Determination of Absolute Configuration of Chiral Molecules Using Vibrational Optical Activity: A Review

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Determination of the absolute handedness, known as absolute configuration (AC), of chiral molecules is an important step in any field related to chirality, especially in the pharmaceutical industry. Vibrational optical activity (VOA) has become a powerful tool for the determination of the AC of chiral molecules in the solution state after nearly forty years of evolution. VOA offers a novel alternative, or supplement, to X-ray crystallography, permitting AC determinations on neat liquid, oil, and solution samples without the need to grow single crystals of the pure chiral sample molecules as required for X-ray analysis. By comparing the sign and intensity of the measured VOA spectrum with the corresponding ab initio density functional theory (DFT) calculated VOA spectrum of a chosen configuration, one can unambiguously assign the AC of a chiral molecule. Comparing measured VOA spectra with calculated VOA spectra of all the conformers can also provide solution-state conformational populations. VOA consists of infrared vibrational circular dichroism (VCD) and vibrational Raman optical activity (ROA). Currently, VCD is used routinely by researchers in a variety of backgrounds, including molecular chirality, asymmetric synthesis, chiral catalysis, drug screening, pharmacology, and natural products. Although the application of ROA in AC determination lags behind that of VCD, with the recent implementation of ROA subroutines in commercial quantum chemistry software, ROA will in the future complement VCD for AC determination. In this review, the basic principles of the application of VCD to the determination of absolute configuration in chiral molecules are described. The steps required for VCD spectral measurement and calculation are outlined, followed by brief descriptions of recently published papers reporting the determination of AC in small organic, pharmaceutical, and natural product molecules.

Index Headings: Absolute configuration; Vibrational optical activity; VOA; Vibrational circular dichroism; VCD; Raman optical activity; ROA; Chiral pharmaceutical molecules; Natural product molecules.
INTRODUCTION

Vibrational optical activity (VOA) is a spectroscopic measure of the differential response of a chiral molecule to left versus right circularly polarized radiation during a vibrational transition. VOA consists of infrared vibrational circular dichroism, known as VCD, and vibrational Raman optical activity, known as ROA. The infrared (IR) or Raman spectrum of a pair of enantiomers is the same but their VCD or ROA spectra are equal in intensity but opposite in sign (mirror images of each other), as shown in Fig. 1 for (1R)-(+) and (1S)-(−)-camphor. The VCD spectrum of a chiral molecule can be calculated using an ab initio density functional theory (DFT) method. The absolute configuration (AC) of a chiral molecule can be determined by comparing the measured VCD or ROA spectrum with the calculated spectrum. After nearly four decades of development since its discovery in the 1970s, vibrational optical activity (VOA) has evolved into a well-established technique used routinely for the AC determination of small organic molecules including natural products and pharmaceutical compounds. VOA has also been successfully applied for the determination of chiral purity (or %EE) of a changing enantiomeric mixture, monitoring reactions that involve chiral molecules, and characterization of biomolecules such as peptides, proteins, carbohydrates, nucleic acids, and even viruses. AC determination has become increasingly important for the pharmaceutical industry because currently more than two thirds of the drugs on the global market are chiral drugs serving myriad therapeutic areas, such as anxiety, indigestion, heartburn, arthritis, AIDS, cancer, allergies, etc. In 2010 nine out of ten top-selling blockbuster drugs, including Lipitor and Plavix, were chiral drugs. With the recent rapid development of biotherapeutic pharmaceuticals, chiral drugs will play an even more important role in saving the lives of patients because all bio-drugs are chiral.

Since the FDA recommended the use of stereochemically pure drugs in 1992, single enantiomer drugs have become the standard in pharmaceutical companies when working with compounds featuring asymmetric centers. Shortening timelines for chiral drug discovery and development usually depends on the efficiency of asymmetric synthesis, enantiomeric purification, and AC determination. Techniques that are prevalent in both academia and the pharmaceutical industry for the determination of the AC of chiral molecules include X-ray crystallography, Mosher’s method (NMR), the chiral liquid crystal NMR technique, and VCD. X-ray crystallography is still considered the most reliable technique, but it requires a single crystal and typically at least one heavy atom. Mosher’s NMR method requires derivatization and is used primarily on alcohols and amines. The chiral-liquid-crystal NMR method generally requires a larger sample size (40 to 50 milligrams) and complex experimental
procedures.27 The VOA technique complements the X-ray and NMR methods well because AC determination by VOA does not require a single crystal, does not entail molecular derivatization or intermolecular interactions, and is suitable for samples in liquid, solid, and gas phases. Dedicated Fourier transform (FT) VCD instrumentation was commercialized first by BioTools with ABB Bomem in 1997,28 and since then many pharmaceutical companies and academic groups have been using VCD as a routine method for the AC determination of chiral molecules in the solution state. More than an estimated 3000 ACs have been determined by VCD in the past few years, and the number is increasing rapidly every year. ROA instrumentation was commercialized by BioTools in 2003 and has been used mainly for the characterization of biomolecules.29 ROA has also been used for the determination of AC,30,31 but its application currently lags behind that of VCD. Recently Gaussian Inc. (Wallingford, CT) implemented ROA calculations in the Gaussian 09 program,32 which should facilitate AC determinations by ROA. Figure 2 shows a comparison of the AC determination of (−)-(S)-(α)-pinene by VCD versus by ROA. It is clear that the AC of (α)-pinene can be determined equally well by either VCD or ROA.

In this Focal Point Review the theoretical background, instrumentation, and methodology for AC determination by VOA are described, and some examples of recent applications of AC determination by VCD are highlighted. ROA is included in the introduction, but the main focus of this review is on AC determination by VCD because very few publications using ROA for AC determination have appeared.

