Reaction between Glutamic Acid and Various Aldehydes.—The composition of the reaction mixtures is given in Table I. For the qualitative demonstration of α-ketoglutaric acid formation 2 ml. of a 0.1% solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid was added to 0.5-ml. aliquots of the reaction mixtures, the solutions allowed to stand for 30 minutes, then extracted with ethyl acetate, and suitable portions of the ethyl acetate extracts chromatographed on paper as described previously.[3,13] The low R_f value of the 2,4-dinitrophenylhydrazone of α-ketoglutaric acid corresponded with that of an authentic sample and allowed it to be readily distinguished from the aldehyde dinitrophenylhydrazones in every case. The results are given in Table I.

Quantitative Keto Acid Determinations in the Reaction of 4-Nitrosalicylaldehyde with Glutamic Acid, Serine and Cysteine.—Composition of the reaction mixtures is given in Table II. The *p*H values were measured after the reaction, and were maintained by use of 0.02–0.04 *M* acetate or bicarbonate buffers in the central *p*H range, and the addition of appropriate amounts of HCl or NaOH at low and high *p*H values, respectively. Quantitative keto acid determinations on 3-ml. aliquots of the above reaction mixtures were carried out according to Metzler and Snell[1]; the results are given in Table II. The keto acid formed in the reactions with serine and cysteine was shown to be pyruvic acid by chromatography and spectral analysis of its 2,4-dinitrophenylhydrazone.[3,13]

Reaction of 4-Nitrosalicylaldehyde with Excess Glutamic Acid, Serine and Cysteine.—Keto acid and ammonia determinations[3] were made on aliquots of the reaction mixtures specified in Table III. Recorded values for recoverable aldehyde were estimated directly by adding 2 ml. of 6 *N* hydrochloric acid to a 5-ml. aliquot of the reaction mixture, extracting with 10.0 ml. of toluene, pipetting off a 5.0-ml. aliquot of the toluene extract, extracting the latter with 15.0 ml. of 1 *N* sodium hydroxide, filtering 10 ml. of the sodium hydroxide extract, and comparing the color intensity of the filtrate with that of a similarly treated standard in an Evelyn colorimeter using a 490 filter. With reaction mixtures containing aldehyde, amino acid and alum, the acidified solution remained yellow-orange in color even after extraction with toluene, apparently due to presence of a relatively stable complex (Schiff base?) between the aldehyde and amino acid. On heating these acidified solutions on a steam-bath for 15 minutes the glutamic acid and serine reaction mixtures became almost colorless whereas the cysteine reaction mixture retained its yellow-orange color. Aldehyde determinations were also run on these "hydrolyzed" solutions (Table III).

The Bratton–Marshall determination[14] for diazotizable aromatic amines was negative when applied directly to the glutamic acid reaction mixtures, but after hydrolysis showed a 9% conversion of 4-nitrosalicylaldehyde to aromatic amine. *m*-Aminophenol was used as the standard. The amount of aromatic amine formed in oxygen and nitrogen atmospheres was identical. A blank containing all reagents except glutamic acid gave a value of 0.5%.

Reaction between Various Amines and Nitro Compounds.—Reaction mixtures similar to those used earlier with glutamic acid, but containing benzylamine or pyridoxamine as the amino compound and *m*-nitrophenol or 4-nitrosalicylaldehyde as the nitrophenol were heated with alum at *p*H 4.0 and 100° for 1–2 hours. Negligible amounts of ammonia were formed.

Reaction between Threonine and 4-Nitrosalicylaldehyde.—This reaction was carried out at *p*H 5 as described in the section on reaction between glutamic acid and various aldehydes. Aliquots of the reaction mixtures were spotted on paper, chromatographed with 77% ethanol as the developing agent, and sprayed with ninhydrin. In the mixture in which alum had been omitted only the threonine spot appeared whereas in the mixture containing alum both threonine and glycine were observed. It has been shown previously[5] that no cleavage of threonine to glycine occurred in the absence of pyridoxal; 4-nitrosalicylaldehyde is an effective substitute for the latter.

2-(2-Hydroxy-4-nitrophenyl)-4-thiazolidinecarboxylic Acid (III).—To 25 ml. of 0.5 *M* acetate buffer (*p*H 4.0) were added 84 mg. (0.5 mmole) of 4-nitrosalicylaldehyde and 160 mg. (1 mmole) of L-cysteine hydrochloride. The mixture was heated to boiling, water added until all the solid had dissolved, and the solution heated on a steam-bath for one hour. On cooling, 98 mg. of fine silky needles was obtained. Recrystallization from water yielded 68 mg. of the dihydrate, m.p. 160° dec. (cor.).

