The Reaction of Phosphorus-Containing Enzyme Inhibitors with Some Hydroxylamine Derivatives

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A previous publication from this laboratory[1] described the rapid reactions of Sarin (isopropyl methylphosphonofluoridate) or DFP (diisopropyl phosphorofluoridate) with several hydroxamic acids. This paper is a continuation of the study of the reaction of some phosphorus-containing enzyme inactivators with hydroxylamine derivatives.

It has been demonstrated that benzohydroxamic phenylcarbamic anhydride ($C_6H_5CONH—O—CONHC_6H_5$) was the predominant product *via* a Lossen rearrangement from the rapid reaction between benzohydroxamic acid and either Sarin or DFP.[1] Benzenesulfonyl chloride and other substances containing strongly acidic groups yielded the same product[2] with benzohydroxamic acid.

Tiemann and his associates had shown that a Beckmann-type rearrangement occurred with amidoximes and benzenesulfonyl chloride to yield the corresponding substituted urea.[3]

$$RC(NH_2)=NOH + C_6H_5SO_2Cl \longrightarrow RNHCONH_2$$

It was of interest to see if Sarin or DFP would react with amidoximes and whether these halophosphates and halophosphonates could effect the above mentioned "Tiemann Rearrangement."

In contrast to the hydroxamic acids, the amidoximes investigated reacted slowly with Sarin and DFP in buffered aqueous solution to yield stable phosphorylated products.[4] The same products were obtained with the corresponding chloro analogs of Sarin and DFP and amidoximes in non-aqueous media in the presence of triethylamine. As a preparative method the latter procedure was far superior to the former with respect to yield. Table I contains the physical and analytical data for some phosphorylated amidoximes.

Tiemann and co-workers[3] were able to isolate substituted ureas by extracting with boiling water the residue obtained from the reaction between benzenesulfonyl chloride and an amidoxime. We have found that when Sarin-phosphorylated benzamidoxime was boiled in water for a short time, no change occurred. However, small amounts of phenylurea, the rearranged product could be obtained after prolonged boiling.

Stieglitz and his students[5] had shown that triphenylmethylhydroxylamines could be arranged to anils. This was accomplished by treating the triphenylmethylhydroxylamine with phosphorus pentachloride in ether or by fusing the triphenylmethylhydroxylamine hydrochloride with phosphorus pentoxide. They also demonstrated that fusion of the benzoate of triphenylmethylhydroxylamine with soda lime at 160° yielded the anil.

We were interested in determining whether the chloro derivatives of Sarin or DFP could also effect this rearrangement. Reaction of isopropyl methylphosphonochloridate with an equimolar amount of triphenylmethylhydroxylamine in benzene in the presence of triethylamine at room temperature yielded benzophenone anil, the Stieglitz rearrangement product.

In all probability the reaction proceeds by an initial phosphorylation of the oxygen of the triphenylmethylhydroxylamine molecule. The result-

TABLE I

PHYSICAL AND ANALYTICAL DATA OF PHOSPHORYLATED AMIDOXIMES

Phosphorylated Amidoxime	M.P., °C.	Anaylses Calc'd				Found			
A = —PO(CH$_3$)OC$_3$H$_7$-*i* B = —PO(OC$_3$H$_7$-*i*)$_2$		C	H	N	P	C	H	N	P
$C_6H_5C(NH_2)$=NOA[a]	108	51.6	6.7	10.9	12.1	51.7	6.5	11.3	12.1
$C_6H_5C(NH_2)$=NOB[b]	86	51.9	7.4	9.3	10.3	52.0	7.1	9.1	10.3
$CH_3C(NH_2)$=NOA[a]	Oil	37.2	7.8		16.0	37.9	7.7		16.6
$CH_3C(NH_2)$=NOB[a]	40	40.2	8.0		13.0	40.5	8.4		13.1
3-Py[d]$C(NH_2)$=NOA[a]	131	46.8	6.2	16.3	12.0	47.0	6.2	16.0	11.8
3-Py[d]$C(NH_2)$=NOB[c]	137–138	47.9	6.7	13.9	10.3	48.3	6.6	13.6	10.2
$C_6H_5C[N(CH_3)_2]$=NOA	<25	55.0	7.41	9.8	10.8	55.4	7.5	9.8	10.3

[a] Recrystallized from chloroform-pet. ether (30–60°). [b] Recrystallized from ether-pet. ether (30–60°). [c] Recrystallized from acetone-pet. ether (30–60°). [d] Py = Pyridyl.