DEFINITIONS OF VIBRATIONAL CIRCULAR DICHROISM AND RAMAN OPTICAL ACTIVITY

Vibrational circular dichroism has only one form, defined as the difference in the absorbance $A(\bar{v})$ of a chiral molecule for left circularly polarized (LCP) versus right circularly polarized (RCP) infrared radiation during vibrational excitation:1

$$\Delta A(\bar{v}) = A_L(\bar{v}) - A_R(\bar{v})$$  \hspace{1cm} (1)

Here the decadic absorbance for unpolarized absorption is given by

$$A(\bar{v}) = [A_L(\bar{v}) + A_R(\bar{v})]/2$$

$$= -\log_{10}[I(\bar{v})/I_0(\bar{v})]$$  \hspace{1cm} (2)
where \( I(\bar{m}) \) and \( I_0(\bar{m}) \) are the transmission intensities with and without the sample, respectively, and where \( \bar{m} \) is the wavenumber frequency of the radiation. VCD is also defined in terms of the molar absorptivity as

\[
\Delta \varepsilon(\bar{m}) = \varepsilon_L(\bar{m}) - \varepsilon_R(\bar{m})
\]

where the molar absorptivity of a sample of path length \( b \) and concentration \( C \) is

\[
\varepsilon(\bar{m}) = A(\bar{m})/bC
\]

In contrast to VCD, ROA has four circular polarization (CP) forms. These are given by the following definitions:\(^1\)

- **ICP-ROA**: \( \Delta I_R(\bar{m}) = I_R^a(\bar{m}) - I_L^a(\bar{m}) \) (5)
- **SCP-ROA**: \( \Delta I^R(\bar{m}) = I_R(\bar{m}) - I_L(\bar{m}) \) (6)
- **DCP I-ROA**: \( \Delta I_I(\bar{m}) = I_R^a(\bar{m}) - I_L^a(\bar{m}) \) (7)
- **DCP II-ROA**: \( \Delta I_{II}(\bar{m}) = I_R^a(\bar{m}) - I_L^a(\bar{m}) \) (8)

Incident circular polarization ROA (ICP-ROA) is the original form of ROA, first measured in 1973\(^6\) and confirmed in 1975,\(^2\) in which the difference in Raman intensity for RCP versus LCP incident radiation states is measured while using fixed non-elliptical (unpolarized or linearly polarized) polarization state \( a \) for the scattered radiation. Scattered circular polarization ROA (SCP-ROA), first measured in 1988,\(^3\) is the difference in Raman intensity for RCP versus LCP scattered radiation while using fixed non-elliptical incident polarization state \( a \). SCP-ROA is the form of ROA used in commercial ROA instruments. Two new forms of ROA, predicted in 1989\(^4\) and measured a few years later,\(^4,5\) are called dual circular polarization in-phase and out-of-phase ROA (DCP I-ROA and DCP II-ROA), which, as seen in Eqs. 7 and 8, are differences in Raman intensity using both incident and scattered circular polarization states in the same measurements.

**METHODOLOGY**

**Method of Absolute Configuration Determination.** For VCD, the AC determination of a chiral molecule is made by comparing the experimental IR and VCD spectra of the unknown sample with those of the corresponding calculated IR and VCD spectra of the molecule using a chosen AC. If the sign and relative intensity of the observed bands in the VCD spectrum of the sample are the same as that of the calculated spectrum, the AC of the sample is the same as the AC chosen for the calculation. If the bands of the observed VCD spectra are the opposite sign of those calculated, the sample has the opposite AC of that used in the calculation.

**Vibrational Optical Activity Instrumentation.** As with infrared spectrometers, there are two types of VCD instruments, dispersive and Fourier transform.\(^36\) Before the advent of the FT-VCD spectrometer in 1979,\(^37\) all VCD instruments used a monochromator that scanned through a certain
Dispersive VCDs are still used for biological samples in the mid-IR region, taking advantage of a stronger source and a narrower spectral range. Shortly after BioTools introduced the first commercial FT-VCD instrument to the market in 1997, Bruker, Jasco, and Thermo-Electron also offered VCD accessories for their FT-IR spectrometers. Despite the different designs for VCD spectrometers, all of them use a photo-elastic modulator (PEM) placed in front of the sample in an FT-IR spectrometer to modulate the IR beam between left- and right-circular polarizations at high frequency. A number of instrumental advances have taken place in the past decade and were incorporated into the ChiralIR-2X FT-VCD spectrometer from BioTools, Inc. These include the use of dual-PEM for baseline stability and artifact suppression, dual source for better signal-to-noise ratio, introduction of digital time-sampling to eliminate external lock-ins and electronic filters, and spectral range extension to 10,000 cm\(^{-1}\). An optical diagram of a Dual-PEM ChiralIR-2X \(^{TM}\) VCD instrument (BioTools, Inc.) is shown in Fig. 3.

Raman optical activity instrumentation originated with the ICP form of ROA, in which the incident light is modulated between right- and left-circu-
larly polarized states and the scattered light is linearly polarized or unpolarized. Instrumentation for this form of ROA is not currently commercially available, but an SCP-ROA spectrometer Chiral-RAMAN™ based on the design of Werner Hug is commercially available from BioTools. This ROA spectrometer depolarizes the incident light completely while the scattered RCP and LCP radiation are measured simultaneously by separating their intensities into different optical paths and are displayed on the upper and lower halves of a multi-channel charge-coupled device (CCD) detector. The ROA spectrum is obtained by subtracting the LCP intensity from the RCP intensity. An

Fig. 6. (Top) Upper panel: optimized geometries, relative energies, and dipole moments for conformers of (2S,2R)-1. Lower panel: comparison of observed IR (lower frames) and VCD (upper frames) spectra of (−)-1 with (a) calculated spectra for (2S,5R)-configuration conformers 5la-1, 5la-2, 5la-3, and 5la-4 and with (b) composite of calculated spectra for (2S,5R)-configuration conformers: 20% 5la-1, 20% 5la-2, 20% 5la-3, and 40% 5la-4. IR and VCD bands unique to the higher-energy conformation 5la-4 are indicated by þ. Adapted from Ref. 98 with permission of John Wiley and Sons.
optical diagram of a Chiral RAMAN-2X™ ROA spectrometer is shown in Fig. 4.

Vibrational Circular Dichroism and Infrared Measurement. To measure VCD and IR spectra of a sample, one must first choose a suitable solvent that has a good spectral window in the IR region of interest. The sample is then dissolved in the solvent and placed in an IR sample cell, which usually has BaF\(_2\) or CaF\(_2\) windows. An IR spectrum is collected to optimize the concentration or path length. In order to have a good signal-to-noise ratio for the measured VCD spectrum, the absorbance of the IR bands should be in the range of 0.2 to 0.8 absorbance. If the tested IR intensities do not fall into this range, then either the concentration or the path length needs to be adjusted to obtain the desired IR absorbance range. The VCD baseline can be corrected by measuring and then subtracting the corresponding VCD spectrum of one of the following, depending on which is available: the enantiomer of the sample (the best), the racemic mixture of the sample and its enantiomer (second best), or the solvent.

