salt (100 g., 0.25 mole), sodium (5.8 g., 0.25 g.-atom) and ethanol (200 g.). Samples of this reaction mixture were taken after 0.5, 2.5, 24, 48 and 120 hr. of refluxing and analyzed by gas–liquid chromatography using an Apiezon column at 145°. A small quantity of benzene was resolved after 30 minutes, but no diethyl ether was detected after 120 hours of reaction. After 24 hours a shoulder appeared on this benzene peak. This shoulder increased when water was added to the reaction sample. After refluxing for 120 hr. the reaction mixture was cooled, filtered and analyzed by mass spectrometry. Approximately 0.07 mole of benzene along with 0.03 mole of butyldiphenylphosphine oxide was found. No ether was detected by either mass spectrometry or infrared analysis.

c. **Sodium Ethoxide and Water.**—Butyltriphenylphosphonium bromide (40 g., 0.10 mole) was treated with sodium (2.3 g., 0.10 g.-atom) in 150 ml. of absolute ethanol under the same conditions as the previous experiment, with the exception that a few milliliters of water was introduced. After refluxing for 24 hr., the clear orange-yellow reaction liquid was distilled at atmospheric pressure. The low boiling distillation cuts (63–78°) were examined by infrared spectroscopy which found them to contain benzene. The distillation residue was extracted with hot benzene and the benzene extract filtered to remove sodium bromide. Upon concentration of the benzene to dryness, 21.1 g. of a crystalline solid was obtained melting at 86–88°. The solid represents 0.082 mole of butyldiphenylphosphine oxide.

d. **Sodium Butoxide.**—Sodium (2.3 g., 0.10 g.-atom) was allowed to react with butanol (100 ml.) and butyltriphenylphosphonium bromide (40 g., 0.10 mole) under the same conditions as the three previous experiments. After 28 hr. of refluxing the mixture was cooled and filtered to remove sodium bromide. The reaction mixture was analyzed by mass spectrometry which found 0.0258 mole of benzene and 0.0026 mole of dibutyl ether plus 0.00645 mole of butyldiphenylphosphine oxide. Upon concentration of the reaction filtrate, 15.3 g. (0.038 mole) of butyltriphenylphosphonium bromide was recovered plus a brown viscous sirup which would not crystallize. Analysis by mass spectrometry of the small volume of gas (450 cc.) displaced during this reaction showed trace amounts of hydrocarbons up to C_6.

Reaction of Tetramethylenebis-(triphenylphosphonium Bromide) with Sodium Butoxide.—Sodium (4.6 g., 0.2 g.-atom) was dissolved in 300 g. of butanol and 74.0 g. (0.1 mole) of tetramethylenebis-(triphenylphosphonium bromide) was added. This mixture was refluxed for 48 hr., then cooled and filtered. A sample of the filtrate was analyzed by gas–liquid chromatography, but showed only a peak for butanol. The filtrate was stripped to half its original volume and 23.0 g. (47.5% yield) of tetramethylenebis-(diphenylphosphine oxide) melting at 260–261° was obtained.[20] The structure of thic compound was further confirmed by infrared analysis.

Reaction of Tetrabutylphosphonium Bromide with Sodium Butoxide. a. **At Atmospheric Pressure.**—Tetrabutylphosphonium bromide (8.7 g., 0.025 mole) was dissolved in 60 ml. of butanol under nitrogen. Sodium (0.5 g., 0.022 g.-atom) was dissolved in an additional 60 ml. of butanol, and the butoxide solution was added to the phosphonium salt. The mixture was refluxed overnight. No gas was evolved during this time. The reaction mixture was analyzed by gas–liquid chromatography, using a 2-meter diisodecyl phthalate column at 125°; only butanol could be detected. This mixture was analyzed by mass spectrometry and contained 0.0063 mole of tributylphosphine, < 0.0075 mole of tributylphosphine oxide, < 0.000121 mole of octane and < 0.00242 mole of dibutyl ether. The original reaction mixture was filtered to remove sodium bromide and the filtrate was stripped to dryness. The residual sirup would not crystallize. It was analyzed for unreacted tetrabutylphosphonium bromide by means of a Volhard titration and 0.0112 mole was found.

