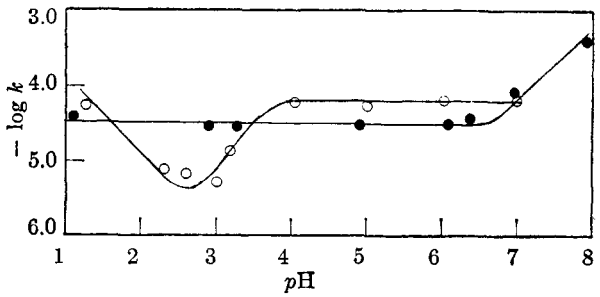

Fig. 1.—The hydrolysis of β-*N*,*N*-dimethylaminoethyl hydrogen phthalate at 75.5°, ●; and of methyl hydrogen phthalate at 109°, ○ (Ref. 6).

carboxyl ionization. It may therefore be concluded that in this *p*H region the bulk of the hydrolysis is due to direct attack of water on the ester group, and that any contribution which intramolecular carboxylate attack may make to the observed hydrolysis rate of I is within the experimental error of the rate constant measurements. If we extrapolate the data from ref. 6 for the fully ionized II at 109° and 84° to a temperature of 75.5°, we find that intramolecular carboxylate attack on the ester group results in a hydrolysis rate constant of 0.079×10^{-5} sec.$^{-1}$. This is less than 3% of the rate constant observed for ester I in the plateau region below *p*H 6.1, so that intramolecular carboxylate attack would not be experimentally detectable in ester I unless it were about four times as efficient as in II. Apparently the cationic group does not produce an effect of this magnitude.

Experimental

β-*N*,*N*-Dimethylaminoethyl Hydrogen Phthalate.—Phthalic anhydride (7.4 g., 0.05 mole) and 17.8 g. (0.2 mole) dimethylaminoethanol were placed in a 250-ml. three-necked round-bottomed flask equipped with a mechanical stirrer. The mixture was stirred at room temperature for 6 hr. The resulting slurry was washed with benzene and recrystallized from ethanol giving small colorless plates, which melt with decomposition 160–170°.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.75; H, 6.36; N, 5.90. Found: C, 61.02; H, 6.46; N, 5.81.

Kinetics.—The hydrolysis reactions were carried out in buffer solutions less than 0.02 *M* in ionized acid. The ionic strength was adjusted to 0.1 *M* by addition of sodium chloride and the *p*H was determined with a Cambridge Research Model *p*H meter. The rate of reaction was studied by observing the disappearance of the peak due to the the ester at 280 mμ on a Beckman DU spectrophotometer. In order to follow the rate of reaction in this manner the free phthalic acid had to be ionized, since unionized phthalic acid has approximately the same extinction coefficient as the ester. Therefore, for most runs, 2-ml. aliquots of the reaction solution (containing 24 mg. ester/100 ml.) were transferred into 2 ml. of 0.5 *M* phosphate buffer (*p*H 6.06), and the optical density (D) determined at 25.3°. Plots of $-\ln (D-D_\infty)$ were linear in time in all cases, and were used to calculate the first-order rate constant. The formation of phthalic acid in the reaction was proved by the similarity between the spectrum of the ester after hydrolysis and phthalic acid at the same *p*H.

Quaternary Derivatives of Nitrogen-Mustards[1]

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Phosphorylated *N*-mustards, prepared by Friedman and Seligman[3] as well as Arnold and Burseaux,[4] are examples of cytotoxic substances with a so-called "hidden" or "toxagenic" *N*-mustard group. According to the hypothesis, the *N*-phoshorylation or urethane formation of the bis(β-chloroethyl)-amine eliminates the basic character of the amine, preventing the formation of intermediary ethylenimonium salts, which are considered the true alkylating agent. The compounds are therefore nontoxic, inactive *in vitro*, and activated only *in vivo* by phosphamidases restoring the secondary amine.

