OPTICAL AND ENZYMATIC CHARACTERIZATION OF AMINO ACIDS

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The susceptibility of a wide variety of α-amino acids to the action of L- and of D-amino acid oxidases from different biological sources has been studied by a number of investigators (1–7). These studies have in general revealed the considerable differences in the rates of oxidation by these enzymes of amino acids of different chemical structure. It would perhaps be superfluous to undertake even a partial reinvestigation and extension of these valuable studies, were it not for the following considerations: (*a*) most of the amino acids employed were racemic, and there is no assurance that in each case the resistant isomer did not affect the initial oxidative rate of the susceptible isomer (8–10);[1] (*b*) no comparative data were presented on the individual diastereoisomers of threonine, hydroxyproline, and isoleucine, while data on uncharacterized, commercially available preparations of DL-isoleucine, as our experience has shown (12), may be considered highly suspect; and (*c*) the resolution of forty-six racemic amino acids conducted in this Laboratory during the past few years and the demonstration of the extremely high optical purity of the isomers obtained (12–40) have provided the opportunity of performing a systematic survey of the relative susceptibility of these individual isomers to L- and to D-amino acid oxidase preparations, and of investigating the properties of amino acids not previously studied by other investigators.

As a further characterization of these isomers, their optical rotations have been determined under nearly identical conditions, both in 5 N HCl and in glacial acetic acid. To our knowledge, optical rotation data in the latter solvent, with the exception of a few isolated cases, have not been generally reported. For many of the amino acids, the magnitude of the rotation values in glacial acetic acid was found to be considerably higher than in HCl solutions, a fact which may be of use in the optical characterization of amino acids available only in very small quantities.

[1] The inhibitory effect of L-leucine on D-amino acid oxidase (8–10) has been noted also by Hellerman, but the extent of this inhibition is considerably less than that exerted by such agents as benzoate (personal communication from Dr. Leslie Hellerman) (*cf.* Hellerman *et al.* (11)).

EXPERIMENTAL

Amino Acids—The resolution of forty-two of the forty-six amino acids was performed by substantially the same procedure; namely, by the asymmetric action of hog kidney acylase or beef pancreas carboxypeptidase on the *N*-acylated DL-amino acid, followed by the separation of the L-amino acid from the *N*-acylated D-amino acid by appropriate measures (12–24, 26, 28–39).[2] Proline could not be resolved in this way, for its *N*-acylated derivatives, lacking a hydrogen on the peptide nitrogen atom, were almost completely resistant to the enzymes mentioned (25). It was resolved by the asymmetric action of an amidase fraction from hog kidney on DL-proline amide, followed by separation of the L-proline and the D-proline amide as the respective *N*-carbobenzoxy derivatives (24). α-Amino-*n*-dodecylic acid (20), like *tert*-leucine (40) and proline, was likewise resolved as the amide by the action of a hog kidney amidase fraction.[3] The diastereoisomers of hydroxyproline were the only isomers prepared non-enzymatically; from hydroxy-L-proline (Nutritional Biochemicals Corporation) as the starting material, there was prepared allohydroxy-L-proline (41) and allohydroxy-D-proline (27), and from the latter hydroxy-D-proline (27).[4]

Manometric methods involving the use of L- and of D-amino acid oxidases and of various decarboxylase preparations have been successfully employed to detect the presence of one optical enantiomorph in the pres-

[2] The application of ion exchange chromatography to the separation of these products has been described (29, 38).

[3] Racemic alanine, histidine, and *S*-benzylcysteine have also been resolved as the amides by an amidase fraction from hog kidney which is apparently different from that which is effective toward proline amide (18). The optical isomers of α-aminododecylic acid reported in this study were donated by Dr. C. G. Baker. The optical isomers of β-phenylserine, prepared by the action of carboxypeptidase on the *N*-trifluoroacetyl derivatives of the respective racemic diastereoisomers (39), were donated by Dr. W. S. Fones.

