heating a solution of 40 mg of phyllanthane in 10 ml of acetic acid and 1 ml of concentrated hydrochloric acid at reflux temperature. The cooled reaction was poured into water, extracted with ether, and the ethereal extract was washed with water, 5% sodium bicarbonate, and water. After drying over anhydrous magnesium sulfate, the solvent was evaporated under reduced pressure to afford an oil which was chromatographed over 1 g of neutral alumina, activity II. Elution with *n*-hexane gave 15 mg of Δ^{12}-ursene (XLI), mp 100–105° after recrystallization from methanol–chloroform.

Stereoselective Syntheses of Optically Active Amino Acids from Menthyl Esters of α-Keto Acids[1]

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Menthyl esters of pyruvic acid, α-ketobutyric acid, and phenylglyoxylic acid were converted to their oximes and Schiff bases of benzylamine. These were hydrogenated catalytically by the use of palladium on charcoal and palladium hydroxide on charcoal. Optically active D-alanine (optical yield 16–25%), D-α-aminobutyric acid (8–21%), and D-phenylglycine (44–49%) were obtained. Possible steric courses of the reactions are discussed.

l-Menthol has been used as an optically active moiety in many types of stereochemical studies.[2] In previous studies the menthyl esters of various α-amino acids have been synthesized by the azeotropic method[3] and from α-amino acid N-carboxyanhydrides.[4]

In this study, the N-hydroxyimino (oxime) and N-benzylimino (benzylamine Schiff base) derivatives of menthyl pyruvate, menthyl α-ketobutyrate, and menthyl phenylglyoxylate were synthesized. These were hydrogenated and hydrogenolyzed by the use of palladium on charcoal (catalyst A) or palladium hydroxide on charcoal[5] (catalyst B) to yield the menthyl esters of alanine, α-amino-*n*-butyric acid, and of phenylglycine. These asymmetrically synthesized menthyl esters of amino acids were saponified by alkali in aqueous alcohol.[6] The liberated, free amino acids were isolated and their optical activities measured to determine the optical yield. However, the isolation and recrystallization procedure resulted in fractionation of the optically active amino acids. The specific rotation of amino acids decreased upon recrystallization and finally the value reached zero after several purification procedures, To avoid the fractionation and to determine the accurate optical purity of the synthesized amino acids, a part of the hydrolyzed reaction mixture was directly treated with 1-fluoro-2,4-dinitrobenzene to yield dinitrophenylamino acids.[7] The resulting DNP-amino acids were isolated chromatographically by the use of a Celite column treated with pH 7 phosphate–citrate buffer.[8] The DNP-amino acids thus obtained were analytically pure without further purification. An advantage of the DNP method was to avoid fractionation completely during the isolation and purification procedures.

Table I shows the summarized results which were obtained by the catalytic hydrogenation procedure. Partially optically active (8–49%) D-amino acids were obtained. However, the optical activities of the amino acids prepared by the use of palladium on charcoal (catalyst A) from the Schiff base of pyruvate and of α-ketobutyrate were found to be zero. The optical activity of α-aminobutyric acid prepared by the use of palladium hydroxide on charcoal (catalyst B) was also zero; however, the DNP-α-aminobutyric acid showed optical activity, $[\alpha]^{25}D$ −7.3°. The latter case can be explained by the fractionation of the product during the isolation procedure. The results show that the hydrogenation reaction of hydroxyimino derivatives (oxime) gave higher optical activity than that of benzylimino derivatives (Schiff base). Menthyl phenylglyoxylate did not form the Schiff base with benzylamine under the azeotropic distillation method which was employed for the other keto esters.

To check the racemization of amino acids during the saponification by alkali, the authentic menthyl esters of D-alanine, D-α-aminobutyric acid, and D-phenylglycine[3,4] were hydrolyzed by the same procedure employed in the hydrolysis of the menthyl esters synthesized in this study. Optical rotations were measured as DNP-amino acid which was separated by column chromatography. Racemization of D-alanine and D-α-aminobutyric acid was slight (4 and 3%, respectively); however, D-phenylglycine lost 77% of its activity during the saponification procedure. The optical purities listed in Table I are corrected by use of the values of standard racemization.

