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Syntheses of Optically Active α-Amino Acids from Esters of α-Keto Acids by Hydrogenolytic Asymmetric Transamination, A Solvent Effect[1]

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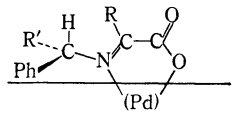
Optically active α-amino acids were synthesized from esters of α-keto acid azomethines by catalytic hydrogenation. Solvent effect of the reaction was studied and the possible steric courses are discussed.

Several studies on the asymmetric syntheses of α-amino acids from their corresponding α-keto acids have been reported.[2-10] Hiskey and Northrop[4] demonstrated the synthesis of optically active amino acids by catalytic hydrogenation and subsequent hydrogenolysis of the Schiff bases of α-keto acids with optically active α-methylbenzylamine (hydrogenolytic asymmetric transamination). The steric course of the hydrogenolytic asymmetric transamination reactions has been studied in this laboratory.[8,9] It was proposed that the possible conformation of the substrate in the catalytic hydrogenation could be a five-membered cyclic complex with the catalyst, as shown in structure I.[8] The



I

proposed intermediate I was supported by the study of a solvent effect of the asymmetric synthesis.[9]

In this investigation, the Schiff bases of α-keto acid esters with optically active amines were subjected to hydrogenation in order to examine the existence of structure I type chelated intermediates in these reactions. α-Keto acids used were ethyl pyruvate and ethyl α-ketobutyrate. Optically active amines used were (S)(−) and (R)(+)-α-methylbenzylamine, (R)(+)-α-ethylbenzylamine, and (R)(+)-1-(1-naphthyl)ethylamine. Solvents used in these reactions were benzene, ethyl acetate, isopropyl alcohol, ethanol, and methanol. Results are summarized in Table 1.

In each six sets of reactions, a solvent effect of the asymmetric synthesis was examined by the use of methanol, ethanol, propanol-2, ethyl acetate, and benzene (dielectric constant ranging from 32.6 to 2.3; Table 1). It was found that when a polar solvent was used, the optical purity of the resulting amino acid was lower and when less polar solvent was used the optical purity increased steadily. These solvent effects in the reactions suggest that the conformations of the substrate molecule changed depending on the solvent used. These results are similar to those obtained in the study of solvent effect in the hydrogenolytic asymmetric transamination between α-keto acids and optically active amine.[9] Therefore, similar conformation of the substrate in the hydrogenolytic asymmetric transamination between optically active amine and α-keto acid ester could be assumed as shown in structure II. Because electrostatic attraction between substrate and catalyst in a less polar solvent is stronger than in a polar solvent and also the solvation of the substrate in the less polar solvent is weak, so that the substrate could react easily with the catalyst to form the intermediate complex II. On the other hand, when polar solvent was used, electrostatic attraction between substrate and catalyst was weaker and the stronger solvation of the

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8) K. Harada and K. Matsumoto, *J. Org. Chem.*, **32**, 1794 (1967).

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TABLE 1. AMINO ACIDS OBTAINED BY HYDROGENOLYTIC ASYMMETRIC TRANSAMINATION BETWEEN ESTERS OF α-KETO ACID AND OPTICALLY ACTIVE AMINES

