Received: 3 September 2021 Revised: 27 September 2021 Accepted: 30 September 2021 DOI: 10.1002/chir.23379

Chirality

RESEARCH ARTICLE

Chiral analysis of pantolactone with molecular rotational resonance spectroscopy

Reilly E. Sonstrom[1,2] | Justin L. Neill[2] | Alexander V. Mikhonin[2] | Reinhard Doetzer[3] | Brooks H. Pate[1]

[1]Department of Chemistry, University of Virginia, Charlottesville, Virginia, USA
[2]BrightSpec, Inc., Charlottesville, Virginia, USA
[3]Competence Center Analytics, BASF SE, Ludwigshafen, Germany

Correspondence
Justin L. Neill, BrightSpec, Inc., Charlottesville, VA 22903, USA.
Email: justin.neill@brightspec.com

Funding information
Virginia Bioscience Health Research Corporation; National Science Foundation, Grant/Award Number: 1904686

Abstract

A molecular rotational resonance spectroscopy method for measuring the enantiomeric excess of pantolactone, an intermediate in the synthesis of panthenol and pantothenic acid, is presented. The enantiomers are distinguished via complexation with a small chiral tag molecule, which produces diastereomeric complexes in the pulsed jet expansion used to inject the sample into the spectrometer. These complexes have distinct moments of inertia, so their spectra are resolved by MRR spectroscopy. Quantitative enantiomeric excess (EE) measurements are made by taking the ratio of normalized complex signal levels when a chiral tag sample of high, known EE is used, while the absolute configuration of the sample can be determined from electronic structure calculations of the complex geometries. These measurements can be performed without the need for reference samples with known enantiopurity. Two instruments were used in the analysis. A broadband, chirped-pulse spectrometer is used to perform structural characterization of the complexes. The broadband spectrometer is also used to determine the EE; however, this approach requires relatively long measurement times. A targeted MRR spectrometer is also used to demonstrate EE analysis with approximately 15-min sample-to-sample cycle time. The quantitative accuracy of the method is demonstrated by comparison with chiral gas chromatography and through the measurement of a series of reference samples prepared from mixtures of (R)pantolactone and (S)-pantolactone samples of known EE.

KEYWORDS

absolute configuration, enantiomeric excess, microwave spectroscopy, molecular rotational resonance, rotational spectroscopy

1 | INTRODUCTION

nutrient, and its prodrug dexpanthenol. (R)-pantothenic acid (usually produced as its more stable salt calcium Pantolactone is a chiral lactone with a five-membered pantothenate) and dexpanthenol are ring-opening prodring and is the corresponding lactone to ω-hydroxy acid ucts of (R)-pantolactone with β-alanine or pantoic acid (2,4-dihydroxy-3,3-dimethylbutyric acid). It 3-hydroxypropylamine, respectively. Both calcium pantois the central intermediate in the synthesis of the vitamin thenate and dexpanthenol are marketed as over-the(R)-(+)-pantothenic acid (Vitamin B_5), an essential counter medications and produced in multi-thousand ton quantities worldwide. Figure 1A shows a survey of the reaction steps.

Since only the (R) stereoisomer of pantothenic acid has desirable biological activity, with the (S) isomer reported to be somewhat antagonistic,[2] pantolactone should be synthesized and made available in pure (R) form. This is achieved commercially either by fermentative production or by chemical synthesis encompassing racemate cleavage. The classical chemical synthesis of Stiller is shown in Figure 1B.[1] It starts from isobutyraldehyde, which undergoes aldol addition of formaldehyde, followed by Strecker synthesis via a cyanohydrin. At the synthetic stage of pantoic acid, racemate cleavage is performed, using optically pure chiral amines, for example, (S)-3-aminomethylpinane as an auxiliary[3] whose salt with (R)-pantoic acid crystallizes more rapidly than the respective diastereomeric salt with (S)-pantoic acid. Upon acidic treatment, dehydration forms (R)pantolactone in an organic phase, while (S)-pantoic acid is recycled and converted back to a racemate. Alternatively, various fermentative syntheses of (R)-pantothenic acid, following the biosynthetic pathway, have been reported and patented and are currently in use.[4–7] In all cases, high optical purity of the products is desired; hence, close control of stereoselectivity during the production process is crucial.

Polarimetry is most commonly used to determine the specific rotation of the product in process solutions. This measurement is fast and may be implemented online; however, the method only produces a single value, which is a sum parameter of all optically active compounds in the sample, weighted by their respective concentrations. Chiral chromatography can overcome this problem,[8,9] but chiral separations are typically slow and unsuitable for determining changes in process conditions on a fast timescale.

In this report, we describe the use of molecular rotational resonance (MRR) spectroscopy to accurately measure the enantiomeric purity of pantolactone. Molecular rotational resonance spectroscopy has been used extensively to characterize the structures of small molecules, including chiral molecules, due to its extremely high sensitivity to molecular structure.[10–12] In MRR, compounds are