**Fig. 7.** Structures of (4S,7R)-(−) and (4S,7S)-(+) 4-isopropylidene-7-methyl-4,5,6,7-tetrahydro-2(1)H-indazoles.

**Fig. 8.** 1H and 2H tautomers of pyrazoles 2a and 2b.

**Fig. 9.** Optimized conformers, relative energies, and Boltzmann populations for pyrazole diastereomer 2a. Reproduced from Ref. 62 with permission of Elsevier.
The IR baseline can be corrected by measuring and then subtracting the IR absorbance spectrum of the solvent from that of the sample.

**Vibrational Circular Dichroism and Raman Optical Activity Calculations. Software for VOA Calculations.** Gaussian, Inc. has pioneered the commercial availability of software for calculating VCD spectra from quantum mechanical ab initio or density functional theory programs since Gaussian 94. These programs employ the magnetic field perturbation (MFP) theory of VCD intensities conceived and implemented by Stephens and Cheeseman, a decade ago to DFT theory with gauge-invariant atomic orbitals (GIAOs), and this approach has become the standard method for the theoretical calculation of VCD spectra. The latest release of Gaussian 09 also contains software for calculating ROA intensities. Other programs are available that contain VOA software, such as Dalton or CADPAC, but these are less user-friendly or less tested than commercially available programs. The Amsterdam Density Functional (ADF) program package recently announced commercially available software for calculating VCD intensities.

Software for ROA calculations is available in readily usable form from Gaussian in versions of 03 and 09. The most recent releases include analytical subroutines for the calculation of all needed ROA tensor derivatives, thereby eliminating the time consuming finite difference derivative steps that restricted the application of ROA to only the smallest of chiral molecules. Now calculations of ROA spectra can be carried out for molecules comparable in size to those highlighted in this review, albeit at some additional computational time for the following reasons. The calculation of ROA spectra is more extensive and complex relative to that for VCD spectra because of the higher-order tensor derivatives needed to form the observable ROA invariants, of which there are two for ROA in the usual far-from-resonance approximation, and the added

**Fig. 10.** Comparison of VCD (upper frame) and IR (lower frame) observed spectra for (−)-diastereomer (right axes) with calculated spectra for diastereomer 2a conformers A and B (left axes). Reproduced from Ref. 62 with permission from Elsevier.

**Fig. 11.** Comparison of the observed IR and VCD spectra for (−)-diastereomer (right axes) with the calculated spectra (left axes, spectra offset for clarity) for 60% 2a-A + 40% 2a-B and with the Boltzmann population weighted sum of calculated spectra for all eight conformers of 2a. Reproduced from Ref. 62 with permission of Elsevier.
sophistication in basis sets needed that must include diffuse functions. Once measured and calculated ROA spectra are acquired for a given molecule, the method of AC determination outlined below is essentially the same for ROA as it is for VCD. Estimates for the additional computational time needed for an ROA calculation, given the results of a VCD calculation that include the determination of the contributing conformers, their equilibrium geometry, and vibrational force fields, are in the range of two to five times longer depending on the particular choice of basis sets and functionals.

**Conformational Search.** The first step for VCD/ROA calculation is to obtain all the possible conformations using molecular mechanics or semi-empirical methods with commercial programs such as the Spartan program from Wavefunction Inc., Hyperchem from Hypercube, Inc., MacroModel from Schrodinger, Inc., or PC Model from Serena Software.

**Geometry Optimization and VCD and IR Calculations.** The lower-energy conformers (within approximately 5 kcal/mol from the lowest-energy conformer) generated from the conformational search are submitted to Gaussian 09 or another program for DFT calculations (or other types) of the geometry optimization and for calculations of the force field, vibrational modes, and VCD and IR intensities. The typical functional and basis set for DFT calculations of VCD is B3LYP/6-31G(d), which has been found to be a good balance between accuracy and cost of computing time. Recently with the development of faster computing power, more and more VCD calculations have been carried out with other functionals and larger basis sets.11

**Simulation of VCD and IR Spectra.** The output of quantum mechanical calculations of IR absorption and VCD is given by a list of the dipole strength \( \Delta i \) for IR intensities and the rotational strength \( R_i \) for VCD intensities for each normal vibrational mode \( i \):

\[
\Delta_i = |\mathbf{l}_i|^2 \quad R_i = \text{Im}(\mathbf{l}_i \cdot \mathbf{m}_i) \quad (9)
\]

where \( \mathbf{l}_i \) is a vector representing the electric dipole transition moment of the molecule and \( \mathbf{m}_i \) is the corresponding magnetic dipole transition moment.1-6 The absolute square of a vector, the square of its length, given in the expression for \( \Delta_i \) is always positive, whereas the scalar dot product of \( \mathbf{l}_i \) and \( \mathbf{m}_i \) can be either positive or negative depending on whether these vectors are pointing roughly in the same or opposite directions. The conversion from a set of dipole or rotational strengths to a full IR or VCD spectrum is given by the expressions

\[
\varepsilon(v) = \frac{v}{9.184 \times 10^{-39}} \sum_i \Delta_i \quad (10)
\]

\[
\Delta\varepsilon(v) = \frac{v}{2.296 \times 10^{-39}} \sum_i R_i \quad (11)
\]

where the line-shape function is typically a normalized Lorentzian function with a half width at half-maximum \( \gamma_i \) for each vibrational mode \( i \) and is given by

\[
f_i(v) = \frac{\gamma_i/\pi}{(v_i - v)^2 + \gamma_i^2} \quad (12)
\]

\[
\int_0^\infty f_i(v) \, dv = 1 \quad (13)
\]

Comparison of the individual conformer IR and VCD spectra with the observed spectra often leads to the identification of the one or two most important conformers, usually those with the lowest calculated energies. The calculated normal mode frequencies are typically scaled by a factor in the range of 0.97 to 0.98 to compensate for the fact that the calculated frequencies are based on a harmonic force field whereas

**Fig. 12.** Structures of M- and P-enantiomer of nonamethoxy cyclotriveratrylene 3.

**Fig. 13.** Structure and isotopic labeling of the P-helical parent Q1A and the isotopomers Q2A.74 Reproduced from Ref. 74 with permission of John Wiley and Sons.
the observed frequencies arise from an anharmonic force field. In addition, solvent effects lead to further frequency differences, and sometimes mode-order differences. In some programs solvent effects can be included in the geometry optimization and IR and VCD calculations.

After the IR and VCD spectra of the contributing conformers have been calculated, they are weighted by their fractional Boltzmann population and summed to produce the final calculated IR and VCD spectra. Analysis of these spectra begins with an assignment of the measured IR spectrum. If the calculated normal modes are numbered from the lowest to the highest frequencies, corresponding numbers can be assigned to the measured IR spectrum based on the frequencies and relative intensities of the bands. This process is more straightforward for the IR spectrum than for the VCD spectrum since all the bands are positive in the IR absorbance spectrum and there is no intensity cancellation like there can be in the VCD spectrum where juxtaposed bands of opposite sign can reduce intensities and change the location of spectral peaks.

