Stereostructure Reassignment and Absolute Configuration of Isoepitaondiol, a Meroditerpenoid from *Stypopodium flabelliforme*

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Careful examination of the published NMR data for isoepitaondiol, a meroditerpenoid from *Stypopodium flabelliforme*, suggests that its published structure **1** must be revised. On the basis of extensive 1D and 2D NMR studies, we now propose that structure **2**, with a *trans-anti-trans-anti-cis* arrangement fits isoepitaondiol diacetate. The relative configuration of **2** was confirmed by single-crystal X-ray diffraction, while the absolute configuration was evidenced by vibrational circular dichroism in combination with DFT B3LYP/DGDZVP calculations.

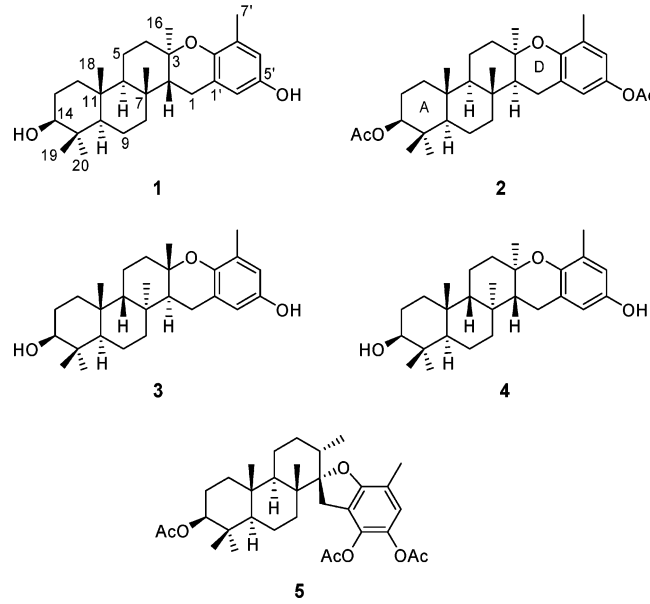
Algae produce a variety of secondary metabolites, often with unusual structures. Among them, the genus *Stypopodium* is a rich source of polycyclic meroditerpenoids biogenetically derived from prenylated hydroquinones.[1] Some polycyclic meroditerpenoids such as stypodiol,[1] epistypodiol,[1] stypotriol,[1] taondiol,[2] epitaondiol,[1,3] 2β,3α-epitaondiol,[4] flabellinol,[4] flabellinone,[4] stypotriolaldehyde,[4] stypohydroperoxide,[4] isoepitaondiol,[5] and 14-ketostypodiol[6] have been isolated from species of the genus *Stypopodium*. In particular, epitaondiol has displayed potent topical anti-inflammatory activity,[7] 2β,3α-epitaondiol has been shown to possess potent sodium channel blocking activity with moderate cytotoxicity toward the NCI-H460 human lung cancer cell line,[4] 14-ketostypodiol diacetate has shown antitumor activity toward prostatic cancer cells,[8] and isoepitaondiol has displayed a stronger radical-scavenging activity in the DPPH test than Trolox or ascorbic acid.[9]

The elucidation of structure **1** for isoepitaondiol has been reported[5] by comparison of its spectroscopic data with those of taondiol analogues. In order to complete this scarce spectroscopic information, we report herein the full assignment of the ^{1}H and ^{13}C NMR spectra of this compound using ^{1}H NMR, ^{1}H−^{1}H COSY, 1D NOE, and NOESY techniques, revealing that the structure of isoepitaondiol diacetate is **2** instead of the originally proposed structure **1** for the nonacetylated molecule.

An independent structural confirmation follows from a single-crystal X-ray diffraction study, while the absolute configuration of the molecule is established after performing a vibrational circular dichroism (VCD) study.

Isoepitaondiol was isolated as its diacetate from an acetylated dichloromethane extract of *S. flabelliforme*. The diacetate was used instead of isoepitaondiol to avoid the possibility of epimerization in a process similar to the described conversion of taondiol into isotaondiol.[10]

In order to avoid NMR signal overlaps that may arise when the spectrum is recorded in $CDCl_3$, the current study of **2** was done in C_6D_6, whereby complete assignment using ^{1}H NMR, ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and sel-pfg-1D NOESY techniques was possible (Tables 1 and 2).



