THE SYNTHESIS OF 1-KETO-3-CARBOMETHOXY-1,2,3,4-TETRAHYDROISOQUINOLINE

Thesis by

Richard Bernard McGriff

In Partial Fulfillment of the Requirements

For the Degree of

Master of Science in Chemistry

California Institute of Technology

Pasadena, California

1959

ACKNOWLEDGEMENTS

I acknowledge my indebtedness to Professor Carl Niemann, my research advisor, for the advice, encouragement, and assistance which made my residence at the California Institute of Technology a stimulating intellectual experience.

I acknowledge my great debt to Dr. Robert Bixler for suggesting this project. I acknowledge a great debt to my other co-workers who created in the laboratory an environment which sustained my scientific curiosity and nourished my creativity.

I acknowledge my graditude to the California Institute of Technology for tuition scholarships and teaching assistantships, the du Pont Company for summer research grants, and the United States Public Health Service for a research assistantship.

ABSTRACT

The preparation of 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline by two routes is described. The first involves hydrolysis of the adduct of α-bromo-o-tolunitrile and acetamidomalinic ester to 1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline. Esterification of this acid yields the desired ester. The second route involves the condensation of phenylalanine with formaldehyde to form 3-carboxy-1,2,3,4-tetrahydroisoquinoline. The benzoyl derivative of this compound is smoothly oxidized by cold permanganate to N-benzoyl (o-carboxyphenyl)alanine, which is converted by acid hydrolysis to 1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline and benzoic acid. Preliminary experiments show that 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline is hydrolyzed by α-chymotrypsin, and indicate that both forms are hydrolyzed, albeit at differing rates.

INTRODUCTION

It has been proposed (1) that 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline can function as a specific substrate of α-chymotrypsin. This compound may be regarded as a member of a larger class of potential substrates which are ester lactams derived from δ or γ carboxy-α-amino acids.

In the ester lactams the α-amino acid side chain and the acyl amino group are incorporated into a five or six membered ring. The possible interactions of the functional groups of the substrate with the active site are limited to those compatible with conformations required by the ring structure. Variation of the substituents on either ring, if reflected in variation of the rate of hydrolysis, will contribute to an understanding of the steric requirements of α-chymotrypsin.

The first compound chosen for synthesis and kinetic study was 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline. This compound resembles N-benzoyl phenylalanine methyl ester, which is known to be a specific substrate of α-chymotrypsin (2). In addition, the benzene ring has three positions available for substitution, thus permitting a study of substituent effects in this portion of the molecule.

The synthesis of 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline as planned at the start of this investigation involved alkylation of diethyl acetamidomalonate with

α-bromo-o-tolunitrile followed by saponification, decarboxylation and removal of the acetyl group by acid hydrolysis. A recent review discusses this general route to amino acids (3).

The alkylation reaction, when run at 50°, gave the ethyl 2-carbethoxy-2-acetamido-3-(o-cyanophenyl) butyrate in 93-99% yield. When the reaction was conducted at 78°, extensive darkening took place and the yield of recrystallized product was ca. 80%. Side reactions involving alkylation of the cyano group (3) become important at the higher temperature.

Saponification of the carboethoxy groups in ethyl 2-carbethoxy-2-acetamido-3-(o-cyanophenyl) butyrate occurs readily. The cyano group, not unexpectedly, resists saponification, and prolonged treatment with 6N base is necessary. The bulkly ortho substituent hinders the approach of the base on the nitrile and thereby markedly inhibits the rate of reaction (4). In order to remove the acetyl group and hydrolyze any residual nitrile, the crude product obtained from the saponification and decarboxylation reaction was refluxed with aqueous 48% hydrobromic acid.

The key intermediate in the synthesis of 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline is the δ-carboxyl-α-amino acid, o-carboxyphenylalanine. This acid spontaneously forms the lactam upon removal of the acyl group. Lactamization of amino acids with aromatic rings fused to the chain is known to be a facile reaction. Bamberger and Dieckmann showed that when aqueous solutions of

o-(β-aminoethyl) benzoic acid or its hydrochloride are evaporated to dryness, hydroisocarbostyril is formed (5). Hydrolysis of 2-phenyl-4-o-carbomethoxybenzylidene oxazolone with alkali gives 1-keto-3-carboxy-3,4-tetrahydroisoquinoline (6). The reduction of diethyl (o-nitrobenzyl) malonate in ethanolic HCl gives hydroisocarboxtyril-3-carboxylic acid (7). In a study of the effect of structure on the polymerization of lactams, Hall found that substituted piperidones have such high cyclization rates that polymerization is very difficult to achieve (8).