Once the IR spectrum is assigned, the same numbers can be used to correlate the measured to the calculated VCD spectra. At this stage, it is now possible to determine whether the AC chosen for the VCD calculation is the same or the opposite of the AC of the sample used for the VCD measurement. If the correspondence is not yet clear, further theoretical analysis is needed to consider a variety of factors such as solvent effects, intermolecular interactions such as dimerization, the use of improved basis sets, or alternative hybrid density functionals. The most serious possible problem, aside from dimerization, is missing an important conformer in the conformational analysis. This latter possibility, and the fact that the VCD from individual conformers can be significantly different from one another due to the extreme sensitivity of VCD to molecular conformation, reinforces the importance of a careful conformational analysis at the outset of the theoretical calculations. A corollary to this statement is that every assignment of AC by VCD includes a determination of the solution-state conformation or solution-state population of the principal conformers of the chiral molecule in question. This additional information is not available from a determination of AC using X-ray crystallography.

If desired, dipole and rotational strength values for the measured IR and VCD spectra can be obtained and compared to the calculated dipole and rotational strengths. This is accomplished by first fitting the IR spectrum with a set of Lorentzian line shapes such that each IR band \( \varepsilon_i(\nu) \) is associated with a Lorentzian band of a given center frequency \( \nu_i \) and spectral width. The area under the band is related to the dipole strength by the expression

\[
D_i = 9.184 \times 10^{-39} \int_0^\infty \frac{\varepsilon_i(\nu)}{\nu} d\nu 
\]

\[
\approx 9.184 \times 10^{-39} \int_{\text{band}} \varepsilon_i(\nu) d\nu \tag{14}
\]

This is just a mathematical inversion of Eq. 10 with support from Eqs. 12 and 13 and the assumption that the frequency of normal mode \( i \) is approximately constant.
over the range of integration of the band. Using the same Lorentzian band positions and widths determined for the IR spectrum, the VCD is fit and the rotational strengths can be extracted in the same way using

\[ R_i = 2.296 \times 10^{-39} \int_0^\infty \frac{\Delta \varepsilon_i(v)}{v} \, dv \]

\[ \approx 2.296 \times 10^{-39} \int_{\text{band}} \Delta \varepsilon_i(v) \, dv \]  \hspace{1cm} (15)

Given a set of experimental and calculated rotational strengths, a plot of calculated versus measured rotational strengths can be constructed such that a perfect fit corresponds to all points lying along a line of slope +1 if the same enantiomer was calculated as was measured in the sample. If the opposite enantiomer was calculated with a perfect fit, the points in the plot would lie along a line of slope -1. From such a plot the quality of the fit of the measured to the calculated VCD data can be ascertained visually. This method of AC quality assessment has been advocated by Stephens.\textsuperscript{51} From such rotational strength correlation plots, statistical measures such as $R^2$ coefficient of determination value can be obtained for both slope equal to +1 and -1 to aid in the level of confidence associated with an assignment of the absolute configuration. The use of statistical measures of comparison of measured and calculated rotational strengths of selected major VCD bands has been reported by Minick.\textsuperscript{52}

The methods of assessing the quality of an assignment of AC by VCD band correlation require (1) assigning measured VCD bands to calculated VCD bands, (2) fitting the selected measured VCD bands to determine their area and individual rotational strengths, and (3) plotting the calculated versus measured rotational strengths and/or calculating $R^2$ coefficients as described above. Steps (1) and (2) are time consuming and require user judgments. An alternative method, in which a quantity called the enantiomeric similarity index (ESI) is defined, has been developed to assess numerically the degree of similarity between a measured and a calculated VCD spectrum without spectral assignment or band area determination.\textsuperscript{53} The ESI is a spectral similarity measure that correlates two sets of spectra with bands at shifted locations. An algorithm using
ESI to evaluate the quality of fit between the observed and the calculated VCD and IR spectra was developed by BioTools in collaboration with Bultinck’s group and is commercially available as a software program called CompareVOA.

Even though there is currently no universally accepted standard for assessing the statistical confidence in the assignment of AC using VCD, it can be said that after literally hundreds of such assignments, there are no cases in which a conflict exists between a thorough VCD analysis leading to a prediction of AC with the determination of AC by other methods, such as X-ray crystallography. In fact, VCD has been used to uncover errors in the assignment of AC by other methods, including X-ray crystallography.52,54

DETERMINATION OF ABSOLUTE CONFIGURATION OF SMALL ORGANIC MOLECULES

Over the past several years there has been a rapid growth in the use of VCD to determine the AC of small chiral organic molecules. A large variety of structural types have been examined, including epoxides and cyclopropanes,55–58 bicyclic and tricyclic structures,59–67 chiral structures with no

![Fig. 17. Structure of perdeuteriophenyl-phenyl-sulfoxide.](image)

![Fig. 18. Left panel: comparison of the B3LYP/TZ2P and B3PW91/TZ2P IR spectra of 4 to the experimental IR spectrum. The assignment of the experimental spectrum is based on the B3LYP/TZ2P spectrum. Right panel: comparison of the B3LYP/TZ2P and B3PW91/TZ2P VCD spectra of (R)-1 and (S)-1 to the experimental VCD spectrum of (+)-4. The assignment of the experimental spectrum is based on the B3LYP/TZ2P spectrum of (S)-1. The calculated IR and VCD spectra have Lorentzian band shapes (τ = 4.0 cm⁻¹). Adapted from Ref. 88 with permission of Elsevier.](image)
chiral center (atropisomer or molecules with axial or helical chirality), sulfur or phosphorus containing compounds, flexible molecules, and other structures that do not fit into any of the above categories. The following examples highlight the versatility of VCD as a powerful tool for the AC determinations of different types of chiral organic structures.

