Determination of the Absolute Configuration of (−)-Mirtazapine by Vibrational Circular Dichroism

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The absolute configuration of the (−)-enantiomer of mirtazapine was determined by means of vibrational circular dichroism (VCD). The observed VCD of (−)-mirtazapine showed excellent correlation with the calculated VCD of the (*R*)-enantiomer. This is in agreement with the absolute configuration as determined by independent synthesis starting from (*R*)-phenylglycine.

Introduction. – Mirtazapine, the structure of which is shown in *Fig. 1*, is a compound with antidepressant therapeutic effects. Mirtazapine is a racemate consisting of two pharmacologically active enantiomers, *both* of which contribute to the therapeutic effect [1][2].

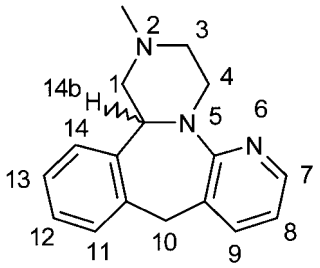

Fig. 1. *Structure of (±)-mirtazapine*

We have determined the absolute configuration of the (−)-enantiomer of mirtazapine by vibrational circular dichroism (VCD) [3–6]. Circular dichroism (CD) is the difference in absorption of left and right circularly polarized light. Only chiral molecules display CD, and enantiomers have CD of equal magnitude but opposite sign. In contrast to VIS/UV CD, which arises from electronic transitions, VCD originates in vibrational transitions. Recently, commercial VCD spectrometers have become available [3]. At the same time, developments in quantum-chemistry software have allowed the reliable calculation of VCD spectra by density-functional theory

(DFT) [7][8]. Comparison of the observed and calculated VCD spectra enables determination of the absolute configuration of chiral molecules without the need to obtain crystals of the compounds suited for single-crystal X-ray analysis. These two developments, VCD instrumentation and software, will increase the applications of VCD to stereochemical problems in general, and the determination of absolute configuration in particular. In addition, the determination of the absolute configuration by VCD also yields a close approximation of the conformation, or conformational distribution, of the sample molecule in solution.

Experimental. – Racemic mirtazapine was obtained as described in [9][10]. The (−)-enantiomer of mirtazapine was obtained from the racemate by dissolution of the racemate with 0.5 equiv. of (−)-dibenzoyltartrate in EtOH and subsequent crystallization by the addition of Et_2O. The compound was treated with aq. base and extracted with an org. solvent to yield the free base [11]. This material had a specific optical rotation of − 528.1° at the sodium D line, at a concentration of 1% in $CHCl_3$.

The (−)-enantiomer was dissolved in $CDCl_3$ at a concentration of 0.10M (4.5 mg/170 µl solvent). The IR and VCD measurements were carried out with a *Chiralir* Fourier transform VCD spectrometer (*Bomem/Bio Tools*, Quebec, Canada) at a full-bandwidth resolution of 8 cm^{-1}, with an IR cell equipped with BaF_2 windows and a path length of 72 µm. The photoelastic modulator was optimized for spectral VCD measurement at 1100 cm^{-1}. The VCD spectra were collected as three blocks of 60 min each. The blocks were averaged to obtain the final VCD spectrum and appropriately subtracted to obtain the estimate of the noise included in the figure with the experimental VCD spectrum. The VCD intensities were calibrated with software associated with the *Chiralir*. The VCD calibration method used by these routines is based on the crossing points of a multiple waveplate, as described in [3]. The VCD baseline was obtained by a small correction from the VCD spectrum of the $CDCl_3$ solvent.

To obtain the calculated VCD and IR spectra, the (*R*)-enantiomer was first built with *Hyperchem* (*HyperCube, Inc.*, Gainsville, FL). The geometry optimization and calculation of vibrational frequencies and IR and VCD intensities were carried out with Gaussian 98 (*Gaussian, Inc.*, Pittsburgh, PA) at the DFT level (B3LYP functional, 6-31G(d) basis set). The VCD calculation incorporated into Gaussian 98 utilizes gauge-invariant atomic orbitals and the magnetic-field-perturbation method for calculating VCD intensities, which has been shown to provide excellent agreement with experiment. The calculations reported here, for a molecule with 39 atoms and 338 basis functions (636 primitive gaussians), were carried out on an *SGI Octane R12K (OCTANE/SE* - 2 × R12K 300 MHz/2MB cache, 1.8 GB memory, 18 GB internal drive). The frequency/VCD intensity calculation required *ca*. 40 h cpu. Each step of the geometry optimization required *ca*. 1 h cpu, and *ca*. 1 week of total computer time was required for both geometry optimization and VCD calculation. The frequency/VCD calculation was repeated on a *Dell 8100* PC with 1.0 GB DRAM, 80 GB hard drive, and a 1.5 GHz processor running Gaussian 98W (the windows version of Gaussian that includes all the features of Gaussian 98), requiring *ca*. 30 h cpu. This molecule required an increase over the default Gaussian 98 memory allocation to at least 128 MB to run to completion on the VCD calculation. The calculated frequencies were scaled uniformly by 0.97, and the calculated IR and VCD intensities were converted to Lorenztian bands with 5 cm^{-1} half-width for visual comparison to the experimental spectra.

