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Raman Optical Activity Spectra and Conformational Elucidation of Chiral Drugs. The Case of the Antiangiogenic Aeroplysinin-1

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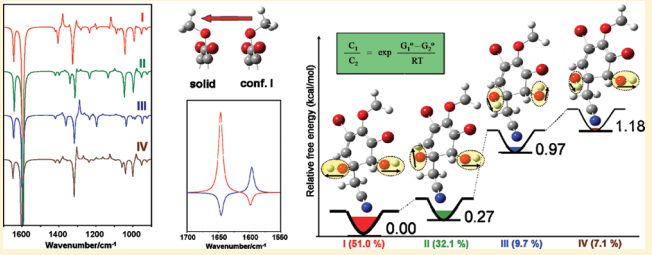
Supporting Information

ABSTRACT: We present the determination of the conformational properties of aeroplysinin-1 in aqueous solution by means of a combined experimental and theoretical Raman optical activity (ROA) and vibrational circular dichroism (VCD) study. Aeroplysinin-1 is an antiangiogenic drug extracted from the sponge *Aplysina cavernicola* which has been proved to be a valuable candidate for the treatment of cancer and other antiangiogenic diseases. Our study shows that this molecule possesses the 1*S*,6*R* absolute configuration in aqueous solution, where only two conformers are present to a significant level. We discuss in detail the relationships between the chiro-optical ROA and VCD features, and the structural properties of various energy accessible conformers are described. The present work is one of the first studies in which both ROA and VCD have been used as complementary tools for the determination of absolute configuration and dominant solution-state conformations of an unknown therapeutically significant molecule.



I. INTRODUCTION

Chiro-optical spectroscopies serve for the unequivocal establishment of stereochemistry and for the accurate elucidation of conformations of molecules. The presence of a stereogenic center (for instance, one or more asymmetric carbons) also determines the tridimensional molecular conformation adopted, in such a way that this stereochemistry/conformation dual property is the origin, for example, of the exclusive role of biological molecules. Recently Raman optical activity (ROA), the Raman branch of vibrational optical activity (VOA), has emerged as a very powerful tool for the analysis of the stereochemistry/conformation property of biomolecules.[1−3] The advantage of ROA relative to the more broadly used vibrational circular dichroism (VCD) is the possibility of accurately analyzing aqueous solutions. Thus, it has been successfully used to investigate the structure of carbohydrates,[4] nucleic acids,[5] and proteins,[6,7] to cite a few examples. Though the ROA spectrum contains the relevant conformational information, the translation of the spectroscopic signal into precise molecular structures requires a library of ROA spectra and associated conformations. As an alternative, quantum chemistry can provide this connection since quantum programs have recently implemented the theoretical estimation of ROA spectra.

Aeroplysinin-1, hereafter referred as APS1 (Figure 1), is a naturally occurring brominated tyrosine metabolite produced by certain marine sponges.[8] It has been demonstrated that APS1 interferes with several key steps of angiogenesis in endothelial cells, including proliferation, migration, capillary tube formation, and the ability to invade and remodel the extracellular matrix.[9] Angiogenesis, the process of generation of new capillaries from a pre-existing vascular bed, has been related to a number of pathological processes such as tumor growth, metastasis, diabetic retinopathy, age-related macular degeneration, psoriasis, or arthritis, among others.[10,11] Thus, the strong antiangiogenic activity of APS1 makes it a valuable drug candidate for the treatment of cancer and other angiogenesis-dependent diseases.

As many other drugs, APS1 is a chirally active molecule owing to the presence of two asymmetric carbon atoms (labeled as 1 and 6 in Figure 1). It is also common for chiral drugs that only one of their stereoisomers is mostly responsible for therapeutic activity. It is therefore important to identify the stereoisomer that is responsible for activity in order to determine how its stereochemistry subtly conducts the specific interactions with biological receptors that trigger drug activity. In this work, we use ROA spectroscopy, combined with Raman spectroscopy, solid-state VCD, and quantum chemistry, to investigate the conformational properties of the active stereoisomer APS1.

