# Optical Activity due to Isotopic Substitution. Vibrational Circular Dichroism and the Absolute Configurations of α-Deuterated Cyclohexanones

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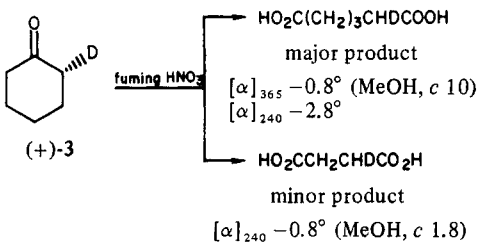
**Abstract:** The first syntheses of optically active [*trans*-2,6-$^2H_2$]cyclohexanone (**1**) and [2-$^3$H]cyclohexanone (**2**) are presented. These compounds are synthesized by use of the enzyme acetoacetate decarboxylase (AAD), and assignments of configurations based on chemical degradation and spectroscopy are described. Circular dichroism spectra for electronic transitions and fundamental vibrational transitions are reported for both enantiomers of **1** from 2000 to 40 000 cm$^{-1}$. The results of observation and calculation of vibrational circular dichroism (VCD) intensities of **1** and its α-monodeuterio analogue, **3**, are presented in both the C–H and C–D stretching regions. Qualitative agreement is obtained between experiment and theory in the C–H region of **1**, confirming the results of independent determinations of the absolute configurations. Measurable VCD in the C–H stretching region was not observed for dilute solutions of either enantiomer of **3** in cyclohexanone–$CCl_4$, although strong VCD bands were observed in the C–D stretching regions of both enantiomers. Only minimal VCD is predicted in the C–D stretching regions of **1** and **3**; however, large observed intensities over a wide frequency range in this region indicate a pronounced and possibly obscuring contribution from overtone and combination bands. The utility of enzymic methods for synthesis of chirally deuterated molecules is described, along with the potential for assignment of absolute configuration by VCD compared with chemical degradation.

In recent years, stereochemical investigations using isotopes as perturbers of molecular symmetry have received increased attention. Of particular significance are studies where the molecular chirality arises solely from an isotopic substitution. This particular aspect has been a focus of electronic optical activity (EOA) investigations, because in the Born–Oppenheimer approximation, isotopic substitution in a parent achiral molecule is not expected to support EOA. Nevertheless, it is now well understood that the observed EOA in such cases arises from vibronic interactions that differ for isotopically substituted and parent molecules. Several workers have now observed the EOA due solely to isotopic substitution,[2-4] and some theoretical models have been developed[5-7] to include vibronic interactions in EOA predictions. In vibrational optical activity (VOA)[8] several reports of VOA due solely to isotopic substitution have appeared.[9-12] More specifically, the vibrational circular dichroism (VCD) in the C–D stretching regions of (–)-[(1*R*)-$^2$H]neopentyl chloride,[9] (–)-[(1*R*)-$^2$H]-1-phenylpropane,[10] and, more recently, (–)-[(1*R*)-$^2$H]neopentyl bromide[11] have been reported. Similarly, (+)-(*S*)-[α-$^2$H]benzyl alcohol and (–)-(1*S*)-4,4-dideuterioadamantan-2-one[12] have been subjected to Raman optical activity (ROA) investigations. The presence of VOA in isotopically chiral molecules is easily conceivable, unlike the EOA, because the vibrations of these molecules are chiral.

Scheme I




In this report we present the VCD in C–H and C–D stretching vibrational regions for [*trans*-2,6-$^2H_2$]cyclohexanone and [2-$^2$H]cyclohexanone, which are optically active due to the deuterium at the α position. The observed spectrum from 2850 to 2950 cm$^{-1}$ forms the first report of VCD in the C–H stretching region for a molecule whose chirality is due to isotopic substitution.

## α-Deuterated Cyclohexanones

The chiroptical properties of cycloalkanones that are optically active solely as a consequence of asymmetric α substitution of deuterium have lately been a subject of special attention. While *trans*-2,5-dideuteriocyclopentanone shows peculiarities in its ORD spectrum,[13] in which the optical rotation changes sign between 436 and 365 nm (a region of no electronic absorption maximum), the α-deuteriocyclohexanones exhibit CD spectra in the UV that are relatively easy to interpret qualitatively.[14,15]

Recently, we have reported a straightforward route to optically active α-deuterio ketones via exchange with water catalyzed by the enzyme acetoacetate decarboxylase (AAD, E.C. 4.1.1.4) from *Clostridium acetobutylicum*.[16] This procedure is particularly useful in preparing *trans*-α,α′-dideuteriocycloalkanones by enzymic exchange of the perprotio compounds with $D_2O$. This procedure affords an excellent yield of [(*trans*-2*R*,6*R*)-$^2H_2$]cyclohexanone,

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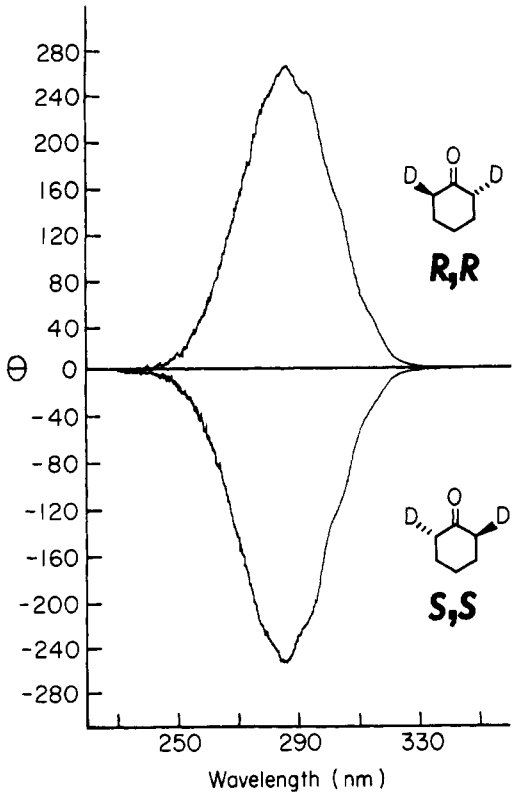


**Figure 1.** UV circular dichroism spectra of both enantiomers of [*trans*-2,6-$^2H_2$]cyclohexanone at room temperature. The spectra are recorded with 1 cm path length and 0.1 M concentration in methanol.