Isoepitaondiol diacetate (**2**) in C_6D_6 showed well-resolved signals for six methyl groups at δ_H 0.66, 0.71, 0.84, 0.91, 1.01, and 2.21. Other well-resolved signals were found for the two *meta*-coupled aromatic protons at δ_H 6.78 and 6.82, the methine protons at δ_H 0.42, 0.58, and 0.95, and the benzylic protons at δ_H 2.54 and 2.69. Experimental sel-pfg-1D NOESY data were incompatible with structure **1** originally proposed[7] for isoepitaondiol. The unambiguous assignment of H-14α in epitaondiol (**3**) and 2β,3α-epitaondiol (**4**) was made on the basis of NMR analysis and confirmed by X-ray crystallography for **4**.[3,4] Irradiation of H-14α of **2** resulted in NOE enhancements of the H-10α and H$_3$-20α signals at δ_H 0.58 and 0.91, respectively, thereby indicating that the three groups are on the same molecular face. A similar situation became evident upon irradiation of H$_3$-20α. In turn, irradiation of the H$_3$-19β signal at δ_H 0.84 (geminal to H$_3$-20α) resulted in enhancement of the H-9β and H-13β signals at δ_H 1.17 and 1.57, respectively, as well as in the H$_3$-18β signal at δ_H 0.66 owing to the angular methyl group. This result strongly suggests a *trans*-diaxial disposition of H-10 and H$_3$-18. While irradiation of H$_3$-18β at δ_H 0.66 results in enhancements of the H$_3$-17β, H$_3$-19β, H-9β, and H-13β signals at δ_H 0.71, 0.84, 1.17, and 1.57, respectively, irradiation of H-6α at δ_H 0.42 resulted in enhancements of H-2α at δ_H 0.95 and H-10α at δ_H 0.58. This set of data is consistent with a *trans* disposition of

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Table 1. ^{1}H and ^{13}C NMR (HMQC) and HMBC Data of **2** in C_6D_6

position	δ_C, mult.	δ_H (*J* in Hz)	ROESY	1D NOESY	HMBC[a]
1α	23.1, CH_2	2.69, dd (18.1, 8.3)	2α, 16	1*β*, 2α, 16	2, 3, 7, 1′, 2′, 6′
1*β*		2.54, d (18.1)	8*β*, 17	1α, 8*β*	
2α	49.9, CH	0.95, d (8.3)	1α, 6α	1α, 1*β*, 6α, 8α, 16	3, 8, 17, 1′
3	75.3, C				
4α	40.8, CH_2	2.14, brd (17.4)	5α, 16	[b]	2, 3, 6
4*β*		1.29, m		[b]	
5α	17.5, CH_2	1.23, m		[b]	3, 5, 7
5*β*		1.61, m		[b]	
6α	59.4, CH	0.42, d (11.5)	2α, 5α, 9α,10α, 12α	2α, 5α, 9α, 10α	2, 4, 5, 10, 11, 17
7	38.5, C				
8α	41.5, CH_2	0.63[c]	6α	[b]	6, 7, 10
8*β*		1.65, m	1*β*, 18	[b]	
9α	17.9, CH_2	1.32, m	6α	[b]	
9*β*		1.17, m	17, 18	[b]	
10α	55.4, CH	0.58, dd (12.2, 1.9)	6α, 14α, 20	[b]	9, 12, 18
11	37.0, C				
12α	38.2, CH_2	0.70, dd (14.2, 5.2)	6α	[b]	6, 10, 13, 14
12*β*		1.45, dd (14.2, 3.6)	13*β*, 18	[b]	
13α	24.0, CH_2	1.72, m	14α	[b]	14, 15
13*β*		1.57, dd (12.2, 3.6)	18	[b]	
14α	80.5, CH	4.70, dd (11.74, 4.9)	10α, 13α, 20	10α, 20	15, 19, 20
15	37.9, C				
16	27.4, CH_3	1.01, s	1α, 4α, 5α	1α, 2α, 4α, 5α, 9α	2, 3, 4
17	15.6, CH_3	0.71, s	1*β*, 9*β*, 18	8*β*, 9*β*, 12*β*	2, 6, 8
18	16.7, CH_3	0.66, s	8*β*, 12*β*, 17, 19	5*β*, 8*β*, 9*β*, 12*β*, 13*β*, 17, 19	6, 10, 11
19	16.6, CH_3	0.84, s	18	9*β*, 13*β*, 18, 20	10, 14, 15, 20
20	28.0, CH_3	0.91, s	10α, 14α	9α, 10, 14α, 19	10, 14, 15, 19
1′	122.5, C				
2′	150.5, C				
3′	127.1, C				
4′	121.4, CH	6.78, d (2.5)		[b]	2′, 5′, 6′, 7′
5′	143.9, C			[b]	
6′	119.0, CH	6.82, d (2.5)		[b]	1, 2′, 4′, 5′
7′	16.1, CH_3	2.21, s		[b]	2′, 3′, 4′
$COCH_3$	170.1, C	1.76, s		[b]	
	20.8, CH_3				
$COCH_3$	168.9, C	1.83, s		[b]	
	20.7, CH_3				

