X-ray Structure Determination, Absolute Configuration and Biological Activity of Phomoxanthone A[‡]

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The dimeric xanthone phomoxanthone A (**1a**), a cytochalasin L-696,474 (**2a**), the corresponding 21-*O*-deacetyl-L-696,474 (**2b**), and 3-nitropropionic acid (**3**) were isolated from the endophytic fungus *Phomopsis* sp. The relative configuration of phomoxanthone A (**1a**) was elucidated by X-ray single-crystal analysis. The comparison of the calculated and measured CD spectra and CD calculation of model compounds revealed that the axial chirality along the biaryl axis dominates the CD spectra of the dimeric xanthone **1a**, and can thus be determined by CD calculation as (a*S*). Knowing the axial chirality, the absolute configurations of the stereogenic centers could be determined from the X-ray data of **1a** as (5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*). In addition, it was demonstrated that in both the solid state (KBr) and solution (methanol/dichloromethane, 4:1) **1a** has the same conformation, i.e. similar dihedral angles along the biaryl axis. Comparison of solid-state CD measurement with calculation of CD data based on X-ray coordinates was introduced for the first time. Phomoxanthone A (**1a**) showed activity against bacteria and phytopathogenic fungi.

Introduction

In connection with our ongoing screening for biologically active secondary metabolites from fungi,[1] we investigated an endophytic *Phomopsis* sp. (internal strain No. 5049), isolated from the stem of *Costus* sp. (Costaceae) growing in the rain forest of Costa Rica. Teleomorph of the mitosporic fungus, *Phomopsis* is *Diaporthe*, an Ascomycete. The fungus was cultivated on biomalt semi-solid agar medium and alternatively in biomalt liquid culture. The crude ethyl ether extracts of both cultures showed good antifungal activity against *Ustilago violacea* and very good antibacterial activity against *Escherichia coli* and *Bacillus megaterium*.

From the *n*-hexane/chloroform solution of the crude extract, a precipitate separated upon cooling. The precipitate was collected and purified by crystallization from dichloromethane/diethyl ether solution to afford yellowish needles of compound **1a**, m.p. 220–225 °C. The compound was optically active {$[a]_D^{22}$ = +82 (c = 0.98 in CH_2Cl_2)} and its structure was elucidated by NMR investigations and confirmed by X-ray single-crystal analysis (vide infra). A literature search showed that the same compound, named phomoxanthone A, was recently isolated from the endophytic fungus *Phomopsis* species (Scheme 1). The published melting point (214–216 °C[2]) and optical rotation {$[a]_D^{25}$ = +99 (c = 0.40, $CHCl_3$)[2]} of phomoxanthone A (**1a**) are in fair agreement with our measured values, confirming that our compound has the same structure and absolute configuration. Isaka et al. determined the relative configuration of the stereogenic centers by NMR experiments but the relative configuration of the axial chirality with respect to the stereogenic centers and the absolute configuration of **1a** have not been elucidated yet. Due to the mixed central and axial chirality elements, the determination of the absolute configuration by CD analysis is not straightforward.

The filtrate of the crystallization was further purified by column chromatography and preparative TLC to afford L-696,474 (**2a**) (10 mg), a cytochalasin acting as a HIV-1 protease inhibitor,[3] first isolated from the bark-inhabiting ascomycete *Hypoxylon fragiforme*.[4,5] In addition, the corresponding 21-*O*-deacetyl-L-696,474 (**2b**) (2 mg), not yet described as a natural product, and 3-nitropropionic acid (**3**) (5.6 mg) were isolated. 3-Nitropropionic acid (**3**) is a toxic metabolite found in Leguminosae as well as in fungi of the genera *Penicillium* or *Aspergillus*.[6] The compound was shown to be active against *Mycobacterium tuberculosis*.[7]

The structure of phomoxanthone A (**1a**) resembles that of secalonic acids (ergochromes)[8] or eumitrins,[9] dimeric xanthones biosynthetically derived from the oxidative cleavage of anthraquinones followed by dimerization.[8] How-

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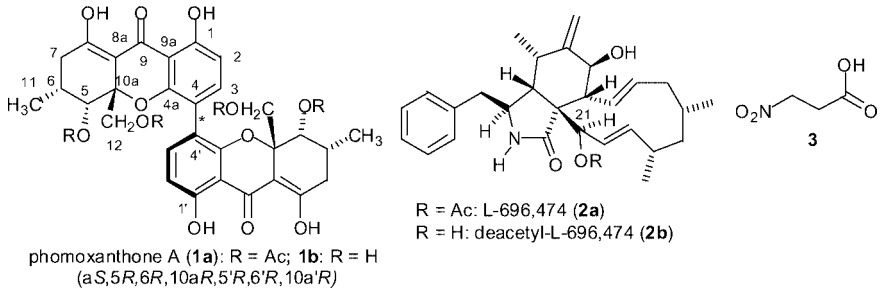


Scheme 1. Structures of compounds **1a**–**3**. *Rotation is restricted along the biaryl axis resulting in axial chirality.

ever, there are three major differences between the structure of **1a** and those of ergochromes or eumitrins: (i) the monomer units of **1a** are coupled through positions 4,4′ in contrast to the 2,2′ coupling of secalonic acids or the 4,2′ coupling of eumitrins, (ii) the ester group at C-10a is reduced to a hydroxymethyl group and converted to its acetate, and (iii) the non-chelated hydroxy groups are acetylated in **1a**.

This paper describes the compounds isolated from the *Phomopsis* sp. with emphasis on the determination of the absolute configuration of the chiral xanthone dimer **1a**, based on single-crystal X-ray analysis and comparison of the quantum-mechanically calculated CD spectrum with the experimental one. The antibacterial and antifungal properties of **1a** were also tested.

