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Phosphorothioates. II. The Effect of the Nucleophilicity of the Attacking Anion upon Rates of Displacement on O,O-Diphenyl Phosphorochloridothioate[1a]

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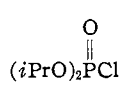
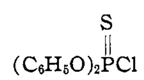
The rates of reaction of O,O-diphenyl phosphorochloridothioate with thirteen anions of oxygen and sulfur have been measured in a "90%" *t*-butyl alcohol–dioxane solution. A plot of acidity of the conjugate acids against the logarithms of the rate constants gives a single curve for almost all anions employed. No differences are observed between anions of sulfur and oxygen. Activation energies parallel rate constants. The anions of benzophenone and acetone oximes are markedly more reactive than would be predicted from their basicities.

Dostrovsky and Halmann have reported that thiophenolate ion is less effective in displacing a chloride ion from O,O-diisopropyl phosphorochloridate (I) than ethoxide or even than phenoxide ion.[2]

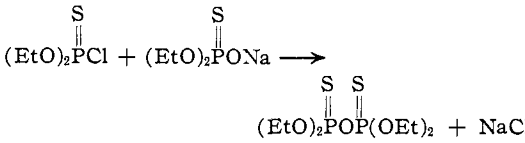

$(i\text{PrO})_2\overset{\overset{\text{O}}{\|}}{\text{P}}\text{Cl}$

I


$(\text{C}_6\text{H}_5\text{O})_2\overset{\overset{\text{S}}{\|}}{\text{P}}\text{Cl}$

II

This report agrees with previous qualitative evidence which suggests that sulfur anions do not exhibit marked nucleophilicity toward phosphorus centers. It has been found, for instance, that thiosulfate ion has little effect on the rate of hydrolysis of Sarin (O-*i*-propylmethanephosphonofluoridate),[3] and that the reaction of O,O-diethyl phosphorochloridothioate with salts of O,O-diethyl phosphorothioic acid yields the O-thiophosphorylated rather than the S-thiophosphorylated products (eq. 1).[4]


$$(\text{EtO})_2\overset{\overset{\text{S}}{\|}}{\text{P}}\text{Cl} + (\text{EtO})_2\overset{\overset{\text{S}}{\|}}{\text{P}}\text{ONa} \longrightarrow (\text{EtO})_2\overset{\overset{\text{S}}{\|}}{\text{P}}\text{O}\overset{\overset{\text{S}}{\|}}{\text{P}}(\text{OEt})_2 + \text{NaCl} \quad (1)$$

These observations are in marked contrast to those made of displacements upon alkyl halides, in which even the most feebly nucleophilic anions of sulfur are more effective displacing agents than the most nucleophilic ions in which oxygen is the reacting element.[5] Thiophenolate ion, one of the most extensively studied sulfur anions, characteristically reacts some 10^3 times as fast as methoxide ion in displacements upon simple alkyl halides, although methoxide is some 10^9 times more basic.[6]

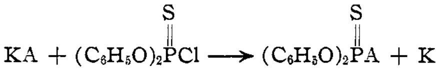
It was therefore of appreciable interest to compare the rates at which sulfur and oxygen anions of equal basicity react at phosphorus centers. Unfortunately, the rapid alcoholysis of phosphorochloridates prevented Dostrovsky and Halmann from determining the actual rate of reaction of I and thiophenolate ion,[2] and other kinetic studies of the reaction of sulfur anions at phosphorus centers do not seem to be available.

This paper reports the results of a kinetic investigation of the reactions of a representative series of anions of sulfur and oxygen at a pentavalent phosphorus center.

Methods and Results.—In order to obtain the desired measurements of rates of attack on *phosphorus* by ions of rather low basicity but high nucleophilicity toward carbon centers,[5] it was necessary to choose a substrate which would not cause displacement at alkyl groups, such as have been found to occur with other phosphates and phosphorothioates,[7] and a solvent which would not cause appreciable solvolysis.

As has been reported in the previous paper in this series,[1] O,O-diphenyl phosphorochloridothioate (II) was chosen as the substrate molecule for kinetic study, and a "90%" mixture of *t*-butyl alcohol and dioxane as the solvent. The potassium salts of *t*-butyl alcohol, pentanethiol, phenol, 2-pyridone, *p*-hydroxyacetophenone, thiophenol, 2-pyridinethiol, 2-hydroxypyrazine, *p*-cyanophenol, acetone oxime, benzophenone oxime, acetic acid and trimethylacetic acid were chosen as nucleophiles.

The reactions of *t*-butoxide and carboxylate anions with II, which follow a more complex course than the other displacements studied, have been considered in a previous paper.[1a] The other reactions all followed simple second-order kinetics, as would be expected from reaction 2.[8]


$$\text{KA} + (\text{C}_6\text{H}_5\text{O})_2\overset{\overset{\text{S}}{\|}}{\text{P}}\text{Cl} \longrightarrow (\text{C}_6\text{H}_5\text{O})_2\overset{\overset{\text{S}}{\|}}{\text{P}}\text{A} + \text{KCl} \quad (2)$$

The kinetic data obtained for typical runs with phenolate and thiophenolate ions are summarized in Table I.

The expected esters were obtained in good yields from synthetic runs using thiophenolate, *p*-acetyl phenolate, 2-pyridinolate, 2-pyrazinolate and pentanethiolate anions. The products of the reaction with potassium 2-pyridinolate and 2-pyrazinolate were clearly the O-thiophosphorylated products, O,O-diphenyl O-2-pyridinyl phosphorothioate (IIIa) and O,O-diphenyl O-2-pyrazinyl phosphorothioate (IIIb), rather than the N-thiophosphorylated products IVa and IVb. Compound IIIa has a very intense peak in the infrared spectrum at 6.32μ and a smaller peak at 6.40μ, as would be

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(1) (a) Previous paper: B. Miller, *J. Am. Chem. Soc.*, **82**, 3924 (1960). (b) Present address: American Cyanamid Co., Agricultural Research Center, Princeton, N. J.

(2) I. Dostrovsky and M. Halmann, *J. Chem. Soc.*, 508 (1953).