[a] Optimized for 8 Hz. [b] Signal not irradiated. [c] Overlapped signal.

Table 2. Selected ^{1}H NMR Shift Comparison for the Original "Isotaondiol Acetate" and Compound **2** in $CDCl_3$

position	δ_H (*J* in Hz)[a]	δ_H (*J* in Hz)[b]
1α	2.94, dd (18.2, 8.1)	2.58, m
1*β*	2.72, d (18.2)	2.58, m
16	1.16, s	1.22, s
17	0.69, s	1.24, s
18	0.88, s	0.97, s
19	0.89, s	0.88, s
20	0.87, s	0.88, s
4′	6.67, brs	6.62, s
6′	6.63, brs	6.62, s
7′	2.16, s	2.11, s
$COCH_3$	2.09, s	2.05, s
$COCH_3$	2.27, s	2.25, s

[a] Current work. [b] From ref 10.

H-6 and H_3-17. Irradiation of H-2α at δ_H 0.95 leads to NOE enhancements of H-6α at δ_H 0.42 and H_3-16α at δ_H 1.01, thereby indicating that the C/D ring junction is *cis*. Additionally, irradiation of H_3-16α at δ_H 1.01 produced an enhancement of the signals owing to H-1α at δ_H 2.69 and H-2α at δ_H 0.95, a result that was corroborated by irradiation of H-1α at δ_H 2.69. The ROESY spectrum of **2** fully confirmed the 1D NOE data (Figure 1). In particular, the H_3-17*β* signal shows cross-peaks with the H-1*β*, H-9*β*, and H_3-18*β* signals, while the H-10α methine proton signal shows a similar cross-peak pattern with the H-6α, H-14α, and H_3-20α signals. Therefore it follows that the stereostructure deduced for isoepitaondiol diacetate is clearly incompatible with previously proposed structure **1**, the difference being the configuration at C-2. Evaluation of the NOE data of isoepitaondiol diacetate (**2**) suggests a *trans-anti-trans-anti-cis* arrangement with chair-type conformations, for the A/B/C ring system.

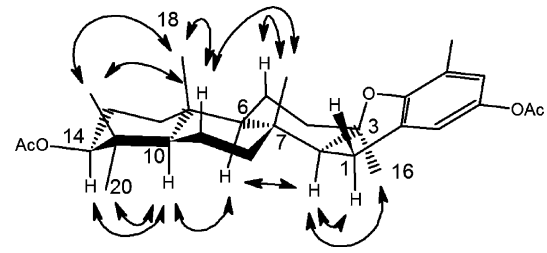

Figure 1. Main correlations in the ROESY NMR spectrum of **2**.

