lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylsulfonyl)-phenyl ester in 10 ml of dimethylformamide, and the resulting solution was stirred overnight. This was poured into 300 ml of water containing 50 ml of 10% citric acid solution and stirred for 2 hr. The precipitate was filtered off, dried, and crystallized from chloroform–ether to yield 0.6 g (73%) of the fully protected heptapeptide: mp 118–120°; $[\alpha]^{25}D$ −25° (*c* 1.0, dimethylformamide); R_f 0.49.

Anal. Calcd for $C_{77}H_{104}N_{10}O_{16}S$: C, 62.1; H, 7.0; N, 9.4. Found: C, 62.4; H, 7.1; N, 9.3.

Optical Purity of N,N′-Dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester (1).—The protected heptapeptide **1** (0.078 g, 5.237×10^{-5} mol) was dissolved in 10 ml of 6 *N* hydrochloric acid–glacial acetic acid (1:1) and heated under reflux at 100–105° for 24 hr. The solution was evaporated to dryness, and the residue was dissolved in 6 *N* hydrochloric acid–glacial acetic acid (4:1) so that the final volume was 2 ml: $[\alpha]^{30}D$ +28.69° (calculated on the basis of the expected amounts of lysine, leucine, isoleucine, alanine, and valine).

A control of 0.0073 g of 4-(methylthio)phenol, 0.0287 g of lysine hydrochloride, 0.0069 g of L-leucine, 0.0069 g of L-isoleucine, 0.0046 g of L-alanine, 0.0061 g of L-valine, 0.0056 g of benzyl alcohol, and 10 ml of 6 *N* hydrochloric acid–glacial acetic acid (1:1) was heated simultaneously with and under the same conditions as those used for the protected heptapeptide **1**. After 24 hr the solution was evaporated to dryness and made up to 2 ml with 6 *N* hydrochloric acid–glacial acetic acid (4:1), $[\alpha]^{30}D$ +29.54°, to give an optical purity of 97.1 ± 5%.

Registry No.—**1**, 17693-03-7; **2**, 17693-04-8; **3**, 17693-05-9; **4**, 17693-06-0; **5**, 17693-07-1; **6**, 17693-08-2; **7**, 17743-96-3; **8**, 17693-09-3; **9**, 17693-10-6; **10**, 17693-11-7; **11**, 17693-12-8; **12**, 17693-13-9; **13**, 17743-97-4; **14**, 17693-14-0; **15**, 17693-15-1; N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine pentachlorophenyl ester, 17693-16-2; *t*-butyloxycarbonyl-L-alanine pentachlorophenyl ester, 17693-17-3; *t*-butyloxycarbonylglycine pentachlorophenyl ester, 17693-18-4; *t*-butyloxycarbonyl-L-isoleucine pentachlorophenyl ester, 17693-19-5; *t*-butyloxycarbonyl-L-leucine pentachlorophenyl ester, 17693-20-8; *t*-butyloxycarbonyl-L-phenylalanine pentachlorophenyl ester, 17693-21-9; *t*-butyloxycarbonyl-O-benzyl-L-tyrosine pentachlorophenyl ester, 17693-22-0; *t*-butyloxycarbonyl-L-valine pentachlorophenyl ester, 17693-23-1; N,N′-di-*t*-butyl oxycarbonyl-L-lysine pentachlorophenyl ester 17693-24-2.

Acknowledgment.—The authors are indebted to the National Science Foundation which supported this investigation, and also the Crown Zellerbach Corp. for samples of 4-(methylthio)phenol.

Notes

The 4-(Methylsulfonyl)phenyl Activated Ester. Susceptibility to Racemization[1]

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In a previous communication[3] it was shown that N-protected amino acid or peptide 4-(methylthio)phenyl esters could be converted by oxidation into 4-(methylsulfonyl)phenyl esters, which were sufficiently activated to be used in peptide synthesis. However, to evaluate the utility of this method, it was necessary to investigate the susceptibility of the activated ester to racemization. Since the most common mechanism is thought to be racemization through the oxazolone, Young's model[4] was chosen for study, because it is especially susceptible to racemization in this manner.

