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## VCD Determination of the Absolute Configuration of Stypotriol

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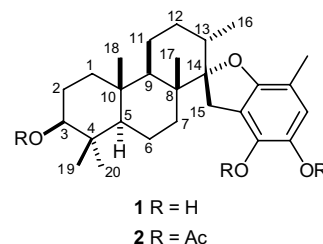
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The absolute configuration of the pentacyclic ichthyotoxin stypotriol, a constituent of *Styopodium zonale*, was deduced to be 3*S*,5*R*,8*R*,9*R*,10*S*,13*S*,14*S*-(-)-**1** by vibrational circular dichroism spectroscopy of the derived triacetate **2** in comparison to DFT B3LYP/DGDZVP calculations. Compound **2**, C<sub>33</sub>H<sub>46</sub>O<sub>7</sub> having 300 electrons, is the largest natural product successfully studied by VCD to date.

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

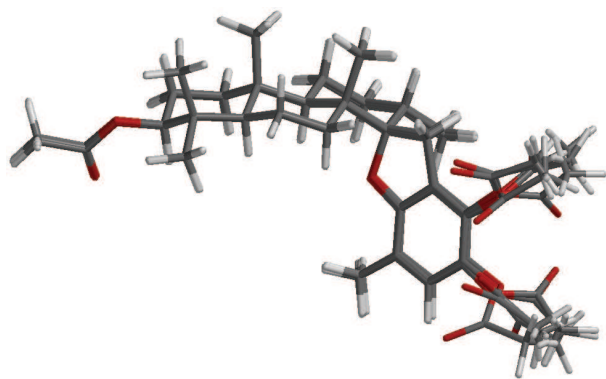
The meroditerpenoid stypotriol (**1**, Figure 1) is a pentacyclic ichthyotoxin of mixed biogenesis first isolated three decades ago from the Caribbean marine brown alga *Styopodium zonale* (Dictyotaceae) collected near Belize [1]. Its structure was determined using spectral and chemical means, and its 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



**Figure 1:** Chemical structures of stypotriol (**1**) and stypotriol triacetate (**2**)

constituents isolated from species of the genus *Styopodium* 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



**Figure 2:** Superposition of the six lower energy conformations of **2**. For conformational distribution and free energy data see Table 2.

independent evidence for the chirality of **1**, and in continuation of our studies for the determination of the absolute configuration of natural products [14] using vibrational circular dichroism (VCD), in the present work we describe the results for stypotriol triacetate **2**, a rather difficult task due to the molecular size of this  $C_{33}H_{46}O_7$  compound that has 300 electrons, since the largest natural product successfully calculated up to now for VCD purposes is ginkgolide B, a  $C_{20}H_{24}O_{10}$  compound with 224 electrons [15]. To meet our goal, we took advantage of DFT B3LYP/DGDZVP calculations, which we have shown to produce equivalent results as those obtained by the frequently used DFT B3LYP/6-31G(d,p) calculations [16], but which consume significantly less computing times [17].

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**.

torsion angle	<b>2a</b>	<b>2b</b>	<b>2c</b>	<b>2d</b>	<b>2e</b>	<b>2f</b>
C1-C10-C5-C6	174.4	174.1	174.3	174.2	174.3	174.3
C1-C10-C9-C8	-170.2	-170.0	-170.1	-170.0	-170.0	-170.3
C1-C10-C9-C11	58.2	58.4	58.2	58.2	58.3	58.2
C4-C5-C10-C9	-167.7	-167.9	-167.6	-167.5	-167.7	-167.7
C7-C8-C9-C11	-177.2	-177.2	-177.2	-177.3	-177.3	-177.2
C7-C8-C14-C15	-56.9	-57.1	-56.7	-57.1	-57.2	-56.9
C7-C8-C14-O	58.6	58.5	58.6	58.6	58.4	58.8
C8-C9-C11-C12	57.6	57.4	57.6	57.7	57.6	57.5
C10-C9-C8-C14	168.2	168.3	168.1	167.9	168.2	168.3
C10-C9-C11-C12	-168.0	-168.2	-168.1	-167.8	-168.0	-168.3
C2-C3-O-C	86.5	86.8	86.5	86.2	87.2	85.9
C3-O-C=O	0.4	0.3	0.2	0.2	0.2	0.7
Co-Cm-O-C	68.7	-69.8	63.6	118.8	-64.2	-119.4
Cm-O-C=O	-1.6	2.0	-7.4	-2.8	8.3	3.3
Cm-Cp-C-O	-83.3	83.0	66.4	128.6	-66.2	-129.4
Cp-O-C=O	-1.4	1.4	-0.4	8.3	0.2	-8.2

<sup>a</sup> Co, Cm, Cp: aromatic carbons *ortho*, *meta* and *para* to the heterocyclic oxygen atom.

