[CONTRIBUTION FROM MEDICAL LABORATORIES OF THE U. S. ARMY CHEMICAL CORPS]

The Kinetics of the Reaction of Isopropyl Methylphosphonofluoridate (Sarin) with Benzohydroxamic Acid[1]

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The reaction between Sarin and benzohydroxamic acid in near neutral aqueous solution has been examined kinetically. A previously postulated reaction series involving the Lossen rearrangement has been supported on a stoichiometric basis and the first reaction in the series, the phosphorylation of benzohydroxamate ion has been shown to be rate determining.

Certain of the very toxic organophosphorus compounds such as the newer insecticides and also the so-called nerve gases act by virtue of their ability to inactivate the enzyme cholinesterase, inactivation being caused by direct phosphorylation of the enzyme.[3] In general these compounds are relatively resistant to aqueous hydrolysis. Thus, Sarin, isopropyl methylphosphonofluoridate, has a half-life in aqueous solution at *p*H 7.6 of approximately 300 minutes. Several groups of compounds have been reported which react rapidly with Sarin under physiological conditions of *p*H and temperature. These include catechols,[4] copper chelates[5] and hydroxamic acids.[6] Certain of the hydroxamic acids have the ability to reactivate cholinesterase which has been inactivated by Sarin[7] and also to act internally as prophylactics.[8] Thus, mice have been protected to a limited extent against Sarin poisoning.

It was the purpose of this study to examine the reaction between Sarin and benzohydroxamic acid in near neutral solution to provide a basis for the comparison of the reactivities of a series of hydroxamic acids with the ultimate objective of gaining insight into the nature of both the direct reaction with Sarin and with the inactivated enzyme.[9]

Based upon the well known acylation of hydroxamic acids[10] and the isolation of O-(N-phenylcarbamyl)-benzohydroxamic acid (II) in yields of 75% or greater under preparative conditions,[6,11] it has been postulated that reaction with phosphoryl halides, sulfonyl halides or acyl halides in alkaline aqueous solution[6,11] or inert solvent containing base[12] proceeds by initial acylation of the hydroxamate ion followed by a Lossen rearrangement

$$(C_6H_5CONHO)^- + PnX \xrightarrow{k_1} C_6H_5CONHOPn + X^- \quad (1)$$

$$C_6H_5CONHOPn + OH^- \xrightarrow{k_{1a}} \underset{\text{I}}{(C_6H_5CONOPn)^-} + H_2O \quad (1a)$$

$$(C_6H_5CONOPn)^- \xrightarrow{k_2} C_6H_5NCO + PnO^- \quad (2)$$

$$C_6H_5NCO + (C_6H_5CONHO)^- \xrightarrow{k_3} \underset{\text{II}}{(C_6H_5CONOCONHC_6H_5)^-} \quad (3)$$

$$2(C_6H_5CONHO)^- + PnX + OH^- \longrightarrow PnO^- + X^- + (C_6H_5CONOCONHC_6H_5)^- \quad (4)$$

Where, Pn = (*i*-PrO)(CH_3)(P=O)-, (*i*-PrO)$_2$(P=O)-, RSO_2-, R(C=O)-; X = Cl, F.

The reaction of major interest, step 1, could be followed kinetically by determination either of the rates of production of phosphonylated hydroxamic acid or fluoride or of the disappearance of Sarin (PnX) or hydroxamate ion. The phosphonylated hydroxamic acid is extremely unstable and not available for analytical study.[13] Determination of fluoride or Sarin[14] at the low concentrations employed are difficult, time-consuming procedures and of only moderate accuracy. Hydroxamate was maintained in large excess and therefore its concentration varied by a relatively small factor during the course of reaction. Hence, inasmuch as there was produced a considerable amount of acid in the over-all reaction and there was available a Beckman Autotitrator which can be set to deliver alkali to maintain constant *p*H, the rate of acid production was measured as a means of studying reaction kinetics. However, in order to employ the rate of acid production as a means of measuring the rate of step 1, it was necessary to establish the validity of the postulated reaction sequence and to demonstrate that step 1 was the limiting step in the sequence. This was accomplished by a confirmatory determination of the rate of fluoride production, independent study of step 3, and also by determination of the stoichiometry of acid production and of hydroxamic acid consumption.

