

Experimental and Calculated Vibrational Circular Dichroism Spectra of an Iminosugar Molecule: (2R, 3R, 4R) 1,4-dideoxy-1,4-imino-D-arabinitol Hydrochloride

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The first vibrational circular dichroism (VCD) measurement of a biologically important iminosugar, (2R, 3R, 4R) 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride is reported. The spectrum was recorded in the mid-IR region (1600 – 1100 cm⁻¹). Similarly to carbohydrate VCD spectra, beside the weak broad signals, a sharp intense negative band was observed around 1144 cm⁻¹. Preliminary DFT calculations were conducted to find the most stable conformers and to report the vibrational modes of the VCD peak. This finding encourages further VCD systematic measurements on iminosugars to establish, if possible, a spectrum-structure relationship.

Keywords: vibrational circular dichroism; density functional theory; iminosugar; carbohydrate; intramolecular hydrogen bond

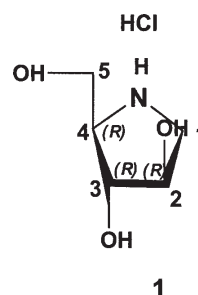
Iminosugars are carbohydrate analogues in which the ring oxygen has been replaced with nitrogen. They play an essential role in many biological and medicinal applications [1-3]. Structurally they are five, six or seven ring compounds with at least three asymmetric centers. Their stereochemical variety can be further widened by substitution of the hydroxyl groups or of the nitrogen atom with other groups which can exponentially increase the number of stereoisomers.

Considering its superior sensitivity to configuration and conformation, chiroptical spectroscopy might be a promising tool for structural analysis of iminosugars. To the best of our knowledge only few of these methodologies were applied to carbohydrates and even less to iminosugars, optical rotation being the most powerful technique in structural analysis, beside the achiral NMR technique [4-6].

From the theoretical point of view, reliable theoretical calculations of chiroptical properties are challenging due to aspects such as flexibility or proper handling of the solvent effects. Orientation of the hydroxyl or hydroxymethyl groups (formation of inter/intra molecular hydrogen bonds) greatly influences the chiroptical data and also the population of each conformer which should be accurately estimated [7-9]. However the usefulness of density functional theory (DFT) and time dependent DFT (TDDFT) in establishing spectrum structure relationships have been proven for carbohydrates [7-10].

Vibrational circular dichroism (VCD) spectroscopy is becoming one of the most convenient and reliable chiral analysis tools having a high theoretical predictive reliability and accessibility to a broad range of vibrations [11, 12]. VCD has been applied to determine the absolute configuration and conformation of chiral molecules [13, 14]. Recently much progress has been made in the field of sugar VCD, including establishing spectrum-structure relationships based on the two intense peaks present in the -CH and respectively in the mid-IR region [4, 7, 8, 15-19].

We report the first VCD measurement and theoretical calculations for the iminosugar molecule 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride **1**.



Experimental part

Materials and methods

Instrumentation

1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride (**1**) was purchased from Sigma - Aldrich and was used without further purification. IR and VCD spectra were measured on a Vertex70 Fourier transform infrared spectrometer coupled with a Bruker PMA 50 accessory. The VCD spectra were recorded with a CaF₂ window for 6h at a resolution of 4 cm⁻¹ at ambient temperature. The concentration was 0.45 mol/L and the path length 210 μm.

DMSO-d₆ (99.9%) was selected as solvent, for best solubility and weak absorption in the region of interest. The VCD and IR spectra were corrected with a solvent spectrum obtained in the same experimental conditions. Spectra were acquired in the mid-IR region, 1600 – 1100 cm⁻¹.

DFT calculations

Theoretical IR/VCD calculations started with a thorough analysis of the conformational freedom of the protonated (2R, 3R, 4R) 1,4-dideoxy-1,4-imino-D-arabinitol. The conformational energy surface was explored initially at semi-empirical level. PM3 method combined with

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simulated annealing technique [20] both implemented in the package Ampac [21] were used to perform the calculations of the relative energies of conformers found in various local minima of this surface. All conformers within roughly 5 kcal/mole of the lowest energy were kept for further DFT calculations.

