played significant infrared absorptions at 2.89 (m), 3.40 (m), 5.73 (s), 5.90 (s), 6.99 (w), 7.23 (m), and 8.05 μ (s).

One gram (6.0 mmoles) of 6-methylthiopurine (IIIb) and 3.0 g. (19.0 mmoles) of D-4-acetoxy-5-hydroxy-2-pentenaldehyde (VIIa) were dissolved in 100 ml. of N,N-dimethylformamide containing 2.0 ml. of triethylamine. The solution was heated at 60–70° for 16 hr. and finally evaporated *in vacuo* at 50° to remove the solvent. The sirupy residue was taken up in 100 ml. of 0.1 *N* hydrochloric acid and heated on the steam bath for 30 min. An examination of the brown solution at this point by ascending paper chromatography (solvent B) revealed the presence of unreacted 6-methylthiopurine ($R_{6\text{-MTP}}$ 1.0) plus strong light blue fluorescent spots at $R_{6\text{-MTP}}$ 0.65 and 0.49, at identical positions with IVb and Vb. Small quantities of light blue fluorescent material which ran ahead of IIIb were present, probably corresponding to acetylated IVb and Vb. The combined yield of IVb and Vb was 78%, estimated spectrophotometrically from eluents of the chromatograms.

A portion of the crude mixture from above was chromatographed over silica gel using solvent B as eluent, as described previously, collecting 23-ml. fractions. Tubes 130–220 contained recovered 6-methylthiopurine (IIIb); tubes 235–310 contained the material of $R_{6\text{-MTP}}$ 0.65; and tubes 335–445 contained the material of $R_{6\text{-MTP}}$ 0.49. A work-up of the combined tubes within each fraction gave compounds IVb and Vb, identical in all respects (infrared spectra, melting points, paper chromatography) with the compounds obtained by direct condensation of 2-deoxy-D-*erythro*-pentose (I) with 6-methylthiopurine (IIIb).

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The Michael addition of IIIb to the α,β-unsaturated aldehyde VIIa could also be carried out in water, although in lower yield. Thus, 0.10 g. of 6-methylthiopurine and 0.30 g. of VIIa were added to 10 ml. of water and the solution was refluxed for 16 hr. Ascending paper chromatography (solvent B) revealed the desired products (IVb and Vb) to be present, along with a considerable quantity of acetylated material. One ml. of 1 *N* hydrochloric acid was added and the mixture was allowed to stand overnight at room temperature. A second chromatography revealed the acetylated material had been largely hydrolyzed by this treatment. The combined yield of compounds IVb and Vb was approximately 54%, estimated spectrophotometrically on eluents of the chromatograms.

Attempted Condensation of 6-Methylthiopurine (IIIb) with D-5-Acetoxy-5,6-dihydro-2-ethoxy-2H-pyran (VIII).—Compound VIII was prepared by the treatment of VIIa with ethyl orthoformate in ethanolic ammonium chloride solution, as described by Gehrke.[18] 6-Methylthiopurine (IIIb, 50 mg.) and VIII (150 mg.) were dissolved in 10 ml. of dry N,N-dimethylformamide containing 0.1 ml. of triethylamine. The resulting solution was heated at 60–70° for 16 hr. Paper chromatography of the colorless solution (solvent B) revealed only unreacted IIIb as a single bright yellow fluorescent spot ($R_{6\text{-MTP}}$ 1.00).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, BRANDEIS UNIVERSITY, WALTHAM, MASSACHUSETTS]

Hydrolysis of D(−)-Ethyl β-Phenyl-β-hydroxypropionate and D(−)-Ethyl β-Phenyl-β-acetamidopropionate by α-Chymotrypsin[1]