b. **At High Pressure.**—Sodium (5.8 g., 0.25 g.-atom) was dissolved in 500 g. of butanol and mixed with 87 g. (0.25 mole) of tetrabutylphosphonium bromide in a stirred 1-liter autoclave. The reactants were heated for 24 hours at 200° and an average pressure of 12 atm. A gas sample was taken after 5 hours of reaction and analyzed by mass spectrometry. Trace quantities of butane and butene (14.4 mole per cent. butane and 22.0 mole per cent. butene in the gas sample) were found. The autoclave was cooled after 24 hr. and the reaction mixture was filtered. The filtrate was analyzed by gas–liquid chromatography. An Apiezon column at 152° resolved only butanol and dibutyl ether. The filtrate was also analyzed by mass spectrometry which found 0.14 mole of tributylphosphine oxide, 0.035 mole of dibutyl ether and 0.0021 mole of octane.

Reaction of Tetraethylphosphonium Iodide with Sodium Ethoxide.—Tetraethylphosphonium iodide (8.5 g., 0.031 mole) was mixed with 20 ml. of absolute ethanol under nitrogen. Sodium (0.7 g., 0.031 g.-atom) was dissolved in an additional 30 ml. of ethanol and the ethoxide solution was added to the phosphonium salt. The mixture was refluxed overnight. No gases were evolved.

Acknowledgment.—We are indebted to Drs. R. Feinland and M. V. Norris for gas chromatographic analyses, and Miss Rosemarie Herberich and A. H. Struck for mass spectrographic analyses. Microanalytical work was carried out under the supervision of Dr. J. A. Kuck and Mrs. E. C. Grim.

(20) Lit. m.p. 257° (see ref. 7).

STAMFORD, CONN.

[CONTRIBUTION FROM THE CHEMISTRY RESEARCH DEPT., AGRICULTURAL DIVISION, AMERICAN CYANAMID CO., STAMFORD, CONN.]

Phosphorothioates. I. The Reaction of Phosphorochloridothioates with Carboxylate and *t*-Butoxide Anions

BY BERNARD MILLER

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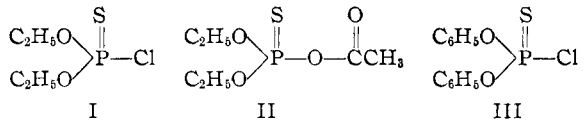
Acetate and trimethylacetate ions react with O,O-diphenyl phosphorochloridothioate with second-order kinetics to give mixed anhydrides, which react extremely rapidly with carboxylate ions to form O,O-diphenyl phosphorothioate anions and carboxylic anhydrides. Potassium *t*-butoxide reacts with O,O-diphenyl phosphorochloridothioate in a similar manner with the consumption of two moles of base. The published report of the preparation of O-acetyl O,O-diethyl phosphorothioate by the reaction of sodium acetate with O,O-diethyl phosphorochloridothioate could not be confirmed.

Introduction

Recently, Zemlyanskii and Malinovskii[1] reported the preparation of a series of acylated phosphorothioates by the reaction of carboxylic acid salts and O,O-diethyl phosphorochloridothioate (I) in ethanol solution. Among the compounds prepared in this manner was O-acetyl O,O-diethyl phosphorothioate (II) which was reported to be a water-soluble solid, m.p. 64°.

(1) N. I. Zemlyanskii and M. S. Malinovskii, *Zhur. Obshchei. Khim.*, **26**, 1677 (1956).



I II III

A few months previously, Kabachnik and his co-workers had shown that the product of the reaction of sodium O,O-diethyl phosphorothioate and acetyl chloride had structure II, rather than the isomeric S-acetylated structure.[2] Their product, however, was a liquid, b.p. 89–90° (4 mm.), $n^{20}D$ 1.4553. These properties are in reasonable agreement with those reported by Arbuzov and Alimov,[3] who prepared II by the reaction of O-acetyl O,O-diethyl phosphite with sulfur.

Some time ago, it was observed in these laboratories that an attempt to prepare II by treating I with sodium acetate in dioxane gave only recovered I and an undistillable tar.

In view of these somewhat inconsistent reports, it seemed of interest to study the reactions of carboxylate ions with phosphorochloridothioates, as part of a general investigation in these laboratories of the kinetics of phosphorothioate reactions.