The lactam of o-carboxyphenylalanine, which is 1-keto-3-carboxy-1,2,3,4-tetrahydroquinoline, was smoothly converted to 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline by esterification with methanol and thionyl chloride (9). The attempted resolution of this ester with α-chymotrypsin gave results which cannot be explained at this time.

Table I summarizes the results of the resolution and isolation experiments. Table II summarizes the results of the kinetic studies utilizing the pH-stat (1, 10). At enzyme concentrations of 10^{-2} (11) the DL-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline is 50% hydrolyzed within a few minutes, presumably to the L-acid and the D-ester. In only one of the resolution experiments was there isolated pure (-)-acid and (+)-ester. No other compound was ever isolated or detected in the spectral studies.

At this stage it was decided that it might be fruitful to investigate the preparation of o-carboxy-L-phenylalanine and its lactam from L-phenylalanine. The preparation of 3-carboxy-tetrahydroisoquinoline is described in the literature (12), and a sequence of reactions involving its preparation from L-phenylalanine and selective oxidation of C-1 would give 1-keto-3-carboxy-tetrahydroisoquinoline. The benzyl side chain can be converted to a benzoyl group by oxidation with permanganate (13). It is also known that oxidation of N-benzoyl piperidine (14) and of N-benzoyl-1,2,3,4-tetrahydroisoquinoline (5) with cold permanganate yields the respective ω-benzoylamido acids.

The sequence of reactions utilized to synthesize 1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline began with formylation of phenylalanine and benzoylation of the adduct. Oxidation of the N-benzoyl acid with weakly alkaline permanganate yielded the N-benzoyl-o-carboxylphenylalanine. This was hydrolyzed with aqueous mineral acid to the lactam and benzoic acid.

The method of Julian (12) was used without modification to prepare the isoquinoline carboxylic acid. DL-phenylalanine was heated with excess formaldehyde in 12N hydrochloric acid for 3.5 hours. Complexes of the amino group and formaldehyde are readily formed; however the slowness of the alkylation of the aromatic ring may necessitate the high temperature, high acidity, and long reaction time (15).

Benzoylation with benzoyl chloride and sodium hydroxide gave the N-benzoyl-3-carboxy-1,2,3,4-tetrahydroisoquinoline in 73% yield.

The oxidation of the N-benzoyl acid in dilute potassium carbonate solution with potassium permanganate gave N-benzoyl-o-carboxyphenylalanine in ca. 50% yield. The low yield indicates that oxidation other than at the 1,2 position occurs. The assignment of the structure of N-benzoyl-o-carboxyphenylalanine to the product isolated is compatible with the neutralization equivalent, analysis, and hydrolysis result.

Hydrolysis of N-benzoyl-o-carboxyphenylalanine gave a mixture of the lactam and benzoic acid. Separation was effected by taking advantages of the differing solubilities of the two products. Benzoic acid is very soluble in ether, while 1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline is practically insoluble. The reaction product was dried over NaOH and H_2SO_4, and extracted with ether. The residue was recrystallized from water, and the melting point, infra red spectrum and neutralization equivalent were the same as for the acid obtained by the acetamido malonic ester alkylation route.

The procedure having been developed with the DL-compound, the reaction sequence was repeated starting with L-phenylalanine. The formaldehyde condensation product had a rotation of α_D^{25} -58.3° (c, 2% 2N HCl), which is higher than that of the starting material α_D^{25} -33.9 (c, 1.90% water). The

N-benzoyl isoquinoline acid possessed a rotation of α_D^{25} -33.7° (c, 2% MeOH). Oxidation gave the N-benzoyl-o-carboxy-L-phenylalanine α_D^{25} -105 (c, 2% MeOH). In the work up of this compound, the basic solution resulting from the oxidation was neutralized to pH 6 before it was concentrated *in vacuo*. This was done in order to avoid the possibility of racemizing the acid by heating it in a basic solution. The hydrolysis of the N-benzoyl dicarboxylic acid gave 1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline, α_D^{25} 41.7° (c, 2% MeOH). Esterification gave the ester, α_D^{25} 75.9° (c, 2% MeOH).