Results and Discussion

The structures of the metabolites isolated from *Phomopsis* sp. were deduced by comparison of the spectroscopic data with those reported in the literature (**1a**,[2] **2a**,[4,5] **3**[7]). Acidic hydrolysis of **1a**, using hydrochloric acid in methanol, afforded deacetyl-phomoxanthone A (**1b**).[2] The structure of the deacetylated cytochalasin (**2b**) was elucidated by comparison with that of its acetate L-696,474 (**2a**).[4,5] In addition to the missing signals for the acetyl group, the upfield shift of the signal of the proton at C-21 from δ = 5.54 ppm in **2a** to δ = 4.17 ppm in the 21-deacylation product (**2b**) confirmed the position of the free hydroxy group in **2b**.

For the elucidation of the absolute configuration of phomoxanthone A (**1a**), the quantum-mechanical calculation of its CD spectrum was performed and the result was compared with the experimental one. This was shown to be an excellent method for the determination of absolute configuration (review: refs.[10–12]), particularly with rigid structures[13,14] and axially chiral natural products such as the biaryl alkaloid ancistrocladeine,[15] phlegmacin-type fungal pigments (dimeric anthraquinones)[16] or natural bicoumarins.[17] For the CD calculation of a compound, knowledge of the relative configuration and low-energy conformers with their relative energies is a prerequisite, since all the conformers present in solution contribute to the observed CD spectrum according to their abundance. For very rigid structures, virtually existing in only one conformation, the structure determined by X-ray single-crystal analysis is of tremendous help. Fortunately, the crystals of phomoxanthone A (**1a**) obtained by recrystallization from dichloromethane/diethyl ether were suitable for X-ray single-crystal analysis. The X-ray data (Figure 1) show not only the relative configuration of the stereogenic centers of the two symmetrical monomer units, but also the relative configuration of the axial chirality with respect to these stereogenic centers, which was not known before.[2]

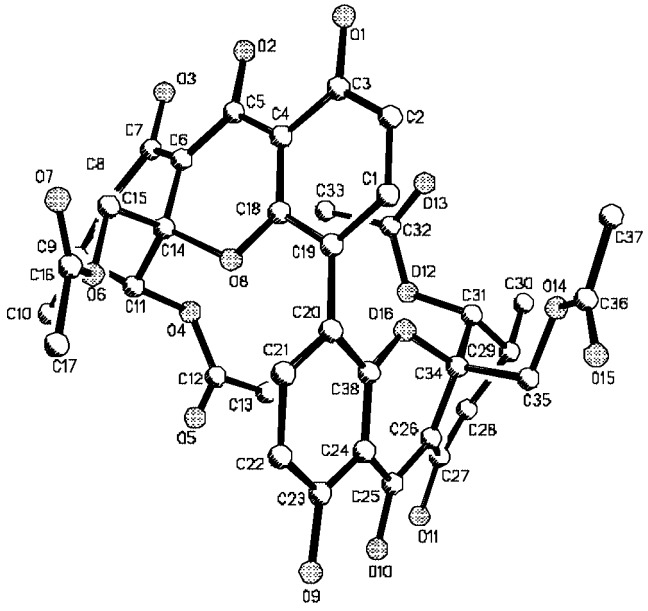


Figure 1. Molecular structure of phomoxanthone A (**1a**) (relative configuration). The dihedral angle C18–C19–C20–C38 (C4a–C4–C4′–C4a′) is 83.3(11)°.

The conformational analysis was performed by combining molecular mechanics and semiempirical (AM1)[18] methods using the MMFF94 force field with Monte-Carlo simulation in water-CT, and rotation of the biaryl axis was allowed only in the *YZ* plane. Eight minimum-energy conformers were computed semiempirically and used up to an energy cut-off of 11 kJ/mol with respect to the lowest energy conformer. These conformers differ in their dihedral angles along the biaryl axis and the orientation of the acetoxymethylene group, while the conformations of the rings are practically the same, reproducing well the X-ray structure. The theoretical calculation of the CD spectra of these eight minimum-energy conformers was carried out by the BDZDO/MCDSPD program package[19] and added up by

means of the Boltzmann statistic to obtain the overall CD spectrum.[20] In order to generate CD spectra from the rotational strengths, they were multiplied by the Gaussian function using 7 nm as halfbandwidth at 1/*e* of $\Delta\varepsilon_{max}$.

As shown in Figure 2, the Boltzmann-weighted CD spectrum of the computed conformers of (a*S*,5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*)-**1a**, and particularly the CD spectrum calculated for the (a*S*,5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*) structure of the X-ray analysis, are in quite good agreement with the experimental spectrum of **1a**. As often observed in these calculations, there is a wavelength shift due to some inaccuracy of the program in the calculation of the UV spectra and a UV correction may be introduced.[10] The match of the calculated and measured CD curves suggested that phomoxanthone A has the (a*S*,5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*) absolute configuration, since the X-ray data have already limited the number of possible structures to two (Figure 3).

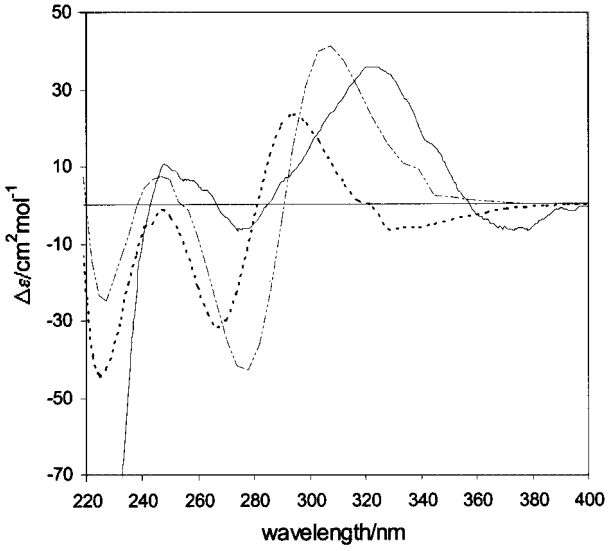


Figure 2. Comparison of the CD spectrum of **1a** measured in MeOH/CH_2Cl_2 (4:1) (−) with those calculated for the X-ray structure of **1a** with (a*S*,5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*) absolute configuration (--- −) or obtained as the weighted average of the computed (a*S*,5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*) conformers (---).

