[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH BETHESDA 14, MD.]

The Interaction of *p*-Phenolate Ions with Side-chain Electrophiles[1]

BY LOUIS A. COHEN AND WILLIAM M. JONES

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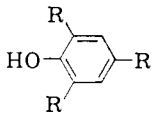
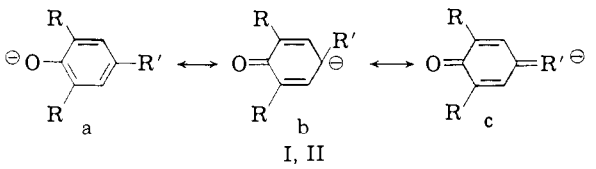
A study of the kinetics of alkaline hydrolysis of N-benzoyl-3,5-di-*t*-butyltyrosine and of N-benzoyl-3,5-di-*t*-butyltyramine reveals the existence of a rate-depressing factor which arises from interaction of the amide carbonyl and the phenolate anion. The effect of the carbonyl as an electrophile in assisting the ionization of the phenolic group supports the kinetic argument.

In the preceding paper of this series,[1] it was shown by rate studies that the alkaline hydrolysis of II was slower than that of I by a factor of 30. The inhibitory effect was attributed to the fact that the resonance-stabilized anion (IIa, b, c) has greater



I, R = H, R′ = CN or $CONH_2$
II, R = *t*-Bu, R′ = CN or $CONH_2$

contributions from IIb and IIc than does the corresponding anion of I from its Ib and Ic forms, and that electrostatic repulsion of a nucleophile attacking R′ is, accordingly, more effective.



I, II

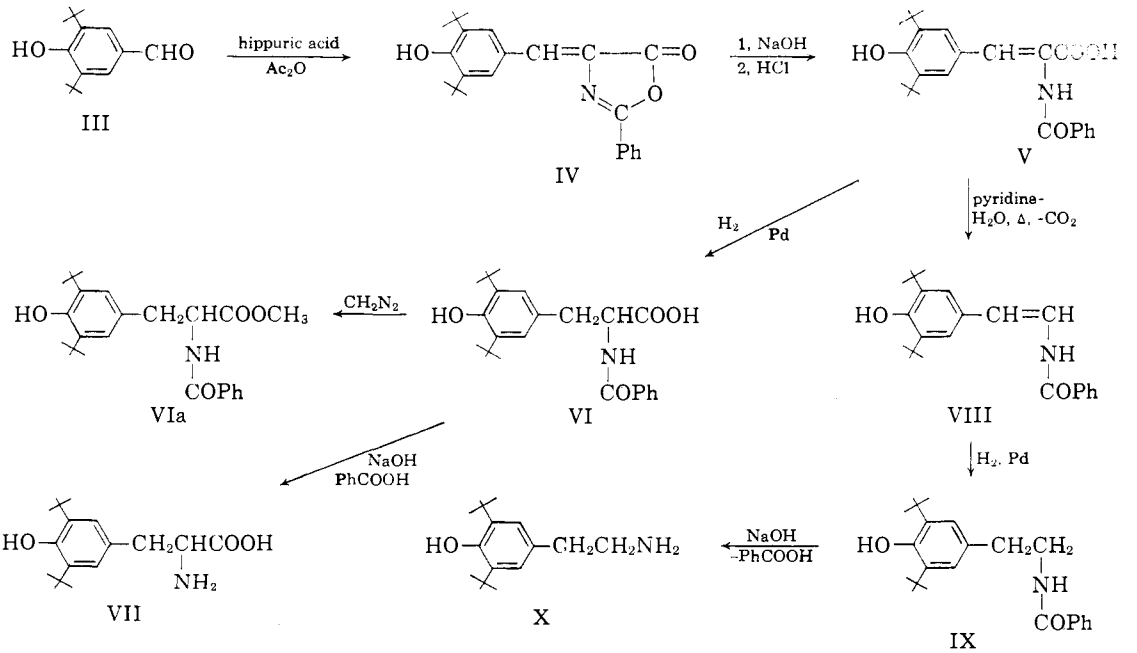
of *t*-butyl groups *ortho* to the phenolate anion on the rates of such reactions. On the basis of our earlier results,[1] one might predict that a rate enhancement should be observed. Although our studies in this area are still in progress, the present report concerns another series in which the effect is quite the converse.