**Tetra-substituted α-Fluoro Cyclohexanones.** Compound 1 (shown in Fig. 5) is one of the chiral tetra-substituted α-fluoro cyclohexanones that are asymmetric catalysts for oxone epoxidation of trans olefins. The AC of the epoxidation products can be predicted by the AC of the catalyst used. Ketone 1 and its analogs were synthesized as racemates, which were then resolved and the relative configuration of each of them was determined by the NMR method. The (−)-enantiomer of ketone 1 was dissolved in CDCl₃ (0.21 M) and placed in a 0.1 mm path length cell with BaF₂ windows. IR and VCD spectra were measured at 4 cm⁻¹ resolution and 5 h collection time for both sample and solvent, with the instrument optimized at 1400 cm⁻¹. All the IR and VCD spectra were baseline corrected by subtracting solvent spectra from those of the sample. The absolute configurations of 1 and its analogs were determined by VCD measurements combined with DFT calculation of VCD using Gaussian 98 with B3LYP functional and 6-31G(d) basis set. The conformational analysis of 1 and its analogs yielded the conformation populations that give the best fit to the experimental data. The absolute configuration of 1 and its analogs were unambiguously determined. The comparison of the experimental spectra with the calculated spectra is shown in Fig. 6.

**4S,7R)-(-) and (4S,7S)-(+)4-Iso-propylidene-7-methyl-4,5,6,7-tetrahydro-2(1)H-indazoles.** Chiral pyrazoles are interesting precursors for the hydrotris(pyrazolyl)borate ligands, which can be used in asymmetric catalysis. Such ligands also have potential use as building blocks for molecular motors. In this collaboration between the groups of Crassous and Nafie, diastereomeric pyrazoles 2a and 2b (shown in Fig. 7) were synthesized, their ACs were determined, and their tautomeric equilibrium was studied by VCD spectroscopy for the first time. Diastereomers 2a and 2b were prepared from dihydrocarvone by a Claisen condensation and then separated by the chiral high-performance liquid chromatography (HPLC) method. The VCD and IR of 2a and 2b were measured in CDCl₃ at 4 cm⁻¹ resolution with 9 h collection time for both sample and solvent, with the instrument optimized at 1400 cm⁻¹. Each of the diastereomers 2a and 2b has two tautomeric forms, shown in Fig. 8.

![Fig. 19. Structure of the 2R, 2'S, 3R, 3'R enantiomer of 2,2'-dinitro-2,2'-biaziridine.](image1)

![Fig. 20. Structures of the three most populated conformers of 5: -udH (63%), -uuC (17%), and -udG (13%) as obtained on the basis of DFT calculations at the B3LYP/6-31+G** level. The population factors are summed over all possible conformations of the CO₂Et groups (H atoms are not reported).](image2)
conformational search and VCD calculations at the DFT level with Gaussian 03 using B3LYP functional and 6-31G(d) basis set revealed that for each of 2a and 2b there are four lowest-energy conformers dominant in solution. For both 2a and 2b two of the four lowest-energy conformers are 1H tautomers and the other two are 2H tautomers. The optimized conformations, relative energies, and Boltzmann populations of 2a are shown in Fig. 9. Comparing the observed VCD and IR spectra with the calculated spectra for the two dominant conformers (Fig. 10) allowed the identification of experimental VCD features that arise from each dominant conformer. The respective (4S,7R)-(-) and (4S,7S)-(+)-absolute configurations of pyrazole diastereomers 2a and 2b could be determined by comparing the experimental with the calculated Boltzmann population weighted sum VCD spectra of the eight lowest-energy conformers (Fig. 11). This detailed VCD study enabled the calculation of the percentage of tautomers 1H and 2H (Fig. 8). The averaged calculated VCD over all tautomers and all conformers closely reproduced the experimental VCD spectrum. The comparison between experiment and theory determined precisely the proportions of each isomer (both tautomers and conformers).

**13C-Labeled Nonamethoxy Cyclo- triveratrylene.** The sensitivity of VOA to the masses of atomic nuclei has been reported for molecules that are chiral only by virtue of isotopic substitution. This collaboration between the groups of Nafie, Luz, and Zimmerman investigated the use of isotopic difference VCD spectra as an aid in the AC determination of chiral molecules for the first time. The enantiomers and the racemates of the parent non-isotopic substituted nonamethoxy cyclotheratrylene (Fig. 12) and the 13C-substituted isotopomer (Fig. 13) were dissolved in CDCl3 and their VCD and IR spectra were measured at 4 cm⁻¹ resolution with 5 h collection, and the instrument optimized at 1400 cm⁻¹. VCD calculations were carried out at the DFT level with Gaussian 03 using a B3LYP functional and a 6-31G(d) basis set. The AC of the parent nonamethoxy cyclotheratrylene and its 13C-labeled isotopomer were determined by comparing the observed VCD and IR spectra with the calculated spectra (shown in Figs. 14 and 15). The isotopic difference VCD and IR spectra were calculated (Fig. 16), and the good fit between the observed and the calculated isotopic difference spectra confirmed the AC assignments previously obtained. Given the quality of fit for Q1A and Q2A, it is of interest to see whether the difference in the measured and the calculated IR and VCD spectra for these two molecules also compare well. The difference spectra shown in Fig. 15 demonstrated that VCD difference spectra for both calculated and measured spectra can be used as a tool to assess the quality of fit of the calculated spectra. Moreover, since only those parts of the IR and VCD spectra affected by the isotopic substitution contribute to the IR and VCD difference spectra, some features in the original that do not agree as well may cancel, leaving a smaller subset of IR and VCD features for comparison. This is the first example of a comparison of measured and calculated VCD difference spectra, and this approach could be useful in the future for AC assignments.

**Perdeuteriophenyl-phenyl-sulfoxide.** Enantiopure sulfoxides are valuable chiral starting material and important chiral auxiliaries in organic synthesis. In the past, the AC of optically pure sulfoxides has been determined by the empirical rule of Mislow et al. However, a recent non-empirical analy-
ysis of a series of ECD spectra of chiral alkyl aryl sulfoxides has found the rule to be wrong for these molecules. In this study, the groups of Stephens and Drabowicz synthesized and determined the AC of an isotopically chiral sulfoxide 4 (Fig. 17) using VCD. The geometry optimization and the VCD and IR spectra were calculated using DFT with B3LYP/TZ2P and B3PW91/TZ2P functional and basis sets. Only one low-energy conformer dominates the solution population and the B3LYP/TZ2P calculated lowest energy conformer is in good agreement with the X-ray determined structure. Based on this study and previous work, the B3LYP/TZ2P equilibrium geometry agrees best with X-ray data and the B3PW91/TZ2P simulated VCD and IR spectra are more accurate than the B3PW91/TZ2P or 6-31G(d) simulated spectra (shown in Fig. 18). Usually for sulfoxide containing molecules, the agreement between the experimental and calculated spectra is not very favorable using the B3LYP functional and 6-31G(d) basis set due to a lower accuracy simulation of the frequency of the S–O stretching mode. In this study, larger basis sets were tested with two different functionals and it was found that B3LYP/TZ2P is a better functional and basis set combination for calculating the geometry and VCD of sulfoxide containing molecules.