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Hydrolysis of DL-ethyl β-phenyl-β-hydroxypropionate by α-chymotrypsin leads to high yields of L(+)-ethyl β-phenyl-β-hydroxypropionate and D(−)-β-phenyl-β-hydroxypropionic acid. Hydrolysis of DL-ethyl β-phenyl-β-acetamidopropionate by α-chymotrypsin leads also to the corresponding L(+) ester and D(−) acid. Kinetic parameters for hydrolysis of D(−)-ethyl-β-phenyl-β-hydroxypropionate are K_m = 0.0062 *M*, k_3 = 0.032 sec.$^{-1}$. Hydrolysis of the L-enantiomorph is much slower and not dependent on concentration. Factors affecting the D-specificity and rates of hydrolysis of these β-substituted esters and the L specificity and rates of hydrolysis of diethyl β-acetamido- and β-hydroxyglutarates and diethyl N-acetylaspartate are discussed. The mechanism previously proposed for preferential hydrolysis of ethyl D-α-acetoxypropionate may not be applied to the D specificity of hydrolysis of the title compounds.

Introduction

The stereospecificity of reactions of α-chymotrypsin has been described in terms of sites on the enzyme oriented complementarily to the four groups oriented tetrahedrally about the α-carbon atom of substrates which are derivatives of α-substituted carboxylic acids.[2–5a,6] While the composition of the complementary sites is uncertain, it has been proposed[4,6] that one is much restricted[5b] and requires a small substrate group, the α-hydrogen; a second may make use of hydrogen bonding, as from the α-acylamido group; a third is relatively nonpolar, associating strongly with β-aryl or other large nonhydrophilic substituent; the fourth may be the reaction site at which presumably is located a serine hydroxyl, which attacks the carbonyl carbon of the group which is undergoing hydrolysis. In these terms, substitution of the α-acetoxyl group of ethyl α-acetoxypropionate for the acetamido group of the corresponding derivative of alanine leads, in the absence of a nonhydrophilic β-substituent, to association of the acetoxyl group at the nonpolar site of the enzyme, inversion of the usual stereospecificity, and more rapid hydrolysis of the D rather than of the L enantiomorph.[4,6] A similar interpretation, also involving productive and nonproductive binding interactions, has been applied to the hydrolysis of D-1-keto-3-carbomethoxytetrahydroisoquinoline by α-chymotrypsin[3,5,7a]; nonproductive, competitive binding interactions had been envisaged previously.[7b]

In our investigation of structural requirements for stereospecificity in these reactions, we found that both the symmetric molecules diethyl α-acetamidomalonate[8] and diethyl β-acetamidoglutarate,[9] and the asymmetric molecules ethyl α-acetamidopropionate[10] and ethyl β-phenyl-β-acetamidopropionate[10] were hydrolyzed stereospecifically by α-chymotrypsin. It appeared that the presence of an α- or β-acetamido group at a center or

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developing center of asymmetry was sufficient to lead to this stereospecificity, and the presence of the typical β-aryl substituent was not required, although its presence does lead to greatly enhanced reactivity. We also found that ethyl β-phenyl-β-hydroxypropionate[11] and dimethyl and diethyl β-hydroxyglutarates[11] were hydrolyzed stereospecifically. The L enantiomorph of ethyl β-phenyl-α-hydroxypropionate was hydrolyzed more rapidly than the D enantiomorph by α-chymotrypsin,[12] while ethyl lactate,[11] diethyl α-hydroxymalonate,[11] and ethyl β-hydroxybutyrate[11] were hydrolyzed without stereospecificity, the latter exceedingly slowly. Thus it appeared that a hydroxyl group could lead to some stereospecificity when substituted for an acetamido group, and that it probably required for this the assistance of a bulky aryl or second carbethoxy group.

The steric sense of the α-chymotrypsin-catalyzed hydrolysis of dimethyl β-hydroxyglutarate appears to be L.[11] The product, (−)-methyl hydrogen β-hydroxyglutarate, obtained in high yield and in high optical purity, was converted to the (−)-1,3-bis(*p*-dimethylaminophenyl)ureide of methyl hydrogen β-acetoxyglutarate which had been obtained from (+)-methyl hydrogen β-acetoxyglutarate, the absolute configuration of which had been assigned[13] by relation to the acetylated methyl ester of (−)-3-D-hydroxypentanoic acid. Quite unexpectedly, it appears that the stereospecific hydrolysis of ethyl β-phenyl-β-hydroxypropionate[11] and ethyl β-phenyl-β-acetamidopropionate[10] proceed by the more rapid hydrolyses of the D enantiomorphs, indicating apparent inversion of the normal stereospecificity.