However, this absolute configuration apparently contradicts a probable configurational assignment made on the basis of the reported CD data of related secalonic acids such as secalonic acids A, D and F (SAA, SAD, SAF) (Scheme 2).

Secalonic acids contain almost the same chromophore system as phomoxanthone A but they have a 2,2′-biaryl linkage in contrast to the 4,4′ coupling of phomoxanthone A. Their (10a*R*,10a′*R*) absolute configurations were correlated with an intensive positive CE at about 330 nm.[22–24] If the same configurational assignment for secalonic acids would be applied to phomoxanthone A, the C-10a and C-10a′ stereogenic centers of phomoxanthone A should be homochiral with the corresponding ones of SAD and SAF, since phomoxanthone A has also a positive CE at 330 nm ($\Delta\varepsilon$ = 13.1 in CH_2Cl_2). This would imply that phomoxanthone A has the (5*S*,6*S*,10a*S*,5′*S*,6′*S*,10a′*S*) absolute configuration, opposite to that obtained from the CD calculation presented in this paper.

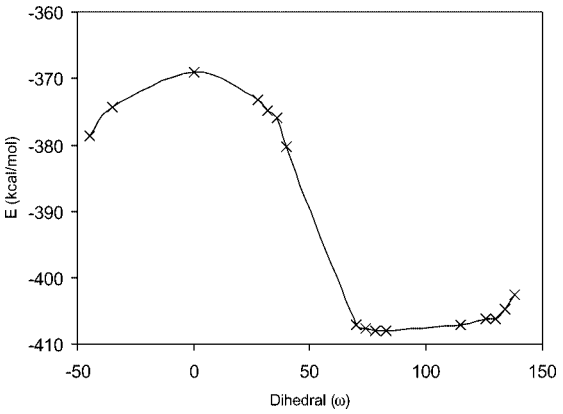
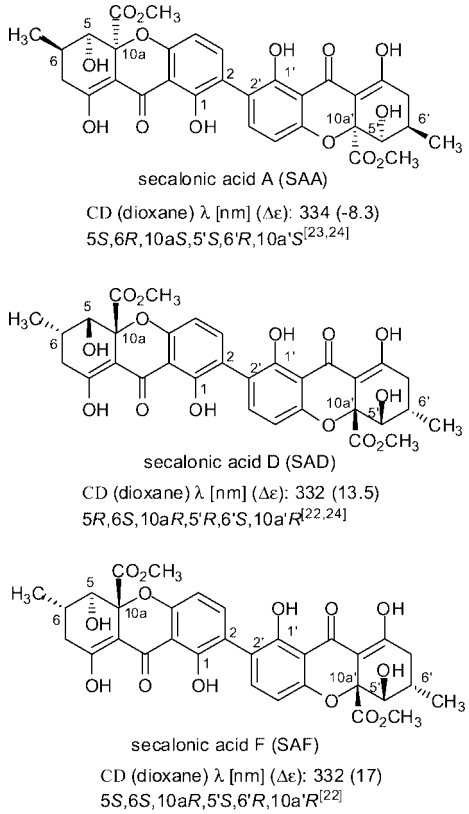


Figure 3. Plot of the energies as a function of the dihedral angles obtained by "coordinate driving" using the AM1 semiempirical model of the Spartan Program.[21] First the dihedral angle was set to 0° and then it was driven from 0° to 180° in 19 steps. After a geometry optimization, their energies were calculated semiempirically and subtraction of the lowest-energy value from the highest-energy one resulted in 37 kcal/mol.



Scheme 2. Structure of secalonic acids SAA, SAD, and SAF and their respective Cotton effects and assigned absolute configurations.

Phomoxanthone A can be viewed as an *ortho*-disubstituted biaryl derivative which, in contrast to secalonic acids, has bulky *ortho* substituents and thus the rotation along the biaryl axis is restricted which leads to atropisomers. The X-ray analysis of SAD gave interplanar angles of opposite

signs for its ethanol and acetic acid solvates, –148.9° and 148.9°, respectively, which supports the notion that there is no atropisomerism in secalonic acids.[25]

In phomoxanthone A, the calculation of the barrier of rotation (37 kcal/mol) unambiguously showed that it is a hindered biaryl and the atropisomers do not interconvert. Thus, phomoxanthone A (**1a**) contains both central and axial chirality, and in this investigation it *was of prime importance to explore which of these chirality elements determine its CD properties* since this also effects its configurational assignment.

CD calculations of three different sets of compounds were carried out to answer this question. Calculations were performed (i) on different rotamers of phomoxanthone A (**1a**), (ii) on the phomoxanthone A monomer (**4**), and (iii) on a biphenyl model compound **5**, carrying most parts of the chromophore of **1a** but lacking the stereogenic centers.

Figure 4 shows the calculated CD spectra of phomoxanthone A rotamers with opposite dihedral angles along the biaryl axis. Although rotamers with opposite dihedral angle are diastereomers, they exhibit almost mirror-image CD spectra as shown in Figure 4, which clearly indicates that it is the axial chirality that predominantly determines the CD properties of phomoxanthones.

