and centrifuged and the clear liquid lyophilized to yield 75 mg of product. This was crystallized from methanol to yield 40 mg (two crops). Recrystallization from methanol gave pure material; 24 mg, mp 105–107°; identical with authentic 5,6-dihydrouridine by mixture melting point and nmr.

5,6-Dihydrothymidine. Catalytic reduction of 2.42 g of thymidine in water in the presence of 0.5 g of 5% rhodium on alumina catalyst in the manner given for uridine above gave, after crystallization of the crude product from isopropyl alcohol, 750 mg of 5,6-dihydrothymidine, mp 123.5–124.5° (lit.[4b] mp 152–153°, presumably a polymorph). The structure was supported by infrared, nmr, and mass spectroscopy.

Anal. Calcd for $C_{10}H_{16}N_2O_5$ (244.2): C, 49.18; H, 6.60; N, 11.47. Found: C, 49.23; H, 6.59; N, 11.74.

1-(β-D-Ribofuranosyl)-4-hydroxytetrahydropyrimidin-2(1H)-one (IV) by Catalytic Reduction of Uridine. A solution of 2.44 g (10 mmoles) of uridine in 75 ml of 0.01 *N* sodium hydroxide was hydrogenated in the presence of 0.5 g of 5% rhodium on alumina catalyst overnight at 50 psi of hydrogen. The hydrogen uptake corresponded to 1.0 mole of hydrogen. An additional 0.5 g of catalyst was added and the reduction continued for 4 hr during which time 0.25 mole of hydrogen was adsorbed. The solution was filtered through a bed of Celite, 1.0 g of fresh catalyst was added, and the solution was reduced an additional 20 hr. The hydrogen uptake during this time was 0.25 mole, making a total uptake of 1.5 moles of hydrogen. The solution was filtered through a bed of Celite (the pH then adjusted to 5.5), concentrated under reduced pressure in a 40° bath, and finally lyophilized. The amorphous solid was dissolved in deuterium oxide and relyophilized and this procedure was repeated. Assuming the anomeric absorption at 5.8 ppm to represent one proton, nmr indicated that there was approximately 45% of dihydrouridine (VI) and 35% of tetrahydrouridine (IV) in the crude product.

Sodium Borohydride Reduction of Dihydrouridine. To an ice-cold solution of 246.2 mg (1.0 mmole) of 5,6-dihydrouridine in 10 ml of water at pH 7.5–8 was added 37.8 mg (1.0 mmole, 4.0 equiv) of sodium borohydride. After 35 min, the reaction mixture was set in the freezer overnight and put in the ice bath the next morning. By tlc (25% MeOH in chloroform) there were two spots moving slower than starting material but all starting material was gone. The excess sodium borohydride was destroyed with acetic acid and the mixture was lyophilized. The crude material was mainly tetrahydrouridine (IV) by nmr and tlc.

Reduction of thymidine under our conditions gave a poorly defined nmr spectrum with absorption at 0.9, 2.0, 3.3, 3.75, and 4.8 ppm.

Acknowledgment. The author wishes to express his appreciation to Dr. Gerald W. Camiener for stimulating the research in this area by his finding that tetrahydrouridine, in particular, is a potent inhibitor of human liver deaminase, and to him and Mr. Robert V. Tao for assaying the reduction products at various stages of their purification. I also wish to recognize Mr. Alfred Koning for valuable technical assistance, Drs. Fred Kagan, Robert C. Kelly, and William J. Wechter for helpful suggestions, Dr. Marvin Grostic for mass spectra interpretations, and Mr. Forrest A. MacKellar and Mr. John F. Zieserl, Jr., for nmr spectra and interpretations.

The Alkaline Hydrolysis of Aromatic Esters of Phosphoric Acid

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Contribution from the Department of Chemistry, University of Chicago,
Chicago, Illinois 60637. *Received July 5, 1967*

Abstract: The five-membered cyclic aromatic phosphate, *o*-phenylene phosphate (I), undergoes alkaline hydrolysis 6×10^6 times faster than does its open-chain analog, diphenyl phosphate (II). This rate acceleration is comparable to those observed in the hydrolyses of five-membered cyclic aliphatic phosphates, five-membered cyclic aromatic sulfates, and five-membered cyclic aromatic sulfonates.