Procedures.—The very rapid solvolysis of phosphorochloridates and phosphorochloridothioates has been a major problem in the study of the displacement reactions of these compounds. Until now, these studies have been limited to strong bases, which can compete successfully with the solvent for the phosphorochloridate molecule.[4]

After a study of possible solvents for kinetic investigations, *t*-butyl alcohol was chosen as the solvent which best combined the ability to dissolve ionic compounds with stability toward strong bases and lack of reactivity toward phosphorochloridothioates. Ten per cent. (by volume) of dioxane was added to lower the inconveniently high melting point of pure *t*-butyl alcohol.

O,O-Diphenyl phosphorochloridothioate (III) rather than the ethyl ester I was chosen for kinetic studies, since previous work has shown that dealkylation of alkyl phosphates may compete significantly with the desired displacements on the phosphorus atom.[5] Even with the phosphorochloridates and phosphorochloridothioates, which might be expected to react at the phosphorus atom rather than at an alkyl group, dealkylation may be a significant side reaction if sufficiently active nucleophiles are employed.[6] As was expected, no evidence of dephenylation was found in any of the reactions of the phenyl ester.

The rates of the displacement reactions were followed by titrating unreacted nucleophile with perchloric acid in glacial acetic acid.[7] The presence of unreacted III did not interfere with these determinations.

(2) M. I. Kabachnik, T. A. Mastryukova, N. I. Kurochkin, N. P. Rodionova and E. M. Popov, *Zhur. Obshchei Khim*, **26**, 120 (1956).

(3) A. E. Arbuzov and P. I. Alimov, *Izvest. Akad. Nauk. S.S.S.R., Otdel. Khim Nauk*, 409 (1951) [*C. A.*, **49**, 160^c (1955)].

(4) I. Dostrovsky and M. Halmann, *J. Chem. Soc.*, 502 (1953).

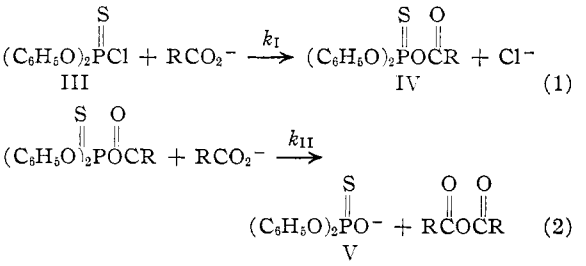
(5) R. F. Hudson and D. C. Harper, *ibid.*, 1356 (1958).

(6) B. Miller and R. W. Young, to be reported.

(7) J. S. Fritz, "Acid–Base Titrations in Nonaqueous Solvents," G. Frederick Smith Chemical Co., Columbus, Ohio, 1952.

Results and Discussion

Unlike most other anions, which reacted with III with simple second-order kinetics,[6] potassium acetate reacted in such a manner that one-half of the original base present, as determined by perchloric acid titration, remained at the completion of the reaction. Interestingly, the amount of base remaining at the end of the reaction was independent of the concentration of III, provided that the initial concentration of III was at least one-half that of the acetate ion. This apparently strange relationship can be explained on the basis of the reaction sequence



III IV (1)

V (2)

If $k_I << k_{II}$, the net result will be the disappearance of one mole of acetate ion and the production of one-half mole of O,O-diphenyl phosphorothioate ion (V). Since V is titrated as a strong base under the conditions employed, one half-mole of base will remain at the end of the reaction.[8]

For a reaction sequence of this sort, the second-order rate constant for the first step, k_I, can be obtained from the equation

$$k_I = \frac{2.303}{t(A_0 - 2P_0)} \log_{10} \frac{AP_0}{PA_0} \quad (3)$$

Where A_0 and P_0 are the initial concentrations of acetate and phosphorochloridothioate, and A and P the corresponding concentrations at time t.

Since one-half mole of V is produced for each mole of acetate consumed, the concentration of titratable base present, B, will be

$$B = A + {}^1/_2(A_0 - A) \quad (4)$$

whence $$A = 2B - A_0 \quad (5)$$

similarly $$P = P_0 - {}^1/_2(A_0 - A) = P_0 - A_0 + B \quad (6)$$

therefore $$k_I = \frac{2.303}{t(A_0 - 2P_0)} \log \frac{P_0(2B - A_0)}{A_0(P_0 - A_0 + B)} \quad (7)$$

The values of k_I obtained from eq. 7 are calculated in Table I for a typical run. The constancy of these values lends strong support to the reaction scheme presented above.