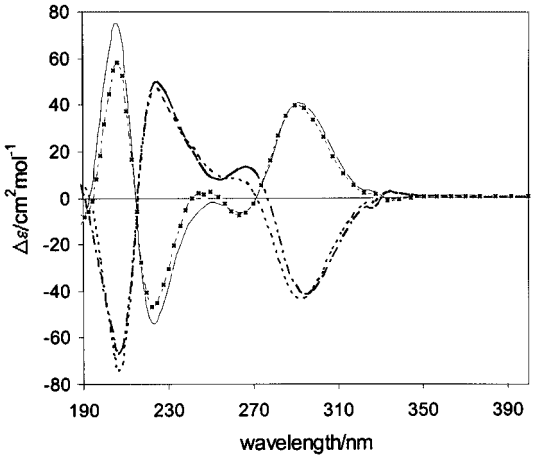
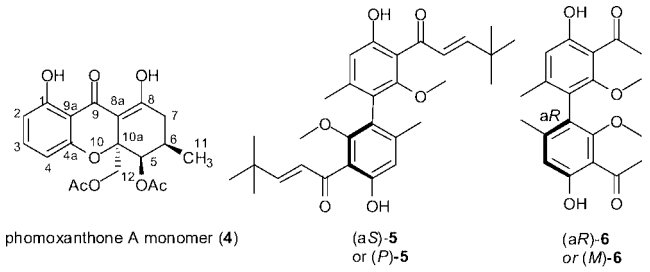


Figure 4. Calculated CD spectra of four low-energy conformers with different dihedral angle –76.09° (— ---), –75.79° (---), 78.07° (—), and 77.48° (-×-) of **1a**.

Next, the CD spectrum of phomoxanthone A monomer **4** (Scheme 3) was calculated (using the Boltzmann-weighted six minimal-energy conformations), which contains the three stereogenic centers, but lacks the axial chirality element. This way the axial chirality contribution was ruled out.

As shown in Figure 5, the calculated CD spectrum of monomer **4** shows only a negative band between 192 and 238 nm with a minimum at 209 nm and a positive CE at 290 nm. The entire curve shows quite a different characteristic from those of the calculated or measured CD curves of phomoxanthone A (**1a**) (Figure 2). Again, this demonstrates the dominating contribution of the axial chirality element on the CD spectra of bianthrones.



Scheme 3. Structures of the phomaxanthone A monomer (**4**), and the biaryls (*P*)-**5** and (*M*)-**6**.

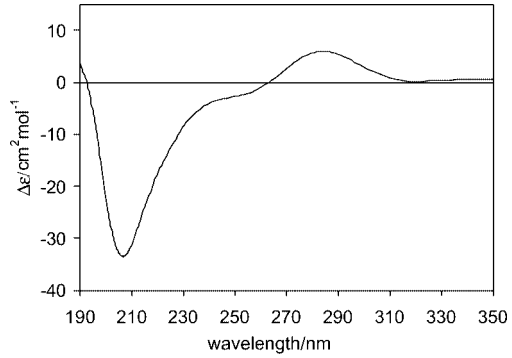


Figure 5. Calculated CD spectrum of monomer building block **4**.

Next, we calculated the CD spectrum of model compound (*P*)-**5** (using the Boltzmann-weighted average of nine minimal-energy conformers), having essential parts of the chromophore and the biaryl system of **1a** but no stereogenic centers in the side chains. The CD spectrum of (*P*)-**5**, shown in Figure 6, has a relatively good agreement with the measured CD spectrum of phomoxanthone A (**1a**), with a maximum and a positive shoulder at 232 and 317 nm, respectively, and a minimum at 256 nm. The hypsochromic shift in the CD spectrum of (*P*)-**5** may be explained by the missing bathochromic substituents in (*P*)-**5** compared to **1a**. This calculation shows again the dominating effect of the axial chirality over the central one in the chiroptical properties of **1a**.

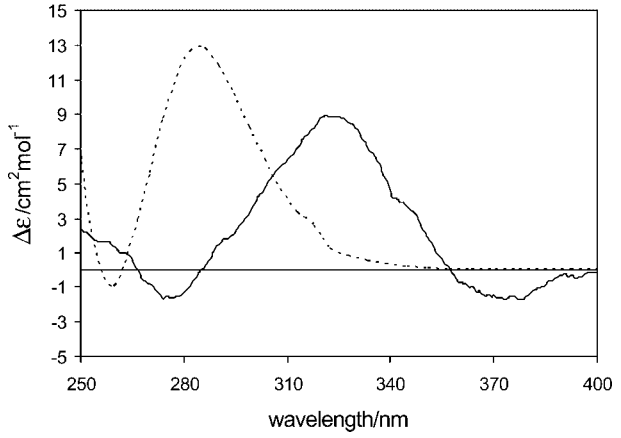


Figure 6. Comparison of the calculated CD spectrum of the biaryl compound (*P*)-**5** (---) and the experimental one (—) of **1a**.

The latter reasoning is supported by the enantiomerically pure biaryl compound (*M*)-**6** [or (a*R*)-**6**, Scheme 3], recently

synthesized by Bringmann and co-workers.[17] (*M*)-**6** has axial chirality and its measured CD spectrum shows a similar pattern but oppositely signed CEs as that of (a*S*)-**5** or (a*S*)-**1a**; i.e. (*M*)-**6** has negative, positive, negative, positive CEs at 310, 267, 241, 211 nm, respectively.

As a conclusion, it was unambiguously confirmed that the axial chirality dominates the chiroptical properties of phomoxanthone A (**1a**) and the good match of the measured CD spectrum with that calculated for (a*S*)-**1a** determines the axial chirality of **1a** as (a*S*) or (*P*). Based on the X-ray data, this also afforded the configurational assignment of the stereogenic centres as (5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*).

