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Product of alaptide synthesis: Determination of the absolute configuration

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ABSTRACT

Alaptide is the active substance of the veterinary dermatological ointment ALAPTID and a potential drug in human medicine. Electronic circular dichroism spectroscopy (ECD), transparent spectral region optical rotation (OR), and *ab initio* calculations were employed to determine the absolute configuration of alaptide. No X-ray structural data determining the absolute configuration were available. It was not possible to employ vibrational circular dichroism spectroscopy (VCD), because alaptide was not sufficiently soluble in common solvents used in VCD spectroscopy to generate reliable spectra. Both ECD spectra and OR values of alaptide solution were in good agreement with predicted data and determined unambiguously the absolute configuration of alaptide synthesized from (*S*)-alanine as being (*S*).

1. Introduction

Alaptide (spirocyclic synthetic dipeptide cyclo(1-amino-1-cyclopentanecarbonyl-L-alanyl) or 8(*S*)-methyl-6,9-diazaspiro[4,5]dekan-7,10-dion, Scheme 1) is a substance discovered in Prague by Šturc and Kasafírek [1]. Being a structural analogue of the hypothalamic factor inhibiting the release of melanocyte-stimulating hormone (MIF), it was found to affect behaviour and learning abilities of rodents, particularly rats and mice [2–4]. In dermatological veterinary medicine, alaptide may be used for the treatment of all warm-blooded animals for local therapy of dermal and mucosal lesions [5], burns, abrasions, frostbites, bedsores, ulcers, dog paws damaged by salted sidewalks etc. It is the active substance of the commercially available veterinary ointment ALAPTID® (Bioveta, Czech Republic).

At present, alaptide is considered as a potential drug in human medicine. Since it is an optically active molecule, it is necessary to inspect the entire synthetic process with the intention to determine unambiguously the absolute configuration of the final product. Except for the recently published X-ray powder diffraction data revealing the unit cell parameters and the space group of alaptide crystals [6], no other structural studies are available. To determine the absolute configuration of a molecule, it is possible to employ vibrational (VCD) or electronic (ECD) circular dichroism spectroscopy, vibrational Raman optical activity (VROA), or transparent spectral region optical rotation (OR), together with *ab initio* calculations [7]. To increase the reliability of the results, it is always feasible to employ more than one of the mentioned techniques [8]. Because of the very low solubility of alaptide in common solvents (~2 mg mL^{-1} in methanol, ~1 mg mL^{-1} in water, ~0.5 mg mL^{-1} in chloroform, ~0.02 mg mL^{-1} in *n*-hexane), it was not possible to obtain reliable VCD spectra of alaptide. Therefore, the results of ECD spectroscopy and OR were compared with the data calculated using time-dependent density functional theory (TD DFT) [9], concretely the B3LYP functional [10]. Our study demonstrates how the absolute configuration of a small pharmaceutically important molecule with low solubility can be determined.

2. Experimental

(*S*)-Alaptide was prepared by cyclization of methylester 1-(L-alanyl-amino)cyclopentane carboxylic acid, which was obtained by deprotection of methylester 1-[*N*-(benzyloxycarbonyl)-L-alanyl-amino]cyclopentane carboxylic acid according to Šturc and Kasafírek [1]. Methylester 1-(*Z*-L-alanyl-amino)cyclopentane carboxylic acid was synthesized from *Z*-protected (*S*)-alanine and methylester cycloleucine hydrochloride using mixed anhydride method. (*R*)-Alaptide was prepared in the same way as (*S*)-alaptide with *Z*-protected (*R*)-alanine as starting material instead of *Z*-protected (*S*)-alanine.

The ECD spectra of alaptide, at a concentration of 1.88 mmol L^{-1} in redistilled water and 1.88 mmol L^{-1} in methanol (analytical grade, Lachner, Czech Republic), were measured in a quartz cuvettes with optical pathlengths of 1 and 10 mm (Starna, USA),

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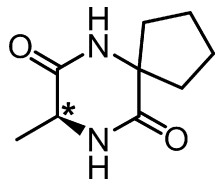

Scheme 1. Structure of alaptide.