In recent publications we have reported that various five-membered cyclic aromatic esters of sulfur-containing acids hydrolyze in alkaline solution at rates which are far greater than those for their acyclic and larger ring analogs. The five-membered cyclic aromatic sulfate, catechol cyclic sulfate, hydrolyzes in alkali with a rate enhancement of 2×10^7 when compared to its open-chain analog, diphenyl sulfate.[1] Furthermore, the five-membered cyclic sulfonate, *o*-hydroxy-α-toluenesulfonic acid sultone, reacts in alkali at a rate which is more than 10^4 times faster than that of the corresponding six-membered compound,[2] β-*o*-hydroxyphenylethanesulfonic acid sultone, and 7×10^5 times faster than that of the open-chain analog, phenyl α-toluenesulfonate.[3] These large rate accelerations are comparable to those described earlier by Westheimer for the alkaline hydrolyses of five-membered cyclic aliphatic esters of phosphorus-containing acids such as potassium ethylene phosphate,[4] methyl ethylene phosphate,[5] and lithium propylphostonate.[6] However, the second-order rate constant for attack by hydroxide ion in the case of the five-membered cyclic aliphatic sulfate, ethylene sulfate, is greater than that for its acyclic analog, dimethyl sulfate, by only a small amount, a factor of about 20.[7]

In view of the contrast between the observations made for the hydrolyses of the five-membered cyclic aliphatic and aromatic sulfates, it is of considerable interest to establish what the lability of a five-membered cyclic aromatic phosphate is relative to its acyclic analog. Accordingly, we have undertaken an investigation of the alkaline hydrolysis of *o*-phenylene phosphate (I) and the open-chain compound, diphenyl phosphate (II), and we report our findings here.

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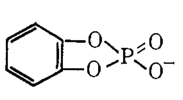

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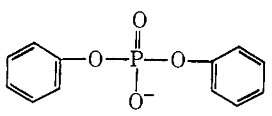

II

Experimental Section

2,2-Dihydro-2,2,2-trichloro-1,3,2-benzodioxaphosphole was prepared by the reaction of catechol with phosphorus pentachloride[8] in a yield of 76%; bp 137-137.5° (16 mm); mp 57.5-59° (lit.[8] mp 61-62°).

o-**Phenylenephosphorochloridate.** The reaction of 2,2-dihydro-2,2,2-trichloro-1,3,2-benzodioxaphosphole with acetic anhydride following the procedure of Khawaja and Reese was used to synthesize this compound.[9] A 74% yield of *o*-phenylenephosphorochloridate, bp 83-87° (0.1 mm) (lit.[9] bp 91° (0.9 mm)), was obtained.

Methyl *o*-**Phenylene Phosphate.** To a solution of 3.8 g (0.12 mole) of methanol and 20 ml of ether, 20.0 g (0.08 mole) of *o*-phenylene phosphorochloridate in 100 ml of ether was added with stirring over a period of 20 min. After removing the solvent, distillation of the residue gave methyl *o*-phenylene cyclic phosphate (12 g, 80% yield); bp 148.5-149° (18 mm); mp 59-60° (lit.[8] mp 58-59°)

Anal. Calcd for $C_7H_7O_4P$: C, 45.11; H, 3.80. Found: C, 44.88; H, 3.84.

The infrared, nmr, and mass spectra obtained for this compound were consistent with its structure.

o-**Phenylene hydrogen phosphate** was synthesized by the reaction of catechol and phosphorus pentoxide following the procedure of Cherbuliez, *et al.*[10] Our product was isolated in 20% yield, bp 237-238° (1 mm).

Anal. Calcd for $C_6H_5O_4P$: C, 41.86; H, 2.91. Found: C, 41.53; H, 3.06.

The infrared and mass spectra were measured and were consistent with the structure of this compound.

Diphenyl Hydrogen Phosphate.[11] A mixture of 94 g (1 mole) of phenol and 77 g (0.5 mole) of phosphorus oxychloride was heated under reflux for 17 hr until the evolution of hydrogen chloride ceased. The reaction mixture was distilled under vacuum twice to give diphenyl phosphorochloridate (80 g, 60% yield), bp 181-182° (17 mm). Hydrolysis of the phosphorochloridate gave diphenyl phosphate, mp 69.5-70.5 (lit.[11] mp 70°).

