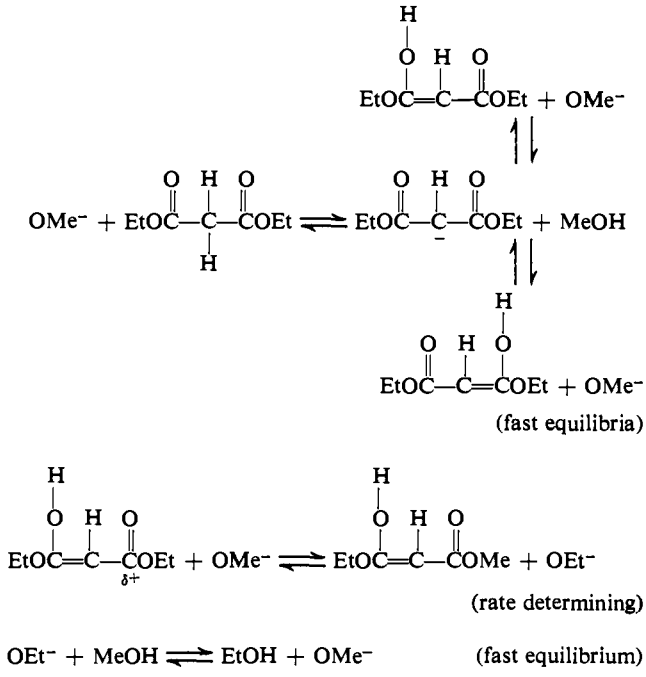
philic attack by methoxide could be the enol molecule, provided the shifting of electrons from the α carbon to enolize one acyl carbon decreases the electron density at the other acyl carbon atom. This would render the nonenolized acyl carbon more electropositive and, hence, more susceptible to nucleophilic attack than any acyl carbon on the keto molecule or the enolate ion. In any event, the enolate ion would not be expected to react appreciably with methoxide because of its charge and limited low concentration. However, enolate would likely participate as an intermediate in fast equilibria with the enol and keto molecular forms. With the molar ratio of methanol to methoxide at least 15,000:1, any equilibria involving enolate, enol, and keto would be shifted toward the molecular species. This means virtually all the malonate could be converted to enol since methoxide is regenerated when enolate becomes enol. The rate of methanolysis should be enhanced with diethyl ethylmalonate because alkyl groups substituted on the α carbon increase the enolization slightly.[16] However, steric effects from the α-substituted ethyl group[17] outweigh any enhancement from increased enolization. The reaction proceeds very slowly with diethyl diethylmalonate because enolization is eliminated and steric hindrance is increased.[18]

A mechanism consistent with the rate law and with enol molecules as the favored reactive specie is summarized in Scheme I. For the formation of dimethyl malonate, the series of reactions is repeated with methyl ethyl malonate in place of diethyl malonate.

Scheme I



Acknowledgment. The authors wish to express their gratitude to Dr. Paul Langford and Dr. Charles Richmond for their helpful suggestions and criticisms during the course of this study.

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Absorption, Rotatory Dispersion, and Circular Dichroism Studies on Some Hydroxy and Amino Acids[1]

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Contribution from the Chemistry Division, Argonne National Laboratory, Argonne, Illinois. *Received February 9, 1967*

Abstract: Comparative data are presented on the absorption, rotatory dispersion, and circular dichroism spectra of lactic, malic, and tartaric acids, as well as alanine, serine, valine, leucine, aspartic and glutamic acids, ornithine, lysine, arginine, proline, and asparagine. Effects are found related to molecular structure and to the state of ionization of the species, with the fully protonated forms as reference states. The influence of vibrational fine structure on the absorption–CD relation is shown, and some examples of the utility of comparing the three spectra directly are pointed out.

In an optically active molecule, all electronic transitions show a difference in absorption coefficient for right and left circularly polarized light, the "circular dichroism." In such a molecule an atomic grouping with a fairly characteristic absorption (a "chromophore") will have associated with it a circular dichroism (CD) with characteristic relation to its absorption spectrum for unpolarized light. The rotatory dispersion (ORD) for some compounds bearing the carboxyl chromophore, namely tartaric acid and the amino acids, recently has been reanalyzed in terms of the Drude equation.[2,3] The Drude parameters are in principle related to the parameters which characterize circular dichroism. One purpose of this investigation is to compare the findings of direct observations in the absorption region of these compounds with the parametric behavior of the Drude analyses in order to evaluate the latter. A more general purpose is to seek