[4] Following the general procedure of Neuberger (41), 11 gm. of allohydroxy-L-proline with $[M]_D$ in water at 25° = −78.0° were obtained from 52 gm. of hydroxy-L-proline, thus an over-all yield of 21 per cent. A yield of similar magnitude was obtained in the analogous conversion of allohydroxy-D-proline to hydroxy-D-proline (27). In both preparations a slight modification of Neuberger's procedure at the C_4 inversion step produced better yields. This consisted of the addition of a slight *excess* of HCl to the cooled alkaline digest of *O*-*p*-toluenesulfonyl-*N*-acetylhydroxyproline, followed by evaporation to dryness *in vacuo*, extraction of the residue with hot acetone, removal of the solvent, refluxing of the oil residue with 2 N HCl for 2 hours, evaporation to dryness *in vacuo*, neutralization of the residue with 2 N LiOH, and repeated recrystallization from aqueous alcohol. By not attempting to isolate the intermediate *N*-acetyl derivative, but proceeding directly to its hydrolysis, the resulting yield is over 90 per cent of the theoretical.

ence of the other with a sensitivity of better than 1 part in 1000 (23).[5] By these means, the following substrates, employed in the present investigation, have been determined to be more than 99.9 per cent optically pure: (*a*) the L isomers of α,β-diaminopropionic acid, serine, allothreonine, hydroxyproline, and allohydroxyproline,[6] (*b*) the D isomers of aspartic, glutamic, and α-aminoadipic acids, ornithine, arginine, histidine, lysine, and the *erythro* isomer of β-phenylserine, and (*c*) the L and D isomers of alanine, α-amino-*n*-butyric acid, valine, norvaline, leucine, norleucine, isoleucine, alloisoleucine, α-amino-*n*-heptylic acid, α-amino-*n*-caprylic acid, α-amino-*n*-nonylic acid, homoserine, pentahomoserine, hexahomoserine, methionine, ethionine, *S*-benzylcysteine, *S*-benzylhomocysteine, citrulline, phenylalanine, tyrosine, tryptophan, aminophenylacetic acid, aminocyclohexylacetic acid, and aminocyclohexylpropionic acid (23–26, 28, 30, 31, 33, 35–39). The L and D isomers of aminocyclohexylpropionic acid have been recently prepared in this Laboratory with the same high degree of optical purity by the action of acylase I on *N*-acetyl-DL-aminocyclohexylpropionic acid (m.p. 178° corrected) (26). The preparations of L-proline contained 0.2 per cent of the D form (24). The optical purity of the remaining substrates described in this report, because of their relative resistance to the oxidases, could not be determined with the sensitivity desired.

The steric purity of the isoleucine preparations, *i.e.* the freedom of L-isoleucine from L-alloisoleucine and of D-isoleucine from D-alloisoleucine, was determined by the action of L-amino acid oxidase on the former pair (42) and by the action of D-amino acid oxidase on the latter pair (12), followed by isolation of the resulting α-keto acids and determination of their respective optical rotations, which for each pair were equal in magnitude and opposite in sign. The determination of the steric purity in this case is limited by the accuracy of the polarimetric method, namely 1 to 2 per cent (23). A similar analysis of the threonine and hydroxyproline diastereoisomers was not possible at this time, due to the resistance of L- and D-threonine and of hydroxy-L-proline and allohydroxy-L-proline, to the action of the respective amino acid oxidases employed, and to the difficulty in isolating the products of the reaction of D-amino acid oxidase on hydroxy-D-proline and allohydroxy-D-proline.

[5] These purity determinations are generally conducted in the presence of relatively large amounts of the oxidases which are considerably greater than those usually employed for the determination of the oxidative rates.

[6] The optical purity of hydroxy-L-proline and of allohydroxy-L-proline, not previously described, was determined as usual with D-amino acid oxidase in the presence and absence of added D enantiomorphs. In each case there was less than 1 part in 1000 of the D enantiomorph, and thus the preparations of the L compounds are considered to be better than 99.9 per cent optically pure.

Optical Rotation of Amino Acid Enantiomorphs—In Table I are collected the molecular rotations of the L-isomers of the amino acids as observed under nearly identical conditions in 5 N HCl and in glacial acetic acid solutions. Each value was obtained from determinations on two to four separate solutions. The glacial acetic acid employed was a good commercial grade which was frozen, the container was inverted and the contents allowed to melt at 25°. When about one-third of the contents had been discarded in this way, the container was stoppered, and the remainder, which was allowed to thaw, was the solvent employed in these studies.

No particular discussion is needed in the case of the molecular rotation values in 5 N HCl, except to point out the familiar circumstance that in several instances the corresponding values under the same conditions in water are higher in absolute magnitude: L-threonine −33.9°, L-phenylalanine −57.0°, L-tryptophan −68.8°, L-proline −99.2°, hydroxy-L-proline −99.6°, allohydroxy-L-proline −78.0°, L-histidine −59.8°, and L-isovaline +13.1°. L-Isovaline does not follow the Lutz-Jirgensons rule (43), for its rotation in water is more positive than in HCl solution.[7] The rotation value given for L-homoserine is based on the freshly prepared solution, for, on standing in acid solution, the compound is transformed into the lactone with an optical rotation of opposite sign (35). Tryptophan and the longer chain amino acids are so insoluble in 5 N HCl that their optical rotations could not be conveniently measured; they were, however, sufficiently soluble in glacial acetic acid.