Since the relative rates of k_I and k_{II} should be strongly dependent upon the ease of attack at the carboxyl group of the intermediate anhydride IV, the reaction of III with potassium trimethylacetate was studied, in the expectation that the steric effect of the trimethylmethyl group would decrease k_{II} sufficiently to allow the isolation of the intermediate mixed anhydride.[9] This expectation was not fulfilled. Just one-half of the starting concentration of titratable base was consumed during the reaction, and a constant value of k_I was again ob-

(8) Compound V does not react with III at a measurable rate under these conditions.

(9) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, p. 212.

TABLE I

RATE CONSTANTS FOR THE REACTION OF POTASSIUM ACETATE WITH O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE AT 58.0°

Sample	t, sec.	$B \times 10^2$, mole/l.	$A \times 10^2$, mole/l.	$P \times 10^2$, mole/l.	$k_I \times 10^3$, moles/l./sec.
1	0	1.487	1.487	0.907	...
2	930	1.444	1.397	.859	3.78
3	2,020	1.404	1.319	.820	3.48
4	3,900	1.333	1.178	.749	3.62
5	6,000	1.272	1.056	.688	3.53
6	9,200	1.203	0.915	.617	3.45
7	13,500	1.123	.757	.538	3.55
8	18,150	1.068	.646	.482	3.45
9	22,500	1.031	.574	.446	3.35
				Av.	3.53 ± 0.09

tained by the use of equation 7. This is illustrated in Table II.

TABLE II

RATE CONSTANTS FOR THE REACTION OF POTASSIUM TRIMETHYLACETATE WITH O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE AT 58.0°

Sample	t, sec.	$B \times 10^2$, moles/l.	$A \times 10^2$, moles/l.	$P \times 10^2$, moles/l.	$k_I \times 10^3$, moles/l./sec.
1	0	1.087	1.087	2.445	
2	915	1.029	0.971	2.387	2.55
3	1,750	0.985	.882	2.343	2.45
4	3,660	.876	.665	2.234	2.86
5	5,355	.824	.561	2.182	2.70
6	7,230	.754	.421	2.112	2.81
7	9,000	.718	.347	2.075	2.85
8	12,060	.661	.235	2.019	2.93
9	15,500	.636	.185	1.994	2.67
10	18,900	.605	.123	1.963	2.71
				Av.	2.73 ± 0.12

The mechanism proposed above to explain the apparent stoichiometry and kinetics of the reaction of III with carboxylate anions was confirmed by the isolation in 96% yield of the phosphorothioate anion V, as its silver salt, from the reaction of potassium trimethylacetate and III. The presence of traces of chloride ion prevented identification of the silver salt of V by its melting point. A comparison of its infrared spectrum (18 peaks) with that of an authentic sample, however, provided an unequivocal identification. Furthermore, trimethylacetic anhydride and trimethylacetic acid were isolated from the reaction mixture, again confirming the reaction scheme postulated above.

TABLE III

RATE CONSTANTS FOR THE REACTION OF POTASSIUM TERT.-BUTOXIDE WITH O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE AT 15.0°

Sample	t, sec.	$B \times 10^2$, moles/l.	$A \times 10^2$, moles/l.	$P \times 10^2$, moles/l.	k_I, mole/l./sec.
1	0	1.601	1.601	1.393	...
2	90	1.412	1.225	1.207	0.117
3	152	1.342	1.085	1.136	.104
4	230	1.242	0.884	1.035	.109
5	312	1.177	.751	0.949	.106
6	390	1.113	.626	.907	.110
7	460	1.076	.549	.869	.110
8	570	1.029	.457	.823	.108
9	675	0.972	.340	.764	.118
10	880	0.903	.203	.696	.131
				Av.	0.113 ± 0.007

A similar stoichiometry was observed in the reaction of III with potassium t-butoxide, for which the following reaction sequence is proposed

$$(C_6H_5O)_2\overset{\overset{S}{\|}}{P}Cl + (CH_3)_3CO^- \xrightarrow{k_I} (C_6H_5O)_2\overset{\overset{S}{\|}}{P}\text{—}OC(CH_3)_3 + Cl^- \quad (8)$$

VI

$$(C_6H_5O)_2\overset{\overset{S}{\|}}{P}OC(CH_3)_3 + (CH_3)_3CO^- \xrightarrow{k_{II}} (C_6H_5O)_2\overset{\overset{S}{\|}}{P}O^- + CH_2{=}C(CH_3)_2 + (CH_3)_3COH \quad (9)$$

VI

The constancy of k_I obtained from eq. 7 may be seen in Table III.

