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Absolute Configurations of Globosuxanthone A and Secondary Metabolites from *Microdiplodia* sp. – A Novel Solid-State CD/TDDFT Approach[‡]

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Dedicated to Prof. Helmut Duddeck on the occasion of his 60th birthday

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The absolute configuration of a potent new antitumor dihydroxanthenone, globosuxanthone A (**1**), active against human solid tumor cell lines and isolated by Gunatilaka et al. from *Chaetomium globosum* Ames[1] and in this study from the endophytic fungus *Microdiplodia* sp., was established by a new methodology. In this new approach, the Cartesian coordinates of the X-ray data serve as input geometry for quantum mechanical calculation of the theoretical CD spectrum. Comparison with the solid-state CD spectrum gives a very good match, since the calculated and the experimentally acquired data are derived from the very same single conformation.

Introduction

Fungal secondary metabolites have served as an important source of lead structures for new drug compounds,[2,3] and in our screening program for new biologically active fungal secondary metabolites we have investigated the metabolites of an endophytic strain, *Microdiplodia* sp. The ethyl acetate extract was subjected to column chromatography and a new metabolite (**1**) was isolated, together with other five known compounds (**2**–**6**) (Scheme 1). The new dihydroxanthenone was preliminary named microdiplodianone (**1**), while the other compounds were identified by comparison with published data as the xanthone derivatives 2-hydroxyvertixanthone (**2**),[4] vertixanthone (**3**),[4] and barleriaquinone II (**4**),[5] the anthraquinone chrysophanol (**5**),[6] and the steroid ergosterol (**6**).[7] The planar structure and relative configuration of microdiplodianone (**1**) was elucidated by extensive 1D and 2D NMR experiments and supported by an X-ray structure analysis. In 2006 we learned that the same dihydroxanthenone (**1**), named globosuxanthone A, had at the same time[8] been isolated from *Chaetomium globosum* Ames, isolated in turn by Gunatilaka et al.[1] from the rhizosphere of the Christmas cactus, *Opuntia leptocaulis* DC (Cactaceae). Compound **1** exhibited significant cytotoxicity against a panel of seven human solid tumor cell lines.

In the meantime, we employed the Cartesian coordinates of the X-ray data to calculate the theoretical CD spectrum and were able to assign the absolute configuration of globosuxanthone A (**1**) by comparing it with the CD spectrum measured in the solid state. This new methodology, in principle, allows elucidation of the absolute configuration of **any** compound, provided that the solid-state structure and CD spectrum measured in the solid state are known. This is a considerable extension of previously employed Boltzmann weighting of several conformations in solution to calculate the CD,[9,10] which with conformationally flexible structures may give particularly unreliable results. We now report in detail on the elucidation of the absolute configuration of globosuxanthone A (**1**) by this new methodology. With respect to a previously described equivalent approach based on semiempirical CD computation,[11] the employment of TDDFT computations is believed to increase accuracy and greatly to expand the scope of the methodology.

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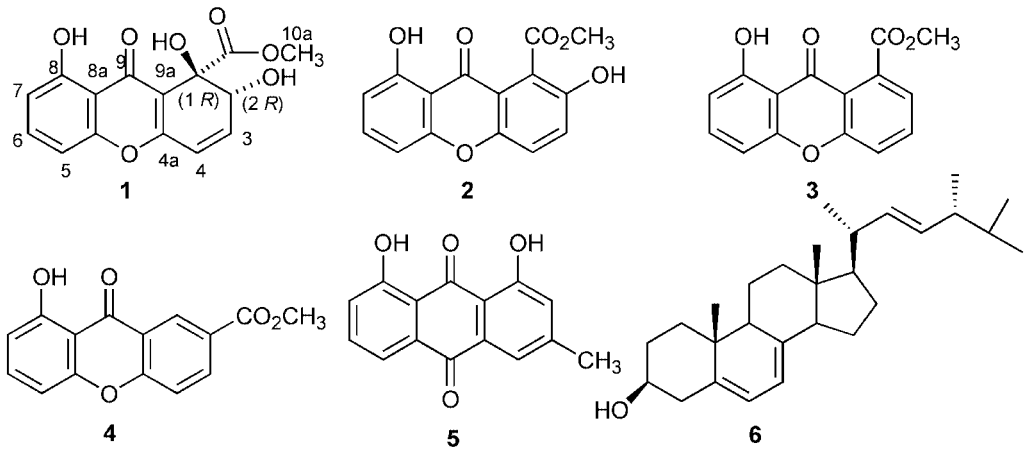