(3) J. Epstein, M. M. Demek and D. H. Rosenblatt, *J. Org. Chem.*, **21**, 796 (1956).

(4) D. G. Coe, B. J. Perry and R. K. Brown, *J. Chem. Soc.*, 3604 (1957).

(5) (a) C. G. Swain and C. B. Scott, *J. Am. Chem. Soc.*, **75**, 141 (1953); (b) J. O. Edwards, *ibid.*, **76**, 1540 (1954).

(6) C. W. L. Bevan and J. Hirst, *Chemistry & Industry*, 1422 (1954).

(7) R. F. Hudson and D. C. Harper, *J. Chem. Soc.*, 1356 (1958).

(8) As a referee has pointed out, conclusions about reaction order can be drawn only for the range of concentrations studied experimentally (*ca.* 0.01 to 0.03 molar in II and anion). Since few of the salts employed have significant solubility at greater concentrations, however, this objection is purely of academic interest.

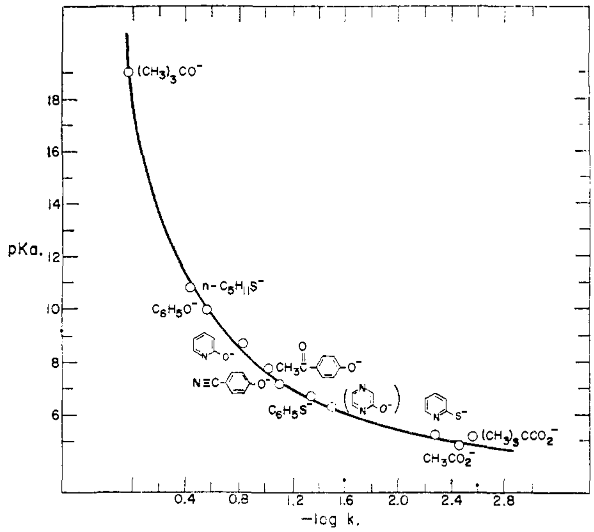
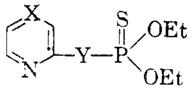
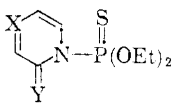


Fig. 1.—Reactions of anions with O,O-diphenyl phosphorochloridothioate at 58°.

expected of a 2-substituted pyridine,[9] and has no significant absorption in the carbonyl region. (2-Pyridone has strong absorption at 6.0–6.1μ.[10]) Similarly, the ultraviolet spectrum of IIIa shows a single maximum at 260 mμ characteristic of a 2-substituted pyridine,[11] while 2-pyridone has a maximum at 298 mμ[11b]; IIIb has infrared peaks at 6.35 and 6.60μ, but is transparent in the 5.8 to 6.2μ region, in which 2-pyrazone absorbs strongly.[10a] Similarly, IIIb has its longest wave length absorption at 271 mμ in the ultraviolet, as do other pyrazinyl derivatives, while 2-pyrazones absorb around 320 mμ.[11b]



IIIa, X = CH, Y = O
b, X = N, Y = O
c, X = CH, Y = S



IVa, X = CH, Y = O
b, X = N, Y = O
c, X = CH, Y = S

Potassium 2-pyridinethiolate reacts to give a viscous liquid whose ultraviolet and infrared spectra are very similar to those of IIIa, indicating that the structure is IIIc, rather than IVc, since 2-pyridinethione derivatives have quite different spectra.[10b,12] Unfortunately, IIIc decomposes rapidly on standing, depositing crystals of 2-pyridinethiol in a gummy matrix of undetermined composition. For this reason, reliable analytical data are not available for IIIc.

In Fig. 1, the logarithms of the rate constants are plotted against the pK_a's (in water) of the conjugate acids of the anions. All of the pK_a's were taken from the literature, with the exception of the values of 2-pyridinethiol, which was calculated from the data of Albert and Barlin[13] using Mason's method,[14] and for p-cyanophenol, which was measured during this work.

TABLE I

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

Sample	t, sec.	[I] × 10^3, moles/l.	[C_6H_5OK] × 10^2, moles/l.	k_2 × 10, l./mole/sec.
1	0	1.048	1.048	. .
2	66	0.920	0.920	2.02[a]
3	124	.789	.789	2.31
4	188	.713	.713	2.57
5	245	.654	.654	2.55
6	305	.595	.595	2.48
7	365	.550	.550	2.59
8	435	.500	.500	2.40
9	515	.472	.472	2.65
10	660	.380	.380	2.54
11	900	.313	.313	2.49
			Av.	2.51 ± 0.08

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

			[C_6H_5SK] × 10^3	k_2 × 10^3
1	0	1.132	1.132	. .
2	150	1.069	1.069	3.47[a]
3	330	0.985	0.985	3.99
4	485	.916	.916	4.48
5	690	.850	.850	4.26
6	870	.790	.790	4.39
7	1050	.742	.742	4.42
8	1230	.699	.699	4.44
9	410	.656	.656	4.53
10	1770	.597	.597	4.46
			Av.	4.37 ± 0.12

[a] Omitted in determining average.

The most striking aspect of this plot is the excellent correlation (remarkably good, considering the very wide range of anion types employed) between reactivity and basicity, and the complete and unique lack of distinction between oxygen and sulfur anions. The increase in reaction rate with increasing basicity is paralleled by a decrease in the energy of activation (Table IV). The *PZ* factor changes proportionately for both oxide and sulfide anions.

The relationships found, however, do not fit a simple Brönsted[15a] or Hammett[15b] linear free energy equation. Most applications of the Hammett and Brönsted relationships, however, have been limited to studies of nucleophiles with rather small ranges of basicity (*i.e.*, substituted phenols or substituted carboxylic acids). It seems probable that extension of some of these studies to include a much wider range of anion basicities would demonstrate a curvature in some of these apparent "straight line" relationships. Along this line, Leahy and Miller have reported a similar curvature in the plot of basicity against reactivity toward 1-chloro-2,4-dinitrobenzene in methanol.[16]

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[9] C. G. Cannon and G. B. B. M. Sutherland, *Spectrochim. Acta*, **4**, 373 (1951).