(1) Based on work performed under the auspices of the U. S. Atomic Energy Commission.
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Table I. Absorption Spectral, ORD, and CD Parameters of Amino Acid Solutions

a. Acid Solution [(+)R-COOH]

Form	λ_a	ϵ_λ	λ_{CD}	$\Delta\epsilon$	$[M]_{max}$	λ_m	λ_0	$\Delta\epsilon/\epsilon$ ($\times 10^2$)	$(A - B)^a$
(D) H_2 tart	212	225	216	−4.4	−6450	228	219	1.95	5.7
(L) H lact	210	70	210	+0.80	+1250	224	213	1.14	...
H_2 mala	208	115	214	0.88	+1400	226.5	215.3	0.77	...
Alanine·H^+	207	45	209	1.04	+1644	224	212	2.3	3.8
Serine·H^+	207	55	209	1.14	+1900	224	212	2.1	3.3
Valine·H^+	206	68	209	1.52	+2880	224	210	2.2	
Leucine·H^+	206	95	209	1.85	+3200	224	210	1.95	3.3
Aspartic·H^+	(208)	(80)	208	0.87	+1625	224	209	(1.0)	7.6
Glutamic·H^+			209	1.38	+2700	224+	209+	...	9.0
Ornithine·$2H^+$			210	1.26	+2400	225	210	...	7.9
Lysine·$2H^+$	209	400	210	1.42	+2800	225	210	0.36	7.1
Arginine·$2H^+$	...	(260)	210	1.60	+3190	225	209	(0.61)	...
Proline·H^+	(207)	52	209	1.0	+517	226	218	1.9	−16.5
Asparagine·H^+	(209)	<210	209	0.72	+1500	225	(207)	>0.34	

b. [(+)R-COO^-] Water Solution

Form	λ_{CD}	$\Delta\epsilon$	$[M]_{max}$	λ_m	λ_0	$(A - B)^a$
H tart$^-$	213	−3.55	−4600	227	217	
tart^{2-}	209.5	−2.2	+4500	204	212	+13.65
			−3000	220		
lact$^-$	215	+0.18	(−1810)	(201)	...	
H mala$^-$	208	1.48	+1680	224	213	
mala^{2-}	207	2.12	+2910	220	210	
Alanine ±	205	0.74	+795	218	206	+0.02
Serine ±	204	0.78	1100	216	204	−2.71
Valine ±	204	0.78	+1520	214	201	
Leucine ±	~200	1.51	1700	211	203	−3.76
Aspartic ±	199	1.02	1080	216	204	+1.20
Glutamic ±	203	1.02	1600	215	204	+3.12
H^+ ornithine ±	200	0.89	1400	215	203	4.18
H^+ lysine ±	202	0.81	1740	216	203	3.67
H^+ arginine ±	≤207	(1.08)	2520	212	203	
Proline ±	214	0.29	(−4300)	(201)	...	−26.4
Asparagine ±						

c. [R−COO^-] Alkaline Solution

Form	λ_{CD}	$\Delta\epsilon$	$[M]_{max}$	λ_m	λ_0	$(A - B)^a$
(Alanine)$^-$	≤212	≥0.38	450	224	215	1.29
(Serine)$^-$						
(Leucine)$^-$	≤213	≥0.83	1650	223	211	2.13
H^+(Aspartic)$^{2-}$	203	1.15	650	218	210	−6.15
(Aspartic)$^{2-}$	201	1.80	2120	218	207	−1.95
H^+(Glutamic)$^{2-}$	202	0.94	1170	216	206	−2.10
(Glutamic)$^{2-}$	210	0.64	1340	222	210	+2.87
H^+(Ornithine)$^-$	213	0.57	1150	225	214	3.44
(Ornithine)$^-$	216	0.49	1150	225	213	3.65
H^+(Lysine)$^-$	214	0.66	1325	225	213	3.59
(Lysine)$^-$	≤212	≥0.86	1320	224	214	3.46
H^+(Arginine)$^-$	211	0.66	1500	224	210	
(Arginine)$^-$	≤216	≥0.61	1400	223	<215	
(Proline)$^-$	~216	0.11	(−3300)	(<214)	...	−29.7
(Valine)$^-$				224	~210	

[a] From Drude equation, $[\alpha]_\lambda = A/(\lambda^2 - \lambda_a^2) - B/(\lambda^2 - \lambda_b^2)$, and ref 2, 3, and 10.

information on the relations between the molecular structures and the optical rotatory and circular dichroic characteristics of their constituent chromophores.

