[Contribution from the Laboratory of Biochemistry, National Cancer Institute, National Institutes of Health

Studies on Diastereoisomeric α-Amino Acids and Corresponding α-Hydroxy Acids. IV. Rotatory Dispersion of the Asymmetric α- and ω-Carbon Atoms of Several Diastereoisomeric Amino Acids

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Received December 22, 1954

The optical rotatory dispersion of 42 L-amino acids was studied at 589, 578, 546, 435, 405 and 365 mμ, and described for the most part in terms of a two constant Drude equation. The empirical relation between the rotatory characteristics of the α-amino acids and the magnitude of λ_0 proposed by Patterson and Brode was tested in this large series of compounds and, in modified form, was found to be generally applicable to amino acids with a single α-asymmetric center as well as to the α-asymmetric center of diastereoisomeric amino acids.

In an earlier paper in this series, the contribution of the individual asymmetric α- and ω-carbon atoms to the total optical rotation at 589 mμ of several diasymmetric amino acids was determined.[1] The present communication extends these calculations to the results of rotatory measurements at wave lengths of 589, 578, 546, 435, 405 and 365 mμ, and, by the use of the Drude equation or appropriate modifications thereof, presents a graphical analysis of the data obtained over this wave length range.

The Drude equation expresses the relation between optical rotation and wave length as $\alpha = \Sigma k_n/\lambda^2 - \lambda_n^2$, where α represents the rotation, k is a constant, λ is the wave length of the light employed for the measurements, and λ_n is a series of wave lengths associated with the spectral absorption bands controlling the dispersion.[2] When a graphical plot of $1/\alpha$ against λ^2 in the visible region of the spectrum yields a straight line, the equation is expressed in its simplest or one-term form, $\alpha = k/\lambda^2 - \lambda_0^2$. If the resultant graph is not a straight line, additional terms must generally be added to express the dispersion linearly to at least a first approximation. The α-amino acids comprise a particularly appropriate series of compounds for the study of rotatory dispersion for the rotation of the asymmetric carbon atom may be expected to be governed appreciably by the position of the anisotropic bands of the α-amino and α-carboxyl groups common to all of these compounds.[3] That certain regularities emerge from the study of the optical dispersion of the α-amino acids was shown by Patterson and Brode who examined some 14 compounds at wave lengths between 440 and 660 mμ.[3] On the basis of the graphical use of the single term Drude equation, these authors suggested that the α-amino acids possessed an $\mathrm{L_s}$-configuration if the following conditions were met: (a) the dispersion was normal, positive, and the value of λ_0 was above 205 mμ, (b) the dispersion was normal, negative, and the value of λ_0 was below 140 mμ, and (c) the dispersion was anomalous (produced by partial rotations of opposite sign) and the sign of rotation changed from negative to positive with decreasing wave length. More recently, the late Dr. Erwin Brand and his co-workers published rotatory dispersion data on five L-amino acids between 250 and 750 mμ employing a two-term Drude equation.[4] They found in all cases that the first term of this equation was positive, and that the λ_0 values calculated were between 220 and 290 mμ. Neither of these two groups of investigators included in their studies any of the diasymmetric α-amino acids.

Before undertaking the study of the rotatory dispersion of each of the asymmetric centers of the diasymmetric α-amino acids, it was considered desirable to first examine the dispersion of the large variety of amino acids available in this Laboratory as a result of the development of enzymatic methods for the resolution of the corresponding racemates.[5] Some 42 L-amino acids of tested optical purity (>99.9%) were studied at wave lengths between 365 and 589 mμ, in part to see whether the Patterson and Brode suggestions were applicable to a wider variety of amino acids than that hitherto studied, and in part to provide a basis of comparison for the analysis of the behavior of the more complex diasymmetric amino acids which was to follow. In most cases the use of the single term Drude equation was sufficient to represent the data obtained, and the observed values of $[\alpha]$ at 589 mμ, and the calculated values of λ_0 from the plot of $100/[\alpha]$ against λ^2, are given in Table I.

If the values for the diasymmetric amino acids, isoleucine, threonine, hydroxyproline, phenylserine and aminotricarballylic acid, and their respective allo stereomers, are excepted from present consideration, it would appear that for positive rotations the values of λ_0 range in water solution from 218 to 348 and in HCl solutions from 199 to 314. For negative rotations in water the values of λ_0 include 151 for histidine, 146 for phenylalanine, and 105 for proline; in HCl solution such values are 198 for cystine and 151 for proline. Several instances where λ_0^2 is negative and λ_0 therefore imaginary occur with negatively rotating amino acids in water (leucine, *t*-leucine, serine, homoserine, methionine, ethionine and tryptophan). A similar situation occurs in the case of the positively rotating δ-hydroxynorvaline in water and tryptophan in HCl. Anomalous dispersion was noted with S-benzylcysteine and phenylalanine in HCl solution. In nearly

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(1) M. Winitz, S. M. Birnbaum and J. P. Greenstein, This Journal, **77**, 716 (1955).

