

Conformational Equilibria and Stereochemical Relationships among Carboxylic Acids¹

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The circular dichroism spectra of certain acyclic carboxylic acids have been surveyed in an effort to study their conformation in solution. In aqueous solution the spectra of many α -hydroxy acids were characterized by two overlapping ellipticity bands: a weak one in the 240-m μ spectral region and a more intense one of opposite sign near 210 m μ . At elevated temperatures or in solvents of low dielectric constant, the band of longer wavelength increased in intensity with an attendant decrease in the short-wavelength band. These properties are compatible with the presence of two structural species in equilibrium, and could be related to the contributions of specific rotational isomers of the acids. Conformations with the hydroxyl and carbonyl groups in an eclipsed alignment are designated as preferred. In contrast to the α -hydroxy acids, carboxylic acids with α -alkyl substituents generally showed only a single dichroic band centered near 210 m μ . In general, coplanarity of the carboxyl group and α substituents has been assumed, and interpretations in terms of the preferred orientation of substituents adjacent to the carboxyl group are suggested. The proposed population of rotational isomers was consistent with the observed effects of solvent polarity, temperature, polarity of substituents, and predicted steric interactions. An empirical rule relating the sign of ellipticity and the conformation of these classes of carboxylic acids has been applied.

Studies using simple α -hydroxy acids such as lactic, malic, and tartaric acid led to some of the early theories of optical activity³ and to assignments of absolute configurations for a variety of compounds. Recently, a number of investigators have reexamined the optical rotatory dispersion (ORD) and circular dichroism (CD) spectra of these compounds, and extended the measurements into the ultraviolet to include the region of absorption of the carboxyl group.⁴⁻¹¹ Although the carboxy sector rule¹² was shown to be suitable for the prediction of preferred alignments of many acids and esters of known conformations accompanied by restricted rotational possibilities,¹³⁻¹⁶ other attempts to correlate ORD and CD data with conformational aspects of carboxylic acids have been confined mainly to studies of carboxyl groups in more rigid systems such as lactones.^{12,17-19} For acyclic carboxylic acids in solution, conformational assignments were considered to be difficult because of the rotation about the single bond between the carboxyl group and adjacent carbon atom.²⁰

More recent studies have, however, uncovered some

fine points in the CD spectra of carboxylic acids that are potentially useful for conformational analysis. We have shown that uronic acid derivatives with equatorial substituents at C-4 of the pyranose ring exhibit two overlapping CD bands which originate from the $n \rightarrow \pi^*$ transition of the carboxyl group.²¹ We interpreted these results to indicate an equilibrium between two structural forms, but we were unable to distinguish between conformational and solvational effects. We also noted that overlapping bands are frequently encountered in the CD spectra of other carboxylic acids.²¹ ORD and CD spectra of lactic acid and related compounds have been reported in numerous⁴⁻¹⁰ studies, but Anand and Hargreaves²² were the first to report the presence of two bands. The weak, longer wavelength band near 245 m μ was assigned to an $n \rightarrow \pi^*$ transition, and the more intense, shorter wavelength band was ascribed to the $\pi \rightarrow \pi^*$ transition. More recently, Barth, *et al.*,²³ have shown this same spectral aspect for some derivatives of lactic acid but attributed both bands to the $n \rightarrow \pi^*$ transition.

Because the electronic transitions are relatively fast compared with the rate of molecular motion, CD offers some inherent advantages over other commonly used methods such as nmr to resolve and study rapidly interconverting rotational isomers in solution. In the present report, the CD spectra of a wide variety of carboxylic acids of known absolute configuration were examined to determine conformational relationships. The effects of solvent, temperature, and pH were studied in order to elucidate underlying factors that define the two-band systems. Some of the structural features common to all compounds eliciting a double ellipticity band have been revealed and the results are explained on the basis of equilibria between rotational isomers about the sp^3 - sp^2 carbon-carbon bond of these acids.