Experimental Section

The ORD, CD, and absorption spectra were plotted with the Durrum-Jasco ORD/UV-5 optical rotatory dispersion recorder with a circular dichroism attachment. All three spectra were obtained on the same sample and chart, without alteration of the wavelength or recording chart settings, so that direct wavelength comparisons could be made. Chart settings were routinely verified with a holmium wavelength standard. The light source was a 450-w Osram xenon arc lamp. The cell lengths used were 1.0 and 5.0 mm. Curves were traced from 250 mμ down to the absorption limit, which ranged between 205 and 195 mμ. Very slow scan speeds were used, and CD and ORD curves were retraced at least once, where there was significant noise. The CD and ORD measurements were made on solutions with optical density generally less than 3 in the wavelength range of significance. The CD scale calibration was monitored with a standard camphorsulfonic acid solution, for which substance the $\Delta\epsilon$ value at 289 mμ was taken to be 2.09.

Nutritional Biochemical Corp. amino acids were used as supplied.[3] D-Tartaric acid (twice purified by ether extraction[2]), Fisher certified reagent sodium tartrate, Pfanstiehl Laboratories CP *l*-malic acid, and Baker analyzed reagent lactic acid were the other substances investigated. The desired pH was obtained by adding Acculute-HCl or Acculute-NaOH solution, as appropriate. Concentrations of carboxylic acid were generally 0.01 *F*; in the cases of malic and lactic acids, 0.02 *F* solutions were also used. The pH was measured to a precision of ±0.01 unit with a Radiometer Model 4 pH meter.

Dilutions of lactic acid made directly into HCl showed $[\alpha]_D$ −18°, indicating the probable presence of lactide. Measurements

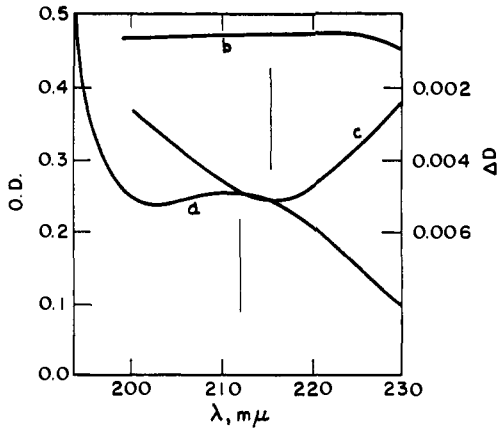

Figure 1. CD and absorption curves, 0.01 *F* D-tartaric acid in 0.25 *F* HCl (1.00-mm path): (a) sample absorption; (b) CD blank (0.25 *F* HCl, ±0.010 ΔD scale); (c) sample CD. (Vertical bars denote wavelengths of extrema.)

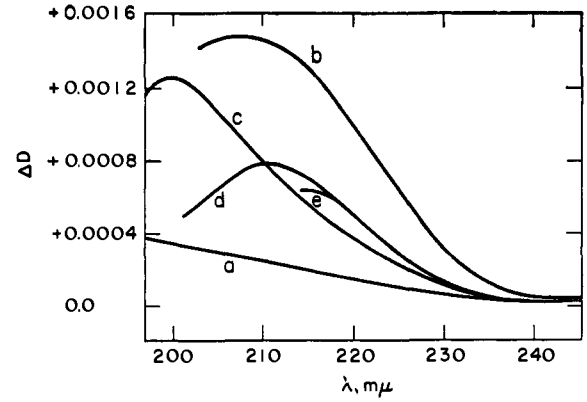

Figure 2. CD curves for various protonation states of L-ornithine (1.00-mm path): (a) CD blank (H_2O); (b) in 0.25 *F* HCl (ornithine·$2H^+$); (c) in H_2O (ornithine·H^+); (d) in 0.01 *F* NaOH (ornithine zwitterion); (e) in 0.25 *F* NaOH (ornithine anion).

reported were therefore made with solutions prepared by first adding NaOH to the lactic acid and then adjusting the solution to the desired acidity. This gave a $[\alpha]_D$ value in HCl of +3.8°, corresponding to the literature value. No significant difference was observed between the two types of solution, in the 200–500-mμ spectral region.

A summary of the parameters of the data obtained is given in Table I. Some characteristic or particularly significant traces are reproduced in Figures 1–3.

Discussion

There exist certain relationships among some of the quantities in Table I which, when recognized, minimize the apparent gaps in the material and make use of the data more effective.