TABLE IV

RATES OF REACTION OF NUCLEOPHILES WITH O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE

Nucleophile, potassium	Temp., °C.	k_I, l./mole/sec.	No. of runs
Acetate	58.0	$3.43 \pm 0.11 \times 10^{-3}$	3
Trimethylacetate	58.0	$2.83 \pm 0.10 \times 10^{-3}$	2
t-Butoxide	15.0	$1.42 \pm 0.18 \times 10^{-1}$	3
	30.3	$3.15 \pm 0.02 \times 10^{-1}$	2
	44.0	6.65×10^{-1}	1

A quantitative yield of the silver salt of V was obtained from the reaction of III and potassium t-butoxide. Since no attempt was made to trap any isobutylene evolved, the reaction of t-butoxide ion with VI to form di-t-butyl ether remains a formal possibility which does not conflict with the available information. The high degree of substitution on both anion and ester, however, would be expected to favor elimination over substitution reactions.[10] The reaction sequence written above, therefore, seems far more probable.

In view of the great reactivity of the mixed anhydride IV with carboxylate ions, it was considered worthwhile to re-examine the Russian report of the preparation of the mixed anhydride II from I and sodium acetate in ethanol solution.[1]

When the published procedure[1] was repeated (on twice the scale) a 57% yield of O,O,O-triethyl phosphorothioate, the solvolysis product of II, was obtained, as well as a 9% yield of its isomeride, O,O,S-triethyl phosphorothioate. Gas–liquid partition chromatography of the solvent obtained from the reaction mixture showed it to contain an appreciable amount of ethyl acetate, which was presumably formed by esterification of acetic acid rather than by ethanolysis of II, since no direct evidence for the presence of O,O-diethyl phosphorothioic acid was obtained.[11]

(10) J. Hine, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, Chap. 7.

(11) The isolation of O,O,S-triethyl phosphorothioate suggests that catalytic amounts of some phosphorothioate salt were present, since O,O,O-triethyl phosphorothioate will not spontaneously rearrange under these conditions, but may rearrange in the presence of sulfur-containing anions; *cf.* G. Hilgetag, G. Schramm and H. Teichmann, *Angew. Chem.*, **69**, 205 (1957); S. Du Breuil and R. W. Young, Abstracts of the 137th Meeting of the American Chemical Society, Atlantic City, N. J., 1959, p. 101T, for previous work on the demethylation of phosphorothioates.

The infrared spectra of the phosphorothioates obtained were free of any carbonyl absorption, nor was there any other definite indication[11] that II was formed during the reaction. This conclusion is consistent with the observation that the presence of sodium acetate does not affect the solvolysis rate of diisopropyl phosphorochloridate.[4]

No attempt was made to investigate the other mixed carboxylic-thiophosphoric anhydrides reported[1] by Zemlyanskii and Malinovskii.

Acknowledgments.—I wish to thank Dr. R. Feinland and Mr. E. Smalley for the vapor-liquid chromatography studies, and Drs. C. A. Streuli and R. W. Young for their most helpful and interesting discussions.

Experimental[12]

Materials.—Glacial acetic acid and 70% perchloric acid (J. T. Baker analyzed reagent) were used as received. Commercial *t*-butyl alcohol was distilled from lithium aluminum hydride, b.p. 81.2–81.5°. Dioxane was purified by Fieser's[13] method and stored under nitrogen in the dark.

A "90%" *t*-butyl alcohol–dioxane mixture was prepared by adding 100 ml. of dioxane to 1 liter of *t*-butyl alcohol at 25°.

Potassium *t*-butoxide was prepared by dissolving metallic potassium in *t*-butyl alcohol–dioxane and titrating aliquots with 0.1000 *N* HCl. Fresh solutions were prepared each day it was used.