Amino acid		Keto acid ester[a]	Amine[b]	Solvent	Catalyst	Yield (%)	Config'n	Free amino acid		DNP-Amino acid	
								$[\alpha]_D^{25}$ 5N HCl	Optical purity (%)[d]	$[\alpha]_D^{25}$ 1N NaOH	Optical purity (%)[e]
Ala	1	Et-Py	(R) (+)-Me	MeOH	$Pd(OH)_2/C$	74	R	−3.1	21	−43.9	31
	2	Et-Py	(R) (+)-Me	EtOH	$Pd(OH)_2/C$	77	R	−4.7	32	−56.0	39
	3	Et-Py	(R) (+)-Me	i-PrOH	$Pd(OH)_2/C$	73	R	−4.7	32	−67.3	47
	4	Et-Py	(R) (+)-Me	AcOEt	$Pd(OH)_2/C$	78	R	−6.5	44	−88.7	62
	5	Et-Py	(R) (+)-Me	Benzene	$Pd(OH)_2/C$	70	R	−7.5	52	−80.8	56
	6	Et-Py	(S) (−)-Me	MeOH	$Pd(OH)_2/C$	75	S	+4.5	31	+46.4	32
	7	Er-Py	(S) (−)-Me	EtOH	$Pd(OH)_2/C$	74	S	+5.9	40	+59.4	41
	8	Et-Py	(S) (−)-Me	i-PrOH	$Pd(OH)_2/C$	79	S	c	c	+64.5	45
	9	Et-Py	(R) (−)-Me	AcOEt	$Pd(OH)_2/C$	73	S	+5.6	39	+91.8	64
	10	Et-Py	(S) (−)-Me	Benzene	$Pd(OH)_2/C$	75	S	+6.7	46	+82.5	57
	11	Et-Py	(R) (+)-Et	MeOH	$Pd(OH)_2/C$	83	R	−1.5	10	−21.0	15
	12	Et-Py	(R) (+)-Et	EtOH	$Pd(OH)_2/C$	82	R	−2.0	14	−33.5	23
	13	Et-Py	(R) (+)-Et	i-PrOH	$Pd(OH)_2/C$	80	R	−3.3	23	−46.3	32
	14	Et-Py	(R) (+)-Et	AcOEt	$Pd(OH)_2/C$	77	R	−7.0	48	−80.8	56
	15	Et-Py	(R) (+)-Et	Benzene	$Pd(OH)_2/C$	73	R	−5.9	41	−72.6	50
	16	Et-Py	(R) (+)-Naph	MeOH	$Pd(OH)_2/C$	83	R	−7.0	49	−82.8	58
	17	Et-Py	(R) (+)-Naph	EtOH	$Pd(OH)_2/C$	82	R	−8.2	56	−93.2	65
	18	Et-Py	(R) (+)-Naph	i-PrOH	$Pd(OH)_2/C$	c	R	−7.3	50	−95.3	66
	19	Et-Py	(R) (+)-Naph	AcOEt	$Pd(OH)_2/C$	68	R	−7.9	54	−118.0	82
	20	Et-Py	(R) (+)-Naph	Benzene	$Pd(OH)_2/C$	70	R	−8.7	60	−100.7	70
	21	Et-Py	(S) (−)-Me	MeOH	Pd/C and $Pd(OH)_2/C$	64	S	+5.1	35	+69.1	48
	22	Et-Py	(S) (−)-Me	EtOH	Pd/C and $Pd(OH)_2/C$	62	S	+4.7	32	+73.2	51
	23	Et-Py	(S) (−)-Me	i-PrOH	Pd/C and $Pd(OH)_2/C$	65	S	+5.5	38	+78.6	55
	24	Et-Py	(S) (−)-Me	AcOEt	Pd/C and $Pd(OH)_2/C$	62	S	+6.4	44	+87.4	61
	25	Et-Py	(S) (−)-Me	Benzene	Pd/C and $Pd(OH)_2/C$	57	S	+5.8	40	+94.2	65
α-NH_2-Bu	26	Et-Bu	(S) (−)-Me	MeOH	Pd/C and $Pd(OH)_2/C$	51	S	+5.5	27	+27.9	28
	27	Et-Bu	(S) (−)-Me	EtOH	Pd/C and $Pd(OH)_2/C$	45	S	+5.5	27	+30.6	31
	28	Et-Bu	(S) (−)-Me	i-PrOH	Pd/C and $Pd(OH)_2/C$	43	S	+5.7	28	+33.5	34
	29	Et-Bu	(S) (−)-Me	AcOEt	Pd/C and $Pd(OH)_2/C$	42	S	+5.7	28	+37.6	38
	30	Et-Bu	(S) (−)-Me	Benzene	Pd/C and $Pd(OH)_2/C$	39	S	+8.3	40	+45.3	46

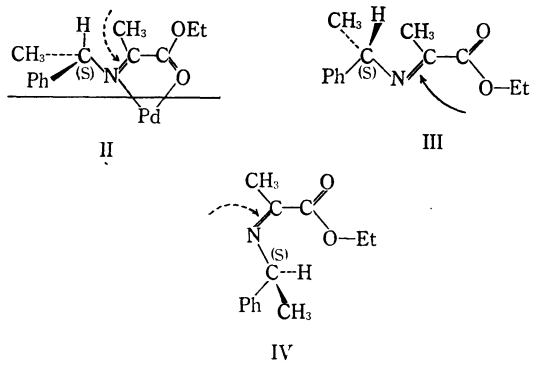
a) Et-Py: ethyl pyruvate; Et-Bu: ethyl α-ketobutyrate.