The steric course of the synthesis could be explained in a way similar to the rules proposed by Cram[9] and Prelog[10] as is shown in Scheme I. The most stable conformation might be structure I since C=O and C=N groups repel each other because of their electric dipoles. The menthyl residue is considered to take a conformation as was proposed by Prelog[10] (Scheme I). The molecules would be absorbed with the less bulky side on a catalyst, and the hydrogen atoms would attack

(1) Sterically Controlled Syntheses of Optically Active Organic Compound. I. Contribution No. 059 of the Institute of Molecular Evolution, University of Miami.

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(5) R. G. Hiskey and R. C. Northrop, *J. Am. Chem. Soc.*, **83**, 4798 (1961).

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(7) F. Sanger, *Biochem. J.*, **39**, 507 (1945); F. C. Green and C. M. Kay, *Anal. Chem.*, **24**, 726 (1952); K. R. Rao aud H. A. Sober, *J. Am. Chem. Soc.*, **76**, 1328 (1954).

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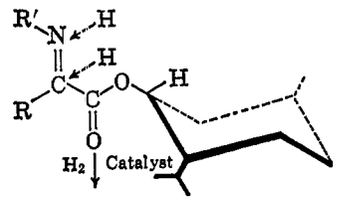
(10) V. Prelog, *Helv. Chim. Acta*, **36**, 308 (1953).

TABLE I

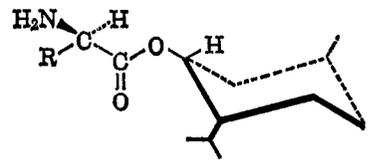
Starting material[a]	Catalyst[b]	Yield, %	Configuration of amino acid	Isolated amino acid,[c] $[\alpha]^{25}D$, deg (c, 5 N HCl)	Optical purity, %	DNP-amino acid,[d] $[\alpha]^{25}D$, deg (c, 1 N NaOH)	Optical purity, %
Py-S	A	79	DL-Ala	0	0	0	0
	B	77	D-Ala	−1.89 (3.38)	13.6	−22.5 (0.71)	16.3
Py-O	A	67	D-Ala	−1.61 (3.90)	11.5	−34.0 (0.81)	24.6
	B	70	D-Ala	−1.53 (3.70)	11.0	−33.8 (0.94)	24.5
B-S	A	74	DL-NH_2-but	0	0	0	0
	B	69	D-NH_2-but	0	0	− 7.32 (0.82)	7.6
B-O	A	64	D-NH_2-but	−2.00 (4.44)	10.3	−19.8 (0.78)	20.5
	B	62	D-NH_2-but	−1.81 (4.40)	9.4	−20.0 (0.87)	20.7
Ph-O	A	88	D-Ph-Gly	−9.50 (2.60)	24.9	+13.3 (0.79)[e]	49.1
	B	84	D-Ph-Gly	−8.69 (2.98)	22.7	+12.0 (0.54)[e]	44.2
Py-S(−)	A	62	L-Ala	+2.56 (3.52)	18.4	+33.2 (0.67)	24.0
	B	57	L-Ala	+2.25 (3.19)	16.2	+26.4 (0.70)	19.1
Py-S(+)	A	67	D-Ala	−8.86 (3.61)	63.9	−91.0 (0.57)	65.8
	B	61	D-Ala	−7.77 (3.41)	55.9	−82.3 (0.58)	59.6

[a] Py-S, benzylamine Schiff base of pyruvate, Py-O; oxime of pyruvate; Py-S(−), (−)-amine Schiff base of pyruvate, Py-S(+), (+)-amine Schiff base of pyruvate; B-S, benzylamine Schiff base of α-ketobutyrate; B-O, oxime of α-ketobutyrate; Ph-O, oxime of phenylglyoxylate. [b] Catalyst A, 5% palladium on charcoal; B, palladium hydroxide on charcoal.[5] [c] L-Ala, $[\alpha]^{25}D$ +14.6° (5 N HCl); L-α-NH_2-but, $[\alpha]^{25}D$ +20.6° (5 N HCl); L-Ph-Gly, $[\alpha]^{25}D$ +168° (5 N HCl). J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids," Vol. 3, John Wiley and Sons, Inc., New York, N. Y., 1961, alanine and α-aminobutyric acid, p 2401; phenylglycine, p 2697. [d] DNP-L-Ala, $[\alpha]^{25}D$ +143.9° (1 N NaOH); DNP-L-NH_2-but, $[\alpha]^{25}D$ +98.8° (1 N NaOH). K. R. Rao and H. A. Sober, *J. Am. Chem. Soc.*, **76**, 1328 (1954). DNP-D-Ph-Gly, $[\alpha]^{25}D$ +119.2° (AcOH). [e] Specific rotation was measured in glacial acetic acid.