Amino Acids in Glacial Acetic Acid Solution—All of the L-amino acids whose molecular rotations in glacial acetic acid solution are given in Table I were soluble in concentrations of 0.5 to 2.0 per cent, except allothreonine and hydroxyproline, whose solubility limit was 0.25 per cent. In some cases gentle warming to 40–50° was found convenient to obtain rapid solution, but none of the compounds crystallized from these solutions on cooling to 25°.

1 gm. of L-methionine was dissolved in 100 ml. of the glacial acetic acid with gentle heating, the solvent was removed by aeration, and the amino acid recovered in 95 per cent yield. It possessed an $[M]_D$ +34.6° for a 2 per cent solution in 5 N HCl at 25°, and upon analysis was as follows: calculated, C 40.2, H 7.4, N 9.4, S 21.5; found, C 40.2, H 7.3, N 9.4, S 21.3. The rotations of freshly prepared solutions in glacial acetic acid were measured for L-alanine, L-leucine, L-valine, L-citrulline, L-ethionine, L-phenylalanine, hydroxy-L-proline, and L-hexahomoserine, and then the solutions were each heated in a boiling water bath for 15 minutes, cooled to 25°, and the rotations measured again. In no case was there any change in the

[7] Fones has recently noted the same phenomenon in the case of the *threo* form of β-phenyl-L-serine (39).

rotation values. The molecular rotation value in glacial acetic acid solution at 25° for acetyl-L-alanine is −51.1° and for acetyl-L-leucine is −57.1°, in contrast with the positive sign of the rotations for L-alanine and for L-leucine in this solvent (Table I).[8] It does not seem likely that the amino acids whose rotations are described in Table I are racemized or acetylated to any extent in glacial acetic acid solution.

In the cases of L-homoserine and L-pentahomoserine, the rotation values given in Table I refer to the freshly prepared solutions. Upon standing for 30 minutes at 25°, no change in these rotation values was evident. On heating at 100° for 30 minutes, the molecular rotation values at 25° were, respectively, −26.2° and +9.3°. On heating at 100° for another 30 minutes, and again cooling to 25°, the rotation values were unchanged. In both cases the respective γ- and δ-lactones were evidently formed, but it required the higher temperature to effect the ring closure.

An interesting distinction in the effect of heating was shown in the cases of L-aminophenylacetic acid and L-aminocyclohexylacetic acid. The former compound is relatively insoluble in glacial acetic acid at 25°, but a 0.10 per cent solution can be obtained after prolonged heating. 200 mg. of L-aminophenylacetic acid were suspended in 200 ml. of glacial acetic acid and the mixture was heated in a boiling water bath for 1 hour, at the end of which period of time complete solution had occurred. The solution was cooled to 25° and the measured optical rotation was found to be zero. The solvent was removed, and the amino acid recovered with 92 per cent yield. At a 1 per cent solution in 5 N HCl at 25°, the rotation was zero. The analysis was as follows: calculated for the free amino acid, C 63.5, H 6.0, N 9.3; found, C 63.2, H 6.0, N 9.3. The amino acid was evidently not acetylated, but did racemize as a result of being heated in the acetic acid. The original material in HCl solution possessed a molecular rotation of +254.0° (Table I). In contrast to these observations, a 0.5 per cent solution of L-aminocyclohexylacetic acid was readily prepared in glacial acetic acid at 25° and the optical rotation measured. The solution was then heated in a boiling water bath for 1 hour, cooled to 25°, and the rotation again measured. No change in the rotation value was noted. It is evident that the complete racemization of the aminophenylacetic acid in hot glacial acetic acid solution must in some way be connected with the presence of a phenyl substituent on the asymmetric carbon atom, for neither aminocyclohexylacetic acid nor phenylalanine is racemized under these conditions. That the aminophenylacetic acid isolated after this treatment

[8] The molecular rotation values of several other acetyl-L-amino acids in glacial acetic acid are for acetyl-L-isoleucine +36.0°, acetyl-L-alloisoleucine +38.2°, acetyl-L-methionine +17.2°, acetyl-*S*-benzyl-L-cysteine −78.5°, acetyl-L-proline −216.0°, and acetylhydroxy-L-proline −207.7°.