(2) P. A. Levene and A. Rothen in H. Gilman's "Organic Chemistry," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1938, pp. 1779–1850.

(3) J. W. Patterson and W. R. Brode, *Archiv. Biochem.*, **2**, 247 (1943).

(4) E. Brand, E. Washburn, B. F. Erlanger, E. Ellenbogen, J. Daniel, F. Lippmann and M. Scheu, This Journal, **76**, 5037 (1954).

(5) J. P. Greenstein, S. M. Birnbaum and M. C. Otey, *J. Biol. Chem.*, **204**, 308 (1953); J. P. Greenstein, *Advances in Protein Chemistry*, **9**, 122 (1954).

TABLE I

ROTATORY DISPERSION DATA FOR L-α-AMINO ACIDS[a]

Amino acid	H_2O as solvent		HCl as solvent[b]	
	$[\alpha]_D$	λ_0[c]	$[\alpha]_D$	λ_0[c]
Alanine	+ 1.8	348	+14.1	258
Butyrine	+ 7.9	282	+20.1	261
Norvaline	+ 7.0	293	+24.1	251
Norleucine	+ 4.7	307	+24.2	244
Aminoheptylic acid	+ 6.7	242	+23.3	247
Aminocaprylic acid	....	...	+23.5	237
Valine	+ 5.6	298	+26.5	253
Isovaline	+11.2	218	+ 6.6	204
Leucine	−10.7	Imag.	+15.6	274
t-Leucine	− 9.1	Imag.	+ 7.4	314
Isoleucine	+12.4	248	+39.5	231
Alloisoleucine	+15.9	270	+39.6	246
Serine	− 7.8	Imag.	+14.5	271
Homoserine	− 8.8	Imag.	....	...
δ-Hydroxynorvaline	+ 6.2	Imag.	....	...
ε-Hydroxynorleucine	+ 3.6	296	+23.5	244
Threonine	−28.5	126	−14.5	Imag.
Allothreonine	+ 9.6	299	+31.7	246
Methionine	− 8.4	Imag.	+23.9	236
Ethionine	− 6.8	Imag.	+23.4	240
Cystine	....	...	−212[d]	198
Homocystine	....	...	+77.1[d]	221
S-Benzylcysteine	....	...	− 4.0[d]	Anom.
S-Benzylhomocysteine	....	...	+24.8[d]	226
Aspartic acid	+ 5.2	257	+24.9	214
Glutamic acid	+11.9	241	+31.5	228
Aminoadipic acid	....	...	+24.1	233
β-Aminoalanine	....	...	+33.1	199
γ-Aminobutyrine	....	...	+31.7	223
Ornithine	....	...	+27.7	226
Lysine	....	...	+25.9	231
Arginine	....	...	+26.9	203
Histidine	−39.2	151	+13.8	270
Phenylalanine	−34.0	146	− 5.9	Anom.
Tryptophan	−33.3	Imag.	+ 6.4[d]	Imag.
Proline	−85.1	105	−54.1	151
γ-Hydroxyproline	−76.7	192	−46.7	167
γ-Allohydroxyproline	−60.0	181	−20.6	106
β-Phenylserine	−33.1	68	−50.3	112
β-Allophenylserine	+ 8.4	294	+82.2	237
Aminotricarballylic acid	−32.8	207	−48.0	204
Alloaminotricarballylic acid	+ 7.5	276	+36.4	205

[a] Optical rotations determined with a photoelectric polarimeter at 589, 578, 546, 435, 405 and 365 mμ with 0.5–2.0% solutions at temperatures between 24 and 28°. Values given in the table refer only to the D or 589 mμ line of sodium. [b] Except where noted concentration of HCl was 5 *N*. [c] λ_0 values determined from the dispersion data by the method of least squares. Where λ_0^2 was negative and λ_0 imaginary the fact was noted by Imag. Where sign of rotation changed with wave length fact noted by Anom. (anomalous dispersion). [d] 1 *N* HCl.