[10] (a) S. F. Mason, *J. Chem. Soc.*, 4874 (1957); (b) A. R. Katritzky and R. A. Jones, *ibid.*, 2947 (1960).

[11] (a) E. F. G. Herington, *Disc. Faraday Soc.*, **9**, 26 (1950); (b) S. F. Mason, *J. Chem. Soc.*, 5010 (1957).

[12] R. A. Jones and A. R. Katrisky, *ibid.*, 3610 (1958).

[13] A. Albert and G. B. Barlin, *ibid.*, 2384 (1959).

[14] S. F. Mason, *ibid.*, 674 (1958).

[15] (a) C. F. Smith, *ibid.*, 521 (1943); (b) L. P. Hammett, "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1940, Chapter VII.

[16] G. D. Leahy and J. Miller, *Chemistry & Industry*, 40 (1953).

TABLE II

REACTION OF O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE WITH ANIONIC NUCLEOPHILES AT 58°

Conjugate acid of anion	pK_a	$k \times 10^3$, l. m.$^{-1}$ sec.$^{-1}$	No. runs	$-\log k$
t-Butyl alcohol	*ca.* 19[a]	121	..[b]	−0.11
1-Pentanethiol	10.66[c]	35.7	..[d]	.448
Phenol	9.94[e]	26.7 ± 0.4	4	.573
2-Hydroxypyridine	8.66[f,g]	14.5 ± .1	2	.840
p-Hydroxyacetophenone	7.75[h]	9.20 ± .05	2	1.035
p-Cyanophenol	7.23[i]	7.75 ± .15	2	1.111
Thiophenol	6.52[j]	4.48 ± .15	3	1.35
2-Hydroxypyrazine	....	3.11 ± .005	2	1.507
2-Pyridinethiol	5.13[g,k]	0.517 ± .009	2	2.29
Trimethylacetic acid	5.05[l]	.283 ± .010	2	2.56
Acetic acid	4.76[l]	.343 ± .011	3	2.47

[a] W. K. McEwen, *J. Am. Chem. Soc.*, 58, 1124 (1936). [b] Extrapolated from rates measured at 15°, 30° and 44° (ref. 1). [c] The pK_a of 1-butanethiol was used (ref. *j*). [d] Extrapolated from rates measured at 30° and 44°. [e] W. H. Fletcher, *J. Am. Chem. Soc.*, 68, 2726 (1946). [f] Ref. 14. [g] Values given are for the aromatic forms of these compounds. [h] J. M. Vanderbelt, C. Henrich and S. G. Vanden Berg, *Anal. Chem.*, 26, 726 (1954). [i] Measured during this work. [j] M. M. Kreevoy, *et al.*, *J. Am. Chem. Soc.*, 82, 3899 (1960). [k] Calcd. from data of ref. 14. [l] N. A. Lange, "Handbook of Chemistry," Seventh Edition, Handbook Publishers, Inc., Sandusky, Ohio, 1949.

TABLE III

REACTION OF O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE WITH ANIONIC NUCLEOPHILES

Conjugate acid of anion	pK_a	*T*, °C.	$k \times 10^3$, l. m.$^{-1}$ sec.$^{-1}$	No. runs	$-\log k$
t-Butyl alcohol	....	30.3	31.5 ± 0.20[a]	2[a]	0.502
1-Pentanethiol	....	30.3	8.24 ± .40	2	1.084
		44.0	17.5 ± .15	3	0.758
Phenol	....	30.3	4.73 ± .08	2	1.325
		44.0	11.6 ± .5	3	0.936
Thiophenol	....	30.3	0.628 ± .024	3	2.20
		44.0	1.68 ± .12	4	1.78
Acetone oxime	12.42[b]	30.3	49.1 ± .1	2	0.309
		20.0	33.3 ± 1.7	2	.477
Benzophenone oxime	12.37[c]	20.0	81.3	1	.090

[a] Ref. 1. [b] C. V. King and A. P. Marion, *J. Am. Chem. Soc.*, 66, 977 (1944). [c] Measured during this work.

TABLE IV

ACTIVATION QUANTITIES FOR REACTION OF ANIONS WITH O,O-DIPHENYL PHOSPHOROCHLORIDOTHIOATE

Conjugate acid of anion	E_a, kcal. mole^{-1}	log *PZ*, l. mole^{-1} sec.$^{-1}$[a]
t-Butyl alcohol	9.80	6.56
1-Pentanethiol	10.27	6.34
Phenol	12.28	7.53
Thiophenol	13.90	7.82
Acetone oxime	6.72	4.51

[a] At 30.3°.

TABLE V

REACTION OF ANIONS WITH *n*-BUTYL BROMIDE AT 58°[a]

Conjugate acid of anion	$k \times 10^3$, l. mole^{-1} sec.$^{-1}$
t-Butyl alcohol	0.0187
Thiophenol	7.07

[a] Results are for a single run in each case.

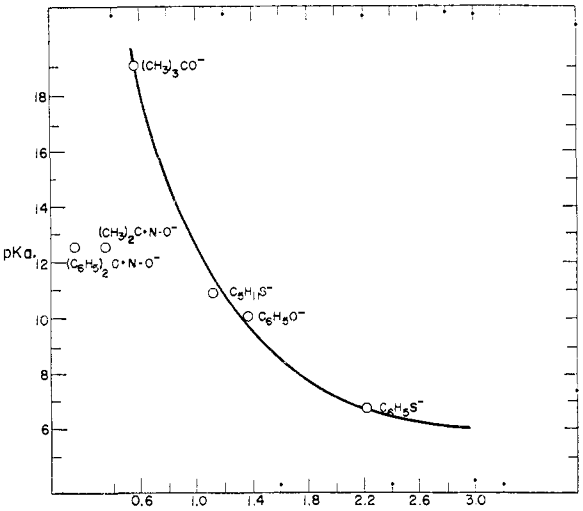

Fig. 2.—Reactions of anions with O,O-diphenyl phosphorochloridothioate at 30.3°.