(1) Hackley, Plapinger, Stolberg, and Wagner-Jauregg, *J. Am. Chem. Soc.*, **77**, 3651 (1955).

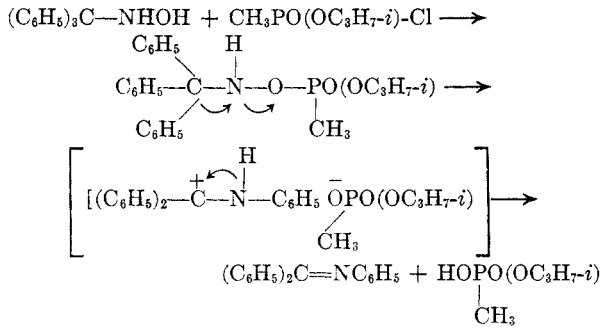
(2) Hurd and Bauer, *J. Am. Chem. Soc.*, **76**, 2791 (1954); Stolberg, Tweit, Steinberg, and Wagner-Jauregg, *J. Am. Chem. Soc.*, **77**, 765 (1955).

(3) Tiemann, *Ber.*, **24**, 4162 (1891); Pinnow, *Ber.*, **24**, 4167 (1891); **26**, 604 (1893).

(4) The rates of reaction of several amidoximes with Sarin and DFP were determined by Mr. B. E. Hackley, Jr., and will be described in a subsequent publication. Only acetamidoxime reacted at a rate comparable to the hydroxamic acids.

(5) Stieglitz and Leech, *Ber.*, **46**, 2147 (1913); *J. Am. Chem. Soc.*, **36**, 272 (1914).

ing unstable phosphorylated product is believed to undergo a concerted change involving loss of phosphonic acid and the simultaneous migration of a phenyl group from carbon to nitrogen.



An initial phosphorylation on the nitrogen rather than on the oxygen of the triphenylmethylhydroxylamine molecule followed by loss of phosphonic acid could also yield the same product.

Triphenylmethylhydroxylamine, when treated with diisopropyl phosphorochloridate under the same conditions as isopropyl methylphosphonochloridate, did not react.

EXPERIMENTAL

Reaction of amidoximes with Sarin, DFP, and the corresponding chloro analogs. The amidoxime[6] was dissolved in the minimum amount of water and the solution was adjusted to *p*H 7.6. An equimolar amount of the fluorophosphate or the fluorophosphonate was added to this solution with stirring and a constant *p*H 7.6 was maintained by titration with alkali from a Beckmann Model K autotitrimeter. When the reaction was completed, the solution was made acid and the product was isolated by filtration or by extraction of the acidic solution with chloroform or ether. Recrystallization from appropriate solvents yielded the phosphonylated products.

The same products could be obtained by reacting 1 mole of amidoxime with 1 mole of chlorophosphate or chlorophosphonate in a non-aqueous solvent in the presence of triethylamine. Several of the phosphorylated products were first isolated only as oils but were obtained crystalline after passage over a column of activated alumnia prior to recrystallization.

The physical and analytical data for the phosphorylated amidoximes are contained in Table I.

Preparation of N,N-dimethylbenzamidoxime. To a stirred solution of benzohydroximyl chloride (15.5 g., 0.1 mole) dissolved in 30 cc. of absolute alcohol maintained at 0° was added 50 cc. of a solution of dimethylamine, (9.9 g., 2.2 moles) in absolute alcohol. The mixture was kept at 0° for 30 minutes and then was allowed to stir at room temperature in a stoppered filtering flask for 24 hours. Part of the alcohol was removed *in vacuo*. Cooling yielded a solid which was filtered and washed with cold alcohol. On recrystallization from alcohol the solid melted at 120°.