Since the X-ray data reflect the solid-state structure of **1a** while the measured CD spectra derive from the solvated structure, it was necessary to correlate these two structures. For this purpose, the solid-state CD spectrum of **1a** was recorded as a KBr disc and compared with the CD spectra measured in different solvent systems. As can be seen from Figure 6, the spectra measured in MeOH/CH_2Cl_2 (4:1) and in the solid state are almost superimposable, confirming that **1a** has identical conformations in the solid state and in MeOH/CH_2Cl_2 (4:1). However, the CD spectra measured in dichloromethane and MeOH/CH_2Cl_2 (4:1) are significantly different, which is in agreement with the assumption that the alcoholic solvent is breaking the chelation of hydroxy groups to carbonyl groups present in dry dichloromethane solution. Any chelation may change the dihedral angle of the biaryl units and thus the shape of the CD curve.

The very good agreement of the calculated CD curve with that of the solid-state CD (see Figures 2 and 7) has also a profound impact on the elucidation of absolute configuration. In solid-state CD spectra, the very same conformation is measured as in the calculation based on the Cartesian coordinates of X-ray data. Thus, the Boltzmann-weighting of a selected set of predominating conformations is not required. Furthermore, the CD spectra of even conformationally flexible compounds, which could be calculated only with a high degree of uncertainty, can be calculated based on X-ray data with greater probability of agreement by the solid-state CD measurement. We are presently actively engaged to apply this methodology to a large set of chiral natural products with known X-ray data.

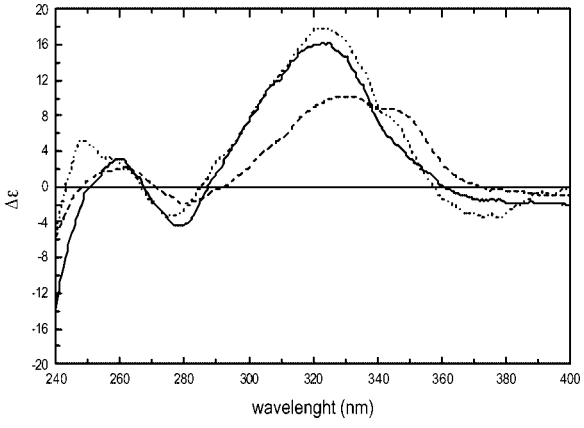


Figure 7. CD spectra of **1a** measured in MeOH/CH_2Cl_2 (4:1) (---), CH_2Cl_2 (···) and as KBr disc (−).

Bioactivity of Phomoxanthone A (1a)

Phomoxanthone A (**1a**) showed a very high activity against the Gram-positive bacterium *Bacillus megaterium* at a concentration of 10 mg/mL (radius of zone of inhibition of 3–4 cm as shown in Table 1) and a moderate activity at a concentration of 1 mg/mL. At a concentration of 10 mg/mL a moderate inhibition of the alga *Chlorella fusca* and the fungus *Ustilago violacea* was displayed. Interestingly, the deacetylated compound **1b** did not show any activity in these tests.

Table 1. Agar diffusion test of phomoxanthone A (**1a**) against *Chlorella fusca* (Chl), *Bacillus megaterium* (Bm), *Ustilago violacea* (Ust.).

Concentration[a]	Chl.	Bm.	Ust.
1 mg/1 mL	–	0.3–0.6[b]	–
10 mg/1 mL	0.2	3–4	0.5–0.8

[a] 1 mg/1 mL or 10 mg/1 mL in a 1:1 mixture of acetic ester/methanol; 50μL applied to discs of 9 mm diameter (Schleicher & Schüll). [b] Radius in cm of inhibition zone.

Phomoxanthone A (**1a**) further showed strong activity in a crop protection screening against a number of harmful fungi (Table 2), in particular against *Pyricularia oryzae*, the dangerous "riceblast" pathogen.

Table 2. Crop protection screening of **1a** against a number of harmful fungi.[a]

ppm[b]	Phytin.	Botrci.	Pyrior.	Septtr.
125	2.2	8.8	0.0	0.9
31	0.4	8.7	0.5	0.3
8	99.2	8.0	0.1	0.4
2	100.0	73.3	42.3	68.5

[a] Phytin.: *Phytophthora infestans*; Botrci.: *Botrytis cinerea*; Pyrior.: *Pyricularia oryza*; Septtr.: *Septoria tritici*. [b] Numbers indicate the percentage of fungal growth at the given concentrations.

Conclusion

In summary, the absolute configuration of the xanthone dimer phomoxanthone A (**1a**) was elucidated to be (a*S*,5*R*,6*R*,10a*R*,5′*R*,6′*R*,10a′*R*) by a combination of X-ray analyis, measured and calculated CD spectra. Furthermore, it was demonstrated by conformational analysis and CD study of related model compounds that **1a** possesses axial chirality which dominates its characteristic CD properties. Comparison of its solid-state CD spectrum with that calculated for the Cartesian coordinates of the X-ray data revealed that **1a** has near identical conformation in the solid state and in MeOH/CH_2Cl_2 (4:1) solution. The method has the potential of elucidating the absolute configurations of all compounds of medium molecular weight by calculating a single solid-state conformation, provided the atomic coordinates are known from X-ray analysis.

Phomoxanthone A (**1a**) shows good activity against Gram-positive bacteria and is highly antifungal, in particular against *Pyricularia oryzae*.