We report here the properties of *N*-mustard derivatives, which are capable of quaternerizing prior to the eventual hydrolysis of *N*-acyl groups. Although, they are not "toxagenic" derivatives of originally active *N*-mustards, nevertheless, they contain quaternerized chloroethylamino groups. These differ from ethylenimonium salts in being less strained six-membered rings of novel structure. *N* - Bis(β - chloroethyl)phosphoramidic dichloride[3] (I) was first treated with four moles of 1,1-dimethylhydrazine, affording the *N* - bis(β - chloroethyl)phosphoramidate di(2,2 - dimethylhydrazidate) (II) as an oily base. On standing at room temperature for a few hours, or warming it several minutes on the steam bath, the compound bisquaternerized to the unusual heterocyclic system 2,7 - dimethyl - 9 - phospha - 9 - oxo - 10 - azapyridazo[5,6 - *e*]pyridazine - 2,7 - bischloromethylate (III), a water soluble crystalline substance.

On the other hand, phenyl-*N*-bis(β-chloroethyl)-phosphoramidic chloride[3] (IV), prepared from I was also treated with dimethylhydrazine to the tertiary base (V), which in turn, yielded the quaternary 1 - methyl - 3 - oxo - 3 - phenoxy - 4-(β - chloroethyl) - 3 - phospha - 1,2,4 - triazine - 1-chloromethylate (VI).

In connection with our investigations about *N*-mustard urethanes,[5] we also prepared the analogous

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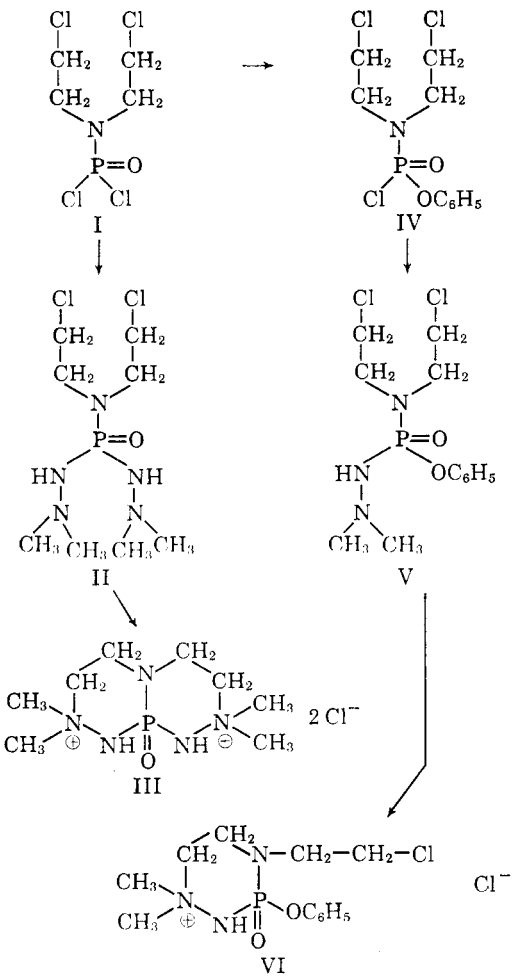
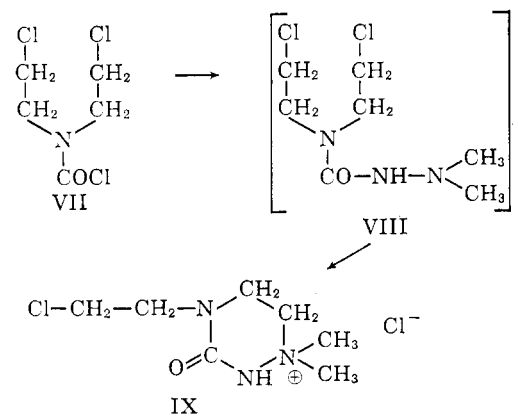
(3) O. M. Friedman and A. M. Seligman, *J. Am. Chem. Soc.*, **76**, 655 (1954).

(4) H. Arnold and F. Burseaux, *Angew. Chemie*, **70**, 539 (1958).



derivative from *N*-chloroformylbis(β-chloroethyl)-amine[6] (VII) with dimethylhydrazine. The reaction in benzene is exothermic, and therefore, besides the dimethylhydrazine hydrochloride, the quaternary salt separates directly. The tertiary urea (VIII) could not be isolated, so the quaternerization was driven to completion by heating, and the 1-methyl-4-(β-chloroethyl)-1,2,4-triazine-3-one-1-chloromethylate (IX) isolated.