Ethyl β-Phenyl-β-hydroxypropionate.—When DL-ethyl β-phenyl-β-hydroxypropionate[11] was treated with α-chymotrypsin at pH 7.8, hydrolysis slowed down markedly at about 50% hydrolysis, and work-up at this point led to high yields of (+)-ethyl β-phenyl-β-hydroxypropionate, $[\alpha]^{22}D$ +40°, and of (−)-β-phenyl-β-hydroxypropionic acid, $[\alpha]^{22}D$ −19.2°. (+)-β-Phenyl-β-hydroxypropionic acid has been converted[14] to optically active ethyl β-phenyl-β-hydroxypropionate, and this to (+)-β-phenyl-β-hydroxypropionamide and the latter, by the Hofmann reaction, to (+)-β-phenyl-β-hydroxyethylamine. This (+)-amine has also been prepared from (+)-mandelic acid *via* (+)-mandelamide.[15,16] Since (+)-mandelic acid belongs to the L series,[17] the (−)-β-phenyl-β-hydroxypropionic acid obtained in our enzymatic hydrolysis of the DL ester belongs to the D series; the D ester had been hydrolyzed preferentially by α-chymotrypsin, and excess L(+) ester had been recovered. It also has been demonstrated[18] by use of the Arndt–Eistert reaction that L-(+)-mandelic acid and (+)-β-phenyl-β-hydroxypropionic acid belong to the same series (L).

We have prepared DL-ethyl β-phenyl-β-hydroxypropionate, converted part of it to DL-β-phenyl-β-hydroxypropionic acid, resolved the acid, converted the L(+) and D(−) acids to the L(+) and D(−) ethyl esters, and examined the action of α-chymotrypsin on the DL, D(−), and L(+) esters. The DL ester behaved as reported previously,[11] leading, after 50% reaction, to high yields of ester $[\alpha]^{22}D$ +42°, and of D(−) acid, $[\alpha]^{22}D$ −18.2. The rotation of the ester, when compared with that of the enantiomorphs (54°), indicated the presence of 89% L(+) and 11% D(−) ester, the D(−) ester appearing to hydrolyze about ten times as rapidly as the L. The D and L enantiomorphs were hydrolyzed separately at pH 7.8 at three concentrations between 2 and 8 × 10^{-3} *M* (Table I); for the D compound a plot[19] of $1/V$ against $1/S$ was linear and led to $K_m = 6.2 \times 10^{-3}$ *M*, $k_3 = 0.032$ $sec.^{-1}$; the L compound was hydrolyzed quite slowly at a rate independent of concentration of substrate, $k_3 = 0.0017$ $sec.^{-1}$, as though it were firmly ($K_m \sim 0$) but ineffectually bound to the enzyme. The rates of hydrolysis of the DL material under these conditions indicated that presence of the L compound interfered somewhat with hydrolysis of the D. The relative rates of hydrolysis of the separate D and L compounds were consistent with the approximate 10 : 1 ratio of rates indicated by the results of the preparative hydrolyses.

TABLE I

HYDROLYSIS OF L(+)- AND D(−)-ETHYL β-PHENYL-β-HYDROXYPROPIONATE BY α-CHYMOTRYPSIN[a]

	Ester, 10^3 *M*	Rate, 10^7, moles $l.^{-1}$ $sec.^{-1}$
L	8.33	2.75
	5.60	2.58
	2.58	2.58
D	7.40	28.2
	5.00	23.2
	2.67	15.8
DL	13.4	21.9
	9.35	15.8
	6.40	14.5
	3.40	12.5

[a] 5 mg./ml., 0.1 *M* NaCl, 25°, pH 7.8.

