VCD Determination of the Absolute Configuration of Stypotriol

Article in Natural product communications · August 2009
Source: PubMed

5 authors, including:

Marcelo A Muñoz
Universidad Austral de Chile
25 PUBLICATIONS 294 CITATIONS

Juana Rovirosa
University of Chile
89 PUBLICATIONS 1,022 CITATIONS

Aurelio San-Martin
250 PUBLICATIONS 1,787 CITATIONS

Pedro Joseph-Nathan
Center for Research and Advanced Studies of the National Polytechnic Institute
272 PUBLICATIONS 2,822 CITATIONS

Some of the authors of this publication are also working on these related projects:

- Natural Products Structures View project
- Evaluation of peripheral biomarkers in the diagnosis of Parkinson’s disease and other proteinopathies View project
The absolute configuration of the pentacyclic ichthyotoxin stypotriol, a constituent of *Stypopodium zonale*, was deduced to be 3S,5R,8R,9R,10S,13S,14S-(−)-1 by vibrational circular dichroism spectroscopy of the derived triacetate 2 in comparison to DFT B3LYP/DGDZVP calculations. Compound 2, C_{33}H_{46}O_{7} having 300 electrons, is the largest natural product successfully studied by VCD to date.

**Keywords:** VCD, stypotriol, absolute configuration.

The meroditerpenoid stypotriol (1, Figure 1) is a pentacyclic ichthyotoxin of mixed biogenesis first isolated three decades ago from the Caribbean marine brown alga *Stypopodium zonale* (Dictyotaceae) collected near Belize [1]. Its structure was determined using spectral and chemical means, and its relative stereochemistry followed from a single crystal X-ray diffraction study of the derived o-quinone stypoldione into which 1 is readily air-oxidized [2]. Later, it was also found as a constituent of *S. flabelliforme* collected at Easter Island [3] but not from the same species collected at Papua New Guinea [4]. Some related marine secondary metabolites belonging to this interesting group of natural occurring spirobenzofuran compounds include [2] the o-quinone stypoldione, stypodiol which lacks for the phenol functionality located meta to the heterocyclic oxygen atom in 1, epistypodiol which is isomeric of stypodiol at C-14, and several other oxidized derivatives [4]. In the case of *S. zonale*, collections made at Belize, at the western Florida Keys and at Palau, Micronesia revealed significant chemical variations of the South Pacific algae in comparison to the American samples [5]. A detailed ecological study of some of the constituents isolated from species of the genus *Stypopodium* has been reported [6], and a wide range of biological activities have been summarized [4] both for isolates of this genus and for some of the same metabolites found in the genus *Taonia*, which include collections from the Aegean Sea [7].

Although there is no direct determination of the absolute configuration of 1, it follows from that of its air-oxidation product (−)-stypoldione which has been synthesized [8-13] several times through multi-step procedures starting from chiral substrates of known stereochemistry. Thus, in order to gain direct and
The six relevant conformers were superimposed, Figure 2, were selected using a Boltzmann distribution based on the DFT energies, these are mainly orientations of two of the three acetate groups, while the pentacyclic molecular scaffold remains rigid, as found for the verticillanes [17], and is evidenced by the torsion angles summarized in Table 1. As shown, the aliphatic acetate group is found in a single preferred disposition with the C=O bond oriented towards the C3-C2 and C3-H3 bonds, as can be seen in all considered low energy conformers, while the two aromatic acetate groups show higher conformational flexibility, having four and six differentiated low energy dispositions at the meta and para positions to the heterocyclic oxygen atoms, respectively.

Since successful VCD studies rely on an accurate prediction of the conformational preferences of the studied molecule, the search for the conformational distribution of triacetate 2 was started using a Monte Carlo-MMFF protocol followed by single point energy calculations at the B3LYP/DGDZVP level of theory. The six relevant conformers 2a-2f (shown superimposed, Figure 2) were selected using a Boltzmann distribution based on the DFT energies, these are mainly orientations of two of the three acetate groups, while the pentacyclic molecular scaffold remains rigid, as found for the verticillanes [17], and is evidenced by the torsion angles summarized in Table 1. As shown, the aliphatic acetate group is found in a single preferred disposition with the C=O bond oriented towards the C3-C2 and C3-H3 bonds, as can be seen in all considered low energy conformers, while the two aromatic acetate groups show higher conformational flexibility, having four and six differentiated low energy dispositions at the meta and para positions to the heterocyclic oxygen atoms, respectively.