2,2'-Dinitro-2,2'-biaziridine. The molecule 2,2'-dinitro-2,2'-biaziridine 5 possesses many useful functional groups, such as an aziridine ring and nitro and carboxylic groups, that can serve as versatile building blocks for the synthesis of a variety of new compounds of interest for asymmetric synthesis of pharmacologically active substrates. In a recent study, a racemic mixture of 5 (Fig. 19) was synthesized and the two enantiomers were separated by chiral HPLC. The AC of the two enantiomers was determined by VCD and further supported by comparison of ECD measurements with TD-DFT calculations of ECD intensities. Due to the flexibility of the sample, approximately 300 possible conformers were analyzed, but the DFT calculation revealed that only a few conformers are populated at room temperature (Fig. 20). Comparing the Boltzmann weighted average calculated (B3LYP/6-31G*) spectra of the 2R, 2'S, 3R, 3'R enantiomers with the spectra measured in CDCl3, the AC of the two enantiomers of 5 were determined unambiguously using VCD (Fig. 21). The comparison of the measured ECD spectra with the TD-DFT calculated ECD spectra reinforced the AC assignment obtained by VCD. The ECD study also indicated that for the ECD simulation the Coulomb-attenuated CAM-B3LYP functional gives better results than the B3LYP functional, especially for N-containing molecules.
This study demonstrated that VCD is suitable not only for rigid molecules but also for flexible molecules. The key step in AC determination of flexible molecules using VCD is the conformational search. With a good strategy and careful survey, it is no longer a challenging task to find all the low-energy conformers in a flexible molecule.

DETERMINATION OF ABSOLUTE CONFIGURATION OF PHARMACEUTICAL MOLECULES

The number of AC determinations by VCD has increased dramatically in the pharmaceutical industry in the past few years into the range of several hundreds of structures per year. However, for proprietary reasons fewer than twenty AC determinations of pharmaceutical compounds via VCD method have been recently reported. The following examples demonstrate that VCD has become a useful tool in assisting the study of structure–activity relationships (SAR) and/or structure–property relationships (SPR) for chiral pharmaceutical compounds.

(R)-Malathion. Malathion 6 (Fig. 22) is one of the most widely used pesticides for suppression of harmful insects, such as the mosquito, and has a flexible liquid-state conformation at room temperature. Several chiral pesticides, including malathion, are liquid at room temperature, and therefore, the absolute configuration of these pesticides cannot be determined by X-ray crystallography in the usual manner. In this study, Izumi and co-workers determined the AC and solution conformation of malathion using VCD spectroscopy and a conformational code that was recently developed by his group. This code can be used in AC analysis for a flexible molecule without a full conformational search using molecular mechanics (MM) calculations. Instead, DFT calculations were carried out for various fragments of (R)-malathion, namely ethyl propionate, (R)-ethyl 2-(methylythio) propanoate, (R)-diethyl 2-(methylthio) succinate, and O,O,S-trimethyl phosphorodithioate. The conformational search and DFT calculation (B3LYP/6-31G(d)) resulted in eight conformers within 1 kcal/mol from the lowest-energy conformer and account for greater than 75% of the calculated population distribution. The predicted most stable conformation of (R)-malathion is shown in Fig. 23. The measured VCD and IR spectra of (−)-malathion (CCl₄, 0.11 M, BaF₂, 72 μm path length) are in good agreement with the population weighted VCD and IR spectra of the eight energetically preferred conformations for (R)-malathion (Fig. 24). The absolute configuration of (−)-malathion was therefore assigned as (R)-malathion from the VCD analysis and corresponds to the reference assignment. The use of a fragment-conformational search with the conformational code highlights the essential conformational features of the molecular fragments and affords insight into the conformational distribution in a solution state for a flexible molecule such as (−)-malathion. This methodology therefore allows the selection time of the predominant conformations of large and flexible chiral molecules to be shortened and the
accuracy of the determination of absolute configuration is significantly improved. Most large molecules consist of the chemical fragments, and each fragment has characteristic conformational distribution patterns. A database of the conformational distribution patterns of numerous common fragments and an associated conformational analysis would be useful for the design of bioactive compounds in the future. This example once again demonstrates the usefulness of VCD for the AC determination of conformationally flexible molecules.

**Histamine H3 Receptor Antagonist GT-2331.** GT-2331, 7 (Fig. 25), is one of the most potent members of a class of chiral drug substances used to regulate the synthesis and release of histamine by the histamine H3 receptor and is an important biomarker for pharmaceutical companies conducting research in this field. In addition to its overall structural features, the bioactivity of this molecule has also been found to be highly dependent on absolute stereochemistry, making the reliable assignment of this property a necessity. Two previous X-ray diffraction studies on this same molecule have assigned ACs that are opposite to each other, leaving its three-dimensional structure uncertain. In view of this, its AC was reinvestigated using VCD. Results from this study provided independent assignment of this important molecule as the (1S,2S)-enantiomer.

A racemic mixture of 7 was synthesized and the racemate was separated into purified enantiomers by preparative supercritical fluid chromatography. VCD and IR spectra were acquired in the mid-IR region (2000–800 cm\(^{-1}\)) at a resolution of 4 cm\(^{-1}\). The (1S, 2S) configuration was chosen for calculations and two tautomeric forms N1–H and N3–H were built. Global conformational searches at the molecular mechanics level followed by DFT calculation using the B3LYP functional with a 6-311G(d,p) basis set predicted one dominant conformer (81\% population) for the N1–H tautomer and six dominant conformers (97\% total population) for the N3–H tautomer and six dominant conformers (97\% total population) for

**FIG. 27.** Structure of (R)-1-[(4-cyano-phenyl)(3-bromo-4-hydroxyphenyl)-methyl]-1H-[1,2,4]triazole.

**FIG. 28.** VCD spectra. Bottom trace: experimental spectrum of 8a (0.256 M CDCl\(_3\)). Middle trace: calculated spectrum for R configuration (weighted average taking into account populations obtained by calculated Gibbs free energies). Upper trace: experimental spectrum of 8b (0.23 M in CDCl\(_3\)). Calculated frequencies have been shifted by 30 cm\(^{-1}\).\(^\text{116}\) Adapted from Ref. 116 with permission of American Chemical Society.