Anal. Calc'd for $C_9H_{12}N_2O$: C, 66.0, H, 7.3. Found: C, 66.0, H, 7.4.

This material was reacted with the chloro derivative of Sarin (Table I).

Rearrangement of Sarin-phosphorylated benzamidoxime to phenylurea. Sarin-phosphorylated benzamidoxime (4.0 g.) was placed in 15 cc. of water and was refluxed for 1 hour. After separating the water solution from an oily material and cooling, 150 mg. of a solid was obtained melting at 147° whose analysis corresponded to that of phenylurea.

Anal. Calc'd for $C_7H_8N_2O$: C, 61.8, H, 5.90, N, 20.6. Found: C, 62.0, H, 5.8, N, 20.7.

The reddish-brown oil which also formed was not identified.

Rearrangement of triphenylmethylhydroxylamine to benzophenone anil. Isopropyl methylphosphonochloridate (1.57 g., 0.01 mole) was added dropwise to a stirred, cooled solution of triphenylmethylhydroxylamine (2.75 g., 0.01 mole) in benzene in the presence of triethylamine (1.01 g., 0.01 mole). The mixture then was allowed to come to room temperature and was kept at room temperature for two hours. The amine hydrochloride was filtered and the solution was concentrated to dryness. The residue was crystallized from absolute alcohol and yielded 1.2 g. of a solid of m.p. 111°. This solid did not contain phosphorus and gave a negative Tollens test. Elemental analysis conformed with that of benzophenone anil.

Anal. Calc'd for $C_{19}H_{15}N$: C, 88.5, H, 5.85, N, 5.43. Found: C, 88.3, H, 5.80, N, 5.10.

In order to prove that the substance of m.p. 111° was the anil, 0.2 g. was hydrolyzed with 18% hydrochloric acid to benzophenone and aniline. The former was isolated as its 2,4-dinitrophenylhydrazone, the latter as its benzenesulfonanilide. Mixture melting point determination with authentic samples of these derivatives gave no depression.

Triphenylmethylhydroxylamine when treated with diisopropyl phosphorochloridate under the same conditions as isopropyl methylphosphonochloridate did not react.

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(6) Acetamidoxime: Michaelis, *Ber.*, **24**, 3439 (1891); Benzamidoxime: Krüger, *Ber.*, **18**, 1053 (1885); Nicotinamidoxime: Nordmann, *Ber.*, **17**, 2746 (1884).

Unsaturated Amines. IX. Through Bis-Enamines to Aromatics[1]

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The preparation of enamines[3] by the reaction of ketones with piperidine and pyrrolidine suggested an application to the synthesis of substituted *p*-phenylenediamines from 1,4-cyclohexanedione. The heating of a mixture of 1,4-cyclohexanedione (I) and pyrrolidine, with collection of the theoretical amount of water, gave a product exhibiting the in-

(1) Article VIII in this series: N. J. Leonard, L. A. Miller, and P. D. Thomas, *J. Am. Chem. Soc.*, **78**, 3463 (1956).

(2) National Science Foundation Fellow, 1954–1955.

(3) C. Mannich and H. Davidsen, *Ber.*, **69**, 2106 (1936); F. W. Heyl and M. E. Herr, *J. Am. Chem. Soc.*, **75**, 1918 (1953); M. E. Herr and F. W. Heyl, *J. Am. Chem. Soc.*, **75**, 5927 (1953); G. Stork, R. Terrell, and J. Szmuszkovicz, *J. Am. Chem. Soc.*, **76**, 2029 (1954); J. L. Johnson, M. E. Herr, J. C. Babcock, R. P. Holysz, A. E. Fonken, J. E. Stafford, and F. W. Heyl, *J. Am. Chem. Soc.*, **78**, 430 (1956).