8,10-Dioxoundecanoic acid: ir 3120 (br), 2940, 2860, 1720, 1690, 1650, 1600, and 1420 cm^{-1}; nmr δ 1.4 (8 H, m), 2.05 (*ca.* 3 H, s), 2.25 (*ca.* 4 H, s), 3.55 (0.3 H, s), 5.55 (*ca.* 1 H, s), and a broad absorption at 11.5.

9,11-Dioxododecanoic acid: ir 3100 (br), 2940, 2850, 1710, 1610, and 1410 cm^{-1}; nmr δ 1.4 (10 H, m), 2.05 (*ca.* 3 H, s), 2.25 (*ca.* 4 H, m), 3.55 (0.4 H, s), 5.55 (*ca.* 1 H, s), and a broad absorption at 11.5.

13,15-Dioxohexadecanoic acid: ir 3100 (br), 2930, 1720, 1650, 1600, 1470, and 1420 cm^{-1}; nmr δ 1.4 (18 H, m), 2.05 (*ca.* 3 H, s), 2.25 (*ca.* 4 H, m), 3.55 (0.4 H, s), 5.55 (*ca.* 1 H, s), and a broad absorption at 11.5.

Registry No.—Acetylacetone, 123-54-6.

References and Notes

(1) This work was supported in part by funds given to the University of Southern Mississippi by the Mississippi Board of Trustees of Institutions of Higher Education for the support of basic research.
(2) NDEA Fellow, 1972–1973.
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(7) Although differences in yields depending on halogen in alkylation of dianions of β-diketones do not appear to be significant in most previously reported cases, there has been at least one other case in which the halogen has made a significant difference: K. G. Hampton and R. E. Flannery, *J. Chem. Soc., Perkin Trans. 1*, 2308 (1973).
(8) Melting points were taken with a Thomas-Hoover melting point apparatus in open capillary tubes and are uncorrected. Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn., and Chemalytics, Inc., Tempe, Ariz. The infrared (ir) spectra were taken with a Perkin-Elmer grating infrared spectrophotometer, Model 257, using KBr pellets; only strong absorptions are reported. The nmr spectra were obtained using a Varian Model A-60D spectrometer and samples dissolved in $CDCl_3$ with $SiMe_4$ as reference.
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(10) Nmr spectra of β-diketones are complicated by extensive keto–enol tautomerism. It is observed that the absorptions may not be of integral intensity in this series of compounds because of this phenomenon. In the spectrum of 4,6-dioxoheptanoic acid, the absorption at δ 2.05 is assigned to the methyl protons in the enolized form; the weaker absorption at δ 2.25 is assigned to the nonenolized form. The peak areas are given in detail for this particular compound and are representative of the effect of the tautomerism on all of these compounds. The intensity of the broad downfield absorption is representative of the hydroxyl protons of both the carboxyl and the enol.

Notes

Resolution of Some 3-(3,4-Dihydroxyphenyl)alanine Precursors with α-Chymotrypsin

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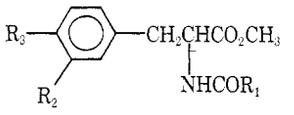
Preferential hydrolysis of aliphatic esters of a variety of L aromatic amino acid esters or their *N*-acyl derivatives by α-chymotrypsin has been exploited to resolve racemic mixtures of these compounds.[1] In an effort to further evaluate the synthetic utility and limitations of this method, we resolved the 3-(3,4-dihydroxyphenyl)alanine (dopa) precursors **1–3** enzymatically and used the enantiomers, plus racemic **4**, to obtain information about the effect of the ring substituents on the rates and stereoselectivities of the reactions.[2]



Compd	R_1	R_2		R_3
1	CH_3	CH_3O		HO
2	C_6H_5	CH_3O		HO
3	CH_3		OCH_2O	
4	CH_3	CH_3O		CH_3O
5	CH_3	H		HO

Erlenmeyer condensation of 3,4-methylenedioxybenzaldehyde or 3,4-dimethoxybenzaldehyde with acetylglycine afforded the corresponding 4-benzylidine-2-methyl-2-oxazolin-5-ones. Methanolysis of the oxazolinone rings produced the ring-substituted methyl *N*-acetyl-α-aminocinnamates. Subsequent catalytic hydrogenation yielded racemic **3** and **4**. A similar procedure was used to synthesize **1** and **2**, except that the starting aldehyde was 4-benzyloxy-3-methoxybenzaldehyde, and with **2**, benzoylglycine replaced acetylglycine. In each case, hydrogenolysis of the benzyl protecting group accompanied reduction of the cinnamate esters.