Figures 1 and 2 are plotted on the tacit assumption that anion basicities in *t*-butyl alcohol are proportional to those in water. This assumption is reasonably valid for anions of similar basicity (even when anions of sulfur and oxygen are compared),[17] but is inaccurate for anions whose basicities are widely different. While little data are available on basicities in *t*-butyl alcohol, available evidence makes it clear that strong acids have their acidities decreased less on transferring from water to non-polar solvents than do weak acids[17,18]; that is, that strong bases will appear relatively stronger in alcohols than in water as compared to weak bases. If proper "correction factors" with which to change basicities in water to basicities in *t*-butyl alcohol–dioxane were available, therefore, their use would increase the curvature of the lines in Figs. 1 and 2. The curvatures of these lines, therefore, cannot be an artifact resulting from the use of an improper acidity scale, but must be an inherent function of the system.

Although the 2-pyrazinolate anion was included among those studies, its reaction rate could not be used to determine the curve of Fig. 1, since the pK_a of 2-hydroxypyrazine cannot be measured by the usual methods[13,14] in view of the presence of a second basic nitrogen atom. The very low scatter of the points in Fig. 1 suggests that the basicities of anions can be determined from this curve, if the rates of reaction of the anions with II are known. The value (6.4) found for the pK_a of 2-hydroxypyrazine by this means seems reasonable.

In view of the recent report by Winstein and his collaborators[19] illustrating the importance of solvent polarity in determining relative rates of displacement reactions, it was considered important to establish that the low dielectric constant of the medium did not produce the marked difference between our system and displacements on alkyl halides.

It was found that potassium thiophenolate reacts with *n*-butyl bromide three hundred and seventy times as fast as does potassium *t*-butoxide, while it is only one-seventieth as reactive toward the thiophosphoryl chloride. Although the nucleophilicity of potassium *t*-butoxide has not been

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(17) (a) G. Schwarzenbach and E. Rudin, *Helv. Chim. Acta*, 22, 360 (1939); (b) W. H. Fletcher, *J. Am. Chem. Soc.*, 68, 2726 (1946).

(18) (a) L. A. Wooten and L. P. Hammett, *ibid.*, 57, 2289 (1935); (b) R. B. Mason and M. Kilpatrick, *ibid.*, 59, 572 (1937).

(19) S. Winstein, L. G. Savedoff, S. Smith, I. D. R. Stevens and J. S. Gall, *Tetrahedron Letters*, No. 9, 24 (1960).

measured in more polar solvents, it is clear that anion polarizability plays its usual preponderant role in carbon displacements when *t*-butyl alcohol is the solvent.[20]

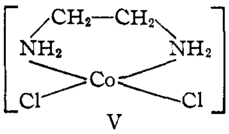
Discussion

We have thus far compared phosphoryl to alkyl halides in considering the effects of sulfur and oxygen nucleophiles. The question arises as to whether other substrates with structural features similar to phosphoryl halides, *i.e.*, central elements other than carbon and the presence of "multiple bonds" or a strong positive charge at the reaction center, would show a similar lack of sensitivity to anion polarizability.

A survey of the literature indicates that most substrates with such gross differences in structure still exhibit marked sensitivity to anion polarizability.

Sulfide ions are far more effective nucleophiles than oxide ions in reactions with aromatic halides, although the relative reactivity is somewhat lower than those found with aliphatic substrates.[5,21] Sulfide ions have been shown to have exceptional nucleophilicity in reactions with carbonium ions,[22] carbenes[23] and benzyne,[24] and in addition to ethylenic[25] and acetylenic[26] bonds. An apparent exception to the rule that sulfide ions exhibit marked nucleophilic powers in reactions at carbon centers is given by Green and Hudson,[27] who found that thiosulfate ion, a very effective nucleophile toward alkyl halides,[2] reacted very slowly with ethyl chloroformate. However, mercaptide anions have been shown to be more reactive than oxygen anions toward several other carboxylic acid derivatives[28] as are other polarizable anions[28] and amines.[28,29]

Far less information is available in regard to nucleophilicities in attack at elements other than carbon. De la Mare and Vernon have shown that thiophenolate is as effective as ethoxide in displacement at a hydrogen atom in *t*-butyl chloride.[30] Ingold and his co-workers have found that the reactivity of a series of anions toward V does not follow the "standard" nucleophilicity values toward carbon. However, polarizable anions (azide, nitrite) are appreciably more reactive than would be predicted from their basicities.[31]



V

The acid-catalyzed hydrolysis of cyclic sulfites (a reaction which proceeds by displacement on the sulfur atom)[32] is accelerated by added salts in the order $Br^- > Cl^- >> HSO4^- > Cl04^-$,[33] an order which agrees with the nucleophilic order toward carbon, but is inversely related to basicity. Though little quantitative data are available, it seems that rates of displacement on bivalent sulfur have little relationship to basicity but are closely related to polarizability and to the nucleophilic order toward carbon.[34]

It appears, then, that phosphates and phosphorothioates are unique in their insensitivity toward anion polarizability, although some other substrates approach this behavior more closely than do saturated carbon centers.

Before considering reasons for the lack of effect of anion polarizability on the reactivity of phosphates, it may be of interest to consider the reason for the effect of anion polarizability on the reactivity of alkyl halides.

The polarizability of an anion can be considered to be the extent to which the center of negative charge is displaced from the center of the atom under the influence of a positive charge.[35] The total energy of the system in which a cation approaches an anion is markedly reduced by the decrease in the distance (due to polarization of the anion) between the cation and the center of negative charge it is approaching. This decrease in energy is partially nullified by the energy required to polarize the anion, but for an anion of high polarizability, at sufficiently small distances from the cationic center, the net decrease in energy is impressive. The polarization energy of an iodide ion two ångstrom units from a point cation of unit charge is 67 kcal./mole; at a distance of three ångstrom units, the polarization energy is 13 kcal./mole.[36] The situation of an ion approaching an alkyl halide molecule is, of course, much less simple, since the halogenated carbon atom can be considered neither a point nor a simple cation. Even in this complex situation, however, polarization of the anion should reduce the net charge separation sufficiently to significantly decrease the activation energy.