Although the Lossen rearrangement of acylated hydroxamic acids has been studied in great detail by

(1) Presented before Maryland Section, Amer. Chem. Soc., Nov. 18, 1955. Permission to publish the information contained in this article has been granted by the U. S. Army Chemical Corps.

(2) To whom inquiries should be forwarded.

(3) H. Michel and S. Krop, *J. Biol. Chem.*, **190**, 119 (1951).

(4) B. J. Jandorf, T. Wagner-Jauregg, J. J. O'Neill and M. A. Stolberg, THIS JOURNAL, **73**, 5202 (1951).

(5) T. Wagner-Jauregg, B. E. Hackley, Jr., T. A. Lies, O. O. Owens and R. Proper, *ibid.*, **77**, 922 (1955).

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

(7) (a) B. J. Jandorf, E. A. Crowell and A. P. Levin, *Federation Proc.*, **14**, 231 (1955). (b) This property was first observed with diisopropyl phosphorofluoridate (DFP)-inactivated cholinesterase. I. B. Wilson and E. K. Meislich, THIS JOURNAL, **75**, 4628 (1953).

(8) M. A. Epstein and G. Freeman, Chem. Corps Med. Labs., Res. Rpt. No. 346 (1955).

(9) Comparison in terms of the Hammett and Brönsted relationships will be reported at a later date.

(10) H. L. Yale, *Chem. Revs.*, **33**, 209 (1943).

(11) M. A. Stolberg, R. C. Tweit, G. M. Steinberg and T. Wagner-Jauregg, THIS JOURNAL, **77**, 765 (1955).

(12) C. D. Hurd and L. Bauer, *ibid.*, **76**, 2791 (1954).

(13) It is noteworthy that the instability of phosphonylated (or phosphorylated) hydroxamic acids is in marked contrast to the stability of the acylated hydroxamic acids. Thus Hauser, *et al.*, observed a consistent inverse relationship between rate of rearrangement of O-acylated hydroxamic acids and the *p*Ka of the acylating acid: C. R. Hauser and W. B. Renfrow, *ibid.*, **59**, 2308 (1937); R. D. Bright and C. R. Hauser, *ibid.*, **61**, 618 (1939). The phosphonylated (or phosphorylated) compounds do not fit into this correlation, decomposing far more rapidly than would be predicted from the acidity of the corresponding acid.

(14) J. Epstein, *Anal. Chem.*, to be published.

Hauser and co-workers,[13] this study provides the first example of an examination of the "complete" reaction.[15] Sarin is particularly suitable for such a study since it is completely water soluble and there is a range of conditions under which it is very reactive toward hydroxamic acids while being relatively resistant to aqueous hydrolysis.

Experimental

A. Reagents.—Benzohydroxamic acid[16] was prepared by the action of hydroxylamine on ethyl benzoate. The acid was crystallized from water and recrystallized repeatedly from a mixture of methylene chloride and ether until a constant melting point of 124–125° was obtained. All acid samples were carefully dried at 80° under vacuum. Commercial haemotoxylin (to be used for the fluoride determination) was repeatedly crystallized from dilute aqueous bisulfite solution until colorless. All inorganic salts were of C.P. grade. Sarin (99% pure) was obtained from the Chemical and Radiological Laboratories, ACC. It was stored in a desiccator over sulfuric acid. Purity was checked regularly by determination of free acid content. All reaction mixtures were prepared from freshly boiled (CO_2-free) distilled water.