Product Analysis. Hydrolysis of Methyl *o*-**Phenylene Phosphate.** Both methyl *o*-hydroxyphenyl phosphate and *o*-hydroxyphenyl phosphate could be obtained as products from the hydrolysis of methyl *o*-phenylene phosphate. Some typical experiments are described.

A. A solution of 0.37 g (0.002 mole) of methyl *o*-phenylene phosphate in 100 ml of 0.1 *N* sodium hydroxide solution was allowed to stand at room temperature for 1 hr and then neutralized with hydrochloric acid. Treatment with S-benzylthiuronium chloride resulted in the formation of di-S-benzylthiuronium *o*-hydroxyphenyl phosphate, mp 157-159°.

Anal. Calcd for $C_{22}H_{27}O_5PS_2$: C, 50.56; H, 5.21. Found: C, 50.70; H, 5.36.

B. Methyl *o*-phenylene phosphate (0.37 g, 0.002 mole) was dissolved in water and the resulting solution was neutralized with sodium hydroxide immediately. After the solution was treated with S-benzylthiuronium chloride, there was obtained S-benzylthiuronium methyl *o*-hydroxyphenyl phosphate, mp 148.5-150°, as well as a trace of an unidentified crystalline material, mp 147-148.5°, which gave a depressed melting point when mixed with S-benzylthiuronium methyl *o*-hydroxylphenyl phosphate.

Anal. Calcd for $C_{15}H_{19}O_5N_2PS$: C, 48.64; H, 5.17. Found: C, 48.60; H, 5.27.

The infrared and nmr spectra of the sample of S-benzylthiuronium methyl *o*-hydroxyphenyl phosphate obtained were consistent with its structure.

C. To an aqueous solution of 0.3 g of S-benzylthiuronium chloride was added 0.3 g of crystalline methyl *o*-phenylene phosphate. After recrystallization of the resulting product, S-benzylthiuronium methyl *o*-hydroxyphenyl phosphate was obtained.

Hydrolysis of *o*-**Phenylene Hydrogen Phosphate.** Product analysis on this hydrolysis reaction was carried out in several ways. Two typical procedures are described.

A. *o*-Phenylene hydrogen phosphate (0.8 g) was dissolved in 10 ml of water and allowed to stand for 1 hr. After removal of the water under vacuum, the residue was dissolved in acetonitrile and the solution was dried over anhydrous sodium sulfate. The acetonitrile was removed by distillation under vacuum to give crystalline *o*-hydroxyphenyl phosphate, 0.55 g, mp 130-139°; after recrystallization from acetonitrile–benzene twice, mp 138-139° (lit.[12] mp 139°).

Anal. Calcd for $C_6H_7O_5P$: C, 37.91; H, 3.71. Found: C, 38.06; H, 3.77.

B. A solution of *o*-phenylene hydrogen phosphate in 0.1 *N* sodium hydroxide was allowed to stand at room temperature for 18 hr. The resulting reaction mixture was treated with S-benzylthiuronium chloride to give S-benzylthiuronium *o*-hydroxyphenyl phosphate, mp 157-159°.

Hydrolysis of Diphenyl Phosphate. A solution of 0.33 g of diphenyl phosphate in 1 *N* sodium hydroxide was heated in a brass-enclosed Teflon reaction cell 100° for 62 hr. After cooling and neutralizing with 1 *N* hydrochloric acid (phenolphthalein being employed as the indicator), the reaction mixture was added to a solution of 0.6 g of S-benzylthiuronium chloride in water to give crystalline S-benzylthiuronium phenyl phosphate, mp 146-148°; after recrystallization twice from ethanol, mp 148.5-149°.

Anal. Calcd for $C_{14}H_{17}N_2O_4PS$: C, 49.41; H, 5.00. Found: C, 48.81; H, 5.27.

Kinetic Methods

The rates of hydrolysis of the phosphate esters were determined spectrophotometrically.

Kinetics of *o*-**Phenylene Phosphate (I) Hydrolysis.** The course of the hydrolysis of this compound was followed by the use of Beckman DU or Gilford spectrophotometers which were maintained thermostatically at 25.0 ± 0.1°. In a typical kinetic run 30 μl of a 3.7×10^{-2} *M* solution of *o*-phenylene hydrogen phosphate in acetonitrile was added to a stoppered cell containing 3 ml of 0.01 *M* sodium hydroxide solution. The cell was shaken vigorously and placed in the cell compartment of the spectrophotometer, and the absorption change with time at 290 mμ was measured.