Results and Discussion. – The optimized conformation of the (*R*)-enantiomer is shown in *Fig. 2*. Other conformations were determined to have unfavorable steric interactions and were not calculated further. For example, moving the aromatic rings back into the plane of *Fig. 2* causes the $-CH_2-$ bridge to move above the plane of *Fig. 2*. This leads to an unfavorable interaction between a C−H bond of this CH_2 group and the methine C*H at the stereogenic center. Energy minimizations at the molecular mechanics level (MM^+ in *HyperChem*) confirmed that other conformations of the six- and seven-membered rings were unfavorable.

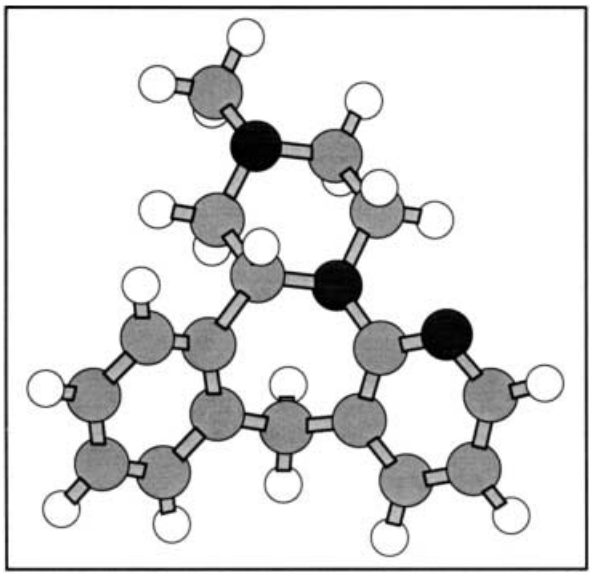

Fig. 2. *Calculated optimized geometry of* (R)*-enantiomer of mirtazapine*

The observed and calculated IR and VCD spectra of mirtazapine are shown together in *Fig. 3*. The calculated VCD spectra are for the (*R*)-enantiomer, while the experimental spectra were measured with the (−)-enantiomer. The bandwidths for the calculated spectrum were selected to match the observed IR data, and the same bandwidth values were used for the calculated VCD spectrum. In this case, a single bandwidth (5 cm^{-1}) was sufficient, but in other spectral regions or other types of molecules, it is sometimes necessary to utilize different bandwidths for different types of modes (*e.g.*, those involving H-bonding) to adequately match the IR data. Uniform scaling of the calculated frequencies by 0.97 brings the most-intense calculated absorption features into close frequency agreement with the experimental spectrum. Vibrational frequencies calculated with DFT methods are typically slightly higher than observed. For the methods employed here, calculated intensities are typically within a factor of two of observed. Identification of absolute configuration requires only a comparison of observed and calculated VCD sign patterns.

In comparing the experimental and calculated VCD spectra, the main focus is on the bands that carry the largest positive or negative VCD intensity. As the magnitude of the intensity decreases, the level of uncertainty increases. For the experimental VCD spectrum, the uncertainty is primarily due to instrumental noise that increases as the magnitude of the IR absorption increases. The VCD bands with the highest level of confidence are generally those that have the highest ratio of VCD to IR intensity, the so-called anisotropy ratio *g*. The calculated VCD spectrum has no noise, *per se*, but bands with small VCD intensity have larger uncertainties in relative magnitude and sign. For example, VCD intensities that arise from nearly orthogonal electric and magnetic dipole transition moments can change sign easily, when the angle between the two transitions changes by a few degrees from less than to greater than 90° or *vice versa*.