II. EXPERIMENTAL METHODS

II.1. Vibrational and Chiro-Optical Spectroscopies. All the spectra were recorded using samples of APS1 isolated and

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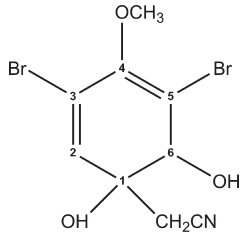

Figure 1. Chemical structure of aeroplysinin-1.

purified from *Aplysina cavernicola*, which were kindly provided by *Instituto Biomar S.A.* (León, Spain). Raman and ROA spectra were recorded simultaneously in aqueous solutions at a concentration of 20 mg/mL, which approaches the therapeutic concentration, using a BioTools ChiralRAMAN spectrometer (BioTools, Inc., Jupiter, FL, USA).[12] An excitation wavelength at 532 nm was used as generated by a Nd:VO_4 laser operating at 700 mW intensity at the sample (measured by a light meter). Backscattering collection of the scattered radiation was used with a spectral resolution of 7 cm^{-1} and acquisition times of 12−16 h. The raw spectra are presented, without smoothing, base lining, or any other data pretreatment.

VCD spectra were measured by means of a Bruker PMA50 optical bench coupled to a Vertex 70 spectrometer. In the PMA50, the infrared radiation is focused by a BaF_2 lens, passing an optical filter (3800−600 cm^{-1} range) and a ZnSe photoelastic modulator (PM, 50 kHz frequency). The light beam is finally collected by a D313/QMTC detector with nondichroic BaF_2 windows. Previous calibration of the PM at a fixed wavenumber is required before recording a VCD spectrum. Typically, calibrations at 1200 and 1800 cm^{-1} allowed us to obtain the VCD signal over the 2100−900 cm^{-1} infrared region. Every VCD spectrum was the result of averaging a minimum of 8000 scans (8 h adquisition time) at a spectral resolution of 4 cm^{-1}.

II.2. Calculation Methods. The Gaussian'03 package of programs[13] was used for DFT quantum chemical calculations. The Becke's three parameter (B3)[14] gradient-corrected exchange functional was used, and the nonlocal correlation was provided by the Lee−Yang−Parr (LYP) expressions.[15,16] Ground state structural properties and vibrational features were calculated using the Dunning's correlation consistent basis set aug-cc-pVDZ,[17,18] which includes the diffuse functions required for an accurate evaluation of the ROA activity of the normal modes. The theoretical spectra were obtained from the DFT intensities in combination with the calculated vibrational wavenumbers, which were unformly scaled with a single scaling factor (0.98) in order to keep unchanged the calculated intensities. Every band was represented by a Gaussian function of 20 cm^{-1} half-height width. All of the calculations employed the polarizable continuum model (PCM)[19,20] to simulate an aqueous environment.

III. RESULTS AND DISCUSSION

The theoretical ROA spectra of the four stereoisomers resulting from the combination of the *R* and *S* forms of the two asymmetric C1 and C6 carbon atoms, namely, 1*R*,6*R*, 1*S*,6*S*, 1*S*,6*R*, and 1*R*,6*S*, are shown in Figure 2. The best matching with the experimental ROA spectrum (see Figure 3 below) is found for the 1*S*,6*R* which is thus considered as the relevant stereoisomer for our study. This assignment agrees with early X-ray diffraction studies on APS1 obtained from sponges belonging