Ethyl β-Phenyl-β-acetamidopropionate.—DL-Ethyl β-phenyl-β-acetamidopropionate was treated with α-chymotrypsin; slow partial hydrolysis took place, leading to (−)-β-phenyl-β-acetamidopropionic acid and (+)-ethyl β-phenyl-β-acetamidopropionate.[10] There have been contradictory interpretations of the steric course of the deamination of some β-amino acids[20,21] and uncertainty about their absolute configurations, but it appears that (+)-β-phenyl-β-aminopropionic acid[22] belongs to the L series. In this correlation[22] (−)-β-phenyl-β-aminopropionic acid was shown to have the same configuration as D(−)-α-phenyl-α-aminoacetic acid, each being related to D(−)-β-phenyl-β-aminoethanol. Subsequent study of the optical rotatory dispersion[23] of the methyl dithiocarbamate and methyl dithiocarbonate of the L(+)-β-phenyl-β-amino- and L(+)-β-phenyl-β-hydroxypropionic acids, respectively, shows that they have negative Cotton effects, opposite in sign to the Cotton effects of the correspondng α-amino and α-hydroxy acids, and that these effects may be useful for assignment of configuration. We have repeated the α-chymotrypsin-catalyzed hydrolysis of the DL-ethyl β-phenyl-β-acetamidopropionate and obtained (−)-β-phenyl-β-acetamidopropionic acid, $[\alpha]^{22}D$ −86°, and (+)-ethyl β-phenyl-β-acetamidopropionate, calcd. $[\alpha]^{22}D$ +57°. We have prepared β-phenyl-β-aminopropionic acid, resolved it through its formyl derivative, isolated L(+)-β-phenyl-β-aminopropionic acid, converted it to L(+)-β-phenyl-β-acetamidopropionic acid, $[\alpha]^{22}D$ +103°, and com-

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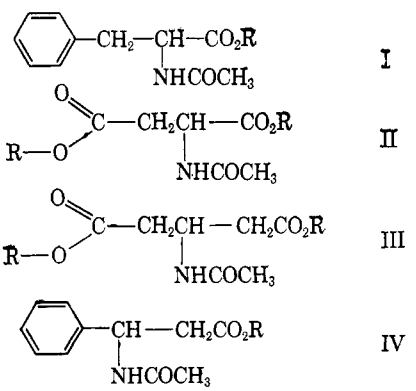
pared it with a sample, $[\alpha]^{22}D$ +95°, kindly provided by Dr. B. Sjoberg, Aktiebolaget Astra, Sodertalje, Sweden. Conversion of the (+)-amino acid to the (+)-acetamido acid establishes that this enzymatic hydrolysis had proceeded in the D sense.

Hydrolysis by α-chymotrypsin of both materials, DL-ethyl β-phenyl-β-hydroxypropionate and DL-ethyl β-phenyl-β-acetamidopropionate, was effectively inhibited by diphenylcarbamyl chloride, giving evidence that the usual active site of α-chymotrypsin was involved in these reactions.[24]

Discussion

The interpretation offered for the more rapid hydrolysis of the D enantiomorph of ethyl α-acetoxypropionate,[4,6] namely that the acetoxy group associates at the β-aryl site and the α-hydrogen and ester group at their usual sites, cannot apply in the present circumstances. It appears that the acetoxyl group generally behaves, in association with α-chymotrypsin, like a nonpolar nonhydrophilic group, and quite differently from hydroxyl and acetamido. Ethyl β-phenyl-β-hydroxypropionate and ethyl β-phenyl-β-acetamidopropionate have β-aryl groups and polar hydroxy and acetamido groups, respectively. Also, other detailed analyses[7] of stereospecificity of reactions of α-chymotrypsin would appear neither to explain nor predict the present observations. Mere change of the position of the hydroxy and acetamido groups from the α- to the β-location in these materials leads to far more rapid hydrolysis of the D enantiomorphs by α-chymotrypsin. But in diethyl β-hydroxyglutarate,[11] and probably also in diethyl β-acetamidoglutarate,[11] stereospecific hydrolysis occurred in the L sense. The location of the substituent with respect to the hydrolyzing ester group is not sufficient to lead to this apparent inversion of stereospecificity.