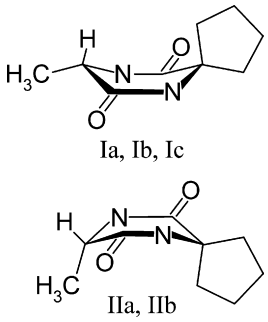

Fig. 1. Schematic representation of the twisted boat conformation of alaptide, with the methyl group in the equatorial (conformers **Ia**, **Ib** and **Ic**) and axial (conformers **IIa** and **IIb**) positions. Hydrogen atoms are omitted for clarity.

using a J-810 spectropolarimeter (Jasco, Japan). The conditions of measurement were as follows: scanning speed, 50 nm min^{-1}; response time, 2s; bandwidth, 1 nm; sensitivity, 100 mdeg; and 6 accumulations. The ECD spectrum of the solvent was used as the baseline and subtracted from the experimental spectra. Optical rotation of alaptide, at a concentration of 2.98 mmol L^{-1} in dimethylsulfoxide (DMSO, analytical grade, Sigma–Aldrich) and 8.78 mmol L^{-1} in methanol, was measured in a cell with an optical pathlength of 100 mm using an AUTOPOL IV polarimeter (Rudolph Research Analytical, USA), at 365, 405, 436, 546, 589, and 633 nm, and 20 °C.

3. Calculations

Conformational analysis of alaptide was carried out by the Conformational Search module of the HyperChem 8 software package [11], using the MM+ force field, with the intention of finding all the conformers generated by changing dihedral angles within both cycles in the molecule of alaptide. The molecular geometries obtained were consequently optimized at the B3LYP/6-31++G** level, using Gaussian 03 software [12]. ECD spectra and OR of all conformers were calculated using the same software package, at the B3LYP/6-31++G** level. All calculations were performed for isolated molecules of alaptide in vacuum.

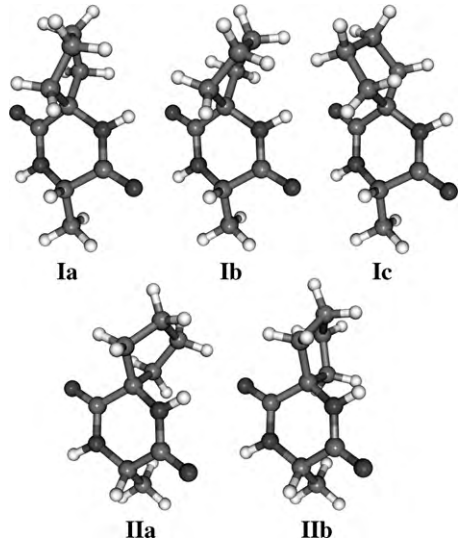

Fig. 2. B3LYP/6-31++G** structures of the alaptide conformers.

Table 1
Relative energies (ΔE) and relative free energies (ΔG) calculated at the B3LYP/6-31++G** level, and relative populations of all conformers of alaptide, determined according to ΔG at room temperature.

Conformer	ΔE (kJ mol^{-1})	ΔG (kJ mol^{-1})	Population
Ia	0.00	1.17	23.4
Ib	0.55	0.00	37.9
Ic	1.92	2.46	13.8
IIa	1.99	3.21	10.2
IIb	2.10	2.29	14.8

For the calculation of ECD spectra, the 20 lowest electronic transitions were calculated for each conformer. The simulated ECD spectrum was obtained using a Gaussian band shape with the exponential half-width (half the bandwidth at 1/*e* peak height) of 0.2 eV. The equilibrium population of conformers at T = 293.15 K was estimated according to their relative free energies (ΔG) calculated at the B3LYP/6-31++G** level, when ΔG included zero-point energy and vibrational, rotational and translational thermal energy corrections.

4. Results and discussion

4.1. Conformational analysis

Alaptide is a relatively small molecule possessing no conformer-generating peripheral groups. Still, it is composed of one six-membered ring and one five-membered ring, with constrained flexibility. Conformational analysis at the molecular mechanics level that was allowed to change simultaneously the dihedral angles within both rings and their mutual orientation disclosed five possible geometries, which were subsequently optimized at

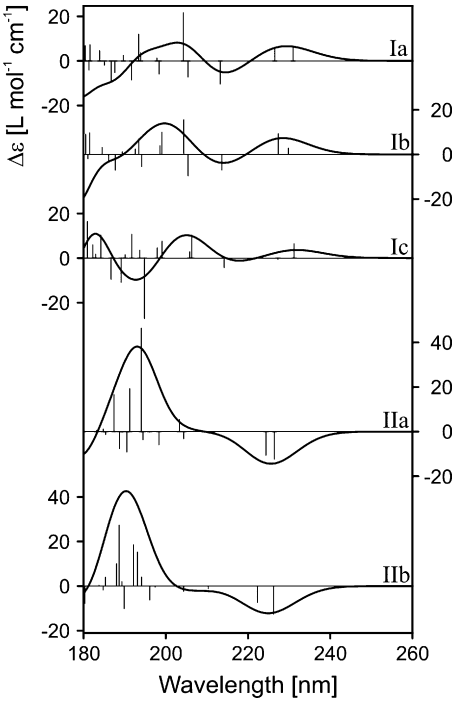

Fig. 3. ECD spectra and rotational strengths of the five conformers of alaptide calculated at the B3LYP/6-31++G** level.

