DOI: 10.1002/ejoc.200800688

Dinemasones A, B and C – New Bioactive Metabolites from the Endophytic Fungus *Dinemasporium strigosum*[‡]

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Dedicated to Professor Dr. Wolfgang Steglich on the occasion of his 75th birthday

Keywords: Endophytic fungi / *Dinemasporium strigosum* / Dinemasones A, B and C / Absolute configuration / Exciton chirality method / Octant rule

Two new bioactive metabolites **1a** and **2a** (dinemasones A, B) were isolated in pure form and one as the diacetate **3b** of dinemasone C (**3a**) from the ethyl acetate extract of *Dinemasporium strigosum* together with the known palmitic acid, ergosterol and the *cis* and *trans* isomers of 4-hydroxymellein. The structures were determined by spectroscopic analysis, notably 2D NMR techniques. Their absolute configurations were established by both their carbonyl n–π^* CD transitions and the exciton chirality method of their respective dibenzoate derivatives. The dinemasones A, B (**1a** and **2a**) showed antibacterial, antifungal, and antialgal activity.

Introduction

As part of our continuing investigations to find new bioactive metabolites from endophytic fungi, we recently investigated the ethyl acetate culture extracts of a culture of theendophytic fungus, *Dinemasporium strigosum*, which had been isolated from the roots of *Calystegia sepium*, and was grown on biomalt solid agar medium. From these extracts, four known compounds were identified as palmitic acid,[2,3] ergosterol,[4] and the *cis* and *trans* isomers of 4-hydroxymellein.[5–9] In addition, two new metabolites with unusual structures, **1a** and **2a**, were isolated in pure form by a combination of repeated chromatography and crystallization. A polar component **3a** was indirectly identified by acetylation of the polar fraction as the *cis*-diacetylated derivative **3b**, produced from the new natural product **3a** (Figure 1). Both the hexahydropyrano[4,3-*b*]pyran-5(7*H*)-one derivative **2a** and the 1,7-dioxaspiro[5,5]undecan-4-one derivative **1a** showed antibacterial, antifungal, and antialgal activity.

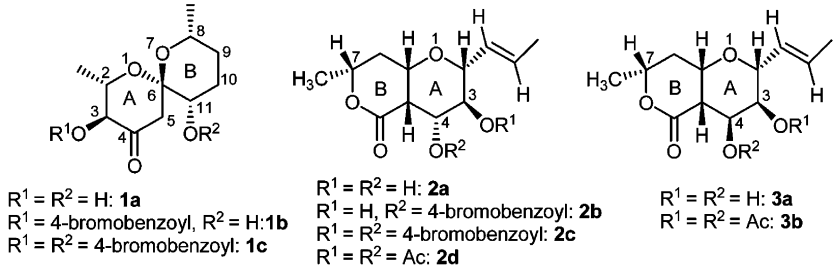

Figure 1. Structures of the three new metabolites and their derivatives isolated from the culture extract of the endophytic fungus *Dinemasporium strigosum*.

[‡] Biologically Active Secondary Metabolites from Fungi, 39. Part 38: Ref.[1]

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Results and Discussion

Compound **1a** of medium polarity displayed eleven carbon resonances in the ^{13}C NMR spectrum, with nine of the C atoms attached to protons as deduced from the HMQC experiment. The ^{1}H NMR and DEPT 135 spectra revealed the presence of four oxygenated methine groups, three methylene groups, and two methyl carbon atoms; the NMR spectroscopic data are compiled in Table 1.

The resonance at δ = 205.8 ppm in the ^{13}C NMR spectrum and the carbonyl band at 1722 cm^{-1} in the IR spectrum were characteristic of the presence of a carbonyl carbon atom. In the ^{1}H NMR spectrum of **1a**, the two one-proton doublets at δ = 2.50 and 3.52 ppm did not show any connectivity with any carbon atom in the HMQC spectrum suggesting the presence of two hydroxy groups in conjunction with the two bands at 3487 and 3425 cm^{-1} in the IR spectrum. The peak at δ = 101.3 ppm (C-6) appeared as a strongly deshielded quaternary carbon atom, typical for a ketal group. Analysis of the 1D and 2D NMR spectra, including COSY, HMQC, and HMBC, led to the assignment of the two partial structures **A** and **B**, as shown in Figure 2. In partial structure **A**, the C-8(CH_3)–C-9–C-10–C-11(OH) portion was assigned by tracing of cross peaks in the COSY spectrum. A tetrahydropyranol ring moiety was disclosed by HMBC correlations (8-H/C-6; 8-CH_3/C-6; 9-H/C-6; 10-H/C-6; 11-OH/C-6). In partial structure **B**, the C-2(CH_3)–C-3(OH)–C-4–C-5 portion was assigned by tracing of cross peaks in the COSY spectrum. A tetrahydropyranone ring moiety was inferred by analysis of the HMBC correlations (2-H/C-4,C-6; 2-CH_3/C-4,C-6; 3-H/C-4; 3-OH/C-4; 5-H/C-4,C-6) and the chemical shift of the C-4 signal (δ_C = 205.8 ppm).

The C-6 carbon of the ketal function is common to both fragments **A** and **B**, suggesting a connection between the partial structures through C-6 as shown in **1a** in Figure 1. This was confirmed by the HMBC correlations of 5-H with C-11 and of 11-H with C-5. Finally, the gross structure of **1a** was confirmed by the mass spectrum with $[M + H]^+$ at *m/z* = 231, suggesting the molecular formula $C_{11}H_{18}O_5$, in agreement with the NMR spectra.

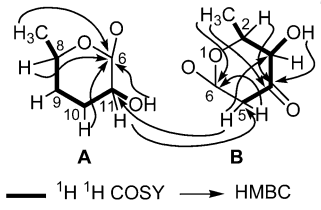

Figure 2. Partial structures **A** and **B** of **1a**.

The relative configuration of **1a** was determined by a combination of the analysis of the coupling constants and by extensive 1D NOE experiments. For this analysis, it was important to note the conformational rigidity of ring A, as seen from the W coupling between 5-H_a and 3-H_a (J = 1.0 Hz). In addition, the large coupling constant of $J_{2,3}$ = 9.5 Hz indicated an antiperiplanar *diaxial* position of these two hydrogen atoms, placing the methyl and hydroxy groups in *trans-equatorial* positions. This was unambiguously confirmed by a strong correlation of the protons of the methyl group at C-2 (δ = 1.47 ppm) with the vicinal proton at C-3 (δ = 3.83 ppm) in the NOE experiment, indicating that they are on the same side of the ring (structure **C**, Figure 3).

The relative configurations of stereogenic centers C-8 and C-11 of ring B and the acetal spiro center at C-6, connecting the two rings, were more difficult to elucidate because the proton coupling system was interrupted by the quaternary spiro center at C-6. However, the problem could be solved by analysis of the entire set of NOE correlations. In this analysis, each stereogenic center at C-6, C-8 and C-11 was changed sequentially, and all the possible relative configurations and conformers were analyzed for unambiguous agreement with the entire set of Overhauser interactions. It was only the relative configuration of **1a** (2*S*,3*S*,6*S*,8*R*,11*S*/2*R*,3*R*,6*R*,8*S*,11*R*; Figure 1) that fitted all the NOE experiments. In this configuration, ring A is fixed in a $_1C^4$ chair conformation that allows the bis(equatorial) arrangement of the C-2 and C-3 substituents. A strong NOE correlation between 2-H_a and 8-CH_3, and be-

Table 1. ^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR spectroscopic data for **1a**.