An alternative explanation, which has since been adopted elsewhere,[37] was offered by Swain and Scott,[2] who suggested that the enhanced nucleophilicity of polarizable anions is due to a polarization of the anion's electrons *away* from the re-

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(20) No attempt was made to characterize the products of the reaction of potassium *t*-butoxide with *n*-butyl bromide. Since elimination to form butene is probably a major side reaction, the ratio quoted above (370 to 1) represents a *minimum* ratio of the rates of reaction of thiophenolate and *t*-butoxide anions.

(21) (a) C. W. L. Bevan and J. Hirst, *J. Chem. Soc.*, 254 (1956); (b) J. F. Bunnett and G. T. Davis, *J. Am. Chem. Soc.*, **76**, 3011 (1954).

(22) C. G. Swain, C. B. Scott and K. H. Lohmann, *ibid.*, **75**, 136 (1953).

(23) J. Hine, *ibid.*, **72**, 2438 (1950).

(24) F. Scardiglia and J. D. Roberts, *Tetrahedron*, **3**, 197 (1958).

(25) C. D. Hurd and L. L. Gershbein, *J. Am. Chem. Soc.*, **69**, 2329 (1947).

(26) W. E. Truce, M. M. Boudakian, R. F. Heine and R. J. McManimie, *ibid.*, **78**, 2743 (1956).

(27) M. Green and R. F. Hudson, *Proc. Chem. Soc.*, 149 (1959).

(28) (a) M. L. Bender, *Chem. Revs.*, **60**, 53 (1960); (b) W. P. Jencks and J. Carriuolo, *J. Am. Chem. Soc.*, **82**, 1778 (1960). The data of reference (b) show that thiosulfate ion, while a poor nucleophile toward carboxylic acid derivatives, is appreciably more reactive than oxygen anions of comparable basicity.

(29) T. C. Bruice and R. Lapinski, *J. Am. Chem. Soc.*, **80**, 2265 (1958).

(30) P. B. D. de la Mare and C. A. Vernon, *J. Chem. Soc.*, 41 (1956).

(31) (a) D. D. Brown, C. K. Ingold and R. S. Nyholm, *ibid.*, 2674 (1953); (b) D. D. Brown and C. K. Ingold, *ibid.*, 2680 (1953).

(32) C. A. Bunton, *et al.*, *ibid.*, 4751 (1958).

(33) C. A. Bunton, P. B. D. de la Mare and J. G. Tillett, *ibid.*, 4754 (1958).

(34) (a) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 583 (1959); (b) A. J. Parker and N. Kharasch, *J. Am. Chem. Soc.*, **82**, 3071 (1960).

(35) J. A. A. Ketelaar, "Chemical Constitution," Second Ed., Elsevier Publ. Co., New York, N. Y., 1958, pp. 72-73.

(36) The polarization energies were calculated from the equation $U = -{}^1/_2\alpha(e^2/r^4)$, where U is the energy derived, α the anion polarizability, e the unit charge and r the distance between charge centers.[35]

(37) A. L. Green, G. L. Sainsbury, B. Saville and M. Stansfield, *J. Chem. Soc.*, 1583 (1958).

action site, thereby reducing the repulsion between the incoming and outgoing anions. Even if one leaves aside the question of the applicability of such an explanation to S$_N$1 reactions, or to such reactions as attack on the mustard cation, this explanation can be dismissed on energetic grounds. Since the polarization energy varies inversely with the fourth power of the distance between the ions,[35] the approximate doubling of the distance involved in the interactions between the two anions (as compared to the distance between the polarizable anion and the reaction center) will reduce the polarization energy to $^1/_{16}$ that previously calculated. It is clear that this value will be insufficient to account for the large reductions in activation energy attributed to anion polarization.[38]

Although the preceeding discussion is phrased in terms of reaction at an alkyl center, the arguments used will hold for any reaction in which the transition state is strongly ionic in character, although the maximum possible polarization energy in each case will very with the anion polarizability and the "bond length" in the transition state. The factor which is of particular interest for the present discussion, however, is the extent to which charge separation actually exists in the transition state. The increase in hydrolysis and substitution rates of phosphorochloridates and phosphinyl chlorides caused by decreasing the solvent polarity or increasing the electron-withdrawing power of substituents clearly demonstrate that the transition states in these reactions is less polar than are the starting materials.[39] Rate information of this type, however, cannot describe the actual nature of the transition state in phosphate displacements.

An attractive explanation for the lack of effect of anion polarizability in these displacements is that the transition state has little ionic character—that is, the nascent bond in the transition state closely resembles the covalent bond of the product rather than the separate charges of an S$_N$1 process. Such a transition state will tend to resemble those processes involving addition (*e.g.*, aromatic nucleophilic substitution and carbonyl reactions), which generally show less susceptibility to anion polarizability than do alkyl substitution reactions.

In view of the far more marked independence of anion polarizability exhibited by the reactions discussed in this paper, bond formation must proceed to a greater extent in displacement in thiophosphates than in any other reaction which has been studied.[40]

An alternative explanation, which cannot yet be completely excluded, is that displacement on pentavalent phosphorus involves a rapid, reversible, addition of the anion, followed by a slow, rate-determining, elimination of halide ion. Since equilibrium processes are not affected by polarizability, the rate of displacement would probably also be independent of polarizability. Dostrovsky and Halmann, however, have shown that hydrolysis of a phosphoryl chloride does not involve equilibration of the phosphoryl oxygen with the solvent,[41] thus strongly suggesting that no equilibrium process occurs. While this argument is not quite conclusive (it is not certain, for instance, that the two oxygens must be indistinguishable in an addition complex) the explanation suggested earlier seems preferable.

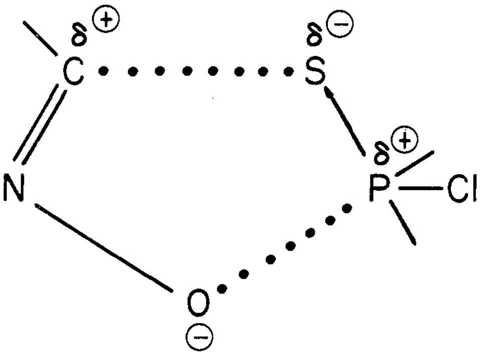

Fig. 3.