Results

The data presented in Table I summarize the position, magnitude, and sign of the ellipticity bands of selected

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TABLE I
 CIRCULAR DICHROISM OF SOME α -HYDROXY ACIDS AND RELATED COMPOUNDS IN AQUEOUS SOLUTION

Compd	Registry no.	Ellipticity maxima and minima					
		pH 2.5				pH 7.0	
		λ_1	$[\theta]_{\lambda_1}$	λ_2	$[\theta]_{\lambda_2}$	λ	$[\theta]_{\lambda}$
L(-)-Malic acid	97-67-6	246	-7	211	3100	207	6300
L(+)-Lactic acid	79-33-4	244	-18	210	2900	215	700
L(+)- α -Hydroxyisocaproic acid	13748-90-8	241	-21	208	4900	204	2200
L(-)- α -Hydroxy- β -methylvaleric acid	23264-19-9	239	-65	208	3500	b	b
L(+)-erythro-2,3-Dihydroxybutyric acid	23334-72-7	241	-82	209	3700	202	1200
L(+)-2,4-Dihydroxy-3,3-dimethylbutyric acid ^a	1112-32-9	240	-70	210	2700	b	b
L(-)-2-Ethoxysuccinic acid ^a	23264-21-3	245	-50	212	2700	210	7700
Dimethyl L(-)-malate ^c	617-55-0	242	-21	211	2600
Ethyl L(+)-lactate ^c	7699-00-5	238	-48	208	4200
L(-)-Chlorosuccinic acid	4198-33-8	222	-2000	200	1700	240	-130
						201	7900

^a Measurements were made on the enantiomers. ^b Multiple overlapping bands were observed for these compounds at neutral pH. ^c Measurements were made at pH 2.5 and 7.0.

 TABLE II
 CIRCULAR DICHROISM OF SOME CARBOXYLIC ACIDS EXHIBITING A SINGLE ELLIPTICITY BAND ABOVE 200 m μ

Compd	Registry no.	pH 2.5		pH 7.0	
		λ	$[\theta]$	λ	$[\theta]$
L(+)-2-Methylbutyric acid	1730-91-2	210	650	195	-1700 ^a
D(+)-Ethylsuccinic acid	4074-24-2	207	3100	208 ^b	-2300
D(+)-Butylsuccinic acid	4254-59-5	207	3200	209 ^b	-2600
D(+)-Isopropylsuccinic acid	1187-70-8	207	3600	209 ^b	-2600
D(+)-Cyclohexylsuccinic acid	3975-92-6	209	5300	206 ^b	-2400
D(+)-Monodeuteriosuccinic acid	10013-03-3	208	150	213 ^b	98
D(-)-Isopropylglutaric acid	3972-39-2	210	2800

^a No ellipticity extrema were observed. ^b These bands were very broad and the locations of the extrema are approximate.

α -hydroxy acids and related compounds in aqueous solution. At pH 2.5 the optically active absorption band near 210 m μ is common to all of the α -hydroxy acids; this band previously has been attributed to the $n \rightarrow \pi^*$ transition of the carboxyl group.^{21,22} In addition, at longer wavelengths, most of the acids also exhibit another ellipticity band of opposite sign and low intensity. The magnitudes of both dichroic bands are independent of concentration (from 0.001 to 1.0 M of acid) and the CD spectra have similar band shapes and energies. For the α -hydroxy acids, the intensity of the 210-m μ band always exceeded that of the long wavelength band by a factor of 30-450. Large background rotations obscured the long-wavelength Cotton effects thus to be expected in the ORD curves of these compounds. Also bands corresponding to these are not easily resolved in their ultraviolet absorption spectra, so that only weak bands near 205 m μ ($\epsilon \cong 60$, per carboxyl group),²⁴ with no obvious vibrational fine structure, are observed in aqueous solution. Because of their opposite signs, however, the two bands are clearly defined in CD spectra.