B. Reaction Rates. 1. Acid Production.—The reaction rates were studied by measuring the rate of addition of standard alkali, taken up by the reaction mixture when maintained at a fixed *p*H by a Beckman Autotitrator. When properly handled this machine was quite satisfactory for the purpose; however several pitfalls must be avoided when one studies relatively fast reactions. Although the machine maintained the *p*H indicated, in many instances this value was not exactly the same as that measured independently with a Beckman Model G *p*H meter, deviations of as great as 0.05 *p*H unit having been noted. It was observed that reaction rates obtained from runs with half-times of less than 7 minutes although "normal" in appearance were difficult to reproduce and led to incorrect results. Erratic behavior was traced to (i) the positioning of the buret tip with respect to the glass electrode and stirrer, (ii) the rate of addition of base as controlled by the anticipation setting,[17] (iii) the efficiency of the stirring action and (iv) control of *p*H. All of these difficulties were surmounted in this work by using an anticipation setting of 0.75, the large stirrer, an auxiliary *p*H meter[18] and by positioning the buret tip slightly forward of and above the circular stirrer blade and directed toward the bottom of the glass electrode *ca.* $^1/_{16}$" distant. Under these conditions we encountered no difficulty in reproducing our rates to within 5–7%, at half-times of 7 minutes and greater.

In a typical rate determination a known quantity of benzohydroxamic acid was dissolved in 0.1 *N* potassium nitrate solution contained in a jacketted beaker through which water of 30.5 ± 0.2° was circulated from a thermostatically controlled bath. The acidity of the solution was adjusted to the desired value while its temperature reached that of the bath. A stock solution containing *ca.* 0.1 ml. of Sarin (99% pure) (*Caution!*),[19] in 250 ml. of water was prepared daily. In the *p*H range of 4 to 6, which the solution assumed, Sarin is sufficiently resistant to hydrolysis so that the stock solution could be kept for several hours at room temperature or for a full day if stored in a refrigerator. An appropriate aliquot was added to the benzohydroxamic acid solution and the quantity of a standard 0.01 *N* sodium hydroxide delivered by the Autotitrator *vs.* time was recorded.

The concentration of the Sarin stock solution was determined in duplicate by treatment of aliquots with excess standard 0.1 *N* sodium hydroxide for 24 hours, and the excess alkali determined by titration with standard acid to a neutral red end-point.

$$(i\text{-PrO})(CH_3)POF + 2OH^- \longrightarrow (i\text{-PrO})(CH_3)POO^- + F^- + H_2O$$

Correction was made for the small amount of free acid contained in the Sarin.

2. Fluoride.—Determination of fluoride was made by the colorimetric method of Hunter and co-workers[20] which is based on the bleaching of a standard blue aluminum–haematoxylin solution. To determine the effect of benzohydroxamic acid on the color absorption of the indicator a series of solutions containing 25 ml. of the standard aluminum haematoxylin reagent and 10-ml. aliquots of benzohydroxamic acid solutions of varying concentrations, both with and without added fluoride, were diluted to 50 ml. and allowed to stand at room temperature for 80 minutes.[21] The absorption of the resulting solution was determined with a Klett-Summerson colorimeter equipped with a #59 filter. These results are recorded in Fig. 1. The effect on color intensity was negligible at benzohydroxamic acid concentrations lower than 10^{-3} *M*. In the presence of 10^{-3} *M* benzohydroxamic acid (also in its absence) a plot of color intensity *vs.* concentration of fluoride (up to 10 μg. F^-/50 ml. test solution) gave a perfectly straight line, in accordance with Beer's law.

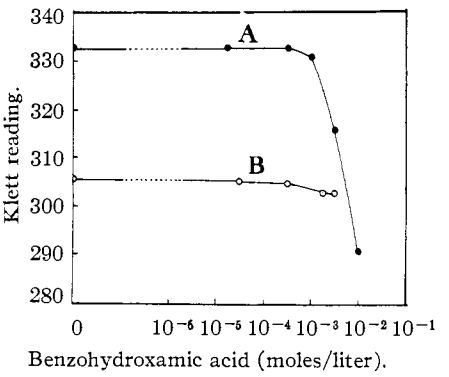


Fig. 1.—Effect of concentration of benzohydroxamic acid on the bleaching of the aluminum–haematoxylin reagent: A, ●–● reagent without added fluoride ion; B, O–O reagent in the presence of 10 μg. of fluoride ion in 50 ml. of test solution.