Scheme 1. Structures of compounds isolated from *Microdiplodia* sp.

Results and Discussion

The endophytic fungus *Microdiplodia* sp. (internal strain no. 7092), was isolated from *Erica arborea* from Gomera and cultivated at room temperature on biomalt solid agar media for 28 d. The culture media (4 L) were then extracted with ethyl acetate to afford a crude extract (5.5 g), and in order to perform an efficient targeted isolation of active metabolites, the ethyl acetate extract was subjected to column chromatography, resulting in the isolation of a number of metabolites.

Globosuxanthone A (**1**) was obtained as yellow crystals and was assigned the molecular formula $C_{15}H_{12}O_7$ by HREIMS. Its IR absorption bands at 3400, 1740, and 1650 cm^{-1} and its MS peaks at *m*/*z* 286 $[M - H_2O]^+$, 276 $[M - CO]^+$, and 245 $[M - COOMe]^+$ suggested the presence of hydroxy, ester, and chelated carbonyl moieties, respectively. Both the ^1H NMR (δ = 3.54 ppm, s, 3 H) and the ^{13}C NMR spectrum (δ = 171.6 and 52.3 ppm) show the presence of a methyl ester, while the ^{13}C NMR signal at 181.8 ppm was assigned to a chelated carbonyl carbon (see Experimental). Preliminary inspection of the ^1H NMR spectrum of globosuxanthone A (**1**) showed two double doublets and one triplet for AMX-type protons at δ = 7.35 (t, *J* = 8.0 Hz), 6.75 (dd, *J* = 8.0, 1.0 Hz), and 6.60 ppm (dd, *J* = 8.0, 1.0 Hz), for 6-H, 5-H, and 7-H, respectively, indicating the monosubstituted ring A. Two coupling double doublets at δ = 6.34 and 6.14 ppm for the 3-H and 4-H alkene protons and a methine proton at δ = 4.64 ppm (dd, *J* = 3.0, 2.5 Hz, 2-H), indicated that compound **1** contains the dihydroxanthenone moiety with a nonaromatic dihydro ring C (Scheme 1).

The partial structure –CH=CH–CH(OH)– in ring C was assigned with the aid of the ^1H–^1H shift correlation (^1H–^1H COSY) spectrum and the HMBC spectrum as shown in Figure 1. The structure was assembled by analysis of the correlation spectra (^1H–^1H-COSY, HMQC) and by HMBC experiments. All these data, together with a UV absorption maximum at 338 nm, confirmed that compound **1** contains a dihydroxanthenone moiety.[12]

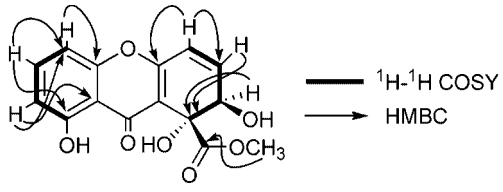

Figure 1. ^1H–^1H COSY and important HMBC correlations for **1**.

The X-ray analysis of **1** (Figure 2) confirmed its connectivity and revealed its relative configuration. Crystals were grown as orange prisms from *n*-hexane/EtOAc (6:4) solution.