the B3LYP/6-31++G** level. For all five conformers, the central six-membered ring adopts exclusively the conformation of a slightly twisted boat. Depending on the position of the methyl group, the resulting conformers can be divided into two groups: the first one, comprising **Ia**, **Ib** and **Ic**, and the second one, comprising **IIa** and **IIb**, with the methyl group being in the equatorial and axial positions, respectively (Fig. 1). The detailed structure of all the conformers is depicted in Fig. 2.

Table 1 shows the relative energies (ΔE) and the relative free energies (ΔG), both of which were calculated at the B3LYP/6-31++G** level, and the relative populations of all conformers, calculated according to ΔG, at room temperature. From Table 1, it is obvious that the order of the first four conformers (**Ia**, **Ib**, **Ic** and **IIa**), according to their relative energies and relative free energies, is different. This is not so surprising, because the difference between the energies of the conformers is very small, and under such circumstances the zero-point energy, thermal energy, and entropy contributions for a particular conformer become significant, and can change the abundance order. Taking ΔG into consideration, the conformers **Ib** and **Ia** have the highest population (37.9 and 23.4%, respectively), and therefore, also the highest impact on the resulting spectrum.

4.2. ECD spectra

Fig. 3 shows the ECD spectra obtained using a Gaussian band shape, together with individual electronic transitions of all five conformers. Among the spectra of the conformers, distinct similarities can be observed within two groups of conformers: those with the methyl group in the equatorial position (**Ia**, **Ib** and **Ic**) and in the axial position (**IIa** and **IIb**). It is apparent that the effect of the geometry of the central six-membered ring on the ECD spectrum is dominant, while the conformation of the cyclopentane ring has only a minor impact.

To compare the calculated and experimental ECD spectra, the calculated spectra were averaged. Fig. 4 shows a weight-averaged spectrum of conformers **Ia**, **Ib**, **Ic**, **IIa** and **IIb**, using weights of 0.234, 0.379, 0.138, 0.102 and 0.148, respectively, together with the experimental spectra of alaptide synthesized from (*R*)- and (*S*)-alanine, measured in water and methanol. Measurement of the ECD spectra of alaptide and its opposite enantiomer allowed the identification of a weak band at ~239 nm; the confirmation of this band was done by measuring both the enantiomers of alaptide in a 10 mm cuvette (inset in Fig. 4).

A very good agreement was observed between the experimental spectrum of water solution of alaptide synthesized from (*S*)-alanine and the calculated spectrum. The bands at ~239, ~212 and ~186 nm in the experimental spectrum were identified with the simulated ones at ~231, ~215 and ~194 nm. Although the positions and intensities of the bands in the simulated and experimental spectra were not exactly the same, the extent of the agreement between the spectra allowed us to determine the absolute configuration of the chiral center of alaptide synthesized from (*S*)-alanine as (*S*). The experimental spectrum of methanolic solution of alaptide synthesized from (*S*)-alanine exhibited bands at ~240 and ~219 nm, which were identified with the simulated ones at ~231 and ~215 nm. The measurement in the spectral region below 210 nm in methanol was not possible due to its high absorption. However, the assignment of the two identified bands confirmed our conclusion regarding the absolute configuration of alaptide.