No.	δ_H (mult., J in Hz)	δ_C	COSY	HMBC
2	4.07 (dq, $J_{2,3}$ = 9.5, $J_{2,2\text{-Me}}$ = 6.0, 1 H)	72.5	2-C*H*$_3$, 3-H	2-*C*H$_3$, C-3, C-4, C-6
2-CH_3	1.47 (d, $J_{2,2\text{-Me}}$ = 6.0, 3 H)	18.7	2-H	C-2, C-3, C-4, C-6
3	3.83 (ddd, $J_{3,2}$ = 9.5, $J_{3,OH}$ = 4.0, $J_{3,5a}$ = 1.0, 1 H)	78.0	2-H, 3-O*H*, 5-H	C-2, 2-*C*H$_3$, C-4
3-OH	3.52 (d, $J_{OH,3}$ = 4.0, 1 H)		3-H	C-2, C-3, C-4
4		205.8		
5	2.90 (d, $J_{5,5}$ = 13.8, 1 H)	45.0	3-H, 5-H	C-3, C-4, C-6, C-11
	2.83 (dd, $J_{5,5}$ = 13.8, $J_{5a,3}$ = 1.0, 1 H)			
6		101.3		
8	3.90 (sext, J = 6.11 H,)	69.9	8-C*H*$_3$, 9-H	C-6, 8-*C*H$_3$, C-10
8-CH_3	1.26 (d, $J_{8,8\text{-Me}}$ = 6.5, 3 H)	20.7	8-H	C-6, C-8, C-9
9e, 10e	1.67 (m, 2 H)	27.6	8-H, 10-H	C-6, C-8, 8-*C*H$_3$, C-10, C-11
10a	1.99 (m, 1 H)	24.8	9-H, 10-H	C-6, C-8, C-9, C-11
9a	1.76 (m, 1 H)	–	9-H, 10-H, 8-H	C-6, C-8, C-9, C-11
11	3.55 (m, $J_{11,10}$ = 7.4, $J_{11,OH}$ = 6.3, $J_{11,10}$ = 3.3, 1 H)	69.7	10-H, 11-O*H*	C-5, C-9, C-10
11-OH	2.50 (d, $J_{OH,11}$ = 6.3, 1 H)		11-H	C-6, C-10, C-11

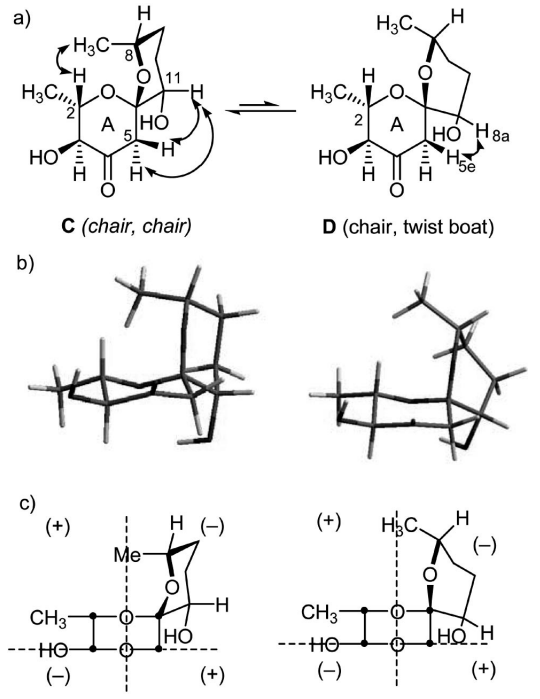

Figure 3. (a) NOE correlations of the major conformer **C** and the minor conformer **D** of (2*S*,3*S*,6*S*,8*R*,11*S*)-**1a**. (b) **C** and **D** MMFF-calculated conformers of (2*S*,3*S*,6*S*,8*R*,11*S*)-**1a** after DFT geometry optimization with populations 95% and 5%, respectively. (c) Octant projection diagrams of conformers **C** and **D** showing negative sum n–π* contribution from the (2*S*,3*S*,6*S*,8*R*,11*S*) enantiomer.

tween 11-H and 5-$H_{a,e}$ is only feasible with axial O-7 and 8-CH_3, which determines the relative configuration of C-6 and C-8. No alternative position of O-7 and 8-CH_3 would allow placement of this remote methyl group in the proximity of 2-H_a. The lack of a large $J_{8,9}$ coupling constant is also in agreement with the axial orientation of the 8-CH_3 group. Moreover, the $J_{10,11}$ = 7.4 Hz coupling constant suggests an equatorial arrangement of 11-OH, which is also supported by equal NOE interaction of 11-H with both 5-H_a and 5-H_e. An MMFF conformational search of (2*S*,3*S*,6*S*,8*R*,11*S*)-**1a** afforded **C** as the major conformational isomer (95% population at room temperature), and in a 2 kcal/mol range it also gave a minor isomer **D**, in which ring B has a twist-boat conformation allowing equatorial arrangement for both the 8-CH_3 and the 11-OH group. In accordance with the calculation, the NOE interactions and coupling constants confirmed that conformer **C** must be the major one, and the weak NOE effect between 8-H_a and 5-H_e derives from the minor conformer **D**.

The absolute configuration of **1a** could be determined on the basis of the sign of its ketone n–π* transition [279 nm ($\Delta\varepsilon$ = –1.2), Figure 4] by applying the octant rule.[10,11] The measured negative n–π* CE must derive from the (2*S*,3*S*,6*S*,8*R*,11*S*) enantiomer, whose ring B is located in the negative upper right (or lower left) octant in both conformers and hence determines the sign of the n–π* transition (Figure 3c). The equatorial 3-OH group lies on the carbonyl symmetry plane and has no contribution, while the positive contribution of the 2-CH_3 group is overcome by the negative contribution of ring B.

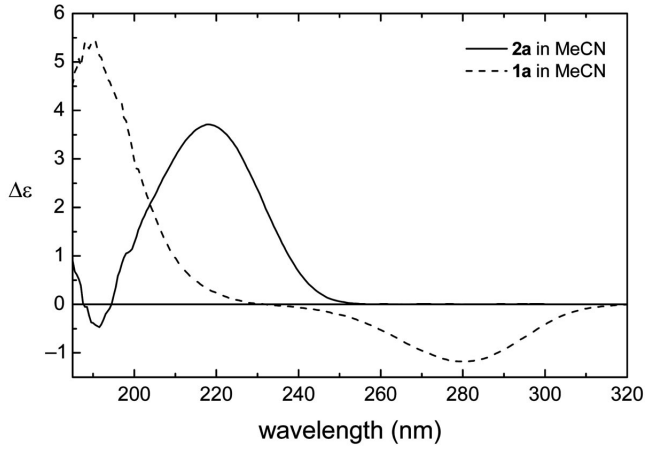

Figure 4. CD spectra of **1a** (broken line) and **2a** (solid line) in acetonitrile.

The 6,6-spiroketal (1,7-dioxaspiro[5.5]undecane) core (without 4-carbonyl group as in **1a**) is quite common in biologically active natural products such as the spirofungins,[12] the reveromycins[13,14] or the olive fly pheromones.[15] However, the corresponding ketones such as dinemasone A (**1a**) are very rarely found in natural products and have remote similarity with the skeleton of the siphonarins A and B, isolated from marine molluscs of the genus *Siphonaria*.[16,17] However, the siphonarins differ considerably from **1a** in their substitution pattern.

The ^{13}C NMR spectrum of compound **2a** displayed twelve carbon resonances. Eleven out of the twelve carbon atoms were attached to protons, as indicated by the HMQC experiment. The ^1H NMR and DEPT spectra revealed the presence of eight methine groups, two of which were olefinic and five oxygenated, in addition to one methylene group and two methyl carbon atoms. The ^1H and ^{13}C NMR chemical shifts are shown in Table 2. The resonance at δ = 173.2 ppm in the ^{13}C NMR spectrum and the carbonyl band at 1732 cm^{-1} in the IR spectrum were characteristic for the presence of a carbonyl carbon atom of an ester or lactone. In the ^1H NMR spectrum of **2a**, the broad singlet at δ = 2.57 ppm and the doublet at δ = 4.04 ppm did not show any connectivity with any carbon atom in the HMQC spectrum. This information, in conjunction with the two bands at 3444 and 3390 cm^{-1} in the IR spectrum, suggested the presence of two hydroxy groups.