(+)-**1**, which is approximately 70% pure, the principal impurities being $d_1$ and $d_3$ analogues. The enantiomeric *S,S*-dideuterio compound, (−)-**1**, was prepared by AAD-catalyzed exchange of [2,2,6,6-$^2H_4$]cyclohexanone with $H_2O$, and the UV CD spectra of these two compounds are shown in Figure 1. This enzymic exchange procedure does not work for cycloheptanone, but it does work with cyclopentanone, although the enzyme becomes denatured before the exchange is complete. Our observation of [*trans*-2,5-$^2H_2$]cyclopentanone corresponds to that reported by Hine and Li,[13] with $[\alpha]_D$ +0.61°, $[\alpha]_{436}$ +0.65°, and $[\alpha]_{365}$ −2.02° (neat). The UV CD spectrum of this compound shows a bisignate feature in the region of electronic absorption, similar to the reported[14] CD spectrum of optically active [2-$^2$H]cyclopentanone.

The deuteriocyclohexanones represent a well-studied case of optical activity resulting from isotopic substitution. Assignment of absolute configuration of **1** by chemical methods was achieved by two independent routes. One route is shown by Scheme I: digestion of (+)-**1** in fuming nitric acid afforded [2-$^2$H]succinic acid as an impurity in the major product, [2-$^2$H]adipic acid.[17] Separation of the deuteriosuccinic acid by repeated recrystallizations enabled us to recover a sample that was barely enough for a measurement of optical rotation. This material was only 10–15 atom % D, as anticipated on statistical grounds, but was levorotatory, indicating an *R* configuration of the chiral center.[18]

An independent assignment of absolute configuration was achieved by preparing the monotritio analogues of (+)- and (−)-**1**, **2**, and converting them to hexanals via Scheme II. The last step in this sequence is an enzymic oxidation of tritiated 1-hexanol with $NAD^+$ and horse liver alcohol dehydrogenase (HLADH), which is known to remove selectively *pro-R* hydrogens from primary alcohols.[19] Using [1-$^{14}$C]-1-hexanol as an internal radioactivity standard, we compared the level of tritium in the 1-hexanol *N*-phenylcarbamate to the level of tritium in the semicarbazone

Scheme II

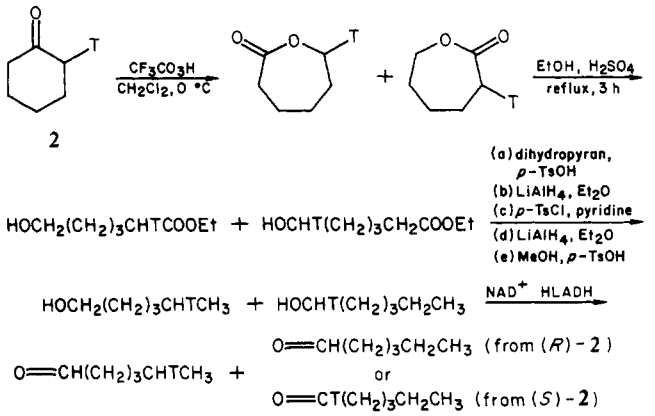


**Table I.** Radioactivity Levels in Derivatives of Optically Active [1-$^{14}$C;1,5-$^3$H]-1-Hexanol Samples Prepared via Scheme II

| | dpm $^3$H/dpm $^{14}$C | | |
|---|---|---|---|
| precursor | *n*-hexyl *N*-phenylcarbamate | hexanal semicarbazone from HLADH oxidn | hexanoic acid from $KMnO_4$ oxidn |
| (+)-2 | 13.8 | 8.4 | |
| (+)-2 | 5.5 | | 3.1 |
| (−)-2 | 10.6 | 10.8 | |
| (−)-2 | 2.5 | | 1.4 |

of $^3$H and $^{14}$C-labeled hexanol recovered from HLADH-catalyzed oxidation.