On the basis of this steriospecific synthesis starting from L-phenylalanine, the (+)-acid and (+)-ester belong to the L-series of amino acids and derivatives thereof. The results summarized in the tables show that the L(-)-ester is hydrolyzed at enzyme concentrations of *ca.* 10^{-1}, whereas the D(+)-ester is hydrolyzed at *ca.* 10^{-3}-10^{-4}. The DL ester at enzyme concentrations at 10^{-1} shows two distinct steps in the hydrolysis reaction. The initial reaction is too fast and consumes 50% of the equivalents of base required for complete saponification. This is followed by the slower hydrolysis of the other enantiomorph.

The postulate is advanced that in the interaction of α-chymotrypsin and 1-keto-3-carbomethoxyiso-1,2,3,4-tetrahydroisoquinoline, the D enantiomorph is hydrolyzed at a more rapid rate than the L. This is a complete reversal of the usual specificity. The finding that both isomers are

hydrolyzed also helps to explain the results of the resolution experiments. In a review of methods of resolving amino acids, Greenstein points out that enzymatic specificity is relative rather than absolute (16). Hydrolysis of both isomers may occur at appreciable rates. It is not possible to predict the relative rates of hydrolysis of a pair of enantiomorphic amino acid derivatives which possess unusual structural features.

EXPERIMENTAL[17,18]

α-Bromo-o-tolunitrile (19).--To 100 grams (0.85 mole) of Matheson o-tolunitrile, heated in an oil bath to 140°, was added 140 g. of bromine over a period of four hours. The bromine was added through a dropping funnel whose tip extended below the surface of the liquid, and the reaction mixture was stirred continuously. Illumination with a #2 photoflood was used to catalyze the reaction. The dark liquid was transferred to a distilling flask, and distilled under diminished pressure. The product boiling at 140-150° 5 mm. solidified in the receiving flask. One recrystallization from 95% ethanol gave the bromide in 65% yield, m.p. 70-71°, lit (19) 71-72°.

Ethyl-2-carbethoxy-2-acetamido-3-(o-cyanophenyl) butyrate.--Anhydrous alcohol was distilled from sodium ethoxide into a flame-dried flask fitted with a reflux condenser, stirrer, and drying tube. After 15 grams (0.7 gram-atom) of sodium had been dissolved in the ethanol, 152 grams (0.7 mole) of diethyl acetamidomalonate was added and the solution stirred at 50° for one hour. An alcoholic solution of 127 grams (0.65 mole) of α-bromo-o-tolunitrile was slowly added to the reaction vessel. After the solution was stirred for five hours at 50°, it was chilled in an ice bath, neutralized with dry hydrogen chloride gas, and the ethanol distilled. The yellow semi-solid remaining after

the alcohol was removed was partitioned between chloroform and water. The chloroform phase was washed with water, aqueous sodium bicarbonate, water, dried over magnesium sulfate and stripped. The residue, m.p. 103-105°, constitutes a nearly quantitative yield of the alkylation product. Recrystallization from chloroform-hexane gave 93-99% of product, m.p. 104-105°. The analytical sample was dried over P_2O_5 at 78° after a further recrystallization from water and melted at 105°.

Analysis: $C_{17}H_{20}N_2O_5$ (333). Calculated, C, 61.4; H, 6.1; N, 8.4. Found, C, 61.6; H, 6.2; N, 8.4.

1-Keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline.-- One hundred grams (0.3 mole) of the diethyl acetamido (o-cyanobenzyl) malonate was saponified by heating with 150 ml. of 6N NaOH in a copper flask under refluxing conditions. After the evolution of ammonia ceased, the solution was acidified to pH 2 and heated to decarboxylate the malonic acid. The slurry was transferred to a glass flask, stripped to dryness and the residue heated under refluxing conditions with 200 ml. of 40% hydrobromic acid. The resulting solution was poured over ice, and the acid precipitated. Three recrystallizations from water gave 20 grams (35%) of the acid, m.p. 235-237°.

Analysis: $C_{10}H_9NO_3$ (191). Calculated, C, 62.8; H, 4.7; N, 7.3; N.E., 191. Found, C, 63.0; H, 4.8; N, 7.1; N.E., 192.