Unlike all other anions used in this study, the anions of benzophenone and acetone oximes react far more rapidly than would be predicted from their basicities (Fig. 2). Oximes belong to a class of "complex" reagents (which include the anions of such compounds as catechols,[42] hydroxamic acids[43,28b] and hydrogen peroxides[8]) which catalyze the ready hydrolysis of both phosphate and carboxylate derivatives.[28b,44] It has been suggested[44] that their efficacy as nucleophiles is due to their high polarizability. In view of the lack of undue reactivity of such highly polarizable anions as mercaptides, however, this explanation, while possibly valid for carboxylate hydrolysis, does not seem plausible for phosphates or phosphorothioates.

Most of the materials in this class are capable of exerting a bifunctional catalysis (push-pull mechanism) in displacement on a phosphate or carboxylate substrate, and some (*e.g.*, catecholate monoanion) clearly react in this manner. It is possible that oximate anions can react similarly with the imido carbon acting as the electrophilic center[45] (Fig. 3).

In support of this mechanism, it was found that the very low activation energy of displacement by acetone oximate anion is partially counteracted by a low *PZ* factor, as would be expected for the rigid structure in Fig. 3. Further discussion of

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(38) It has been suggested (*e.g.*, E. S. Gould, "Mechanism and Structure in Organic Chemistry," Henry Holt and Co., New York, N. Y., 1959, p. 260; also ref. 19) that at least part of the nucleophilicity of larger anions derives from the greater ease of desolvation of these ions, rather than their polarizability. Although this factor may contribute to the total decrease in activation energy, it seems difficult to account for the differences in susceptibilities of different substrates on this basis.

(39) I. Dostrovsky and M. Halmann, *J. Chem. Soc.*, 516 (1953).

(40) Dr. R. F. Hudson, of the Cyanamid European Research Institute, Geneva, has reached a similar conclusion, though based on a quite different argument. We wish to thank Dr. Hudson for discussing his elegant studies with us prior to publication.

(41) I. Dostrovsky and M. Halmann, *J. Chem. Soc.*, 1004 (1956).

(42) J. W. Churchill, M. Lapkin, F. Martinez and J. A. Zaslowsky, *J. Am. Chem. Soc.*, **80**, 1944 (1958).

(43) B. E. Hackley, R. Plapinger, M. Stolberg and T. Wagner-Jauregg, *ibid.*, **77**, 3651 (1955).

(44) A. L. Green and B. Saville, *J. Chem. Soc.*, 3887 (1956).

(45) The imido carbon of an oximate anion is actually a nucleophilic, rather than electrophilic, center. If, however, the earlier argument as to the nature of nucleophilic displacement on pentavalent phosphorus is accepted as correct, it will be seen that the imido carbon, in the transition state, will have the normal electrophilic character of carbonyl derivatives.

the reactions of oximate and related anions will be reserved for a succeeding paper.

Acknowledgments.—I wish to thank Dr. R. W. Young of this Laboratory for his valuable advice and encouragement, and Professor E. Miller of the Department of Metallurgy, New York University, for most interesting discussions.

Experimental

Kinetic Studies.—Reaction rates were followed by titration of unreacted nucleophile with perchloric acid in glacial acetic acid, as before.[1] Potassium chloride precipitated as the reaction progressed. Although precipitation normally did not begin until some time after the start of the reaction, no changes in rate constant were observed at the points where potassium chloride first became visible. No other salts were added to the reactions. The concentrations of I and of the nucleophile were in the range 0.01 to 0.03 *N* at the start of each run. Rate constants were determined by the use of the equations

$$k = \frac{2.303}{t(A_0 - B_0)} \log 10 \frac{AB_0}{BA_0}$$

or

$$k = \frac{A_0 - A}{t(A_0A)}$$

depending on the relative concentrations of the two reactants.

Materials.—O,O-Diphenyl phosphorochloridothioate, solvents and titrants were prepared or purified as before.[1] Baker and Adamson reagent grade **phenol** was distilled under nitrogen, b.p. 105° (27 mm.), m.p. 39.5–41.0°. Eastman Kodak Co. white label **thiophenol** was distilled through a 14-inch, glass bead packed column; b.p. 164.5–165°, n^{25}D 1.5864. Eastman Kodak Co. white label ***n*-amylmercaptan** was distilled, b.p. 124–125°. Eastman Kodak Co. technical ***p*-hydroxyacetophenone** was recrystallized from ether; m.p. 108–109°.

2-Pyridone and 2-Pyridinethiol.—The procedure used was modified from the reported method[46] by the use of NaHS·2H$_2$O instead of KHS, and 2-chloropyridine instead of 2-bromopyridine. These modifications are not recommended to succeeding workers.

Propylene glycol (500 ml.) and sodium hydrosulfide dihydrate (203 g., 2.2 moles) were stirred in a 1-liter 3-necked flask until the solid had dissolved, and then heated to 155°. 2-Chloropyridine (86.8 g., 0.76 mole) was added drop by drop over a 15-minute period. The temperature dropped to 145°, while vigorous refluxing occurred. The temperature was kept at 145–150° for 21 hours, and the mixture was then cooled to room temperature, filtered under vacuum, and the residual salt (49.4 g., 0.84 mole after washing) washed with ethanol, which was added to the filtrate. The solution was distilled at 25 mm. until the vapor temperatures reached 100°.

The residue was cooled and dissolved in water (Caution: appreciable exotherm). The solution was acidified with glacial acetic acid, extracted with chloroform, and the extracts dried over sodium sulfate. Evaporation of the solvent left 115.5 g. of light red oil, which crystallized on standing overnight in the ice-box. Washing with a small amount of benzene and filtration gave 28.7 g. of crude 2-pyridinethiol as bright yellow needles, m.p. 81–103°. Two recrystallizations from benzene gave 9.1 g. m.p. 124–127° (reported[46] m.p. 125–128°).

The mother liquors from the first crystallization were evaporated and the residue chromatographed on 650 g. of neutral alumina. The products (10.0 g.) obtained by elution with benzene, ether and chloroform were discarded, and the fraction (25.7 g.) obtained by elution with methanol was crystallized by rubbing with benzene; m.p. 101–104°. Four recrystallizations from carbon tetrachloride gave 6.3 g. of α-pyridone, m.p. 106–107.5° (reported[47] m.p. 106–107°).