b) (S) (−)-Me, (S) (−)-α-methylbenzylamine; R(+)-Me, (R) (+)-α-methylbenzylamine; (S) (−)-Et, (S) (−)-α-ethylbenzylamine; R(+)-Et, (R) (+)-α-ethylbenzylamine; R(+)-Naph, (R) (+)-1-(1-naphthyl)ethylamine.

c) The samples are lost.

d) Defined as $[\alpha]_D$ obsd/$[\alpha]_D$ lit × 100. (S)-Alanine, $[\alpha]_D^{25}$ +14.6° (5N HCl); (S)-α-Amino-*n*-butyric acid, $[\alpha]_D^{25}$ +20.6° (5N HCl). J. P. Greenstein and Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley & Sons, Inc., New York, N. Y. (1961) (alanine, p. 1819; α-amino-*n*-butyric acid, p. 2401).

e) Defined as $[\alpha]_D$ obsd/$[\alpha]_D$ lit × 100. DNP-(S)-ala, $[\alpha]_D$ +143.9° (1N NaOH); DNP-(S)-α-NH_2-But, $[\alpha]_D$ +98.8° (1N NaOH). K. R. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, **76**, 1328 (1954).



substrate interfered with the attraction between substrate and catalyst to form the intermediate complex II. Therefore, it could be assumed that the conformation of substrate in polar solvent could be more in the form of structures III and IV. Structure IV is rather sterically hindered so that the structure III could be increased in proportion by the use of polar solvent. Structures II and III would be adsorbed at the less bulky side of the molecule and the hydrogenation takes place. When (S)-amine was used, (S)- and (R)-amino acid would be expected from structures II and III respectively. Therefore the use of polar solvent increases the proportion of structure III, and this would result in the formation of amino acid of lower optical purity. From the results obtained, structure II could be the major conformation throughout the reactions.

Figure 1 shows the relationship between the dielectric constants of the solvents used and the optical activities of synthesized amino acids. The effect of the catalyst, palladium hydroxide on charcoal or palladium on charcoal, was observed. By the use of palladium hydroxide on charcoal,[4] optical activity of amino acids dropped when benzene was used as a solvent (Fig. 1). Optical activities of amino acids increased rapidly when less polar solvents were used. On the other hand, by the use of palladium on charcoal optical activities of amino acids obtained increased slowly in the less polar solvents. A decrease in optical activity of amino acid by the use of benzene was not observed. High optical activity of amino acid (82%) was observed when R(+)-1-(1-naphthyl)-ethylamine was used. The reason for this is understandable from the steric effect in the intermediate complex II.

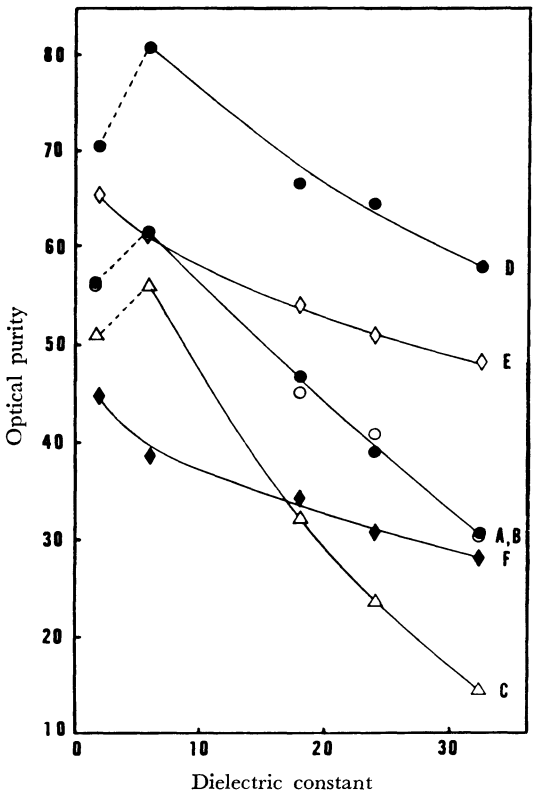
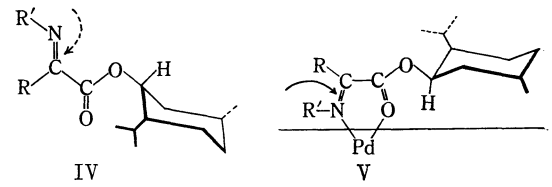