1-Keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline.--Esterification of 1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline was accomplished by the method of Bremner and Huber (9). To 50 ml. of methanol in a flask equipped with addition funnel, stirrer, and drying tube was added dropwise 4.3 ml. (0.6 mole) of thionyl chloride. The flask was cooled in an ice-salt bath and stirred during the addition of the thionyl chloride and 10 grams (0.052 mole) of the acid. After the acid had been added, the slurry was allowed to slowly come to room temperature and give a clear solution. This was heated at 40° for 1 hour, and the solvent removed *in vacuo* to give an oil. The oil was taken up in chloroform, washed with water, aqueous sodium bicarbonate, water, and dried over sodium sulfate. Removal of the solvent gave a solid that was recrystallized from chloroform-hexane m.p. 114-115°.

(-)-1-Keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--To a solution of 7.14 grams of the above ester in 250 ml. in 30% aqueous methanol was added 100 mg. of chymotrypsin in 1 ml. of water. A stream of nitrogen was passed over the surface of the liquid, and the pH maintained between 7-7.5 by addition of 1N sodium hydroxide. The asymmetric hydrolysis is complete in an hour. The solution was acidified to pH 2, allowed to stand in the refrigerator. The precipitated acid was collected on a filter and purified by dissolution in bicarbonate, filtration and reprecipitation with hydrochloric

acid. The 2.52 grams (82%) of the (-)-acid had a specific rotation of α_D^{25} -39.6 (c, 1.99% methanol) and melted at 233-235°. An attempt to duplicate this result with all conditions held constant gave the same acid α_D^{25} -12.5% (c, 2.00% methanol).

(-)-1-Keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline.--Esterification of the (-)-acid as described for the DL-acid gave 70% of the ester, m.p. 89-90°, α_D^{25} -75.8 (c, 4.06% methanol).

(+)-1-Keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline.--Extraction with chloroform of the solution resulting from the resolution of the DL-ester, followed by drying of the organic phase with sodium sulfate and removal of the solvent gave the desired compound. The (+)-ester was recrystallized from chloroform-hexane and has m.p. 98-99°, α_D^{25} 78.6 (c, 4.33% methanol). The duplicate experiment gave (+)-ester α_D^{25} 16.2 (c, 4.12% methanol).

(+)-1-Keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--A 100 milligram sample of the (+)-ester, α_D^{25} 79.4 (c, 2.00% methanol) was dissolved in 10 ml. of dioxane and 10 ml. of 1N NaOH added. Twelve hours later, the solution was treated with Norite, filtered, and 10 ml. of 1N hydrochloric acid added. The acid was collected, washed, dried and melted 233-235°, α_D^{25} 45.6 (c, 2.11% methanol).

Analysis: $C_{10}H_9NO_3$ (191). Calculated, C, 62.8; H, 4.7; N, 7.3. Found, C, 60.5; H, 4.6; N, 6.5.

Two recrystallizations from water gave ca. 40 milligrams, m.p. 234-235°, α_D^{25} 43.2 (c, 2.00% methanol).

Found, C, 62.8; H, 4.8; N, 7.2.

3-Carboxy-1,2,3,4-tetrahydroisoquinoline.--The method of Julian, et. al. (12) was used. A mixture of 37.5 grams of DL-phenylalanine, 85 ml. of formalin, and 388 ml. of conc. hydrochloric acid was heated on the steam bath for 0.5 hour. The addition of 37.5 ml. of formalin and 75 ml. of hydrochloric acid was followed by heating for an additional three hours. The reaction flask was cooled in an ice bath and the white precipitate collected on a sintered glass funnel. The filter cake was taken up in 500 ml. hot water, 1000 ml. of methanol added, and the solution adjusted to pH 5 with 10% aqueous ammonia. The acid crystallized as shimmering platelets, m.p. 313-316(dec.), lit(12) 331°, 33 grams, 80% yield.

N-benzoyl-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--The method shown Steiger to give no racemization of optically active amino acids was used (20). Thirty three grams (0.186 mole) of the above acid was placed in a one liter three necked flask, and suspended in 744 ml. of 0.25N sodium hydroxide. The flask was immersed in an ice-salt bath and stirred rapidly. To the suspension of the sodium salt of the acid were added dropwise 26 grams (0.186 mole) of benzoyl chloride and 43 ml. of 2N sodium hydroxide over 0.5 hour. The mixture was stirred an additional 0.25 hour, treated with Norite,

filtered, and acidified to pH 2 with 6N hydrochloric acid. The gummy solid which precipitated was collected on a sintered glass filter, and the filtrate concentrated *in vacuo* to yield another crop. The two fractions were separately recrystallized from acetone-water and combined to give 38 grams (73%) of the N-benzoyl acid, m.p. 170-172°. Neutralization equivalent calculated, 281; found, 280.