The second carbethoxyl group in the glutarates led to greatly increased reactivity, the β-hydroxy- and β-acetamidoglutarates hydrolyzing far more rapidly than the β-hydroxy-[11] and β-acetamidobutyrates,[10] which were very inert. The carbethoxyl groups also led to increased stereospecificity. The effect of the second carbethoxy group, β to an α- instead of a β-acetamido group was examined, and diethyl N-acetylaspartate (II) proved to be an excellent substrate[25] for α-chymotrypsin, but not quite as reactive as ethyl N-acetyl-β-phenylalaninate (I). From the formulas of the acetamido derivatives I–IV, it appears that the second carbethoxyl group in the aspartate (II) may associate with α-chymotrypsin at precisely the position at which the β-phenyl of the alaninate (I) associates. This places the α-acetamido and hydrolyzing ester groups at their normal positions and high reactivity and normal stereospecificity[25] result. The second carbethoxyl group in the glutarate (III) may associate with the enzyme similarly, placing the acetamido group and the methine hydrogen at the normal positions for these groups leading to normal stereospecificity, but placing the hydrolyzing ester group one methylene away from the nucleophilic group of the enzyme. Distortion is required for reaction, and low activity results.[26] In ethyl β-phenyl-β-acetamidopropionate (IV), and in the corresponding hydroxy compound, association of the phenyl group at its normal site is dominant, leaving the acetamido or hydroxyl group one methylene displaced from the normal acylamido site. Several possibilities may then be considered to account for the observed D stereospecificity: (1) The β-acetamido or hydroxyl group may find at this position a hydrogen bonding site which is not normally called upon and which prefers a D configuration. (2) The β-acetamido or hydroxyl may associate at the normal α-acylamido site causing a distortion in the enzyme, and this distorting association may occur preferentially with the D substrate. (3) The association may occur preferentially with the L substrate, but it distorts the enzyme and makes the nucleophilic site inaccessible to the ester. The L substrate may thus be bound firmly but ineffectively. The D ester may associate essentially only through its phenyl group, the acetamido group of "incorrect" configurations may be little involved and distortion is minimal and hydrolysis results. The stereospecificity would result from factors which diminish the reactivity of the L enantiomorph rather than increase the reactivity of the D. The limited kinetic data which we have on the ethyl β-phenyl-β-hydroxypropionate seem more consistent with this third possible explanation; the reactivity of the ethyl β-phenyl-β-acetamidopropionate is quite low, and it has not seemed feasible to examine it kinetically. Experiments are in progress in which the study of related compounds as competitive inhibitors may allow a choice to be made among the several interpretations.



Experimental

α-Chymotrypsin was from Worthington, was recrystallized three times, and was salt free. A sample was dried at 100° to allow for correction of weight due to water content. Melting points are uncorrected.