The geometry was optimized with Gaussian 09 [22] on an isolated molecule in solution (DMSO), at density functional theory level using B97D functional [23] and 6-311++G** basis set. Solvent effects were simulated using the polarizable continuum model with the integral equation formalism variant (IEFPCM). We chose to work with B97D functional because it describes better than B3LYP (a common functional for carbohydrates) the long range interactions and consequently the hydrogen intramolecular bonds. Moreover the 6-311++G** basis set was proven to be large enough to calculate the relative energies of carbohydrates, therefore we consider it to be reliable in this case [24].

Boltzman populations were calculated using ΔG values at 298 K. IR and VCD spectra were constructed from calculated dipole and rotational strengths assuming lorentzian band shapes of 4 cm^{-1} at half-width. For comparison to experimental data, the calculated frequencies were scaled by 0.975.

Results and discussions

IR and VCD spectra can provide useful stereochemical information only if the signals are significantly strong. In figure 2 the IR and VCD spectra of the five-member ring iminosugar **1** are given. To the best of our knowledge this is the first example of VCD spectrum of an iminosugar.

As expected, the IR spectrum shows a broad signal, which most probably is due to the effect of the intra- and/or intermolecular hydrogen bonding or the conformational flexibility of the ring. The formation of intermolecular hydrogen bonds is highly probable because of the three

OH groups on the ring. The generation of dimers, trimers or higher order clusters can cause a dramatic change in both IR and VCD spectra when going from a monomer to higher order clusters in solution, as was shown for phenylpropionic acid or simple alcohols [13, 25].

In the VCD spectrum of compound **1**, beside the broad and weak patterns we identified a sharp, intense, negative band at around 1144 cm^{-1} . This is a signal that might be independent of the inter/intramolecular hydrogen bonds. It appears at approximately the same frequency with the so called "glycoside band", characteristic for a large number of carbohydrates (mono-, di-, polysaccharides) and which is independent of the inter/intra molecular hydrogen bonds [7, 15, 18, 19].

A complete analogy with the vibrational modes of carbohydrates responsible for the "glycoside band" is not appropriate, because this band is present only for the sugar anomers with an axial glycosidic linkage, regardless of the sugar type. In the VCD spectrum of sugars, the vibrational mode corresponding to the "glycoside band" is assigned to the C1-H1 deformation coupled with the C-O1 stretching vibration [7]. This type of linkage cannot be a characteristic feature of iminosugars, which lack the hydroxyl (methoxy) substituent in position 1. We also cannot talk in this case about the C-O1 stretching. The corresponding band of **1** might have a contribution from the C1-H deformation coupled with other vibrational modes as presented in table 1.

Moreover, for carbohydrates, the negative signal was assigned to the axial glycosidic sugar in the 4C_1 conformation, while the positive one was attributed to the 1C_4 form [7]. Therefore, from this band two important stereochemical informations were extracted: the anomeric configuration and the pyranose ring conformation [7]. In the case of furanose sugars a generalized spectrum – stereochemistry relationship is not yet established [4].

	Relative energy ΔG (kcal/mol)	ν_{calc} (cm^{-1})	Rotational Strengths $10^{-44}\text{esu}^2\text{cm}^2$	Description
Conformer C1	0	1148.5	-48.27	C ₁ H ₂ twisting, C ₅ H bending, (C ₂) O-H bending, C ₄ H bending, NH ₂ twisting, (C ₃) O-H bending,
Conformer C2	0.016	1149.5	-7.67	C ₁ H ₂ twisting, (C ₅) O-H bending, C ₂ H rocking, C ₅ H rocking, C ₄ H rocking, C ₃ H rocking, (C ₃) O-H bending,
Conformer C3	1.02	1148.6	46.18	C ₁ H ₂ twisting, C ₄ H bending, C ₅ H bending, (C ₅) O-H bending, NH ₂ twisting, (C ₂) O-H bending, (C ₃) O-H bending.