The CD of carboxylic acids with α -alkyl substituents in place of the hydroxyl groups are shown in Table II. A single ellipticity band near 210 m μ characterizes the CD spectra of these compounds, and in these instances no longer wavelength band is discernible. The differences in the rotational strengths among these compounds, especially the alkyl succinic acids, are consistent with the observations using ORD.⁵ It is also significant that the signs of the 210-m μ ellipticity bands of the alkyl succinic acids in the D configuration are the same as those of the corresponding hydroxy, ethoxy, and chlorosuccinic acids in the L configuration.

Ionization of the carboxyl group induces substantial modifications in the ellipticity associated with it. Thus the ionization of the α -hydroxy acids generally results in the disappearance of the longer wavelength band (Table I). In addition, the sign of the ellipticity of most of the α -alkyl acids is inverted by the ionization of the carboxyl group (Table II). In contrast with the results obtained for the protonated forms, substituted succinic acids in the ionized form all display ellipticities of the same order of magnitude. Ionization of these compounds may be conveniently monitored by CD, and typical titration curves are shown in Figure 1. The known pK values of ethylsuccinic acid (pK₁ = 4.0, pK₂ = 5.7) and 2-methylbutyric acid (pK₁ = 4.8) correspond to the observed inflections in the curves. An intramolecular hydrogen bond has been postulated previously for the monoanion of substituted succinic acids,^{25,26} and in this context the major CD changes accompany the ionization of the second carboxyl group.

Kresheck²⁷ has recently ascribed the change in sign of the CD band accompanying the ionization of ascorbic acid to conformational changes. Such an explanation, however, cannot be applied for the CD changes induced by the ionization of the carboxyl group of the compounds in the present study. The ionized carboxyl group is inherently different from, and more symmetrical than, the protonated form. Changes in characteristics and sign of the ellipticity bands are not unexpected, since the entire asymmetric system is altered.

The effect of solvent on the CD of these compounds may be seen from the spectra shown in Figure 2. The same pattern was observed for most of the other α -hy-

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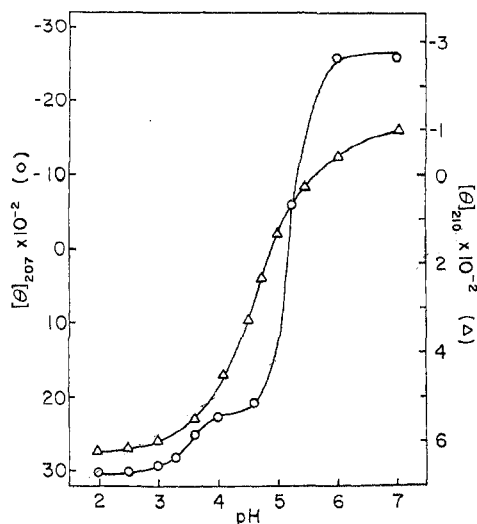


Figure 1.—Titration curves for D-ethylsuccinic acid (O) and L-2-methylbutyric acid (Δ). Measurements were made at the wavelength of maximum ellipticity of the acids at pH 2.0.

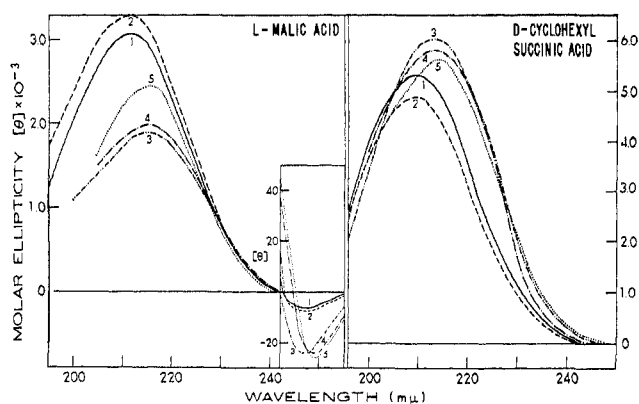


Figure 2.—Circular dichroism spectra of L-malic acid and D-cyclohexylsuccinic acid in various solvents. The solvents indicated are: 1, water; 2, trifluoroethanol; 3, acetonitrile; 4, heptane-dioxane (4:1); 5, dioxane.