The acute toxicity[1] of the two phosphates was surprisingly low. The LD_{50} of III was 1600 mg./kg. (mouse, single intraperitoneal injection), that of VI was 1000 mg./kg. The cancerostatic screening[7] on Sarcoma 180 revealed, however, that both compounds (SK-25,029 and SK-25,030) were completely inactive in doses up to 500 mg./kg. This might be attributed to the assumption, that a six membered quaternary salt is much more stable than an ethylenimonium salt, is therefore incapable of splitting at the C—N bond, and acting as an alkylating agent. The single chloroethyl group in VI can not quaternerize; it is therefore inactive, too.

Experimental[8]

2,7-Dimethyl-9-phospha-9-oxo-10-azapyridazo-[5,6-e]-pyridazine-2,7-chloromethylate (III).—A 1.30-g. sample of *N*-bis(β-chloroethyl)phosphoramidic dichloride[3] and 1.52 ml. of 1,1-dimethylhydrazine were dissolved in 15 ml. of dry benzene. The solution became warm, and dimethylhydrazine hydrochloride started to crystallize. After standing overnight, the salt was filtered off, and the solution evaporated under reduced pressure, affording 1.00 g. (65.4%) of a colorless viscous oil.

Heating this on a steam bath, it solidified immediately to a hard mass. After 30 min., it was triturated with a small amount of boiling ethanol, chilled, filtered, and recrystallized from methanol-ether, yielding 0.47 g. (31.7%) bis-quaternary salt, m.p. 212–215° (dec.)

Anal. Calcd. for $C_8H_{22}Cl_2N_5OP$: C, 31.4; H, 7.2; Cl, 23.2; N, 22.9. Found: C, 31.3; H, 7.4; Cl, 22.9; N, 23.1.

1-Methyl-3-oxo-3-phenoxy-4-(β-chloroethyl)-3-phospha-1,2,4-triazine-1-chloromethylate (VI).—A 15.8-g. sample of phenyl-*N*-bis(β-chloroethyl)phosphoramidic chloride[3] and 8.0 ml. of 1,1-dimethylhydrazine in 100 ml. dry benzene was refluxed for 1 hr., chilled, filtered, and the solution evaporated under reduced pressure. The resulting oil was heated on the steam bath for 30 min., solidifying immediately. This was triturated with acetone, filtered, and recrystallized from ethanol, yielding 6.08 g. (35.8%) of colorless crystals, m.p. 214–215° (dec.).

Anal. Calcd. for $C_{12}H_{20}Cl_2N_3O_2P$: C, 42.4; H, 5.9; Cl, 21.0; N, 12.3. Found: C, 42.5; H, 6.1; Cl, 20.8; N, 12.5.

1-Methyl-4-(β-chloroethyl)-1,2,4-triazine-3-one-1-chloromethylate (IX).—A 2.04-g. sample of chloroformylbis-(β-chloroethyl)amine[6] and 1.6 ml. of 1,1-dimethylhydrazine were dissolved in 10 ml. of dry benzene. The solution became hot. After 1 hr. at room temperature, the benzene was evaporated under reduced pressure from the semisolid reaction mixture, and heated for 20 min. at 95°. It was triturated with cold ethanol, filtered, and recrystallized from a large amount of ethanol, yielding 0.52 g. (22.8%) crystals, m.p. 209–210° (dec.).

Anal. Calcd. for $C_7H_{15}Cl_2N_3O$: C, 36.8; H, 6.6; Cl, 31.3; N, 18.4. Found: C, 36.7; H, 6.7; Cl, 31.4; N, 18.3.

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(7) The cancerostatic screening was performed by the Sloan-Kettering Institute for Cancer Research, Rye, N. Y. We wish to thank Dr. C. C. Stock for making the data available to us.

(8) All melting points are uncorrected. Microanalyses were done by Dr. C. Daessle Microanalytical Laboratory (Montreal), and Dr. G. Papineau-Couture and his staff of Ayerst, McKenna & Harrison Co. Ltd. (Montreal), to whom thanks are due.