TABLE I

Properties of Optical Isomers of α-Amino Acids

Amino acid	Mol. wt.	L isomers			D isomers	Bibliographic references to preparation of isomers
		$[M]_D$ in*		Oxidation† by L-amino acid oxidase	Oxidation‡ by D-amino acid oxidase	
		5 N HCl	Glacial acetic acid			
Alanine	89.1	+13.0	+29.4	0.9	10.5	(18, 25, 28)
α-Amino-*n*-butyric acid	103.1	+21.2	+43.3	49	7.1	(17, 25)
Valine	117.1	+33.1	+72.6	9.0	9.9	(25)
Norvaline	117.1	+29.2	+41.0	181	6.7	(17, 25, 37)
Isovaline	117.1	+9.7	+26.3	0	0	(29)
Leucine	131.1	+21.0	+29.5	225	7.8	(25)
Norleucine	131.1	+32.1	+47.9	192	13.6	(17, 23, 25)
Isoleucine	131.1	+53.5	+64.2	71	14.8	(12, 13, 25)
Alloisoleucine	131.1	+53.1	+55.7	0.8	5.9	(12, 13, 25)
α-Amino-*n*-heptylic acid	145.2	+33.8	+47.9	142	14.6	(20, 36)
α-Amino-*n*-caprylic acid	159.2	+36.6	+50.9	102	6.1	(20, 36)
α-Amino-*n*-nonylic acid	173.2		+58.0	61	4.0	(36)
α-Amino-*n*-decylic acid	187.2		+58.0	35	1.0	(36)
α-Amino-*n*-undecylic acid	201.2		+58.3	13	0.2	(36)
α-Amino-*n*-dodecylic acid	215.2		+52.3	1.0	0	(20)
Serine	105.1	+15.9		0	2.8	(25)
Homoserine	119.1	+21.8§	+14.3§	8.0	3.2	(35)
Pentahomoserine	133.1	+38.3	+46.6§	53	1.8	(31)
Hexahomoserine	147.1	+34.9	+57.4	201	3.2	(31)
Threonine	119.1	−17.9	−35.7	0	0.3	(25)
Allothreonine	119.1	+36.3	+45.3‖	0.9	2.6	(19, 25)
Methionine	149.2	+34.6	+29.8	243	15.6	(25)
Ethionine	163.2	+38.7	+29.3	242	8.5	(25)
Cystine	240.2	−557.4		63	1.4	(18, 28)
Homocystine	268.2	+209.2		84	0.9	(35)
S-Benzylcysteine	211.2	−42.2	−52.8	155	3.5	(18, 28)
S-Benzylhomocysteine	225.2	+61.3	+36.0	108	8.3	(35)
Aspartic acid	133.1	+33.8		0.1	4.6	(25)
Glutamic "	147.1	+46.8		0.2	0.3	(22, 25)
α-Aminoadipic acid	161.1	+40.3		17	0	(33)
α,β-Diaminopropionic acid¶	104.1	+35.4		0	2.4	(30, 34)
α,γ-Diaminobutyric acid¶	118.1	+37.4		0.1	0.6	(32)
Ornithine¶	132.2	+37.5		0.3	0.8	(21, 25, 32)
Lysine¶	146.2	+37.9		0.4	0	(17, 25)
Histidine¶	155.2	+18.3	+11.6	35	0.8	(18, 28)

TABLE I—*Concluded*

Amino acid	Mol. wt.	L isomers			D isomers	Bibliographic references to preparation of isomers
		[M]D in*		Oxidation† by L-amino acid oxidase	Oxidation‡ by D-amino acid oxidase	
		5 N HCl	Glacial acetic acid			
Arginine¶	174.2	+48.1	+51.3	18	1.0	(21, 28)
Citrulline	175.2	+42.4	+30.7	98	0.9	(21, 25)
Phenylalanine	165.1	−7.4	−12.4	185	9.6	(16, 25)
Tyrosine	181.2	−18.1		185	11.1	(16, 37)
Tryptophan	204.1		−69.4	199	3.0	(16)
Proline	115.1	−69.5	−92.1	0	20.3	(24)
Hydroxyproline	131.1	−66.2	−100.9‖	0	5.0	(27)
Allohydroxyproline	131.1	−24.7	−39.3	0	6.1	(27, 39)
Aminophenylacetic acid	151.2	+254.0		5.0	4.0	(26)
Aminocyclohexyl-acetic acid	157.2	+55.8	+102.2	4.0	7.5	(26)
Aminocyclohexylpropionic acid	171.2	+25.7	+46.2	92	4.4	(26)
β-Phenylserine (*threo*)	181.2	−88.1	−92.6	0.7	0	(39)
" (*erythro*)	181.2	+147.3	+108.7	140	0	(39)
tert-Leucine	131.1	+11.8	+47.2	0	0	(40)
Cysteine	121.1	+7.9	+15.7			(28)

* Molar rotations calculated as specific rotations multiplied by the molecular weight and divided by 100; temperature 24–26°. The concentrations, except where indicated, were 0.5 to 2.0 per cent. A 2 d.cm. tube was employed. The values are expressed as degrees.