Analysis of 1D and 2D NMR spectra including COSY, HMQC, and HMBC led to the assignment of structure **E** as shown in Figure 5. In this structure, the C-2(C-1′–C-2′–C-3′)–C-3(OH)–C-4(OH)–C-4a–C-8a–C-8–C-7(CH_3) portion was assigned by tracing of cross peaks in the COSY spectrum. A tetrahydropyranone ring moiety was disclosed by HMBC correlations (4a-H/C-5; 8a-H/C-5; 7-CH_3/C-5) and the chemical shift of the C-5 signal (δ_C = 173.2 ppm). A tetrahydropyrandiol ring was deduced from an HMBC correlation of 8a-H to C-2, confirming the fusion of rings

Table 2. ^{1}H (500 MHz, $CDCl_3$) and ^{13}C (125 MHz, $CDCl_3$) NMR spectroscopic data for **2a**.

no.	δ_H (mult., J in Hz)	δ_C	COSY	HMBC
2	3.57 (t, $J_{2,3} = J_{2,1'} = 8.1$, 1 H)	81.1	3-H, 1′-H	C-3, C-4
3	3.66 (t, $J_{3,4} = J_{3,2} = 8.1$, 1 H)	72.1	2-H, 3-O*H*, 4-H	C-4
3-OH	2.57 (br. s, 1 H)		3-H	
4	3.71 (m, 1 H)	73.4	3-H, 4-O*H*, 4a-H	C-3, C-5, C-8a
4-OH	4.04 (d, $J_{OH,4} = 11.2$, 1 H)		4-H	C-4
4a	3.02 (t, $J_{4a,4} = J_{4a,8a} = 3.8$, 1 H)	44.8	4-H, 8a-H	C-3, C-4, C-5, C-7, C-8a
5		173.2		
7	4.38 (sept, $J_{7,8} = 12.0$, $J_{7,7} = 6.3$, $J_{7,8} = 3.91$, H)	72.7	7-CH_3, 8-H	
7-CH_3	1.44 (d, $J_{7,7} = 6.3$, 3 H)	20.5	7-H	C-5, C-7, C-8, C-8a
8	2.48 (ddd, $J_{gem} = 15.2$, $J_{8,8a} = 9.1$, $J_{8,7} = 3.9$, 1 H)	36.9	7-H, 8-H, 8a-H	C-7, C-8a
	1.81 (ddd, $J_{gem} = 15.2$, $J_{8,7} = 12.0$, $J_{8,8a} = 3.0$, 1 H)			
8a	4.16 (sext, $J_{8a,8} = 9.1$, $J_{8a,4a} = 3.8$, $J_{8a,8} = 3.0$, 1 H)	71.2	4a-H, 8-H	C-2, C-5, C-7
1′	5.52 (ddd, $J_{1',2'} = 15.4$, $J_{1',2} = 8.1$, $J_{1',3'} = 1.4$, 1 H)	127.7	2-H, 2′-H, 3′-CH_3	C-3′
2′	5.89 (sext, $J_{2',1'} = 15.4$, $J_{2',3'} = 6.4$, 1 H)	131.4	1′-H, 3′-CH_3	C-2
3′	1.78 (dd, $J_{3',2'} = 6.4$, $J_{3',1'} = 1.4$, 3 H)	18.0	1′-H, 2′-H	C-1′, C-2′

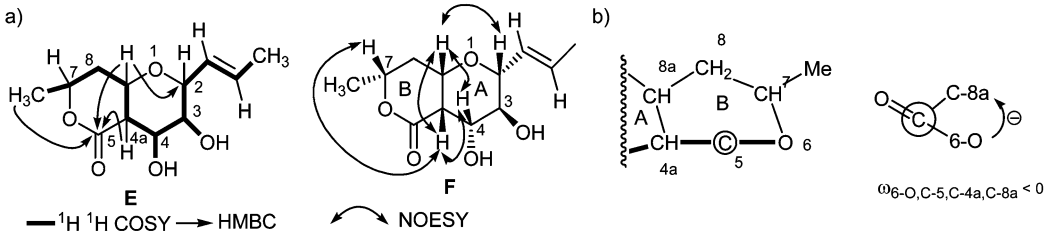

Figure 5. (a) Connectivities (**E**) and NOESY correlations in the relative configuration (**F**) of **2a**. (b) Absolute conformation of ring B and $\omega_{6\text{-O,C-5,C-4a,C-8a}}$ torsional angle in (2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*)-**2a**.

A and B at C-4a and C-8a. Finally, the gross structure of **2a** was confirmed by the mass spectrum with $[M + H]^+$ at $m/z = 243$, suggesting the molecular formula $C_{12}H_{18}O_5$, in agreement with the NMR spectra.

The relative configuration of **2a** was determined by analysis of the correlations in the NOESY spectrum (Figure 5a) and coupling constants (Table 2). The cross peaks of 2-H, 4-H, and 4a-H with 8a-H and of 4a-H and 7-H with 4a-H indicated that the protons 2-H, 4-H, 4a-H, 7-H, and 8a-H are on the same side of the rings (Structure **F**, Figure 5). These NOESY interactions and analysis of the proton-proton coupling constants (Table 2) with characteristic *trans*-diaxial coupling constants of $J_{3,4} = J_{3,2} = 8.1$ Hz allowed the determination of the relative configuration of **2a** showing *cis* annelation of the two rings, the equatorial orientation of all four substituents, and chair conformation for ring A and a boat conformation for ring B. The coupling constant of $J_{8,8a} = 9.1$ Hz is only possible when ring B is in a boat conformation with an equatorial arrangement for its methyl group. This was further confirmed by the absence of NOE interaction between 8a-H and 7-H and by an NOE interaction between 4a-H and 7-H. This long-distance correlation is only possible if ring B adopts a boat conformation.

In the CD spectrum of **2a**, a positive Cotton effect (CE) was measured for the lactone n–π* transition at 218 nm ($\Delta\varepsilon = -3.7$), which allowed us to decide between the two possible absolute configurations: (2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*) or (2*S*,3*R*,4*S*,4a*R*,7*S*,8a*R*). In δ-lactones, the lactone ring is chiral due to its preferred helicity, and it determines the sign of the lactone n–π* transition.[18] Semiempirical rules describe the correlation between the helicity or conformation of the δ-lactone ring and the sign of the n–π* CE; the negative $\omega_{6\text{-O,C-5,C-4a,C-8a}}$ torsional angle or the boat conformation shown in Figure 5b is manifested in a positive n–π* CE.[18] Since **2a** has a positive n–π* CE, its ring B of boat conformation adopts the conformation shown in Figure 5b with negative a $\omega_{6\text{-O,C-5,C-4a,C-8a}}$ torsional angle, which on the basis of the known relative configuration determines the absolute configuration as (2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*).

A literature survey confirmed that **2a**, named dinemasone B, is a new natural product. This hexahydropyrano[4,3-*b*]pyran-5(7*H*)-one structure is extremely rarely found in nature and the skeleton, albeit embedded in a larger structural array, is only found in the antibiotic FR 182876, produced by a *Streptomyces* species,[19] and in hexacyclinic acid, isolated from *Streptomyces cellulosae* spp.[20] Such a structure has never been found to be synthesized by a fungus.

Both metabolites **1a** and **2a** were treated with 4-bromobenzoyl chloride to afford the respective mono- and bis(4-bromobenzoates) **1b**, **1c** and **2b**, **2c** (Figure 1) with the hope of establishing the absolute configuration by single-crystal X-ray analysis with a heavy atom incorporated. Unfortunately, none of the four bromobenzoates was suitable for X-ray analysis. However, the NMR spectroscopic data of these derivatives confirmed the relative configuration of **1a** and **2a**. In addition, the absolute configurations of **1a** and **2a** also could be determined by the exciton chirality method[21] from their respective dibenzoate derivatives **1c**

and **2c**. The diol **2a** was also acetylated to the diacetate **2d** for comparison with the acetylation product **3b** (see below).

The dibenzoate derivative **1c** showed a weak negative exciton-coupled CD couplet around 245 nm [252 ($\Delta\varepsilon$ = –5.0), 234 nm (7.3), Figure 6a], which derives from the coupling of the two benzoate ^1L_a electric transition moments lying parallel with their CH–O ester bond.[21] According to the negative couplet, the two ^1L_a electric transition moments have a counter-clockwise arrangement or negative chirality. Based on their similar coupling constants, the conformers **C** and **D** of (2*S*,3*S*,6*S*,8*R*,11*S*)-**1a** were also considered prevalent for the dibenzoate **1c**. In conformer **C**, the $\omega_{3\text{-O},3\text{-C},11\text{-C},11\text{-O}}$ projection angle is –160.3° (negative chirality), which results in a weak negative exciton-coupled interaction between the ^1L_a electric transition moments of the two *equatorial p*-bromobenzoate groups (interaction is zero at 180° angle). In conformer **D**, the $\omega_{3\text{-O},3\text{-C},11\text{-C},11\text{-O}}$ projection angle was also negative (–148.2°), and thus the observed weak negative CD couplet predicts the (2*S*,3*S*,6*S*,8*R*,11*S*) absolute configuration of **1a**, in agreement with that determined by the octant rule.