**FIG. 29.** ECD spectra. Dark blue trace: experimental spectrum of 8a. Light blue trace: experimental spectrum of compound 8b. Black trace: calculated spectrum for R configuration (average over eight conformers with calculated population). The spectra were recorded on 10\(^{-4}\) M solutions in the range 190–240 nm and on 10\(^{-3}\) M solutions in the range 240–340 nm.\(^\text{116}\) Adapted from Ref. 116 with permission of American Chemical Society.
the N3–H tautomer. The combination of all the conformers of N1–H and N3–H resulted in the lowest-energy N1–H conformer being 90–95% populated. However, when comparing the Boltzmann weighted IR and VCD spectra with the experimental data, the agreement is not satisfactory, indicating that the simulated spectra based on gas-phase Boltzmann distribution of the conformers are not accurate for this molecule in solution state. Comparing the experimental IR with the calculated IR of each conformer suggested that the N3–H tautomer was underestimated using gas-phase free energies. The mole fractions of N1–H and N3–H were estimated empirically using the relative intensities of bands at 1595 cm⁻¹ and 1575 cm⁻¹. Based on those intensities the mole fractions of N1–H and N3–H were estimated to be 0.65 and 0.35, which is in good agreement with the literature reported. The simulated IR and VCD spectra predicted for a 65:35 mixture of N1–H and N3–H tautomers are in good agreement with the experimental spectra (Fig. 26). This study indicated that for molecules that have strong intermolecular interactions, the Boltzmann distribution based on gas-phase free energies may not be accurate. When dealing with this type of molecule one should carry out a very careful population analysis to determine the more accurate populations of each conformer.

1-[(4-Cyanophenyl)(3-bromo-4-hydroxyphenyl)methyl]-1H-[1,2,4]triazole. 1-[(4-Cyanophenyl)(3-bromo-4-hydroxyphenyl)methyl]-1H-[1,2,4]triazole 8 is the synthetic precursor for 1-[(4-cyanophenyl)(3-bromo-4-sulfamoyl oxyphenyl)methyl]-1H-[1,2,4]triazole, which was found to be the most potent of the achiral and racemic aromatase

![Figure 30](image_url)

**Fig. 30.** Structure of (1R, 5S) azabicyclo[3,2,1]octane derivative 9.

![Figure 31](image_url)

**Fig. 31.** Left panel: observed VCD (upper frame) and IR (lower frame) spectra of 9 compared with those of the calculated spectra of the eight lowest-energy conformers of (1R, 5S). Right panel: observed VCD (upper frame) and IR (lower frame) spectra of 9 compared with those of the calculated Boltzmann averaged spectra of the eight lowest-energy conformers of (1R, 5S).
inhibitors for the treatment of hormone-dependent breast cancer in postmenopausal women. The two enantiomers \(8a\) and \(8b\) were separated by semi-preparative chiral HPLC. After an extensive effort to obtain crystals suitable for X-ray analysis failed, the AC of each enantiomer \(8a\) and \(8b\) were determined by VCD and reinforced by electronic circular dichroism (ECD). The experimental VCD spectra of \(8a\) and \(8b\) and the calculated Boltzmann averaged VCD (B3LYP/6-31G*) for the \(R\) configuration of \(8\) are shown in Fig. 28. The ECD measurement in conjunction with TDDFT (B3LYP/6-31G*) calculations reinforced the AC assignment obtained from VCD study (Fig. 29).

Azabicyclo[3,2,1]octane Derivatives. A series of biaryl amides containing an azabicyclooctane amine headpiece were synthesized and evaluated as mixed arginine vasopressin (AVP) receptor antagonists that are being investigated for use in the treatment of hyponatremia, hypertension, congestive heart failure, liver cirrhosis, and any state of excessive retention of water.\(^{120}\) The SAR study revealed that the (1\(S\), 5\(R\)) stereoisomers had significantly higher affinity for the vascular and renal receptors than their enantiomers.\(^{120}\) In some cases the eutomer was greater than 1000-fold more active than the corresponding distomer in the vascular-receptor assay.\(^{120}\) Therefore, AC determination in a timely manner played an important role in understanding the SAR and/or SPR of these analogs. The enantiomers of each compound were synthesized and separated by chiral supercritical fluid chromatography. The VCD and IR spectra of both enantiomers were measured in CDCl\(_3\) (0.8 M) using a 100 \(\mu\)m path length cell with BaF\(_2\) windows; 8 h collection for both enantiomers; instrument optimized at 1400 cm\(^{-1}\) with 4 cm\(^{-1}\) resolution. The (1\(R\), 5\(S\)) configuration was built and the conformational search was carried out using HyperChem (Hypercube, Inc.).

![Fig. 32. Structures of chromanes 10-1 and 10-2.](image_url)

![Fig. 33. Calculated VCD spectra, optimized structures, and relative energies of the four lowest-energy conformers of (R)-10-1.](image_url) Reproduced from Ref. 142 with permission of Elsevier.
The resulting conformers were calculated for geometry optimization and IR and VCD intensities at DFT level using Gaussian03 \(^{100}\) with B3LYP functional and 6-31G(d) basis set. The Gaussian calculation resulted in eight low-energy conformers that have energies within 1 kcal/mol from the lowest-energy conformer. These conformers differ by the orientation of the carbonyl and the aromatic groups. The comparison of the observed VCD and IR spectra with the overlay of the calculated spectra of the eight low-energy conformers (the left panel of Fig. 31 for compound 9 (structure shown in Fig. 30)) demonstrated the sensitivity of VCD to the conformational changes. The AC of each enantiomer was determined by comparing the measured VCD and IR spectra with those of the calculated Boltzmann averaged spectra of the (IR, 53) configuration (the right panel of Fig. 31 for compound 9). The ACs of other analogs of compound 9 were determined by comparing the observed VCD of each unknown compound with that of compound 9. This study provided a useful example for the determination of AC for analogs used in SAR and/or SPR studies in drug discovery.

**DETERMINATION OF ABSOLUTE CONFIGURATION OF NATURAL PRODUCT MOLECULES**

Within the past four years more than thirty papers have been published on AC determination of natural products. Most of these natural product molecules feature more than one chiral center and their relative stereochemistries were determined by X-ray or NMR methods. The majority of these molecules were investigated by the groups of Stephens\(^{11,123-128}\) and Joseph-Nathan,\(^{129-139}\) but additional groups in natural-product research have begun to take advantage of VCD for AC determinations.\(^{140-149}\) Nafie published an extensive review on the AC determination of natural products by VCD in 2008,\(^{10}\) and as a result only a few more recent examples are described in this section.