N-*t*-Butyloxycarbonyl-L-leucine 4-(methylthio)phenyl ester (**1**), was prepared from N-*t*-butyloxycarbonyl-L-leucine and 4-(methylthio)phenol using DCC. Treatment of **1** with hydrogen chloride in glacial acetic acid yielded L-leucine 4-(methylthio)phenyl ester hydrochloride (**2**), which was benzoylated to give N-benzoyl-L-leucine 4-(methylthio)phenyl ester (**3**). Oxidation of **3** with excess hydrogen peroxide in glacial acetic acid for 12 hr gave N-benzoyl-L-leucine 4-(methylsulfonyl)phenyl ester (**4**). Under these oxidative conditions the 4-(methylthio)phenyl ester is converted completely[3] into the 4-(methylsulfonyl)-phenyl ester as shown by infrared data. The presence of the optically active 4-(methylsulfinyl)phenyl ester was inferred to be absent. Thus it was considered that a comparison of the optical activity of the total acid hydrolysate of compounds **3** and **4** would indicate the amount of optical retention during this conversion. To this end N-benzoyl-L-leucine 4-(methylthio)phenyl ester (**3**) and N-benzoyl-L-leucine 4-(methylsulfonyl)-phenyl ester (**4**) were hydrolyzed using 6 *N* hydrochloric acid–glacial acetic acid (1:1) mixture, under identical conditions. Comparison of the the specific rotations of the hydrolysates of **3** and **4** showed that nearly 100% optical purity had been maintained.

In order to study the susceptibility of the 4-(methylsulfonyl)phenyl-activated ester to racemization in the presence of base, solutions of the ester **4** and tertiary amine (in 1:2 molar ratio) were mixed together in a 1-dm polarimeter tube; changes in optical rotation were observed on a Carl Zeiss polarimeter.

The general mechanism proposed[5,6] for racemization through the formation of an oxazolone provides the rate expression

$$\frac{-d[L]}{dt} = k_1[B]([L] - [D])$$

(1) This is the third article in this series. For the previous paper see B. J. Johnson and E. G. Trask, *J. Org. Chem.*, **33**, 4521 (1968).

(2) To whom any correspondence should be sent.

(3) B. J. Johnson and P. M. Jacobs, *Chem. Commun.*, 73 (1968).

(4) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).

(5) M. Goodman and L. Levine, *J. Amer. Chem. Soc.*, **86**, 2918 (1964). M. Goodman and W. J. McGahren, *ibid.*, **87**, 3028 (1965).

(6) M. W. Williams and G. T. Young, *J. Chem. Soc.* 3701 (1964).

Since the base concentration was in a large excess it can be considered as a constant, thus integration yields

$$\ln \frac{[L_0]}{[L] - [D]} = k_1[B]t$$

where $k_1[B]$ is the pseudo-first-order rate constant k_I and k_1 is the second-order rate constant k_{II}. Thus a plot of ln ([L] − [D]) or ln α_{obsd} will give a straight line of slope $-k_{II}[B]$.

The half-time of the pseudo-first-order reaction is then given by

$$t_{1/2} = \ln 2/k_I = \ln 2/k_{II}[B]$$

Experimentally, plots of ln α_{obsd} *vs.* time gave straight-line curves; thus pseudo-first-order kinetics are followed at a ratio of the activated ester to the base of 1:2. The results are shown in Table I. As expected,[7] the reaction was found to be faster in dioxane–water than in chloroform.

TABLE I

RACEMIZATION OF N-BENZOYL-L-LEUCINE 4-(METHYLSULFONYL)PHENYL ESTER IN THE PRESENCE OF TERTIARY AMINE

Amine[a]	Solvent	T, °C	$k_I \times 10^3$, sec^{-1}[b]	$k_{II} \times 10^3$, sec^{-1}[c]	$t_{1/2}$, min[d]
TEA	Chloroform[e]	24.0	0.63 ± 0.09	1.26 ± 0.17	18.3
	Chloroform	33.7	1.1 ± 0.15	2.2 ± 0.29	10.4
	80% dioxane–water	32.1	6.82 ± 0.10	13.64 ± 1.99	1.7
TBA	Chloroform	33.0	No reaction after 24 hr		

[a] TEA = triethylamine; TBA = tribenzylamine. Amine concentration 0.5 *M*, ester concentration 0.25 *M*. [b] Pseudo-first-order rate constant. [c] Second-order rate constant = $k_I/[B]$. [d] Time for optical rotation to drop by one-half. [e] Contains 0.75% ethanol.

Bodanszky,[8] studying the *p*-nitrophenyl ester, used the same ratio of ester to base, but at lower concentration. Since the pseudo-first-order half-life is inversely proportional to the base concentration, it was necessary to convert the half-times of racemization into the same base concentration in order to compare the 4-(methylsulfonyl)phenyl ester to the *p*-nitrophenyl ester. Bodanszky[8] reported a half-time of 30 min at 24° for the racemization of the *p*-nitrophenyl ester in chloroform in the presence of 0.1 *M* triethylamine; at this base concentration, the 4-(methylsulfonyl)phenyl ester would have a half-life of approximately 90 min.