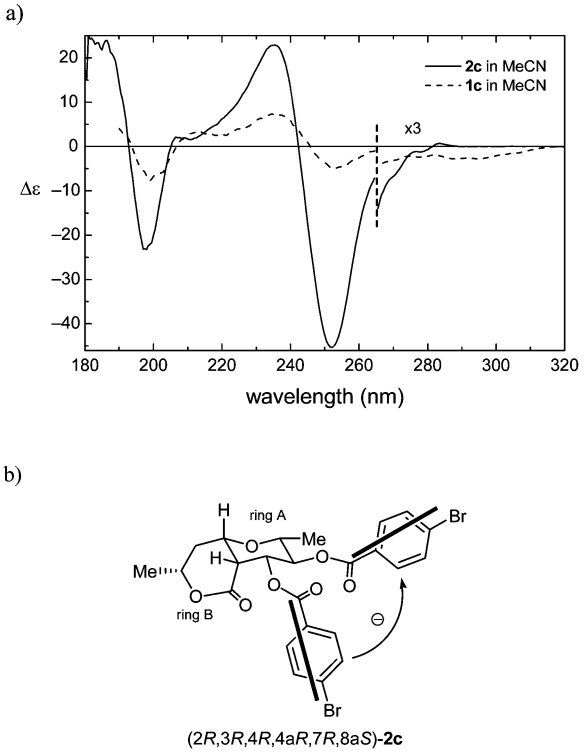

Figure 6. (a) CD spectra of **1c** (broken line) and **2c** (solid line) in acetonitrile. CD data are multiplied by 3 in the high-wavelength region. (b) (2*R*,3*R*,4*R*,4a*R*,7*R*,8a*S*)-**2c** showing the negative chirality between the ^1L_a electric transition moments of the *p*-bromobenzoate chromophores.

A strong positive CD couplet [252 ($\Delta\varepsilon$ = –45.34), 235 nm (22.90); Figure 6a] was observed in the CD spectrum of **2c**, which derives from the coupling between the ^1L_a transitions of the two adjacent equatorial *p*-bromobenzoate chromophores of negative chirality as shown in Figure 6b. The negative exciton couplet defines the negative chirality of the two ester bonds, which on the basis of the known relative configuration, unambiguously determines the absolute configuration of **2c** as (2*R*,3*R*,4*R*,4a*R*,7*R*,8a*S*). This absolute configuration corroborates the (2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*) absolute configuration of **2a** determined by the δ-lactone helicity rule, in agreement with the exciton chirality method in ring A of **2c**.

It is also noteworthy that two other CD couplets were also observed in the CD spectrum of **2c**; A positive one around 280 nm and a negative one around 195 nm. The positive CD couplet around 280 nm [283 ($\Delta\varepsilon$ = 0.23), 278 nm (–0.41)] belongs to the ^1L_b bands of the benzoates. Since the direction of the electric transition moment of the ^1L_b transition is perpendicular to that of the ^1L_a transition, the chirality of the ^1L_b transitions is opposite to that of the ^1L_a transitions, and thus positive CD couplets were measured. The negative couplet around 195 nm [198 ($\Delta\varepsilon$ = –23.25), 186 nm (23.88)] belongs to the ^1B transition of the benzoates whose electric transition moment has the same orientation as that of the ^1L_a transition, which results in a negative couplet.

Except for the configuration at C-4, the relative configuration of **3b** remained the same as that of **2d**; we therefore tentatively suggest the (2*R*,3*S*,4*S*,7*R*,8a*S*) absolute configuration for the diol **3a** based on the co-occurrence in the same fungus.

The isolation of metabolites from the polar fractions obtained by silica gel chromatography was hampered by the presence of dark brown polymeric material. The entire polar fraction was therefore subjected to acetylation in the hope that polar hydroxy groups would be converted into less polar esters, which can be purified more easily. From this experiment, a nonseparable mixture of two acetylated derivatives **2d** and **3b** was isolated (Figure 1). To compare the data of the stereoisomers, a pure sample of **2a** was independently acetylated to the diacetate **2d**. Comparison of the spectra showed that this compound **2d** was the major isomer in a 1:2.4 mixture of the diacetates **2d** and **3b** and the NMR spectra of **3b** were easily analyzed by subtraction of the **2d** signals. As expected, the spectra of the two diastereoisomeric diacetates were very similar. The most striking difference was the large $J_{3,4}$ = 9.8 Hz coupling, demonstrating the *trans*-diaxial relationship of these protons and thus the *cis* configuration of the 3- and 4-OAc groups as shown in **3b** in Figure 1. In addition, the HH-COSY and HMBC spectra showed the same connectivity as in **2a** (Figure 5). A small sample of the **2d**/**3b** mixture was treated with aqueous alkali, in the hope of obtaining a pure sample of **3a** from the saponification. However, most of the material decomposed, and the material was not sufficient for isolation of pure **3a**. Thus, the presence of the *cis*-diol **3a**, named dinemasone C, could only be deduced indirectly (but unambiguously) from its acetylated product **3b**. The four known metabolites palmitic acid,[2,3] ergosterol,[4] and the two diastereomers of 4-hydroxymellein[5] were identified by comparison of their spectroscopic data with those published in the literature.

Antimicrobial Activity

Metabolite **1a** was tested for antibacterial, antifungal, and antialgal activities (Table 3) by the agar diffusion assay method,[22,23] metabolite **2a** in a microtiter array. For the latter test, 200 μL of medium (CP for *Chlorella fusca*, MPY for *Microbotryum violaceum*, NB for *Bacillus megaterium*[22]), 50 μL of suspension of the test organism, and 10 μL of test substance, dissolved in a mixture of acetone and methanol (1:1) at a concentration 10 mg/mL, were pipetted into each well of a microtiter plate. At the higher concentration, **1a** exhibited considerable activity against the Gram-positive bacterium *Bacillus megaterium*, the fungus *Microbotryum violaceum*, and the alga *Chlorella fusca*, whereas at the lower concentration it only exhibited good antifungal activity. Compound **2a** was active against all the test organisms at a low concentration. In fact, the antifungal and antibacterial activities of **2a** are very promising.

Table 3. Biological activity of pure metabolites **1a** and **2a** against microbial test organisms.[a]

Metabolites	Concentration [mg/mL]	*Bacillus megaterium*	*Microbotryum violaceum*	*Chlorella fusca*
1a	1	0	6	0
	5	6	8	7
Penicillin	1	18	0	0
Tetracycline	1	18	0	10 gi[b]
Nystatin	1	0	20	0
Actidione	1	0	50	35
Acetone	1	0	0	50
2a[c]	0.4	42[d]	58[d]	15[d]

[a] Concentration: 50 μL at a concentration of 1 μg/μL (= 0.05 mg of test substance/test filter disc) of **1a** and of the control substances were tested in an agar diffusion assay; numbers for the agar diffusion test indicate radius of zone of inhibition in mm. [b] gi = growth inhibition, i.e. there was some growth within the zone of inhibition. [c] **2a** was tested in a microtiter array. [d] Numbers indicate % inhibition compared to the non-inoculated control.

Conclusions

Three new metabolites, dinemasones A, B and C (**1a**, **2a**, **3a**) were identified as secondary metabolites produced by the endophytic fungus *Dinemasporium strigosum*. With dinemasone A (**1a**), the family of the bioactive 1,7-dioxaspiro[5.5]undecanes, which are not uncommon in nature, is extended, whereas the hexahydropyrano[4,3-*b*]pyran-5(7*H*)-one structure of dinemasones B and C (**2a**, **3a**) is extremely rare. Their relative configurations were elucidated by extensive NMR experiments, and their absolute configurations were established by both their carbonyl n–π* CD transition and the exciton chirality method of their respective dibenzoates **1c** and **2c**. The lactone **2a** showed promising antifungal activity at low concentrations.

Experimental Section

General: For general methods and instrumentation see ref.[24] For microbiological methods and conditions of culture see ref.[23] The ^{1}H (200 and 500 MHz) and ^{13}C NMR (50 and 125 MHz) chemical shifts are reported in ppm. Hydrogen connectivity (C, CH, CH_2, CH_3) information was obtained from DEPT 135 experiments. ^{1}H and ^{13}C peak assignments were based on 2D NMR analysis (COSY, HMQC and HMBC). CD spectra were recorded with a J-810 spetropolarimeter. Column chromatography was performed by using silica gel (Merck). Preparative TLC was performed on silica 20×20 cm TLC plates (Macherey and Nagel); compounds were detected by spraying with cerium-molybdenum spray reagent followed by heating.