Anal. Calcd. for C_5H_5NO: C, 63.2; H, 5.27; N, 14.74. Found: C, 63.35, 63.30; H, 5.44, 5.58; N, 14.35, 14.41.

2-Hydroxypyrazine.—Concentrated hydrochloric acid diluted with an equal volume of water (approx. 350 ml. of diluted acid) was added slowly with rapid stirring to 330 g. of technical sodium pyrazinolate.[48] (Care must be taken to avoid over-acidification, which may cause extensive decomposition.) The mixture was cooled to 5°, the solids were removed by filtration, and recrystallized from isopropyl alcohol. The yield was 81.5 g., m.p. 188–189° (reported[49] m.p. 187–188°). Titration with tetrabutylammonium hydroxide in pyridine[50] indicated a purity of 100.0 ± 0.3%.

O,O-Diphenyl 0–2-Pyridinyl Phosphorothioate.—O,O-Diphenylphosphorochloridothioate (3.52 g., 12.38 mmoles) was dissolved in 175 ml. of *t*-butyl alcohol–dioxane solution. α-Pyridone (11.96 g. 1.259 mmoles) was dissolved in 100 ml. of solvent, and 25 ml. of 0.495 *N* potassium *t*-butoxide solution (12.38 mmoles) added. The two solutions were mixed and kept at 58° for 14 hours. Evaporation of the solvent left a brown oil, which was dissolved (except for the salt present) in a benzene–petroleum ether mixture, and chromatographed on 25 g. of Woelm neutral alumina, to give 2.99 g. (8.77 mmoles, 73%) of colorless oil, which could not be crystallized. The ultraviolet spectrum (in methanol) had λ_{max} 260 mμ (ε 4330), shoulders at 255 and 265 mμ; λ_{min} 231 mμ (ε 1000).

Anal. Calcd. for $C_{17}H_{14}O_3PSN$: C, 59.58; H, 4.11; P, 9.02; N, 4.07; S, 9.35. Found: C, 60.18, 60.22; H, 4.33, 4.22; P, 8.85; N, 3.92; S, 8.96.

O,O-Diphenyl S-2-Pyridinyl Phosphorodithioate.—2-Pyridinethiol (1.381 g., 12.43 mmoles) was dissolved in 100 ml. of *t*-butyl alcohol–dioxane, and 25 ml. of 0.495 *N* potassium *t*-butoxide solution (12.38 mmoles) added. The solution was added to a solution of O,O-diphenyl phosphorochloridothioate (3.52 g. 12.38 mmoles) in 125 ml. of solvent and kept at 58° for 14 hours. The mixture was cooled, filtered, and evaporated to give 4.8 g. of yellow oil. This was chromatographed on 25 g. of Woelm neutral alumina to give 3.19 g. (8.87 mmoles, 71%) of colorless oil, which turned a bright yellow within a few hours in the ice-box, and deposited crystals of 2-mercaptopyridine on standing for 3 days. The ultraviolet spectrum had λ_{max} 268 mμ, (ε 5520). Except for the slight displacement of the maximum, the spectrum was very similar to that of O,O-diphenyl O-2-pyridinyl phosphorothioate.

O,O-Diphenyl 0-2-Pyrazinyl Phosphorothioate.—O,O-Diphenyl phosphorochloridothioate (3.063 g., 10.78 mmoles) and sodium pyrazinolate (1.30 g., 10.96 mmoles) were dissolved in 250 ml. of *t*-butyl alcohol and stirred for 6 hours at 58°. The solvent was evaporated to give 3.45 g. of yellow oil. This was chromatographed on 25 g. of Woelm acid-washed alumina, yielding 2.58 g. (7.53 mmoles, 70%) of colorless oil. The ultraviolet spectrum had λ_{max} 270 mμ (ε 6040).

Anal. Calcd. for $C_{16}H_{13}O_3PSN$: C, 55.85; H, 3.81; P, 9.00; N, 8.14; S, 9.31. Found: C, 56.04; H, 3.85; P, 9.13; N, 8.16; S, 9.41.

O-(*p*-Acetylphenyl) O,O-Diphenyl phosphorothioate.—Ten ml. of 0.450 *N* potassium *tert*-butoxide solution were added to a solution of *p*-hydroxacetophenone (0.6145 g., 4.52 mmoles) in 100 ml. of *t*-butyl alcohol–dioxane, and a solution of O,O-diphenyl phosphorochloridothioate (1.279 g., 4.50 mmoles) in 100 ml. of solvent was added. The solution was kept at 58° for 2 hours, and the solvent then evaporated. The residue was taken up in methylene chloride, filtered and evaporated to give 1.70 g. of light yellow oil. This was chromatographed on 10 g. of Woelm acid-washed alumina to give 1.52 g. (3.70 mmoles, 85%) of colorless oil.

Anal. Calcd. for $C_{20}H_{17}PSO_4$: C, 62.50; H, 4.55; P, 8.06; S, 8.34. Found: C, 62.22, 62.76; H, 4.96, 5.00; P, 8.14; S, 8.18.

O,O-Diphenyl S-*n*-Pentyl Phosphorodithioate.—To 1-pentanethiol (0.603 g., 5.80 mmoles) in 50 ml. of *t*- butyl alcohol was added 25 ml. (4.97 mmoles) of 0.198 *N* potassium *t*-butoxide solution. O,O-Diphenyl phosphorochloridothioate (1.401 g., 4.93 mmoles) in 200 ml. of *t*-butyl alcohol

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[46] J. R. Thirtle, *J. Am. Chem. Soc.*, **68**, 342 (1946).

[47] W. Koenigs and G. Koerner, *Ber.*, **16**, 2152 (1883).

[48] M. E. Hultquist, U. S. Patent 2,805,223 (1957).

[49] J. Weijlard, M. Tishler and A. E. Erickson, *J. Am. Chem. Soc.*, **67**, 802 (1945).