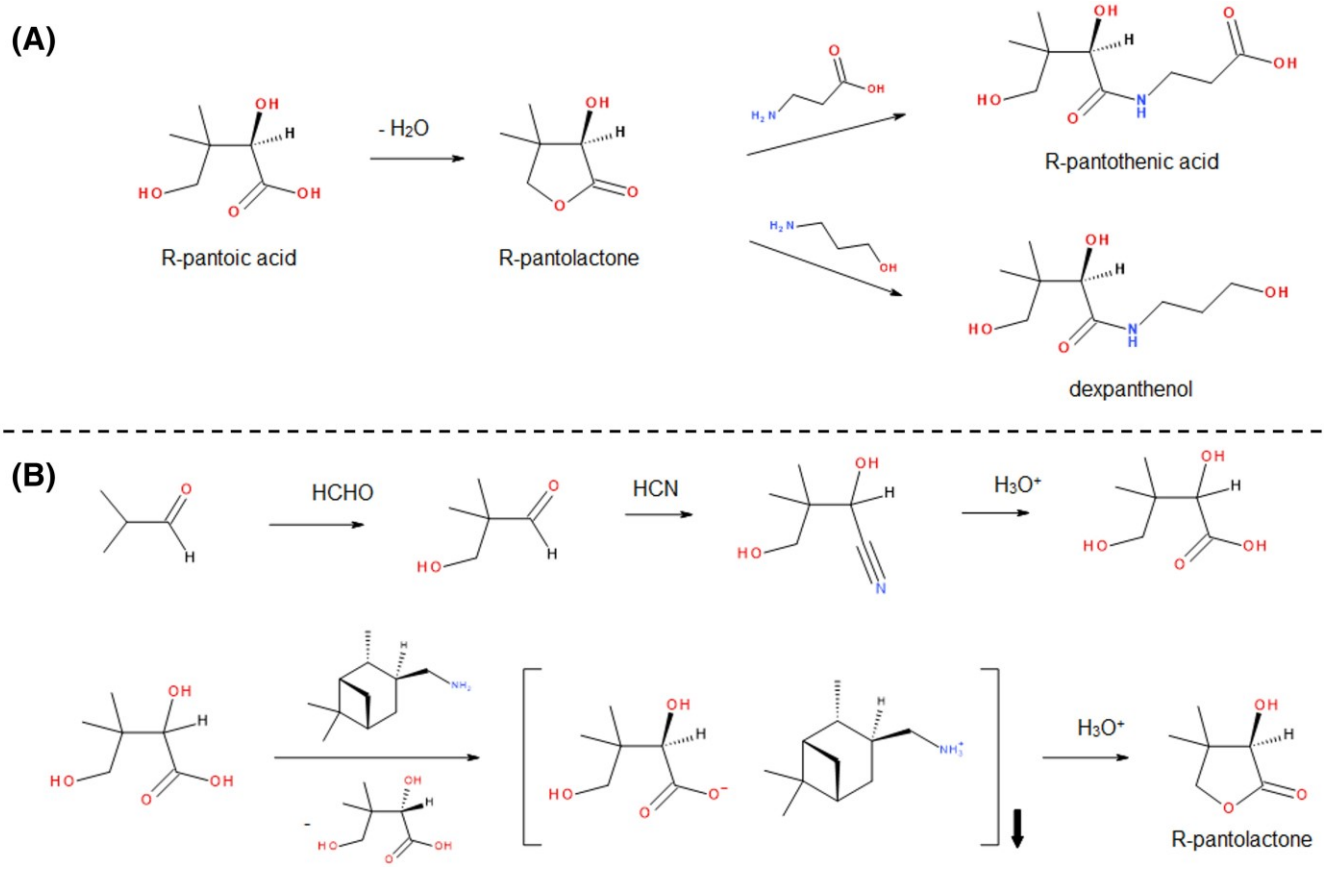


FIGURE 1 Synthesis of (R)-pantolactone and relevant pharmaceutical products. (A) Synthetic route to (R)-pantothenic acid and dexpanthenol via pantolactone. (B) Synthesis of (R)-pantolactone using (S)-3-aminomethylpinane as auxiliary. See Stiller et al.[1] for more details

characterized through their three rotational constants (inversely proportional to the three moments of inertia in the principal axis system for molecular rotation).[13] Two

key technological developments have enabled the practical application of MRR to monitoring enantiomeric purity. The first is that measuring broadband rotational spectra, necessary for the characterization of new species, became orders-of-magnitude faster with the invention of chirped-pulse Fourier transform microwave spectroscopy[14,15] in which broadband linear frequency sweeps are used to excite numerous rotational transitions in a gas-phase sample at once. This greatly reduces the measurement time and sample consumption for the spectroscopy measurements required to determine the parameters for structural characterization. The second is that two methodologies have been developed for rapid, analyte-specific determination of enantiomeric excess in a mixture: three-wave mixing and chiral tagging. In three-wave mixing rotational spectroscopy, a sequence of excitation pulses is applied to the sample to generate a single-frequency signal where the phase determines the dominant enantiomer of the sample and the amplitude is proportional to the enantiomeric excess.[16–18] Chiral tagging, meanwhile, uses in situ chiral derivatization through the formation of gas-phase diastereomeric complexes between a small, chiral molecule of known enantiopurity (the tag), and the analyte.[19–21] The complexes form through non-covalent interactions between the tag and analyte and are generated in the pulsed jet expansion used to inject samples into MRR spectrometers. In this paper, we employ the chiral tagging method for enantiomeric analysis of pantolactone.

The primary advantages of MRR for this application are that it is completely analyte specific and is capable of determining chiral purity directly within a mixture (including one where other chiral components are present). Because samples can be analyzed directly, this method can often achieve faster response times than typical chiral chromatography analyses. Here, we describe the development of a method for enantiomeric analysis of pantolactone using a broadband chirped-pulse MRR spectrometer using chiral tagging, followed by transfer to routine, online capable monitoring with a targeted MRR instrument. The results obtained with this method, using both instruments, were validated by comparison to chiral gas chromatography.

2 | EXPERIMENTAL METHODS

2.1 | MRR spectroscopic measurements

Two MRR spectrometer designs were used in this study. The broadband spectrometer design is a chirped-pulse Fourier transform microwave (CP-FTMW) spectrometer operating from 2–8 GHz.[12,14] Results from two different instruments using this design, one at the University of Virginia (UVa) and one at BrightSpec, are reported and provide insight on the transferability of the analysis between different instruments. In the CP-FTMW spectrometer design, an arbitrary waveform generator (AWG) is used to generate broadband chirped pulses with linear frequency sweep, which are amplified by a traveling wave tube amplifier (TWTA) and broadcast by a high-gain horn antenna across a vacuum chamber at approximately 10^6 Torr. Gas-phase molecules present in the chamber during the excitation pulse are polarized by this radiation and subsequently emit coherent radiation at all transition frequencies in the pulse bandwidth. This broadband signal dephases primarily through Doppler shifts resulting from the velocity distribution of the gas sample and the signal resembles the well-known free induction decay (FID) signal of a Fourier transform nuclear magnetic resonance (NMR) instrument. The FID signals are collected by a second horn antenna, amplified, and digitized directly on a fast oscilloscope.

Samples are introduced into the vacuum chamber through pulsed supersonic nozzles (General Valve) with 0.9 mm diameter. In the experiments presented here, three nozzles oriented in parallel are operated simultaneously to increase measurement sensitivity. The nozzles are operated at 3 Hz (UVa) or 5 Hz (BrightSpec), and on each valve pulse, a total of eight broadband spectra are recorded. A typical measurement consists of 10^5–10^6 broadband FID acquisitions signal-averaged in the time domain, and Fourier transformed to produce the MRR spectrum. The nozzles have been modified to include a reservoir to hold liquids or solids, with the capability to heat the reservoirs to increase the sample vapor pressure.[22] Pantolactone is heated to 80C for optimal signals. The tag molecule is pre-mixed into neon carrier gas at 0.1% concentration, and this mixture passes over the heated sample reservoirs to entrain the analyte vapor as the gas flows to the pulsed nozzle orifice. Two tag molecules are used in this work: propylene oxide and trifluoropropylene oxide. The backing pressure of the neon gas mixture was of approximately 30 psi absolute pressure.