The high stability of the N-benzoyl-L-leucine *p*-nitrophenyl ester in the presence of tribenzylamine has also been reported.[8] We therefore investigated the effect of tribenzylamine on the racemization of N-benzoyl-L-leucine 4-(methylsulfonyl)phenyl ester. At 33°, a solution of the two showed no change in optical rotation after 24 hr. This can be ascribed to the weaker basicity and steric hindrance of the amine. From these results it has been concluded that the conversion of the 4-(methylthio)phenyl ester into the activated 4-(methylsulfonyl)phenyl ester is not accompanied by racemization. However, the resulting activated ester, like other commonly used activated esters, are subject to racemization in the presence of excess strong base.

(7) M. Goodman and W. J. McGahren, *J. Amer. Chem. Soc.*, **88**, 3887 (1966).

(8) M. Bodanszky and A. Bodanszky, *Chem. Commun.*, 591 (1967).

Experimental Section[9]

N-*t*-Butyloxycarbonyl-L-leucine 4-(methylthio)phenyl Est er (1).—N,N′-Dicyclohexylcarbodiimide (8.7 g, 0.0042 mol) was added to a solution of N-*t*-butyloxycarbonyl-L-leucine (9.3 g, 0.004 mol) and 5.6 g of 4-(methylthio)phenol in methylene chloride (150 ml). After stirring or 12 hr at room temperature, the solvent was removed under reduced pressure to give a solid. This was dissolved in ethyl acetate, filtered, washed successively with 10% citric acid and water, and dried (Na_2SO_4). Evaporation of this solution afforded a solid which was crystallized from hexane to yield 7.7 g (54.5%) of the 4-(methylthio)phenyl ester: mp 68–69°; $[\alpha]^{31}D$ −49.6° (*c* 1.19 in methanol).

Anal. Calcd for $C_{18}H_{27}NO_4S$: C, 61.2; H, 7.65; S, 9.1. Found: C, 61.4; H, 7.6; S, 8.8.

L-Leucine 4-(Methylthio)phenyl Ester Hydrochloride (2).—To 40 ml of 1 *N* hydrogen chloride in glacial acetic acid was added 4.5 g of N-*t*-butyloxycarbonyl-L-leucine-4-(methylthio)-phenyl ester. The solution was left at room temperature for 20 min and then evaporated under reduced pressure to give an oil. The oil was triturated with anhydrous ether to give 3.4 g (93%) of the hydrochloride, mp 197° dec. Recrystallization from methanol–ether raised the melting point to 201° dec; $[\alpha]^{31}D$ +20.4° (*c* 0.24 in methanol).

Anal. Calcd for $C_{13}H_{20}ClNO_2S$: C, 53.9; H, 6.9; Cl, 12.2. Found: C, 54.0; H, 7.05; Cl, 12.0.

N-Benzoyl-L-leucine 4-(Methylthio)phenyl Ester (3).—L-Leucine 4-(methylthio)phenyl ester hydrochloride (2) (2.1 g, 0.0112 mol) was suspended in 50 ml of ethyl acetate containing 1.6 g (0.0112 mol) of benzoyl chloride. A solution of 3.6 g (0.0336 mol) of sodium carbonate in 25 ml of water was added, and the two-phase mixture was stirred vigorously for 30 min. The aqueous layer was extracted with 100 ml of ethyl acetate; the combined organic phases were washed with 0.5 *N* hydrochloric acid, dried (Na_2SO_4), and concentrated under reduced pressure to give an oil; upon addition of hexane, 3.9 g (95%) of the ester was obtained, mp 135–136°. Recrystallization from ethyl acetate–hexane gave 3.2 g (78%) of pure product: mp 134–136°; $[\alpha]^{30}D$ −34.6° (*c* 0.68 in acetic acid).

Anal. Calcd for $C_{20}H_{23}NO_3S$: C, 67.2; H, 6.4; S, 9.0. Found: C, 67.4; H, 6.4; S, 9.0.

N-Benzoyl-L-leucine 4-(Methylsulfonyl)phenyl Ester (4).—N-Benzoyl-L-leucine 4-(methylthio)phenyl ester (4.9 g, 0.0137 mol) was dissolved in 50 ml of glacial acetic acid, and 15 ml of 30% hydrogen peroxide was added. The solution was left at room temperature for 12 hr and then poured into 600 ml of water. The precipitated 4-(methylsulfonyl)phenyl ester was collected and dried (5.1 g, 98%): mp 134–138°; ν_{max}^{Nujol} 1310, 1150 cm^{-1} (sulfone[10]); there was no absorption at 1050 cm^{-1} attributable to the sulfoxide.[10] It was recrystallized from methylene chloride–hexane which raised the melting point to 146°, $[\alpha]^{31}D$ −30.0° (*c* 0.65 in acetic acid).