Computational Methods: Conformational searches were run by employing MMFF, with standard parameters implemented in Spartan 06.[25] The obtained minima were then optimized with DFT at the B3LYP/6-31G(d) level by using Gaussian 03W.[26]

Isolation of Secondary Metabolites: *Dinemasporium strigosum*, internal strain no. 6744, which had been isolated following surface sterilization from the roots of the herbaceous plant *Calystegia sepium* growing on the shores of the Baltic Sea, Wustrow, Germany, was cultivated at room temperature for 21 d on biomalt solid agar medium. The culture medium was extracted three times with ethyl acetate to obtain the crude extract (16.0 g). The crude extract was subjected to column chromatography for fractionation on silica gel by using gradients of petroleum ether/dichloromethane, then dichloromethane, followed by a gradient of dichloromethane/methanol, and finally methanol to afford a total of 15 fractions. These fractions were screened by TLC on silica gel under UV light and by spraying with cerium-molybdenum spray reagents. Methanol was added to the crude solid mass of the column fraction of petroleum ether/60% CH_2Cl_2 and kept at –20 °C for several hours. After that, the solvent was filtered off to give palmitic acid,[2,3] (35.0 mg) as a white powder. The crude mass of the column fraction of petroleum ether/75% CH_2Cl_2 after crystallization from CH_2Cl_2/acetone gave white fine needles of ergosterol[4] (8.0 mg). The column fraction of petroleum ether/55% CH_2Cl_2 was subjected to preparative TLC on silica gel (CH_2Cl_2/MeOH/AcOH, 100:0.6:0.2) to obtain 2.3 mg of the *cis* and *trans* isomers of 4-hydroxymellein[5–9] as a mixture of two diastereomers. Chromatography of a polar fraction (CH_2Cl_2/1–2% MeOH) on preparative layer silica gel plates (CH_2Cl_2/acetone/AcOH, 100:4:0.2) followed by crystallization from CH_2Cl_2/Et_2O/pentane afforded dinemasone A (**1a**) (14.6 mg) as white fine needles. The crude mass of another polar fraction (CH_2Cl_2/2% MeOH) after crystallization from CH_2Cl_2/Et_2O gave white fine needles of dinemasone B (**2a**) (59.0 mg). Compound **1a** was treated with 4-bromobenzoyl chloride to afford, after the usual workup, the mono- and bis(4-bromobenzoates) of **1b** and **1c**. In a similar way, the diacetate **1d** was obtained by usual acetylation of **1a**. Dinemasone B (**2a**) was also treated with 4-bromobenzoyl chloride to afford the mono- and bis(4-bromobenzoates) of **2b** and **2c**. The NMR spectroscopic data of all the benzoates contributed to reconfirm their respective parent structures. Some very polar fractions obtained by the silica gel chromatography (CH_2Cl_2/1.5–3% MeOH) showed very poor resolution of their compounds on TLC. A portion of the mixed polar fractions was subjected to acetylation by using acetic anhydride/pyridine in dichloromethane with the hope that polar hydroxy groups would be converted into less polar esters, which can be purified more easily. A new metabolite, dinemasone C (**3a**), was indirectly identified from the acetylated derivatives **2d/3b**.

(2*S*,3*S*,6*S*,8*R*,11*S*)-3,11-Dihydroxy-2,8-dimethyl-1,7-dioxaspiro-[5,5]undecan-4-one (Dinemasone A, 1a): Colorless crystals, m.p. 149 °C. $[\alpha]_D^{25} = -80.4$ ($c = 0.68$, $CHCl_3$). $R_f = 0.29$ (CH_2Cl_2/2.9% MeOH). CD (MeCN, $c = 7.3 \times 10^{-4}$): λ ($\Delta\varepsilon$) = 279 (–1.2), 190 (5.4) nm. IR (KBr): $\tilde{\nu}$ = 3487, 3425, 3383, 2976, 2947, 2916, 2866, 1722, 1454, 1402, 1381, 1279, 1254, 1227, 1167, 1134, 1088, 1074, 1063,

1001, 989, 930, 885 cm^{-1}. ^{1}H and ^{13}C NMR: Table 1. EIMS (70 eV, 200 °C): *m*/*z* (%) = 230 (1) [M$^+$], 241 (3), 188 (52), 186 (39), 168 (62), 145 (100), 129 (57), 127 (95), 111 (34), 87 (77), 57 (54), 43 (67). HREIMS (EI, 70 eV): calcd. for $C_{11}H_{18}O_5$ 230.1154, found 230.1153.

(2*S*,3*S*,6*S*,8*R*,11*S*)-11-Hydroxy-2,8-dimethyl-4-oxo-1,7-dioxaspiro-[5,5]undec-3-yl 4-Bromobenzoate (1b) and (2*S*,3*S*,6*S*,8*R*,11*S*)-2,8-Dimethyl-4-oxo-1,7-dioxaspiro[5,5]undec-3,11-yl Bis(4-bromobenzoate) {Dibenzoate of 3,11-Dihydroxy-2,8-dimethyl-1,7-dioxaspiro[5,5]undecan-4-one (1c)}: To a stirred solution of **1a** (5.6 mg, 0.024 mmol) in dry pyridine (2.0 mL) was added *p*-bromobenzoyl chloride (16.3 mg) and 4-(dimethylamino)pyridine (10.0 mg). The reaction mixture was stirred at room temperature for 1.5 h (TLC monitoring) and was then neutralized by addition of 1 N HCl. The mixture was extracted with CH_2Cl_2, washed with water, dried with anhydrous Na_2SO_4, filtered, and the solvents were evaporated to dryness. The resulting mixture was purified by preparative TLC on silica gel (1 mm, 1 development, CH_2Cl_2) followed by recrystallization (CH_2Cl_2/Et_2O) to afford **1b** (1.9 mg, 20.8%) and **1c** (8.6 mg, 58.3%) as gums.

Data for 1b: $[\alpha]_D^{25}$ = –68.7 (*c* = 0.15, $CHCl_3$). R_f = 0.53 (CH_2Cl_2/2.9% MeOH). UV ($CHCl_3$): λ_{max} (lg ε) = 268 nm (3.25) nm. IR (KBr): $\tilde{\nu}$ = 3504, 2926, 2854, 1745, 1728, 1591, 1456, 1275, 1104, 1012, 754 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.29 (d, $J_{8,8}$ = 6.4 Hz, 3 H, 8-C*H*$_3$), 1.46 (d, $J_{2,2}$ = 6.2 Hz, 3 H, 2-C*H*$_3$), 1.64 (m, 1 H, 9-H), 1.71 (m, 1 H, 9-H), 1.78 (m, 1 H, 10-H), 2.03 (m, 1 H, 10-H), 2.55 (d, $J_{OH,11}$ = 4.8 Hz, 1 H, 11-OH), 2.86 (dd, J_{gem} = 13.9, $J_{5,3}$ = 0.8 Hz, 1 H, 5-H), 2.98 (d, J_{gem} = 13.9 Hz, 1 H, 5-H), 3.64 (m, 1 H, 11-H), 3.91 (m, 1 H, 8-H), 4.59 (qq, $J_{2,3}$ = 10.1, $J_{2,2}$ = 6.2 Hz, 1 H, 2-H), 5.17 (dd, $J_{3,2}$ = 10.1, $J_{3,5}$ = 0.8 Hz, 1 H, 3-H), 7.63 (d, $J_{3'5',2'6'}$ = 8.6 Hz, 2 H, 3′-H, 5′-H), 7.97 (d, $J_{2'6',3'5'}$ = 8.6 Hz, 2 H, 2′-H, 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (2-*C*H$_3$), 20.9 (8-*C*H$_3$), 25.1 (C-10), 27.3 (C-9), 45.8 (C-5), 69.2 (C-11), 69.3 (C-2), 70.1 (C-8), 78.8 (C-3), 101.1 (C-6), 128.1[a] (C-4′), 128.7[a] (C-1′), 131.5[b] (C-2′, C-6′), 131.8[b] (C-3′, C-5′), 164.7 (-*C*OO), 198.3 (C-4) ppm. EIMS (70 eV, 200 °C): *m*/*z* (%) = 414 (5) [M$^+$ + 2], 412 (5) [M$^+$], 333 (14), 329 (83), 327 (83), 284 (5), 282 (5), 228 (11), 226 (11), 185 (100), 183 (100), 168 (50), 157 (31), 155 (32), 127 (32), 85 (38), 44 (43), 29 (14). [a,b] Identical superscripts represent interchangeable assignments.