A reaction mixture was prepared as previously described and 10-ml. aliquots were withdrawn at appropriate times for the determination of fluoride content. The consumption of alkali was noted simultaneously. In Table I the raw data from a single run are recorded, corrections having been made for the aliquots withdrawn. The final colorimetric end-point of the reaction could not conveniently be determined. Hence, the first-order rate constant for production of fluoride was ascertained by the statistical procedure of Schultz,[22] which does not require a knowledge of the end-point. This method is similar in principle to the Guggenheim procedure[23] but has the advantage of greater accuracy. Also, it provides a measure of the internal consistency of the data. Its application is particularly useful when rate data can be obtained for only a limited part of the reaction.

C. Determination of pK_a of Benzohydroxamic Acid.—A solution of benzohydroxamic acid in 0.1 *N* KNO_3 was half neutralized by addition of the calculated quantity of 0.1 *N* sodium hydroxide, and serially diluted by addition of 0.1 *N* KNO_3. The *p*H values of the resultant solutions were determined at once with a Beckman Model G *p*H meter. The values obtained as apparent *pK*'s of the acid were plotted against the concentration as shown in Fig. 2. At low con-

(15) The importance of a study of the "complete" reaction has been pointed out with respect to the related Beckmann rearrangement. D. E. Pearson and F. Ball, *J. Org. Chem.*, **14**, 118 (1949).

(16) A. H. Blatt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 67.

(17) That setting was considered optimum which produced the maximum rate of addition of standard alkali (0.01 *N*) to a dilute solution of acetic acid in 0.1 *N* potassium nitrate, without over-titrating.

(18) Some of the data were obtained before the importance of the auxiliary *p*H meter was realized. Hence the results contain greater scatter than the system is capable of attaining.

(19) Sarin is *extremely* toxic in both the liquid and vapor state and must be manipulated with great caution in a hood of large capacity.

(20) G. J. Hunter, B. J. MacNulty and E. A. Terry, *Anal. Chim. Acta*, **8**, 351 (1952).

(21) The presence of benzohydroxamic acid seemed to slow the reaction between aluminum–haematoxylin and fluoride. With an 80 minute reaction time we found that complete reaction was assured.

(22) H. Schultz, *J. Am. Stat. Soc.*, **28**, 139 (1933).

(23) A. A. Frost and R. G. Pearson, "Kinetics and Mechanism," John Wiley and Sons, Inc., New York, N. Y., 1953, p. 48.

TABLE I

RATE OF HYDROLYSIS OF SARIN BY BENZOHYDROXAMIC ACID[a]

Acid production		Fluoride production	
Time, min.	Alkali delivered,[b] ml.	Time, min.	R[c]
0	0.00	0	0
2	.20	5	1.3
5	.45	10	3.0
7.5	.65	15	5.1
12	.97	20	6.1
15	1.16	25	7.2
17.5	1.34	35	8.9
20	1.50	45	11.2
23	1.70	55	13.5
25	1.89	75	15.3
30	2.22	95	17.8
45	3.04	115	20
65	3.90		
∞	6.16[d]		
$k_{obs} \times 10^4 = 2.36$ sec.$^{-1}$		$k_{obs} \times 10^4 = 2.22$ sec.$^{-1}$	

[a] Run 17, Table II. [b] 0.0153 N NaOH, volume corrected for aliquots of reaction mixture withdrawn for fluoride analysis. [c] Difference between reading on Klett at zero time and time of sampling. [d] Theoretical value, based on 2.78 moles acid/mole Sarin.

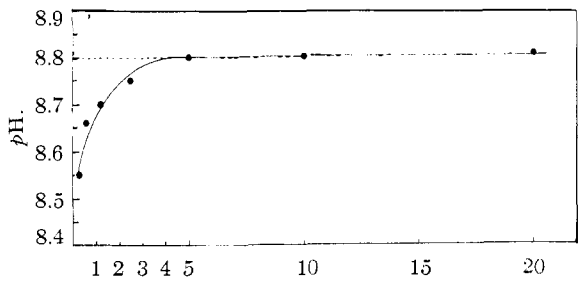


Fig. 2.—Variation of the apparent pK_a of benzohydroxamic acid with concentration at constant ionic strength. Circles represent pH of half-neutralized solutions of acid in 0.1 N potassium nitrate.