Anal. Calcd for $C_{20}H_{23}NO_5S$: C, 61.7; H, 5.9; N, 3.6. Found: C, 61.6; H, 5.9; N, 3.7.

Optical Purity of N-Benzoyl-L-leucine 4-(Methylthio)phenyl Ester (3) and N-Benzoyl-L-leucine 4-(Methylsulfonyl)phenyl Ester (4).—N-Benzoyl-L-leucine 4-(methylthio)phenyl ester (3) (0.5 g, 0.00136 mol) was dissolved in 4 ml of glacial acetic acid–6 *N* hydrochloric acid (1:1) and heated to 100–105° for 24 hr. The solution was evaporated to dryness, and the residue was dissolved in glacial acetic acid so that the final volume was 5 ml: $[\alpha]^{25}D$ +11.90°. N-Benzoyl-L-leucine 4-(methylsulfonyl)-phenyl ester (4) (0.5 g, 0.00129 mol, mp 146°) was hydrolyzed concurrently with and under exactly the same conditions as those used for the ester 3. After 24 hr, the solution was evaporated to dryness and made up to 5 ml with glacial acetic acid, $[\alpha]^{25}D$ +12.18°, to give an amount of optical purity retained of 98 ± 3%.

Kinetic Studies on Racemization.—To 3 ml of a 0.5 *M* solution of N-benzoyl-L-leucine 4-(methylsulfonyl)phenyl ester in a 1-dm polarimeter tube was added 3 ml of a 1.0 *M* solution of a purified tertiary amine. It was considered to be zero time when the last of the amine solution had been added; a stopwatch was used for timing. Readings on the polarimeter were begun after suffi-

(9) Microanalyses were carried out by Dr. S. M. Nagy, Massachusetts Institute of Technology, Cambridge, Mass. Melting points were taken with a Mel-Temp apparatus. Optical rotations were taken with a Carl Zeiss precision polarimeter.

(10) L. J. Bellamy, "Organic Sulfur Compounds," Vol. 1, N. Kharasch, Ed., Pergamon Press, New York, N. Y., 1961, p 48.

cient mixing to ensure homogeneity. The half-shade angle on the instrument was set at 5°, which normally gives an error of ±0.02° in α_{obsd}; since the readings had to be made rapidly, an error of ±0.04° was assigned. Error in the pseudo-first-order rate constant was evaluated by the method of limiting slopes. Results are summarized in Table I.

A. Triethylamine in Chloroform.—Runs were made at two temperatures: 33.7° ± 0.3 and 24.0° ± 0.1.

B. Triethylamine in 80% Dioxane–Water.—In this case the ester was dissolved in pure dioxane and the amine in 60% dioxane–water; the temperature was 32.1° ± 0.2.

C. Tribenzylamine in Chloroform.—There was no change in optical rotation after 24 hr at 33°.

D. Tribenzylamine Hydrochloride in 20% Methanol–Chloroform.—There was no change in optical rotation after 24 hr at 33°.

Registry No.—**1**, 17659-10-8; **2**, 17659-11-9; **3**, 17659-18-6; **4**, 17730-92-6.

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Synthesis of Optically Active Alanine from Oxaloacetic Acid by Hydrogenolytic Asymmetric Transamination[1]

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Hiskey and Northrop published a method for synthesizing optically active α-amino acids from the corresponding α-keto acids. They employed optically active α-methylbenzylamine and subsequent catalytic hydrogenation and hydrogenolysis.[2] In the previous study from this laboratory, the possible steric courses of the asymmetric synthesis have been studied.[3,4] Also, the formation of optically active amino acids from α-keto acids and optically active α-phenylglycine in alkaline aqueous solution by catalytic hydrogenation and subsequent hydrogenolysis has been studied.[5]

In this investigation, reactions of oxaloacetic acid with (*S*)(−)-α-methylbenzylamine and with (*S*)(−)-α-ethylbenzylamine in alcoholic solution were used to obtain optically active aspartic acid. However, the resulting amino acid was found to be only optically active α-alanine (optical purity 69 and 52%, respectively). No aspartic acid was identified in the reaction product. Therefore, very fast decarboxylation of oxaloacetic acid during the reaction is inferred.