To demonstrate the capability for MRR to perform rapid, routine enantiomeric analyses, the BrightSpec IsoMRR instrument was used. This instrument utilizes a Fabry-Perot resonator based on the designs of Balle and Flygare[23] and Suenram et al.[22] The advantage of this instrument for routine measurements is that it achieves significantly higher sensitivity across a narrow spectral bandwidth. Therefore, once the transition frequencies to be measured are known from analysis on the broadband spectrometer, comparable sensitivity can be achieved with significantly less

measurement time and sample consumption than in the broadband instrument. Two papers have described the application of this instrument to problems in chiral analysis (diastereomer and enantiomer analysis), including in process.[24,25] In the IsoMRR instrument, one of the mirrors is mounted on a motorized translation stage, which automatically tunes the cavity length to achieve resonance with each spectral transition selected for measurement. In this study, pantolactone solutions were injected manually via syringe through a septum onto a liner filled with deactivated glass wool. The outlet of the liner was connected via a heated transfer line into a modified reservoir nozzle, which had a similar design to that used in the broadband studies. A neon gas flow, with the chiral tag mixed in, was used as the carrier gas to convey the volatilized analytes into the vacuum chamber. The same pulsed nozzle design is used as in the broadband studies, with a repetition rate of 10 Hz and the acquisition of 5 FID spectra per nozzle injection cycle. After the injection of each sample, the liner was heated to first vent the solvent at or above its boiling point (typically dichloromethane, 40C) and then heated to 80C to volatilize the pantolactone. An automatically controlled vent valve is installed in the system to allow for solvents to be removed without running the pulsed nozzle. This vent valve can also be used at the end of each measurement to vent off any remaining analytes before the next sample injection. The transfer line and nozzle were kept to a higher temperature than the inlet (typically 100C) to maintain volatilized analytes in the gas phase.

2.2 | Gas chromatography

Chiral GC-MS was performed to validate the MRR results. Separation of enantiomers was achieved using a Restek Rt-βDEXsm (30 m, 0.25 mmID, 0.25 μm df) column. One microliter of diluted pantolactone was injected into the inlet (230C) and introduced to the column using helium as a carrier gas with a split ratio of 10:1 and a flow rate of 1.0 ml/min. The oven was held at 40C for 1 min and then increased to 160C at 1C/min.

2.3 | Materials

Racemic pantolactone (TCI America, achiral purity >95%), (R)-D-pantolactone (MilliporeSigma, >99%), and (S)-L-pantolactone (MilliporeSigma, >97%) were used without further purification. The chiral tags used were as follows: Racemic propylene oxide (PO) (TCI America, >99%), (R)-propylene oxide (TCI America, >98%), (S)propylene oxide (TCI America, >98%), racemic 3,3,3-trifluoro-1,2-epoxypropane—or trifluoropropylene oxide (TFPO)[26]—(Synquest Labs, 98%), and (S)-3, 3,3-trifluoro-1,2-epoxypropane (MilliporeSigma, 97%). Dichloromethane (MilliporeSigma, >99.8%) was used as the solvent for injection of pantolactone standards onto the IsoMRR instrument. Note that all purity levels reported here are achiral purity as provided by the manufacturer. Enantiomeric purity of each chiral tag was determined by MRR through complexation to (R)3-butyn-2-ol. The chiral tag EE values are (S)-propylene oxide: 99.7%, (R)-propylene oxide: 99.2%, and (S)-TFPO:
94.1%.

3 | COMPUTATIONAL METHODS

All calculations were performed using Gaussian 09.[27] For each diastereomeric complex, a number of candidate structures are generated using chemical intuition based on the fact that the strongest non-covalent interaction is expected to be hydrogen bond formation between the hydroxyl group of pantolactone and the ring oxygen in PO or TFPO. Geometries were optimized using the B2PLYP-D3BJ/def2-TVZP model chemistry to determine the rotational constants and dipole moment magnitude and direction. The D3BJ dispersion correction of Grimme and coworkers[28–30] has been demonstrated to have superior performance in the calculation of the structural parameters, to which MRR is highly sensitive. For the pantolactone monomer, a B3LYP-D3BJ/6-311++G(d,p) level of theory was used. The results of these quantum chemistry calculations are summarized in the supporting information.

4 | RESULTS AND DISCUSSION

4.1 | Broadband spectroscopic measurements and assigning absolute configuration

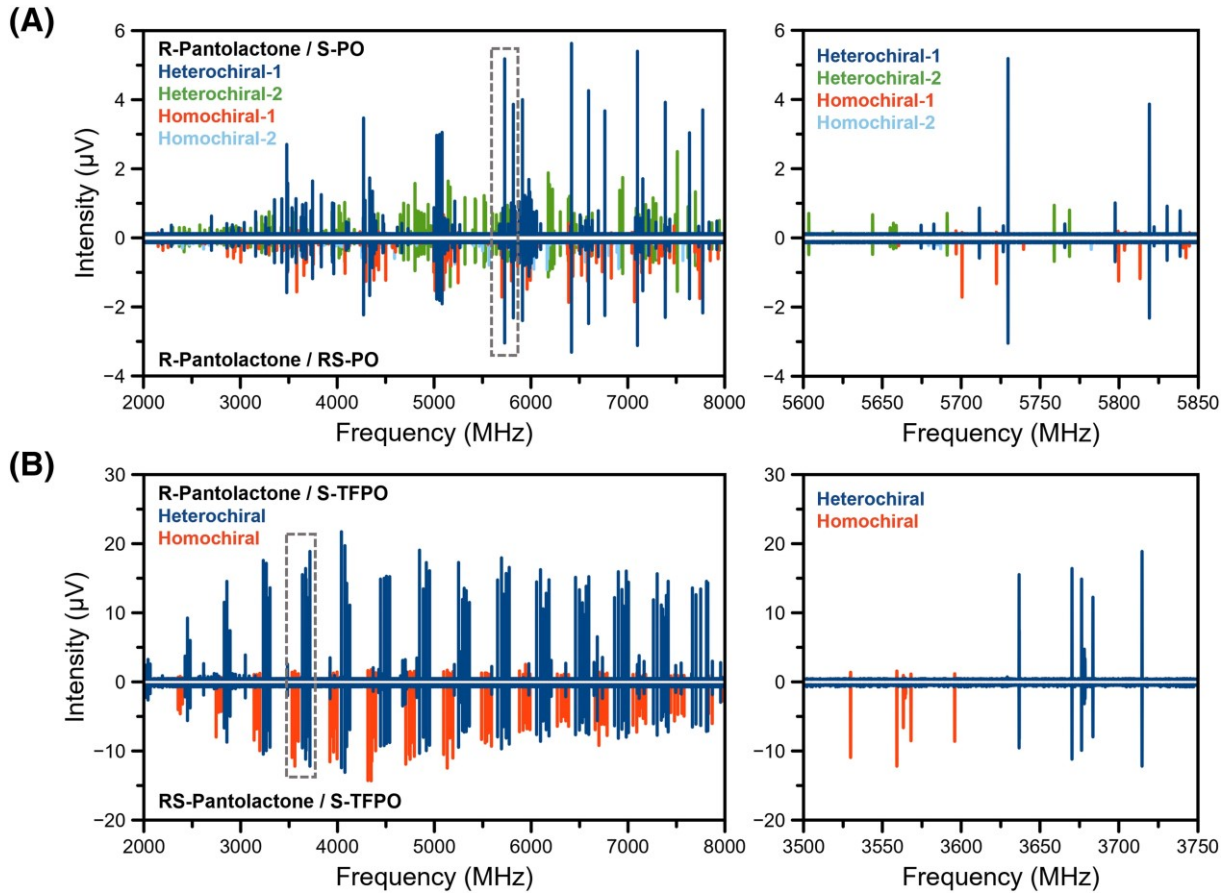
The MRR spectrum of the pantolactone monomer was first characterized through its measurement without any chiral tag present. Quantum chemical calculations revealed that isolated pantolactone has two conformations; this is also observed experimentally in the rotational spectrum of the related compound γ-butyrolactone.[31] The dimethylated carbon on the ring is puckered, while the other four atoms in the ring lie nearly in a plane. The two conformers differ in the direction of the puckering of the envelope. Calculations