As expected from Scheme II, half of the tritium was in an unexchangeable position in the labeled *n*-hexanols. When *n*-hexanol samples were oxidized to hexanoic acid chemically, they lost only half of their radioactivity, which is consistent with the fact that conversion of **2** puts roughly half of the tritium into the 5 position of 1-hexanol, from which it cannot be removed by enzymic or known chemical methods of oxidation. The other half of the tritium label is in the 1 position of the 1-hexanol. Three 1-hexanol samples were prepared, the first from a sample of racemic **2**. The second 1-hexanol sample was prepared by degradation of a sample of **2** which had been prepared by AAD-catalyzed exchange of cyclohexanone with tritiated water. Since this sample of **2** has the same absolute configuration as (+)-**1**, we shall designate it as (+)-**2**, although its optical rotation was never observed. The third *n*-hexanol sample was prepared from **2** that had been prepared by AAD-catalyzed exchange of racemic **2** with $H_2O$. Because this sample of **2** has the same absolute configuration as (−)-**1**, we shall designate it as (−)-**2**, although its optical rotation was never observed either. When oxidized with HLADH and $NAD^+$ in the presence of acetaldehyde, 1-hexanol from racemic **2** lost 23% of its tritium label, while 1-hexanol from (+)-**2** lost 39% of its tritium label. 1-Hexanol from (−)-**2** lost none of its tritium label when converted to hexanol by HLADH-catalyzed oxidation. These results, as well as the results of chemical oxidation of radiolabeled *n*-hexanol samples to hexanoic acid, are summarized in Table I. Therefore, we assign the *R* configuration to (+)-**2** and, hence, the *R,R* configuration to (+)-**1**; we assign the *S* configuration to (−)-**2** and, hence, the *S,S* configuration to (−)-**1**. This assignment is corroborated by the work of Professor John M. Schwab of Catholic University, who has degraded a sample of (−)-**1** to [1-$^2$H]-*n*-pentyl camphanate and has examined the $^1$H NMR and $^2$H NMR spectra in the presence of lanthanide shift reagents. Using a correlation developed by Gerlach and Zagalak, Schwab concludes that (−)-**1** has the *S,S* configuration,[20] a result in agreement with our assignment.

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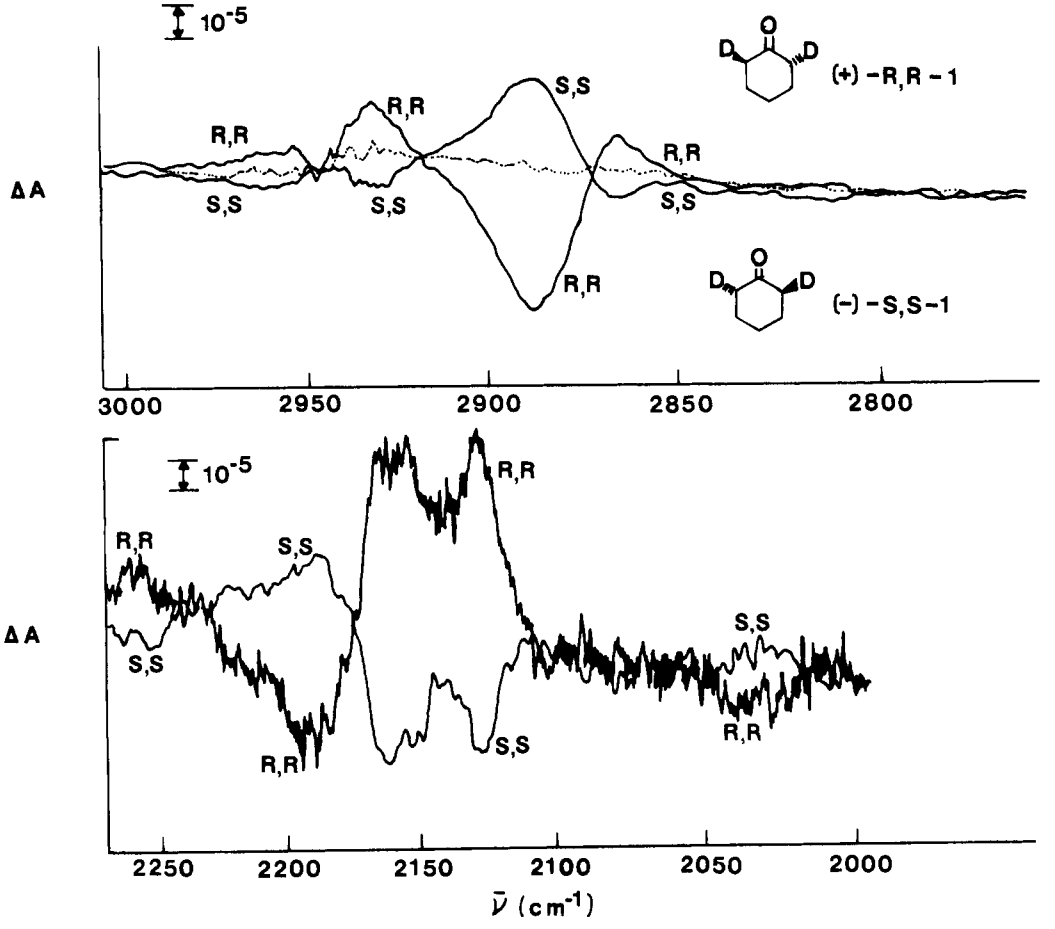


**Figure 2.** VCD spectra in C–H and C–D stretching regions for both enantiomers of [*trans*-2,6-$^2H_2$]cyclohexanone. The spectra are recorded with 300 μm path length and 0.22 M concentration in carbon tetrachloride. The dotted line represents the spectrum obtained with optically inactive cyclohexanone in carbon tetrachloride.

Use of AAD to synthesize optically active α-deuterio ketones has enabled us to prepare gram quantities of the monodeuterated cyclohexanone (+)-**3** simply by limiting AAD-catalyzed exchange of cyclohexanone with $D_2O$ to approximately 20 atom % $^2H$. Under these conditions, the recovered material contains a $d_0$:$d_1$:$d_2$ ratio of 25:5:1, $[\alpha]^{25}_D$ +0.37° (neat), which is suitable for chiroptical studies. The enantiomer, (−)-**3**, was prepared by allowing a sample of (−)-**1** to exchange with water in the presence of AAD. Under these conditions, a sample containing a $d_0$:$d_1$:$d_2$ ratio of 27:9:1 was obtained, which had $[\alpha]^{25}_D$ −0.24° (neat). The magnitude of the observed rotation of this (−)-**3** was about half of that expected, which we attribute to partial racemization during distillation. The observed rotations for **1**, $[\alpha]^{25}_D$ +3.7° for (+)-**1** and $[\alpha]^{25}_D$ −3.9° for (−)-**1**, are of a magnitude roughly twice the rotation that we would calculate for pure (+)-**3**. Our assignment of absolute configurations agrees with the assignment by Djerassi et al., based on their synthesis of (+)-**3** from (−)-nopinone.[15]