The rates of alkaline hydrolysis of N-benzoyltyrosine, N-benzoyltyramine and of their 3,5-di-*t*-butyl analogs VI and IX were determined in 2 *N* sodium hydroxide (50% ethanol) at reflux. The latter compounds were synthesized according to the scheme of Chart I.

Experimental[3]

N-Benzoyltyramine.—A solution of 13.7 g. (0.1 mole) of tyramine and 20 ml. of triethylamine in 100 ml. of chloroform was cooled to 0° and 11.6 ml. (0.1 mole) of benzoyl chloride was added dropwise with stirring over 30 minutes. The solution was stirred an additional hour at 25°, washed with *N* hydrochloric acid, *N* sodium bicarbonate, dried over sodium sulfate and concentrated. The solid residue was recrystallized from ethanol–water to afford 20 g. (83%) of N-benzoyltyramine, m.p. 164.5–165° (lit.[4] 162°).

CHART I



Since it has been shown by several investigators that anions such as Ib can serve as nucleophiles in internal displacement reactions at sites along the side chain,[2] it was of interest to examine the effect

(1) Paper IV of a series on phenol–dienone tautomerism; for paper III, *cf.* L. A. Cohen and W. M. Jones, *J. Am. Chem. Soc.*, **84**, 1625 (1962).

(2) *Cf.* A. S. Dreiding, *Helv. Chim. Acta*, **40**, 1812 (1957); S. Winstein and R. Baird, *J. Am. Chem. Soc.*, **79**, 756 (1957); R. Baird and S. Winstein, *ibid.*, **79**, 4238 (1957).

(3) Melting points were taken on a Kofler block and are uncorrected. Ultraviolet spectra were measured on a Cary spectrophotometer, model 14. The authors thank Mr. H. G. McCann and his associates of this Institute for performing the microanalyses.

(4) G. Barger, *J. Chem. Soc.*, **95**, 1123 (1909).

Anal. Calcd. for $C_{16}H_{15}NO_2$: C, 74.66; H, 6.27; N, 5.81. Found: C, 74.52; H, 6.37; N, 5.74.

N-Benzoyl-L-tyrosine was prepared according to published procedures[5]; m.p. 164–165° (lit.[5] 163–164°).

2-Phenyl-4-(3,5-di-*t*-butyl-4-hydroxybenzal)-5-oxazolone (IV).—A mixture of 23.4 g. (0.1 mole) of 3,5-di-*t*-butyl-4-hydroxybenzaldehyde (III),[6] 22.4 g. (0.125 mole) of hippuric acid, 10.3 g. (0.125 mole) of fused sodium acetate and 75 ml. of acetic anhydride was heated on steam for 6 hours and the resulting deep orange solution chilled in ice for 1 hour. The crystalline precipitate was collected by suction filtration, washed thoroughly with 50% ethanol and dried *in vacuo* to give 23 g. (60%) of the oxazolone, m.p. 210–215°. Recrystallization from methylene chloride–ligroin (90°) afforded deep yellow prisms, m.p. 214–215°.

Anal. Calcd. for $C_{24}H_{27}NO_3$: C, 76.36; H, 7.21; N, 3.71. Found: C, 76.05; H, 7.35; N, 3.72.

α-Benzamido-3,5-di-*t*-butyl-4-hydroxycinnamic Acid (V).—To a solution of 18.9 g. (0.05 mole) of the oxazolone IV in 150 ml. of ethanol was added a solution of 8 g. of sodium hydroxide in 100 ml. of water. The cherry-red solution was heated on steam for 30 min. with exclusion of air, chilled in ice, diluted with 300 ml. of water and acidified to *p*H 1 with 6 *N* hydrochloric acid. The granular precipitate was collected, washed thoroughly with water and dried to give 19 g. (96%) of an almost colorless product, m.p. 202–204°. After recrystallization from methylene chloride–ligroin (90°) or from ethanol–water, the compound melted at 203–205°.

