), 248 sh (6.65), 239 (7.95), 224 (–4.50), 207 (11.08). UV (MeOH): λ_{max} (lg ε): 326 sh (3.61), 313 sh (3.79), 299 (3.94), 288 sh (3.86), 226 (4.82). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3455, 3069, 2983, 1243, 1129, 1070, 942, 731. For ^1H and ^{13}C NMR spectroscopic data see Tables 1 and 2. EIMS: *m/z* (%) = 340 (80) [M^+], 322 (90), 286 (10), 160 (100), 131 (30), 115 (40), 69 (20), 44 (75), 28 (35). HREIMS: *m/z* (%) = 340.13107 (calcd. for $C_{20}H_{20}O_5$ 340.13026).

Crystal Structure Determination of Palmarumycin M_1 (1a):[31] $C_{20}H_{20}O_5$, Mr = 340.4, orthorhombic, space group $P2_12_12_1$, a = 6.1280(9), b = 11.5772(17), c = 22.372(3) Å, V = 1587.2(4) Å^3, Z = 4, D_x = 1.424 g/cm^3, $F(000)$ = 720, T = 120(2) K. Bruker-AXS SMART

APEX CCD,[32] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.10 mm^{-1}, colorless platelike crystal, size 0.40 × 0.20 × 0.02 mm^3, 16375 intensities collected $1.8 < \theta < 28.2°$, $-8 < h < 8$, $-15 < k < 15$, $-29 < l < 29$. Structure solved by direct methods,[32] full-matrix least-squares refinement[32] with 2270 independent reflections based on F^2 and 229 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with $U = 1.5\,U_{iso}$(O) or $1.2\,U_{iso}$(C). **1a** crystallizes in the non-centrosymmetric space group $P2_12_12_1$; however, in the absence of significant anomalous scattering effects, the Flack[33] parameter is essentially meaningless. Accordingly, Friedel pairs were merged. Refinement converged at $R_1[I > 2\sigma(I)]$ = 0.048, wR_2(all data) = 0.065, S = 0.916, max(δ/σ) < 0.001, min/max height in final ΔF map –0.22/0.22 e/Å^3. Figure 2 shows the molecular structure.

4,5,8-Triacetylpalmarumycin M_1 (1b): Palmarumycin M_1 (**1**) (5 mg) was dissolved in pyridine (1 mL) and acetic anhydride (0.5 mL) was added. The reaction mixture was stirred for 15 h at ambient temperature. The solvent was evaporated at reduced pressure to afford crude **1a**, which was purified by preparative TLC using dichloromethane/ethyl acetate (5:1), yielding **1a** as colorless gum. For ^{1}H and ^{13}C NMR spectroscopic data see Table 1 and Table 2. EIMS: *m/z* (%) = 466 (25) [M$^+$], 406 (30), 286 (80), 167 (72), 149 (100), 113 (16), 44 (60). HREIMS: *m/z* 466.16299 (calcd. for $C_{26}H_{26}O_8$ 466.16278).

***p*-Bromobenzoate of 1a:** To a solution of 10 mg of **1** in 1 mL of pyridine were added 50 mg of *p*-bromobenzoyl chloride and 50 mg of DMAP, and the mixture was left to stand for 24 h. To the reaction solution was added 50 mL of AcOEt, and the mixture was washed with diluted HCl, saturated $NaHCO_3$, and water, successively. The reaction product was purified by TLC to give 4,5-dibenzoate (7 mg). ^{1}H NMR spectroscopic data see Table 1. CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 1.6 \times 10^{-4}$): 328 (2.26), 321 (0.38), 316 (–0.35), 307 sh (–1.38), 300 sh (–2.77), 294 sh (–3.25), 284 (–4.01), 276 sh (–2.39), 251 (48.17), 234 sh (–16.61), 227 (–19.03), 214 sh (–6.84), 199 (59.16). CD (KCl, λ [nm] ϕ [mdeg]), 31 µg and 253 mg of KCl: 329 (1.33), 324 (0.49), 317 (–0.33), 307 (–1.09), 301 (–1.77), 295 (–1.90), 288 (–1.98), 256 (19.24), 234 (–10.26), 199 (17.48). UV (MeCN): λ_{max} (lg ε): 327 sh (3.62), 313 sh (3.81), 299 (3.97), 283 sh (3.88), 243 (4.62), 226 (4.89), 198 (4.97).