### VCD Spectra and Theoretical Predictions

The VCD spectra were recorded on the dispersive instrument at Syracuse which has been described elsewhere.[21] The only modifications are the incorporation of $CaF_2$ modulator and a high-pressure xenon lamp in the place of ZnSe modulator and tungsten–halogen lamp, respectively. Figure 2 shows the VCD spectra in the C–H and C–D stretching regions for both enantiomers of **1**. VCD spectra for (−)-**1** were also obtained in separate scans with improved signal-to-noise ratio, which resulted in the replotted spectra in Figure 3 for the C–H stretching region. There are two broad infrared absorption bands centered near 2948 and 2866 cm$^{-1}$. The former contains the absorption due to antisymmetric $CH_2$ and α-C–H stretching motions, while the latter contains the absorption due to symmetric $CH_2$ stretching motions. The VCD spectrum exhibits two negative peaks on the high-frequency side and one on the low-frequency side. Between these negative features is a largely positive VCD band with a distinct high-frequency shoulder. Clearly, from the band positions, the origin of VCD can be traced to both antisymmetric and symmetric C–H stretching motions.

For a more detailed interpretation, we have calculated the VCD intensities, using the fixed partial charge (FPC) model.[22] First, we have carried out a vibrational analysis for cyclohexanone-$d_0$, using the force field of Fuhrer et al.[23] and reproduced the reported frequencies. We have utilized the partial charges employed in our previous calculations on (+)-(3*R*)-methylcyclohexanone[24] and first obtained zero rotational strengths for all vibrations of cyclohexanone-$d_0$. Then, with use of the same force constants, geometry, and charges, the VCD calculations were performed for the molecules of the present investigation. The calculated results are compared with experimental values in Table II. The simulated spectrum, using a 15 cm$^{-1}$ fwhm Lorentzian band shape, is overlayed with the replotted experimental spectrum in Figure 3.

From a comparison of the results in Table II and Figure 3, several interesting points can be noted. The interaction of one α-C–H stretch with in-phase and out-of-phase antisymmetric β-$CH_2$ stretching motions renders the VCD in these $CH_2$ modes a bisignate feature. The negative component of the bisignate feature, reinforced by the negative VCD contribution from the α-C–H stretch, gives a resultant negative VCD on the high-frequency side. The second α-C–H stretch, along with the anti-

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Table II. Observed and Theoretical IR and VCD Parameters for C-H and C-D Stretching Motions of [*trans*-(2*S*,6*S*)-$^2H_2$]Cyclohexanone (1)

| frequency, $cm^{-1}$ | | | $\epsilon$ | | $\Delta\epsilon \times 10^3$ | | |
|---|---|---|---|---|---|---|---|
| | obsd | | | | | | |
| calcd | IR | VCD | calcd | obsd | calcd | obsd | |
| | | 2958 | | | | −0.34 | |
| 2945 | 2948 | | 43.75 | 110.9 | 0.56 | | antisym $\beta$-$CH_2$ stretch |
| 2941 | | | 18.79 | | −0.98 | | antisym $\beta$-$CH_2$ stretch |
| | | 2938 | | | | −0.55 | |
| 2935 | | | 5.15 | | −0.46 | | $\alpha$-C-H stretch |
| 2932 | | | 7.10 | | 0.78 | | $\alpha$-C-H stretch |
| 2917 | | 2919 | 48.65 | | 0.20 | 0.25 | antisym $\gamma$-$CH_2$ stretch |
| | | 2890 | | | | 2.57 | |
| 2877 | | | 29.60 | | −0.06 | | sym $\beta$-$CH_2$ |
| 2876 | | | 27.20 | | −0.00 | | sym $\beta$-$CH_2$ |
| | 2866 | 2868 | | 53.45 | | −0.49 | |
| 2853 | | | 27.31 | | −0.02 | | sym $\gamma$-$CH_2$ stretch |
| | | 2259 | | | | −2.5 | |
| | 2213 | 2224 | | 7.2 | | 2.7 | |
| | 2198 | 2194 | | 7.3 | | 3.7 | |
| 2171 | | | 1.20 | | −0.08 | | $\alpha$-C-D stretch |
| 2166 | | 2166 | 0.80 | | 0.07 | −5.7 | $\alpha$-C-D stretch |
| | 2160 | 2160 | | 4.9 | | −5.3 | |
| | 2133 | 2130 | | 5.8 | | −6.3 | |