Anal. Calcd. for $C_{24}H_{29}NO_4$: C, 72.88; H, 7.39; N, 3.54. Found: C, 72.62; H, 7.32; N, 3.51.

The methyl ester of V was prepared with methanol–hydrogen chloride and crystallized from methylene chloride–ligroin (90°); m.p. 197°.

Anal. Calcd. for $C_{25}H_{31}NO_4$: C, 73.32; H, 7.63; N, 3.42. Found: C, 73.33; H, 7.57; N, 3.30.

N-Benzoyl-3,5-di-*t*-butyl-DL-tyrosine (VI).—To a solution of 15.8 g. (0.04 mole) of V in 150 ml. of purified dioxane was added 3 g. of 10% palladium–charcoal. The mixture was hydrogenated at atmospheric pressure until a test sample no longer showed an olefinic absorption peak at 315 mμ in the ultraviolet. In several runs, complete hydrogenation required 3–5 days. The solution was filtered and concentrated to a colorless glass. After crystallization from methylene chloride–ligroin (65°), the product was obtained as plates, m.p. 98°, resolidifying and remelting at 160–162°. The acid was recrystallized from the same solvent pair, m.p. 104°, 167–168°. The material was dried at 25° *in vacuo* for 24 hours, without significant loss of solvate.

Anal. Calcd. for $C_{24}H_{31}NO_4 \cdot CH_2Cl_2$: C, 62.24; H, 6.89; N, 2.90. Found: C, 63.58; H, 6.59; N, 3.18.

After exposure to 140° *in vacuo* for 48 hours and 165° for 1 hour, the compound melted at 184–185°.

Anal. Calcd. for $C_{24}H_{31}NO_4$: C, 72.51; H, 7.86; N, 3.52. Found: C, 72.67; H, 7.88; N, 3.47.

The compound was crystallized from several other solvent pairs, in each case in a solvated form.

The methyl ester (VIa) was obtained either by catalytic hydrogenation of the methyl ester of the olefinic acid V or by the action of diazomethane on VI. Hydrogenation of the methyl ester of V proceeded more rapidly than that of the acid and a product free of olefinic impurity was more readily obtained. The ester crystallized from methylene chloride–ligroin (65°), m.p. 121.5–122.5°, in a solvent-free state.

Anal. Calcd. for $C_{25}H_{33}NO_4$: C, 72.96; H, 8.08; N, 3.40. Found: C, 72.78; H, 7.97; N, 3.78.

3,5-Di-*t*-butyl-DL-tyrosine (VII).—A solution of 1 g. of VI in 100 ml. of 2 *N* sodium hydroxide was refluxed for 6 days with exclusion of air in an alkali-resistant flask.[7] The mixture was acidified to *p*H 3 with 6 *N* hydrochloric acid and the solution concentrated to a dry powder. The material was extracted several times with methylene chloride to remove unchanged VI and benzoic acid. The solid was then extracted with hot benzene until the residue was negative to ninhydrin. The benzene extract was concentrated to approximately 10 ml. and diluted to turbidity with ligroin (65°). After storage of the solution at 0° for 24 hours, 100 mg. of the amino acid was obtained as fine needles, m.p. 135°, 205°. Analysis suggested the material to be a hydrate. It was dried 48 hours at 100° *in vacuo* and 24 hours at 135°; m.p. 203–205°. Analysis at this point indicated a hemihydrate which did not lose water with further drying at 150°.

Anal. Calcd. for $C_{17}H_{27}NO_3 \cdot {}^1/_2H_2O$: C, 67.52; H, 9.33; N, 4.63. Found: C, 67.70; H, 8.95; N, 4.70.

The amino acid is readily soluble in water, alcohol, acetone, ethyl acetate and in warm ether or benzene.

In a number of attempts to hydrolyze VI under acidic conditions, the *t*-butyl groups were invariably lost, tyrosine being obtained.