Anal. Calcd. for $C_{10}H_{10}O_5N_2S\cdot 2H_2O$: C, 39.2; H, 4.6; N, 9.15. Found: C, 39.4; H, 4.6; N, 9.1.

(13) D. E. Metzler, J. Olivard and E. E. Snell, THIS JOURNAL, **76**, 644 (1954).

(14) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).

AUSTIN, TEXAS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, AND THE DEPARTMENT OF SURGERY, BETH-ISRAEL HOSPITAL, AND HARVARD MEDICAL SCHOOL]

Preparation of N-Phosphorylated Derivatives of Bis-β-chloroethylamine[1a]

BY ORRIE M. FRIEDMAN[1b] AND ARNOLD M. SELIGMAN

RECEIVED JULY 6, 1953

In order to re-examine the distribution of phosphamidase in normal and malignant tissues with substrates of a type that could be used as possible chemotherapeutic agents, a number of N-phosphorylated derivatives of bis-(β-chloroethyl)-amine were prepared. Treatment of the amine hydrochloride with phosphorus oxychloride afforded the dichlorophosphamide III from which the triamidophosphonate IV was obtained with ammonia. Reaction of the dichlorophosphamide III with one molar equivalent of phenol gave the corresponding phenylchlorophosphamide (V). When V was treated successively with ammonia, ethanol, *t*-butyl alcohol and neopentyl alcohol intermediates were obtained from which the corresponding amido and alkyl ester monobasic acids IX, XIII, XIV and XV were prepared by replacement of the phenyl group by hydrogenolysis over platinum. When V was treated with benzyl alcohol followed by hydrogenolysis over palladium the phenyl ester monobasic acid VIII was obtained. By deamidation of the corresponding phenylamidophosphamide VI with nitrous acid VIII was also obtained.

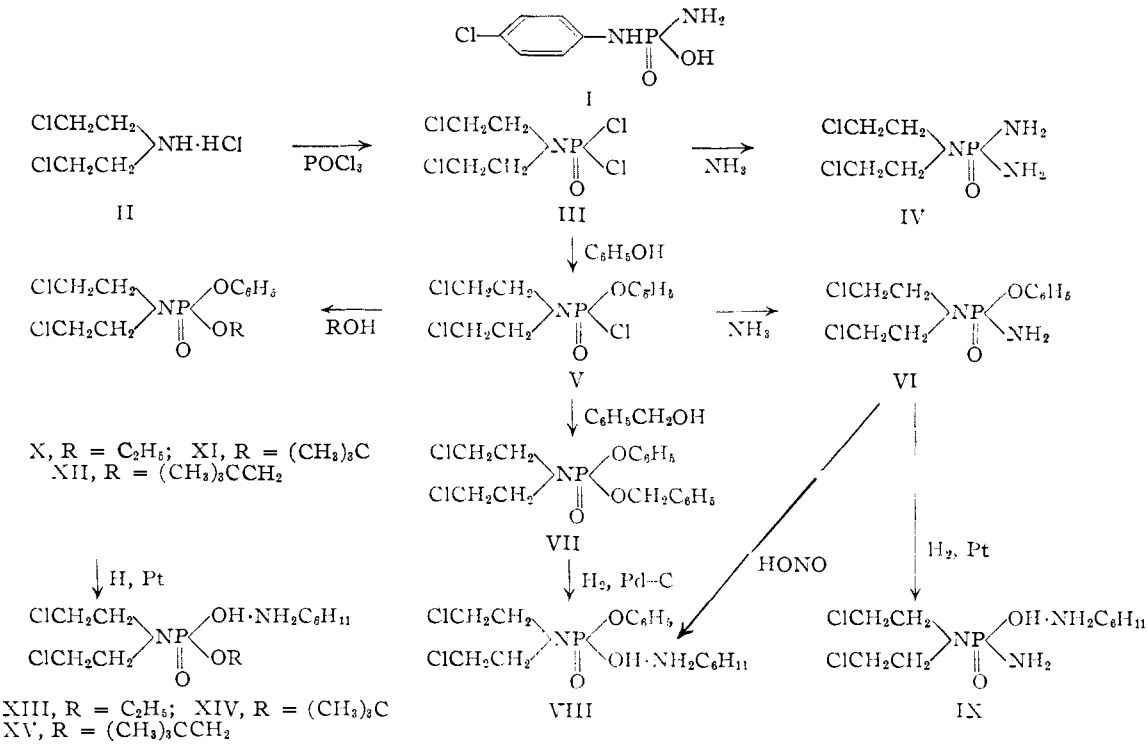
Phosphamidase activity has been ascribed to various preparations from plant and animal sources,[2] although the natural substrate is unknown. Based on an earlier claim for the synthesis of *p*-chloroanilinophosphoric acid[3] methods have been developed for estimation of phosphamidase activity quantitatively by the titration of phosphoric acid liberated enzymatically[3]; and histochemically at *p*H 5.4–5.8 by the formation of lead phosphate at the site of enzymatic action in tissue sections.[4] From histochemical studies with this substrate