droxy acids and esters and α -alkyl acids studied, and the representative compounds shown were selected to illustrate the types of effects observed. In general, an increase in the ratio of the long-wavelength ellipticity magnitude compared with that of the short-wavelength ellipticity is observed for the α -hydroxy acids in the nonaqueous solvents. The solvent effect is more pronounced for the α -ethoxy acid (not shown) and these effects are similar to, but not of comparable magnitude with, those observed previously for the uronic acids.²¹ The red shift of the 210-m μ CD band in aprotic solvents is characteristic of an $n \rightarrow \pi^*$ transition.^{28,29} CD is not as reliable as ultraviolet absorption spectra to define these frequency shifts, however, since overlapping bands of opposite sign often distort the spectra. For these carboxylic acids it is likely that a $\pi \rightarrow \pi^*$ transition overlaps the shorter wavelength end.³⁰ Indeed, the beginning of an intense negative

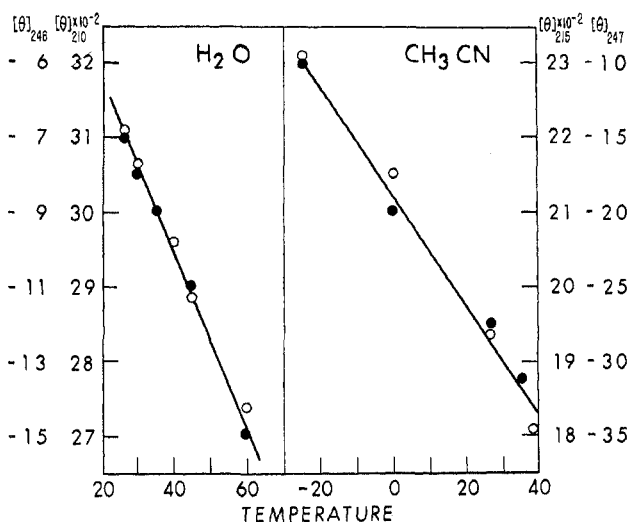


Figure 3.—Temperature-dependence studies; molar ellipticities of L-malic acid in water and acetonitrile. The open circles represent the longer wavelength band and the dark circles represent the short-wavelength band in both solvents.

band below 190 m μ has been observed in the CD spectrum of L-(+)-lactic acid in water, and some residual ellipticity above 210 m μ is obtained in concentrated H₂SO₄.

The temperature-dependence data for malic acid (Figure 3) illustrate typical effects observed with the hydroxy acids. For all of the α -hydroxy acids in Table I, an increase in temperature induces an increase in the intensity of the long-wavelength band near 240 m μ and a concomitant decrease in the band near 210 m μ , and a decrease in temperature induces the opposite effect. Similar results were obtained with lactic acid derivatives.²³ This behavior implies that the two bands are closely interrelated and are probably associated with a single electronic transition. By extrapolation of the data in Figure 3 to a zero value of the longer wavelength band, an estimated value of the intensity of the isolated short-wavelength band may be obtained. Based on the assumption that each band represents a single structural form, approximate equilibrium constants at the various temperatures were calculated and approximate ΔG°_{25} values were obtained for L-malic acid. Typical free-energy differences between the two forms (240 \rightleftharpoons 210 m μ) were $\Delta G^\circ_{25} = -1.1$ kcal/mol in water and $\Delta G^\circ_{25} = -0.8$ kcal/mol in acetonitrile. For the α -alkyl acids, the 210-m μ band generally diminishes in intensity at the higher temperatures. For example, $[\theta]_{218}$ of D-cyclohexylsuccinic acid in acetonitrile is +7500° at -10° and +5600° at 50°.

