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ScienceDirect
Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 18 (2007) 925–930

Absolute configuration of hypothemycin and 5′-*O*-methylhypothemycin from *Phoma* sp.—a test case for solid state CD/TDDFT approach☆

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Received 31 January 2007; accepted 12 April 2007

Abstract—Two metabolites, the known antitumor macrolide hypothemycin **1** and its new 5′-*O*-methyl ether, 5′-*O*-methylhypothemycin **2**, and the known steroid ergosterol **3**, were isolated from a *Phoma* sp. The structures were elucidated by means of spectroscopic data analysis, and the absolute configuration of hypothemycin **1** was confirmed by single crystal X-ray analysis in combination with the new solid-state CD/TDDFT methodology. Since the solid-state CD spectrum shows contributions from intermolecular interactions in the crystal, **1** represents a critical test case for our solid-state CD/TDDFT approach.

1. Introduction

Hypothemycin is a 14-membered β-resorcylic acid macrolactone which has been isolated from the fungal fermentations of *Hypomyces trichothecoides*,[2,3] *Hypomyces subiculosis*,[4] *Coriolus versicolor*,[5] and *Aigialus parvus*.[6] The macrolide exhibited moderate antimalarial and antifungal activity, as well as cytotoxicity against various murine and human cell lines.[5,7] It has also been demonstrated to suppress the growth of murine and human tumor cells transplanted into the backs of mice.[8] Both in vitro and in vivo studies by Zhao et al.[9] and Schirmer et al.[7] have identified hypothemycin as a potent and selective inhibitor of the threonine/tyrosine-specific kinase, MEK, and other protein kinases that contain a conserved cysteine residue in their ATP binding site. Since many of these kinases play an important role in signal transduction pathways that regulate cell proliferation, cell differentiation, and apoptosis,[10–12] their aberrant activation can lead to uncontrolled cell proliferation and transformation.[13] Hence, compounds that are specific inhibitors of these target proteins are promising candidates as anticancer agents. Due to the biological importance of the molecule, two independent syntheses have recently been published.[14,15] In a series of preliminary screenings, the culture extract of *Phoma* sp. displayed excellent fungicidal activity, particularly against *Phytophthora infestans*, as well as moderate algicidal, antibacterial, antialgal, and herbicidal activities. To obtain preliminary structural information on the active constituents, the ethyl acetate extract of the fungal cultivation was subjected to column chromatography. We isolated three metabolites, the known macrolide, hypothemycin **1**, a new hypothemycin analogue, 5′-*O*-methylhypothemycin **2**, and the known steroid ergosterol **3**. The absolute configuration of hypothemycin **1** had been tentatively determined by X-ray analysis using anomalous dispersion of oxygen atoms,[5] and then by X-ray analysis of an aigialomycin C4-bromobenzoyl derivative, chemically correlated to hypothemycin.[6] Later, the absolute configuration was also confirmed by stereospecific total synthesis of **1**.[14,15] Having

☆ Biologically Active Secondary Metabolites from Fungi, Part 30. Part 29 lit.[1]

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doi:10.1016/j.tetasy.2007.04.005

independently determined the relative configuration of **1** by X-ray analysis, we applied our solid state CD/TDDFT methodology[1,16–18] to test the new procedure on a molecule endowed, from our perspective, with two interesting properties. First, the 14-membered ring may be expected to be rather conformationally flexible. In such a case, the conventional approach referring to the solution state requires the entire set of CD spectra from all conformations to be computed by TDDFT[19,20] or other quantum mechanical methods,[21,22] weighted by the Boltzmann procedure, and then compared with the solution CD spectrum.[23] In contrast, in our solid-state CD/TDDFT approach, the Cartesian coordinates of the X-ray data serve as input geometry for TDDFT calculation of the theoretical CD spectrum, which is then compared with the CD spectrum recorded in the solid state. In this way, the whole conformational search step is skipped and any geometrical uncertainty avoided. Moreover, theoretical and experimental spectra are expected to give a good match,[1,16–18] since the calculated and the experimentally acquired data are derived from the very same single conformation of the solid state. This approach was first introduced by some of us employing semi-empirical calculations of CD spectra,[16] and later improved by switching to TDDFT calculations which greatly broadened the scope of this method.[1,17,18]

The main prerequisite for the application of the present approach is that the solid-state CD be essentially of a molecular origin, since the calculation is run on a single molecule. Therefore, CD effects intrinsic to the solid state must be negligible, which may arise, for example, from intermolecular couplings between molecules tightly packed in the crystal.[24,25] The analysis of hypothemycin **1** thus offers the possibility to test the validity of the method in the presence of strong chromophores such as β-resorcylate, which in principle may give efficient couplings of the exciton and/or of the magnetic–electric types,[26] potentially limiting our CD/TDDFT approach.