Stereoselective hydrolysis of DL-**1** and DL-**3** by α-chymotrypsin was accomplished in aqueous suspensions of the esters at pH 7.0. The enzyme was essentially unreactive toward DL-**2** under similar conditions. However, in 15% v/v acetonitrile–water the reaction proceeded at a measurable rate. Results of subsequent kinetics studies in solution indicate that the observed order of reactivity in the heterogeneous mixtures (**1** > **3** > **2**) parallels the solubilities of the esters in water. The time course of production of *N*-acylamino acid was followed by addition of 1 *N* NaOH such that neutral pH was maintained. Uptake of base continued until the amount required to account for hydrolysis of one enantiomer had been added and the reactions stopped. Unreacted ester was isolated by continuous extraction with EtOAc, or with **2**, by filtration. Decreasing the pH of the aqueous layer to 3 followed by continuous extraction produced the *N*-acylamino acids corresponding to **1–3**. HBr-catalyzed hydrolysis of a portion of the isolated unreactive isomers of esters **1–3** gave D-dopa. Thus, the enzyme preferentially hydrolyzes the L esters. The *N*-acyl-L amino acids were reconverted to their methyl esters with thionyl chloride in methanol. A high degree of optical purity of the resolved enantiomers is suggested by their nearly equal and opposite specific rotations and behavior in enzyme kinetics studies. α-Chymotrypsin was

Table I
Chymotryptic Reactivities of Some Methyl Esters[a]

Compd	Expts[b]	10^3 S_0,[c] M	10^7 E_0,[c] M	k_c/K_m, M^{-1} sec^{-1}
L-1	13	5.0–28.0	2.11	2060 ± 85
L-2	15	1.0–6.80	2.11	12,572 ± 1400
L-3	14	1.5–15.0	4.05	5265 ± 438
L-5	24	2.0–25.0	2.10	10,095 ± 684

[a] At 25°, pH 7.0 in 15% v/v acetonitrile–water. Ionic strength 0.1 M (NaCl). [b] Number of points in Lineweaver–Burk plot. [c] S_0 and E_0 are initial substrate concentration ranges and enzyme concentrations, respectively.

unreactive in slurries of DL-4 in water or 15% v/v acetonitrile–water and the DL mixture was used for kinetics studies.

Chymotryptic reactivities of L-1, L-2, and L-3 in solution were measured by the specificity constant k_c/K_m. This ratio is the most accurate reflection of α-chymotrypsin specificity for a substrate.[3] It has the units of a second-order rate constant. Values of k_c/K_m, listed in Table I, are compared with that found for N-acetyl-L-tyrosine methyl ester (L-5), a specific α-chymotrypsin substrate. α-Chymotrypsin exhibited no reactivity toward 5×10^{-3} M solutions of the D isomers of 1–3 and DL-4, even at enzyme concentrations of $\sim 10^{-4}$ M.

Because the reactivities of the four esters for which specificity constants were measured fall within a sixfold range, it may be concluded that introduction of a methoxy substituent into the meta position of L-5 has little effect on chymotryptic reactivity. In contrast, the enzyme is sensitive to small changes in substrate structure at the para position, since DL-4 is unreactive but L-3 is nearly as reactive as L-5. The inability of α-chymotrypsin to hydrolyze DL-4 is consistent with the report that the enzyme is inert toward para-substituted N-acyl-L-tyrosines.[4] This low reactivity is thought to be due to unfavorable steric interactions of the substrate aromatic ring with the enzyme.[4] With L-3 these interactions evidently are diminished enough to permit efficient hydrolysis to occur. Of the dopa precursors tested here, however, DL-1 is more suitable than DL-2 or DL-3 for large-scale resolutions, primarily because of its higher solubility in water.