Fig. 1. Relationship between optical activities of synthesized amino acids and dielectric constants of the solvents used.
A. ala, reactions 1—5 (see Table 1)
B. ala, reactions 6—10
C. ala, reactions 11—15
D. ala, reactions 16—20
E. ala, reactions 21—25
F. α-NH_2-Bu, reactions 26—30

In the previous studies, hydrogenation of oximes and Schiff bases of α-keto acid *l*-menthyl esters has been studied. It was discussed that the conformation of the substrate might be transoidal, as shown in structure IV.[6,8] From the results obtained in the present investigation, it could also be possible



to explain the steric course by a cisoidal conformation which forms chelate intermediate with the catalyst as shown in structure V. The menthyl group would be situated as in structure V because of the steric hindrance between menthyl residue and catalyst. Then, the structure V could be adsorbed on the catalyst at the less bulky side of the molecule, and hydrogenation would take place. From structure V, R-amino acid would be expected, which agrees with the experimental results.[6]

In order to examine the newly postulated intermediate (V) in the hydrogenation of α-keto acid *l*-menthyl ester oxime, a similar study of solvent effect was carried out. The substrates used were *l*-menthyl pyruvate oxime, *l*-menthyl α-ketobutyrate oxime, and the Schiff base of *l*-menthyl pyruvate

TABLE 2. AMINO ACIDS OBTAINED FROM α-KETO ACID MENTHYL ESTERS[a)]

Amino acid		Keto acid esters[b)]	Solvent	Yield[c)] (%)	Config'n	Free amino acid		DNP-Amino acid	
						$[\alpha]_D^{25}$ (5N HCl)	Optical purity (%)[d)]	$[\alpha]_D^{25}$ (1N NaOH)	Optical purity (%)[e)]
Ala	31	Py-M-OX	MeOH	87	R	−2.6	18	−28.2	20
	32	Py-M-OX	EtOH	82	R	−2.5	17	−31.3	22
	33	Py-M-OX	i-PrOH	73	R	−2.4	17	−23.6	16
	34	Py-M-OX	AcOEt	74	R	−2.5	17	−28.1	20
	35	Py-M-OX	Benzene	75	R	−3.4	23	−32.2	22
α-NH_2-Bu	36	Bu-M-OX	MeOH	85	R	−3.3	16	−16.4	17
	37	Bu-M-OX	EtOH	71	R	−3.6	18	−22.4	23
	38	Bu-M-OX	i-PrOH	81	R	−2.7	13	−15.4	16
	39	Bu-M-OX	AcOEt	54	R	−3.4	17	−17.2	17
	40	Bu-M-OX	Benzene	60	R	−4.2	20	−22.2	23
Ala	41	Py-M-(R)-Me	MeOH	46	R	−8.1	55	−82.0	57
	42	Py-M-(R)-Me	EtOH	51	R	−8.1	56	−84.5	59
	43	Py-M-(R)-Me	i-PrOH	50	R	−7.5	52	−65.5	46
	44	Py-M-(R)-Me	AcOEt	48	R	−7.9	54	−66.7	46
	45	Py-M-(R)-Me	Benzene	47	R	−8.6	59	−88.8	62