Experimental Section

General: Melting points were measured with a Büchi SMP-20 apparatus and are not corrected. NMR spectra were recorded with a Bruker AMX-600 spectrometer (^{1}H: 600 MHz; ^{13}C: 150 MHz) or AMX-300 (^{1}H: 300 MHz; ^{13}C: 75 MHz). The CD spectra were recorded with a J-810 spectropolarimeter and the concentrations for the CD measurements are given in mol/dm^3. For the solid-state CD measurement, the disc was prepared by mixing 241.1 mg of KBr (Aldrich, 98%, heated at 100 °C) and 64 μg of **1a** with a Perkin–Elmer vibrating mill for 5 min. Then the mixture was pressed at 10 t with a Perkin–Elmer press under vacuum to obtain a transparent disc for CD measurements. Four slightly different spectra were recorded by rotating the disc in 90° intervals, which were averaged to produce the overall solid-state spectrum of **1a**. The value of $\Delta\varepsilon$ was calculated by using the molar concentration (mol·L^{-1}) of the sample in KBr and the thickness of the disc measured by a micrometer as the path length.[26] For other general methods, instrumentations and culture conditions, see ref.[27]

Isolation: Two cultures (each 3 L) of the endophytic *Phomopsis* sp. (internal strain No. 5049) isolated from the stem of *Costus* sp. (Costaceae) growing in a rain forest in Costa Rica, were grown on biomalt semisolid agar and malt-soya media for 70 d. The cultures were then homogenized in a Waring blender and extracted three times with 500 mL each of ethyl acetate. The two extracts did not show significant differences by TLC analysis or biological activity and were therefore combined. The combined ethyl acetate extracts were dried (Na_2SO_4), filtered, and the solvent was removed to yield 3.0 g of a gummy residue. The residue was partly dissolved in a mixture of 15 mL of *n*-hexane and 1 mL of chloroform. On cooling the soluble fraction overnight, the dimeric xanthone phomoxanthone A (**1a**) crystallized, which was purified by recrystalization from dichloromethane/diethyl ether to yield 101 mg of pure **1a**. The insoluble fraction (ca. 2.3 g) was again partly dissolved in 8 mL of chloroform. The soluble fraction was separated by column chromatography on silica gel (dichloromethane/1–3% methanol; first column cgromatography, then TLC) to yield the cytochalasins **2a** (10 mg) and **2b** (2.0 mg). The insoluble part of the chloroform extraction was stirred with 10 mL of dichloromethane. The dichloromethane solution was chromatographed on silica gel to yield 3-nitropropionic acid (**3**) (5.6 mg).

Data for Phomoxanthone A (1a): M.p. 220–225 °C (ref.[2] m.p. 214–216 °C). $[\alpha]_D^{22}$ = +82, (c = 0.98 in CH_2Cl_2) {ref.[2] $[\alpha]_D^{25}$ = +99, (c = 0.40 in $CHCl_3$)}. CD (CH_2Cl_2; $c = 1.7\times10^{-4}$): λ ($\Delta\varepsilon$) = 346 sh (11.1), 330 (13.1), 279 (–2.5), 261 (2.8), 254 sh (2.1), 228 sh (–45.6), 212 nm (–65.2 cm^{2}mmol^{-1}). CD (MeOH/CH_2Cl_2, 4:1; $c = 1.5\times10^{-4}$): λ ($\Delta\varepsilon$) = 378 (–3.4), 344 sh (7.8), 321 (17.8), 274 (–3.3), 258 sh (3.1), 248 (5.2), 226 (–51.9), 210 nm (–75.9 cm^{2}mmol^{-1}). CD (KBr disc, $c = 9.7\times10^{-4}$): λ ($\Delta\varepsilon$) = 401 (–0.6), 343 sh (5.6), 324 (16.3), 278 (–4.4), 260 (3.1), 228 sh (–48.9), 212 nm (–67.7 cm^{2}mmol^{-1}). IR: $\tilde{\nu}$ = 3060, 2969, 1747, 1614, 1585, 1469, 1220, 1045, 917 cm^{-1}. UV (CH_2Cl_2): λ (lg ε) = 345 (78), 325 (86), 270 (47) nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.99 (d, 3 H, J = 6.0 Hz, 11-H), 1.98 (s, 3 H, 14-H), 2.04 (s, 3 H, 16-H), 2.33 (m, 2 H, 6-H), 2.37 (m, 1 H, 7-H), AB signal [$\Delta\delta$ = 0.11, δ_A = 4.27 (d, 1 H, ^2J = 12.76, 12a-H), δ_B = 4.16 (d, 1 H, ^2J = 12.76 Hz, 12b-H)], 5.41 (s, 1 H, 5-H), 6.56 (d, 1 H, J = 9.0 Hz, 2-H), 7.43 (d, 1 H, J = 9.0 Hz, 3-H), 11.48 (s, 1 H, 1-OH), 14.07 (s, 1 H, 8-OH) ppm. ^{13}C NMR (150 MHz, $CDCl_3$): δ = 17.91 (q, C-11), 20.84 (q, C-14), 21.07 (q, C-16), 27.98 (d, C-6), 33.60 (t, C-7), 64.99 (t, C-12), 70.77 (d, C-5), 80.78 (s, C-10a), 100.53 (s, C-8a), 106.67 (s, C-4), 109.92 (d, C-2), 115.78 (s, C-9a), 141.57 (d, C-3), 154.25 (s, C-4a), 161.93 (s, C-1), 169.99 (s, C-15), 170.47 (s, C-13), 178.08 (s, C-8), 188.16 (s, C-9) ppm. HRMS (EI): calcd. for $C_{38}H_{38}O_{16}$ 750.21599; found 750.21463.

X-ray Structure Determination: Data were collected with a Bruker-AXS P4 diffractometer with graphite monochromator, λ(Mo-K_α) = 0.71073 Å. The compound crystallized in a non-centrosymmetric space group; however, in the absence of significant anomalous scattering effects, the Flack parameter[28] is essentially meaningless. Accordingly, Friedel pairs were merged. The structure was solved by direct methods, full-matrix least-squares refinements based on F^2, all but the H atoms were refined anisotropically. The H atoms were positioned at idealized positions riding on their attached C or O atoms, with isotropic displacement parameters U_{iso}(H) = 1.5·U_{eq}(CH_3 or OH) or 1.2·U_{eq}(C). All CH_3 groups were allowed to rotate but not to tip. There are two independent but geometrically identical molecules per asymmetric unit, as well as two solvent water molecules. Pertinent crystallographic data are given in Table 3, Figure 1 shows the molecular structure. Programs used: Bruker package.[29] CCDC-192082 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Table 3. Crystallographic data of **1a**.