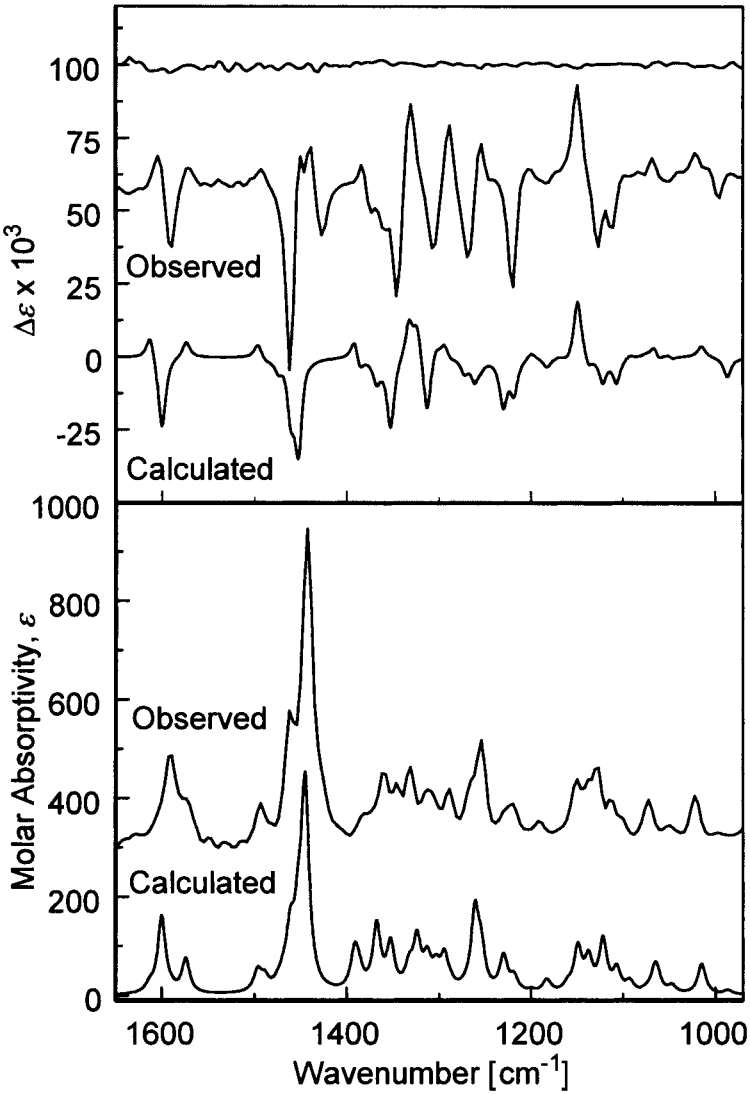

Fig. 3. *Observed IR and VCD spectra the sample of (−)-mirtazapine compared to calculated spectra for the* (R)-*entantiomer* (5 cm^{-1} half-width Lorentzian bands, frequencies scaled by 0.97). Uppermost trace is VCD noise. Observed spectra offset for clarity.

As a result, comparison of measured and calculated VCD spectra in regions of high experimental noise and for small VCD intensities should not be regarded as carrying a high level of significance.

The comparison then is based on the overall pattern of the relative magnitudes and signs of the corresponding VCD bands in the measured and calculated spectra. In the case of absolute configuration, once a good correlation is established, one needs only to

decide whether the signs are all the same or opposite. As a result, knowledge of the absolute configuration used for the calculated VCD spectrum yields the desired assignment.

The overall calculated IR-intensity pattern agrees remarkably well with experiment, consistent with the expectation of only one dominant solution conformation. The calculated VCD data are also in excellent agreement with experiment. For the (*S*)-enantiomer, all the VCD bands have opposite sign (but the same magnitude) compared to those calculated for the (*R*)-enantiomer. The agreement in sign and relative intensities between the observed and calculated VCD spectra in *Fig. 3*, thus, unambiguously leads to the assignment of the configuration of (−)-mirtazapine as (*R*), and simultaneously identifies the solution conformation of this molecule in $CDCl_3$ as shown in *Fig. 2*.

An overlay of the solution structure determined as described with the conformation as determined by X-ray [12] is shown in *Fig. 4*. Except for some slight differences around the *N*-Me group, the structures are identical. Moreover, qualitative comparison with the $CDCl_3$ solution conformation determined by NMR [12][13] confirms all main features found in the present study: the chair conformation of the piperazine ring, the equatorial position of the *N*-Me group, the tetragonal atom N(5), the *anti*-relationship between the N(5) lone pair and H−C(14b) (see *Fig. 1*).

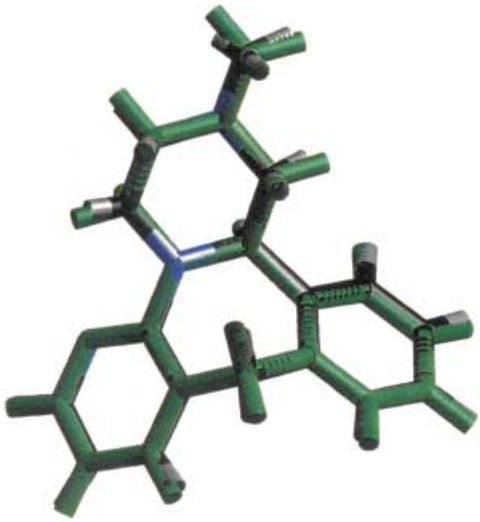

Fig. 4. *Overlay between X ray conformation* (green) *and the conformation* (grey) *used to calculate the IR and VCD spectra*

Conclusions. – The observed VCD spectrum of the (−)-enantiomer is in excellent agreement with the calculation for the (*R*)-configuration, and, thus, the absolute configuration is assigned as (−)-(*R*)-mirtazapine. The quality of the correspondence is partly due to the rigid structure of mirtazapine, which results in the presence of one single conformer in solution and substantially simplifies the calculations of the VCD spectrum. The absolute configuration reported here is in agreement with that determined independently by synthesis starting from (−)-D-phenylglycine [11].

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