Registry No.—L-1, 51703-90-3; D-1, 51703-91-4; DL-1, 16024-50-3; L-2, 51593-50-1; D-2, 51593-51-2; DL-2, 51593-52-3; L-3, 51593-53-4; D-3, 51593-54-5; DL-3, 51703-92-5; L-5, 2440-79-1; 6,[5] 51593-55-6; 7,[5] 51593-56-7; 10,[5] 51593-57-8; 11,[5] 51593-58-9; 14,[5] 51593-59-0; 15,[5] 30037-41-3; 16,[5] 28104-71-4; α-chymotrypsin, 9004-07-3; 4-benzyloxy-3-methoxybenzaldehyde, 2426-87-1; acetylglycine, 543-24-8; acetic anhydride, 108-24-7; N-acetyl-L-tyrosine, 537-55-3.

Supplementary Material Available. Details of the syntheses, resolutions, and kinetic procedures, including elemental analyses and ir and nmr spectral data, will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JOC-74-2291.

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Mild Cleavage of a Peptide Bond through the Assistance of the Neighboring Phenylazo Moiety

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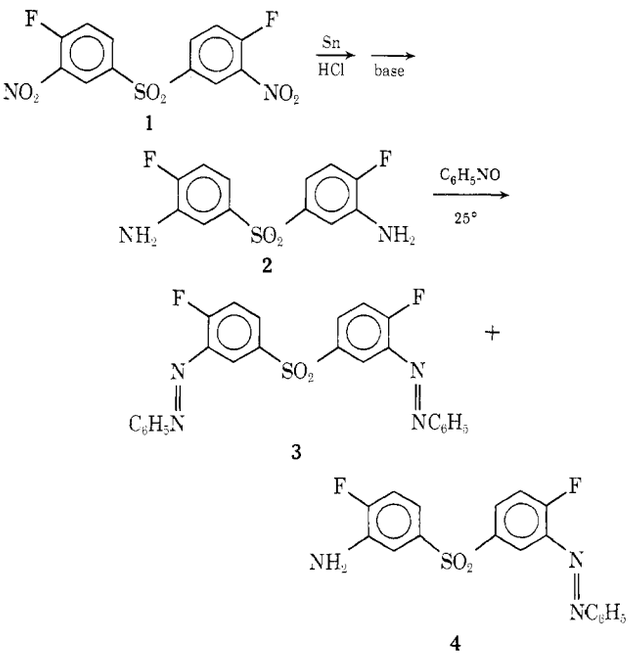
The facile removal of an amino-protecting group through the participation of an adjacent o-phenylazophenoxyacetyl moiety was recently reported from our laboratory.[1] The work involved the cleavage of a neighboring amide bond after reduction of the azo group. The success of this procedure led us to propose that the phenylazo moiety might be useful in another research program, the object of which is the development of a new procedure for the stepwise degradation of peptide chains. We report here the successful use of the phenylazo group as an effective participant in the rupture of the peptide bond of glycylglycine.

The commercially available[2] protein cross-linking[3] reagent, bis(4-fluoro-3-nitrophenyl) sulfone (1), was used as the starting material. It was easily reduced to the corresponding bis(aminofluorophenyl) sulfone, 2, which was, in turn, condensed with nitrosobenzene to provide two phenylazo products, 3 and 4, in 28 and 36% yields, respectively



(2.2 mol of nitrosobenzene per mole of 2). Decreasing the relative amount of nitrosobenzene increased the yield of the monophenylazo product, 4, to a high of 40%. However, using a greater excess of nitrosobenzene (up to 3.7 mol per mole of 2) never resulted in a larger yield of the bisphenylazo product, 3.

3-Amino-4,4′-difluoro-3′-phenylazophenyl sulfone (4) was used in the remainder of this work because, first, it was always obtained in greater quantity than was 3 and, second, adequate purification of sufficient amounts of 3 was much more tedious and generally required high-pressure liquid chromatography even after 3 was partially separated from 4.

Intermediate 4 was easily condensed with the dipeptide, glycylglycine (5), in a nucleophilic aromatic substitution which afforded 4-(4′-fluoro-3′-aminophenylsulfonyl)-2-phenylazo-N-phenylglycylglycine (6). Reduction of the azo group in 6 with potassium borohydride at room tempera-