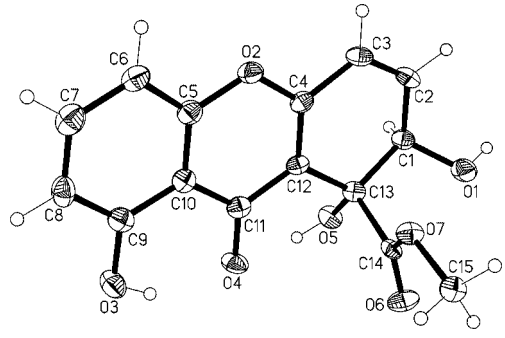

Figure 2. Molecular structure of globosuxanthone A (**1**) in the crystal state.

The absolute configuration of **1** was determined by comparison of its solid-state CD spectrum, measured with a KCl disc, with the TDDFT CD spectrum calculated from the X-ray coordinates.[8] The solution CD spectra in methanol and in an $MeCN/CH_2Cl_2/MeOH$ (1:1:1) solvent system showed distinct differences, especially above 320 nm (Figure 3), mainly due to the contributions of different conformers. The solid-state CD spectrum is also different from its solution counterparts, although the signs and relative intensities of the major bands are preserved, so its origin is primarily "molecular", and no significant crystal lattice contributions are apparent.[13,14] In the interpretation of solid-state CD, only one conformer has to be considered, and

its coordinates were known from the X-ray analysis, thus opening the way to a full calculation of the circular dichroism of **1** through the use of its solid-state structure as input geometry, without the necessity for any preliminary conformational analysis.[9] The time-dependent DFT method[15–17] was employed for the excited states calculations with two different hybrid-type functionals (B3LYP and PBE0) and a triple-zeta split valence basis set (TZVP).[17,18] The spectra calculated with the assumption of a (1*R*,2*R*) configuration are shown in Figure 4, together with the experimentally acquired solid-state spectrum. It is apparent that the DFT-computed curves reproduce the main experimental features above 250 nm very well, in terms of position, sign, and relative intensity of bands, including the weakest ones (shoulders at 303 and 326 nm). The first six computed transitions (see vertical bars reproducing the rotational strengths in Figure 4) are well below the estimated ionization potential; they are mainly due to excitations from the five highest occupied Kohn–Sham orbitals to the two lowest virtual orbitals, both of which have negative eigenvalues. With these conditions fulfilled, TDDFT calculations are known to be accurate enough[15–17,19] for safe use in configurational assignment.[20–23]

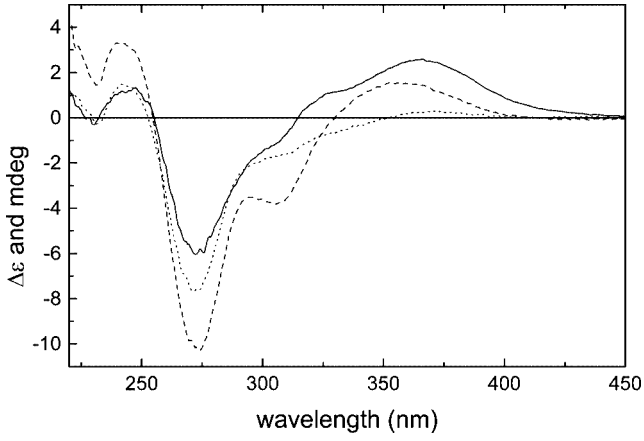

Figure 3. CD spectra of **1** measured in methanol (dotted line), in MeCN/CH_2Cl_2/MeOH 1:1:1 (dashed line), and as KCl disc (solid line, in mdeg units).