β-Benzamido-3,5-di-*t*-butyl-4-hydroxystyrene (VIII).—A solution of 3.96 g. (0.01 mole) of V in 50 ml. of pyridine was diluted with 50 ml. of water and the solution refluxed for 12 hours with exclusion of air.[8] The mixture was chilled and the tan, crystalline precipitate collected, 2.1 g., m.p. 215–218°. The material was recrystallized from methylene chloride–ligroin (65°) or from ethanol–water (after decolorizing with Norite) to afford 1.75 g. (50%), m.p. 220–221°. By acidification of the aqueous pyridine mother liquors, unreacted V could be recovered for use in subsequent runs.

Anal. Calcd. for $C_{23}H_{29}NO_2$: C, 78.59; H, 8.32; N, 3.99. Found: C, 78.75; H, 8.39; N, 4.03.

N-Benzoyl-3,5-di-*t*-butyltyramine (IX).—To a solution of 3.5 g. (0.01 mole) of VIII in 100 ml. of 95% ethanol was added 1 g. of 10% palladium–charcoal and the mixture subjected to hydrogenation at atmospheric pressure until a test sample no longer showed olefinic absorption at 325 mμ in the ultraviolet. The solution was filtered and concentrated to a colorless solid. Recrystallization from methylene chloride–ligroin (65°) afforded 3.2 g. of IX, m.p. 120–121°.

Anal. Calcd. for $C_{23}H_{31}NO_2$: C, 78.14; H, 8.84; N, 3.96. Found: C, 78.03; H, 8.83; N, 3.73.

3,5-Di-*t*-butyltyramine (X).—A solution of 1.77 g. (0.005 mole) of IX in 100 ml. of ethanol was diluted with 50 ml. of 4 *N* sodium hydroxide. The mixture was refluxed for 72 hours in an alkali-resistant flask with exclusion of air. After acidification to *p*H 1, the solution was concentrated to remove ethanol, chilled and the precipitate of unreacted IX collected (0.35 g.). When the aqueous filtrate was made alkaline, X separated as a colorless solid. Two recrystallizations from ethanol–water afforded 0.95 g., m.p. 112–113°.

Anal. Calcd. for $C_{16}H_{27}NO$: C, 77.06; H, 10.91; N, 5.62. Found: C, 77.09; H, 11.11; N, 5.72.

Kinetic Runs.—Reaction mixtures were made up by adding 0.005 mole of compound to 100 ml. of 2 *M* sodium hydroxide in 50% ethanol. The solutions were thus 0.05 *M* with respect to compound and contained a forty-fold ratio of alkali. Corning alkali-resistant glass, No. 7280, was used for the reaction vessel, which was fitted with a 2-neck adapter. One neck supported a condenser followed by a mineral oil trap and the other neck was sealed with a rubber stopper containing a stainless steel hypodermic needle which reached almost to the bottom of the reaction vessel. Samples could thus be withdrawn by syringe without opening the system to the atmosphere. The system was flushed with nitrogen and the solution heated at reflux (82°) in an oil-bath kept at 125°. At various time intervals, portions of approximately 1.5 ml. were withdrawn and cooled to 25° in small flasks. From each sample two aliquots (0.25 ml. each) were transferred to 10-ml. volumetric flasks containing 0.25 ml. of 2 *N* acetic acid and the solutions diluted to volume with water. For ninhydrin assay, tubes containing 1 ml. of the neutralized, diluted solution and 1 ml. of ninhydrin–stannous chloride reagent[9] were heated for 20 minutes in a boiling water-bath. After dilution with 8 ml. of 1-propanol–water (1:1), color intensity at 570 mμ was measured. Ninhydrin color yields[10] (Table I) were determined

(5) S. W. Fox and C. W. Pettinga, *Arch. Biochem. & Biophys.*, **25**, 13 (1950); K. F. Itschner, E. R. Drechsler, C. Warner and S. W. Fox, *ibid.*, **53**, 294 (1954); S. W. Fox, *J. Am. Chem. Soc.*, **68**, 194 (1946).

(6) L. A. Cohen, *J. Org. Chem.*, **22**, 1333 (1957).

(7) In order to obtain a colorless, crystallizable product, it is essential that VI be totally free of the olefinic acid V.