indicated that the conformer that allows the hydroxyl group to adopt an equatorial conformation is more stable by 8.6 kJ/mol, and in fact, the MRR spectrum of pantolactone is observed to be dominated (at least 99%) by a single conformer with rotational constants in excellent agreement with this OHequatorial conformer. The experimental spectrum of the pantolactone monomer with pure neon backing gas, where no chiral tag complexes are present, is used to mask all monomer transition frequencies in subsequent chiral tag measurements so that transitions arising from complexes between the tag and analyte can be isolated. Dimers of the analyte, which for pantolactone have been studied computationally and experimentally in the condensed phase,[32,33] are removed in this spectral isolation process. In-house library spectra of the pure chiral tag samples are used to mask transitions associated with the tag and any related complexes (e.g., dimers or trimers of the tag).

The chiral analysis strategy using broadband MRR spectroscopy involves making two measurements. The first measurement requires that either the analyte or the chiral tag, or both, is racemic so that both homochiral and heterochiral diastereomers of the 1:1 tag complexes are generated in equal amounts. (Note: In this work, a homochiral complex is defined as the diastereomer where both the tag and analyte have the same Cahn-IngoldPrelog designation.) In most cases, there are several lowenergy isomers for the complexes so that more than one rotational spectrum will be observed for each diastereomeric complex. The second measurement, where the goal is determination of the chiral purity of the analyte, uses a high enantiopurity tag sample. In this measurement, if the analyte has an enantiomeric excess, the spectra for the chiral tag complexes will change intensity. From the intensity variation between the racemic and enantiopure tag measurements, it is possible to separate the spectral transitions observed in the racemic measurement into two groups, one for the isomers of each diastereomeric complex, based on whether the transitions increased or decreased in intensity between the two measurements. Comparison of the spectroscopic parameters to theoretical estimates is used to establish whether each group corresponds to homochiral or heterochiral complexes. Each diastereomeric complex exists as a pair of enantiomers, and the designation only provides the relative stereochemistry of the analyte and tag. The homochiral denotes either R-analyte /R-tag or S-analyte /S-tag, and the heterochiral complex can be R-analyte/S-tag or S-analyte/Rtag. The absolute configuration is determined by using a chiral tag of known enantiopurity. For example, if the analyte has an excess of the enantiomer with the same Cahn-Ingold-Prelog label as the tag used, the homochiral complexes will show increased intensity in the enantiopure tag measurement, while the heterochiral complexes will decrease, as compared to the racemic measurement. The opposite will occur if the analyte and tag have opposite labels. It is also possible that larger complexes (e.g., the analyte complexed to two tag molecules) may form in the pulsed jet; however, because each complex geometry has a unique spectrum that is fully resolved from that of the analyzed 1:1 complexes, their presence does not affect the enantiomeric analysis.

Assignment of the analyte's absolute configuration by chiral tagging requires high-confidence pairing of a computed chiral tag complex structure with a measured rotational spectrum. This pairing uses the agreement between the theoretical rotational constants calculated using the equilibrium geometry and the experimental rotational constants from the spectral analysis. When more than one spectrum is observed for the homochiral and heterochiral complexes, consistency between the set of experimental and theoretical rotational constants for multiple low-energy isomers can be used to support the assignments. All conformers of the analyte and tag, if applicable, should be considered in the calculations, even those not observed in the monomer spectra, as the energy preferences may change considerably upon complexation, favoring geometries that in the isolated state are unfavorable. This was recently observed in the case of
alaninol.[25]

The measurement strategy is illustrated in the chiral tagging spectra of pantolactone with PO and TFPO, given in Figure 2. The replacement of the CH_3 group in PO with a CF_3 group in TFPO can change the bonding geometries, as well as the dipole moment and number of observed isomers for each of the complexes; therefore, both tags were assessed. With PO, a total of four isomers were assigned, two of which had higher intensity with the enantiopure tag, while the other two had lower intensity. For TFPO, only two strong isomers were identified, one for each diastereomeric complex. The spectroscopic fit results are given in Table 1, where these spectra are designated Homochiral 1 and Heterochiral 1 and are shown to be in good agreement with the rotational constants of the lowest energy isomers of the chiral tag complex from quantum chemistry. In this complex, the hydroxyl group in pantolactone forms a hydrogen bond with the oxygen atom in propylene oxide, while the carbonyl oxygen of pantolactone is bifurcated between two C—H bonds from the propylene oxide tag, as shown in Figure 3A. However, this set of theoretical homochiral and heterochiral complex geometries has nearly equal rotational constants, and this lowers the confidence in pairing the experimental spectra with a specific geometry. The second pair of assignments, Homochiral 2 and Heterochiral 2, matches the



second lowest energy isomers identified in the quantum chemistry analysis, with geometries also shown in

FIGURE 2 Chiral analysis of (R)-pantolactone using (A) propylene oxide (at BrightSpec) and (B) trifluoropropylene oxide (at UVa). The left panels show the comparison of the masked spectra of the assigned complexes between the enantiopure and racemic measurements. The right panel is a zoomed in portion of the spectra to illustrate how the enantiomeric excess is determined, by observing the change in intensity of the assigned spectra between the two measurements

TABLE 1 Experimental and calculated rotational parameters for observed pantolactone-propylene oxide complexes

	Experiment[a]	Calculated	% Diff. (Exp Calc)	Experiment[a]	Calculated	% Diff. (Exp Calc)
	Homochiral 1			Heterochiral 1		
A (MHz)	1548.9890 (5)	1,543.7203	+0.34	1,558.92389 (43)	1,529.7772	+1.87
B (MHz)	384.22842 (19)	387.3886	0.82	380.79141 (11)	386.2120	1.42
C (MHz)	349.92519 (24)	352.1701	0.64	349.95089 (11)	354.9789	1.44
E (hartree)		653.771815			653.771687	
E (kJ/mol)[b]		0			1.6	
	Homochiral 2			Heterochiral 2		
A (MHz)	1,269.77652 (38)	1,248.5282	+1.67	1,421.72962 (31)	1,414.9858	+0.47
B (MHz)	469.23629 (23)	479.4049	2.17	420.847960 (93)	426.8636	1.43

C (MHz)	445.18406 (21)	454.5594	2.11	386.38942 (11)	391.1734	1.24
E (hartree)		653.771422			653.772309	
E (kJ/mol)[b]		1.0			0	

Note: Standard errors in the experimental rotational constants are given in parentheses in units of the last digit. Calculations performed at a B3LYP-D3BJ/ def2-TZVP level of theory.