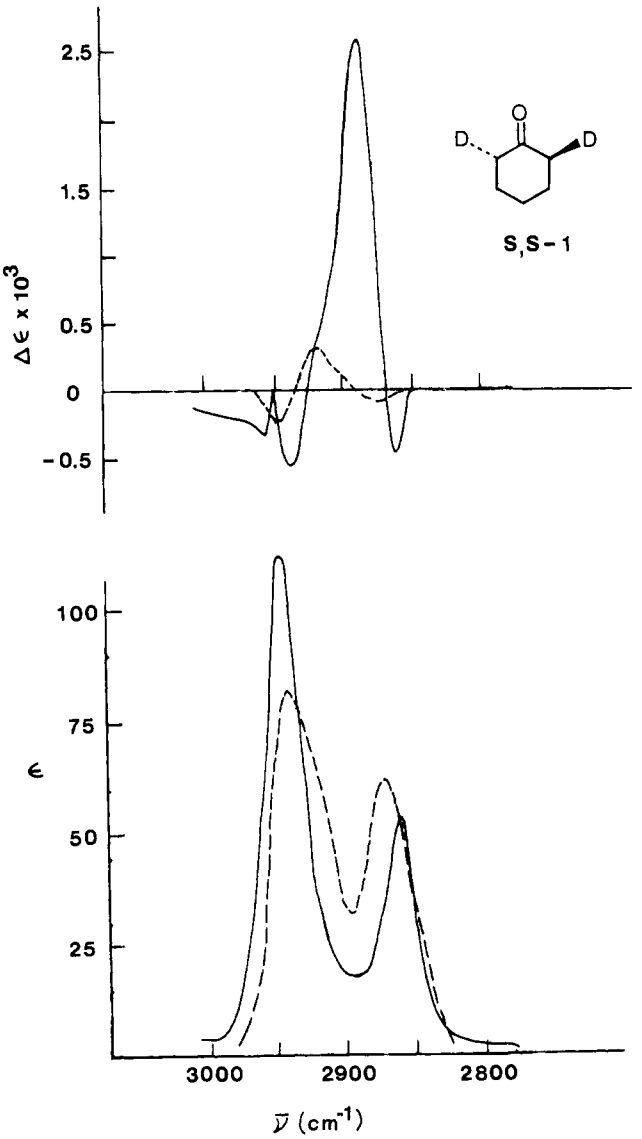


**Figure 3.** VCD and IR absorption spectra in the C–H stretching region for [(*trans*-2*S*,6*S*)-$^2H_2$]cyclohexanone. Path length and concentration employed are 215 $\mu$m and 0.55 M in $CCl_4$, respectively. The original spectra are replotted in $\Delta\epsilon$ and $\epsilon$ units. The calculated spectra, using the FPC model, are represented by dashed lines.

symmetric $\gamma$-$CH_2$ stretching mode, gives rise to a positive VCD contribution, which closely corresponds to the shoulder on the intense VCD peak. All symmetric $CH_2$ stretching motions give rise to negative VCD contributions, corresponding to the observed negative VCD on the low-frequency side. Therefore, the predicted sign features (negative–positive–negative) match the experimental observation and provide a qualitative understanding of the origin of VCD in the C–H stretching region. However, the magnitudes do not agree closely, especially since the analogue of the large experimental VCD is not present in the calculated spectrum. The VCD intensity of this particular peak at 2890 $cm^{-1}$, though, may have been enhanced through resonance interactions or may be due to an overtone of a bending mode, and the very small infrared absorption associated with this peak supports the latter possibility. If this is true, the harmonic FPC model then is unable to predict this large positive VCD, and anharmonic calculations may be required. On the other hand, the calculated infrared absorption intensities (with charges scaled to fit the absorption spectrum of 3-methylcyclohexanone[24]) match the observed values very well.

In the C–D stretching region, one would expect a simple VCD spectrum corresponding to the two $\alpha$-C–D stretching motions. However, the observed spectrum in Figure 4 contains multiplet features that go beyond a simple description. Similar complexity was noted in this region for (−)-[(1*R*)-$^2$H]-1-phenylpropane.[10] According to our FPC calculations, the two C–D stretching motions should have nearly the same frequency with nearly equal and opposite signed VCD, and therefore no measurable VCD. On the contrary, a substantial amount of VCD is present in the 2250–2100 $cm^{-1}$ region.

The calculated frequencies for C–D stretching motions are 2166 and 2171 $cm^{-1}$, suggesting that the observed VCD around 2160 $cm^{-1}$ might be due to a C–D stretching motion. An alternate explanation may emphasize that the frequencies are not well predicted and that the observed absorption peaks at 2213 and 2198 $cm^{-1}$ are due to C–D stretching motions. This later possibility appears less probable, because the force field employed here has successfully predicted the observed frequencies for symmetrically deuterated cyclohexanones.[23] In any event, all the other absorption bands and the corresponding VCD must be originating from overtone and combination bands. Hence, there is a need for further identification and interpretation in this region, beyond a simple FPC calculation with harmonic vibrational analysis.

These conclusions are further substantiated by the observed VCD spectrum of (+)-**3** shown in Figure 4. The observed absorption band at 2160 $cm^{-1}$ and the associated VCD could be assigned to the lone C–D stretching motion, on the basis that the calculated frequencies for the two chair conformations of this molecule are 2170 and 2167 $cm^{-1}$. The the observed intensity