### Table 1: Selected torsion angles (in deg) of the six lowest energy conformations of 2.

<table>
<thead>
<tr>
<th>torsion angle</th>
<th>2a</th>
<th>2b</th>
<th>2c</th>
<th>2d</th>
<th>2e</th>
<th>2f</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1-C10-C5-C6</td>
<td>174.4</td>
<td>174.1</td>
<td>174.3</td>
<td>174.2</td>
<td>174.3</td>
<td>174.3</td>
</tr>
<tr>
<td>C1-C10-C9-C8</td>
<td>-170.2</td>
<td>-170.0</td>
<td>-170.1</td>
<td>-170.0</td>
<td>-170.0</td>
<td>-170.3</td>
</tr>
<tr>
<td>C1-C10-C9-C11</td>
<td>58.2</td>
<td>58.4</td>
<td>58.2</td>
<td>58.2</td>
<td>58.3</td>
<td>58.2</td>
</tr>
<tr>
<td>C4-C5-C10-C9</td>
<td>-167.7</td>
<td>-167.9</td>
<td>-167.6</td>
<td>-167.5</td>
<td>-167.7</td>
<td>-167.7</td>
</tr>
<tr>
<td>C7-C8-C9-C11</td>
<td>-177.2</td>
<td>-177.2</td>
<td>-177.2</td>
<td>-177.3</td>
<td>-177.3</td>
<td>-177.2</td>
</tr>
<tr>
<td>C7-C8-C14-C15</td>
<td>-56.9</td>
<td>-57.1</td>
<td>-56.7</td>
<td>-57.1</td>
<td>-57.2</td>
<td>-56.9</td>
</tr>
<tr>
<td>C7-C8-C14-O</td>
<td>58.6</td>
<td>58.5</td>
<td>58.6</td>
<td>58.6</td>
<td>58.4</td>
<td>58.8</td>
</tr>
<tr>
<td>C8-C9-C11-C12</td>
<td>57.6</td>
<td>57.4</td>
<td>57.6</td>
<td>57.7</td>
<td>57.6</td>
<td>57.5</td>
</tr>
<tr>
<td>C10-C9-C8-C14</td>
<td>168.2</td>
<td>168.3</td>
<td>168.1</td>
<td>167.9</td>
<td>168.2</td>
<td>168.3</td>
</tr>
<tr>
<td>C2-C3-O-C</td>
<td>86.5</td>
<td>86.5</td>
<td>86.5</td>
<td>86.5</td>
<td>86.7</td>
<td>85.9</td>
</tr>
<tr>
<td>C3-O-C-O</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
<td>0.2</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Co-Cm-O-C</td>
<td>68.7</td>
<td>-69.8</td>
<td>63.6</td>
<td>118.8</td>
<td>-64.2</td>
<td>-119.4</td>
</tr>
<tr>
<td>Cm-O-C=O</td>
<td>-1.6</td>
<td>2.0</td>
<td>-7.4</td>
<td>-2.8</td>
<td>8.3</td>
<td>3.3</td>
</tr>
<tr>
<td>Cm-Cp-C-O</td>
<td>-83.3</td>
<td>83.0</td>
<td>66.4</td>
<td>128.6</td>
<td>-66.2</td>
<td>-129.4</td>
</tr>
<tr>
<td>Cp-O-C=O</td>
<td>-1.4</td>
<td>1.4</td>
<td>-0.4</td>
<td>8.3</td>
<td>0.2</td>
<td>-8.2</td>
</tr>
</tbody>
</table>

* Co, Cm, Cp: aromatic carbons ortho, meta and para to the heterocyclic oxygen atom.

Molecular mechanics energy of conformers obtained from the Monte Carlo analysis, in kcal/mol relative to 2b with E_MMFF = 133.227 kcal/mol. The absolute value is G_H0 for conformer 2b calculated at 298 K and 1 atm. b Molecular mechanics population in %. c Sum of electronic and zero-point DFT/B3LYP/DGDZVP energies in kcal/mol relative to conformer 2b calculated at 298 K and 1 atm. d For conformer 2b the absolute values are E_H0 = -1811.300234 au, E_298 = -1811.259911 au, E_298 + H_298 = -1811.258967 au. e For conformer 2a the absolute value is G_298 = -1811.374792 au. f DFT population in % calculated from ΔG_298 values.

Nevertheless one molecular conformation, in which both aromatic acetate groups are oriented to opposite sides, accounts for 51% of the conformational distribution, the second conformer accounts for almost 17%, and two further conformers account each for around 10% of the conformational distribution. Details regarding the energetic profile of the conformers and their relative abundances are summarized in Table 2.