Trimethylacetic acid (Eastman, m.p. 33–35°) was converted to the potassium salt by reaction *in situ* with a stoichiometric amount of potassium *t*-butoxide. Potassium acetate (Baker and Adamson reagent grade) was dried at 100° for 12 hours before use.

O,O-Diphenyl Phosphorochloridothioate.—To a solution of 56.14 g. (1.0 mole) of potassium hydroxide in 400 ml. of methanol was added 94.6 g. (1.0 mole) of phenol. The solvent was evaporated under vacuum and the residue dried by heating on a steam-bath at 1.0 mm. for 2 hours.

In a 1-liter round-bottomed flask were placed 400 ml. of anhydrous ether and 56.5 g. (0.333 mole) of thiophosphoryl chloride. The mixture was cooled in ice and stirred constantly, while 88.3 g. (0.664 mole) of powdered potassium phenoxide were added in portions over a 1-hour period. No appreciable rise in temperature occurred. The ice-bath was removed, and the mixture allowed to come to room temperature, and then to reflux gently without external heating. When the refluxing stopped (about 0.5 hour) the mixture was refluxed for an additional hour, and then cooled in ice and filtered free of potassium chloride. The ether was evaporated and 50 ml. of hexane was added to the mixture, which was stirred and placed in the ice-box. After standing overnight, 73 g. (0.257 mole, 78%) of white crystals, m.p. 59–66°, was obtained. Three recrystallizations from hexane gave 26.9 g. m.p. 66–67°. *Anal.* Calcd. for $C_{12}H_{10}O_2PSCl$: C, 50.62; H, 3.54; P, 10.88; Cl, 12.45. Found: C, 50.65, 50.34; H, 3.58, 3.70; P, 10.59; Cl, 12.22.

Potassium O,O-Diphenyl Phosphorothioate.—To 9.14 g. (0.032 mole) of O,O-diphenyl phosphorochloridothioate was added 3.70 g. (0.065 mole) of potassium hydroxide in 100 ml. of 50% dioxane–water. The mixture was shaken mechanically for 30 minutes, and was then evaporated on the steam-bath under vacuum. The residue was extracted with acetone and the insoluble salt discarded. Evaporation of the acetone left a pinkish gum, which began to crystallize on addition of a few drops of methanol. The product was rubbed with benzene, cooled in the ice-box, and filtered, yielding 8.10 g. (90%) of white crystals, m.p. 145–158°. Recrystallization from acetone–benzene gave 6.5 g. of white needles, m.p. 165–167° dec. The mother liquors yielded an additional 0.75 g., m.p. 165–167°.

Silver O,O-Diphenyl Phosphorothioate.—To a solution of 1.0 g. (0.487 mmole) of potassium O,O-diphenyl phosphorothioate in 20 ml. of water was slowly added an aqueous silver nitrate solution. Addition was continued until no further precipitation occurred. The white flocculent precipitate was filtered, washed several times with water, and stored in a dark bottle. The yield was 1.32 g. (0.482 mmole, 99%), m.p. 222–224° dec.

Kinetics.—Solutions of the nucleophile and of O,O-diphenyl phosphorochloridothioate in 50 ml. of *t*-butyl alcohol–dioxane were prepared, and allowed to come to thermal equilibrium in a thermostat filled with dibutyl phthalate and maintained within 0.1° of the desired temperature. The two solutions were then rapidly mixed. Ten ml. aliquots were removed at intervals with quick draining pipets and poured into 25-ml. portions of glacial acetic acid. The acetic acid solution was then titrated with a solution of perchloric acid in glacial acetic acid (*ca.* 0.02 *M*) which had previously been standardized against potassium acid phthalate, and the titration followed by a Beckman zeromatic *p*H meter.[7] Careful plotting of e.m.f. *vs.* volume delivered was necessary, because of the leveling effect of the *t*-butyl alcohol present.

Isolation of Products. (a) Reaction of III with Potassium Trimethylacetate.—Trimethylacetic acid (1.546 g., 14.95 mmoles) was dissolved in 50 ml. of *t*-butyl alcohol–dioxane and 50 ml. (15.35 mmoles) of 0.307 *N* potassium *t*-butoxide solution was added. O,O-Diphenyl phosphorochloridothioate (2.195 g., 7.73 mmoles) was dissolved in 150 ml. of solvent. The two solutions were equilibrated at 58° and then mixed. After standing for 23 hours, the mixture was cooled to room temperature, filtered free of precipitated salt and distilled through a 6-inch wire gauze packed column to remove solvent. The residue was dissolved in methylene chloride, washed with water and dried over magnesium sulfate.