Data for 1c: $[\alpha]_D^{25}$ = –48.2 (*c* = 0.6, $CHCl_3$). R_f = 0.69 (CH_2Cl_2/1.4% MeOH). UV ($CHCl_3$): λ_{max} (lg ε) = 272 (3.37) nm. CD (MeCN, *c* = 3.1 × 10^{-4}): λ ($\Delta\varepsilon$) = 283 (0.2), 278 (–0.4), 252 (–45.3), 235 (22.9), 206 (2.1), 198 (–23.2), 186 (23.9) nm. IR (KBr): $\tilde{\nu}$ = 2927, 2854, 1747, 1728, 1591, 1485, 1456, 1398, 273, 1242, 1173, 1117, 1103, 1012, 982, 847, 754 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.38 (d, $J_{8,8}$ = 6.5 Hz, 3 H, 8-C*H*$_3$), 1.40 (d, $J_{2,2}$ = 6.2 Hz, 3 H, 2-C*H*$_3$), 1.74 (m, 1 H, 9-H), 1.87 (m, 1 H, 9-H), 1.99 (m, 1 H, 10-H), 2.18 (m, 1 H, 10-H), 2.82 (dd, J_{gem} = 13.7, $J_{5,3}$ = 0.8 Hz, 1 H, 5-H), 2.94 (d, J_{gem} = 13.7 Hz, 1 H, 5-H), 4.06 (m, 1 H, 8-H), 4.54 (qq, $J_{2,3}$ = 10.1, $J_{2,2}$ = 6.2 Hz, 1 H, 2-H), 5.13 (dd, $J_{11,10}$ = 7.5, $J_{11,10}$ = 3.9 Hz, 1 H, 11-H), 5.16 (d, $J_{3,2}$ = 10.1 Hz, 1 H, 3-H), 7.63[a] (d, $J_{3''5''}$ = $J_{2''6''}$ = 8.7 Hz, 2 H, 3′′-H, 5′′-H), 7.65[a] (d, $J_{3'5'}$ = $J_{2'6'}$ = 8.7 Hz, 2 H, 3′-H, 5′-H), 7.96[b] (d, $J_{2''6''}$ = $J_{3''5''}$ = 8.7 Hz, 2 H, 2′′-H, 6′′-H), 7.98[b] (d, $J_{2'6'}$ = $J_{3'5'}$ = 8.7 Hz, 2 H, 2′-H, 6′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.7 (2-*C*H$_3$), 21.0 (8-*C*H$_3$), 22.6 (C-10), 34.1 (C-9), 46.7 (C-5), 69.1 (C-2), 70.0 (C-8), 71.3 (C-11), 78.9 (C-3), 100.0 (C-6), 128.1[c] (C-4′′), 128.4[c] (C-1′′), 128.7[c] (C-4′), 129.0[c] (C-1′), 131.3[d] (C-2′′, C-6′′), 131.5[d] (C-2′, C-6′), 131.8[d] (C-3′′, C-5′′), 131.9[d] (C-3′, C-5′), 164.6 (-*C*OO), 165.2 (-*C*OO), 198.0 (C-4) ppm. EIMS (70 eV): *m*/*z* (%) = 598 (1) [M$^+$ + 4], 596 (2) [M$^+$ + 2], 594 (1) [M$^+$], 554 (1), 552 (2), 550 (1), 517 (2), 515 (2), 396 (5), 394 (5), 352 (3), 350 (3), 284 (2), 282 (2), 183 (100), 157 (8), 155 (9), 104 (13), 85 (12), 43 (5). [a–d] Identical superscripts represent interchangeable assignments.

(2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*)-Hexahydro-3,4-dihydroxy-7-methyl-2-[(1*E*)-prop-1-enyl]pyrano[4,3-*b*]pyran-5(7*H*)-one (2a): $[\alpha]_D^{25}$ = –27 (*c* = 0.97, CH_2Cl_2). R_f = 0.23 (CH_2Cl_2/3.8% MeOH). CD (MeCN, *c* = 8.9 × 10^{-4}): λ ($\Delta\varepsilon$) = 218 (3.7), 191 (–0.5) nm. IR (KBr): $\tilde{\nu}$ = 3444, 3390, 2960, 2916, 2854, 1745, 1732, 1385, 1259, 1232, 1205, 1063, 1014, 958, 796 cm^{-1}. ^{1}H and ^{13}C NMR: Table 2. EIMS (70 eV, 175 °C): *m*/*z* (%) = 242 (16) [M$^+$], 224 (8), 188 (13), 172 (28), 113 (100), 100 (23), 84 (40), 71 (38), 41 (16). HREIMS (EI, 70 eV): calcd. for $C_{12}H_{18}O_5$ 242.11543, found 242.11567.

(2*R*,3*R*,4*R*,4a*R*,7*R*,8a*S*)-Octahydro-3-hydroxy-7-methyl-5-oxo-2-[(1*E*)-prop-1-enyl]pyrano[4,3-*b*]pyran-4-yl 4-Bromobenzoate (2b) and Dibenzoate of (2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*)-Hexahydro-3,4-dihydroxy-7-methyl-2-[(1*E*)-prop-1-enyl]pyrano[4,3-*b*]pyran-5(7*H*)-one (2c): To a stirred solution of **2a** (7.5 mg, 0.031 mmol) in dry pyridine (2.0 mL) were added *p*-bromobenzoyl chloride (21.0 mg) and 4-(dimethylamino)pyridine (10.0 mg). The reaction mixture was stirred at room temperature for 1.5 h (TLC monitoring) and was then neutralized by addition of 1 N HCl. The mixture was extracted with CH_2Cl_2, washed with water, dried with anhydrous Na_2SO_4, filtered, and the solvents were evaporated to dryness. The resulting mixture was purified by preparative TLC on silica gel (1 mm, 1 development, CH_2Cl_2/0.6% MeOH) followed by recrystallization (CH_2Cl_2/Et_2O) to afford **2b** (6.3 mg, 50%) and **2c** (6.0 mg, 32%) as fine needles.

Data for 2b: M.p. 143 °C. $[\alpha]_D^{25}$ = –11.9 (*c* = 0.59, $CHCl_3$); R_f = 0.33 (CH_2Cl_2/1.4% MeOH). UV ($CHCl_3$): λ_{max} (lg ε) = 268 (3.35) nm. IR (KBr): $\tilde{\nu}$ = 3498, 2925, 2854, 1755, 1747, 1714, 1590, 1485, 1398, 1284, 1275, 1196, 1120, 1068, 1012, 962, 762 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.30 (d, $J_{7,7}$ = 6.0 Hz, 3 H, 7-C*H*$_3$), 1.69 (dd, $J_{3',2'}$ = 6.5, $J_{3',1'}$ = 1.3 Hz, 3 H, 3′-C*H*$_3$), 1.69 (ddd, J_{gem} = 15.3, $J_{8,7}$ = 12.1, $J_{8,8a}$ = 2.7 Hz, 1 H, 8-H), 2.03 (br. s, 3-OH), 2.39 (ddd, J_{gem} = 15.3, $J_{8,8a}$ = 9.4, $J_{8,7}$ = 3.6 Hz, 1 H, 8-H), 3.26 (dd, $J_{4a,4}$ = 4.9, $J_{4a,8a}$ = 3.1 Hz, 1 H, 4a-H), 3.63 (t, $J_{2,3}$ = $J_{2,1'}$ = 9.2 Hz, 1 H, 2-H), 4.15 (t, $J_{3,4}$ = $J_{3,2}$ = 9.2 Hz, 1 H, 3-H), 4.22 (sext, $J_{8a,8}$ = 9.4, $J_{8a,4a}$ = 3.1, $J_{8a,8}$ = 2.7 Hz, 1 H, 8a-H), 4.26 (m, 1 H, 7-H), 5.09 (dd, $J_{4,3}$ = 9.2, $J_{4,4a}$ = 4.9 Hz, 1 H, 4-H), 5.45 (ddd, $J_{1',2'}$ = 15.4, $J_{1',2}$ = 9.2, $J_{1',3'}$ = 1.3 Hz, 1 H, 1′-H), 5.83 (qq, $J_{2',1'}$ = 15.4, $J_{2',3'}$ = 6.5 Hz, 1 H, 2′-H), 7.51 (d, $J_{3''5'',2''6''}$ = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.90 (d, $J_{2''6'',3''5''}$ = 8.5 Hz, 2 H, 2′′-H, 6′′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 18.0 (3′-*C*H$_3$), 20.3 (7-*C*H$_3$), 37.3 (C-8), 43.9 (C-4a), 68.4 (C-3), 71.7 (C-8a), 72.1 (C-7), 74.3 (C-4), 82.0 (C-2), 127.4 (C-1′), 128.6[a] (C-4′′), 128.6[a] (C-1′′), 131.6[b] (C-2′′, C-6′′), 131.8[b] (C-3′′, C-5′′), 132.4 (C-2′), 166.1 (*C*OO), 168.5 (C-5) ppm. EIMS (70 eV, 200 °C): *m*/*z* (%) = 426 (5) [M$^+$ + 2], 424 (5) [M$^+$], 356 (3), 354 (3), 309 (3), 202 (7), 200 (7), 185 (67), 183 (69), 154 (100), 113 (58), 112 (82), 71 (24), 55 (16). [a,b] Identical superscripts represent interchangeable assignments.