Although these are small changes to the overall conformational preferences of 2, they are followed by large differences in the vibrational spectra calculated for each preference due to the techniques high sensitivity to small conformational changes. Thus to ascertain good quality data, and to obtain solid stereochemical conclusions it is necessary to generate a weighed plot that can be compared to the experimental spectrum. This comparison is shown in Figures 3 and 4, which depict the IR and VCD spectra, respectively. Detailed
evaluation of the IR spectra is important since it allows determination the anharmonicity factor for the comparison. For this purpose it has to be taken into account that the experimental IR and VCD frequencies derive from an anharmonic force field, while the calculated frequencies derive from a harmonic force field and therefore the later has to be scaled by the anharmonicity factor. This factor is determined from the corresponding IR spectra wherein all bands have the same phase. The bands in the calculated spectrum are numbered and assigned to the bands in the measured spectrum using described methodology [15], which in the current case provided an anharmonicity factor of 0.99. As can be observed, good coincidence exists between the experimental and theoretical plots of both vibrational spectra, which evidences the quality of the mathematical model used and the selection of the correct enantiomer for the calculations. It is also relevant to notice that bands in the 1700-1800 cm\(^{-1}\) region of VCD spectra, which correspond to the stretching vibrations of carbonyl groups present in a molecule, are known to be affected by solute-solvent interactions and are quite difficult to predict [18].

Nevertheless, in the present case the sign of both bands were correctly predicted by the current calculations.

The good agreement observed between the experimental and theoretical VCD spectra, allows to conclude that the absolute configuration of the meroditerpenoid stypotriol 1, of its acetylated derivative 2 and of the air-oxidation product (-)-stypoldione is 3\(S\),5\(R\),8\(R\),9\(R\),10\(S\),13\(S\),14\(S\) as depicted in Figure 1.

**Experimental**

**Algal collection:** The brown alga *Stypopodium flabelliforme* was collected by scuba diving between 5 and 10 m from the vicinity of Hanga Roa, Easter Island, Chile, in 2005. A voucher specimen, number IPA050395 is in deposit at Museo Nacional de Historia Natural, Santiago, Chile where Prof. M. Eliana Ramirez kindly confirmed its identity.

**Spectral determinations:** A sample of 7.2 mg of 2 was dissolved in 700 µL of CDCl\(_3\) 99.8 atom D % and the solution was placed in an NMR sample tube to verify the identity and purity of the molecule by \(^1\)H and \(^13\)C NMR measurements on a Varian Mercury 300 spectrometer. After evaporation of the solvent, the compound was dissolved in 150 µL of CDCl\(_3\) 100% atom D and the solution was placed in a BaF\(_2\) cell with a path-length of 100 µm. The IR and VCD measurements were performed on a BOMEM-Bio Tools Chiral/R FT-VCD spectrophotometer and data were acquired at a resolution of 4 cm\(^{-1}\) during 10 h.

**Extraction and isolation:** Wet specimens of *S. flabelliforme* (2.0 kg) were frozen for transportation and later repeatedly extracted with dichloromethane to afford 60.5 g of a crude organic extract which was immediately acetylated with acetic anhydride-pyridine. A portion of the acetylated extract was separated by repeated column chromatography on silica gel using an n-hexane/EtOAc/MeOH gradient to yield 1.1 g of stypotriol triacetate 2 the spectral properties of which (\(^1\)H and \(^13\)C NMR, IR and UV) were in agreement with reported data [3].

**Molecular modeling and VCD calculation:** The conformational distribution of 2 was obtained using a stochastic Monte Carlo conformational search based on molecular mechanics (MMFF), using the Spartan'04 software package [19] by considering an energy cutoff of 10 kcal/mol that provided 22 conformers. Single point energy calculations at the
B3LYP-DGDZVP level of theory where then performed for all conformations derived from the search and a Boltzmann distribution was used to obtain relative abundances from the DFT energies. The geometry of the six more stable conformations, accounting for 99.9% of the entire conformational distribution, were then further optimized at the same DFT level of theory, followed by vibrational calculations from which frequencies, dipole and rotational strengths were obtained. Plots using the later data were produced considering Lorentzian bands with half-widths of 6 cm\(^{-1}\) and an anharmonicity factor of 0.99. The frequencies and intensities were obtained by Lorentzian fitting using the PeakFit software [20], and all DFT calculations were performed using the Gaussian 03W software package [21]. The DFT B3LYP/DGDZVP optimization process required between 20 to 40 calculation cycles of 2.6 h per conformer, followed by 100 h of vibrational calculations, also per conformer, when using a personal computer with 2 Mb RAM running at 3.0 GHz to account for a total of more than 1000 h computational time.

Acknowledgment - Financial support from Proyecto Anillo ACT-38, Proyecto CSIC-UCHILE 02/07-08 and from CONACYT-Mexico (Grant 61122) is acknowledged.

References