The water wash was treated with silver nitrate solution to give 2.75 g. (7.38 mmoles, 96%) of creamy white solid, m.p. 217–220° dec. The infrared spectrum was identical (Nujol mull) with that of silver O,O-diphenyl phosphorothioate.

The methylene chloride solution was filtered and evaporated to give 1.66 g. of reddish oil, which was distilled at 3 mm. to yield 0.63 g. of colorless liquid, b.p. 52°. Gas–liquid chromatography (using a 6-ft. silicone column at 160° and a helium flow rate of 67 ml. per min.) separated the product into two components, which were collected in carbon tetrachloride and shown by infrared spectra to be trimethylacetic acid and trimethylacetic anhydride (ν_{max} 1750 and 1815 cm.$^{-1}$).

(b) Reaction of III with Potassium t-Butoxide.—O,O-Diphenyl phosphorochloridothioate (0.662 g., 2.33 mmoles) was dissolved in 50 ml. of *t*-butyl alcohol–dioxane, and 10 ml. (4.55 mmoles) of 0.455 *N* potassium *t*-butoxide solution were added. After standing at room temperature for 30 minutes the mixture was centrifuged to give a clear solution, and a solution of silver nitrate in 50% ethanol–water was added. An immediate precipitate formed, which was filtered and dried, m.p. 209–213° dec. The yield of silver O,O-diphenyl phosphorothioate was 900 mg. (2.43 mmoles, 106%). Its infrared spectrum in Nujol mineral oil was identical with that of authentic material.

Reaction of Sodium Acetate and O,O-Diethyl Phosphorochloridothioate. (a) In Ethanol.—Absolute ethanol (60 ml.) was added to a mixture of O,O-diethyl phosphorochloridothioate (15.0 g., 0.079 mole) and sodium acetate (4.95 g., 0.061 mole). The mixture was stirred and refluxed for 1 hour, and was then cooled in ice and filtered. The solvent was evaporated under vacuum and collected in a Dry Ice trap. Gas–liquid chromatography (using a 1-meter polyglycol column at 80°) showed the solvent to be 93.9% ethanol and 5.9% ethyl acetate.

The non-volatile residue was a mobile liquid which did not crystallize on standing in the ice-box. It was distilled at 1.75 mm. to give O,O,O-triethyl phosphorothioate (8.35 g., 0.045 mole, 57%), b.p. 61–67°, $n^{22}D$ 1.4554, and 1.32 g. (0.007 mole, 9%) of a product which had b.p. 77–84°, $n^{22}D$ 1.4543. Gas–liquid chromatography on a 12-ft. silicone column at 217° showed this to be a mixture containing 32% O,O,O-triethyl phosphorothioate and 66% O,O,S-triethyl phosphorothioate. Neither fraction exhibited any carbonyl absorption in the infrared region.

(b) In Dioxane.—Purified dioxane (300 ml.), potassium

(12) Elementary analyses were done by Dr. J. A. Kuck and his associates. Melting points are uncorrected. Infrared spectra were taken on a Perkin–Elmer Infracord or a Beckman model IR4 spectrophotometer.

(13) L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1955, 3rd Edition, p. 284.

acetate (19.90 g., 0.2 mole) and O,O-diethyl phosphorochloridothioate (37.8 g., 0.2 mole) were stirred at 75° for 5 hours. The mixture was cooled to room temperature and filtered. Evaporation of the solvent left 42.4 g. of yellow oil, which was distilled at 0.75 mm., to give 8.20 g. of recovered O,O-diethyl phosphorochloridothioate, b.p. 73–78°. The pot residue thickened to a viscous, undistillable tar during the distillation.