(1) (a) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Department of Health, Education and Welfare, and in part by a research grant from Mrs. Albert B. Lasker. (b) School of Science, Brandeis University, Waltham, Mass.

(2) J. B. Sumner and K. Myrback, "The Enzymes," Academic Press, Inc., New York, N. Y., 1952. Vol. I, Chapt. 11, p. 405, J. Roche.

(3) P. Otto, *Ber.*, **28**, 617 (1895).

(4) (a) M. Ichihara, *J. Biochem.* (*Japan*), **18**, 87 (1933); (b) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **69**, 407 (1948).



Gomori[4] reported that enzymatic activity was higher in a number of malignant tumors than in normal tissues. Rorig,[5] however, has shown that the substrate used by Ichihara and Gomori, prepared by Otto's procedure,[2] did not have the structure assigned to it but was in fact the diamido derivative I rather than a dibasic acid. Furthermore, the ease with which this substance undergoes spontaneous hydrolysis at *p*H 5.0[6] casts some doubt on the reliability of the results of the histochemical studies. We considered it worthwhile to re-examine the question of the abundance of phosphamidase activity in malignant tissue as compared to normal tissues, with substrates which could be used as possible chemotherapeutic agents should Gomori's results[4] represent the true state of affairs. Since phosphorylated nitrogen mustards would be expected to be devoid of mustard action as a consequence of loss of basicity of the nitrogen atom in the phosphamide bond, enzymatic hydrolysis of this bond would liberate nitrogen mustard within cells in proportion to their phosphamidase activity. If malignant cells were, indeed, rich in phosphamidase activity, more nitrogen mustard could be delivered to them by the intravenous injection of a suitable N-phosphorylated nitrogen mustard than by injection of a tolerated dose of nitrogen mustard itself.[7]

We used the readily available bis-(β-chloroethyl)-amine hydrochloride (II) in order to develop practical methods for the synthesis of phosphorylated nitrogen mustards with which both to study the role of various substituents on phosphorus in altering susceptibility to spontaneous and enzymatic hydrolysis, and to determine the distribution of phosphamidase in normal and malignant tissues. More potent secondary nitrogen mustards[8] will be used in studies of inhibition of tumor growth. The rate of hydrolysis of several of the compounds herein reported in buffered solutions and in tissue homogenates was estimated by the colorimetric determination of phosphate.[9]

When bis-(β-chloroethyl)-amine hydrochloride (II) was heated with an excess of phosphorus oxychloride, the dichlorophosphamide III was obtained after distillation as a crystalline solid in good yield. Treatment of the dichlorophosphamide III in chloroform with ammonia gas gave N,N-di-(2-chloroethyl)-phosphorotriamidate (IV).[10] Reaction of the dichlorophosphamide III in benzene with one molar equivalent of phenol in the presence of triethylamine gave phenyldi-(2-chloroethyl)-phosphoramidic chloride (V). Treatment of the chlorophenylphosphamide V in benzene with ammonia gas afforded phenyl N,N-di-(2-chloroethyl)-phosphorodiamidate (VI) as a crystalline solid. When the chlorophenylphosphamide V was refluxed with benzyl alcohol and triethylamine in benzene, benzyl phenyl di-(2-chloroethyl)-phosphoramidate (VII) was obtained as a crude product. The benzyl group was removed selectively with hydrogen to give phenyl hydrogen di-(2-chloroethyl)-phosphoramidate isolated as a beautiful crystalline cyclohexylamine salt VIII. The

(5) K. Rorig, THIS JOURNAL, **71**, 3561 (1949).

(6) H. Holter and Si-Oh Li, *Compt. rend. trav. lab., Carlsburg, sir. Chim.*, **27**, 393 (1951); *C. A.*, **46**, 10216[h] (1952).