† Rattlesnake (*Crotalus adamanteus*) venom. Rates in terms of micromoles of oxygen consumed per hour per mg. of N.

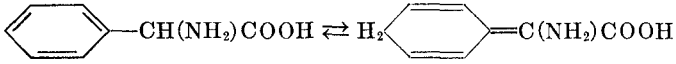
‡ Hog kidney preparation. Rates in terms of micromoles of oxygen consumed per hour per mg. of N.

§ Freshly prepared solutions.

‖ 0.25 per cent solutions, compounds insoluble at 0.5 per cent.

¶ As the free base.

was actually in the DL form was shown, not alone by the absence of optical rotation in HCl, but also, and by more stringent proof, by the fact that the substance was equally oxidized to 50 per cent by L-amino acid oxidase and by D-amino acid oxidase, respectively. The original L-aminophenylacetic acid was not affected at all by D-amino acid oxidase (26). It is possible that the aminophenylacetic acid is racemized by the following mechanism:



This reaction does not apparently occur in alkaline solution at 25°, for

the molecular rotation of L-aminophenylacetic acid for a 1 per cent solution in 1 N NaOH is +187.5°. No change in this value was observed after the solution had stood for 10 hours at 25°.

It is difficult to know just how much water is present in the glacial acetic acid employed as the solvent in these studies, but the effect of added water was investigated as follows. 1.0 per cent solutions of L-leucine were prepared in glacial acetic acid, in 100, 99, 98, 97, 96, 95, and 90 per cent acetic acid solutions. The molecular rotations noted were, respectively, as follows: +29.5°, +29.5°, +29.5°, +29.0°, +22.0°, +21.0°, and +21.0°. L-Aminophenylacetic acid in 90 per cent acetic acid gave close to the same molecular rotation value as in 5 N HCl, namely +250° (Table I).

The above considerations have been presented at some length because of the care needed when the use of a relatively new solvent for the optical characterization of the amino acids is suggested. It will be noted from Table I that the magnitude of the molecular rotation values for many of the amino acids is higher in glacial acetic acid than in HCl solutions. In these cases it might be more convenient and economical of material to use the former solvent. A few amino acids such as serine, cystine, homocystine, the dicarboxylic amino acids, the hydrochlorides of the diamino acids, and tyrosine are too insoluble in glacial acetic acid. Attempts to measure the optical rotation of amino acids in glacial propionic acid failed because of their very low solubility in this solvent.

Optical Rotation of Glycyl-L-amino Acids—As first pointed out by Lutz and Jirgensons (43), a general characteristic of the L-amino acids is their possession of a more positive optical rotation value in HCl than in water solutions. Two exceptions to this observation are L-isovaline and the *threo* isomer of β-phenyl-L-serine (39). If this comparison is based upon the rotation values in water and in glacial acetic acid, it is noted that three amino acids, namely L-threonine, L-tryptophan, and hydroxy-L-proline, have each practically the same molecular rotation value in both solvents, and that all the rest, now including L-isovaline but still excluding the *threo* isomer of β-phenyl-L-serine, have more positive rotations in the latter solvent.

When the optical rotations of a series of glycyl-L-amino acids (37, 44) are compared in water, in HCl, and in glacial acetic acid solutions (Table II), it is observed that the values for some of the compounds are more positive in HCl than in water solution, whereas for others the reverse is the case. However, when this comparison is based upon the rotation values in water and in glacial acetic acid, *all* of the peptides studied possess a more positive rotation in the latter solvent than in the former (Table II). It would appear that the Lutz-Jirgensons "rule" may with more general applicability be employed to characterize the optically active amino acids

in water and in glacial acetic acid rather than in water and in HCl solutions.[9]

The effect of introducing a glycyl radical in the acyl position of various L-amino acids on the molecular rotation values in the three different sol-