Crystal Structure Determination of 4,5-Dibenzoate of 1a (1c):[31] $C_{34}H_{26}Br_2O_7$, CH_3OH, Mr = 738.4, monoclinic, space group $P2_1$, a = 8.077(2), b = 11.442(3), c = 16.413(5) Å, β = 93.432(5)°, V = 1514.0(7) Å^3, Z = 2, D_x = 1.620 g/cm^3, F(000) = 748, T = 120(2) K. Bruker-AXS SMART APEX CCD,[32] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 2.73 mm^{-1}, colorless platelike crystal, size 0.30 × 0.25 × 0.03 mm^3, 15569 intensities collected $2.2 < \theta < 28.2°$, $-10 < h < 10$, $-15 < k < 15$, $-21 < l < 21$. Semi-empirical absorption correction from equivalents with SADABS.[32] Structure solved by direct methods,[32] full-matrix least-squares refinement[32] with 7394 independent reflections based on F^2 and 409 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with $U = 1.5\,U_{iso}$(O) or $1.2\,U_{iso}$(C). One enclosed CH_3OH solvent molecule per asymmetric unit. Refinement converged at $R_1[I > 2\sigma(I)]$ = 0.052, wR_2(all data) = 0.108, S = 0.889, max(δ/σ) < 0.001, min/max height in final ΔF map –0.48/0.35 e/Å^3. Figure 3 shows the molecular structure.

Decaspirone C (2): Colorless gum. $[\alpha]_D^{25}$ = +173 (c = 0.06, CH_3OH) ref.[2] $[\alpha]_D^{25}$ = +89 (c = 0.58, CH_3OH). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3455, 3069, 2983, 1668, 1243, 1129, 1070, 942, 770 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2. EIMS: *m/z* (%) = 338 (60) [M$^+$], 279 (30), 252 (25), 167 (45), 149 (80), 84 (90), 49 (100). HREIMS: *m/z* 338.11557 (calcd. for $C_{20}H_{18}O_5$ 338.11542).

Palmarumycin M_2 (3): Colorless gum. $[\alpha]_D^{25}$ = +355 (c = 0.007, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3457, 3069, 2985, 1732, 1275, 1129, 1050, 922, 831 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Tables 1 and 2. EIMS: *m/z* (%) = 338 (20) [M$^+$ – H_2O], 279 (30), 252 (25), 167 (45), 149 (80), 84 (90), 57 (100).

Papyracillic Acid A (4): Colorless crystals, m.p. 115–117 °C. $[\alpha]_D^{25}$ = –2.3 (c 0.06, CH_2Cl_2) ref.[14] m.p. 97–99 °C, $[\alpha]_D$ = 0 (c = 1.0 in methanol). CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 3.0 \times 10^{-4}$): 226 (1.59), 196 (–3.05). CD (KCl, λ [nm] ϕ [mdeg]), 87 µg and 239 mg of KCl: 229 (2.54), 195 (–10.411). CD (KBr, λ [nm] ϕ [mdeg]) 54 µg **4** and 240 mg of KBr: 229 (8.44) negative below 212 nm but could not be measured below 208 nm due to high absorbance. UV (MeCN): λ_{max} (lg ε): 224 (4.01). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3450, 2921, 1770, 1640, 1360, 1210, 940, 860 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Tables 3 and 4. EIMS: *m/z* (%) = 209 (10) [M$^+$ – OH], 184 (20), 166 (100), 151 (65), 139 (80), 106 (40), 39 (20).

Crystal Structure Determination of Papyracillic Acid A (4):[31] $C_{11}H_{14}O_5$, Mr = 226.2, monoclinic, space group $P2_1$, a = 5.6613(8), b = 9.5105(14), c = 10.7207(16) Å, β = 96.175(3)°, V = 573.9(1) Å^3, Z = 2, D_x = 1.309 g/cm^3, F(000) = 240, T = 120(2) K. Bruker-AXS SMART APEX CCD,[32] graphite monochromator, λ(Mo-K_α) = 0.71073 Å, μ = 0.10 mm^{-1}, colorless crystal, size 0.40 × 0.40 × 0.08 mm^3, 4657 intensities collected $1.9 < \theta < 28.3°$, $-7 < h < 7$, $-12 < k < 11$, $-14 < l < 14$. Structure solved by direct methods,[32] full-matrix least-squares refinement[32] with 1514 independent reflections based on F^2 and 147 parameters, all but H atoms refined anisotropically, H atoms refined with riding model on idealized positions with U = 1.5 U_{iso}(O and methyl-C) or 1.2 U_{iso}(C). **4** crystallizes in the non-centrosymmetric space group $P2_1$; however, in the absence of significant anomalous scattering effects, the Flack[33] parameter is essentially meaningless. Accordingly, Friedel pairs were merged. Refinement converged at $R_1[I > 2\sigma(I)]$ = 0.044, wR_2(all data) = 0.102, S = 1.05, max(δ/σ) < 0.001, min/max height in final ΔF map –0.18/0.34 e/Å^3. Figure 5 shows the molecular structure.