[a] Quartic distortion parameters were also used in this fit, but are not presented here. These results can be found in the supporting information.

[b] Energy relative to the minimum of all geometries assessed for that diastereomeric complex.

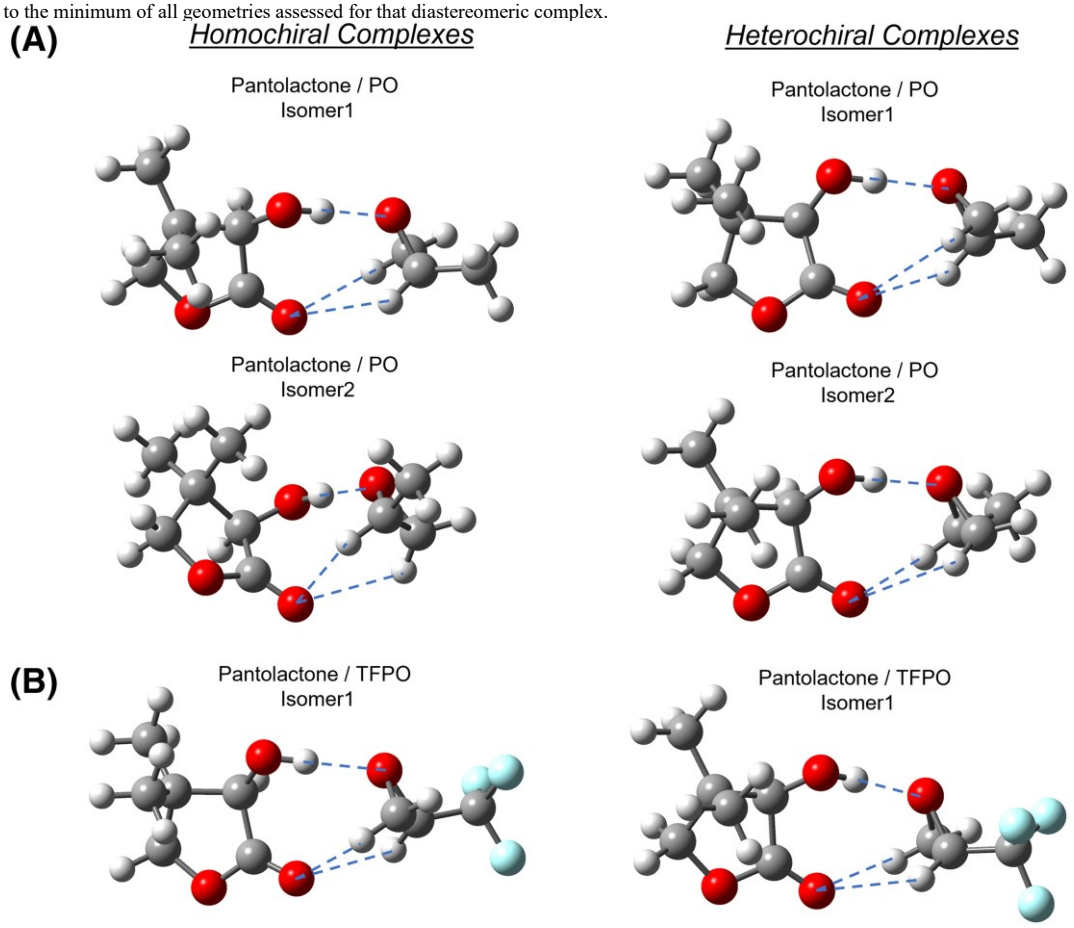


FIGURE 3 Optimized geometries of the assigned complexes of pantolactone-PO and pantolactone-TFPO. Structures were optimized using the B3LYP-D3BJ/def2-TZVP level of theory

TABLE 2 Experimental and calculated rotational parameters for observed pantolactone-trifluoropropylene oxide complexes

	Experiment[a]	Calculated	% Diff. (Exp - Calc)	Experiment[a]	Calculated	% Diff. (Exp - Calc)
	Homochiral			Heterochiral		
A (MHz)	1,196.110 (51)	1,169.4107	+2.23%	1,143.05150 (42)	1,144.1682	0.10%
B (MHz)	201.673590 (74)	204.8048	1.55%	208.642810 (32)	209.1411	0.24%
C (MHz)	194.326580 (72)	197.4271	1.60%	199.962690 (35)	200.1666	0.10%

Note: Standard errors in the experimental rotational constants are given in parentheses in units of the last digit. Calculations performed at a B3LYP-D3BJ/def2TZVP level of theory. [a]Quartic distortion parameters were also used in this fit but are not presented here. These results can be found in the supporting information.

Figure 3A, but there is a much larger distinction between the rotational constants for the homochiral and heterochiral complexes. In particular, the rotational constants for the Homochiral 2 spectrum have no close match in the family of structures identified for heterochiral complexes, as seen in the supporting information. This allows the assignment of the homochiral and heterochiral complex geometries and the absolute configuration of the measured pantolactone sample. Interestingly, both isomers of the homochiral complex favor pantolactone in its higher-energy conformer, while the heterochiral complex favors the lower-energy conformer.

The rotational constants for the observed complex for pantolactone with TFPO are presented in Table 2.

Because of the heavier fluorine mass, greater differentiation is observed between the two structures (3–5% difference in rotational constants), and so the correlation of experimental assignment with calculated structure can be made securely using this spectrum (Table 2). The calculated structures of the observed pantolactone-TFPO complexes that are identified in the spectrum are presented in Figure 3B. Both of these complexes are the lowest-energy structures that were calculated. For the chiral tagging experiments with both PO and TFPO, the absolute configuration assignments by MRR spectroscopic analysis of the diastereomeric tag complexes are in agreement with the absolute configuration of the commercial samples.

4.2 | Enantiomeric excess measurements using the broadband MRR spectra

The enantiomeric excess is determined from the intensity ratios of the rotational spectroscopy transitions assigned to homochiral and heterochiral chiral tag complexes when an enantiopure tag sample is used. If the tag sample were 100% enantiopure, then the intensities in the rotational spectrum of a homochiral complex would be solely due to the population of a single enantiomer, with the intensity of the heterochiral complex solely due to the population of the other enantiomer. In this case, the EE determination would be analogous to the analysis of a fully resolved chiral chromatogram. However, quantitative analysis requires including the actual enantiopurity of the tag sample. In addition, the instrument response function is needed to account for intensity variations across the full measurement bandwidth of the spectrometer.