2. Results and discussion

The endophytic fungus *Phoma* sp. (internal strain no. 7133) was isolated from *Senecio kleinii* from Gomera (Spain) and cultivated at room temperature on biomalt solid agar medium for 28 days. The 4 L culture medium was then extracted with ethyl acetate to afford 4.3 g of a crude extract. The crude extract of *Phoma* sp. showed excellent fungicidal activity, particularly against *Phytophthora*, as well as moderate algicidal, antibacterial, antialgal, and herbicidal activities. Column chromatography on silica gel of the fungal ethyl acetate extract led to the isolation of three metabolites, **1**–**3**.

Hypothemycin **1** (Fig. 1), molecular formula $C_{19}H_{22}O_8$, showed maxima in the UV spectrum near 220, 265, and 307 nm, typical of the 4-methoxy, resorcylic acid lactone macrolide chromophore present in the radicicol derivatives.[3] The IR spectrum showed the presence of a highly chelated lactone carbonyl peak at 1620 cm^{-1}. Comparison of the ^1H and ^{13}C NMR spectroscopic data of **1** with those of hypothemycin, a macrolide previously isolated from *Coriolus versicolor*,[5] proved the identity of the compounds. The structure of hypothemycin **1** was further confirmed by X-ray diffraction analysis of a single crystal obtained from EtOAc and *n*-hexane (Fig. 2). As the atomic coordinates derived by Agatsuma et al.[5] are not available from the CCDC database, we have deposited our data therein.[27]

The solution and solid-state CD spectra of hypothemycin **1** were very similar (Fig. 3), although some differences emerged. In the solid-state CD spectra recorded as KCl or KBr disc, a new negative CD transition appeared at 226 nm, and three transitions were observed above 285 nm instead of the 305 nm trough and 333 nm peak of the solution CD spectrum. The individual solid-state CD spectra were obtained as the average of four slightly different CD spectra recorded with 90° rotation of the KCl or

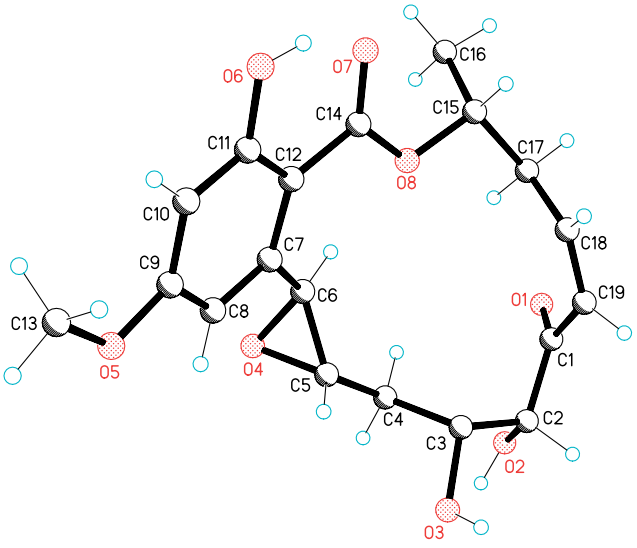
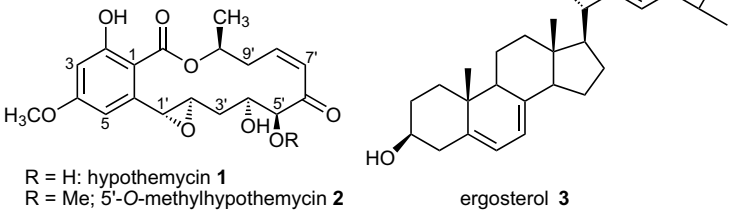


Figure 2. Molecular structure of hypothemycin **1** in the crystal.



R = H: hypothemycin **1**
R = Me; 5'-*O*-methylhypothemycin **2**

ergosterol **3**

Figure 1. Structures of compounds isolated from *Phoma* sp.