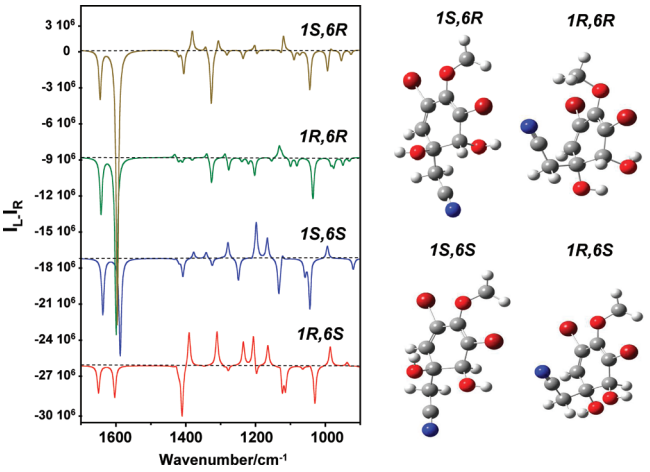

Figure 2. Theoretical ROA spectra of the four stereoisomers of APS1. In all cases the ROA activities were calculated over the B3LYP/aug-cc-pVCZ fully optimized structures shown to the right.

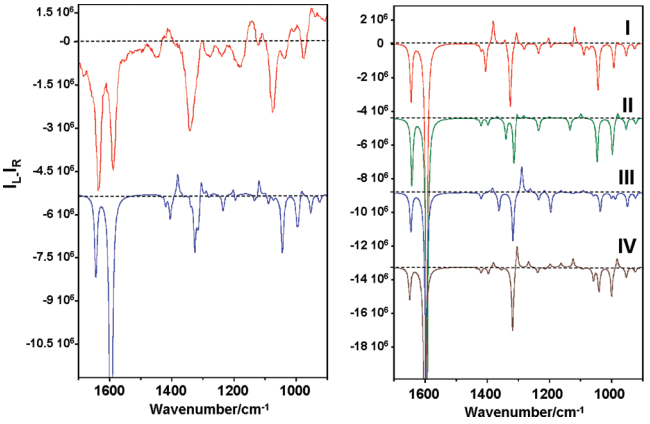

Figure 3. (Left) Experimental in aqueous solution (red) and averaged B3LYP/aug-cc-pVDZ (blue) ROA spectra of APS1. (Right) Calculated ROA spectra of the four conformers of 1*S*,6*R*-APS1 (see Figure 4).

to the genus *Aplysina*[21,22] (note that we studied APS1 obtained exclusively from the sponge *Aplysina cavernicola*).

Figure 3 (left panel) displays the experimental and theoretical ROA spectra of APS1 in aqueous solution. The theoretical spectrum was obtained after a conformational study of the 1*S*,6*R* isomer of APS1 This study was carried out in four steps:

(i) Optimization of the structure of APS1 until the minimal energy point was reached by allowing all the geometrical parameters to vary independently.

(ii) Energy scanning over the full variation of the bonds C1−OH, C1−CH_2CN, C4−OCH_3, and C6−OH. The analysis of the four energy profiles indicated that the molecular energy was largely dependent on the two hydroxyl rotors. The energy profiles obtained for the four rotors are included in Figure S1 of the Supporting Information.

(iii) Two-rotor simultaneous energy scanning over the C1−OH and C6−OH bonds to obtain the potential energy surface. This allowed us to detect the more stable conformers. Four minima were found in the potential energy surface (considering a cutoff of 2 kcal/mol), which represent the target conformers responsible of the ROA spectra.

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(iv) Structural optimization and force field calculation of the selected conformers and evaluation of the Raman and ROA spectra. Complete sets of structural parameters can be consulted in the Supporting Information (Tables S1−S4).