L(+)- and D(−)-Ethyl β-Phenyl-β-hydroxypropionate.—Ethyl bromoacetate (65.5 g., 0.40 mole) and 52 g. (0.49 mole) of benzaldehyde were treated with 32 g. (0.50 g.-atom) of zinc as described in the literature,[27] leading to **ethyl *dl*-β-phenyl-β-hydroxypropionate**, 37 g. (0.19 mole), 48% yield, b.p. 146–148° (3 mm.). This compound (33 g., 0.17 mole) was boiled under reflux for 4.5 hr. in 300 ml. of 5% potassium hydroxide and was allowed to stand overnight. The solution was acidified with hydrochloric acid and evaporated to dryness *in vacuo*, and the residue was extracted with ether. The extract was dried and concentrated, leading to **DL-β-phenyl-β-hydroxypropionic acid**, 23 g. (0.14 mole), 82% yield, m.p. 92–93° from chloroform, lit.[28] m.p. 93–94°. Brucine (16.2 g., 0.042 mole) was added to a hot solution of 16.5 g. (0.10 mole) of this compound in 120 ml. of ethyl acetate, and the solution was allowed to cool.[28,29] Crystals were collected and the filtrate was set aside. The salt was recrystallized six times from ethyl acetate, yielding 8.11 g. (0.014 mole, 33%), m.p. 99–108° dec., lit.[28] m.p. 89–108° dec. The brucine salt was treated with 100 ml. of *N* hydrochloric acid, the solution was taken to dryness, the residue was extracted with ether, and the ether was dried and concentrated, leading to **L(+)-β-phenyl-β-hydroxypropionic acid**, 1.70 g. (0.010 mole), 25% yield, m.p. 117–118° from chloroform, lit.[30] m.p. 116°, $[\alpha]^{22}D$ +21.1°, 2.2% in ethanol; lit.[28] $[\alpha]^{21}D$ +18.2°. The ethyl acetate filtrate which had been set aside was extracted with

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N hydrochloric acid, dried, and concentrated leading to D(−)-β-phenyl-β-hydroxypropionic acid, 1.60 g. (0.0096 mole), 23% yield, m.p. 118–119° from chloroform, lit.[29] m.p. 115–116, $[\alpha]^{22}D$ −18.9, 2.3% in ethanol. Diazoethane was prepared from 3 g. (0.02 mole) of ethyl N-nitrosourethane in 100 ml. of ether and was added to a boiling solution of 15 g. of potassium hydroxide in 60 ml. of absolute ethanol and 100 ml. of ether. Distillation of diazoethane and ether was continued until the distillate was colorless, ether being added to the distillation flask. D(−)-β-Phenyl-β-hydroxypropionic acid (1.0 g., 0.0060 mole) was added to the diazoethane solution, magnesium sulfate was added, and the solution was allowed to stand overnight. The solution was filtered, concentrated, and distilled leading to **D(−)-ethyl β-phenyl-β-hydroxypropionate**, 0.34 g. (0.0018 mole), 30% yield, b.p. 75° (0.05 mm.), $[\alpha]^{22}D$ −54.9°, 3.5% in chloroform. **L(+)-Ethyl β-phenyl-β-hydroxypropionate** was prepared similarly from 1 g. of the L(+) acid, yielding 0.40 g. (35%), b.p. 68–70° (0.03 mm.), lit.[29] b.p. 90–91° (<0.1 mm.), $[\alpha]^{22}D$ +53.2°, 3.4% in chloroform; lit.[29] +19.17° (neat). The infrared spectra were identical for the D, L, and DL esters, and the partially active ester (L) recovered from the enzymatic hydrolysis of the DL ester, below.

Enzymatic Hydrolysis of Ethyl β-Phenyl-β-hydroxypropionate. A.—A suspension of 1.96 g. (0.0101 mole) of the DL ester in a solution of 0.326 g. of α-chymotrypsin in 12 ml. of 0.1 *M* Na_2HPO_4 and 19 ml. of water was allowed to react at pH 7.8 in a pH stat at 27° for 21 hr., 5.10 ml. of *N* sodium hydroxide being consumed in 21 hr., the reaction then being quite slow. The suspension was extracted with ether, the extracts were dried and concentrated leading to optically active [excess L(+)] unhydrolyzed ester. The aqueous solution was brought to pH 2, taken to dryness under vacuum, and the residue was extracted with acetone, leading to active acid. A total of 12.8 g. (0.066 mole) of DL ester was treated in this way. The samples of recovered ester were combined and distilled to yield 5.4 g. (0.028 mole, 84%), b.p. 80–82° (<0.1 mm.), $[\alpha]^{22}D$ +41.8, 3.1% in chloroform; lit.[29] $[\alpha]D$ +19.17°, neat.