every case, the λ_0 value of a positively rotating amino acid in water is greater than that for the same positively rotating amino acid in HCl solution. Only proline furnished an appropriate example of an amino acid with a negative rotation in both water and HCl, and in this instance the λ_0 in water is less than that in HCl. It is difficult to assign a particular physical significance to values of λ_0 when the experimental data are obtained at wave lengths so relatively far from the region of absorption, but it is of interest to observe the apparent regularities associated with this figure. Thus, in accord with the suggestion of Patterson and Brode, the positively rotating L-amino acids possess λ_0 values of 200 mμ or more. The relatively few appropriate examples of the negatively rotating L-amino acids indicate that their values of λ_0 are 200 mμ or less. It appears reasonable therefore to adopt a slightly modified form of the Patterson and Brode suggestion as a means of determining the optical configurations of α-amino acids, whereby the λ_0 value of 200 mμ is set as the dividing line between positively rotating L-amino acids with $\lambda_0 > 200$ and negatively rotating L-amino acids with $\lambda_0 < 200$. The D-enantiomorphs will of course have the same λ_0 values but opposite signs of rotation. The Patterson–Brode rule based upon rotatory dispersion in a single solvent thus emerges as a companion to another empirical generalization based upon optical rotatory power for the determination of optical configuration in α-amino acids, namely, that of Lutz–Jirgensons which depends upon shifts in the direction of optical rotation in water and in acid solutions.[6]

Diastereoisomeric Amino Acids.—Inspection of the data in Table I for isoleucine, threonine, hydroxproline, phenylserine and aminotricarballylic acid and for their allo stereomers indicates that isoleucine, alloisoleucine, threonine, allothreonine, hydroxyproline, allohydroxyproline, allophenylserine and alloaminotricarballylic acid follow both the Lutz–Jirgensons and Patterson–Brode rules for the optical behavior of L-amino acids. The optical behavior of phenylserine follows the Patterson–Brode but not the Lutz–Jirgensons rule, and that of aminotricarballylic acid follows neither rule. As pointed out in an earlier paper in this series,[1] the exceptional position of phenylserine in respect to the Lutz–Jirgensons rule could be clarified on the basis that the optical rotation of the molecule was the sum of the nearly independent rotations of the α- and β-optical centers. By the use of this principle of optical superposition it was shown that the optical behavior of the α-asymmetric center of phenylserine did in fact follow the Lutz–Jirgensons rule, for the calculated value of this center in HCl solution was more positive than in water solution.[7]

The case of aminotricarballylic acid is more complex, for there is at present no unequivocal evidence other than rotatory bearing on the optical configuration of this compound. There are four possible diastereoisomeric pairs of this compound which may be designated *l,l*-allo, *d,l*-allo, *l,d*-allo and *d,d*-allo, where *l* and *d* refer to the sign of optical rotation.[1] Analysis of the contribution of the partial rotations of the α- and β-asymmetric centers permitted the elimination of the first two of these possible pairs for the optical behavior of their α-cen-

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(6) O. Lutz and B. Jirgensons, *Ber.*, **63**, 448 (1930); **64**, 1221 (1931); **65**, 784 (1932).

(7) For a discussion of the exceptional position of isovaline in respect to the Lutz–Jirgensons rule see footnote 1.

TABLE II

ROTATORY DISPERSION DATA FOR DIASTEREOISOMERIC AMINO ACIDS[a]

	H_2O as solvent				HCl as solvent			
	α-Carbon atom		ω-Carbon atom		α-Carbon atom		ω-Carbon atom	
L-Amino acid	λ_0	Slope[d]	λ_0	Slope[d]	λ_0	Slope[d]	λ_0	Slope[d]
Isoleucine	263[b]	0.025	216[c]	−0.219	243[b]	0.009	333[c]	−0.827
Threonine	Imag.[c]	.....	217[c]	− .018	307[b]	.046	185[c]	− .014
γ-Hydroxyproline	189[c]	−0.005	230[c]	− .041	150[c]	− .009	198[c]	− .025
β-Phenylserine	Imag.[c]	− .019	185[c]	− .015	297[b]	+ .024	209[c]	− .005
Aminotricarballylic acid	163[c]	− .024	218[c]	− .016	198[c]	− .061	205[c]	− .008

[a] Data calculated from Table I. [b] Positive rotation. [c] Negative rotation. [d] Least square slopes calculated from the experimental data.