For example, the absorption peak of the carboxyl group can be seen directly only in the fully protonated (acid) form, as on ionization it becomes masked by the tail of more intense absorptions at shorter wavelength. However, its position can be inferred from that of the associated circular dichroism, which is seen in all but a few cases. It must be pointed out that generally the peak of the CD in the acid solutions falls definitely at longer wavelength than the absorption maximum (*e.g.*, Figure 1 for tartaric acid), rather than at the same wavelength. This difference implies that there is a distinct vibrational fine structure in the transitions. A clear analog is in the camphor carbonyl-group absorption. In effectively nonpolar solvents, the absorption spectrum, ORD, and CD all show components whose resolution is lost in polar solvents.[4–6] There is a 1:1 correspondence between the components of the absorption spectrum and the CD spectrum, but the intensity progression is not the same in the two series.[7] Consequently, in polar solvents in which the resolution of the components is lost, the envelope of the CD spectrum has its maximum shifted to longer wavelengths than the maximum of the absorption curve. (See also older data on hydrocarbon solutions with poor resolution.[8])

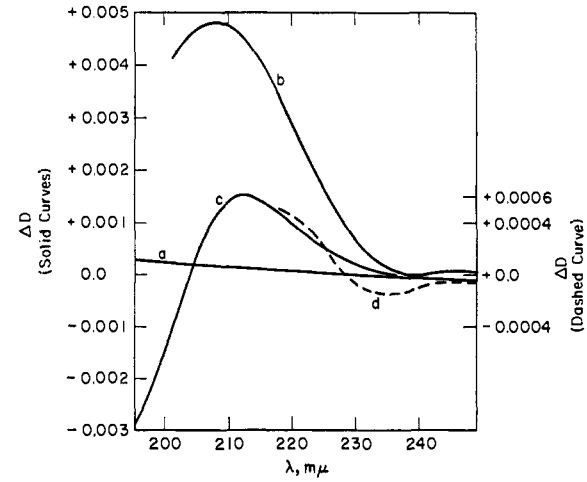

Figure 3. CD curves for various protonation states of L-proline (5.00-mm path): (a) CD blank (H_2O); (b) in 0.25 *F* HCl (proline·H^+); (c) in H_2O (proline zwitterion); (d) in 0.25 *F* NaOH (proline anion).

An overlapping, very strong peak of the opposite sign may cause an apparent shift in λ_{CD}, in some cases, clearly illustrated by the proline zwitterion (Figure 3). A useful check against this possibility is furnished by a comparison of the wavelength for zero rotation in the ORD curve (λ_0) with λ_{CD}; an abnormally large difference will be seen, as the ORD is more sensitive than the CD to such factors.

One can also infer the width of a masked absorption peak from the other parameters. The wavelength of the Cotton extremum, λ_m, occurs at about the inflection of the CD peak. The difference, $\lambda_m - \lambda_{CD}$, gives a measure of the CD peak width, and, inferentially, that of the absorption peak. It is apparently characteristic, for example, that the carboxyl width measure is greater for the amino acids (15–16 mμ) than for the hydroxy acids (12–14 mμ).

Inferences of the effective intensity for the carboxylate absorption have a definite upper limit (that due to the masking absorption), but within this one may tentatively assume that it remains essentially constantly proportional to the CD intensity (*i.e.*, that $\Delta\epsilon/\epsilon$ is constant). Finally, in the alkaline solutions, for some of which excessive absorption makes it impractical to observe

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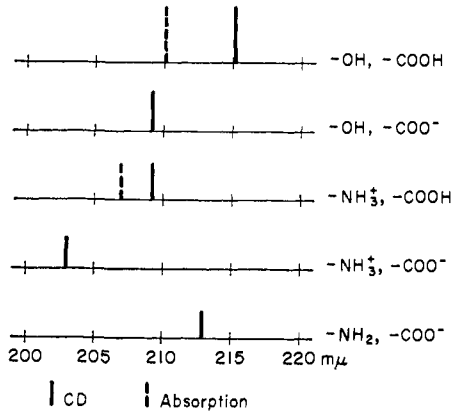

Figure 4. Positions of CD and absorption peak maxima for carboxyl groups of hydroxy and amino acids, as a function of ionization state. Heights of bars give qualitative indication of relative intensities, state to state. Relations are read "from the bottom up;" *i.e.*, the $-NH_2$, $-COO^-$ relation holds even if additional $-NH_3^+$, groups are present; the $-NH_3^+$, $-COO^-$ relation even if additional -COOH groups are present, etc.

even the CD directly, its wavelength variations may be inferred from those of λ_0 and λ_m, in parallel with those for which direct observation is possible.