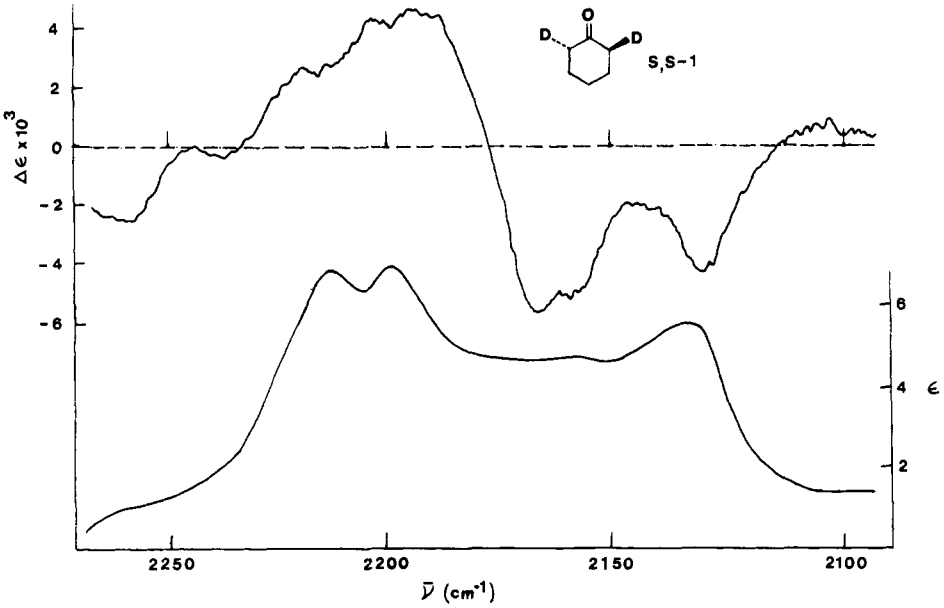


**Figure 4.** VCD and IR absorption spectra in the C–D stretching region for [(*trans*-2*S*,6*S*)-$^2H_2$]cyclohexanone. Path length and concentration employed are 300 μm and 1.1 M in $CCl_4$, respectively. Base line is deduced from the mirror image spectra shown in Figure 2.

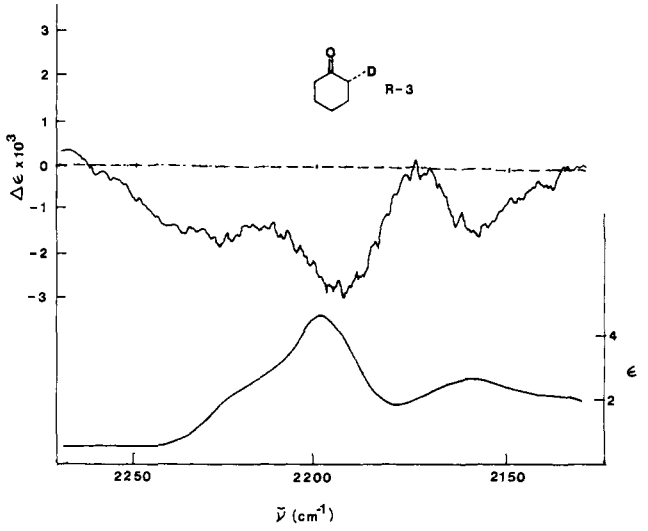


**Figure 5.** VCD and IR absorption spectra in the C–D stretching region for (+)-[(2*R*)-$^2$H]cyclohexanone. Path length and concentration respectively are 260 μm and 2.39 M in cyclohexanone. The base line is obtained from the mirror image spectra of the enantiomers.

between 2250 and 2200 $cm^{-1}$ remains to be explained in terms of multiquantum transitions. We did not observe any measurable VCD in the C–H stretching region for this molecule, which can be understood on the basis that there are two equally probable chair conformations that will yield opposing contributions. To a major extent this hypothesis is borne out by our FPC calculations, summarized in Table III, where the resultant Δε values can be seen to average out to small values, when appropriate band width (≈15 $cm^{-1}$) is introduced.

## Summary

We have shown that when molecular chirality is introduced through deuterium substitution, the VCD associated with C–H stretching motions is more readily interpreted than that associated with C–D stretching motions. Also the presence of at least two oscillators at the same frequency appears to give larger VCD due to the presence of interoscillator coupling. As we have pointed out elsewhere,[25] the coupling of ring methylene modes may serve as valuable markers in the study of the influence of differing substituents. This coupling is seen in **1** where the VCD arises not only from the chiral centers, but also from achiral centers (namely the β and γ positions), which contribute intensity. This is also found to be true in (+)-(3*R*)-methylcyclohexanone[24] and in 4-substituted cyclohexenes.[25] In the C–D stretching region, we find that the spectra are more complicated due to the presence of significant VCD from overtone and combination bands, and as a result, a simple harmonic FPC model is inadequate for the understanding of the observed VCD in this stretching region.

The qualitative agreement between predicted and observed VCD in the C–H stretching region of **1** (as well as agreement of the absence of VCD in this region for **3**) demonstrates the potential for using VCD to assign absolute configurations of molecules whose optical activity results from asymmetric isotopic substitution. As we[16] and others[14] have shown, enzymic reactions provide an excellent route for converting achiral materials to single enantiomers of chirally deuterated compounds in the laboratory. The enzymic reactions provide excellent yields in a few steps, as

Table III. Theoretical VCD Strengths for C–H and C–D Stretching Motions of [(2*R*)-$^2$H]Cyclohexanone (3)

| I[a] | | II[b] | | | |
|---|---|---|---|---|---|
| ν, $cm^{-1}$ | $R \times 10^{44}$, esu² cm² | ν, $cm^{-1}$ | $R \times 10^{44}$, esu² cm² | Δε × $10^3$ [c] | assignment |
| 2965 | 1.52 | 2965 | −1.63 | −0.03 | antisym α-$CH_2$ stretch |
| 2944 | −0.72 | 2945 | −1.43 | −0.58 | antisym β-$CH_2$ stretch |
| 2940 | −2.49 | 2941 | 6.21 | 1.01 | antisym β-$CH_2$ stretch |
| 2935 | 2.02 | | | 0.55 | α-C–H stretch |
| | | 2932 | −3.08 | −0.83 | α-C–H stretch |
| 2917 | −1.40 | 2917 | 0.72 | −0.18 | antisym γ-$CH_2$ stretch |
| 2900 | 0.81 | 2900 | −0.88 | −0.02 | sym α-$CH_2$ stretch |
| 2877 | 0.21 | 2877 | 0.03 | 0.06 | sym β-$CH_2$ stretch |
| 2876 | 0.03 | 2876 | −0.02 | $0.00_3$ | sym β-$CH_2$ stretch |
| 2853 | 0.01 | 2853 | 0.06 | 0.02 | sym γ-$CH_2$ stretch |
| | | 2170 | 0.01 | $0.00_2$ | α-C–D stretch |
| 2167 | 0.09 | | | 0.02 | α-C–D stretch |

[a] Deuterium in axial position. [b] Deuterium in equatorial position. [c] These values are obtained from the total rotational strengths (sum of I and II) by using the Lorentzian band shape with a 15 $cm^{-1}$ band width.