To clarify the decarboxylation during the asymmetric synthesis, several amines and solvent systems were used. Benzylamine resulted in racemic alanine

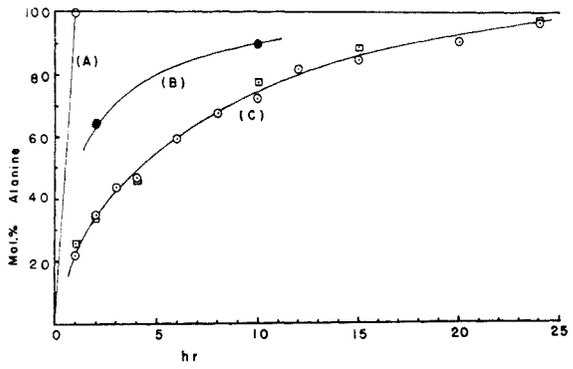
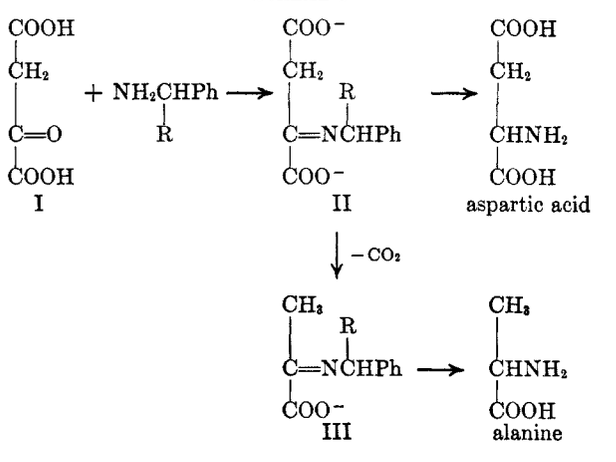


Figure 1.—Decarboxylation during the reductive amination of oxaloacetic acid. (A) oxaloacetic acid (1.32 g, 0.01 mol) + (*S*)(−)-α-methylbenzylamine (3.63 g, 0.03 mol). (B) Oxaloacetic acid (0.66 g, 0.05 mol) + pyridoxamine dihydrochloride (1.2 g, 0.005 mol). (C) Oxaloacetic acid (1.32 g, 0.01 mol) + (*R*)(−)-phenylglycine (1.51 g, 0.01 mol). ⊙, determined by amino acid analyzer; ⊡, determined by DNP method.

in an alcoholic solution, the same as the optically active α-methyl- and α-ethylbenzylamine did. When optically active (*S*)(+)-or (*R*)(−)-α-phenylglycine was used in the reaction with oxaloacetic acid in aqueous solution, the products were found to be a mixture of (*S*)(+)-alanine–(*S*)(+)-aspartic acid or (*R*)(−)-alanine–(*R*)(−)-aspartic acid. The decarboxylation rate in this reaction is relatively slow compared with that in the reaction with α-alkylbenzylamine in alcoholic solution. The observed results are shown in Figure 1, in which the ratios of the resulting alanine and aspartic acid, depending on time in the reaction, are presented. The summarized results of yield and optical purity are presented in Table I.

The inferred route of this reaction is shown in Scheme I. Oxaloacetic acid reacts with benzylamines to form

SCHEME I



the Schiff base (II). The structure II might lose its β-carboxyl group easily to convert it into the Schiff base of pyruvic acid (structure III).[6] In the reaction with benzylamine, α-alkylbenzylamine, or α-(1-naphthyl)-ethylamine, the decarboxylation rate seems to be very fast in alcoholic solution. When an aqueous solvent was used, decarboxylation was not so fast that the re-

(1) Sterically controlled synthesis of optically active organic compounds VII. Part VI: K. Harada and K. Matsumoto, *J. Org. Chem.*, **33**, 4467 (1968). Contribution No. 079 from the Institute of Molecular Evolution, University of Miami.

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

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

(4) Part VI.[1]

(5) K. Harada *Nature*, **212**, 1571 (1966); K. Harada, *J. Org. Chem.*, **32**, 1790 (1967).

(6) The decarboxylation reaction mechanism could be similar to those of enzymatic β-decarboxylation proposed by A. Meister, J. S. Nishimura, and A. Novogradsky, "Chemical and Biological Aspects of Pyridoxal Catalysis," E. E. Snell, P. M. Fasella, A. Braunstein, and A. Rossi Fanelli, Ed., The Macmillan Co., New York, N. Y., 1963, p 229.