Empirical formula	$C_{38}H_{40}O_{17}$
Formula mass	768.7
Temperature [K]	200(2)
Wavelength [Å]	0.71073
Crystal system	monoclinic
Space group	$P2_1$
Unit cell dimensions	a = 11.179(4) Å b = 16.541(6) Å, β = 91.68(1)° c = 20.589(6) Å
Volume	3806(2) Å^3
Z	4
$D_{calcd.}$ [Mg/cm^3]	1.342
Absorption coefficient	0.107 mm^{-1}
F(000)	1616
Crystal size [mm]	0.48 × 0.45 × 0.06
θ range for data collection [°]	2.33–25.90
Index ranges h, k, l	–13/1, –1/20, –25/25
Reflections collected	9427
Independent reflections	7599 [R(int) = 0.038]
Absorption correction	ψ-scans
Max./min. transmission	0.993/0.951
Refinement method	full-matrix least squares on F^2
Data/restraints/parameters	7599/1/1000
Goodness-of-fit on F^2	0.958
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.068, wR_2 = 0.163
R indices (all data)	R_1 = 0.150, wR_2 = 0.229
Largest diff. peak/hole [e·Å^{-3}]	0.81/–0.31

Deacetylation of Phomoxanthone A (1a): A solution of **1a** (5.0 mg) in 2 mL of methanol was treated with 2 drops of TMSCl to generate hydrochloric acid. The mixture was stirred overnight and the solvent was then removed at reduced pressure. The residue was purified by TLC to afford 3.2 mg of the deacetylation product **1b** as yellow crystals; m.p. 213–214 °C (ref.[2] m.p. 208–210 °C).

Data for 1b: $[\alpha]_D^{22}$ = +27.8 (c = 0.13 in CH_2Cl_2) {ref.[2] $[\alpha]_D^{25}$ = +14 (c = 0.87 in $CHCl_3$)}. CD (CH_2Cl_2; $c = 2.7\times10^{-4}$): λ ($\Delta\varepsilon$) = 355 (–1.2), 330 (5.5), 272 (–1.5), 257 (–1.0), 226 sh (–8.7), 215 nm (–11.3

$cm^2 mmol^{-1}$). CD (MeOH/CH_2Cl_2, 4:1; $c = 1.6 \times 10^{-4}$): λ ($\Delta\varepsilon$) = 365 (–0.7), 335 (5.7), 261 (–4.1), 237 (2.2), 210 nm (–11.2 $cm^2 mmol^{-1}$). IR: $\tilde{\nu}$ = 3429, 2956, 2876, 1731, 1644, 1463, 1434, 1387, 1272, 1033 cm^{-1}. UV (CH_2Cl_2): λ_{max} (lg ε) = 329 (4.0), 269 (3.65) nm. ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.14 (d, 3 H, J = 6.7 Hz, 11-H), 2.11 (m, 1 H, J = 6.3, J = 11.0 Hz, 6-H), 2.33 (dd, 1 H, J = 11.0, J = 19.2 Hz, 7a-H), 2.50 (dd, 1 H, J = 6.3, J = 19.2 Hz, 7b-H), AB signal [$\Delta\delta$ = 0.45, δ_A = 3.84 (d, 1 H, ^2J = 13.0 Hz, 12a-H), δ_B = 3.39 (d, 1 H, ^2J = 13.0 Hz, 12b-H)], 5.29 (d, 1 H, J = 0.7 Hz, 5-H), 6.60 (d, 1 H, J = 8.6 Hz, 2-H); 7.27 (d, 1 H, J = 8.6 Hz, 3-H); 11.55 (s, 1 H, 1-OH); 14.01 (s, 1 H, 8-OH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 17.77 (q, C-11), 28.48 (d, C-6), 32.80 (t, C-7), 64.37 (t, C-12), 68.87 (d, C-5), 84.41 (s, C-10a); 100.72 (s, C-8a), 107.14 (s, C-4); 110.85 (d, C-2), 116.25 (s, C-9a), 139.23 (d, C-3), 154.17 (s, C-4a), 161.98 (s, C-1), 179.70 (s, C-8), 188.03 (s, C-9) ppm. HRMS (EI): calcd. for $C_{30}H_{30}O_{12}$ 582.17404; found 582.17373.