**Chromanes.** Two chromanes, 10-1 and 10-2 (Fig. 32), were isolated from Peperomia obtusifolia as racemic mixtures and resolved using chiral HPLC.\(^{142}\) The initial stereochmical determination was based on an empirical rule that the sign of the \(\lambda_4\) band of the ECD can predict the AC of the dihydropyran ring. Experimentally, the chromane 10-1 was dissolved in CDCl\(_3\), FIG. 34. Comparison of the VCD and IR spectra of the measured (+)-10-1 with the calculated VCD and IR spectra of the Boltzmann average of the four lowest-energy conformers of the corresponding (R)-10-1. The comparison establishes the absolute configuration of this molecule as (+)-(R)-10-1.\(^{142}\) Reproduced from Ref. 142 with permission of Elsevier.

FIG. 35. Structure of salvileucalin A (11a) and salvileucalin B (11b).
in a BaF2 cell (100 μm path length) and the VCD spectra were measured. A conformational search using MM+ and MMFF force fields in Hyperchem (Hypercube, Gainsville, FL) and Spartan (Wavefunction, Irvine, CA) resulted in 53 conformers with relative energy less than 3 kcal/mol above that of the most stable conformer. The geometry optimization and IR and VCD intensities of these conformers were calculated using Gaussian 09 at the DFT level with the B3LYP functional and 6-31G(d) basis set. Among the ten low-energy conformers with relative energy within 1.4 kcal/mol from the lowest-energy conformer, four conformers representing 75% of the Boltzmann distribution were selected to construct the calculated IR and VCD spectra (Fig. 33). Comparison of the observed spectra with the DFT calculations revealed that the absolute configuration of the chromane 10-1 is (R) (Fig. 34), opposite to the previous assignment based on the observed sign of the 1Lb ECD band and the empirical rule. ECD calculations based on time-dependent DFT (TDDFT) (B3LYP/6-311++G(2d,2p)//B3LYP/6-31G(d) showed that both P- and M-helicity of the heterocyclic ring could result in the same sign for the ECD 1Lb band, which results in the failure of the empirical rule in this case. The quality of the fit of the observed VCD/IR spectra with those of the calculated spectra was evaluated by the CompareVOA algorithm, and a high confidence level (enantiomeric similarity index (ESI) = 76.6 for chromane 10-1) further confirmed the correctness of the new AC assignment by VCD.

**Salvileucalin B.** Salvileucalin B 11b (Fig. 35) is the first natural product having a caged carbon framework that exhibits high cytotoxicity against human A549 and HT-29 cells. In this study Salvileucalin B and its genetically related analog Salvileucalin A were isolated from *Salvia leucantha* (Mexican bush sage), their structure skeletons were elucidated by extensive NMR studies, and their relative configurations were determined by X-ray crystallography. The absolute configurations of Salvileucalin A and B were determined by VCD spectroscopy in combination with DFT calculations carried out at the level of B3LYP functional and 6-31G(d) basis set. The comparison of the observed VCD and IR spectra of Salvileucalin A with the calculated spectra is shown in Fig. 36. For molecules that have multiple chiral centers in a fused ring system, such as Salvileucalin A and B, the best strategy for AC determination is to first use other methods such as NMR or X-ray to establish the relative stereochemistry, then use VCD to determine the AC. This will significantly reduce the time spent on the calculation of different possible configurations.

**Isoepitaondiol.** The structure of isoepitaondiol 12a (Fig. 37), a meroditer-
penoid isolated from *Stypopodium flabelliforme* was reassigned as the diacetate to be 12b (Fig. 37). The relative configuration was determined by X-ray crystallography and extensive NMR studies. The absolute configuration was determined by VCD in comparison to DFT calculations with a B3LYP/DGDZVP basis set. The IR and VCD spectra were measured at a resolution of 4 cm$^{-1}$. Conformational searches were carried out using a Monte Carlo guided protocol using the X-ray coordinates of 12b as the input data. The 13 conformers obtained from the MMFF molecular mechanics searches were calculated at DFT level for geometry optimization using a B3LYP/DGDZVP basis set. The two lowest energy conformers were Boltzmann weighted to simulate the calculated IR and VCD spectra. The absolute configuration was unambiguously determined by comparing the calculated with the observed VCD spectra (Fig. 38). This is another example of AC determination of a molecule bearing several chiral centers on a fused ring system using the methods of NMR, X-ray, and VCD.

**CONCLUSIONS**

Vibrational optical activity, and especially VCD, has evolved to become a powerful tool for the determination of the AC of chiral molecules in the solution state. Further advancements in VOA instrumentation now allow more accurate VOA measurements with minimal artifacts that might interfere with the interpretation of the experimental data. In addition, the continued development and upgrade of the computing power and VOA software, together with the availability of higher level functionals and basis sets, make the prediction of VOA spectra even more accurate and reliable for the unambiguous determination of AC in chiral organic molecules. Compared to other more traditional techniques such as X-ray and NMR, the VOA method does not require a single crystal or derivatization and therefore is more straightforward, efficient, and convenient in practice. The other advantage of VOA is that along with the determination of AC, the solution-state conformational populations via conformational analysis are obtained by determining the most important conformers included in the VOA calculation or by comparing the experimental VOA spectra with the calculated spectra of each conformer. Although many groups have used VCD, ECD, and/or optical rotatory dispersion (ORD) together for AC determination, the VCD method is much easier and more reliable because vibrational transitions have much narrower bandwidths than electronic transitions and VCD spectra are much better resolved than ECD spectra. Additionally vibrational rotational strengths only depend on the wavefunction of the ground electronic state, whereas electronic rotational strengths depend on wavefunctions of both ground and excited electronic states, and the calculation of the ground electronic state wavefunction is more accurate than that of any of the excited electronic states.

The recent examples of AC determination by VCD described here demonstrate that VCD has been well adopted in a variety of research areas including the basic study of chirality, asymmetric synthesis or catalysis, drug screening, pharmacology, and natural products. With the development of the Compare-VOA algorithm for evaluating the quality of fit between the observed and the calculated spectra, reliable statistical measures for AC determination by VCD will further enhance the growth in the application of this methodology. Although it is not yet widely recognized, the availability of ROA instrumentation and quantum chemistry software make possible the use of ROA as a closely related and complementary tool for determination of AC in the solution state that is only marginally less effective than its popular cousin VCD. In the future, it is likely that both VCD and ROA will be used together in concert or separately according to ease of sampling to determine AC in chiral molecules to a level of confidence that is even higher than that currently enjoyed today by VCD alone.

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*Fig. 38. Experimental and Boltzmann weighted calculated DFT B3LYP/DGDZVP VCD spectra of isoepitaondiol diacetate (12b).* Adapted from Ref. 138 with permission of American Chemical Society.
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