(8) *Cf.* L. A. Cohen and W. M. Jones, *J. Am. Chem. Soc.*, **82**, 1907 (1960); A. Galat, *ibid.*, **72**, 4436 (1950).

(9) S. Moore and W. H. Stein, *J. Biol. Chem.*, **176**, 367 (1948).

(10) The wide variation in color yield will be discussed in a later publication.

for stock solutions of the pure compounds. Intensity values for each pair of aliquots were averaged, agreement being better than 3% in almost all cases.

TABLE I

NINHYDRIN COLOR YIELDS (RELATIVE TO LEUCINE)

Leucine	1.00	Phenethylamine	0.25
Phenylalanine	0.90	Tyramine	.22
Tyrosine	.90	X	.88
VII	.92		

Results and Discussion

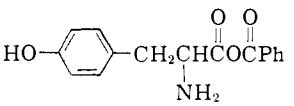
Rate constants were determined graphically and are summarized in Table II. (Pseudo)-first-order rate laws were obeyed over the entire extent of hydrolysis, which varied from 20–60%.

TABLE II

(PSEUDO)-FIRST-ORDER RATE CONSTANTS FOR ALKALINE HYDROLYSIS

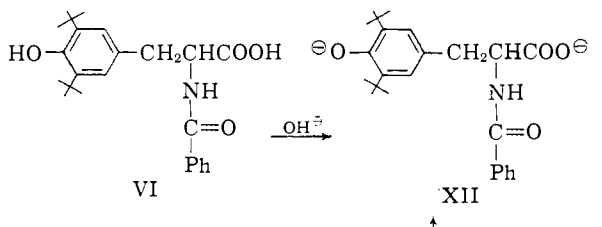
N-Benzoyl-	$k \times 10^6$, min.$^{-1}$	Rel. rate
Phenylalanine	108	1
Tyrosine	115	1.06
Di-*t*-butyltyrosine (VI)	50	0.46
Phenethylamine	2110	19.5
Tyramine	2430	22.5
Di-*t*-butyltyramine (IX)	357	3.3

It is immediately evident that the carboxylate anion exerts a strong electrostatic retarding effect on the rate of alkaline hydrolysis; thus, N-benzoylphenethylamine reacts at 20 times the rate of N-benzoylphenylalanine.[11] It is also apparent that participation of the carboxyl group in accelerating hydrolysis *via* intermediates such as XI is not significant.[12]

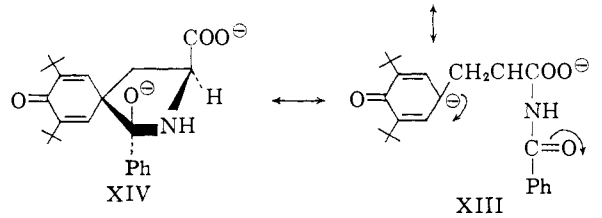


XI

The rate of hydrolysis of VI is approximately one-half that of N-benzoyltyrosine, the decrease being attributed to the sequence of events VI-XIV.



VI XII



XIV XIII

The transformation to the labile spirodienone XIV would, in effect, decrease the concentration of N-benzoyl species available for attack by external base. The effect is accentuated in the tyramine series (IX) since formation of the spirodienone XV is not as electrostatically unfavorable as is the formation of XIV. Although electrostatic repulsion may be avoided by placing the two anions in a *trans* relationship, as in XVI, the bulk effect of *cis* phenyl and carboxyl is equally unfavorable.[13]