TABLE II

Molecular Rotations of Glycyl-L-amino Acids in Different Solvents

Peptide	Mol. wt.	$[M]_D$ in*			$[M]_D$ of L-amino acid in H_2O†
		H_2O	5 N HCl	Glacial acetic acid	
Glycyl-L-alanine	146.1	−74.5	−92.0	−58.4	+2.4
Glycyl-L-aminobutyric acid	160.1	−49.6	−60.0	−24.0	+9.6
Glycyl-L-valine	174.1	−34.6	−21.8	+4.4	+7.6
Glycyl-L-norvaline	174.1	−47.9	−57.5	−20.9	+8.8
Glycyl-L-isovaline	174.1	0	−12.4	+8.7	+13.1
Glycyl-L-leucine	188.1	−68.3	−68.7	−56.4	−14.4
Glycyl-L-norleucine	188.1	−30.1	−42.7	−15.0	+9.8
Glycyl-L-isoleucine	188.1	−26.5	−5.6	+32.0	+17.7
Glycyl-L-alloisoleucine	188.1	−9.8	0	+28.2	+23.6
Glycyl-L-serine	162.1	−11.7	+13.8	‡	−7.9
Glycyl-L-threonine	176.1	−28.5	+5.3	+10.6	−33.9
Glycyl-L-methionine	206.2	−21.0	−47.2	−6.8	−14.9
Glycyl-L-aspartic acid	190.1	+23.8	+9.5	‡	+7.3
Glycyl-L-glutamic acid	204.2	−13.9	−28.6	+20.4	+21.3
Glycyl-L-phenylalanine	222.2	+92.2	+63.3	+127.8	−57.0
Glycyl-L-tyrosine	238.2	+104.8	+76.2	+142.9	‡
Glycyl-L-tryptophan	261.2	+35.3	+89.6	+150.2	−68.8

* Concentrations 0.5 to 2.0 per cent. All the compounds readily dissolved at 25°; temperature 24–26°. A 2 d.cm. tube was employed.

† Concentrations, 1.0 to 5.0 per cent.

‡ The compounds were too insoluble.

vents has been calculated by subtracting the values for the amino acids from those of the peptide while maintaining the signs (Table III). There is a fair agreement among the values in the three solvents for each of the

[9] The molecular rotation values at 25° in water, 5 N HCl, and glacial acetic acid are, respectively, for glycylglycyl-L-alanine −73.8, −91.8, and −40.6, and for glycyl-L-alanylglycine −129.4, −116.8, and −101.6. Both compounds have a more positive rotation in glacial acetic acid solution than in water. However, when L alanine is the acyl group, the reverse holds, for the molecular rotations in the same order are for L-alanylglycine +73.7, +18.3, and +36.5, and for L-alanylglycylglycine +74.4, +63.0, and +71.1. Whether the higher peptides of other amino acids possess the same characteristics as those of L-alanine must await further investigation. Certain of these peptides of L-alanine were generously donated by Dr. E. Brand, who had made an extensive series of studies related to this problem (45–47).

unsubstituted amino acids, *i.e.*, the first nine in Table III which have only an α-amino, α-carboxyl, and aliphatic hydrocarbon side chain. All possess a negative sign, and, with the exception of isovaline, the absolute values are not far from the same order of magnitude in all three solvents. With β substitution of the amino acid residues, the agreement in the different solvents, with the exception of serine, is not good, and, in the case of threonine and the aromatic ring-substituted compounds, the sign of the difference values is positive. In the case of aspartic acid, this sign is positive in

TABLE III

Effect of Substitution of Glycyl Residue in L-*Amino Acids on* $[M]_D$* *in Various Solvents*

L-Amino acid	H_2O	HCl	Glacial acetic acid
Alanine	−77	−105	−87
Aminobutyric acid	−60	−81	−67
Valine	−42	−54	−68
Norvaline	−57	−87	−62
Isovaline	−13	−22	−17
Leucine	−54	−90	−87
Norleucine	−40	−75	−63
Isoleucine	−44	−60	−32
Alloisoleucine	−33	−53	−28
Serine	−3	−2	
Threonine	+5	+23	+46
Methionine	−6	−81	−37
Aspartic acid	+17	−24	
Glutamic "	−35	−75	
Phenylalanine	+149	+70	+140
Tyrosine		+94	
Tryptophan	+104		+219

* The figures in the table are based upon the equation $[M]_D$ for the glycyl-L-amino acid minus $[M]_D$ for the L-amino acid, the signs being maintained throughout. The values are based on Tables I and II.

water and negative in HCl solutions. It is therefore possible to state, tentatively, that only for the simpler, aliphatic, monoaminomonocarboxylic acids does substitution of a glycyl residue on the α-amino group produce nearly the same effect, both in sign and in order of magnitude, on the molecular rotation values in each of the three solvents studied.