N-benzoyl-(o-carboxyphenyl) alanine.--To 11.2 grams (0.04 mole) of the N-benzoyl isoquinoline acid and 5.5 grams (0.040 mole, 0.08 equivalents) of potassium carbonate in one liter of water was added 12.2 grams of potassium permanganate suspended in 250 ml. of water. The mixture was stirred for nine hours at ambient temperature, sodium sulfite added to remove excess permanganate, and the solution filtered through Celite to remove the finely divided manganese dioxide. Concentration *in vacuo* to a small volume was followed by acidification to precipitate the acid. The dicarboxylic acid was recrystallized from acetone to give 6.66 gram (53%), m.p. 204-207. The analytical sample was recrystallized from acetone, m.p. 210-211°.

Analysis: $C_{17}H_{15}NO_5$ (333). Calculated, C, 65.2; H, 4.8; N, 4.5; N.E. 156. Found, C, 64.9; H, 5.1; N.E. 150.

1-Keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--A 6.7 gram sample of N-benzoyl-(o-carboxyphenyl) alanine in 100 ml. of 6N hydrochloric acid was held at reflux overnight. The solution was cooled, filtered, and the mineral acid and

water removed *in vacuo*. The residue and the filtrate were combined, dried, and extracted with three 30 ml. portions of ethyl ether. The ether insoluble material was recrystallized from water to give 3.28 grams (31%) of the acid. The melting point, 234-235°, neutralization equivalent, 191, mixed melting point, and spectrum in a Nujol mull show this to be the same product as that formed *via* the acetamido malonic ester route.

1-Keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline.--Esterification of the acid as described above gives the ester in 85% yield, m.p. 114-115°. The infra-red spectrum in chloroform is superimposable on the spectrum of the ester obtained *via* the acetamido malonic ester route.

Analysis: $C_{11}H_{11}NO_3$ (204). Calculated, C, 64.4; H, 5.4; N, 6.8. Found, C, 64.4; H, 5.5; N, 6.7.

L-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--Treatment of three grams of L-phenylalanine with formaldehyde and hydrochloric acid as described for the DL compound gave 1.2 gm (40%) of the L-acid, m.p. 274° (dec.), α_D^{25} -58.3 (c, 2.17% methanol). The L-acid is more soluble than the DL-acid and the yield is improved to 60% by precipitating the acid from the hydrochloric acid-formaldehyde solution with methanol. The analytical sample was recrystallized from aqueous methanol.

Analysis: $C_{10}H_{11}NO_2$ (177). Calculated, C, 67.8; H, 6.3; N, ~~7.1.~~ 7.9 Found, C, 67.9; H, 6.45; N, 7.0.

L-N-benzoyl-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--The benzoylation of the above L-acid was carried out as described for the DL acid in 72% yield, m.p. 163-164. The analytical sample was recrystallized from acetone-water, m.p. 164°, $[\alpha]_D^{25}$ -33.7 (c, 1.99% methanol).

Analysis: $C_{17}H_{15}O_3N$ (281). Calculated, C, 72.6; H, 5.4; N, 5.0. Found, C, 72.6; H, 5.4; N, 5.0.

L-N-benzoyl-(o-carboxyphenyl) alanine.--Oxidation of the cyclic N-benzoyl acid, 1.35 gram (4.8 millimole) with 1.02 grams (6.44 millimole) of potassium permanganate in 250 ml. of 2.91×10^{-3} M potassium carbonate solution was allowed to proceed for six hours. Sodium sulfite was added, the solution freed of manganese dioxide, and the filtrate adjusted to pH 6. The solution was concentrated *in vacuo*, acetone added, and acidified to pH 2 with hydrochloric acid. The precipitated salt was removed by filtration, and the solution concentrated until crystallization began. There was obtained 0.87 gram (58%) of the oxidation product, m.p. 100-102°, $[\alpha]_D^{25}$ -105 (c, 2% methanol).

Analysis: $C_{17}H_{15}NO_5$ (313). Calculated, C, 65.2; H, 4.8; N, 4.5. Found, C, 65.2; H, 4.8; N, 4.5.