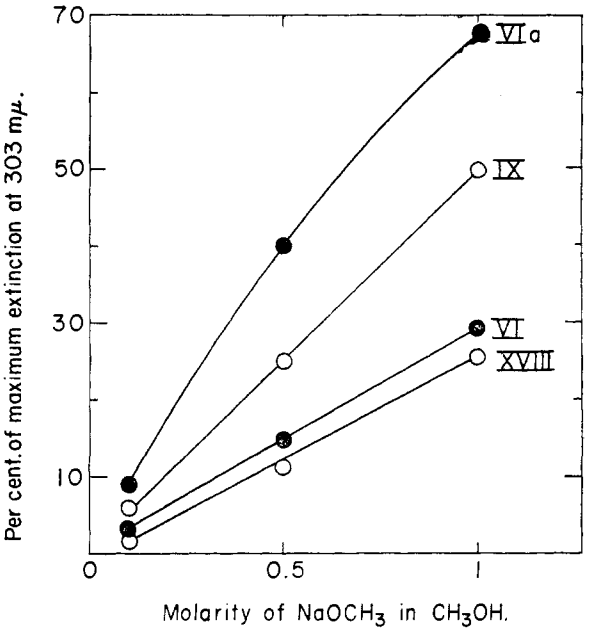
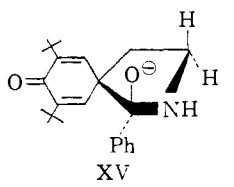
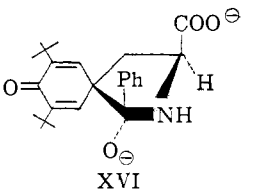


Fig. 1.—Ionization of 3,5-di-*t*-butylphenols in sodium methoxide: compounds, 3×10^{-4} *M*; λ_{max}^{303} estimated for VIa by extrapolation according to R. S. Stearns and G. W. Wheland, *J. Am. Chem. Soc.*, 69, 2025 (1947).



XV



XVI

In the preceding paper,[1] the inhibitory effect of *t*-butyl groups on the alkaline hydrolysis of II was attributed to the combined effect of IIb and IIc in an unknown ratio. Separation of the two effects has been achieved in the present case, and it is evident from the results that IIb can contribute measurably to the rate depression.

An interesting consequence of the interaction of the phenolic system and the benzamido side chain is revealed by spectral studies. Di-*t*-butylphenols without *p*-electronegative substituents show the spectral shift from phenol to phenolate ion only in very alkaline media.[6,14] Since we have proposed[1,6] that such resistance to anion formation is the result of steric hindrance to solvation of the negative charge between the bulky *t*-butyl groups, it

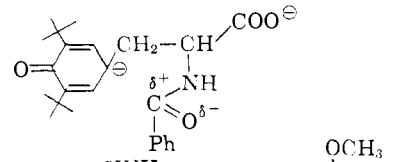
(11) From one kinetic study, the electrostatic retarding factor for the alkaline hydrolysis of monomethyl succinate may be estimated to be approximately 6: C. K. Ingold, *J. Chem. Soc.*, 2170 (1931).

(12) *Cf.* M. L. Bender, *Chem. Revs.*, 60, 87 (1960).

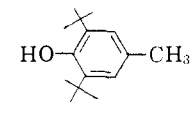
(13) Although dipole interaction (*cf.* XVII) could also account for the kinetic data, we feel that the preceding stereochemical argument lends support to the covalent bond formulation (at least at the reaction temperature). In any event, it should be emphasized that such proposals are tentative.

(14) N. D. Coggeshall and A. S. Glessner, Jr., *J. Am. Chem. Soc.*, 71, 3150 (1949).

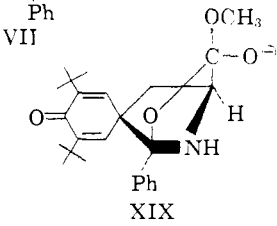
follows that any modification of the phenolate hybrid which serves to stabilize electron density at the *para* carbon atom should facilitate ionization. Whether by electrostatic effects alone, as in XVII, or by covalent bond formation as well, as in XIV, the benzamido carbonyl may serve as an electrophile to promote anion solvation at sites other than the phenolic oxygen. Such an argument is supported by the spectral data of Fig. 1, IX being more acidic than VI and both more acidic than the corresponding cresol XVIII. The fact that the ester VIa is more readily ionized than the free acid VI supports the argument used to relate the hydrolysis rates of VI and IX. Ionization constants for a variety of unhindered phenols with appropriate side chains were determined spectrophotometrically; in each case, the pK was found to lie within the range 10.0 to 10.1. Thus, it seems unlikely that the variations shown in Fig. 1 need



XVII



XVIII



XIX

be ascribed to inductive effects resulting from side-chain modification. We, therefore, attribute the enhanced ionization of VIa to the existence of an additional locus for charge solvation, as in XIX or its dipolar analog.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF CALIFORNIA, BERKELEY, CALIF.]