For MRR chiral tag analysis, the instrument response function is obtained from the transition intensities in the racemic measurement as described in the preceding section. The normalized signal intensity for each transition is

$$I_{norm} = \frac{I_{enatiopure\ tag}}{I_{racemic\ tag}} \quad (1)$$

The ratio of the normalized signals for a pair of transitions, one from a spectrum of a homochiral complex and one from a heterochiral complex, is

$$R = \frac{I_{norm,homo}}{I_{norm,hetero}} \quad (2)$$

This ratio is then used to calculate a quantity that is related to the tag and analyte enantiomeric excess:

$$\frac{R-1}{R+1} = ee_{tag}ee_{analyte} \quad (3)$$

In this expression, (ee) is fractional value of the enantiomeric excess, related to the more common definition by EE = 100 x (ee). Note that the determination of the analyte EE requires accurate knowledge of the enantiomeric excess of the tag sample.

A broadband MRR spectrum contains many rotational transitions for the homochiral and heterochiral complexes. Therefore, a large number of EE determinations can be made

using all pairs of transitions from the homochiral and heterochiral tag complex spectra. For example, if there are four strong transitions observed for each complex (N = 4), then there are 16 possible transition pairs that can be used for EE determinations using Equations 1–3. The reported EE value uses the mean value of all determinations, and the measurement uncertainty (σ) is calculated from the width of the histogram
($w_{histogram}$), quantified as the sample standard deviation (half-width at half-height) from all determinations, divided by the square root of the number of transitions used for each complex:

$$\sigma = \frac{w^{histogram}}{\sqrt{N}} \qquad (4)$$

The histograms for analysis of (R)-pantolactone, analyzed with both PO and TFPO, is presented in Figure 4. Corresponding histograms for (S)-pantolactone with each tag can be found in the supporting information. In addition, the analyses using PO as the tag were performed on two different CP-FTMW instruments by different operators. Table 3 shows the enantiomeric excess of each sample measured by broadband MRR. Table 3 also reports the EE determination using chiral gas chromatography which is reported to validate the MRR analysis and excellent agreement is observed between the MRR measurements and the chiral GC results.

We performed two additional measurements using the UVa instrument to assess the performance at lower
EE values. Two mixtures of the commercial samples were prepared: a 2:1 mixture of (R)-pantolactone:(S)pantolactone and a 1:2 mixture of these samples. The EE of the mixtures was calculated using the mass of each component and the EE obtained from the chiral GC analysis. The calculated EE values (%R–%S) for the mixtures are 32.5% and 29.1%, respectively, while the MRR anal-

ysis results are and 31.6(9)% and 29.7(7)%, which agree with the calculated mixture EE within the measurement uncertainty. The histograms for the EE analysis of these two mixtures are shown in the supporting information.

4.3 | Targeted MRR spectroscopy of pantolactone complexes

As described above, the broadband MRR analysis determines both the absolute configuration and enantiomeric

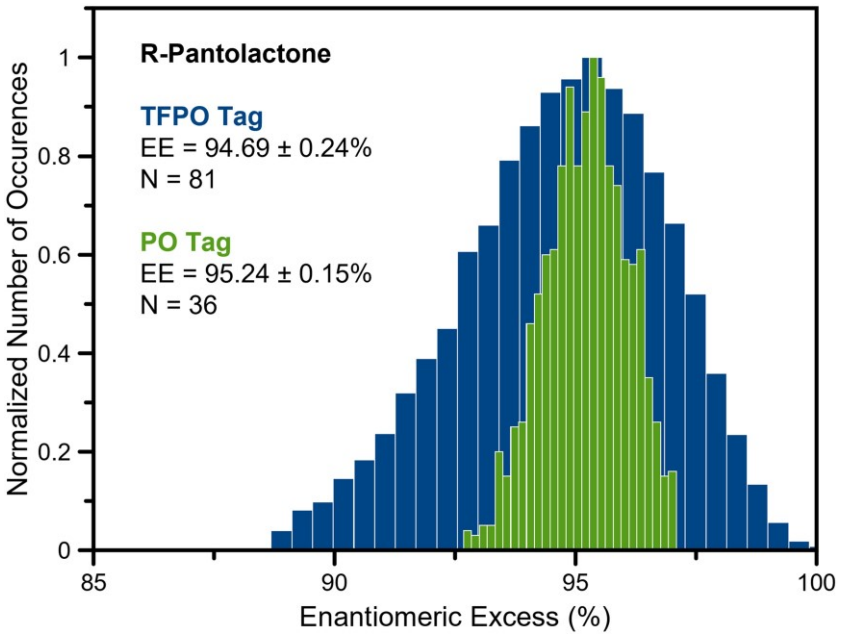


FIGURE 4 Histograms showing the enantiomeric excess determination of (R)pantolactone using both PO and TFPO. These measurements were performed using the broadband spectrometer at the University of Virginia

TABLE 3 Comparison of broadband MRR, IsoMRR, and GC-MS measurements of pantolactone enantiomeric excess

	Broadband MRR[a] PO tag (BrightSpec) (N= 16)	Broadband MRR[a] PO tag (UVa) (N= 36)	Broadband MRR[a] TFPO tag (UVa) (N= 81)	IsoMRR[b]	Chiral GC-MS[b]
(R)-pantolactone	95.65 ± 0.17%	95.24 ± 0.15%	94.69 ± 0.24%	95.6 ± 0.5%	95.9 ± 0.5%
(S)-pantolactone	92.19 ± 0.3%	91.38 ± 0.17%	91.57 ± 0.12%	92.7 ± 0.8%	90.8 ± 0.5%

[a] See the text for the description of how the uncertainty is determined.
[b] The Chiral GC-MS error comes from the standard deviation of three sample injections. For the IsoMRR data, a single run consists of three to five measurements of each line; the results of which are averaged together.

excess from a single analyte sample of unknown enantiopurity. However, the broadband measurements require several hours, so it is desirable to have the capability for faster measurements that also require less sample. After the chiral tagging analysis has been completed once for a particular analyte by broadband MRR, the complex transitions are known, and so a targeted approach is much more efficient. Therefore, we used the IsoMRR spectrometer to demonstrate more rapid (15 min) pantolactone measurements.

As the IsoMRR spectrometer used in this study had a frequency range of 6–18 GHz, propylene oxide was used as the tag for targeted enantiomeric excess measurements due to its stronger line intensities in this frequency range. The strongest transitions of each of the two complexes were first assessed on the IsoMRR instrument to determine which lines give the best sensitivity. We selected the 6_{34}–5_{24} transition at 10,324.9 MHz for the Homochiral 1 complex and the 8_{36}–7_{26} transition at 11,840.15 MHz from the Heterochiral 2 complex. In this instrument, the excitation power is also adjusted to achieve optimal sensitivity for each line. With racemic propylene oxide as the tag, the typical signal-to-noise ratio for each complex was approximately 20:1 in 20 s (200 total nozzle pulses, 1,000 FID acquisitions).