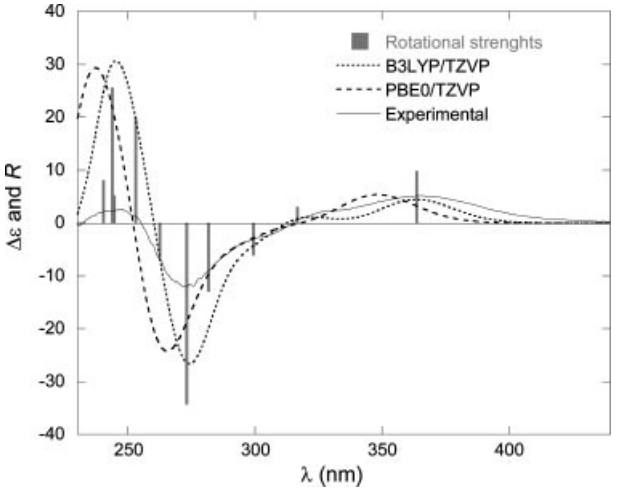

Figure 4. CD spectra of **1**: experimental as KCl disc (solid line, multiplied by two), and computed with TDDFT with use of B3LYP/TZVP (dotted line) and PBE0/TZVP (dashed line). Vertical bars represent B3LYP-computed rotational strengths in 10^{-40} cgs units.

The dihydroxanthenone globosuxanthone A (**1**), isolated from *Chaetomium globosum* Ames[1] and in this study from the endophytic strain *Microdiplodia sp.*, is therefore assigned as methyl (1*R*,2*R*)-1,2,8-trihydroxy-9-oxo-2,9-dihydro-1*H*-xanthene-1-carboxylate.

Conclusions

A novel methodology has been employed to assign the absolute configuration of a natural compound. It relies on comparison between the compound's solid-state CD spectrum and the ab initio computed one with use of the solid-state structure as input geometry. With respect to the standard methods based on full CD spectra computations, this technique has the advantage of skipping a thorough conformational analysis in solution, which often represents the most demanding step and may require concurrent use of various spectroscopic and computational techniques. The absolute configurations of compounds with conformationally very flexible skeletons may now therefore be more easily determined by CD spectra computations. The main prerequisites for the application of the current method appear to be: 1) a crystalline sample and determination of its X-ray structure, 2) measurement of an artefact-free solid-state CD spectrum, and 3) a mainly molecular origin of the solid-state CD, with minimal interference from crystal lattice contributions (CD signals intrinsic to the solid state).[13,14] Once these conditions are fulfilled, the reasons to prefer this present approach are twofold. Firstly, there is a direct and safe relationship between the molecular structure and the observed spectroscopic property, and secondly, the impact of structure-determination steps is minimized, especially for flexible systems in which multiple conformations need to be determined and their relative energies accurately computed. In principle, the absolute configuration of most chiral crystalline compounds with known solid-state structures from X-ray structure analysis may now be elucidated by this new method. Further investigation into the scope and applicability of the solid-state CD/TDDFT approach is ongoing.

Experimental Section

General Experimental Procedures: For general methods and instrumentation see ref.[24] and for microbiological methods and conditions of culture see refs.[25,26] The CD spectra were recorded on a J-810 spectropolarimeter and the concentrations for the CD and specific rotation measurements are given in mol dm^{-3} if not otherwise stated. For the solid-state CD measurement, the disc was prepared by mixing KCl (optical grade, heated at 100 °C, 180.3 mg) and **1** (130 μg) with the aid of a Perkin–Elmer vibrating mill for 5 min, and the mixture was then pressed under vacuum at ten tons with a Perkin–Elmer press to provide a transparent disc for CD

measurement. Three slightly different spectra were recorded by rotating the disc at approximately 120° intervals, and these were averaged to produce the overall solid-state spectrum of **1**.

Extraction and Isolation: The endophytic fungus *Microdiplodia* sp., internal strain No. 7092, was isolated from *Erica arborea* from Gomera and cultivated on biomalt solid agar medium (4 L, 5% w/v) at room temperature for 28 d.[26] The culture medium was then extracted with ethyl acetate to afford a residue (5.5 g) after removal of the solvent under reduced pressure. The extract was separated into three fractions by column chromatography (CC) on silica gel (190 g), with use of gradients of *n*-hexane/ethyl acetate (90:10, 50:50, 0:100).