The Kinetics of the Acid-catalyzed Isomerization of *cis*-Cinnamic Acid[1-3]

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RECEIVED SEPTEMBER 29, 1961

cis-Cinnamic acid is smoothly isomerized to *trans*-cinnamic acid by moderately concentrated sulfuric acid. In the region 45–75% sulfuric acid, the rate of isomerization follows Hammett's acidity function h_0 with unit slope. Evidence is presented to show that the course of the reaction follows an addition–elimination sequence, with β-phenyl-β-hydroxypropionic acid being formed as an unstable intermediate.

Introduction

For some time we have been interested in a variety of acid-catalyzed reactions. Most recently, detailed studies of the isomerization of *cis*-benzalacetophenones[3,5,6] have been reported. Two different mechanisms were found to be operative depending upon the nature of the substituents in the benzene rings. One of these mechanisms involved addition of water to form the hydroxy-enol, while the other mechanism involved the direct rotation about the carbon–carbon double bond in the oxonium salt of the substituted benzalacetophenone.

The present paper presents a study of the acid-catalyzed isomerization of *cis*-cinnamic acid to *trans*-cinnamic acid. This study was undertaken to obtain additional information regarding reaction mechanisms to which the use of the acidity function H_0 might be applied.

The cinnamic acid system offers several advantages for a study designed to test proton addition mechanisms[7] for acid-catalyzed reactions. It is known from the thermal isomerization of methyl cinnamate[8] that the *cis–trans* isomerization proceeds to completion. In considering possibly useful related compounds, β-phenyl-β-hydroxypropionic acid is well known and has also been resolved. Further, the stereomutation reaction will be free of any complications of α–β,β–γ equilibria, which would not be the case with many aliphatic compounds.

We desired to obtain a body of information, which would establish with a high degree of certainty the mechanism for the isomerization of *cis*-cinnamic acid; and then finally to consider the acidity dependence (H_0 or "non-H_0") of the reaction in the light of these results.

In this and the immediately following papers, we present our experimental results and then finally the conclusions which we have drawn from these studies.

Experimental

Preparation of Materials.—Methyl *cis*-cinnamate was prepared by hydrogenation of methyl phenylpropiolate using a Lindlar catalyst. Hydrolysis of methyl *cis*-cinnamate afforded ***cis*-cinnamic acid**, which was crystallized to constant m.p. from ligroin; m.p. 67.5–68.0° (lit.[9] 68°), neut. equiv. 147.2 (calcd. 148.15).

Kinetic Procedures.—The progress of the reaction was followed by the increase in ultraviolet absorption at an appropriate wave length, usually 300 mμ, using a Beckman DU spectrophotometer. A weighed amount of *cis*-cinnamic

(1) This research was supported in part by a grant from the Petroleum Research Fund administered by the American Chemical Society. Grateful acknowledgment is hereby made to the donors of this fund.

(2) Presented in part at the 8th Conference on Organic Reaction Mechanisms, Princeton, N. J., September, 1960.

(3) Paper XIII in the Series Carbonyl Reactions; previous paper, D. S. Noyce and M. J. Jorgenson, *J. Am. Chem. Soc.*, **88**, 2525 (1961).

(4) Union Carbide and Carbon Fellow, 1956–1957.

(5) D. S. Noyce, W. A. Pryor and P. A. King, *J. Am. Chem. Soc.*, **81**, 5423 (1959).

(6) D. S. Noyce, G. L. Woo and M. J. Jorgenson, *ibid.*, **83**, 1160 (1961).

(7) F. A. Long and M. A. Paul, *Chem. Revs.*, **57**, 935 (1957), review much of the literature relative to such mechanisms.

(8) G. B. Kistiakowsky and W. R. Smith, *J. Am. Chem. Soc.*, **57**, 269 (1935).

(9) C. Liebermann, *Ber.*, **23**, 2510 (1890).