Table 1
DESCRIPTION OF THE THEORETICAL BAND AT THE APPROXIMATE FREQUENCY 1148 cm^{-1} ((C₂)O-H INDICATES THE CARBON 2 TO WHICH THE OH GROUP IS ATTACHED)

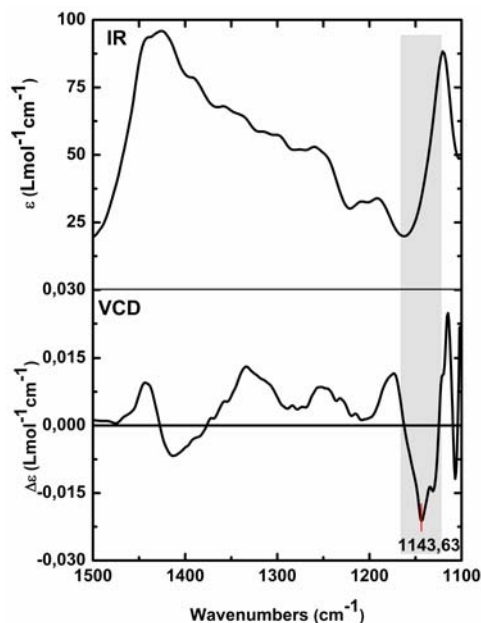


Fig. 1. IR and VCD spectra in DMSO-d6 ($c = 0.45 \text{ M}$, $l = 210 \mu\text{m}$) of (2*R*,3*R*,4*R*) 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride (1). Data collection time (6 h); resolution (4 cm^{-1})

A preliminary computational approach was also performed on the positively charged 1,4-dideoxy-1,4-imino-D-arabinitol. As emphasized before, this is challenging due to various aspects of these systems (five and six ring iminosugars) which are difficult to handle. The high flexibility of the five member ring molecule results in a high number of possible conformers. Two main conformations are possible for the ring, the envelope (E) and the twisted (T) form. For each 10 possible conformations are possible, which present a very low interconversion barrier [26]. Because of the free rotation of the hydroxyl groups, thousands of conformers are possible. However, the intramolecular hydrogen bonds stabilize preferentially certain conformations reducing the number of conformers. In our theoretical approach we considered the isolated molecule and we did not consider intermolecular interactions.

After the screening process at semiempirical level using the simulated annealing technique, twenty conformers were selected. Further, based on the predicted Gibbs energies, calculated at DFT level with the B97D/6-311++G**, it was found that positively charged (2*R*,3*R*,4*R*)-1,4-dideoxy-1,4-imino-D-arabinitol exists predominantly in six main conformations summing 99% of the Boltzman weighted populations. The two most stable conformers represent 77% of the total population. To the best of our knowledge for the iminosugar class there is no study that evaluates different density functionals and basis sets, but as in the case of carbohydrates, it is expected that the stability, the relative energy and the shape of the spectra of various conformers of an iminosugar compound varies with the change of basis functions and correlation levels [24]. However this is not the purpose of our study, so these aspects might be the subject of a future works.

Figure 2 presents the structures of the three most stable conformers (85.3%) and their corresponding VCD spectra in the region where the more intense experimental peak appears. The first two are stabilized by intramolecular hydrogen bonds, the difference between them being the relative orientation of two hydroxyl groups. The difference in energy between them is very small (0.16 kcal/mol). The hydroxyls of the third most stable conformer do not form any intra-molecular hydrogen bond, therefore this