Fresh stock solutions of *o*-phenylene phosphate in acetonitrile were prepared regularly. In some kinetic experiments the alkaline reaction mixtures were degassed under high vacuum or flushed with nitrogen, but it was found that no difference exists between the kinetic results obtained under these conditions or in the presence of air.

Kinetics of Diphenyl Phosphate (II) Hydrolysis. The base-catalyzed hydrolysis of diphenyl phosphate was conducted in brass-enclosed Teflon reaction cells placed in an oil bath maintained thermostatically at 75 or 100°. In a typical kinetic run, the reaction solution was prepared by mixing 0.5 ml of a 5×10^{-2} *M* solution of diphenyl phosphate in acetonitrile, 25 ml of a 1 *N* NaOH solution, and 6.25 ml of a 4 *M* sodium perchlorate solution and adjusting the volume to 50 ml by the addition of distilled water. Aliquots (5 ml) of this solution were placed in separate reaction cells. These cells were sealed and placed in an oil bath. The reaction was quenched by cooling the cells rapidly, the solutions were transferred to spectrophotometer cells, and measurements on the absorption at 288 mμ were made using a Beckman DU spectrophotometer maintained at 25.0°.

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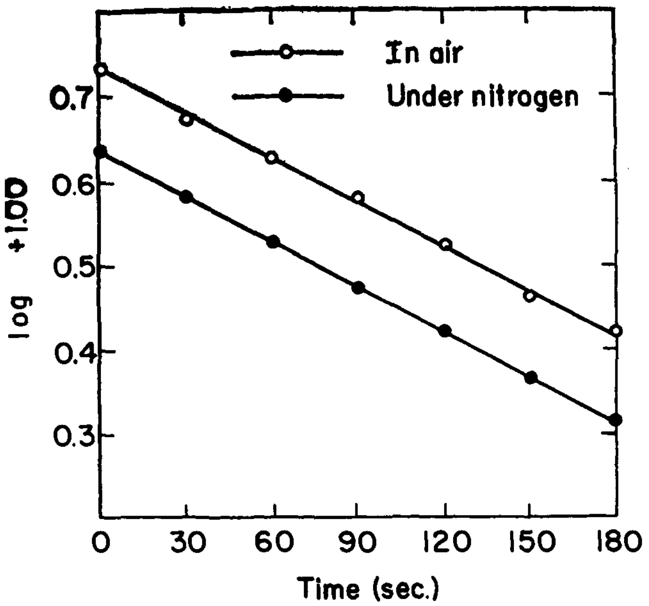

Figure 1. Plot of the data in Tables I and II.

Kinetic Results

Methyl *o*-Phenylene Phosphate. We hoped originally that the rate of the hydrolysis of this compound in alkaline solution could be studied. However, our preliminary kinetic experiments indicated that the cyclic triester reacted too rapidly for us to make measurements by either automatic titration or by conventional ultraviolet absorption spectrophotometry. For this reason the kinetic results we are reporting here are confined to a comparison of the rates of hydrolysis of the diesters, *o*-phenylene phosphate (I) and diphenyl phosphate (II).

***o*-Phenylene Phosphate (I).** The results obtained in typical kinetic runs carried out on the hydrolysis of I in air and under nitrogen are tabulated in Tables I and II and plotted in Figure 1. Pseudo-first-order rate constants observed in the presence of excess base were shown to be dependent on the hydroxide ion concentration as illustrated in Table III and Figure 2. From the data given in Table III and Figure 2, the second-order rate constant, k_{OH}, for the base-catalyzed hydrolysis of *o*-phenylene phosphate was found to be 6.93 × 10^{-1} M^{-1} sec^{-1} at ionic strength 0.40. The value of k_{OH} obtained at an ionic strength of 0.01 was different, 4.05 × 10^{-1} M^{-1} sec^{-1}. Therefore, the effect of ionic strength on the value of k_{OH} was examined and the data obtained are summarized in Table IV and illustrated in Figure 3. The product, *o*-hydroxyphenyl phosphate, was found to be stable under the conditions employed in the rate studies on *o*-phenylene phosphate.