We prepared a total of six standards for analysis: the two commercially obtained pantolactone samples ([R] and [S]), along with four additional mixtures. Each sample mixture was weighed on a balance, and in the figures below, the true weighed ratio is used. Each solution was then dissolved at approximately 100 mg/ml in solvent grade dichloromethane to enable injection. The typical solution injection volume was 20 µl, so approximately 2 mg of pantolactone was used in each measurement. For each injection, we alternately measured each of the two complexes between three and five times, depending on the sample composition, to assess the measurement reproducibility. The reported value is the average of these measurements, with the uncertainty estimated by the standard error of the measurements. For the measurements presented herein, (R)-propylene oxide was

used as the chiral tag. The intensity calibration was performed using racemic propylene oxide, and applying Equations 1–3 to determine the enantiomeric excess for each sample. However, in the case of the IsoMRR, there is only a single transition to consider for each complex.

The results of this study are presented in Figure 5. Here, the measured enantiomeric excess is compared to the gravimetrically prepared samples. The results from the chiral GC analyses were used to compute the actual enantiomeric excess for each of the samples. While the IsoMRR instrument can perform EE measurements more rapidly and with less sample consumption than the broadband spectrometer, the measurement uncertainty is higher because only a single line is being measured for each of the two complexes. Additionally, because the lines of the two complexes are being measured sequentially rather than simultaneously as in the broadband spectrometer, any variations in the sample or complexing conditions will lead to additional errors in the EE determination. Where possible, performing several measurements in a row on the same injection is preferable to reduce this possibility. In the measurements presented here, we found that the signals of the two complexes were stable between measurements (typically within 5% on the major complex). The best-fit line to these results shows the slope is very near 1 (1.0143) and the intercept very near 0 (1.02%), as expected, demonstrating that the IsoMRR instrument can accurately determine EE without a calibration sample. Finally, we note that the IsoMRR method required about 15 min, significantly faster than the chiral GC method used (2 h), and with comparable uncertainty; however, the chiral GC method required much less sample.

While the samples analyzed here were pure (containing only pantolactone and dichloromethane, aside from any possible impurities in the commercial samples), enantiomeric excess measurements by MRR can still be performed accurately in the presence of other impurities. Due to the high resolution of molecular rotational transitions in these instruments, the impurities are highly unlikely to overlap with the transitions of interest. In the case, there is an overlap observed, there are numerous other transitions of the diastereomeric complexes that could be used in the analysis, and as described above, any pair can be used for the enantiomeric determination. Additionally, quantification of other impurities in the sample could also be performed in the same instruments.

5 | CONCLUSION

We have presented a method for the accurate determination of the enantiomeric excess of pantolactone, using molecular rotational resonance spectroscopy. Complexes of pantolactone with small chiral tag molecules are produced in a pulsed jet system to convert the enantiomers into diastereomeric complexes, and the enantiomeric excess is determined through the relative populations of the two complexes. Measurements using broadband MRR spectroscopy show quantitative agreement with the results from chiral GC. In addition, the EE determination is shown to be the same when two different tags (propylene oxide and trifluorpropylene oxide) are used in the measurement. Measurement speed is an important consideration in many applications of chiral analysis. Broadband MRR spectroscopy requires relatively long measurement times for the EE analysis. However, it is possible to use the chiral tag methodology in targeted MRR spectrometers that offer

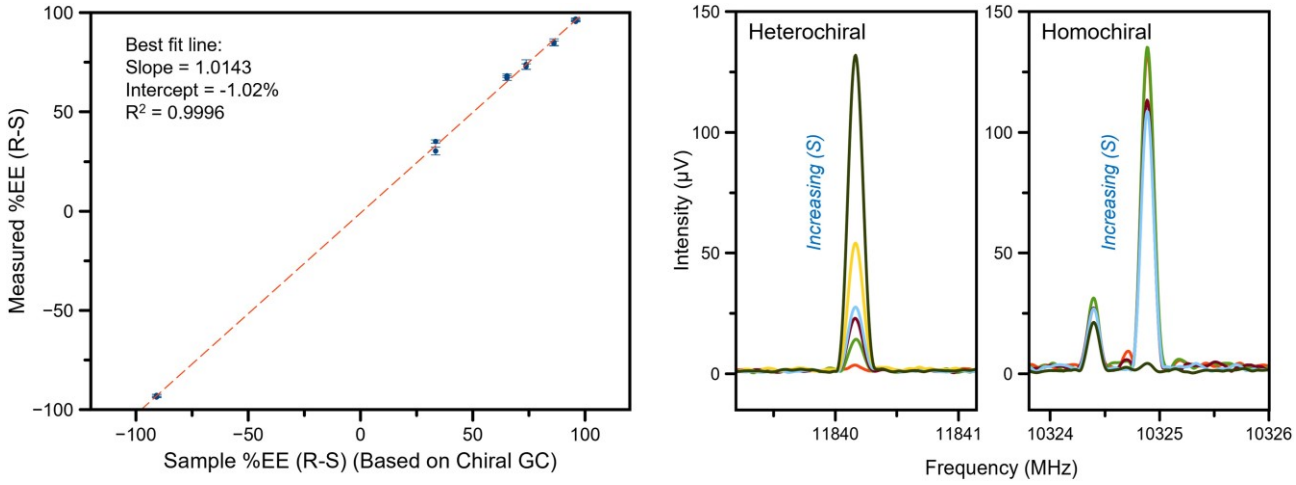


FIGURE 5 Targeted enantiomeric excess measurements of pantolactone, using (R)-propylene oxide as chiral tag. Left: plot of expected EE (%R–%S) against measured EE. The x axis uses the chiral GC results for the two commercial samples (Table 3) and the measured weights for each in the mixtures. The red dashed line is the best fit line to the data. The right panels show the measured data from one injection of each of the six samples. The line at 10,324.4 MHz in the right-most panel is not associated with the pantolactone-PO complex

significant reductions in measurement time and sample consumption. The accuracy of EE determinations in these measurements that target a single transition pair of the homochiral and heterochiral tag complexes is demonstrated. The chiral tagging method is broadly applicable for small molecules in chemical and pharmaceutical production.

ACKNOWLEDGMENTS

This work was supported by the Chemistry Division of the National Science Foundation through award 1904686. The work was also supported by a Catalyst grant from the Virginia Bioscience Health Research Corporation. Quantum chemistry calculations were performed with a research allocation from the University of Virginia high-performance computing group (using Rivanna).

DATA AVAILABILITY STATEMENT

The data from this study are available from the corresponding author upon reasonable request.