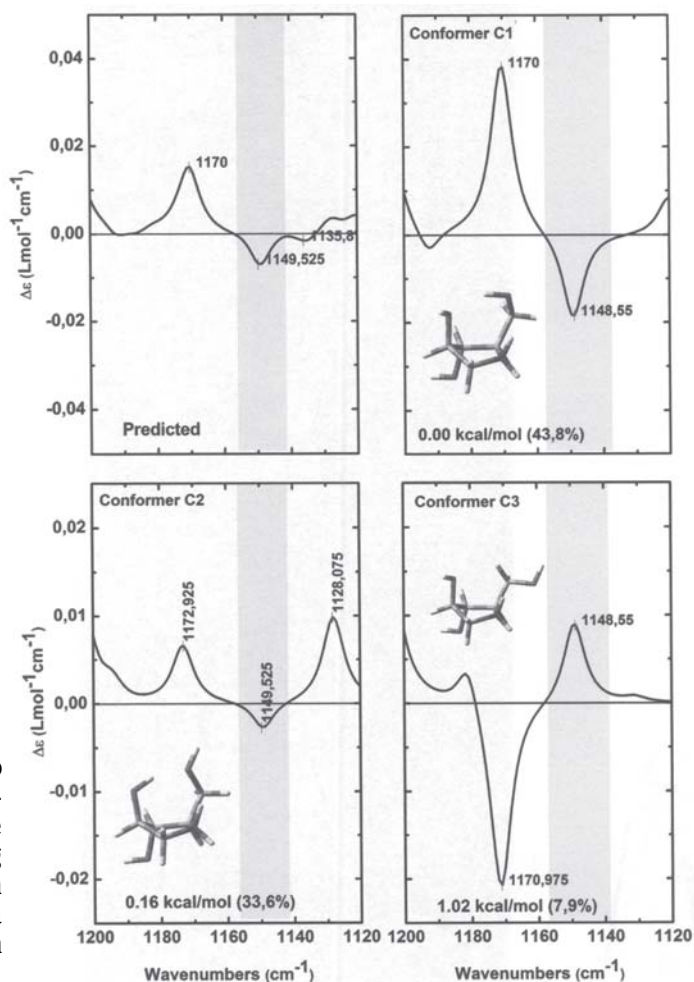


Fig. 2. The mediated spectra and the structure of three most stable conformers (C1, C2, C3) with their relative free energies and populations calculated at DFT level with B97D functional and 6-311++G** basis set and their calculated VCD spectra in the vicinity of the sharp, intense experimentally identified band ($1200 - 1120 \text{ cm}^{-1}$). Color scheme: red – oxygen, blue – nitrogen, grey – carbon, white hydrogen

conformer is less stable ($\approx 1 \text{ kcal/mol}$). They have all an envelope ring conformation.

The mediated IR spectrum (not shown here) does not agree well with the experimental one, mostly due to the inter-molecular interactions occurring in solution as we mentioned before. The same incompatibility is found in the VCD spectra, but we have identified the peaks around 1149 cm^{-1} which correspond to the peak found in the experimental measurement (fig. 2).

It is to be observed in the VCD spectrum (fig. 2) that the negative peak (at 1148.5 cm^{-1}) exhibited by the most stable conformer (C1) is stronger than the one exhibited by the second most stable conformer (C2) and the peak in the mediated spectrum. The VCD spectrum of the third most stable conformer (C3) exhibits a positive band at the same frequency.

In table 1 the normal mode (NM) assignments for the identified band for each conformer are described. The proposed assignment of the calculated NM is useful for understanding the signal presented in figure 1. Table 1 shows that the VCD band observed in the experimental spectrum at approx. 1144 cm^{-1} might contain important contributions from the ring CH_2 twisting, a mode that appears for all three conformers and a number of deformation vibrations (CH and OH) that are characteristic for each conformer. NH_2 twisting participates as well. The contribution of CH

bending to important VCD bands was pointed out in the VCD literature [27, 28].

More work is required both experimental and theoretical to elucidate better the nature of vibrational modes.

Conclusions

In the VCD spectrum of (2*R*, 3*R*, 4*R*) 1,4-dideoxy-1,4-imino-D-arabinitol hydrochloride we identified among the broad and weak signals, a negative, sharp and intense signal around 1144 cm⁻¹.

A preliminary computational approach was performed at DFT level using the B97D/6-311++G** combination. The first three most stable conformers, summing up 85.3% of the Boltzman population, show calculated VCD bands similar to that of the experimental spectrum. The characterization of the identified peak was performed at theoretical level and the vibrational modes as the C₂H₂ twisting coupled with a series of CH and OH bending and rocking modes were assigned.

The presence of the signal at 1144 cm⁻¹ stimulates us to conduct in the near future an extensive VCD study on various iminosugars (five and six membered) to construct if possible a comprehensive iminosugar VCD database which may lead to the first structure - VCD spectrum relationship for iminosugars.

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