centrations of benzohydroxamic acid considerably lower pK_a values are observed than at higher concentrations. This effect is due in part to hydrolysis of the conjugate base[24] and also to the absorption of CO_2 which effect would be more pronounced in the more dilute solutions.[25] A pK_a value of 8.80 was taken for benzohydroxamic acid in 0.1 N potassium nitrate solution.[26]

Results

Stoichiometry of Reaction.—As indicated in Fig. 3, which represents a typical plot of acid production when the reaction is carried to completion, there is produced 2.7 moles of acid per mole of Sarin. The "blank" value for acid production, in 0.1 M potassium nitrate solution of benzohydroxamic acid, 6.13×10^{-4} M, was 2.2×10^{-5} mole of acid produced per hour at pH 7.6. This was due only in part to the hydrolysis of benzohydroxamic acid which was found to occur at pH 7.6 to the extent of

(24) For a thorough discussion of this phenomenon see T. B. Smith "Analytical Processes," 2nd Ed., Longmans, Green and Co., Inc., New York, N. Y., 1940.

(25) Similar deviations at low concentrations were observed for substituted benzohydroxamic acids and for phenol.

(26) W. M. Wise and W. W. Brandt reported the pK_a of benzohydroxamic acid as 8.88, THIS JOURNAL, **77**, 1058 (1955).

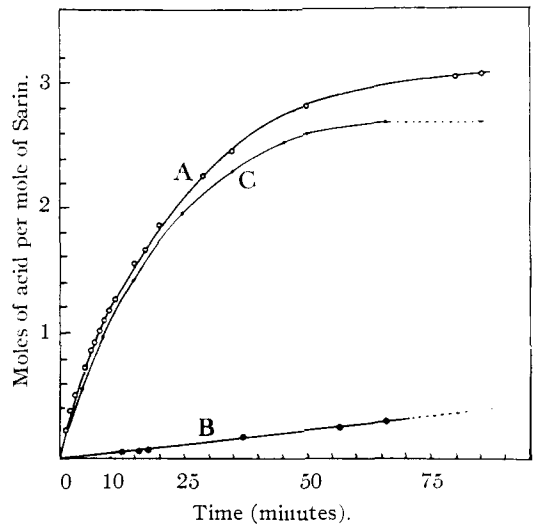


Fig. 3.—Curve A, O–O, represents the acid production *vs.* time relationship in a typical reaction between Sarin (6.34×10^{-5} M) and benzohydroxamic acid (6.30×10^{-4} M) at pH of 7.6, t of 30.5° in 0.1 N potassium nitrate solution. Curve B, ●–● "blank" rate of acid production. Curve C, ●–●, corrected rate of acid production.

2.6% per hour.[27] The remaining acid was probably due to absorption of carbon dioxide from the atmosphere.

Benzohydroxamic acid was destroyed during the course of reaction. When reaction between 18.5 μmoles of Sarin and 163 μmoles of benzohydroxamic acid in 275 ml. of 0.1 M potassium nitrate solution, at pH 7.6, was permitted to go to completion, it was found that 1.67[28] ± 0.16 mole of the hydroxamic acid was destroyed per mole of Sarin[27] (corrected for the spontaneous hydrolysis of hydroxamic acid).

Kinetics of Reaction. A. Acid Production.—In all of the runs, the concentration of benzohydroxamic acid was maintained in large excess over that of the Sarin. The raw rate data yielded excellent first-order lines, up to 80–85% of total reaction, assuming 2.78 moles of acid produced at the end-point when plotted by conventional methods. Guggenheim plots,[23] which are not dependent on a knowledge of the end-point value yielded substantially identical rate constants. First-order plots calculated for a typical run by the two methods are given in Fig. 4. The value of the first-order rate constant in each case was determined from the slope of the straight line, as per equations 7 and 8, by a least squares calculation.

$$\text{Conventional: } \log \frac{V_\infty}{V_\infty - V_t} = \frac{k_t}{2.303} \qquad (7)$$

$$\text{Guggenheim: } \log \frac{V_{15}}{V_{t+15} - V_t} = \frac{k_t}{2.303} \qquad (8)$$

Where V_t, V_{15}, V_{t+15} and V_∞ are volume of standard alkali delivered to maintain constant pH at times = t, 15, t + 15 minutes and ∞ (end-point value), respectively.