Data for Cytochalasin 2a: M.p. 204–207 °C (ref.[5] m.p. 201 °C). $[a]_D^{25}$ = +9 (c = 0.58 in CH_2Cl_2). IR: $\tilde{\nu}$ = 2950, 2921, 1741, 1691, 1454, 1230, 1027, 966, 700 cm^{-1}. UV (CH_2Cl_2): λ_{max} (lg ε) = 269 (35) nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.92 (d, 3 H, J = 7.0 Hz, 11-H), 1.00 (d, 3 H, J = 7.0 Hz, 23-H), 1.01 (d, 3 H, J = 7.0 Hz, 22-H), 1.38 (m, 1 H, 16-H), 1.36 (m, 1 H, 17b-H), 1.60 (m, 1 H, 17a-H), AB signal [$\Delta\delta$ = 0.21, δ_A = 1.96 (dd, 1 H, ^2J = 11.0, ^3J = 4.9 Hz, 15a-H), δ_B = 1.75 (dd, 1 H, ^2J = 11.0, ^3J = 10.9 Hz, 15b-H)], 2.08 (m, 1 H, 18-H), 2.12 (t, 1 H, J = 4.0, J = 4.0 Hz, 4-H), 2.21 (s, 3 H, 25-H), AB signal [$\Delta\delta$ = 0.04, δ_A = 2.64 (dd, 1 H, ^2J = 13.4, ^3J = 4.3 Hz, 10a-H), δ_B = 2.60 (dd, 1 H, ^2J = 13.4, ^3J = 10.0 Hz, 10b-H)], 2.87 (m, 1 H, 5-H), 2.88 (m, 1 H, J = 10.1, J = 10.5 Hz, 8-H), 3.24 (m, 1 H, J = 4.3, J = 4.0, J = 10.0 Hz, 3-H), 3.82 (d, 1 H, J = 10.5 Hz, 7-H), 5.10 (s, 1 H, 12a-H), 5.30 (m, ^2J = 16.4 Hz, 1 H, ^3J = 10.9, ^3J = 4.9 Hz 14-H), 5.35 (s, 1 H, 12b-H), 5.45 (s, 1 H, 2-H), 5.54 (d, 1 H, J = 2.4 Hz, 21-H), 5.70 (m, ^3J = 16.4 Hz, 1 H, ^3J = 5.2, ^4J = 2.1 Hz, 19-H), 5.76 (m, ^3J = 16.4 **Hz**, 1 H, ^3J = 10.5, ^4J = 1.0 Hz, 13-H), 5.96 (dd, 1 H, ^3J = 16.4, ^3J = 2.4 Hz, 20-H), 7.13 (dd, 2 H, J = 7.1, ^4J = 1.4 Hz, 28/30-H), 7.22 (t, 1 H, 29-H), 7.33 (dd, 2 H, J = 7.1, ^4J = 2.2 Hz, 27/31-H) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ = 14.31 (q, C-11), 20.90 (q, C-25), 22.13 (q, C-22), 25.30 (q, C-23), 33.01 (d, C-5), 33.42 (d, C-16), 34.27 (d, C-18), 42.55 (t, C-15), 45.74 (t, C-10), 47.34 (d, C-8), 48.44 (t, C17), 50.70 (d, C-4), 51.86 (s, C-9), 53.76 (d, C-3), 69.60 (d, C-7), 78.62 (d, C-21), 113.89 (t, C-12), 125.49 (d, C-20), 127.10 (d, C-29), 127.51 (d, C-13), 128,99 (d, C-27/31), 129.04 (d, C-28/30), 135.91 (d, C-19), 137.56 (s, C-26), 138.43 (d, 14), 148.17 (s, C-6), 170.13 (s, C-24), 174.31 (s, C-1) ppm.

21-*O*-Deacetyl-L696,476 (2b): M.p. 132–134 °C. $[a]_D^{25}$ = +16 (c = 0.4 in CH_2Cl_2). IR: $\tilde{\nu}$ = 3373, 2948, 2921, 1684,1436,1028, 970, 700 cm^{-1}. UV (CH_2Cl_2): λ_{max} (lg ε) = 283 (25), 280 (25) nm. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 0.98 (d, 3 H, J = 7.0 Hz, 22-H), 1.01 (d, 3 H, J = 7.0 Hz, 23-H), 1.06 (d, 3 H, J = 6.7 Hz, 11-H), 1.37 (m, 1 H, 16-H), AB signal [$\Delta\delta$ = 0.31, δ_A = 1.72 (m, 1 H, 17a-H), δ_B = 1.41 (m, 1 H, 17b-H)], AB signal [$\Delta\delta$ = 0.21, δ_A = 1.97 (m, 1 H, 15a-H), δ_B = 1.76 (m, 1 H, 15b-H)], 2.15 (m, 1 H, 18-H), AB signal [$\Delta\delta$ = 0.34, δ_A = 2.92 (dd, 1 H, J = 13.4, J = 10.4 Hz, 10a-H), δ_B = 2.58 (dd, 1 H, J = 13.4, J = 3.7 Hz, 10b-H)], 2.61 (dd, 1 H, J = 4.7, J = 2.4 Hz, 4-H), 2.81 (t, 1 H, 8-H), 2.91 (m, 1 H, 5-H), 3.27 (m, 1 H, 3-H), 3.82 (d, 1 H, J = 11.0 Hz, 7-H), 4.17 (br. s, 1 H, 21-H), 5.10 (s, 1 H, 12b-H), 5.25 (m, 1 H, J = 15.5, J = 11.0, J = 4.7 Hz, 14-H), 5.33 (s, 1 H, 12a-H), 5.45 (s, 1 H, 2-H), 5.76 (dd, 1 H, J = 15.5, J = 10.0 Hz, 13-H), 5.90 (m, 1 H, J = 16.4, J = 6.5, J = 2.2 Hz, 19-H), 6.19 (dd, 1 H, J = 16.4, J = 2.7 Hz, 20-H), 7.23 (t, 1 H, J = 6.6, J = 1.4 Hz, 27-H), 7.30 (m, 2 H, J = 7.4, J = 1.4 Hz, 25/29-H), 7.45 (m, 2 H, 26/28-H) ppm. ^{13}C NMR (600 MHz, $CDCl_3$): δ = 14.22 (q, C-11), 22.47 (q, C-22), 25.22 (q, C-23), 33.10 (d, C-5), 33.52 (d, C-16), 34.17 (d, C-18), 42.33 (t, C-15), 45.76 (t, C-10), 46.16 (d, C-8), 48.33 (t, C17), 50.18 (d, C-4); 53.13 (s, C-9), 53.87 (d, C-3), 69.79 (d, C-7), 77.26 (d, C-21), 113.63 (t, C-12), 131.23 (d, C-20), 128.94 (d, C-29/25), 127.08 (d, C-13), 128,04 (d, C-27), 129.15 (d, C-28/26), 134.32 (d, C-19), 137.81 (s, C-24/18), 137.81 (d, 14), 148.6 (s, C-6), 175.81 (s, C-1) ppm. HRMS (EI): calcd. for $C_{28}H_{37}O_3N$ 435.27734; found 435.27701.

3-Nitropropionic Acid (3): M.p. 62–64 °C (ref.[30] m.p. 64–65 °C).

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