Figure 4 summarizes the relative Gibbs free energies and relative concentrations (C_i) at 298.15 K calculated by supposing a thermodynamic equilibrium (see equation inserted) among the more energy accessible conformers. The averaged ROA spectrum (Figure 3) was obtained by summing the individual spectra conveniently weighted by their concentrations. As can be observed, the pattern and sign of the dichroic bands are successfully estimated for all the measurable ROA bands, with the exception of the peaks around 1600 cm^{-1} which correspond to the stretching modes of the two double bonds. The experimental ROA spectrum is mainly dictated by contribution of the most stable conformer, **I**, of 1*S*,6*R*-APS1; however some details also arise from the higher energy conformers. In all cases, the distant positions of the C−OH groups in the 1*S*,6*R*-APS1 diastereomer prevent the formation of intramolecular hydrogen bonds whichever relative dihedral angles are adopted (see Tables S1−S4 in the Supporting Information for calculated values of relevant bond lengths and angles) what is a relevant structural feature as for the interaction, for instance, with polar substrates through H bonds. Judging from the geometries, there is partial conjugation between the two double bonds which confers some rigidity to this molecular fragment resulting in two chiral carbon atoms, C1 and C6, above and below the molecular plane.

A selection of the more relevant eigenvectors for conformer **I** has been included in Figure S2 of the Supporting Information. They show that the two O−H bending vibrations cannot be assigned to two specific wavenumbers (they are widely coupled with C−H bending modes). Between 1500 and 900 cm^{-1} most of the observed ROA signals are accurately predicted in wavenumber and in (±) sign. The most intense features in this region are the peaks at 1342 (−) and 1073 (−) cm^{-1}; the former can be assigned to the C6−H and C2−H bending modes, while the second band corresponds to the stretching vibration of the C1−CH_2CN bond. The description of the ROA spectrum in terms of vibrational modes is a certainly new approach to establishing spectroscopy-conformation relationships.

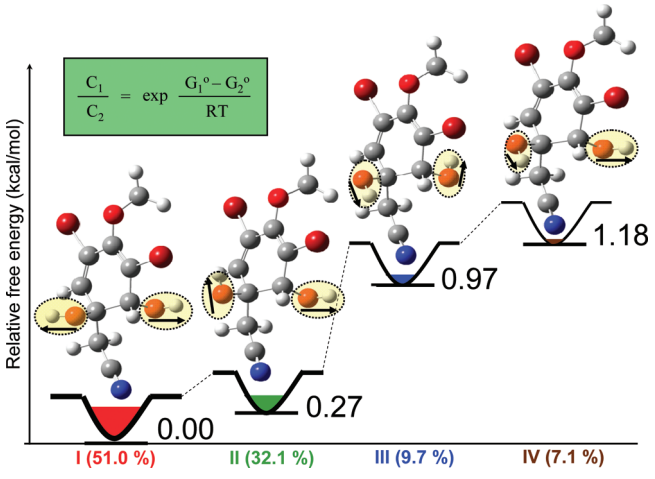

Figure 4. Conformations of the four lower energy conformers of (1*S*,6*R*)-APS1. In yellow, the relative orientation of the two hydroxyls is highlighted.

It is worth mentioning that efforts are still needed in order to rationalize chiro-optical signals in terms of molecular conformations and to connect optical activity and normal mode description. Here we show that this methodology is successful in order to establish meaningful structure−activity relationships. To get a complete understanding of the ROA spectrum, the corresponding normal Raman spectra, together with a list of relevant vibrational wavenumbers and assignments, are included in Figure S3 and Table S5 of the Supporting Information, respectively. The two C=C stretching bands are significantly less intense for **III** and **IV** than than for **I** and **II**, which could suggest a relationship between the optical activity of these bands and the interaction between these double bonds, in the sense that the stronger this interaction in the conformers is, the greater the ROA intensity. The bisignate couplet observed in the experimental ROA spectrum at 1448 (−) and 1411 (+) cm^{-1} is only correctly predicted in the case of **I**. These bands are assigned to OH, CH, and CH_2 bending vibrations and are strongly localized around the two stereogenic centers. Meanwhile, the spectrum of **II** fits well with the experimental spectrum in the 1200−900 cm^{-1} region, especially concerning the delocalized vibrations, such as the one associated with the negative feature at 1135 cm^{-1}. The ROA spectra of **III** and **IV** display more significant deviations, which is consistent with the lower concentrations predicted for these conformers in solution.