Stereostructure **2** has the relative configuration reported for isotaondiol,[10] obtained by base-catalyzed isomerization of taondiol over 35 years ago. The available ^{1}H NMR data of that early isotaondiol diacetate,[10] measured at 100 MHz in $CDCl_3$, are compared in Table 2 to our current measurements of compound **2**. Since these two sets of data clearly differ, it follows that the published structure for isotaondiol[10] is in need of revision. The complete ^{1}H and ^{13}C NMR assignments of **2** in $CDCl_3$ are given in the Supporting Information.

Independent confirmation for the structure of isoepitaondiol was obtained after performing a single-crystal X-ray diffraction study of the corresponding diacetate **2**. The pertinent crystal data, collection details, structure solution, and refinement are given in the Experimental Section, while Figure 2 shows the molecular perspective in the solid state in which the conformation of the individual six-membered rings can be envisaged.

A single -crystal X-ray diffraction study of **4** revealed[4] that an absolute configuration assignment based on the Flack parameter failed, as evidenced by the use of Mosher esters. Therefore, and considering that vibrational circular dichroism (VCD) has been

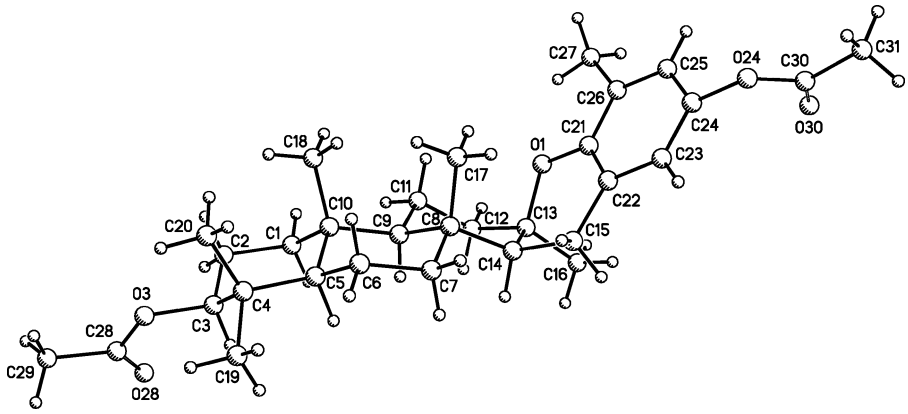

Figure 2. Perspective view of the X-ray crystal structure of isoepitaondiol diacetate (**2**). Atom numbering is as usual for steroids and terpenes.

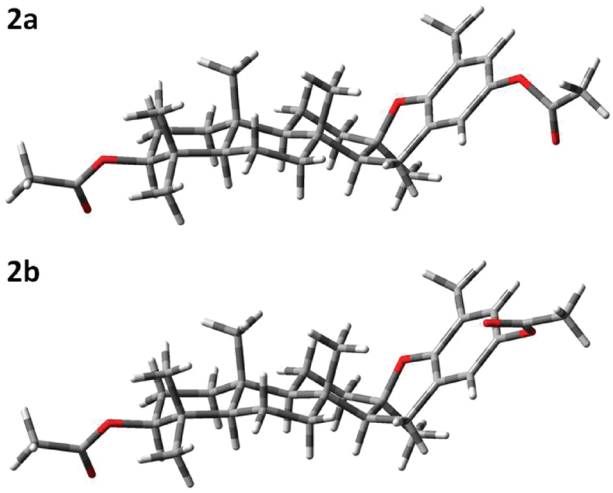

Figure 3. The two more stable conformers of isoepitaondiol diacetate (**2**).

shown to be a reliable methodology for the determination of the absolute configuration of a variety of natural products,[11] and in continuation of our studies[12] using this methodology, we decided to determine the absolute configuration of **2** using VCD. We could anticipate this task would not be trivial due to the molecular size. Compound **2** is a $C_{31}H_{44}O_5$ molecule with 270 electrons, only surpassed by stypotriol triacetate (**5**), a $C_{33}H_{46}O_7$ compound with 300 electrons that was studied by VCD recently.[13]