Anal. Calcd. for $C_{11}H_{14}O_3$: C, 68.0; H, 7.28. Found (by C. Fitz): C, 67.9; H, 7.3.

The samples of the acid hydrolysis product, β-phenyl-β-hydroxypropionic acid, containing excess D(−) acid were combined to yield 5.0 g., 100% crude yield; after crystallization from chloroform the melting point was 116–117°, $[\alpha]^{22}D$ −18.2, 2.2% in ethanol; lit.[30] m.p. 116°, $[\alpha]D$ −18.4°, 2.4% in ethanol.

The D(−) and L(+) Esters. B.—The ester was added to 15 ml. of 0.1 *M* sodium chloride under nitrogen at 25°, brought to pH 7.8, and followed in the pH stat for 30 min. α-Chymotrypsin, 0.100 g. in 5 ml. of water, was added, the pH was restored to 7.8, and the consumption of 0.1 *N* NaOH followed. Initial zero-order rates were determined at several concentrations of ester and are indicated in Table I. Corrections have been applied for consumption of alkali by the esters and enzyme alone.

L(+)-β-Phenyl-β-acetamidopropionic Acid.—Malonic acid (90 g., 0.86 mole), ammonium acetate (135 g., 1.75 moles), benzaldehyde (90 g., 0.86 mole), and 200 ml. of 95% ethanol were boiled under reflux for 6 hr., cooled, and filtered. The solid was dissolved in 1.5 l. of boiling water, treated with 4.2 l. of ethanol, cooled in ice, filtered, and washed with 95% ethanol, leading to **β-phenyl-β-aminopropionic acid**, 54 g. (0.33 mole), 38% yield, m.p. 215–216°, lit.[31] m.p. 216°. This product, 50 g. (0.30 mole), was heated three times[32] on the steam bath for 3 hr. with 75 g. of 95% formic acid and was evaporated to dryness. The residue was dissolved in water and extracted with ethyl acetate. The extract was treated with Norite and sodium sulfate, filtered, and concentrated to dryness, leading to **N-formyl-β-phenyl-β-aminopropionic acid**, 42 g. (0.21 mole), 68% yield, m.p. 127.5–128.5 from water, lit.[32] m.p. 128°. This compound, 0.21 mole, and 68 g. (0.21 mole) of quinidine were dissolved in 350 ml. of boiling methanol and allowed to crystallize. The salt was collected and recrystallized from methanol, leading to the **quinidine salt of L(+)-N-formyl-β-phenyl-β-aminopropionic acid**, 34 g. (0.065 mole), 62% yield, m.p. 192–193°, $[\alpha]^{22}D$ +181°, 5% in ethanol. A solution of 26 g. (0.050 mole) of this salt in 130 ml. of ethanol was treated with 52 ml. of *N* sodium hydroxide and concentrated to about 40 ml. *in vacuo*. The quinidine was filtered, and the filtrate was treated with 12 ml. of 5 *N* hydrochloric acid, leading to **L(+)-N-formyl-β-phenyl-β-aminopropionic acid**, 7.5 g. (0.039 mole), 36% yield, softens at 134°, m.p. 144–145°, lit.[32] m.p. 142–143°; $[\alpha]^{22}D$ +103°, 0.64% in ethanol; lit.[32] $[\alpha]D$ +115°. This compound (7.0 g., 0.036 mole) was boiled under reflux for 1 hr. in 125 ml. of 10% hydrochloric acid and concentrated to dryness *in vacuo*, and the residue was crystallized from methanol–ether leading to the hydrochloride of **L(+)-β-phenyl-β-aminopropionic acid**, 6.0 g. (0.030 mole), 83% yield. This was dissolved in water and treated with 30 ml. of *N* sodium hydroxide at 40°, leading to **L(+)-β-phenyl-β-aminopropionic acid**, 2.2 g. (0.013 mole), 44% yield, m.p. 232–233°, lit.[32] m.p. 234–235°; $[\alpha]^{22}D$ +10.6°, 0.65% in water; lit.[32] $[\alpha]^{20}D$ +6.9°; $[\alpha]^{22}D$ −10.2°, 1.5% in *N* sodium hydroxide; reported[32] $[\alpha]^{20}D$ −9.1°. L(+)-β-phenyl-β-aminopropionic acid, 1.0 g. (0.0060 mole), in 15 ml. of acetic anhydride was boiled under reflux for 3 hr. and concentrated *in vacuo*. The residue was taken up in chloroform and shaken with water, leading to **L(+)-β-phenyl-β-acetamidopropionic acid**, 0.64 g. (0.0031 mole), 51% yield, m.p. 198–199°, melting point of sample from Dr. B. Sjoberg, 200–202°; m.m.p. 199–200°, $[\alpha]^{22}D$ +103°, 1% in ethanol; $[\alpha]^{22}D$ of sample from Dr. Sjoberg, +95°.