a) All hydrogenation reactions were carried out by the use of 5% palladium on charcoal.
b) Py-M-OX: *l*-menthyl pyruvate oxime; Bu-M-OX: *l*-menthyl α-ketobutyrate oxime; Py-M-(R)-Me: Schiff base of *l*-menthyl pyruvate with R(+)-α-methylbenzylamine.
c) Reactions 31—40: the yields are calculated from oximes of α-keto acid menthyl esters. Reactions 41—45: the yields are calculated from *l*-menthyl pyruvate.
d) Defined as $[\alpha]_D$ obsd/$[\alpha]_D$ lit × 100. (S)-Alanine, $[\alpha]_D^{25}$ +14.6° (5N HCl); (S)-α-amino-*n*-butyric acid, $[\alpha]_D^{25}$ +20.6° (5N HCl). J. P. Greenstein and Winitz, "Chemistry of the Amino Acid," Vol. 3, John Wiley & Sons, Inc., New York, N. Y. (1961) (alanine, p. 1819; α-amino-*n*-butyric acid, p. 2401).
e) Defined as $[\alpha]_D$ obsd/$[\alpha]_D$ lit × 100. DNP-(S)-ala, $[\alpha]_D$ +143.9° (1N NaOH); DNP-(S)-α-NH_2-Bu, $[\alpha]_D$ +98.8° (1N NaOH). K. R. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, **76**, 1328 (1954).

with (R)(+)-α-methylbenzylamine. Results are summarized in Table 2.

However, in these reactions, the solvent effect observed was rather small compared with that of the previous study[9)] or with the results summarized in Table 1. Also observed was the discontinuation of optical purities of amino acids in these series of reactions (Fig. 2). The optical purities increased by the use of ethanol compared to that use of methanol. However, optical purities dropped by the use of isopropyl alcohol and increased steadily by the use of less polar solvent. The factors which affect the optical purity of amino acids in these reactions seem to be complex and it is difficult to explain these results at the present time. However, from the results obtained from the hydrogenolytic transamination summarized in Tables 1 and 2 the existence of intermediate complex structure V seems likely.

On the other hand, catalytic reduction of α-keto acid esters with optically active alcohols has been discussed and the reduction was found to follow the Prelog rule.[11)] The results obtained in the present study suggest that the formation of α-hydroxy acid from α-keto acid ester with optically active alcohol by catalytic hydrogenation could also be explained by the chelated intermediate hypothesis.[8,9)] The study on the synthesis of optically active α-hydroxy acid from α-keto acid amide with optically active amine also supports the chelation hypothesis.[12)]

Optically active free amino acids were obtained by the use of a Dowex 50 column. However, these amino acids still contain some impurities. To avoid fractionation of optically active isomers during the purification process, all amino acids were treated with 2,4-dinitrofluorobenzene.[13)] The resulting DNP-amino acids were purified by the use of celite column[14)] treated with pH 7.0 citrate buffer without fractionation of partially optically active amino acids. Therefore, the values of optical purities of DNP-amino acids are more reliable and consistent (Table 1).

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12) K. Harada and K. Matsumoto, Presented at the 1st American Peptide Symposium at Yale University (1968).

13) F. Sanger, *Biochem. J.*, **39**, 507 (1945); K. Rao and H. A. Sober, *J. Amer. Chem. Soc.*, **76**, 1328 (1954).

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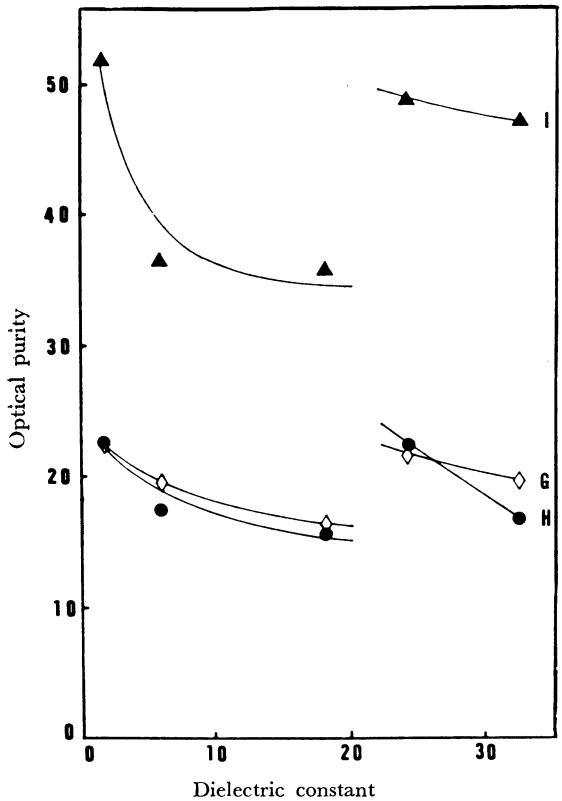