Table I. Sample Run: Base-Catalyzed Hydrolysis of I at 25.0°[a]

Time, sec	Absorbance at time t, A_t	Concn of I remaining at time t, $A = A_\infty - A_t$	Log A + 1.00
0	0.160	0.540	0.732
30	0.220	0.480	0.681
60	0.275	0.425	0.628
90	0.318	0.382	0.582
120	0.365	0.335	0.525
150	0.410	0.290	0.462
180	0.435	0.265	0.423
∞	0.700	. . .	. . .

[a] 0.01 N NaOH, initial concentration of ester *ca.* 3 × 10^{-4} M, reaction run in the presence of air.

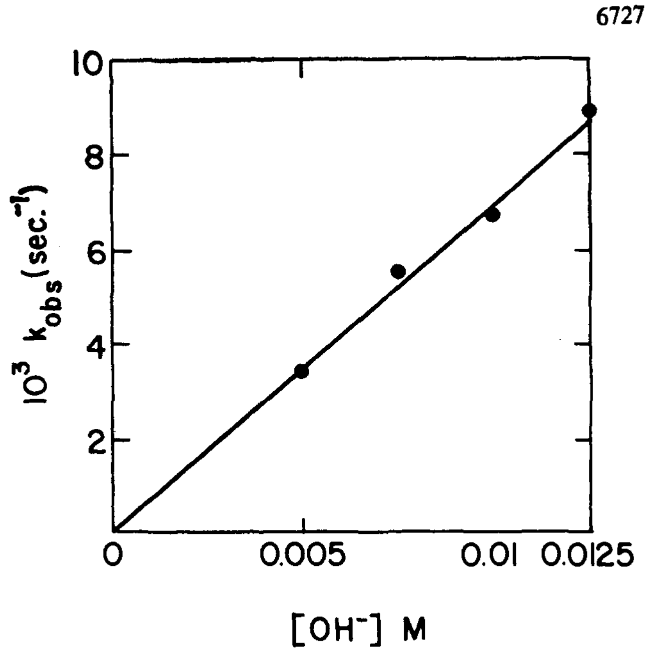

Figure 2. Plot of the data in Table III.

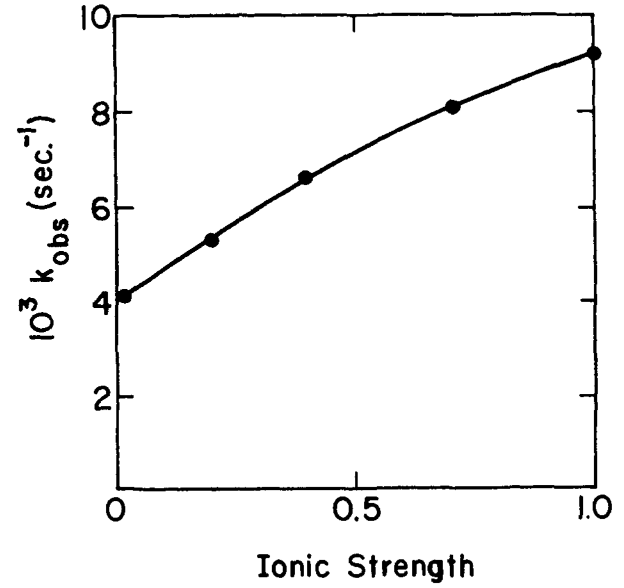

Figure 3. Plot of the data in Table IV.

Diphenyl Phosphate (II). The kinetic results obtained for the base-catalyzed hydrolysis of diphenyl phosphate at an ionic strength of 1.0 (maintained with sodium perchlorate) and at temperatures of 75 and 100° are summarized in Table V. The second-order rate constant, k_{OH}, for the catalysis by hydroxide ion was found to be 0.7 × 10^{-5} M^{-1} sec^{-1} at 75° and 3.25 × 10^{-5} M^{-1} sec^{-1} at 100°.

Discussion

The results obtained at 75 and at 100° and extrapolated to 25.0° gave a value of 1.56 × 10^{-7} M^{-1} sec^{-1} for

Table II. Sample Run: Base-Catalyzed Hydrolysis of I at 25.0°[a]

Time, sec	Absorbance at time t, A_t	Concn of I remaining at time t, $A = A_\infty - A_t$	Log A + 1.00
0	0.277	0.433	0.637
30	0.330	0.380	0.580
60	0.373	0.337	0.528
70	0.413	0.297	0.473
120	0.447	0.263	0.420
150	0.477	0.233	0.367
180	0.501	0.209	0.320
∞	0.710	...	...