(27) Determined colorimetrically by the disappearance of benzohydroxamic acid determined as the ferric chloride complex; S. Hestrin, *J. Biol. Chem.*, **180**, 249 (1949).

(28) Average of three runs.

The results obtained by variation of the concentrations of reactants and the *p*H of the reaction medium (Table II) indicates a first-order dependence upon Sarin and benzohydroxamate ion. Over a fairly wide concentration range it was possible to show that the reaction yielded reasonably constant bimolecular rate constants (Table II). The reaction rate constants were substantially unaffected by added salts (Table II).

TABLE II

KINETICS OF REACTION OF SARIN WITH BENZOHYDROXAMIC ACID[a]

Run	*p*H	QHA,[b] *M* × 10⁴	(A⁻),[c] *M* × 10⁴	Sarin, *M* × 10⁴	k_{obs} × 10⁴, sec.⁻¹ Obsd.	Cor.[d]	k_0, l./mole sec.
1	7.6	13.0	0.781	0.59	17.8	15.8	20.2
2	7.6	12.7	.763	1.16	14.9	13.8	18.1
3	7.6	13.0	.781	0.59	18.8	16.6	21.2
4	7.6	12.7	.763	1.16	16.1	15.0	19.7
5	7.6	13.0	.781	1.16	15.1	14.0	17.9
6	7.6	12.7	.763	1.21	15.8	14.7	19.3
8	7.6	13.0	.781	0.61	16.9	14.8	19.0
9	7.6	13.0	.781	0.61	17.3	15.2	19.5
10	7.6	12.7	.763	1.21	16.1	15.0	19.7
11	7.4	6.39	.246	0.67	5.97	5.23	21.2
12	7.6	6.39	.384	.67	8.50	7.61	19.8
13	7.4	6.39	.246	.67	5.80	5.02	20.4
14	7.6	6.39	.384	.67	8.20	7.41	19.3
15	7.4	6.59	.254	.70	6.03	5.25	20.6[e]
16	7.4	6.59	.254	.70	6.06	5.29	20.8[e]
17	6.9	10.1	.124	.115	2.58	2.36	19.0
							20.9 ± 1.2[e]

[a] Unless specified all reactions were run in 0.1 *N* KNO_3. [b] QHA = moles of benzohydroxamic acid added to 1 liter of reaction mixture = (HA) + (A⁻). [c] Molar concentration of benzohydroxamate ion. [d] Corrected for hydrolysis of benzohydroxamic acid. [e] Reactions 15 and 16 run in 0.05 and 0.25 *N* KNO_3, respectively. These values have not been included in calculation of the average.

B. Fluoride Production.—Early attempts, using the thorium-lake method for the analysis for fluoride ion,[29] at these extremely low concentrations of reactants proved unsuccessful.[30] Similarly the zirconium–alizarin method[31] proved to be too tedious and erratic. However, with the reservations discussed in the Experimental section, the aluminum–haematoxylin method[20] was found to be sufficiently sensitive and accurate to be applicable in our system. However, it was far more time consuming than was the determination of rate of acid production. Hence, after ascertaining that fluoride and acid liberation occurred at substantially identical rates in a single run, all further studies were made by acid determination.

The observed first-order rate constants for acid and fluoride production (Table I and Table II, run 17) were shown to be of no significant difference by Students' "*t*" test ($t = 0.0247$, $n = 22$, $p > 0.9$). Their 19/20 (90%) confidence limits were calculated as $2.27 - 2.50 \times 10^{-4}$ and $1.88 - 2.55 \times 10^{-4}$ sec.$^{-1}$, respectively.