ORCID

Justin L. Neill https://orcid.org/0000-0003-0964-3275

Alexander V. Mikhonin https://orcid.org/0000-0003-4910-5615

REFERENCES

1. Stiller ET, Harris SA, Finkelstein J, Keresztesy JC, Folkers K.Pantothenic acid. VIII. The total synthesis of pure pantothenic acid. J Am Chem Soc. 1940;62(7):1785-1790.
2. Kimura S, Furukawa Y, Wakasugi J, Ishihara Y, Nakayama A.Antagonism of (L)-()-pantothenic acid on lipid metabolism in animals. J Nutr Sci Vitaminol. 1980;26(2):113-117.
3. Hoffmann W, Himmele W, Paust J, von Fraunberg K, Siegel H,Pfohl S. Resolution of racemic pantolactone. DK134937C, 1974.
4. Leonardi R, Jackowski S. Biosynthesis of pantothenic acid andcoenzyme A. EcoSal Plus. 2007;2(2). https://doi.org/10.1128/ecosalplus.3.6.3.4
5. Hermann T, Witteck B, Rieping M, Kruse D. Production of Dpantothenic acid, optionally as salt and/or contained in feed additive, by fermenting Enterobacteriaceae strain in which specific nucleotide sequences have been amplified. DE10128780A1, 2001.
6. Yocum RR, Patterson TA, Pero JG, Hermann T. Microorganisms and processes for enhanced production of pantothenate. US7291489B2, 2003.
7. Hikichi YS, Miki HC, Moriya TS, Nogami IN, Yamaguchi TT.Production of D-pantoic acid and D-pantothenic acid. DE6933325T2, 1993.
8. Patil RA, Weatherly CA, Armstrong DW. Chapter 11—chiral gas chromatography. In: Polavarapu PL, ed. Chiral Analysis. Second ed. Elsevier; 2018:468-505.
9. Wahab MF, Weatherly CA, Patil RA, Armstrong DW. Chapter 12—chiral liquid chromatography. In: Polavarapu PL, ed. Chiral Analysis. Second ed. Elsevier; 2018:507-564.
10. Wilson EB. Microwave spectroscopy in chemistry. Science. 1968;162(3849):59-66.
11. Pate BH. Taking the pulse of molecular rotational spectroscopy.Science. 2011;333(6045):947-948.
12. Pérez C, Lobsiger S, Seifert NA, et al. Broadband Fourier transform rotational spectroscopy for structure determination: the water heptamer. Chem Phys Lett. 2013;571:1-15.
13. Gordy W, Cook RL. Microwave Molecular Spectra. 3rd ed. New York: John Wiley & Sons, Inc.; 1984.
14. Brown GG, Dian BC, Douglass KO, Geyer SM, Shipman ST,Pate BH. A broadband Fourier transform microwave spectrometer based on chirped pulse excitation. Rev Sci Instrum. 2008; 79(5):053103.
15. Park BG, Field RW. Perspective: the first ten years of broadband chirped pulse Fourier transform microwave spectroscopy. J Chem Phys. 2016;144(20):200901.
16. Patterson D, Schnell M, Doyle JM. Enantiomer-specific detection of chiral molecules via microwave spectroscopy. Nature. 2013;497(7450):475-477.
17. Shubert VA, Schmitz D, Pérez C, et al. Chiral analysis usingbroadband rotational spectroscopy. J Phys Chem Lett. 2016;7(2): 341-350.
18. Domingos SRR, Pérez C, Schnell M. Sensing chirality with rotational spectroscopy. Annu Rev Phys Chem. 2018;69(1):499-519.
19. Pate BH, Evangelisti L, Caminati W, et al. Chapter 17—quantitative chiral analysis by molecular rotational spectroscopy. In: Polavarapu PL, ed. Chiral Analysis. Second ed. Elsevier; 2018:679-729.
20. Xie F, Seifert NA, Jäger W, Xu Y. Conformational panoramaand chirality controlled structure-energy relationship in a chiral carboxylic acid dimer. Angew Chem Int Ed. 2020;59(36): 15703-15710.
21. Domingos SR, Pérez C, Marshall MD, Leung HO, Schnell M.Assessing the performance of rotational spectroscopy in chiral analysis. Chem Sci. 2020;11(40):10863-10870.
22. Suenram RD, Grabow JU, Zuban A, Leonov I. A portable,pulsed-molecular-beam, Fourier-transform microwave spectrometer designed for chemical analysis. Rev Sci Instrum. 1999; 70(4):2127-2135.
23. Balle TJ, Flygare WH. Fabry-Perot cavity pulsed Fourier transform microwave spectrometer with a pulsed nozzle particle source. Rev Sci Instrum. 1981;52(1):33-45.
24. Neill JL, Yang Y, Muckle MT, et al. Online stereochemcial process monitoring by molecular rotational resonance spectroscopy. Org Process Res Dev. 2019;23(5):1046-1051.
25. Neill JL, Mikhonin AV, Chen T, Sonstrom RE, Pate BH. Rapidquantification of isomeric and dehalogenated impurities in pharmaceutical raw materials using MRR spectroscopy. J Pharm Biomed Anal. 2020;189:113474.
26. Marshall MD, Leung HO, Wang K, Acha MD. Microwave spectrum and molecular structure of the chiral tagging candidate,

3,3,3-trifluoro-1,2-epoxypropane and its complex with the argon atom. Chem A Eur J. 2018;122(19):4670-4680.

27. Frisch MJ, Trucks GW, Schlegel HB, et al. Gaussian 09, Revision

33. Ghidinelli S, Abbate S, Koshoubu J, Araki Y, Wada T, Longhi G. Solvent effects and aggregation phenomena studied by vibrational optical activity and molecular dynamics: the case of pantolactone. J Phys Chem B. 2020;124(22):4512-4526.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Sonstrom RE, Neill JL, Mikhonin AV, Doetzer R, Pate BH. Chiral analysis of pantolactone with molecular rotational resonance spectroscopy. Chirality. 2022;34(1):114-125. doi:10.1002/chir.23379

E.01. Wallingford, CT: Gaussian, Inc.; 2009.
28. Grimme S, Steinmetz M. Effects of London dispersion correction in density functional theory on the structures of organic molecules in the gas phase. Phys Chem Chem Phys. 2013; 15(38):16031-16042.
29. Grimme S, Hansen A, Brandenburg JG, Bannwarth C. Dispersion-corrected mean-field electronic structure methods. Chem Rev. 2016;116(9):5105-5154.
30. Grimme S, Ehrlich S, Goerigk L. Effect of the damping function in dispersion corrected density functional theory. J Comput Chem. 2011;32(7):1456-1465.
31. Legon AC. The microwave spectra and ring configuration ofγ-butyrolactone and γ-crotonolactone. J Chem Soc D. 1970; 13(13):838-838.
32. Polavarapu PL, Covington CL. Wavelength resolved specificoptical rotations and homochiral equilibria. Phys Chem Chem Phys. 2015;17(33):21630-21633.