It is possible to extract the following over-all relations of the absorption and circular dichroism peak wavelengths (see also graphical summary, Figure 4).

(a) The substitution of an -OH group by an $-NH_3^+$ group moves the absorption and associated polarized light spectra to shorter wavelength.

(b) Ionization of the first carboxyl group (in these cases, it is also the one at the asymmetric center)[9] shifts the carboxyl absorption to lower wavelengths, and more for the amino acids than for the hydroxy acids. The apparent exceptions (lactic acid and proline, Figure 3) result from the displacement of the net CD by intense circular dichroisms of the opposite sign which are centered at wavelengths below 200 mμ. The presence of a second carboxyl, whether in its ionized or associated form, seems to have a secondary effect on the observed carboxyl CD.

(c) Removal of a proton from the $-NH_3^+$ group at the asymmetric carbon (this is again the one with lower[9] p*K*) moves the absorption sharply to longer wavelengths, approximating those of the hydroxy acid anions. Ionization of a second basic group, when present, makes a relatively minor shift in general.

One molecular spectroscopic inference from these relations is that the presence of charge (particularly positive charge) in a molecule tends to increase the separation of ground and excited states, at least for the carboxyl chromophore, and that the effect is even greater when both positive and negative charges are present. These are not minor influences; the shifts for ornithine, for example, amount to about 16 mμ.

A second point which should be made is that, even within as homogeneous a group as we are considering here (aliphatic hydroxy or amino acids), the small structural differences between different molecules may give significant differences in the observed characteristics for the carboxyl group. Factors are at work which have not been clearly described as yet.

(9) D. M. Greenberg, "Amino Acids and Proteins," Charles C. Thomas, Springfield, Ill., 1951.

The relative intensities of the carboxyl CD, $\Delta\epsilon/\epsilon$, appear to be slightly greater for the amino acids than for the hydroxy acids, though in general both the molar absorption and the molar circular dichroism are less for the amino acids. (Compare the apparent greater peak width above, however.) In general, the CD values for amino acids and hydroxy acids decrease with ionization, but both aspartic acid and its hydroxy analog, malic acid, show an apparently unique increase in the $\Delta\epsilon$ value with ionization. The $\Delta\epsilon/\epsilon$ values for the carboxyl group are definitely less than the relative intensities (0.06–0.25) found for some carbonyl absorptions, but some 5–25 times the intensity for the phenyl chromophore in some phenylalkylamines, for example.

When both ORD and CD data are available on the same sample, as in these studies, information on transitions not in the wavelength region of observation can be obtained, as well as on those directly observed. If the observed transition (here, the carboxyl absorption) were spectrally isolated, λ_0 would be expected to coincide with λ_{CD}. In the presence of a rotatory contribution from shorter wavelengths, however, λ_0 will be less than λ_{CD} if the additional rotation is of the same sign as [M], the molar rotation at the Cotton extremum. If it is of the opposite sign, λ_0 will be greater than λ_{CD}. Tartaric and lactic acids, alanine, and serine have λ_0 greater than λ_{CD} by about 3 mμ, and for proline the difference is as great as 9 mμ. In the last case, the contribution of negative rotation from the far-ultraviolet is so strong that the net rotation in the visible region is negative, even though the carboxyl $\Delta\epsilon$ is greater than for aspartic acid, which has a large positive rotation.[10] For the other acids, λ_0 is either the same as λ_{CD} or exceeds it by less than 1 mμ. The steepness of the rotatory dispersion in the λ_0 region is sufficient that 1–2 mμ may correspond to a considerable difference in rotation value.

The numerical ratio, $[M]/\Delta\epsilon$, gives another perspective on this relation. Tartaric acid, with $\lambda_0 - \lambda_{CD}$ of 3 mμ, has a ratio of 1500° per unit of dichroic extinction. The ratio is 1500–1600° for the other acids with $\lambda_0 - \lambda_{CD}$ about 3 mμ, and increases with decrease in $\lambda_0 - \lambda_{CD}$ to about 2000° for $\lambda_0 = \lambda_{CD}$. Proline, with its strong negative contribution from the far-ultraviolet and $\lambda_0 - \lambda_{CD}$ of 9 mμ, has a ratio less than 600°.