The longer wavelength band was absent in the CD of some N-methyl- α -amino acids such as N-methyl-L-alanine ($[\theta]_{201} = +3200^\circ$), N-methyl-L-glutamic acid ($[\theta]_{202} = +4500^\circ$), and N-methyl-L-proline ($[\theta]_{208} = +1800^\circ$), and we were unable to detect the 248-m μ band that was previously reported for L-alanine at neutral pH.³¹ Several complex α -hydroxy acids such as tartaric,³² gluconic, and α -hydroxyglutaric acid

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were atypical and also showed only single bands. The structural features of these classes of compounds as well as a detailed account of the α -halo acids will be presented in future reports. It may be noted that 3-methyladipic acid, because its asymmetric center is removed from the direct proximity of the carboxyl chromophore, exhibits a weak ellipticity band ($[\Theta]_{209} = +650$). Also, in contrast to the great majority of α -hydroxy acids studied, β -hydroxybutyric acid exhibited a single CD band at 210 $m\mu$.

Discussion

Most of the α -hydroxy mono- and dicarboxylic acids exhibited similar CD spectra with an optically active $n \rightarrow \pi^*$ transition near 210 $m\mu$ and an additional band at longer wavelengths. It is particularly difficult to rationalize the presence of an $n \rightarrow \pi^*$ transition for the carboxyl group at the long wavelengths of 239–246 $m\mu$, but the overall appearance of the CD curves may be deceiving. The non-Gaussian nature and low intensity of the long-wavelength band suggest an appreciable overlap with the more intense 210- $m\mu$ band. Therefore the apparent location of this long-wavelength band most probably does not define the actual spectral region of the transition. It has been shown that the superimposition of overlapping bands of opposite sign and with maxima separated by 1–20 $m\mu$ yields a curve which appears to have two bands separated by 28–32 $m\mu$.³³ Thus the observed frequency shifts of overlapping bands depend on their relative magnitudes and extent of overlap, but the actual frequency difference is generally much less than the observed value. For uronic acids we have shown that the energy differences between the two transitions are indeed much less than the measured values.²¹ For the α -hydroxy acids, the observed spectral separations of ca. 35 $m\mu$ are also exaggerated, and both extrema are probably centered about the $n \rightarrow \pi^*$ transition of the carboxyl group.

Certain possible explanations for the presence of the two overlapping CD bands may be rejected readily for the acids studied here. Monomer-dimer equilibria have often been described for carboxylic acids^{34,35} and, because of the coupled transition moments, the optical activity of carboxyl groups in some cyclic systems is substantially different from that of the open-chain forms.^{36,37} An interpretation based on the presence of associated forms may, however, be ruled out in the present study, since no concentration dependence was observed and the acids and esters both gave similar spectra. An intramolecular hydrogen bond between the α -hydroxyl and carbonyl group to give a five-membered ring is unfavored and furthermore need not be considered, since the α -alkoxy acids also show the two bands. In addition, it is difficult to rationalize the results in terms of hydrogen bonding to solvents or an equilibrium between solvated and hypothetical non-

solvated forms.³⁸ If the long-wavelength band of the α -hydroxy acids is indeed a result of the presence of a nonsolvated species, the α -alkyl acids would also be expected to exhibit this long-wavelength band in hydrocarbon or nonpolar solvents. This effect, however, was not observed, and indeed the entire concept of a nonsolvated form has recently been considered to be unsatisfactory as an explanation of multiple CD bands.³⁹

For carboxylic acids and esters, the interaction of the nonbonding electrons of the oxygen atom attached to the carbonyl group with the π orbitals of the carbonyl has been postulated to raise the energy of the antibonding π^* orbital and cause the split of the bonding π orbital into two new orbitals.⁴⁰ An appreciable overlap of the lowest energy $\pi \rightarrow \pi^*$ band with the $n \rightarrow \pi^*$ band is reported in the present study. There are no indications, however, that the $\pi \rightarrow \pi^*$ transition, or some other previously undefined transition moments, account for the long-wavelength band near 240 $m\mu$. On the contrary, the observation that experimental conditions that induce an increase in the 240- $m\mu$ band always cause an attendant decrease in the other band suggests that both bands are associated with the same electronic transition. In all probability the latter is the $n \rightarrow \pi^*$ transition.