Papyracillic Acid Methyl Acetal (5): Colorless gum. $[\alpha]_D^{25}$ = –42.1 (c = 0.03, CH_2Cl_2); ref.[14] m.p. 116-l 18 "C. $[\alpha]_D$ = –42 (c = 1.3 in chloroform). CD (MeCN, λ [nm] ($\Delta\varepsilon$), $c = 2.7 \times 10^{-4}$): 224 (11.71), 195 (–19.58). CD (KCl, λ [nm] ϕ [mdeg]), 87 µg **5** and 239 mg of KCl: 227 (46.84), 198 (–82.24). UV (MeCN): λ_{max} (lg ε): 223 (4.01). IR (KBr): $\tilde{\nu}_{max}$ (film) = 2940, 1770, 1640, 1460, 1360, 1210, 950, 870 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 3 and Table 4; CIMS: *m/z* (%) = 241 (25) [M + 1], 209 (30), 166 (100), 139 (15), 123 (18), 68 (26), 43 (50).

Compound 6: Colorless crystals: m.p. 106–107 °C. $[\alpha]_D^{25}$ = –1.5 (c = 0.05, CH_3OH). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3465, 2925, 1770, 1640, 1360, 1210, 940, 860 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 3 and Table 4. HREIMS: *m/z* 244.0378 (calcd. for $C_{11}H_{16}O_6$ 244.0947).

Microsphaeropsin A (7): Colorless gum. $[\alpha]_D^{25}$ = –2.5 (c = 0.003, CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 3487, 2918, 1460, 1377, 1206, 1000 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 5. HREIMS: *m/z* 266.15167 (calcd. for $C_{15}H_{22}O_4$ 266.15132).

Microsphaeropsin B (8): Colorless gum, $[\alpha]_D^{25}$ = –43.3 (c = 0.06 CH_2Cl_2). IR (KBr): $\tilde{\nu}_{max}$ (film) = 2929, 1672, 1460, 1382, 1243, 1020 cm^{-1}. For ^{1}H and ^{13}C NMR spectroscopic data see Table 6. HREIMS: *m/z* 248.14125 (calcd. for $C_{15}H_{20}O_3$ 248.14125).

Computational Section: Conformational searches were run employing Molecular Merck force field (MMFF), with standard parameters and convergence criteria, implemented in Spartan'06, Wavefunction, Inc, Irvine CA. All minima thus found for compound **5** were optimized with DFT at B3LYP/6-31G(d) level.

DFT and TDDFT calculations were run with Gaussian'03W, Revision B.05, Gaussian, Inc., Pittsburgh PA. The input geometries of **4** for TDDFT calculations were obtained from the solid-state structure upon re-optimization, using the DFT method at B3LYP/6-31G(d) level, of (a) the H-atoms' positions, (b) all bond lengths. TDDFT calculations were executed employing the hybrid functional B3LYP with TZVP basis set.[24] All computed transitions responsible for the spectra shown in Figure 6 had energies below the estimated ionization potentials.[25] CD spectra were generated using rotational strengths computed with dipole-length gauge formulation to which a Gaussian band-shape was applied with 2,500 cm^{-1} half-height width, corresponding to 14 nm at 235 nm. Rotational strengths computed for most transitions with dipole-velocity gauge formulation differed from dipole-length values by less than 5% for both **4** and **5**.

Exciton DeVoe-type calculations were run with a Fortran program written by W. Hug[34] using the X-ray geometry of **1c**, and parameters (transition frequencies, dipoles polarizations) extracted from the absorption spectra of **1a** and **1c** and from TDB3LYP/TZVP calculations on methyl 4-bromobenzoate and 1,8-dihydroxynaphthalene [B3LYP/6-31G(d) geometries]; see Supporting Information.

Supporting Information (see also the footnote on the first page of this article): Exciton DeVoe-type calculations on compound (4*S*,5*S*)-**1c**, showing the effect of degenerate (benzoate/benzoate) and non-degenerate (naphthalene/benzoate) couplings (Figure S1).

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