The atom coordinates of the X-ray analysis of **2** were used as input data for a conformational distribution of isoepitaondiol diacetate using a Monte Carlo guided search within the molecular mechanics force field. The resulting 13 low-energy conformers in the first 10 kcal/mol were reduced to only two after DFT single-point energy calculations at the B3LYP/DGDZVP level of theory, representing 99.95% of the entire conformational distribution. These low-energy conformers were then submitted to further geometry reoptimizations, and their free energies were calculated at room temperature, followed by vibrational calculations at the same level of theory. The optimized conformers **2a** and **2b** given in Figure 3 show chair dispositions of the three fused cyclohexane rings, along with a combination of low-energy orientations of the acetate groups. While the acetate group attached to the aliphatic ring showed only one low-energy orientation, identical to the aliphatic acetate group of stypotriol triacetate[13] (**5**) with the C3−O−C═O dihedral angle value of 0.4° in the lowest energy conformer in both molecules, two stable preferences were present for the aromatic acetate group. As seen in Table 3, the energy difference between the more energetic conformation (**2b**) and the lowest energy conformation

Table 3. Calculated Relative Energies (kcal/mol), Relative Free Energies, and Abundances (%) of the Two More Stable Conformers of Isoepitaondiol Diacetate (**2**) Using a Monte Carlo Guided Search and Geometry Optimization Calculations at the MMFF and DFT B3LYP/DGDZVP Levels of Theory

conf	E_{MMFF}[a]	$\%_{MMFF}$	ΔG_{OPT}[a]	$\%_{OPT}$[b]
2a	0.00	51.84	0.00	73.8
2b	0.04	48.16	0.61	26.2

[a] Relative to the lowest energy conformer in the molecular mechanics force field (E_{MMFF} **2a** = 120.95 kcal/mol) and DFT (ΔG_{OPT} **2a** = −993659.97 kcal/mol) levels of theory. [b] Calculated using the optimized free energies of the relevant conformers.

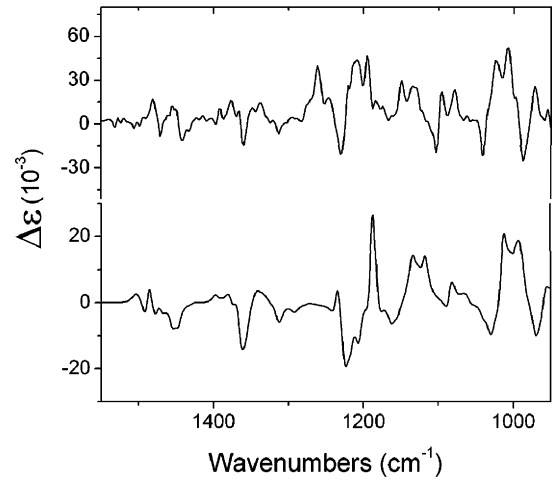

Figure 4. Experimental (top) and Boltzmann-weighted calculated (bottom) DFT B3LYP/DGDZVP VCD spectra of isoepitaondiol diacetate (**2**).

(**2a**) was 0.61 kcal/mol, accounting for a Boltzmann distribution of 73.8% for **2a** and of 26.2% for **2b** at 25 °C. The vibrational calculations, frequency values, dipolar strengths, and rotational strengths for each vibrational mode were obtained for each conformation, and weighed plots of IR and VCD spectra were generated considering their respective abundances. This calculated VCD spectrum is compared in Figure 4 with the corresponding experimental trace for **2**, where an obvious resemblance between them reveals the absolute configuration of the natural sample is the one used for the vibrational calculations and corresponds to that depicted for **2**.

In conclusion, we established structure **2** for isoepitondiol diacetate from detailed NMR studies and verified it by a single-crystal X-ray diffraction study, while the absolute configuration of the molecule followed from a VCD study. Stereostructure **2** has

the relative configuration reported[10] for isotaondiol, obtained by alkaline treatment of taondiol. Because the ^{1}H NMR data of both compounds measured in $CDCl_3$ are different, it follows that at least the structure of isotaondiol is in need of revision. Also, a meroditerpenoid with stereostructure **1** remains to be isolated from nature or prepared in the laboratory.