The evidence presented here is entirely compatible with an established equilibrium between two structural or conformational forms of these α -hydroxy (alkoxy or halo) acids in solution. We consider an interpretation on the basis of conformation to be easily justifiable and the most reasonable. It is well known that the intensity and sign of an ellipticity band are directly contingent upon the alignment of substituents with respect to the transition dipole moment.^{37,41} Since all of the acids studied have the identical carboxyl chromophore, the three-dimensional structure of the carboxyl group and its adjacent substituents must be considered in any interpretation of the CD spectra.⁴² The atoms in the C_α -COOH group have been shown to be coplanar or very nearly coplanar on the basis of X-ray diffraction studies of carboxylic acids.⁴³ Also, staggered conformations are ordinarily favored on an energetic basis, and according to the "bent bond" or "banana type" concept of the double bond,⁴⁴ structures with α substituents eclipsing the double bond would be preferred (dihedral angles of less than 10° are generally found for these carboxylic acids^{45,46}). For an α -hydroxy acid or related compound in the L configuration, a threefold barrier to rotation about the sp^3 - sp^2 carbon-carbon single bond may therefore be assumed and, as a first

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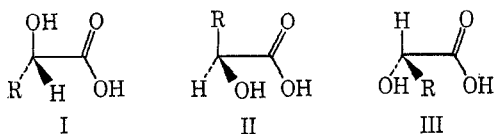
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approximation, the preferred alignment of substituents about the carboxyl group may be represented as follows.



On the basis of nmr studies of α -halo aldehydes and ketones⁴⁷ and infrared spectra of α -halo esters,⁴⁸ structures with eclipsed halo substituents and carbonyls have been suggested as the preferred conformations. The present results with the α -hydroxy acids may be interpreted in terms of structure I as the favored rotational isomer, and also as the isomer responsible for the predominant CD band centered near 210 $m\mu$. Structure III is the least favored, and it is therefore suggested that structure II generates the longer wavelength band, whereas the contribution of structure III is probably insignificant and cannot presently be assessed.

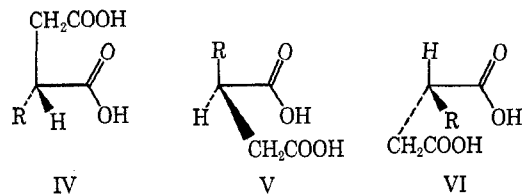
The above structural assignments of rotational isomers are consistent with the results of the temperature- and solvent-dependency studies. An increase in temperature should increase the population of the less favored isomer. This conclusion is compatible with the observed increased intensity of the longer wavelength CD band and decreased intensity of the shorter wavelength band as the temperature is increased (Figure 3). Lowering the temperature induces opposite effects. In addition, an increase in the ratio of the intensity of the shorter wavelength band, compared with the magnitude of ellipticity at the long-wavelength extremum, generally accompanies an increase in solvent polarity (Figure 2). These results, consistent with a preponderance of the more polar rotamer I in the more polar solvents, also shows that the shorter wavelength band is associated with rotamer I. Accordingly, in solvents of lower polarity, the population of rotamers II or III are increased.

Further verification of the foregoing structural interpretations may be deduced from our earlier studies of uronic acids.²¹ For these compounds, adjacent substituents limit the rotational possibilities of the carboxyl group. If one assumes that structure I is the favored form for carboxylic acids of this type, the ring oxygen and carbonyl of the uronic acids would be in an eclipsed conformation. For galacturonic acid derivatives this is indeed the preponderant structure, and only a single ellipticity band was observed.²¹ On the other hand, this carboxyl conformation for glucuronic and mannuronic acid derivatives gives rise to a nonbonded interaction with the adjacent equatorial hydroxyl group at C-4. It is reasonable that other conformations may prevail for the latter compounds, and indeed multiple CD bands have been observed.