To get further insights on the conformational properties of the 1*S*,6*R* stereoisomer of APS1, we attempt to record the VCD spectrum in the solid state. Unfortunately, the ROA technique is not yet developed for solid state spectroscopy. Important alterations of the molecular structure in the solid state can be expected due to the conformational constrains imposed by intermolecular interactions. Figure 5 shows the VCD spectrum of solid APS1 which is compared with (i) the theoretical VCD spectrum calculated on the crystalline structure reported for APS1 (from sponges belonging to the genus *Aplysina*) and (ii) the theoretical VCD spectrum calculated for conformer **I**. The experimental spectrum is better reproduced by the first of these two approaches. This is particularly evident for the two bisignate couplets observed around 1600 and 1100 cm^{-1}, for which the

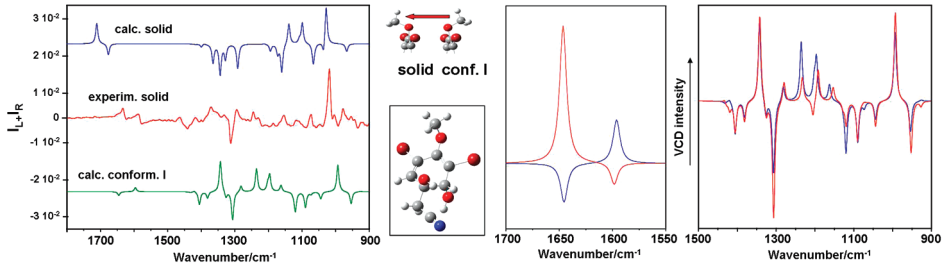

Figure 5. (Left panel) VCD spectra of APS1. (Right panels) Theoretical VCD spectrum of conformer **I** (red) and of the same structure reorienting the methoxy groups such as in the solid (blue).

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theoretical spectrum on the conformer **I** predicts the wrong sign. One of the most noticeable structural differences between the solid and conformer **I** is the different orientation of the methoxy group, such as represented in Figure 5. Recalculating the VCD spectrum of conformer **I** with the methoxy at the solid state position, the sign of the 1600 cm^{-1} bands is corrected. As a result 1*S*,6*R*-APS1 seems to contain a rather rigid skeleton due to the cis-diene (the six member ring and the bromines) and four "flexible" attachments (see the conformational flexibility of the methoxy as a example) with the hydroxyl groups marking the shape of the relevant conformers.

IV. CONCLUSIONS

The present work is one of the first studies in which both ROA and VCD have been used as complementary tools for the determination of absolute configuration and dominant solution-state conformations of an unknown therapeutically significant molecule. We have applied a chiro-optical vibrational study to the determination of the conformations of biomolecules based on ROA spectroscopy and its theoretical estimation over the most energetic accessible conformers. This is relevant since it allows assessment of the real conformation of biomolecules in close-to physiological conditions. This feature of ROA largely surpasses the existing dichroic spectroscopic techniques, most of them impeded for studies in water. In this sense, we show a nice example of the complementary use of VCD and ROA (in the conditions that VCD is not operative, ROA is, and vice versa). This study in APS1 has led to the elucidation of its conformation in water and to the visualization of the subtle interplay among the flexible groups of the molecule, in particular the two polar hydroxyls. The relative orientation of these groups such as deduced here could conduct specific interactions with biological targets and being involved in the antiangiogenic activity. Our next work on the use of ROA is directed to the elucidation of the conformational structure of APS1 when coupled to the active substrates under in vivo conditions.

■ ASSOCIATED CONTENT

Supporting Information. Details of optimized geometrical parameters, calculated wavenumbers, predicted assignments, conformational analysis, Raman spectra, and vibrational eigenvectors for the lower energy conformers of aeroplysinin-1. This material is available free of charge via the Internet at http://pubs.acs.org.

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