(7) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman and G. Wolf, *Ann. Surg.*, **130**, 333 (1949).

(8) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **76**, 658 (1954).

(9) To be published elsewhere in collaboration with Dr. A. M. Rutenburg.

(10) The nomenclature is in accord with the recommendations of the American Chemical Society Commitee on Nomenclature, A.C.S. Official Reports, *Chem. Eng. News*, October (1952).

same compound VIII was also obtained from VI by treatment with amyl nitrite in acetic acid. When phenyl N,N-di-(2-chloroethyl)-phosphorodiamidate (VI) was hydrogenated over platinum the phenyl group was replaced by hydrogen to give the monobasic acid, hydrogen N,N-di-(2-chloroethyl)-phosphorodiamidate, which was isolated as a crystalline cyclohexylamine salt (IX).

Attempts to prepare the dibasic acid, di-(2-chloroethyl)-phosphoramidic acid, either by deamination oft he corresponding triamidophosphonate (IV) with amyl nitrite in acetic acid, or by hydrogenolysis of the phenylamidophosphonate VIII were unsuccessful. A previous attempt to obtain the dibasic acid by hydrogenolysis of the dibenzyl ester, dibenzyl di-(2-chloroethyl)-phosphoramidate, was similarly unsuccessful.[11] The product is apparently difficult to isolate because of instability.

Phenyl di-(2-chloroethyl)-phosphoramidic chloride (V) reacted readily with ethanol, *t*-butyl alcohol or neopentyl alcohol in the presence of triethylamine. Hydrogenolysis of the crude products X, XI and XII, respectively, over platinum afforded the corresponding monobasic acids XIII, XIV and XV isolated as crystalline cyclohexylamine salts. The hydrogenolysis of the *t*-butyl ester XI was carried out in *t*-butyl alcohol to prevent the possibility of ester exchange with the alcoholic solvent.

Of the phosphorylated bis-(β-chloroethyl)-amines in which at least one of the groups on phosphorus is either amino or hydroxyl (IV, VI, VIII, IX, XIII, XIV and XV), spontaneous hydrolysis over the *p*H range 4.5–8.5 was extensive with the monobasic diamido acid (IX), was less with both the triamidophosphonate (IV) and the monobasic *t*-butyl acid ester (XIV), and essentially negligible with the other four compounds in this series (VI, VIII, XIII and XV).[9] The results of enzymatic studies with these compounds will be given in detail elsewhere.[9]

Experimental[12]

Di-(2-chloroethyl)-phosphoramidic Dichloride (III).—A suspension of 50 g. of bis-(β-chloroethyl)-amine hydrochloride[13] in 130 cc. of distilled phosphorus oxychloride, b.p. 105.5–107.5°, was heated under reflux for 12 hours until complete solution resulted. The excess phosphorus oxychloride was removed by distillation and the residue distilled at reduced pressure. The fraction, b.p. 123–125° (0.6 mm.), was collected as a water-clear fluid which crystallized as a solid mass on cooling, 58 g., m.p. 54–56°. The product crystallized from acetone–petroleum ether, after standing in the cold, in large prisms, m.p. 54–56°.

Anal. Calcd. for $C_4H_8NPOCl_4$: C, 18.55; H, 3.09. Found: C, 18.60; H, 3.25.

N,N-Di-(2-chloroethyl)-phosphorotriamidate (IV).—Ammonia gas was bubbled rapidly for 30 minutes through a solution of 50 g. of the dichlorophosphamide III in 500 cc. of chloroform. The precipitate that formed was collected and was extracted with 750 cc. of boiling acetone for ten minutes. When the mixture was filtered hot, ammonium chloride was separated on the filter and the product crystallized from the filtrate when stored in the cold overnight. A second crop was obtained from the mother liquors on concentration and cooling. The combined product recrystallized from acetone as transparent needle-like crystals 30 g. (72%), m.p. 126–127°.

Anal. Calcd. for $C_4H_{12}N_3POCl_2$: C, 21.83; H, 5.51; N, 19.10. Found: C, 22.23; H, 5.61; N, 18.97.