Fig. 2. Relationship between optical activity of synthesized amino acids and dielectric constants of the solvents used.
G. ala, reactions 31—35 (see Table 2)
H. α-NH_2-Bu, reactions 36—40
I. ala, reactions 41—45

Experimental[15]

Alanine from Ethyl Pyruvate and S (—)-α-Methylbenzylamine. Ethyl pyruvate (1.16 g, 0.01 mol) and S(—)-α-methylbenzylamine (1.21 g, 0.01 mol) were dissolved in 20 m*l* of benzene at room temperature. After about ten minutes the solution became cloudy by separation of water. Anhydrous sodium sulfate was added and the mixture was stirred for one hour at room temperature. Sodium sulfate was removed by filtration and the sodium sulfate was washed with benzene. The combined benzene solution was evaporated to dryness under reduced pressure on a water bath (45°C). A light yellow oil was obtained. This was dissolved in 30 m*l* of methanol and the solution was subjected to hydrogenation and hydrogenolysis by the use of 1.0 g of palladium hydroxide on charcoal[4] for 12 hr. The first mole of hydrogen was absorbed in a few minutes. However, the second mole of hydrogen for hydrogenolysis required a much longer time. After the reaction was over, the catalyst was removed by filtration. The catalyst was washed with hot methanol several times. The combined methanol solution was evaporated under reduced pressure at 40°C. The residue was hydrolyzed under reflux with 60 m*l* of 3N hydrochloric acid for 4 hr. The hydrolyzate was extracted once with ether and the hydrochloric acid solution was evaporated to dryness under reduced pressure. Water was added to the residue and it was evaporated again to minimize the remaining free hydrochloric acid. The residual amino acid hydrochloride was dissolved in 10 m*l* of water and the solution was applied to a Dowex 50×2 column (hydrogen form, 100—200 mesh, 25×1.8 cm). The acidic component was washed with water, then alanine was eluted with 1N aqueous ammonia. Fractions containing alanine were combined and were evaporated to dryness. Free alanine, 0.67 g, was obtained. This is pure on paper chromatograph. $[\alpha]_D^{25}=+4.5°$ (*c* 4.0, 5N HCl), optical purity 31%. After one recrystallization with water and ethanol, elemental analysis was carried out.

Found: C, 40.65; H, 8.01; N, 15.58%. Calcd for $C_3H_7ON_2$: C, 40.44; H, 7.92; N, 15.72%.

A part of the unrecrystallized alanine (100 mg) was converted to DNP-alanine in a conventional manner.[13] DNP-Alanine obtained was purified by the use of celite column treated with pH 7 citrate buffer (0.2M).[14] These procedures are similar to those described in earlier reports.[6,8]

DNP-Alanine, mp 172—174°C dec., $[\alpha]_D^{25}=+46.4°$ (*c* 1.1, 1N NaOH), optical purity 32%.

Found: C, 42.42; H, 3.66; N, 16.64%. Calcd for $C_9H_9N_3O_6$: C, 42.36; H, 3.55; N, 16.47%.

Other experiments were carried out in the same way as described above. Results are summarized in Table 1.

Amino Acids from α-Keto Acid *l*-Menthyl Ester. The experimental procedures for the synthesis of optically active amino acids from α-keto acid *l*-menthyl esters were similar to those already described in the earlier papers[6,8] except for the use of different solvents. Alanine and α-aminobutyric acid obtained by saponification of menthyl esters were converted to their DNP-derivatives. These DNP-amino acids were similarly purified by the use of celite column.[14] Results are summarized in Table 2.

This work was supported by grant number NGR 10-007-052 from the National Aeronautics and Space Administration.

15) All hydrogenations and hydrogenolyses were carried out by the use of the Parr 3910 shaker type hydrogenation apparatus. All optical rotations were measured by the use of Durrum-JASCO ORD/UV-5 automatic spectropolarimeter. Some of the optical rotations of free amino acids were measured by the use of the Rudolph model 80 polarimeter with a PEC-101 photometer.