Data for 2c: M. p. 200 °C. $[\alpha]_D^{25}$ = –95.6 (*c* = 0.16, $CHCl_3$). R_f = 0.69 (CH_2Cl_2/1.4% MeOH). UV ($CHCl_3$): λ_{max} (lg ε) = 267 (3.72) nm. CD [MeCN, *c* = 3.1 × 10^{-4}]: λ ($\Delta\varepsilon$) = 306 sh (–0.5), 295 sh (–0.9), 288 (–0.9), 277 sh (–0.8), 252 (–5.0), 234 (7.3), 213 (3.4), 199 (–7.8) nm. IR (KBr): $\tilde{\nu}$ = 2924, 2854, 1741, 1720, 1589, 1483, 1398, 1286, 1263, 1201, 1174, 1115, 1103, 1070, 1012, 962, 756 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.42 (d, $J_{7,7}$ = 6.1 Hz, 3 H, 7-C*H*$_3$), 1.62 (dd, $J_{3',2'}$ = 6.6, $J_{3',1'}$ = 1.6 Hz, 3 H, 3′-C*H*$_3$), 1.84 (dtd, J_{gem} = 15.4, $J_{8,7}$ = 12.1, $J_{8,8a}$ = 2.8 Hz, 1 H, 8-H), 2.53 (ddd, J_{gem} = 15.4, $J_{8,8a}$ = 9.3, $J_{8,7}$ = 3.6 Hz, 1 H, 8-H), 3.45 (dd, $J_{4a,4}$ = 5.0, $J_{4a,8a}$ = 3.2 Hz, 1 H, 4a-H), 3.95 (t, $J_{2,3}$ = $J_{2,1'}$ = 7.8 Hz, 1 H, 2-H), 4.38 (m, 1 H, 7-H), 4.38 (sext, $J_{8a,8}$ = 9.3, $J_{8a,4a}$ = 3.2, $J_{8a,8}$ =

2.8 Hz, 1 H, 8a-H), 5.45 (dd, $J_{4,3}$ = 9.6, $J_{4,4a}$ = 5.0 Hz, 1 H, 4-H), 5.51 (ddd, $J_{1',2'}$ = 15.3, $J_{1',2}$ = 7.8, $J_{1',3'}$ = 1.6 Hz, 1 H, 1′-H), 5.78 (qq, $J_{2',1'}$ = 15.3, $J_{2',3'}$ = 6.6 Hz, 1 H, 2′-H), 5.87 (t, $J_{3,4}$ = $J_{3,2}$ = 9.6 Hz, 1 H, 3-H), 7.54 (d, $J_{3'',5'',2'',6''}$ = 8.5 Hz, 2 H, 3′′-H, 5′′-H), 7.54 (d, $J_{3''',5''',2''',6'''}$ = 8.5 Hz, 2 H, 3′′′-H, 5′′′-H), 7.80[a] (d, $J_{2'',6'',3'',5''}$ = 8.5 Hz, 2 H, 2′′-H, 6′′-H), 7.90[a] (d, $J_{2''',6''',3''',5'''}$ = 8.5 Hz, 2 H, 2′′′-H, 6′′′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.8 (3′-*C*H_3), 20.4 (7-*C*H_3), 37.3 (C-8), 44.1 (C-4a), 69.4 (C-3), 71.9 (C-4), 72.0 (C-7), 72.0 (C-8a), 80.7 (C-2), 126.6 (C-1′), 128.1[b] (C-1′′), 128.3[b] (C-4′′), 128.5[b] (C-1′′′), 128.7[b] (C-4′′′), 131.1[c] (C-2′′, C-6′′), 131.5[c] (C-2′′′, C-6′′′), 131.8 (C-3′′′, C-5′′′), 131.8 (C-3′′, C-5′′), 132.4 (C-2′), 164.3 (*C*OO), 165.8 (*C*OO), 167.7 (C-5) ppm. EIMS (70 eV, 200 °C): *m*/*z* (%) = 610 (2) [M^+ + 4], 608 (3) [M^+ + 2], 606 (2) [M^+], 408 (8), 406 (8), 338 (12), 336 (12), 223 (68), 183 (100), 155 (9), 104 (10), 43 (4). [a–c] Identical superscripts represent interchangeable assignments.

(2*R*,3*S*,4*S*,4a*S*,7*R*,8a*S*)-3,4-Diacetoxy-hexahydro-7-methyl-2-[(1*E*)-prop-1-enyl]pyrano[4,3-*b*]pyran-5(7*H*)-one (2d) and (2*R*,3*S*,4*R*,4a*S*,7*R*,8a*S*)-3,4-Diacetoxy-hexahydro-7-methyl-2-[(1*E*)-prop-1-enyl]pyrano[4,3-*b*]pyran-5(7*H*)-one (3b): To a suspension of 249 mg of a polar fraction (CH_2Cl_2/1.5–3% MeOH) in CH_2Cl_2 (5 mL) and pyridine (2 mL) were added acetic anhydride (0.6 mL) and DMAP (15 mg), and the mixture was stirred at room temperature for 5 h (TLC control). The mixture was worked up by dilution with 2 N HCl and extraction with CH_2Cl_2 (30 mL × 3). The combined extracts were washed with water, dried (Na_2SO_4), and concentrated in vacuo to give a semisolid residue (323 mg). Flash chromatography of the crude extract followed by repeated preparative TLC on silica gel (toluene/20% ethyl acetate) afforded a mixture of **3b** and **2d** (**2d**/**3b** = 2.4:1; 9.6 mg) as colorless gum. In a similar way, 3 mg of **2a** was acetylated to yield 3.6 mg of pure **2d**, identical with the major compound in the mixture.

Data for 3b: *Optical rotation: $[\alpha]_D^{25}$ = +3.7 (*c* 0.94, $CHCl_3$). *R_f = 0.28 (CH_2Cl_2/1.4% MeOH). *UV ($CHCl_3$): λ_{max} (lg ε) = 270 (2.31), 271 (2.30), 274 (2.29) nm. *IR (KBr): $\tilde{\nu}$ = 2925, 1755, 1747, 1732, 1373, 1246, 1230, 1198, 1059, 962 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.41 (d, $J_{7,7}$ = 6.2 Hz, 3 H, 7-CH_3), 1.70 (dd, $J_{3',2'}$ = 6.5, $J_{3',1'}$ = 1.7 Hz, 3 H, 3′-CH_3), 1.75 (dtd, J_{gem} = 15.3, $J_{8,7}$ = 12.0, $J_{8,8a}$ = 3.3 Hz, 1 H, 8-H), 1.99 (s, 3 H, 3-OCOCH_3), 2.17 (s, 3 H, 4-OCOCH_3), 2.44 (ddd, J_{gem} = 15.3, $J_{8,8a}$ = 9.3, $J_{8,7}$ = 3.6 Hz, 1 H, 8-H), 2.88 (t, $J_{4a,4}$ = $J_{4a,8a}$ = 3.3 Hz, 1 H, 4a-H), 4.08 (dd, $J_{2,3}$ = 10.0, $J_{2,1'}$ = 7.7 Hz, 1 H, 2-H), 4.32 (m, 1 H, 7-H), 4.44 (tt, $J_{8a,8}$ = 9.3, $J_{8a,8}$ = $J_{8a,4a}$ = 3.3 Hz, 1 H, 8a-H), 5.11 (dd, $J_{3,2}$ = 10.0, $J_{3,4}$ = 3.3 Hz, 1 H, 3-H), 5.38 (ddd, $J_{1',2'}$ = 15.3, $J_{1',2}$ = 7.7, $J_{1',3'}$ = 1.7 Hz, 1 H, 1′-H), 5.79** (1 H, 2′-H), 5.83 (t, $J_{4,3}$ = $J_{4,4a}$ = 3.3 Hz, 1 H, 4-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.9 (3′-*C*H_3), 20.5 (7-*C*H_3), 20.7 (3-OCO*C*H_3), 20.9 (4-OCO*C*H_3), 36.7 (C-8), 44.6 (C-4a), 67.0 (C-4), 67.8 (C-3), 67.9 (C-8a), 72.2 (C-7), 76.0 (C-2), 127.4 (C-1′), 131.5 (C-2′), 169.0 (C-5), 169.2 (3-O*C*OCH$_3$), 169.6 (4-O*C*OCH$_3$) ppm. *EIMS (70 eV, 200 °C): *m*/*z* (%) = 326 (5) [M^+], 283 (10), 266 (34), 224 (34), 223 (72), 213 (34), 196 (32), 171 (33), 154 (64), 113 (76), 112 (60), 71 (43), 43 (100). *HREIMS (EI, 70 eV): calcd. for $C_{16}H_{22}O_7$ 326.13656, found 326.13657. * Data for mixed diacetates **3b** and **2d**. ** *J* value could not be provided due to overlapping with another signal.