Phenyl-di-(2-chloroethyl)-phosphoramidic Chloride (V).—To a solution of 52 g. of the dichlorophosphamide (III) and 20 g. of phenol in 300 cc. of dry benzene heated to reflux was added dropwise over a period of 45 minutes a solution of 32 cc. of dry triethylamine in 100 cc. of dry benzene. Reflux was maintained by heat for three hours after addition was complete. After several hours at room temperature to complete precipitation, triethylamine hydrochloride was separated from the mixture on a filter. The benzene was distilled from the filtrate and the residue fractionated at reduced pressure. The product was obtained as a slightly yellow oil, b.p. 167–169° (0.2 mm.), 47.5 g. (75%).

Anal. Calcd. for $C_{10}H_{13}NPO_2Cl_3$: C, 37.94; H, 4.14; N, 4.43. Found: C, 38.21; H, 4.23; N, 4.51.

Phenyl N,N-Di-(2-chloroethyl)-phosphorodiamidate (VI).—Ammonia gas was bubbled through a solution of 1.0 g. of the chlorophenylphosphamide V in 20 cc. of dry benzene for 20 minutes, until the precipitation of ammonium chloride was complete. The ammonium chloride was separated by filtration. The benzene filtrate, concentrated to a volume of about 10 cc. and diluted to cloudiness with petroleum ether, gave on storage at 5° a first crop of pure product as fluffy white needles, 0.5 g., m.p. 57–58°. A second crop, 0.1 g., m.p. 57–59°, was obtained by concentration and further dilution of the mother liquor with petroleum ether.

Anal. Calcd. for $C_{10}H_{15}N_2PO_2Cl_2$: C, 40.42; H, 5.09; N, 9.43. Found: C, 39.72, 40.23; H, 5.11, 5.41; N, 9.80.

Cyclohexylammonium Phenyl Hydrogen Di-(2-chloroethyl)-phosphoramidate (VIII). A. From the Benzylphenylphosphamide (VII).—A solution of 4.8 g. of the chlorophenylphosphamide V, 1.65 cc. of benzyl alcohol and 1.7 cc. of triethylamine in 75 cc. of toluene was heated under reflux for three days. After cooling and standing, the mixture was filtered to remove 1.9 g. of triethylamine hydrochloride. The toluene was distilled from the filtrate leaving a residue of 4.3 g. of benzyl phenyl di-(2-chloroethyl)-phosphoramidate (VII) as a light brown sirup. Without further purification the crude product (VII) was hydrogenated at slight pressure over 0.5 g. of palladium–charcoal in 40 cc. of absolute ethanol at room temperature. The mixture took up 210 cc. of hydrogen in 2.5 hours and 1.4 cc. of cyclohexylamine was added. The ethanol was removed by distillation at reduced pressure leaving a solid residue which on recrystallization from methanol–ethyl acetate gave 1.7 g. of crystalline product. A second crop, 1.0 g., of product was obtained by concentration of the mother liquor. Repeated crystallization of the combined product from methanol–ethyl acetate gave feathery white needles, m.p. 184–185°.

Anal. Calcd. for $C_{16}H_{27}N_2PO_3Cl_2$: C, 48.37; H, 6.85; N, 7.05. Found: C, 49.06, 48.36; H, 6.70, 7.04; N, 6.83.

B. From the Phenyldiamidophosphonate (VI).—Amyl nitrite, 0.27 cc., was added dropwise to 0.63 g. of phenyldiamidophosphonate (VI) in 5 cc. of glacial acetic acid. The addition was accompanied by an evolution of nitrogen which ceased after ten minutes when the mixture was evaporated to dryness at room temperature under reduced pressure. The oily residue in solution in ether–petroleum ether when treated with cyclohexylamine gave a white precipitate, 0.65 g., m.p. 183–192° (soft 150°). When recrystallized from methanol–acetone the crude product gave a first crop of material, m.p. 213–214°, which by mixed m.p. proved to be dicyclohexylammoniumphenylphosphoric acid, m.p. 211–217°. The second and largest fraction, after recrystallization from methanol–ethyl acetate, melted 182–183° and gave no depression in m.p. when mixed with the phenylphosphamide (VIII). A third crop of crystalline material m.p. 136–138°, was obtained in small amount.

Cyclohexylammonium Hydrogen N,N-Di-(2-chloroethyl)-phosphorodiamidate (IX).—The phenyldiamidophosphonate (VI), 0.65 g., in 30 cc. of absolute alcohol was hydrogenated over 0.2 g. of platinum oxide under slight positive pressure at room temperature. The mixture took up 198 cc. of hydrogen in 30 min. when uptake had essentially stopped. Cyclohexylamine, 0.25 cc., was added immediately and the solvent removed by distillation at reduced pressure. The white crystalline residue was washed onto a filter with ether; 0.55 g. (80%), m.p. 125–126°.