Discussion

In the reaction between benzohydroxamate ion and Sarin, according to equation 4, it can be seen that there are converted two moles of acid which

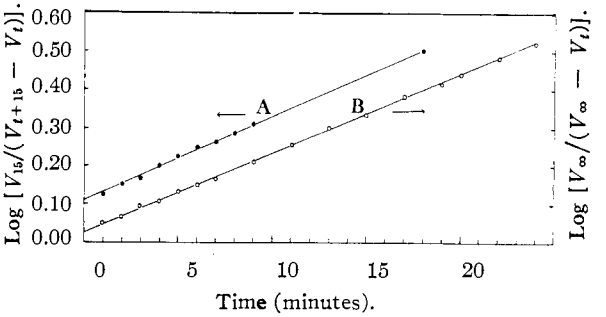


Fig. 4.—Comparison of the Guggenheim method with the conventional first-order plot for a typical rate run: curve A, Guggenheim plot; curve B, conventional plot.

are incompletely ionized under our reaction conditions 2($C_6H_5CONHOH$) and one neutral molecule (Sarin) to two moles of completely ionized acid (isopropyl methylphosphonic acid and hydrofluoric acid) and one mole of incompletely ionized acid (II). Hence, the quantity of acid titrated during reaction at constant *p*H is a function of the *p*H of the reaction medium and is related to the fraction which is neutralized of each of the weakly acidic reagents and products.

According to equation 4 the total quantity of acid per mole of Sarin produced, Q_{acid}, at constant *p*H is defined by

$$Q_{acid} = 2 + \left[\frac{(A^-)}{(HA) + (A^-)}\right]_{II} - 2\left[\frac{(A^-)}{(HA) + (A^-)}\right] \; C_6H_5CONHOH \quad (5)$$

From

$$K_a = \left[\frac{(A^-)(H^+)}{(HA)}\right]$$

we can convert equation 5 to

$$Q_{acid} = 2 + \left[\frac{K_A}{(H^+) + K_A}\right]_{II} - 2\left[\frac{K_A}{(H^+) + K_A}\right] \; C_6H_5CONHOH \quad (6)$$

The pK_a values of II and benzohydroxamic acid, in 0.1 *M* potassium nitrate solution, are 6.4[11] and 8.8, respectively. Hence, the quantities of acid which would be produced at *p*H 7.6, 7.4 and 6.9 are 2.82, 2.83 and 2.73 moles, respectively, per mole of Sarin.

Correction for the side reaction of Sarin with aqueous solvent which yields two moles of completely ionized acid with a first-order rate constant[32] of *ca.* 4×10^{-5} sec.$^{-1}$ at *p*H 7.6 would reduce the quantity of acid expected at *p*H 7.6 from 2.82 to 2.78 moles. If phenyl isocyanate were to react completely with water instead of hydroxamate ion (as indicated in equation 3) it would yield 2.75 moles of acid per mole of Sarin[33] instead of 2.78 moles for the over-all reaction. Hence, this side reaction which accounts for 25% of the reaction of phenyl isocyanate (see below) will not significantly alter the quantity of acid produced. The value experimentally observed of 2.7 moles of acid produced per mole of Sarin may be considered to be in

(29) "Anal. Chem. of the Manhattan Project," McGraw–Hill Book Co., New York, N. Y., 1945, p. 235.

(30) S. Seltzer, unpublished observations.

(31) G. C. Gainer, *Can. J. Res.*, **23B**, 275 (1945).

(32) Calculated from the experimentally determined half-time of 275–300 minutes.

(33) At *p*H 7.6, 18.5 μmoles of phenyl isocyanate in 250 ml. of water hydrolyzed to yield 15.1 ± 0.5 μmole of acid (81 mole %).

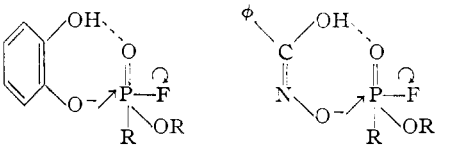
good agreement with the theoretical value of 2.78 moles.