The more polar fractions (500 mg) were separated by silica gel column chromatography with elution with *n*-hexane/ethyl acetate (6:4) to give crude compound **1**, together with pure compound **2** (6.5 mg). The crude compound **1** was then recrystallized from ethyl acetate/*n*-hexane to give the pure natural product **1** (20 mg). Fraction B (400 mg) was separated by CC on silica gel (9 g) with *n*-hexane/ethyl acetate (8.5:1.5) to give crude compounds **3**, **4**, **5**, and **6**. Subsequently, each crude fraction was further purified by further CC to give compounds **3** (15 mg), **4** (4 mg), **5** (12 mg), and **6** (55 mg).

Globosuxanthone A (1): Yellow crystals, m.p. 152 °C; ref. m.p. >233 °C (dec.).[1] $[\alpha]_D^{25}$ = –50, (c = 1.21·10^{-3} in MeOH/$CHCl_3$ 5:3); ref. specific rotation $[\alpha]_D^{25}$ –29.0 (c = 0.2 g/100 mL, DMSO).[1] CD (KCl disc, c = 4.7·10^{-3}): λ ($\Delta\varepsilon$) = 366 (2.58), 326 sh (1.07), 307 sh (–1.05), 272 (–6.04), 247 nm (1.32). CD (MeOH, c = 1.1·10^{-4}): λ ($\Delta\varepsilon$) = 371 (0.29), 311 sh (–1.57), 271 (–7.69), 241 nm (1.47). CD (MeCN:CH_2Cl_2/MeOH 1:1:1, c = 1.7·10^{-4}): λ ($\Delta\varepsilon$) = 366 sh (1.44), 352 (1.54), 305 sh (–3.81), 274 (–10.29), 242 nm (3.31). ^{1}H NMR (500 MHz, $CDCl_3$ + CD_3OD): δ = 7.35 (t, J = 8.5 Hz, 1 H, 6-H), 6.75 (dd, J = 8.5, 1.0 Hz, 1 H, 5-H), 6.60 (dd, J = 8.5, 1.0 Hz, 1 H, 7-H), 6.34 (dd, J = 10.5, 3.0 Hz, 1 H, 3-H), 6.14 (dd, J = 10.5, 2.5 Hz, 1 H, 4-H), 4.64 (dd, J = 3.0, 2.5 Hz, 3 H, 2-H), 3.54 (s, 3 H, COOCH_3) ppm. ^{13}C NMR (125 MHz, $CDCl_3$ + CD_3OD): 181.8 (C-9), 171.6 (*C*OOCH$_3$), 160.4 (C-4a), 159.9 (C-8), 155.3 (C-10a), 142.0 (C-3), 135.5 (C-6), 119.7 (C-4), 113.4 (C-9a), 111.4 (C-7), 110.4 (C-8a), 107.1 (C-5), 78.3 (C-1), 73.5 (C-2), 52.3 (COO*C*H$_3$) ppm. IR $\tilde{\nu}_{max}$ = ($CHCl_3$ + MeOH): 3400 (OH), 1740 (COO), 1650 (CO). UV λ_{max} (logε, MeOH): 340 nm (3.50), 267 (4.24). EIMS (rel. int.): m/z 304 $[M]^+$ (30), 286 $[M - H_2O]^+$ (18), 276 $[M - CO]^+$ (10), 245 $[M - COOCH_3]^+$ (100), 227 (57), 215 (18), 171 (10), 137 (10), 79 (10), 63 (5), 39 (10).

Computational Section: TDDFT calculations were run with the Gaussian ′03 program (Gaussian, Inc., Pittsburgh PA, 2003). The input geometry was obtained by starting from the solid-state structure, in which only the H-atoms' positions had been re-optimized by the DFT method at B3LYP/6-31G(d) level. The two native hybrid functionals B3LYP and PBE0 and Ahlrich's TZVP basis set were employed for the TDDFT calculations. CD spectra were generated by use of dipole length-computed rotational strengths for consistency with the current literature;[10,19,21] dipole-velocity values differed by less than 10% for the main transitions, with use of both functionals. To calculate full CD spectra, a Gaussian band-shape was applied to the computed rotational strengths[10,19,21] with 2500 cm^{-1} half-height width (corresponding to 33 nm at 365 nm).

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