Experimental Section

General Experimental Procedures. NMR spectra were recorded on an AVANCE 400 Bruker spectrometer, equipped with a 5 mm inverse multinuclear detection pulsed field gradient probe heat (1H-BB1, PFG-ZGRD, Z8202/0253), operating at 400.132 MHz for ^{1}H and 100.623 MHz for ^{13}C. The VCD spectrum was measured on a BioTools Chiral*IR* FT spectrophotometer equipped with dual photoelastic modulation using 6.6 mg of **2** in 150 μL of 100% atom-D $CDCl_3$ placed in a BaF_2 cell with 100 μm path length acquiring data at a resolution of 4 cm^{-1} during 4 h.

Algal Collection. The brown alga *Stypopodium flabelliforme* was collected intertidally near Hanga Roa, Rapa Nui, at Easter Island (South Pacific Ocean), Chile, in March 2007 at 5−10 m depth by scuba diving. Voucher specimen number 2207 is deposited at Museo Nacional de Historia Natural, Santiago, Chile, where its identity was confirmed by Prof. M. Eliana Ramirez.

Extraction and Isolation. Isoepitaondiol diacetate (**2**) was isolated from the seaweed *S. flabelliforme* as recently reported.[14] Crystals were grown by slow evaporation from acetone−methanol and showed a mp of 169−171 °C.

Single-Crystal X-ray Analysis of Isoepitaondiol Diacetate (2). A crystal measuring 0.30 × 0.26 × 0.18 mm was mounted on a Nonius Bruker CAD4 diffractometer. The crystal was orthorhombic, space group $P2_12_12_1$, with cell dimensions $a = 9.053(2)$ Å, $b = 10.170(6)$ Å, $c = 30.108(7)$ Å, $V = 2772(1)$ Å^3, $\rho_{calcd} = 1.190$ g/cm^3 for $Z = 4$, $C_{31}H_{44}O_5$, MW = 496.7, and $F(000) = 1880$ e. A total of 2235 reflections were collected using graphite-monochromated Cu Kα radiation ($\lambda = 1.54184$ Å). The data were corrected for background, Lorentz polarization, and absorption ($\mu = 0.626$ mm^{-1}), while crystal decay was negligible. The structure was solved by direct methods using SHELX97. For the structural refinement, the non-hydrogen atoms were treated anisotropically, and the hydrogen atoms were refined isotropically. A total of 2235 reflections were collected within a θ range of 2.94−59.95° for $0 \le h \le 10$, $0 \le k \le 10$, $2 \le l \le 31$. The unique reflections were 2118, the observed reflections were 1955, and final discrepancy indices, refining 341 parameters, were $R_F = 3.9\%$ and $R_w = 11.6\%$. The final difference Fourier map was essentially featureless, the highest residual peaks having densities of 0.195 e/A^3. Crystallographic data are deposited with number 753734 at the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK. Fax: +44-(0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk.

VCD Calculations. Isoepitaondiol diacetate (**2**) showed 13 conformations in the first 10 kcal/mol when a Monte Carlo guided search algorithm was used in the molecular mechanics force field as implemented in the Spartan '04 software package.[15] In order to obtain more accurate energies, these conformations were subjected to single-point energy calculations at the DFT B3LYP/DGDZVP level of theory, reducing the relevant conformer to only two that account for 99.95% of the entire conformational distribution. The geometries of these conformations were then optimized at the B3LYP/DGDZVP level of theory, and the vibrational frequencies, dipole strengths, and rotational strengths were calculated[16] using the same level of theory. The later values were then converted to molar absorptivity units, and plots against frequency were produced to obtain comparable IR and VCD spectra. A scale factor of 0.97, obtained from comparison between calculated and experimental IR spectra, was used over the calculated frequencies to account for the harmonic force field used during the calculations, whereas the experimental frequencies arise from an anharmonic force field.[11]

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Supporting Information Available: Copies of 1D and 2D NMR spectra and X-ray atomic coordinates for isoepitaondiol diacetate (**2**). This material is available free of charge via the Internet at http://pubs.acs.org.

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