L-1-keto-3-carboxy-1,2,3,4-tetrahydroisoquinoline.--Hydrolysis of the N-benzoyl dicarboxylic acid as described for the DL compound gave the L-lactam, m.p. 230-230°, $[\alpha]_D^{25}$ 41.4 (c, 2.0% methanol).

L-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline.--Esterification of the L-acid with methanol and thionyl chloride gave the L-ester, m.p. 88-90°, α_D^{25} 75.9 (c, 1.90% methanol). The rotation and hydrolysis behavior indicate that this is 95% L-ester.

TABLE I

The Resolution of DL-1-keto-3-carbomethoxy-1,2,3,4-tetrahydroisoquinoline[a]

Run	pH	grams DL-ester	Vol. Solvent[b]	Enzyme Conc.[c]	Rotation (-)-acid[d]	Rotation (+)-ester
1	8.0	0.628	75	0.735	-[e]	-[e]
2	8.0	3.0	100	-[e]	-30	-[f]
3	7.0	11	750	0.133	-30	-[f]
4	7.0	28	900	0.221	-32.5	-[f]
5[g]	7.0	8.07	250	0.250	-40.3[l]	49.2[j]
6[g]	7.0	7.14	250	0.400	-39.7[m]	78.6[j]
7[g]	7.0	7.0	250	0.400	-17.2	16.2[j]
8[g]	7.0	2.5[h]	60	0.87	-12.5	79.3[d]
9[g]	7.0	2.4[i]	60	0.87	-37.6	79.4[d]
10[g]	7.0	2.0	100	0.400	-40.4	79.9[d]
11[g,k]	7.0	2.6	100	0.400	-19.5	78.9[d]

a. All runs were carried out in a beaker of appropriate size. The solutions were stirred with a magnetic stirrer and the pH measured with a Beckman model G pH meter equipped with extension electrodes.

b. The solvent is 30% methanol. The ester was dissolved in methanol and distilled water added to 30% (vol./vol.) methanol-water.

c. In milligrams per milliliter.

d. Concentration 2% in methanol.

e. Not recorded.

f. The (+)-esters combined from runs 2-4 α_D^{25} 3.23 (c, 10% methanol).

TABLE I (Continued)

g. A stream of nitrogen was passed over the surface of the solution to exclude CO_2.
h. The (-)-ester obtained in run 7.
i. The (+)-ester obtained in run 7.
j. Concentration 4% in methanol.
k. Prepared by reaction sequence starting with DL-phenylalanine.
l. Reesterification gave the (-)-ester α_D^{25} -78.8 (c, 4% methanol.
m. Reesterification gave the (-)-ester α_D^{25} -75.8 (c, 4% methanol).

TABLE II

The α-Chymotrypain-catalyzed Hydrolysis of 1-Keto-3-Carbomethoxy-1,2,3,4-tetrahydroisoquinoline[a]

Date[b]	Origin[c]	S_o[d]	E_o[e]	μeq. base[f]	% Hydrolysis[g]	Time[h]
9-2-58	2-4, (-)[i]	7.90	14.5	39.0	50	3
9-2-58	2-4, (+)[j]	8.60	14.5	30.8	36	3
9-5-58	2-4, (-)[i]	8.16-16	15.8	81.7-136.1	49.4[k]	3
10-23-58	6, (+)	3.92	15.0	0.23	0.59	8
10-23-58	5, (+)	3.96	15.0	8.60	21.70	8
10-23-58	6, (-)	3.93	15.0	42.0	108	2
10-23-58	6, (-)	3.93	1.50	42.0	108	18.4
9-24-58	DL[l]	8.37	14.3	41.2	49.3	4.8
9-24-58	DL[l]	8.37	1.43	26.1	31.4	8
12-16-58	5, (-)	8-16	.852	24-32	8-16[m]	8
1-20-59	DL[l,n]	7.85	14.3	6.63	8.45	9
1-20-59	DL[l]	7.85	14.3	36.4	46.2	3
4-29-59	DL[o]	8.20	12.0	42.8	52.3	3
4-29-59	L[p]	8.08	12.0	5.41	6.70	4
5-12-59	L	7.12	146.4	16.9	23.8	8
5-12-59		1.76	146.4	15.7	89.6	33
5-12-59	11, (+)	2.51	14.6	0.93	3.7	8
5-12-59	5, (-)	5.4	1.46	15.6	28	8
5-12-59	5, (-)	45.5	1.46	28.9	6.35	8