SCHEME I

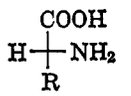


I



II

NaOH



III, R = Me, Et, Ph
R' = OH, Ph−CH_2
D configuration

the double bond from the backside of the plane of the paper (Scheme I). The resulting hydrogenated and hydrogenolyzed α-amino acids have D configuration.

When optically active (−)- and (+)-α-methylbenzylamine[11,12] [(−)-amine, (+)-amine] were used instead of benzylamine, the results were complex. Table I shows the summarized results. When (−)-amine was used, L-amino acids were obtained (19–24% optically active) and (+)-amine gave D-amino acids (60–66% optically active). This suggests that the steric contribution of the N-methylbenzylimino group attached at the α-carbon atom is larger than that of the menthyl group. In the reaction, the menthyl group and optically active (+)-methylbenzyl residues would cooperate with each other to cause higher optical purity of the product, whereas the menthyl group and the (−)-methylbenzyl residue would result in lower optical purity as is shown in Table I. These reactions were similar to those described recently by Hiskey on pyruvyl L- or D-alanine.[13]

(11) W. Theilacker and H. Hinkler, *Chem. Ber.*, **87**, 690 (1954).
(12) W. Leithe, *Ber.*, **64**, 2831 (1931).

Experimental Section[14]

Menthyl Pyruvate.—Pyruvic acid (59 g), *l*-menthol (110 g), and *p*-toluenesulfonic acid monohydrate (3.0 g) were dissolved in benzene (200 ml) and the mixture was refluxed for 3 hr with a Dean–Stark separator. After the reaction was over, the benzene solution was washed with sodium bicarbonate solution and the solvent was evaporated. The residual oil was distilled: yield, 110 g (73%); bp 83–85° (1 mm); $[\alpha]^{25}D$ −84.1° (*c* 1.88, absolute ethanol).

Menthyl α-Ketobutyrate.—The ester was prepared the same way as above: yield, 58%; bp 119–120° (2 mm); $[\alpha]^{25}D$ −79.3° (*c* 3.08, absolute ethanol).

Menthyl Phenylglyoxylate.—The ester was prepared as above: yield, 70%; mp 71–72°; $[\alpha]^{25}D$ −46.2° (*c* 0.66, absolute ethanol).

Anal. Calcd for $C_{18}H_{24}O_3$: C, 74.97; H, 8.39. Found: C, 74.61; H, 8.21.

Menthyl Pyruvate Oxime.—A mixture of menthyl pyruvate (7.2 g), hydroxylamine hydrochloride (7.5 g), pyridine (38 ml), and absolute ethanol (38 ml) was refluxed for 2 hr in a water bath. After the reaction was over, pyridine and ethanol were evaporated under reduced pressure. Water (30 ml) was added to the residue and the mixture was extracted with ether. The ether solution was dried and evaporated. The residual syrup was used for further experiments: yield, 7.0 g (90%).

Methyl α-Ketobutyrate Oxime.—The oxime was prepared as above: yield, 90%; mp 90–93° [ether and petroleum ether (bp 30–60°)]; $[\alpha]^{25}D$ −76.9° (*c* 1.54, absolute ethanol).

Anal. Calcd for $C_{14}H_{25}NO_3$: C, 65.85; H, 9.87; N, 5.49. Found: C, 66.09; H, 9.94; N, 5.43.

Menthyl Phenylglyoxylate Oxime.—The oxime was prepared as above: yield, 70%; mp 133–135° (ether and petroleum ether); $[\alpha]^{25}D$ −22.4° (*c* 0.80, absolute ethanol).

Anal. Calcd for $C_{18}H_{25}NO_3$: C, 71.26; H, 8.31; N, 4.62. Found: C, 71.31; H, 8.47; N, 4.63.

(13) R. G. Hiskey and Ralph C. Northrop, *J. Am. Chem. Soc.*, **87**, 1753 (1965).
(14) All temperature measurements were uncorrected. All optical rotation measurements were carried out by the use of the Rudolph Model 80 polarimeter with PEC-101 photometer.