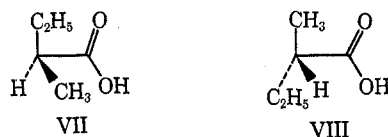
The wavelength differences between the two ellipticity bands may be small, and since the electronic interactions have not been elucidated in the present study, reasons for these differences cannot be established. It has been shown, however, that the non-

bonding distances between a carbonyl of a carboxylic acid and an eclipsed α -hydroxyl group is *ca.* 0.1–0.3 Å shorter than the corresponding distance between a carbonyl and alkyl substituent.⁴⁶ Conceivably, therefore, a slight overlap with a low-energy empty orbital of the hydroxyl, ethoxyl, or halo group on the α carbon stabilizes the antibonding orbital of the carbonyl, lowers the energy of the excited state, and gives the longer wavelength band. The energy levels of rotamers II and III would be degenerate if this were the case. In structure I this stabilization is also possible with the nonbonding electrons on the carbonyl and no spectral shift is expected. A similar interaction has been proposed for axial α -hydroxy, alkoxy, and halo ketones.^{49,50} Although solvational effects cannot account for the gross character of the CD spectra, it is also possible that the mode of solvent binding to the chromophore may differ for these conformers and thereby account for the frequency shifts.

Rotational isomerism for carboxylic acids with α -alkyl substituents are more difficult to elucidate, as only a single CD band can be resolved for these compounds. This does not preclude the possibility that rotational isomers exist, since a wavelength shift for rotamers would not be expected if the proposed interactions with the excited states are required. Indeed, with the alkyl-substituted succinic acids an increase in intensity of the 210- $m\mu$ CD band is observed as the size of the α substituent is increased (Table II) or as the temperature is lowered. This probably indicates a greater preponderance of the favored rotational isomer as the size of the alkyl substituent is increased or the temperature lowered. Structures IV, V, and VI may



be considered to represent the three preferred rotamers of the D-alkylsuccinic acids; IV is the preferred isomer with VI as the least stable. As the bulkiness of the R group increases, structure V may become even less favored because of nonbonded interactions with the carbonyl group, and structure IV would thus be accommodated to an even greater extent. The increase in magnitude of ellipticity in the order R = ethyl or butyl > isopropyl > cyclohexyl is consistent with this interpretation. The energy difference between the two favored isomers of L-(+)-2-methylbutyric acid (VII and VIII) is probably not appreciable, and rapid



interconversion between the two forms (with a slight preference for structure VIII) may be anticipated. These two forms should contribute rotational strengths of opposite sign, and thus it is not surprising that the resultant low-intensity CD band is observed (Table II).

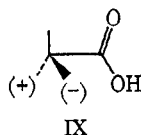
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Several limitations and approximations in the approach suggested here are recognized. Thus any partial double bond character of both carbon-oxygen bonds of the carboxyl group are not taken into consideration in structures I-VIII. Also the possible deviations from the staggered conformations shown to give other rotational isomers are not considered. In addition, the optically active transitions of carboxyl groups are difficult to compare with those of the well-characterized keto groups because of the inherent electronic differences induced by the additional oxygen atom bonded directly to the carbonyl. Rigorous theoretical relationships are difficult to establish. It is significant, however, that some of the preferred conformations suggested here are consistent with the conformational assignments for analogous acids in the crystalline state.^{45,46} In the assumed structures represented in I-VIII, the substituent eclipsing the double bond lies in a nodal plane of the nonbonding electrons on the carbonyl oxygen and therefore may not contribute appreciably to the observed optical activity associated with the $n \rightarrow \pi^*$ transition. On the basis of this assumption, a simple empirical rule may be proposed for the carboxylic acids (or esters) viewed as shown in the structure IX. Substituents projecting in the direction



toward the viewer contribute negative ellipticity at 210 $m\mu$ and substituents projecting away contribute positive ellipticity. This hypothesis explains why the alkyl succinic acids in the D configuration and favoring conformer IV have the same 210- $m\mu$ ellipticity signs as the α -hydroxy-, alkoxy-, and halosuccinic acids in the L configuration, which exist mainly as rotamer I.