TABLE II (Continued)

Date[b]	Origin[c]	S_o[d]	E_o[e]	μeq. base[f]	% Hydrolysis	Time[h]
6-5-59	DL[o]	4.15	163	21.3, 3.0[g]	51, 7.2[g]	1.5, 8[g]
6-5-59	6, (-)	4.36	163	43.2	99	3
6-5-59	DL[o]	4.15	1.63	19.4	46.7	9
6-5-59	6, (-)	4.36	1.63	35.0	80	8
6-5-59	L[p]	3.93	1.63	1.69	4.3	8
6-5-59	L[o]	3.93	163	4.0	10.1	8

a. The experimental conditions were as follows. Unless otherwise noted, the runs were carried out in aqueous solutions of 10 ml. at 25.0°, pH 7.90, and 0.1M in sodium chloride, and titrated under nitrogen.

b. Month-day-year.

c. Run number in Table I, direction of rotation of ester in methanol, or configuration related to L-phenylalanine.

d. In units of 10^{-3}M.

e. In units of 10^{-3} mg. protein nitrogen per ml.

f. Calculated by multiplying scale reading at time given by scale factor by normality of sodium hydroxide added.

g. Calculated by converting S_o into μeq. per 10 ml., and dividing this quantity into 100 times the μeq. base added.

h. In minutes the reaction was followed.

TABLE II (Continued)

i. The combined (-)-esters from runs 2-4, Table I had a_D^{25} -3.82 (c, 10% methanol).
j. The combined (+)-esters from runs 2-4, Table I had a_D^{25} 3.23 (c, 10% methanol).
k. Average extent of hydrolysis of four runs between the concentration limits given in column 3.
l. The DL-ester synthesized by a reaction sequence beginning with alkylation of diethyl acetamido malonate.
m. For four different concentrations over the range given in column 3.
n. This run was carried out in 30% (vol./vol.) methanol-water, pH 7.90, and no added salt.
o. Synthesized by a reaction sequence beginning with DL-phenylalanine.
p. Synthesized by a reaction sequence beginning with L-phenylalanine.
q. The first figures are for the initial, rapid hydrolysis; the second are for the slower hydrolysis as calculated from the point where the first reaction ceased.

REFERENCES

1. Bixler, R., Ph.D. Thesis, California Institute of Technology, 1957.
2. Smoke, J. E., and Neurath, H., Arch. Biochem., 21, 337 (1949).
3. Cope, A. C., Holmes, H. L., House, H., and Adams, R., Ed. "Organic Reactions" IX, J. Wiley, New York, N.Y., p. 107.
4. Taft, R. W. and Newman, M. S., Ed. "Steric Effects in Organic Chemistry" J. Wiley, New York, 1956, p. 648.
5. Bamberger, E. and Dieckmann, W., Ber., 26, 1205 (1893).
6. Bain, O., Perkins, W., Robinson, R., J. Chem. Soc., 105, 2392 (1922).
7. Reissert, A., Ber., 29, 633 (1896); Leuchs, H. and Katinszky, H., ibid., 55, 710, (1922).
8. Hall, H. K., J. Am. Chem. Soc., 80, 6404 (1958).
9. Brenner, M. and Huber, W., Helv. Chim. Acta, 36, 1109 (1953).
10. Bixler, R. L. and Niemann, C., J. Am. Chem. Soc., 81, 1412 (1949).
11. All enzyme concentrations are in units of mg. protein-nitrogen per ml.
12. Julian, R. L., Karper, W. J., Magnani, A., Meyer, E., J. Am. Chem. Soc., 70, 180 (1948).

13. Fuson, R. C., "Advanced Organic Chemistry," J. Wiley, New York, N.Y. 1950, p. 247.

14. Schotten, C. and Schlomann, H., Ber., 24, 3687 (1891).

15. French, D. and Edsall, J. T., Advances in Protein Chemistry, 3, 283 (1947).

16. Greenstein, J. P., Advances in Protein Chemistry, 9, 121 (1953).

17. All melting points are corrected.

18. Analyses by Dr. A. Elek.

19. Fuson, R. C., J. Am. Chem. Soc., 48, 830 (1926).

20. Steiger, R. E., J. Org. Chem., 9, 396 (1944).