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compared to the lengthy procedures required to synthesize similar compounds chemically from optically active precursors. Thus far, assignment of absolute configurations of enzymatically synthesized molecules has often required tedious degradative procedures, such as described here and elsewhere for a half-dozen deuterated compounds.[16] It is hoped that this work will lead to further extensions of enzymatic methods in organic synthesis and to development of VCD as a reliable tool for assignment of absolute configurations.

## Experimental Section

Acetoacetate decarboxylase (AAD) was prepared by using the published procedure[26] in the laboratory of F. H. Westheimer with the able assistance of Mr. Jerome V. Connors. UV CD spectra and optical rotations at wavelengths <365 nm were recorded on a Cary Model 60 spectropolarimeter. Optical rotations at 365, 436, and 589 nm were measured on a Perkin–Elmer Model 141 polarimeter. Except where otherwise noted, reported absolute rotations and molar ellipticities are observed values and are not corrected to 100% enantiomeric excess.

Commercial tritiated water (New England Nuclear Corp.), [1-$^{14}$C]-1-hexanol (U.S. Radiochemical Corp.), and deuterium oxide (Stohler Isotope Co.; BioRad Laboratories, Ind.: 99.7 atom % D) were used without further purification. Commercial grades of cyclopentanone (MCB), cyclohexanone (MCB), acetaldehyde (Aldrich), and *N*-morpholino-1-cyclohexene (Aldrich) were purified by distillation from calcium hydride. Horse liver alcohol dehydrogenase (1.8 IU/mg) and $NAD^+$ were obtained from Sigma and used without further purification. All other reagents and solvents were commercial grades and were used without further purification.

**Enantiomers of 1.** Our synthesis of [(*trans*-2*R*,6*R*)-$^2$H$_2$]cyclohexanone, (+)-**1**, has previously been described.[16] This material, purified by distillation at atmospheric pressure, bp 150–155 °C, shows $[\alpha]^{25}_D$ +3.71° (neat) and the following 70 V mass spectrum: *m*/Z (rel intensity) 102 (4), 101 (19), 100 (44), 99 (5), 98 (1), 85 (3), 84 (9), 83 (6), 82 (6), 81 (4), 73 (6), 72 (23), 71 (40), 70 (24), 69 (4), 58 (9), 57 (20), 56 (100), 55 (69), 54 (4), 45 (11), 44 (39), 43 (78), 42 (46), 41 (18), 40 (23), 39 (17). Its antipode, [(*trans*-(2*S*,6*S*)-$^2$H$_2$]cyclohexanone, (−)-**1**, was synthesized from 2,2,6,6-tetradeuteriocyclohexanone, ≈90 atom % D, which had been prepared by two sequential exchanges of cyclohexanone with $D_2O$ catalyzed by 0.2 M $K_3PO_4$. A solution of 2.0 g of the tetradeuteriocyclohexanone in 40 mL of 0.05 M potassium phosphate buffer (pH 5.95) was mixed with a solution of 2 mg of AAD in 1.5 mL of buffer and set on a shaking table at 28 °C. The rotation at 365 nm was monitored periodically, and after 2.5 h the solution had reached its peak optical activity, $\alpha_{365}$ −1.09°, whereupon it was extracted with ether, the ethereal phase separated and dried over sodium sulfate, and the solvent removed by flash distillation at atmospheric pressure. Distillation of the remaining material afforded 1.4 g of (−)-**1**, bp 149–152 °C, $[\alpha]^{25}_D$ −3.96° (neat); 70 V mass spectrum: *m*/*z* (rel intensity) 102 (2), 101 (11), 100 (60), 99 (14), 98 (2.5), 85 (3), 84 (10), 83 (7), 82 (6), 81 (5), 73 (3), 72 (22), 71 (47), 73 (36), 69 (7), 58 (6), 57 (21), 56 (100), 55 (24), 54 (5), 45 (8), 44 (30), 43 (23), 42 (45), 41 (22), 40 (24), 39 (21).

**Degradation of (+)-[2-$^2$H]Cyclohexanone to [2-$^2$H]Adipic and [2-$^2$H]Succinic Acids.** A 17-g sample of (+)-[2-$^2$H]cyclohexane, $[\alpha]^{25}_D$ +2.1° (neat), prepared by swirling 30 g of cyclohexanone with 80 mL of buffered $D_2O$ containing 13 000 IU AAD for 22 h followed by distillation was digested in 60 mL of 90% fuming nitric acid in a 4-L Erlenmeyer flask on a steam bath. After the violent reaction had subsided, 21 mL of water was added and heating continued for another 0.5 h. The reaction mixture was allowed to cool and then filtered; a portion of the residue was recrystallized twice from concentrated nitric acid to afford white crystals of [2-$^2$H]adipic acid, approximately 50 atom % D. Water was removed from the filtrate from the reaction mixture under aspirator pressure and the residual brown solid recrystallized three times from acetone followed by two recrystallizations from water to afford 44 mg of levorotatory [2-$^2$H]succinic acid, mp 186–187 °C, which showed an unchanged melting point when mixed with an authentic sample of succinic acid.