[CONTRIBUTION FROM THE DEPARTMENT OF MICROBIOLOGY, COLLEGE OF PHYSICIANS AND SURGEONS, COLUMBIA UNIVERSITY, NEW YORK, NEW YORK]

Studies on the Reactivation of Diethylphosphorylchymotrypsin[1]

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The reactivation of a lyophilized preparation of diethylphosphoryl (DEP) chymotrypsin by approximately forty nucleophilic agents (oximes and hydroxamic acids) was examined, using a rapid colorimetric assay for chymotrypsin. The hydroxamic acids tested were synthesized by the usual methods as well as by two new synthetic techniques. With the exception of three compounds, all of the nucleophilic agents possessed activities which were related to their dissociation constants. The three exceptions, N-phenylbenzohydroxamic acid, N-phenylnicotinohydroxamic acid and pyridine 4-aldoxime dodeciodide, showed enhanced activities. The effect of *p*H on the reactivation process was also studied as well as the stability of DEP-chymotrypsin relative to chymotrypsin. Kinetic data were calculated on the basis of the catenary reaction DEP-chymotrypsin → chymotrypsin → denatured chymotrypsin. Preparations of DEP-chymotrypsin could be completely reactivated even after standing several months.

Introduction

α-Chymotrypsin reacts stoichiometrically with certain organic phosphates,[2,3] such as tetraethylpyrophosphate (TEPP) and diisopropyl fluorophosphate (DFP), with a resulting loss of enzymic activity. Considerable experimental evidence points to a phosphorylation of the hydroxyl group of a serine residue located at the active site of the enzyme.[4] A number of other esterases[5,6] and phosphoglucomutase[7] are similarly affected. In the case of DFP-inactivated cholinesterase, Wilson and others[8,9] were able to design effective nucleophilic reactivators as a result of a careful study of the specificity of the enzyme. Wilson also recently reported that pyridine aldoximes could slowly reactivate chymotrypsin inhibited by TEPP.[10] Earlier work by Cunningham has established that chymotrypsin, inactivated by diethyl *p*-nitrophenylphosphate,[11] can be partially reactivated by hydroxylamine, and while this paper was in preparation, Green and Nicholls reported the reactivation of Sarin-inactivated chymotrypsin by several oximes and hydroxamic acids.[12]

We are reporting the results of a study of the reactivation of lyophilized preparations of TEPP-inactivated chymotrypsin by a series of oximes and hydroxamic acids. In order to facilitate this study, a rapid assay method for chymotrypsin has been developed, which has made possible the screening of more than 40 compounds. Furthermore, two new methods have been devised for the synthesis of hydroxamic acids directly from carboxylic acids using, in one case, phosphorus pentoxide as the condensing agent, and, in the other, a mixed anhydride intermediate for the preparation of hexano- and N-phenylnicotinohydroxamic acids, respectively.

Experimental

Enzymes.—Three times crystallized α-chymotrypsin which had been dialyzed salt-free and lyophilized was purchased from Worthington Biochemical Co., Freehold, New Jersey.

Diethylphosphoryl-α-chymotrypsin (DEP-chymotrypsin) was prepared by the method of Jansen, *et al.*[2] The dialyzed and lyophilized material retained 0.1–0.7% of the initial activity.

Nucleophilic Reagents.—A number of oximes and hydroxamic acids were purchased from Distillation Products Industries, Rochester 3, New York: acetone oxime, benzaldoxime, cyclohexanaldoxime, methoxyamine, N-methyl-N-nitroso-aniline, N-phenylbenzohydroxamic acid, phenylglyoxaldoxime, 1-phenyl-1,2-propanedione dioxime, 1-phenyl-1,2-propanedione-2-oxime.

We are indebted to Drs. I. B. Wilson and S. Ginsburg of the Department of Biochemistry for the following compounds: nicotinohydroxamic acid methiodide, pyridine-4-aldoxime dodeciodide, 4-pyridine-1,2-ethanedione-2-oxime methiodide, 3-pyridine-1,2-ethanedione-2-oxime methiodide, pyridine-2-aldoxime heptiodide, pyridine-2-aldoxime methiodide, pyridine-2-aldoxime heptiodide, pyridine-2-aldoxime methiodide, pyridine-3-aldoxime methiodide, pyridine-4-aldoxime methiodide, pyridine-4-aldoxime pentiodide.

The following compounds were prepared in this Laboratory according to methods described in the literature: acetohydroxamic acid,[13] *o*-aminobenzohydroxamic acid,[14] *p*-aminobenzohydroxamic acid,[15] benzohydroxamic acid,[16]

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