[a] 0.01 N NaOH, initial concentration of phosphate *ca.* 3 × 10^{-4} M, reaction run under nitrogen.

Table III. Pseudo-First-Order Rate Constants for the Base-Catalyzed Hydrolysis of *o*-Phenylene Phosphate at 25.0°[a]

Hydroxide ion concn, M	$10^3 k_{\text{obsd}}$, sec^{-1}
0.0050	3.49
0.0075	5.53
0.0100	6.72
0.0125	8.92

[a] Ionic strength 0.40. When the initial concentrations of the phosphate ester were varied over the range 3.7 × 10^{-4} to 7.64 × 10^{-4} M, the agreement between the observed first-order rate constants was generally better than 5%.

Table IV. Effect of Ionic Strength on the Base-Catalyzed Hydrolysis of *o*-Phenylene Phosphate at 25.0°

Ionic strength[a]	$10k_{\text{OH}}$
0.01	4.05
0.20	5.30
0.40	6.72
0.70	8.12
1.00	9.21

[a] The ionic strength was adjusted with sodium perchlorate. The initial concentrations of the phosphate esters were varied over the range 3.7 × 10^{-4} to 7.64 × 10^{-4} M. The hydroxide ion concentration was maintained at 0.01 M in these runs.

Table V. Base-Catalyzed Hydrolysis of Diphenyl Phosphate. Pseudo-First-Order Rate Constants[a]

Hydroxide ion concn, M	Temp, °C	$10^5 k_{\text{obsd}}$, sec^{-1}
1.00	100	3.42
0.75	100	2.68
0.50	100	1.36
0.25	100	0.61
1.00	75	0.70

[a] Ionic strength 1.0. The concentration of diphenyl phosphate was varied over the range 2 × 10^{-4} to 5 × 10^{-4} M. At a given hydroxide ion concentration the agreement between observed first-order rate constants was better than 10%.

k_{OH} for the alkaline hydrolysis of diphenyl phosphate at an ionic strength of 1.0. The second-order rate constant measured at 25.0° and at an ionic strength of 1.0 for the hydroxide ion catalyzed hydrolysis of *o*-phenylene phosphate was 9.21 × 10^{-1} M^{-1} sec^{-1}. Therefore, it can be concluded that the five-membered cyclic aromatic ester, *o*-phenylene phosphate, undergoes alkaline hydrolysis about 6 × 10^6 times as fast as does the acyclic compound, diphenyl phosphate. This rate acceleration is similar in magnitude to that reported by Westheimer[4] for the hydroxide ion catalyzed hydrolysis of the five-membered cyclic aliphatic ester, ethylene phosphate, relative to its acyclic analog, dimethyl phosphate.

Acknowledgment. This study was supported by Agricultural Research Service, U. S. Department of Agriculture, Grant No. 12-14-100-9145 (71), administered by Northern Utilization Research and Development Division, Peoria, Ill. We wish to express our appreciation to Mr. K. W. Lo for his assistance with some of the kinetic measurements.

Intramolecular Catalysis in a Nonhydroxylic Solvent and Its Relationship to the Enzymatic Active Site

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Contribution from the Department of Chemistry, East Tennessee State University, Johnson City, Tennessee 37601. *Received May 24, 1967*

Abstract: Evidence is presented to show that intramolecular catalysis of ester aminolysis is possible in a nonhydroxylic solvent and that the required acid–base catalytic species can be derived from the reactants themselves. This evidence is used in support of a new concept of the structure and function of the active sites of certain classes of enzymes.

There are apparently no reported studies of intramolecular catalytic processes in nonhydroxylic media. We wish to report here the results of one such study, that of the amidation of methyl salicylate by *n*-butylamine in dry dioxane, and to show how these results support a new concept of the structure and function of the active sites of certain types of enzymes.

The *o*-hydroxyl group in salicylic acid derivatives has been shown by Bender[2] and by Bruice[3] to function in its phenoxide form as an intramolecular catalytic

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(1) Taken in part from the M.S. Thesis of W.-K. Kwok, East Tennessee State University, 1963, and from work to be submitted by Y. Kim in his M.S. Thesis, East Tennessee State University.

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