Experimental Section

Solvents.—The dioxane (Fisher Scientific Co.) and trifluoroethanol (Halocarbon, Inc.) were dried and distilled prior to use, and the acetonitrile and heptane (Eastman) were spectro grade and employed without further purification. The pH of each aqueous solution was adjusted by the addition of small amounts of HCl or NaOH. Identical CD spectra were obtained when low ionic strength buffers were used to adjust the pH.

Carboxylic Acids.—Samples of L-(−)-malic, L-(+)-lactic, L-(+)- α -hydroxyisocaproic, L-(+)- α -hydroxyglutaric, and L-(−)- α -hydroxy- β -methylvaleric acids were obtained from Sigma Chemical Co., 3-methyladipic acid from Aldrich, and the N-methyl amino acids from Cyclo Chemical Co. We are most grateful to Dr. A. Fredga and Dr. W. Klyne for supplying the samples of the D-(−)-ethyl-, butyl-, isopropyl-, cyclohexyl-, and ethoxysuccinic acids. We also thank Dr. A. Hayashi for the L-(+)-*erythro*-2,3-dihydroxybutyric acid, and Dr. S. Kumar for the L-(+)- β -hydroxybutyric acid.

L-(−)-Chlorosuccinic and D-(+)-monodeuteriosuccinic acids were prepared by us for earlier studies.^{51,52} Pantoyl lactone (K & K Chemical Co.) was hydrolyzed to give the pantoic acid. Dimethyl L-(−)-malate (bp 240–245°, n_D^{20} 1.442, d_4^{20} 1.23, $[\alpha]_D -16^\circ$) was prepared by esterification of the L-(−)-malic acid in methanol and H_2SO_4 , and L-(+)-2-methylbutyric acid (bp 175°, n_D^{20} 1.410, d_4^{20} 0.94, $[\alpha]_D +21^\circ$) was prepared by alkaline permanganate oxidation of L-(−)-isoamyl alcohol (Aldrich). The optical purity of each compound was checked by measurement of its $[\alpha]_D$ value and comparison with values reported in the literature.

Methods.—CD measurements were made using a Cary Model 60 spectropolarimeter with a 6001 CD accessory. The instrument records the angle of ellipticity Θ in degrees, and the molecular ellipticity $[\Theta]$ was calculated using the relationship $[\Theta] = \Theta M / 10lc$ (deg cm^2 /decimol), where M is the molecular weight, c is the concentration (g/ml), and l is the path length in centimeters. The slit width of the polarimeter was programmed to give half-band widths of less than 1.5 $m\mu$ through the entire spectral range, and all molar ellipticities were obtained using solutions with absorption values of less than 2.0. Strain-free cylindrical cells with quartz windows of 20–22 mm diameter and path lengths of 0.005, 0.02, 0.1, 1.0, and 5.0 cm were used; the shorter path length cells were employed mainly for the concentration-dependence studies and the studies in dioxane. A jacketed 1-cm cell with quartz windows of 14-mm diameter was employed for the temperature-dependence studies. All other measurements were made at 27°, the temperature of the cell compartment. Corrections for density or refractive index changes as a function of temperature were not made, since these factors were relatively small compared with changes in ellipticity.

The ellipticity values at the long-wavelength extrema were obtained from at least two independent measurements. A typical result may be represented as follows: for a 5% malic acid solution in water ($\Theta_{246} = -6.9$ deg cm^2 /decimol) in a 1-cm cell, the observed ellipticity value, Θ , was 0.026°; the noise level of the instrument under these conditions was *ca.* 0.001° and the reproducibility was better than 0.002°.

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