Action of L- *and of* D-*Amino Acid Oxidases on Amino Acid Optical Isomers*—The action of L- and D-amino acid oxidase preparations on the various L and D isomers is described in Table I. The former enzyme was employed as follows. 10 μM of the substrate at pH 7.2 (5 μM in the cases of cystine and homocystine), dissolved or suspended in 2 ml. of 0.1 M tris(hydroxymethyl)aminomethane buffer, pH 7.2, and 0.1 ml. of catalase

solution containing approximately 0.6 unit were added. The side arm contained 0.3 ml. of enzyme solution, prepared by dissolving the appropriate amount of rattlesnake venom (*Crotalus adamanteus*) in 0.2 M tris(hydroxymethyl)aminomethane buffer at pH 7.2. The center well contained filter paper soaked in 20 per cent KOH. The D-amino acid oxidase (12) was employed as follows. 10 μM of the substrate (5 μM in the cases of cystine and homocystine) were dissolved or suspended in 2 ml. of 0.05 M sodium pyrophosphate buffer at pH 8.2. The side arm contained 0.4 ml. of hog kidney D-amino acid oxidase preparation (12). Again the center well contained KOH. With both enzymes, the atmosphere above the reaction mixture was air, each compound was studied at least in triplicate, and the oxidative rates, expressed in terms of micromoles of oxygen consumed per hour per mg. of protein N, were determined graphically from the initial linear relation between time and oxidation. The rates so obtained were standardized by the inclusion of L-methionine in the L-amino acid oxidase and of D-alanine in the D-amino acid oxidase runs.[10] In all cases the values were corrected for the enzyme blanks, which generally were very small. The long chain α-amino acids, from α-aminoheptylic to α-aminododecylic, were very insoluble and difficult to wet, and in these cases a few drops of Tween 20 solution (polyethylene sorbitan monolaurate) were added to disperse the compounds. Tween itself had no effect on either the L- or the D-amino acid oxidase preparations.

Considering first the action of L-amino acid oxidase, many amino acids, reported by Bender and Krebs (6) to be resistant to cobra venom, are found to be oxidizable by rattlesnake venom (Table I). These include alanine, α-aminobutyric acid, α-aminoundecylic acid, α-aminododecylic acid, valine, isoleucine, aspartic acid, glutamic acid, α-aminoadipic acid, histidine, ornithine, lysine, and cystine. While some amino acids in this list are relatively weakly oxidized by the rattlesnake venom preparation, others, like isoleucine, α-aminoadipic acid, histidine, and cystine, are very appreciably oxidized (Table I). The experimental conditions employed by Bender and Krebs and those employed in the present report are, however, quite different, and any extensive comparison of the respective results would not be warranted.

Under the experimental conditions employed in Table I, the oxidation of L-isoleucine is considerably greater than that of L-alloisoleucine, and L-allothreonine is more readily oxidized than is L-threonine. The *erythro*

[10] The considerably lower value for the oxidation rate of L-methionine by rattlesnake venom report d earlier (32) was very probably due to the use of phosphate buffer which, as shown by Kearney and Singer (48), has an inhibitory effect on the L-amino acid oxidase activity from this source. The appreciable rate of oxidation of D-aspartic acid by the hog kidney D-amino acid oxidase preparation (Table I) may be due to the presence of some D-aspartic acid oxidase (49).

form of β-phenylserine is oxidized at a rate some 200-fold that of the corresponding *threo* form. None of the L-proline derivatives are affected by the venom oxidase. However, these data, together with the remainder in Table I, are obtained under the conditions specified, and different relative results may be observed when the conditions are altered. Thus, when the substrate concentrations of the following amino acids were raised 5-fold, *e.g.* to 50 μM, while all the other conditions remained the same, the following rates of oxidation were obtained: L-isoleucine 117, L-alloisoleucine 14, L-threonine 0.2, L-allothreonine 3.7, L-α,β-diaminopropionic acid 0.5, and