ters did not follow the Lutz–Jirgensons rule. Anticipating data to be given below, it is of interest to note that these pairs also may be eliminated from consideration for their α-centers do not follow the Patterson–Brode rule either. The choice between the *l,d*-allo and *d,d*-allo pairs of diastereomers was made tentatively on the basis of the optical rotatory behavior of the α-asymmetric center of natural *d*-isocitric acid which had been obtained by deamination of *l*-aminotricarballylic acid.[8] The diastereomeric pair, *l,d*-allo, was that finally chosen.[1] However, as noted in Table I, the optical behavior of *l*-aminotricarballylic acid follows neither the Lutz–Jirgensons nor the Patterson–Brode rule. When the optical rotation value for this compound is referred to the partial rotations of its α- and β-asymmetric centers, it is found that not only does the α-center follow the Lutz–Jirgensons rule as noted before,[1] but the Patterson–Brode rule as well. The pertinent data are given in Table II. The partial rotations of each asymmetric center of aminotricarballylic acid, isoleucine, threonine, hydroxyproline and phenylserine were calculated from the experimental values at each wave length by the method previously described,[1] and λ_0 calculated for each center from least-square slopes of the Drude equations. With one exception, the one-term form of this equation was sufficient to represent the data. The rotation of the α-carbon atom of isoleucine in HCl solution appeared to be best described by a two term equation $\alpha = -1.992/\lambda^2 - 0.04654 + 1.676/\lambda^2$. The partial rotations of the β-carbon atoms of isoleucine in HCl at the three highest wave lengths were too small in magnitude to be included in an accurate calculation of λ_0, and only the values obtained at the three lowest wave lengths were therefore employed. In addition to values of λ_0, the slopes of the Drude plots also are given in Table II.

Inspection of Table II indicates that the Patterson–Brode generalization is applicable to the determination of the configuration of the α-asymmetric center of the diastereoisomeric amino acids, for with the L-series studied values of λ_0 for positively rotating α-centers were above 200 mμ and those with negatively rotating centers possessed λ_0 values less than 200 mμ. In some cases, however, as in that of aminotricarballylic acid, additional evidence is necessary to assign unequivocally an L- or D-configuration to the α-center of a diastereomeric amino acid. Thus, although the *l,d*-allo diastereomeric pair of aminotricarballylic acid follows in the optical behavior of its α-asymmetric center both Lutz–Jirgensons[1] and Patterson–Brode (Table II) rules, so too does the *d,d*-allo combination, for the λ_0 value of the positively rotating α-center of these compounds in water and in HCl, respectively, is 218 and 205. The λ_0 values of the α-centers of the *l,l*-allo and *d,l*-allo pairs in water are, respectively 218 for a negative rotation and 163 for a positive rotation, in HCl solution they are 205 for a negative rotation and 198 for a positive rotation, and thus in no case is the Patterson–Brode rule followed.

All of the ω-asymmetric centers described for the compounds in Table II possess a negative rotation, and the values of λ_0 calculated range from 185 to 333. The generalization noted does not apply to optically active centers other than the α in amino acids. Consideration of the slopes described in Table II reveals the strikingly greater order of magnitude associated with the β-carbon of isoleucine compared with that of either α- or ω-centers in the other diasymmetric amino acids studied. This is a reflection in part of the much smaller magnitude of the rotation values of the β-center in isoleucine, which at 589 mμ are −1.8° in water and −0.2° in HCl.

Experimental

The forty-two L-amino acids used in the present investigation were all prepared in this Laboratory.[5] The instrument employed was a Rudolph photoelectric polarimeter of high precision equipped with glass filters for isolating the sodium D line at 589 mμ, and lines of the mercury arc spectrum at 578, 546, 435, 405 and 365 mμ. The actual rotations were observed by the method of symmetrical angles, with the aid of a photomultiplier tube connected to a photometer. Calibration of the instrument was effected by means of two quartz control plates, with a maximum error at any of these wave lengths of 0.22%. Replicate readings were reproducible to ±0.003°. Measurements were made on 0.5 to 2.0% solutions of the compounds in water and in HCl, and at temperatures between 24 and 28°. Solutions were prepared of the carefully dried materials in 5-ml. volumetric flasks. The readings which were taken in a 2-dm. polarimeter tube were corrected with respect to zero points obtained with solvent blanks. The rotatory dispersion of each compound required several hours of investigation, and hence such compounds as homoserine which rapidly lactonize in acid solution could not be studied in this medium. For nearly all of the compounds studied, except those noted whose dispersion was anomalous, the plot of 100/[α] against λ^2 was quite linear, and λ_0 values could be calculated to within 1%.

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(8) J. P. Greenstein, N. Izumiya, M. Winitz and S. M. Birnbaum, THIS JOURNAL, **77**, 707 (1955).