Data for 2d: ^{1}H NMR (500 MHz, $CDCl_3$): δ = 1.39 (d, $J_{7,7}$ = 6.2 Hz, 3 H, 7-CH_3), 1.69 (dd, $J_{3',2'}$ = 6.4, $J_{3',1'}$ = 1.8 Hz, 3 H, 3′-CH_3), 1.76 (dtd, J_{gem} = 15.2, $J_{8,7}$ = 12.1, $J_{8,8a}$ = 2.9 Hz, 1 H, 8-H), 2.01 (s, 3 H, 3-OCOCH_3), 2.11 (s, 3 H, 4-OCOCH_3), 2.45 (ddd, J_{gem} = 15.2, $J_{8,8a}$ = 9.2, $J_{8,7}$ = 3.7 Hz, 1 H, 8-H), 3.32 (dd, $J_{4a,4}$ = 5.0, $J_{4a,8a}$ = 2.9 Hz, 1 H, 4a-H), 3.72 (t, $J_{2,3}$ = $J_{2,1'}$ = 8.7 Hz, 1 H, 2-H), 4.23 (tt, $J_{8a,8}$ = 9.2, $J_{8a,8}$ = $J_{8a,4a}$ = 2.9 Hz, 1 H, 8a-H), 4.32 (m, 1 H, 7-H), 5.04 (dd, $J_{4,3}$ = 10.0, $J_{4,4a}$ = 5.0 Hz, 1 H, 4-H), 5.43 (ddd, $J_{1',2'}$ = 15.3, $J_{1',2}$ = 7.9, $J_{1',3'}$ = 1.8 Hz, 1 H, 1′-H), 5.48 (t, $J_{3,2}$ = $J_{3,4}$ = 9.8 Hz, 1 H, 3-H), 5.79 (ddd, $J_{2',1'}$ = 15.3, $J_{2',3'}$ = 6.4, $J_{2',2}$ = 0.7 Hz, 1 H, 2′-H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 17.8 (3′-*C*H_3), 20.3 (7-*C*H_3), 20.8 (3-OCO*C*H_3), 21.0 (4-OCO*C*H_3), 37.2 (C-8), 43.8 (C-4a), 68.5 (C-3), 71.5 (C-4), 71.8 (C-8a), 71.9 (C-7), 80.8 (C-2), 127.0 (C-1′), 131.9 (C-2′), 167.8 (C-5), 169.1 (3-O*C*OCH$_3$), 171.2 (4-O*C*OCH$_3$) ppm.

Acknowledgments

K. K., M. H. S., B. S. and S. D. thank the BASF AG and the Bundesministerium für Bildung und Forschung (BMBF) (project no. 03F0360A), and S. A. and T. K. thank the Hungarian Scientific Research Fund (OTKA) and the National Office for Research and Technology (NKTH) for financial support (T-049436, NI-61336 and K-68429). We thank Kathrin Meier and Qunxiu Hu for excellent technical assistance.

[1] K. Krohn, M. H. Sohrab, S. Draeger, B. Schulz, *Nat. Prod. Commun.* **2008**, *3*, in press.
[2] D. R. Lide, *Handbook of Chemistry and Physics*, CRC Press LLC, Boca Raton **1997**, pp. 3–183.
[3] Y. H. Kuo, S. M. Lee, J. S. Lai, *Chin. Pharm. J.* **2000**, *52*, 27–34.
[4] G. Goulston, E. I. Mercer, L. J. Goad, *Phytochemistry* **1975**, *14*, 457–462.
[5] R. L. Edwards, D. J. Maitland, C. L. Oliver, M. S. Pacey, L. Shields, A. J. S. Whalley, *J. Chem. Soc., Perkin Trans. 1* **1999**, 715–719.
[6] M. Devys, M. Barbier, J.-F. Bousquet, A. Kollmann, *Z. Naturforsch., Teil C* **1992**, *47*, 779–781.
[7] Y. Izawa, T. Hirose, T. Shimizu, K. Koyama, S. Natori, *Tetrahedron* **1989**, *45*, 2323–2335.
[8] D. C. Aldridge, S. Galt, D. Giles, W. B. Turner, *J. Chem. Soc. C* **1971**, 1623–1627.
[9] U. Höller, G. M. König, A. D. Wright, *J. Nat. Prod.* **1999**, *62*, 114–118.
[10] D. A. Lightner, *The Octant Rule in Circular Dichroism – Principles and Applications* (Eds.: N. Berova, K. K. Nakanishi, R. W. Woody), Wiley-VCH, New York, **2000**, vol. 2, chapter 10, p. 261.
[11] E. L. Eliel, S. H. Wilen, L. N. Mander, *Stereochemistry of Organic Compounds*, John Wiley & Sons, New York, **1994**, chapter 13, p. 1022.
[12] A. Hoeltzel, C. Kempter, J. W. Metzger, G. Jung, I. Groth, T. Fritz, H.-P. Fiedler, *J. Antibiot.* **1998**, *51*, 699–707.
[13] H. Takahashi, H. Osada, H. Koshino, T. Kudo, S. Amano, S. Shimizu, M. Yoshihama, K. Isono, *J. Antibiot.* **1992**, *45*, 1409.
[14] M. Ubukata, H. Koshino, H. Osada, K. Isono, *J. Chem. Soc., Chem. Commun.* **1994**, 1877–1878.
[15] R. Baker, R. Herbert, A. H. Parton, P. E. Howse, Q. T. Jones, W. Francke, W. Reith, *J. Chem. Soc., Chem. Commun.* **1980**, 52.
[16] J. E. Hochlowski, J. C. Coll, D. J. Faulkner, J. E. Biskupiak, C. M. Ireland, Z. Qi-tai, H. Cun-heng, J. Clardy, *J. Am. Chem. Soc.* **1984**, *106*, 6748–6750.
[17] I. Paterson, A. S. Franklin, *Tetrahedron Lett.* **1994**, *35*, 6925–6928.
[18] a) W. Klyne, P. M. Scopes, "The Carboxyl and Related Chromophores" in *Fundamental Aspects and Recent Developments in Optical Rotatory Dispersion and Circular Dichroism* (Eds.: F. Ciardelli, P. Salvadori), Heiden, London, **1973**, pp. 126ff; b) S. Stončius, U. Berg, E. Butkus, *Tetrahedron: Asymmetry* **2004**, *15*, 2405–2413.
[19] S. Yoshimura, B. Sato, S. Takase, H. Terano, *J. Antibiot.* **2004**, *57*, 429–435.

[20] R. Hoefs, M. Walker, A. Zeeck, *Angew. Chem. Int. Ed.* **2000**, *39*, 3258–3261.
[21] N. Berova, K. Nakanishi, "Exciton Chirality Method: Principles and Applications" in *Circular Dichroism – Principles and Applications* (Eds.: N. Berova, K. Nakanishi, R. W. Woody), Wiley-VCH, New York, **2000**, vol. 2, chapter 12, pp. 337–382.
[22] B. Schulz, J. Sucker, H.-J. Aust, K. Krohn, K. Ludewig, P. G. Jones, D. Döring, *Mycol. Res.* **1995**, *99*, 1007–1015.
[23] W. Zhang, K. Krohn, H. Egold, S. Draeger, B. Schulz, *Eur. J. Org. Chem.* **2008**, accepted.
[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] Spartan 06: Wavefunction, Inc., www.wavefun.com.
[26] Gaussian 03W: M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, J. A. Pople, Gaussian, Inc., Wallingford CT, www.gaussian.com.

Received: July 11, 2008
Published Online: October 13, 2008