According to equation 4, two moles of hydroxamic acid should be consumed per mole of Sarin. The competing side reaction between aqueous solvent and Sarin will reduce this value by an amount which will depend on both *p*H and concentration of hydroxamic acid. Under the conditions of this study the side reaction between Sarin and solvent occurs to only a minor extent. On the other hand, the side reaction between phenyl isocyanate and aqueous solvent (competitive with reaction 3) is appreciable. In the absence of Sarin, the reaction of phenyl isocyanate (18.5 μmoles) with an excess of benzohydroxamic acid (163 μmoles) in 275 ml. of 0.1 *M* potassium nitrate at 7.6 consumed only 13.2 ± 0.2 μmole of hydroxamic acid, 0.72 mole of hydroxamic acid per mole of phenyl isocyanate. Hence, according to the postulated reaction series, the reaction of Sarin and benzohydroxamic acid under the same conditions, should consume 1.72 moles of hydroxamic acid per mole of Sarin, which is in close agreement with the observed value of 1.67 moles.

It was considered highly unlikely that reaction 3, the step which involves the very reactive phenyl isocyanate molecule would contribute to the kinetic picture. Under the conditions of this study, phenyl isocyanate was observed to react with water or benzohydroxamic acid at an immeasureably rapid rate.[34] Also, the rate of acid production from the aqueous hydrolysis of benzohydroxamic acid under our reaction conditions is zero order (Fig. 3B), and the pseudo-first-order side reaction between Sarin and aqueous solvent is so small in magnitude that it can be safely ignored.

Thus the experimental data clearly support the postulated reaction series and indicate that step 1, the phosphonylation reaction, is rate determining. Hence, it is established that the rate of production of acid can be employed satisfactorily for comparison of the relative reactivity of Sarin with a series of hydroxamic acids.[35]

It is interesting to note that the rate of reaction of Sarin with benzohydroxamate ion is very large as compared with the rate observed in reaction with anions of other weak acids. This suggests the operation of powerful stereoelectronic effects in the former reaction and is perhaps analogous in process to that proposed for the rapid reaction of catechol (as compared to phenol) with DFP[36] (*cf.* structures A and B) and Sarin.[37]



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(34) At *p*H 7.6, a half-time of 25 sec. was observed with the Autotitrator when 10 δ of phenyl isocyanate in 10 ml. of acetone was added to 300 ml. of 0.1 *N* KNO_3 solution. However, this represents the limiting rate of operation of the Autotitrator rather than the actual rate of reaction.

(35) Barring an inversion in the relative rates of steps 1 and 2, which would be evidenced by a change in the over-all kinetic picture.

(36) B. J. Jandorf, T. Wagner-Jauregg, J. J. O'Neill and M. A. Stolberg, THIS JOURNAL, **73**, 5202 (1951).

(37) J. Epstein, D. H. Rosenblatt and M. M. Demek, *ibid.*, **78**, 341 (1956).

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Effects of Hydrostatic Pressure upon Sedimentation in the Ultracentrifuge

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Effects of hydrostatic pressure upon the sedimentation process in the ultracentrifuge are investigated mathematically on the basis of the Lamm sedimentation equation without the diffusion term. In accordance with a recent article by Oth and Desreux, the sedimentation coefficient, *s*, is assumed to vary linearly with pressure. First the case in which *s* is dependent only on pressure is considered in detail, and the concentration gradient curve for an illustrative case is computed using the analytical solution obtained, in order to show the characteristic feature of pressure-dependent sedimentation. Methods for correcting measured sedimentation coefficient values to those at a pressure of one atmosphere are shown. Consideration is then extended to the case of *s* dependent both on pressure and concentration. Because of the complexity of the general solution obtained, its numerical calculation is not attempted. On the basis of these results Oth and Desreux's treatment of a similar problem is criticized.

At speeds of rotation usually employed in velocity sedimentation measurements, a large pressure difference, which may amount to several hundred atmospheres, is produced between the meniscus and the bottom of the cell. Since the viscosity and density of the solvent and the specific volume of the solute may vary with pressure, it is expected that sedimentation processes in such a field of high pressure gradient should differ more or less from those in a field of uniform pressure. In order to attain a high precision in the evaluation of the molecular weight of a given substance by means of sedimentation measurements, a correction must be made to sedimentation data with respect to this pressure effect, along with, among other things, the elimination of the concentration effect by means of extrapolation to infinite dilution.

This problem was first considered by Mosimann

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