D-(−)-**Alanine from Oxime.**—Methyl pyruvate oxime (2.40 g) was dissolved in ethanol (40 ml). The solution was hydrogenated with 1.0 g of catalyst (B) at room temperature for 8 hr. The catalyst was removed by filtration. The filtrate was mixed with 30 ml of 10% aqueous sodium hydroxide solution and was allowed to stand for 3 days at room temperature. Water (30 ml) was added and the solvent was evaporated to about 30 ml under reduced pressure. Ether extraction was carried out to remove the unreacted menthyl ester and menthol. The aqueous solution was acidified with 6 *N* hydrochloric acid and evaporated to dryness *in vacuo*. The dried residue was extracted with 50 ml of absolute ethanol. The alcoholic solution was kept in a freezer overnight and the precipitated inorganic salt was filtered. To the filtrate, pyridine was added to precipitate alanine. After the suspension was kept in a freezer overnight, alanine, 0.63 g (70%), was obtained: $[\alpha]^{25}D$ −1.53° (*c* 3.70, 5 *N* HCl).

Anal. Calcd for $C_3H_7NO_2$: N, 15.72. Found: N, 15.42.

DNP-Alanine.—A part of the hydrolyzed solution (including about 150 mg of alanine) was treated with 1-fluoro-2,4-dinitrobenzene (0.50 g) and sodium bicarbonate (0.50 g) by the usual method.[7] DNP-alanine was separated by celite column chromatography.[8] The celite (45 g) was treated with pH 7 phosphate–citrate buffer (0.2 *M*) (22.5 ml). The charged DNP-alanine was developed with a mixture of chloroform and ether (4:1). The band corresponding to DNP-alanine was cut off, dried, and extracted with 2% sodium bicarbonate solution. The solution was acidified and extracted with ethyl acetate. The solvent was evaporated. Crystallized DNP-alanine was used for measurement of optical rotation: mp 172–175° dec; $[\alpha]^{25}D$ −33.8° (*c* 0.94, 1 *N* NaOH).

Anal. Calcd for $C_9H_9N_3O_6$: N, 16.47. Found, N, 16.31.

D-(−)-α-**Aminobutyric Acid from Oxime.**—D-(−)-α-Aminobutyric acid was prepared from menthyl α-ketobutyrate oxime (2.54 g) by the use of catalyst B in the manner described above: yield, 0.64 g (62%); $[\alpha]^{25}D$ −1.81° (*c* 4.40, 5 *N* HCl).

Anal. Calcd for $C_4H_9NO_2$: N, 13.59. Found: N, 13.33.

DNP-D-(−)-α-aminobutyric acid showed $[\alpha]^{25}D$ −20.0° (*c* 0.87, 1 *N* NaOH); mp 139–142° dec.

Anal. Calcd for $C_{10}H_{11}N_3O_6$: N, 15.61. Found: N, 15.33.

D-(−)-**Phenylglycine from Oxime.**—D-(−)-Phenylglycine was prepared from the oxime of menthyl phenylglyoxylate (3.0 g) by the use of catalyst B in the manner described above: yield, 1.27 g (84%); $[\alpha]^{25}D$ −8.69° (*c* 2.98, 5 *N* HCl).

Anal. Calcd for $C_8H_9NO_2$: N, 9.27. Found: N, 9.10.

DNP-D-(−)-phenylglycine[15] showed $[\alpha]^{25}D$ +12.0° (*c* 0.54, AcOH).

D-(−)-**Alanine from Benzylamine Schiff Base.**—Methyl pyruvate (2.26 g) and benzylamine (1.10 g) in benzene (30 ml) were refluxed with a Dean–Stark separator for 30 min to remove resulting water. After evaporation of benzene, the residue was dissolved in 40 ml of ethanol. Catalyst B (1.0 g) was added to the solution and hydrogenation was carried out at room temperature for 6 hr. The reaction mixture was treated in the same way as described in the hydrogenation of the oximes. After alkaline hydrolysis, 0.69 g (77%) of alanine was obtained: $[\alpha]^{25}D$ −1.89° (*c* 3.38, 5 *N* HCl).

(15) DNP-phenylglycine did not crystallize. The *c* value was determined colorimetrically by the use of DNP-alanine as a standard. DNP-phenylglycine is racemized in 1 *N* NaOH. Specific rotation was measured in glacial acetic acid.