[50] We wish to thank Dr. C. A. Streuli for performing this determination.

was added, the mixture was kept at 58° for 2 hours, evaporated, extracted with methylene chloride, washed with water and dried over $MgSO_4$. Evaporation left 1.62 g. of yellow oil, which was distilled at 8μ pressure to give 1.57 g. (4.46 mmoles, 90%) of colorless liquid, b.p. 110–115°, n^{25}_D 1.5698.

Anal. Calcd. for $PS_2O_2C_{17}H_{21}$: C, 57.85; H, 5.97: P, 8.76; S,18. 14. Found: C, 57.88; H, 5.96; P, 8.61; S, 18.53, 17.83.

O,O,S-Triphenyl Phosphorodithioate.—To 0.575 g. (5.22 meq.) of freshly distilled thiophenol in 50 ml. of *t*-butanol was added 20 ml. (4.80 meq.) of 0.240 *N* potassium *t*-butoxide soln. O,O-Diphenyl phosphorochloridothioate (1.21 g.. 4.25 meq.) was added, and the mixture shaken and kept at 60° for 6 hours. The solvent was evaporated, the residue extracted with methylene chloride, and the methylene chloride extract washed with water and dried over $MgSO_4$. Evaporation of the solvent left 1.57 g. of yellow oil, which was evaporatively distilled at 5μ pressure to give 1.28 g. (3.58 meq., 84%) of colorless liquid, n^{25}_D 1.6340, which solidified on standing to give white prisms, m.p. 66–70°. Recrystallization from methanol raised the m.p. to 71.5–73.0°.

Anal. Calcd. for $PS_2O_2C_{18}H_{15}$: C, 60.40; H, 4.20: P, 8.66; S, 17.89. Found: C, 60.38, 60.51; H, 3.96, 4.11; P, 8.56, 8.79; S, 18.00, 18.20.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE, NEW YORK, NEW YORK]

Tritiation of Oxytocin by the Wilzbach Method and the Synthesis of Oxytocin from Tritium-labelled Leucine[1]

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The preparation of tritium-labelled oxytocin is described. The first approach leading to *randomly* labelled oxytocin involved the tritiation of a sample of synthetic oxytocin by the gas exposure technique of Wilzbach. After purification of the tritiated material by countercurrent distribution, a product was obtained with a specific radioactivity of 12 microCuries per mg. and a potency of 330 units per mg. All of the component amino acids were found to contain radioactivity as would be expected. *Specifically* labelled oxytocin was prepared by a total synthesis in which tritium-labelled L-leucine, obtained by tritiation of N-triphenylmethyldehydro-L-leucine, served as the starting material. The synthesis of the desired nonapeptide was accomplished *via* the tetrapeptide S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide which was coupled by means of dicyclohexylcarbodiimide with the pentapeptide S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparagine. Treatment of the product with sodium in liquid ammonia and subsequent aeration of the reduced nonapeptide yielded biologically active material which was purified by countercurrent distribution. A hormone preparation was obtained with a specific radioactivity of 130 microCuries per mg. and with a biological potency equal to that of our best preparations of nonradioactive oxytocin (approximately 500 units per mg.).

Early in our studies of oxytocin it was realized that an isotopically labelled preparation was desirable for certain chemical and biological investigations. Our first approach almost three years ago to a radioactive preparation was through random labelling of the hormone by the tritium gas exposure method of Wilzbach.[3,4] In this paper we wish to report some results with the application of this method to oxytocin and to present the synthesis of specifically labelled oxytocin.

For the preparation of randomly labelled oxytocin a sample of synthetic oxytocin, possessing approximately 450 U.S.P. units per milligram, was exposed to tritium for seven days. The resulting material, after having been dissolved in dilute acetic acid and lyophilized, was assayed again and found to possess about 330 units per milligram. The preparation was then repeatedly lyophilized from dilute acetic acid in an attempt to remove exchangeable tritium. Through electrophoresis on paper, it was found that only a small fraction of the radioactivity in the sample was bound to the hormone.

The bulk of the lyophilized material was subjected to two consecutive countercurrent distributions in the system butanol–ethanol–0.05% acetic acid (4:1:5). After 425 transfers the fraction present in the tubes representing the central portion of the peak of biological activity was removed and lyophilized. The material was then subjected to a further 100 transfer countercurrent distribution. The distribution curves for the biological activity, radioactivity and the color developed by the Folin–Lowry reaction agreed with the calculated distribution curve. From the active peak of the second countercurrent distribution, after concentration and lyophilization, a product was obtained in about 40% over-all yield having a specific radioactivity of 12.4 microCuries per mg. and a biological potency of 330 units per mg. After hydrolysis and paper chromatography according to the method of Levy and Chung[5] all of the amino acids were found to contain radioactivity. Glycine and tyrosine appeared to be somewhat more highly radioactive than the others.

The availability of *specifically* labelled oxytocin would obviously be of particular advantage in certain types of chemical, enzymic and metabolic studies of the hormone. The preparation of spe-

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[2] (a) Smith-Mundt-Fulbright Fellow under the auspices of the Institute of International Education. (b) Deceased February 3, 1959.

[3] K. E. Wilzbach, *J. Am. Chem. Soc.*, **79**, 1013 (1957).

[4] The application of the Wilzbach method to the random labelling of another posterior pituitary hormone, lysine-vasopressin, has been reported in a preliminary communication by Fong and co-workers (C. T. O. Fong, I. L. Schwartz, E. A. Popenoe, L. Silver, M. A. Schoessler, *J. Am. Chem. Soc.*, **81**, 2592 (1959)). After exposure of lysine-vasopressin to tritium, they obtained after purification an extremely low yield (0.1%) of tritiated lysine-vasopressin with a specific activity of approximately 300 micro Curies per milligram. By use of a modified Wilzbach procedure employing an electric discharge for tritium labelling, a preparation of arginine-vasopressin with high specific radioactivity and full biological activity has been more recently reported (C. T. O. Fong, L. Silver, D. R. Christman and I. L. Schwartz, *Proc. Natl. Acad. Sci. U. S.*, **46**, 1273 (1960)).

[5] A. L. Levy and D. Chung, *Anal. Chem.*, **25**, 396 (1953).