**[2-$^3$H]Cyclohexanone (2).** Racemic **2** was prepared by mixing 2.5 mL of *N*-morpholinocyclohexene, 0.5 mL of tritiated water (100 mCi/mL), and 1.5 mL of concentrated hydrochloric acid and separating the organic layer, which was mixed with unlabeled cyclohexanone and distilled. A sample of (−)-**2** was prepared by exchange of 1.0 g of racemic **2** with 1 mL of buffered $H_2O$ containing 700 IU of AAD for 6 h, followed by separation of the organic layer and a second exchange with 2 mL of buffered $H_2O$ containing another 700 IU of AAD. A sample of (+)-**2** was prepared by swirling 5.0 g of cyclohexanone with 20 cm$^3$ of tritiated water (buffered at pH 5.8) containing 2000 IU of AAD for 1.2 h at 30 °C, followed by separating the organic layer and distilling the solution.

**Conversion of 2 to [1,5-$^3$H]-1-Hexanol.** Baeyer–Villiger oxidations of samples of **2** were performed in $CH_2Cl_2$ over solid $Na_2HPO_4$, using the procedure of Emmons and Lucas.[27] After oxidation was complete, the solvent was distilled off, unlabeled ϵ-caprolactone added to the residue, and distillation continued under vacuum. The distilled [2,6-$^3$H]caprolactone was then dissolved in absolute ethanol, concentrated sulfuric acid added, and the mixture refluxed for 2–4 h to convert it to ethyl [2,6-$^3$H]-6-hydroxyhexanoate.[28] Approximately half of the ethanol was removed by distillation at atmospheric pressure, the reaction mixture cooled and diluted with approximately 6 volumes of ether and washed repeatedly with saturated aqueous $NaHCO_3$, and distillation continued until the head temperature reached 40 °C. Then a sample of unlabeled ethyl 6-hydroxyhexanoate was added to the remaining ethanolic solution and distillation continued at atmospheric pressure until the head temperature reached 170 °C. The product was isolated by further distillation under vacuum, and the fraction boiling between 100 and 110 °C (2 torr) was reacted with dihydropyran in benzene in the presence of *p*-TsOH, followed by reduction of the THP ether with lithium aluminum hydride to afford [1,5-$^3$H]-6-hydroxyhexyl tetrahydropyranyl ether, which was tosylated and reduced with lithium aluminum hydride to yield the THP ether of [1,5-$^3$H]-1-hexanol. The THP group was removed by refluxing the solution in methanol with a trace of *p*-TsOH, and the radiolabeled 1-hexanol was diluted with unlabeled 1-hexanol and distilled at atmospheric pressure.

**HLADH-Catalyzed Oxidation of [1,5-$^3$H]-1-Hexanol.** A 70–175 μL sample of [1,5-$^3$H]-1-hexanol was mixed with 5–10 μL of [1-$^{14}$C]-1-hexanol (5–10 nCi). A 50–60 μL portion was added to 25 mL of 0.1 M $(NH_4)_2CO_3$ buffer (pH 10.1) containing 10–14 mg of $NAD^+$, which has been degassed by bubbling nitrogen through it. A 4.5 mg sample of HLADH was quickly dissolved in the solution and 0.2 mL of acetaldehyde added. The reaction mixture was allowed to sit under a nitrogen blanket for 18 h and then worked up by three extractions with a total of 100 mL of ether. Then 0.3 g of unlabeled hexanal was added to the remaining reaction mixture and the aqueous solution extracted three times more with another 100 mL of ether. The ether layers were combined and the solvent removed by distillation at atmospheric pressure. The residue was converted to the semicarbazone, which was purified by 3–4 recrystallizations from 30% aqueous ethanol. From the specific activity of $^{14}$C in the recovered semicarbazone, conversion of optically active, radiolabeled 1-hexanol to hexanal was estimated to be ≈50%.

A 10–60 μL portion of each radiolabeled 1-hexanol sample was added to 0.8 mL of unlabeled 1-hexanol, which was heated with 0.5 mL of phenyl isocyanate to yield the double-labeled *n*-hexyl *N*-phenylcarbamate, which was recrystallized 3–4 times from petroleum ether. The $^3$H/$^{14}$C ratios for the *n*-hexyl *N*-phenylcarbamates and the corresponding hexanal semicarbazones were determined by liquid scintillation counting in a Beckman Model 200 LSC.

**Chemical Oxidation of [1,5-$^3$H]-1-Hexanol.** Samples of tritiated 1-hexanol were diluted with [1-$^{14}$C]-1-hexanol, and each double-labeled sample divided into two portions. One portion was added to unlabeled 1-hexanol and converted to [1-$^{14}$C;1,5-$^3$H]-1-hexyl *N*-phenylcarbamate by reaction with phenyl isocyanate. A 100-μL sample of the other portion was oxidized with a solution of 250 mg of $KMnO_4$ in 1 mL of 11% aqueous NaOH, the excess $KMnO_4$ was destroyed by addition of $NaHSO_3$, and the reaction mixture was extracted with ether. After removal of the ether by distillation, the residue was dissolved in the minimum amount of water, the pH adjusted to 7, and silver hexanoate precipitated by titration with 1.25 M $AgNO_3$ solution. The precipitate was collected by filtration and then added to hydrochloric acid, where the acid was extracted with ether. The ether was allowed to evaporate in a scintillation vial, and the $^3$H/$^{14}$C ratio was determined by liquid scintillation counting and compared to the $^3$H/$^{14}$C ratio of the corresponding *n*-hexyl *N*-phenylcarbamate.

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