Anal. Calcd for $C_3H_7NO_2$: N, 15.72. Found: N, 15.60.

DNP-D-(−)-alanine.—DNP-alanine was prepared and isolated in the manner described above: $[\alpha]^{25}D$ −22.5° (*c* 0.71, 1 *N* NaOH); mp 173–176° dec.

D-(−)-α-**Aminobutyric Acid from Benzylamine Schiff Base.**—D-(−)-α-Amino acid was prepared from menthyl α-ketobutyrate (2.40 g) and benzylamine (1.10 g) by the use of catalyst B as described above: yield, 0.71 g (69%); $[\alpha]^{25}D$ 0° (*c* 3.50, 5 *N* HCl).

Anal. Calcd for $C_4H_9NO_2$: N, 13.59. Found: N, 13.33.

DNP-D-(−)-α-aminobutyric acid showed $[\alpha]^{25}D$ −7.31 (*c* 0.82, 1 *N* NaOH); mp 137–140° dec.

L-(+)-**Alanine from the Schiff Base of** (−)-α-**Methylbenzylamine.**—The Schiff base was prepared from menthyl pyruvate (2.26 g) and (−)-α-methylbenzylamine (1.21 g, $[\alpha]^{25}D$ −42.3° in benzene) by the azeotropic method as described earlier. The Schiff base was dissolved in 40 ml of ethanol containing 1.0 g of catalyst B and hydrogenation was carried out. The reaction mixture was treated in the same way as mentioned earlier: L-(+)-alanine, 0.55 g (62%); $[\alpha]^{25}D$ +2.56° (*c* 3.52, 5 *N* HCl).

Anal. Calcd for $C_3H_7NO_2$: N, 15.72. Found: N, 15.77.

DNP-L-(+)-alanine showed $[\alpha]^{25}D$ +33.2° (*c* 0.67, 1 *N* NaOH); mp 174–175° dec.

D-(−)-Alanine was prepared from Schiff base of (+)-α-methylbenzylamine ($[\alpha]^{25}D$ + 41.5° in benzene) in the same way as above. Yield and specific rotations of alanine and DNP-alanine are listed in Table I.

Hydrolysis of D-Alanine Menthyl Ester.—D-Alanine menthyl ester hydrochloride[3,4] [2.47 g, $[\alpha]^{25}D$ −72.0° (*c* 1.74, absolute ethanol)] was dissolved in 50 ml of ethanol. To this was added 10% sodium hydroxide solution (40 ml) and the mixture was allowed to stand at room temperature for 3 days. The reaction mixture was treated as described in earlier experiments. D-Alanine, 0.83 g (93.3%), was isolated: $[\alpha]^{25}D$ −14.4° (*c* 2.22, 5 *N* HCl). The optical rotation did not change by recrystallization from water and ethanol.

DNP-D-alanine showed $[\alpha]^{25}D$ −138.2° (*c* 0.64, 1 *N* NaOH); mp 174–176° dec.

Hydrolysis of D-Aminobutyric Acid Menthyl Ester.—D-α-Aminobutyric acid menthyl ester hydrochloride[3,4] [2.78 g, $[\alpha]^{25}D$ −74.5° (*c* 0.77, absolute ethanol)] was hydrolyzed in the same condition. D-(−)-α-Aminobutyric acid, 1.00 g (96.7%), was obtained: $[\alpha]^{25}D$ −19.3° (*c* 4.0, 5 *N* HCl). After recrystallization, the specific rotation rose to −19.7°.

DNP-D-α-aminobutyric acid showed $[\alpha]^{25}D$ −96.5° (*c* 0.52, 1 *N* NaOH); mp 136–138° dec.

Hydrolysis of D-Phenylglycine Menthyl Ester.—D-Phenylglycine menthyl ester hydrochloride[4] [0.70 g, $[\alpha]^{25}D$ −75.1° (*c* 1.07, absolute ethanol)] was hydrolyzed as above. D-(−)-Phenylglycine, 0.32 g (98%), was obtained: $[\alpha]^{25}D$ − 38.2° (*c* 1.59, 5 *N* HCl).

DNP-D-phenylglycine showed $[\alpha]^{25}D$ +27.1° (*c* 0.78, AcOH).

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