**Table 2:** Molecular mechanics relative energy and population, and DFT thermochemical parameters and population for the six minimum energy conformers of **2**.

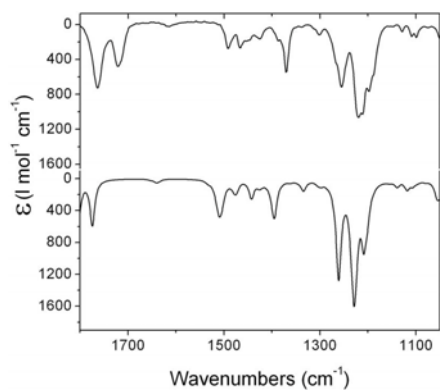
conf.	$\Delta E_{MMFF}^a$	$p_{MMFF}^b$	$\Delta E_0^{c,d}$	$\Delta E_{298}^d$	$\Delta H_{298}^d$	$\Delta G_{298}^e$	$p_{DFT}^f$
<b>2a</b>	0.07	21.83	0.23	0.75	0.75	0.00	51.00
<b>2b</b>	0.00	24.42	0.00	0.00	0.00	0.66	16.79
<b>2c</b>	1.85	1.07	0.36	0.79	0.79	0.90	11.24
<b>2d</b>	1.46	2.09	1.13	1.65	1.65	0.97	9.85
<b>2e</b>	1.77	1.24	0.53	0.90	0.90	1.18	6.96
<b>2f</b>	1.35	2.52	1.31	1.76	1.76	1.48	4.17

<sup>a</sup> Molecular mechanics energy of conformers obtained from the Monte Carlo analysis, in kcal/mol relative to **2b** with  $E_{MMFF} = 133.227$  kcal/mol.

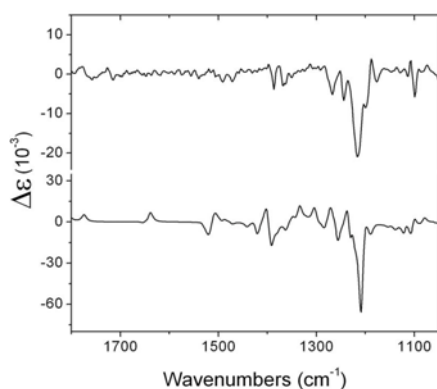
<sup>b</sup> Molecular mechanics population in %. <sup>c</sup> Sum of electronic and zero-point DFT/B3LYP/DGDZVP energies in kcal/mol relative to conformer **2b** calculated at 298 K and 1 atm. <sup>d</sup> For conformer **2b** the absolute values are  $E_0 = -1811.300234$  au,  $E_{298} = -1811.259911$  au,  $E_{298} + H_{298} = -1811.258967$  au. <sup>e</sup> For conformer **2a** the absolute value is  $G_{298} = -1811.374792$  au. <sup>f</sup> DFT population in % calculated from  $\Delta G_{298}$  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 conformations 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



**Figure 3:** Theoretical Boltzmann weighted (bottom) and experimental (top) IR spectra of **2**. Frequencies of the theoretical spectrum are scaled using an anharmonicity factor of 0.99.



**Figure 4:** Theoretical Boltzmann weighted (bottom) and experimental (top) VCD spectra of **2**. Frequencies of the theoretical spectrum are scaled using an anharmonicity factor of 0.99.

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  $\text{cm}^{-1}$  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 *Styopodium 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 Ramírez kindly confirmed its identity.