On ionization, the [M] decreases, as would be expected from the decrease in $\Delta\epsilon$, but the $[M]/\Delta\epsilon$ values are lower than for the protonated forms. This indicates that with the hypsochromic shift of λ_{CD} there is a greater contribution from the far-ultraviolet of rotation of the opposite sign, either because the two transitions are now closer, the far-ultraviolet one is now stronger, or both. In the cases of proline zwitterion, tartrate ion, and lactate ion, direct evidence for new Cotton extrema in the 200-mμ region is seen. The proline also shows part of a strong negative CD, with its peak somewhere below 195 mμ.

Ionization of the second carboxyl in aspartic acid gives another example. A shift of λ_{CD} occurs from 199 mμ for the zwitterion to 203 mμ. The shift in λ_m is smaller than this while that in λ_0 is larger; at the same time [M] is greatly decreased while $\Delta\epsilon$ itself is increased.

(10) The difference of the Drude rotatory parameters ($A - B$) serves as a useful indicator of the sign and magnitude of rotation in the visible region.[2,3]

These shifts would seem to reflect primarily an increased contribution from a far-ultraviolet component of opposite sign. This is borne out by the strongly negative rotation in the visible,[3] whereas the rotation of the zwitterion is positive, and the increased $\Delta\epsilon$ and bathochromic shift of λ_{CD} should otherwise both drive the rotation even more positive.

The sign of the carboxyl CD, in the examples given here, reflects the dextro or levo absolute configuration of the molecular species in the Fischer convention. Tartaric acid, belonging to the D configuration, has the opposite sign for the carboxyl CD to the other acids, which are all of the L configuration. The Drude fitting of the rotatory dispersion of tartaric acid and the amino acids has shown a similar correlation of the sign of the longer wavelength Drude term with the configuration.[2,3] In the case of the phenyl chromophore, however, it has been observed[11] that the sign of the Cotton effects and CD in the 268-mμ absorption region, in a series of phenylamines, is determined by the configuration, but that substitution of hydroxyl for the amino group reverses the sign of the phenyl group rotation for the same configuration. Such influences must be investigated for the carboxyl group also, though in this specific comparison (hydroxyl *vs.* amino) there seems to be no difference in the sign of the carboxyl group dichroism.

Proline in alkaline solution shows a unique phenomenon. A broad negative CD appears in addition to the normal positive carboxyl CD at 216 mμ, at an apparent wavelength of 236 mμ. The $\Delta\epsilon$ value is -0.024. It is not possible to say certainly whether this represents a new absorption transition near that wavelength, or whether it represents the tail of the strong negative CD and accompanying absorption peak already noted at shorter wavelengths. If it is the latter, it must be an exceedingly broad peak.

Inferences have been drawn as to λ_{CD} values from Drude parameters found to fit ORD dispersions remote from the absorption region, for some of these systems.[2,3] For HCl solutions of tartaric acid[2] or the amino acids,[3] the longer wavelength Drude parameters (positive term for the amino acids, negative for tartaric acid) fall in general 2–8 mμ lower than λ_{CD}. The shorter Drude wavelength is very close to the longer.[2,3] Similar relations hold between Drude parameters and λ_{CD} for the solution species with one proton less than in HCl solution. These relations, therefore, resemble the "metastable" Drude solution for synthetic rotatory dispersion data found in model computations.[12] This behavior must be linked with the strength of the rotatory contributions from the farther ultraviolet, in some fashion. Nevertheless, the longer wavelength Drude term does reflect the behavior of the carboxyl CD, in direction and approximate amount of shift. Rotatory intensity relations are much more complicated, particularly with the involvement of the contributions from the far-ultraviolet (see above). The situation is different from that with the carbonyl absorption of camphor,[4] with the peak near 290 mμ spectrally far removed from the next absorptions, near or below 200 mμ. In that case the far-ultraviolet contribution constitutes a minor correction to the contribution from the carbonyl peak, at wavelengths above 300 mμ.

Some measurements have been made by others of the rotatory dispersion of amino acids, with greater or lesser penetration into the Cotton region,[13–15] and of malic acid.[16] Insofar as conditions are comparable, measurements on the same compound are in essential agreement. Some circular dichroism measurements on amino acids have also been reported.[17] Aside from minor differences ascribable to slight variance in composition with respect to the various protonated forms, the two sets of λ_{CD} and $\Delta\epsilon$ values are in perhaps surprising match. No comparable simultaneous comparison of the ORD, CD, and absorption spectra has come to our attention, however.

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