Enzymatic Hydrolysis of DL-Ethyl β-Phenyl-β-acetamidopropionate.—DL-Ethyl β-phenyl-β-acetamidopropionate was prepared as described previously,[10] m.p. 39–40°, lit.[10] m.p. 40.5–41.5°. This compound (0.504 g., 2.14 mmoles) was suspended in 15 ml. of water and brought to pH 7.8 in a pH stat, under nitrogen. Consumption of sodium hydroxide was nil. α-Chymotrypsin (0.106 g. in 5 ml. of water) was added, the pH was brought to 7.8, and consumption of alkali was followed, 0.360 ml. of *N* sodium hydroxide being consumed in 50 hr. Additional enzyme (0.097 g.) was added, 0.182 ml. of additional sodium hydroxide was required in the next 45 hr.; 25% hydrolysis resulted with 50% of one enantiomorph. The mixture was extracted with ether. The aqueous solution was brought to pH 2 with hydrochloric acid and lyophilized; the residue was extracted with acetone, the acetone was evaporated, and this residue was shaken with chloroform and water and crystallized from water to yield D(−)-β-phenyl-β-acetamidopropionic acid, 0.0312 g., (0.15 mmole), 28% yield based on consumption of alkali, m.p. 200–201°, $[\alpha]^{22}D$ −86°, 0.57% in ethanol. The previously reported rotation $[\alpha]^{22}D$ −175° was in error by a factor of two, due to use of an incorrect length of polarimeter tube in the calculation. The infrared spectrum of the recovered acid taken in potassium bromide was identical with those of the samples synthesized in this laboratory and that of B. Sjoberg. The original ether extract was decolorized with charcoal and evaporated, leading to an oil, 0.351 g., 93% recovery of unhydrolyzed ester. Its infrared spectrum in chloroform was identical with that of the racemic starting material. It was optically active, [α] observed was +0.42°, 2.2% in chloroform; $[\alpha]^{22}D$ +57°, calculated for 50% hydrolysis of one enantiomorph.

Effect of Diphenylcarbamyl Chloride.—A solution of 0.080 g. (3.2 μmoles) of α-chymotrypsin in 5.0 ml. of distilled water was added to 15 ml. of 0.1 *M* sodium chloride, and to this was added 0.064 ml. of 0.1 *M* diphenylcarbamyl chloride (Eastman Kodak Co.) in methanol. The solution was brought to and maintained at pH 7.8 for 20 min., with stirring. The substrate, DL-ethyl β-phenyl-β-hydroxypropionate was added (0.0229 g., 1.15 mmoles), and the rate consumption of alkali was observed, 0.0003 ml./min. of 0.1 *N* NaOH. Under identical conditions in the absence of the poison, the rate was 0.0100 ml./min. DL-Ethyl β-phenyl-β-acetamidopropionate was examined under the same conditions, 0.0200 g. (0.85 mmole) of substrate consuming 0.1 *N* NaOH at a rate of 0.0013 ml./min. in the absence of the poison, and at essentially zero rate in the presence of the poison.

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