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

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## References

- [1] Gerwick WH, Fenical W, Fritsch N, Clardy, J. (1979) Stypotriol and stypoldione; ichthyotoxins of mixed biogenesis from the marine alga *Styopodium zonale*. *Tetrahedron Letters*, 145-148.
- [2] Gerwick WH, Fenical W. (1981) Ichthyotoxic and cytotoxic metabolites of the tropical brown alga *Styopodium zonale* (Lamouroux) Papenfuss. *Journal of Organic Chemistry*, **46**, 22-27.
- [3] Rovirosa J, Sepulveda M, Quezada E, San-Martin A. (1992) Isoepitaondiol, a diterpenoid of *Styopodium flabelliforme* and the insecticidal activity of stypotriol, epitaondiol and derivatives. *Phytochemistry*, **31**, 2679-2681.
- [4] Sabry OMM, Andrews S, McPhail KL, Goeger DE, Yokochi A, LePage KT, Murray TF, Gerwick WH. (2005) Neurotoxic meroditerpenoids from the tropical brown alga *Styopodium flabelliforme*. *Journal of Natural Products*, **68**, 1022-1030.
- [5] Gerwick WH, Fenical W, Norris JN. (1985) Chemical variation in the tropical seaweed *Styopodium zonale* (Dictyotaceae). *Phytochemistry*, **24**, 1279-1283.
- [6] Hay ME, Fenical W, Gustafson K. (1987) Chemical defense against diverse coral-reef herbivores. *Ecology*, **68**, 1581-1591.
- [7] Gil B, Ferrandiz ML, Sanz MJ, Terencio MC, Ubeda A, Rovirosa J, San Martin A, Alcaraz MJ, Paya M. (1995) Inhibition of inflammatory responses by epitaondiol and other marine natural products. *Life Sciences*, **57**, PL25-PL30.
- [8] Abad A, Agullo C, Arno, M, Cunat AC, Meseguer B, Zaragoza RJ. (1998) An efficient stereoselective synthesis of stypodiol and epistypodiol. *Journal of Organic Chemistry*, **63**, 5100-5106.
- [9] Abad A, Agullo C, Arno M, Cunat AC, Meseguer B, Zaragoza RJ. (1996) An efficient stereocontrolled synthesis of (-)-stypoldione. *Synlett*, **9**, 913-915.
- [10] Mori K, Koga Y. (1995) Diterpenoid total synthesis. XXXI. Synthesis and absolute configuration of (-)-stypoldione, a metabolite of *Styopodium zonale*. *Liebigs Annalen*, **10**, 1755-1763.
- [11] Falck JR, Chandrasekhar S, Manna S, Chiu CC, Mioskowski C, Wetzel I. (1993) Total synthesis of the spiro-o-benzoquinonefuran (-)-stypoldione. *Journal of the American Chemical Society*, **115**, 11606-11607.
- [12] Mori K, Koga Y. (1992) Synthesis and absolute configuration of (-)-stypoldione. *Bioorganic & Medicinal Chemistry Letters*, **2**, 391-394.
- [13] Begley MJ, Fish PV, Pattenden G, Hodgson ST. (1990) Synthesis of marine toxins. A biomimetic approach to the novel spirobenzoquinonefuran stypoldione. *Journal of the Chemical Society, Perkin Transactions 1*, 2263-2271.
- [14] Torres-Valencia JM, Chávez-Ríos OE, Cerda-García-Rojas CM, Burgueño-Tapia E, Joseph-Nathan P. (2008) Dihydrofurochromones from *Prionosciadium thapsoides*. *Journal of Natural Products*, **71**, 1956-1960.
- [15] Nafie LA. (2008) Vibrational circular dichroism: a new tool for the solution-state determination of the structure and absolute configuration of chiral natural product molecules. *Natural Product Communications*, **3**, 451-466.
- [16] Freedman TB, Cao X, Dukor RK, Nafie LA. (2003) Absolute configuration determination of chiral molecules in the solution state using vibrational circular dichroism. *Chirality*, **15**, 743-758.
- [17] Cerda-García-Rojas CM, García-Gutiérrez HA, Hernández-Hernández JD, Román-Marín LU, Joseph-Nathan P. (2007) Absolute configuration of verticillane diterpenoids by vibrational circular dichroism. *Journal of Natural Products*, **70**, 1167-1172.
- [18] Debie E, Bultinck P, Herrebout, W, van der Veken B. (2008) Solvent effects on IR and VCD spectra of natural products: an experimental and theoretical VCD study of pulegone. *Physical Chemistry Chemical Physics*, **10**, 3498-3508.
- [19] Spartan '04. Wavefunction: Irvine, CA, 2004.
- [20] PeakFit v4.12 for Windows, SeaSolve Software Inc.: San Jose, CA, 2003.
- [21] Gaussian 03W. Gaussian Inc.: Pittsburgh, PE, 2003.