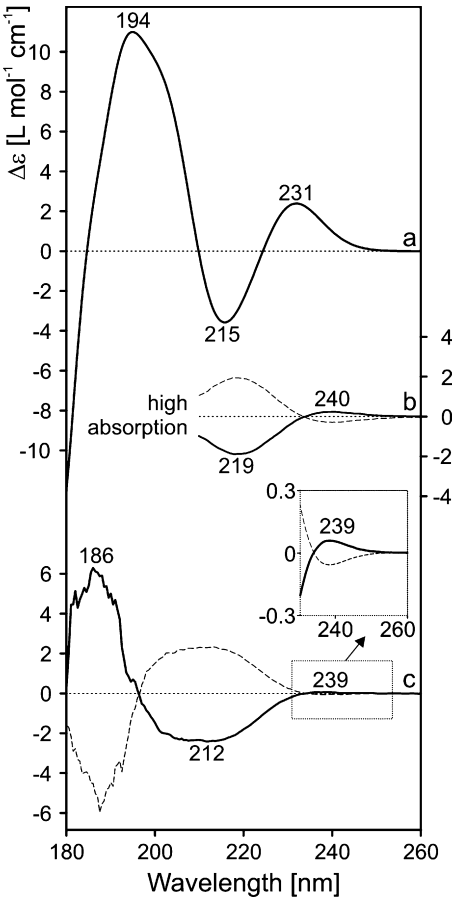

Fig. 4. Comparison of the experimental spectra of alaptide synthesized from (*S*)-alanine (full line) and (*R*)-alanine (dashed line) measured in a 1 mm cuvette in water (c) and methanol (b) with the simulated spectrum of (*S*)-alaptide (a) obtained as a weight-average of spectra of five conformers. Inset: detail of the band at 239 nm, spectrum measured in water in a 10 mm cuvette.

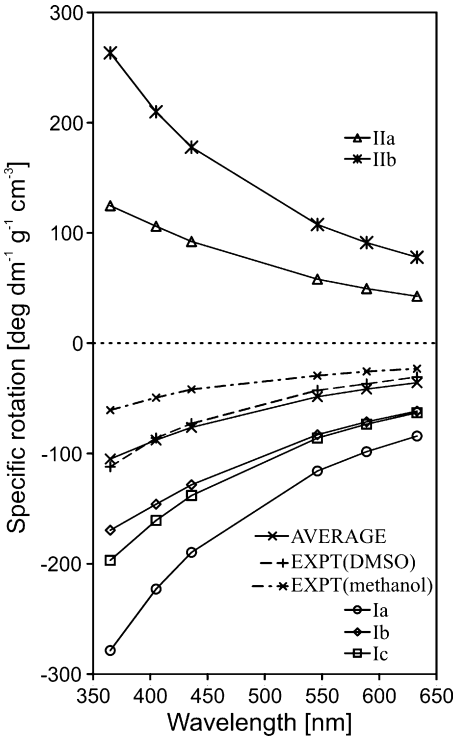

Fig. 5. Calculated OR values of the conformers **Ia**, **Ib**, **Ic**, **IIa** and **IIb**, together with their population-weighted average in comparison with experimental values.

4.3. Transparent spectral region optical rotation

Fig. 5 shows the experimental specific rotations of alaptide synthesized from (*S*)-alanine, measured at 365, 405, 436, 546, 589, and 633 nm, together with the calculated optical rotations of five conformers of (*S*)-alaptide and their weight-averaged values. Similar to the ECD spectra, the values of the optical rotation of a particular conformer were mostly affected by the geometry of the central six-membered ring, whereas the impact of the cyclopentane ring conformation was negligible. Therefore, optical rotations of **Ia**, **Ib** and **Ic** were negative for all the calculated wavelengths, while optical rotations of **IIa** and **IIb** were positive. Optical rotations of all five conformers were weight-averaged according to their populations at room temperature. Averaged optical rotation values, having negative sign for all wavelengths, were in excellent agreement with experimental data measured in DMSO. The experimental OR values of alaptide measured in methanol were lower compared to the predicted values but still in a reasonably good agreement with the simulation. The results of OR were consistent with the assignment of the chiral center of alaptide obtained by the ECD method.

5. Conclusion

Conformational analysis of alaptide led to five conformers, all significantly populated at room temperature. The central six-membered ring of alaptide adopts exclusively the conformation of a slightly twisted boat. Alaptide conformers can be divided into two groups, with the methyl group being in equatorial (**Ia**, **Ib** and **Ic**) and the axial (**IIa** and **IIb**) positions. Distinctively similar ECD spectra and optical rotation values can be observed for conformers within each of the two groups.

Based on the extent of agreement between the experimental and calculated ECD spectra, and optical rotations, alaptide synthesized from (*S*)-alanine was determined to have the (*S*) configuration. Nevertheless, it should be pointed out that both groups of conformers (**Ia**, **Ib**, **Ic** and **IIa**, **IIb**) with dissimilar ECD spectra and almost opposite optical rotations are significantly populated. Therefore, the reliability of the result is always given by the correctness of the determination of the conformer population. This work demonstrates the possibility to determine the absolute configuration of molecules whose solubility is too low for VCD spectroscopy, which is usually employed as the first method of choice and is generally the most reliable one.

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