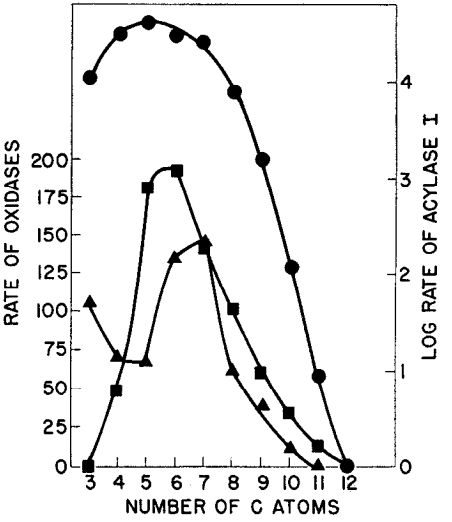


FIG. 1. The effect of increasing straight side chain length for the homologous series of amino acids from alanine to α-aminododecylic acid. ■, rates of oxidation with *Crotalus adamanteus* L-amino acid oxidase on the L isomers; ▲, rates of oxidation × 10 with hog kidney D-amino acid oxidase on the D isomers; and ●, log rate of hydrolysis with hog kidney acylase I of the *N*-chloroacetyl-L-amino acids (36).

L-serine 0.5, in terms of micromoles of oxygen absorbed per hour per mg. of N. Thus in all cases the absolute magnitude of the rates was increased with the higher substrate concentrations, and, in the case of the isoleucine diastereoisomers, the ratio of the oxidative rates of L-isoleucine to L-alloisoleucine, instead of being about 90:1 with 10 μM of each substrate, was now about 8:1. When phosphate buffer was used in place of the tris(hydroxymethyl)aminomethane buffer at pH 7.2, the following rates with 50 μM each of the amino acids (with all other conditions the same) were obtained: L-isoleucine 46 and L-alloisoleucine 14. In the presence of phosphate as contrasted with the "tris" buffer, a considerable inhibition in the oxidative rate of L-isoleucine was observed, whereas the oxidative rate of L-alloisoleucine was unchanged.

A lesser effect was noted on increasing the substrate concentrations to 50 μM in the presence of the D-amino acid oxidase preparation. Under such conditions the oxidative rates were D-isoleucine 13.5, D-alloisoleucine 8.7, D-aminoadipic acid 0.6, D-threonine 0.6, and D-allothreonine 4.5. Again, the rates for the diastereoisomers of isoleucine were closer in value at the higher substrate concentration, and a small but definite oxidative rate was noted with D-aminoadipic acid and D-threonine.

Upon consideration of the straight chain α-amino acid homologues from L-alanine to L-α-aminododecylic acid, there appears to be a maximal rate of oxidation at L-α-aminocaproic acid (norleucine) (Fig. 1). Plotted in the same figure are the hydrolytic rates with acylase I of the corresponding *N*-chloroacetyl-L-amino acids, from chloroacetyl-L-alanine to chloroacetyl-L-α-aminododecylic acid, and it is noted that there is a maximal rate likewise in the same general region (20, 36). With D-amino acid oxidase, the oxidative rate decreases upon passing from the C_3- to the C_4- and C_5-amino acids, rises progressively to the C_6- and C_7-amino acids, and thereafter progressively diminishes to D-α-aminododecylic acid which, under the conditions employed, is apparently inert (Table I; Fig. 1).

SUMMARY

The L and D isomers of forty-six amino acids of high optical purity have been characterized by their molecular rotations in 5 N HCl and in glacial acetic acid solutions, and by their susceptibility to *Crotalus adamanteus* L-amino acid oxidase and hog kidney D-amino acid oxidase, respectively.

In many cases, the absolute magnitude of the optical rotations was higher in glacial acetic acid than in 5 N HCl. All of the amino acids studied were optically stable in glacial acetic acid solutions, except for aminophenylacetic acid which racemized under conditions in which its saturated analogue, aminocyclohexylacetic acid, retained its optical activity.

L-Threonine, L-tryptophan, and hydroxy-L-proline have the same molecular rotations and the same sign in water and in glacial acetic acid solutions. All of the other L-amino acids studied, including L-isovaline and excluding only the *threo* isomer of β-phenyl-L-serine, have more positive rotations in the latter solvent. Determination of the molecular rotations of several glycyl-L-amino acids revealed that, whereas some of them possessed more positive values in HCl than in water solution while with others the reverse was the case, all of them possessed more positive values in glacial acetic acid solution than in water. The effect on the molecular rotation of the L-amino acid residue by introduction of the *N*-glycyl radical has been calculated for three different solvents.

The rates of oxidation of the L and D isomers of the various amino acids have been reported under specified conditions. The effect of alteration of the substrate concentration in a few cases is described. Among the

homologous series of straight chain aliphatic α-amino acids from C_3 to C_{12}, an optimal rate of oxidation by L-amino acid oxidase occurs at about C_6. A comparison with a similar optimum, which occurs in the relative rates of hydrolysis of the *N*-acylated derivatives of these same L-amino acids by acylase I, has been drawn.

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