(11) O. M. Friedman, D. L. Klass and A. M. Seligman, THIS JOURNAL, **76**, 916 (1954).

(12) Microanalysis by S. M. Nagy and his associates, Microchemical Lab., M.I.T., all melting points are corrected.

(13) K. Ward, THIS JOURNAL, **57**, 914 (1935).

Anal. Calcd. for $C_{10}H_{23}N_3PO_2Cl_2$: C, 37.63; H, 7.26; N, 13.16. Found: C, 37.18; H, 7.66; N, 13.14.

Cyclohexylammonium Ethyl Hydrogen Di-(2-chloroethyl)-phosphoramidate (XIII).—A mixture of 1.0 g. of the chlorophenylphosphamide (V) and 0.5 cc. of triethylamine in 20 cc. of absolute ethanol was heated under reflux for 3.5 hours. The excess ethanol was removed by distillation at reduced pressure. Extraction of the oily solid residue with dry ether left 0.45 g. of triethylamine hydrochloride. From the extract by distillation of the ether there was obtained crude **ethyl phenyl di-(2-chloroethyl)-phosphoramidate (X)** as a light-colored oil. This product X without purification, treated with hydrogen over 0.3 g. of platinum oxide in 20 cc. of absolute alcohol at slight positive pressure at room temperature, took up 344 cc. of hydrogen in 65 minutes. Cyclohexylamine, 0.4 cc., was added. When the mixture was distilled to dryness at reduced pressure, a solid crystalline residue remained. The product, dissolved in 10 cc. of acetone diluted with 10 volumes of petroleum ether and stored at 5° overnight, precipitated as a white crystalline solid, 0.85 g., m.p. 140°. After two recrystallizations from acetone, it melted 146–147°. A second impure crop was obtained by further dilution of the acetone solution with petroleum ether; 0.2 g., m.p. 135–138°.

Anal. Calcd. for $C_{12}H_{27}N_2PO_3Cl_2$: C, 41.27; H, 7.79; N, 8.02. Found: C, 41.59; H, 8.16; N, 8.08.

Cyclohexylammonium *t*-Butyl Hydrogen Di-(2-chloroethyl)-phosphoramidate (XIV).—A mixture of 2.0 g. of the chlorophenylphosphamide (V) and 1.0 cc. of dry triethylamine in 20 cc. of *t*-butyl alcohol was heated under reflux for 30 hours. Distillation of the mixture at reduced pressure to remove excess *t*-butyl alcohol left a residue of oily solid which on extraction with dry ether left 0.5 g. of triethylamine hydrochloride as a crystalline solid. From the ether extract on evaporation of the solvent there was obtained 2.15 g. of ***t*-butyl phenyl di-(2-chloroethyl)-phosphoramidate (XI)** as an oil. The crude product (XI) was hydrogenolyzed with 0.5 g. of platinum oxide in 30 cc. of *t*-butyl alcohol. The uptake of hydrogen, 650 cc., was rapid and in excess of the required amount at the end of one hour when the reaction was stopped. Cyclohexylamine (1.0 cc.) was added to the mixture which was distilled at reduced pressure to remove excess solvent. Extraction of the residue with acetone left a solid crystalline residue, 0.35 g., of cyclohexylamine hydrochloride. The concentrated acetone extract when diluted with ten volumes of petroleum ether gave, after storage for a day in the cold, 1.1 g. of crystalline material. The product was obtained after two recrystallizations from acetone as fine white needles, m.p. 131–133°.

Anal. Calcd. for $C_{14}H_{31}N_2PO_3Cl_2$: C, 44.56; H, 8.28; N, 7.43. Found: C, 45.14; H, 8.38; N, 7.37.

Cyclohexylammonium Neopentyl Hydrogen Di-(2-chloroethyl)-phosphoramidate (XV).—A mixture of 20 g. of the chlorophenylphosphamide (V), 1.0 cc. of triethylamine and 2.5 g. of neopentyl alcohol in a flask fitted with a condenser was heated on the steam-bath for 8 hours. The mixture was diluted with 30 cc. of dry benzene to completely precipitate triethylamine hydrochloride, 0.85 g., which was separated on a filter. The filtrate gave, after distillation of the benzene, crude **neopentyl phenyl di-(2-chloroethyl)-phosphoramidate (XII)**, 2.3 g., as an oil. The crude product XII in 25 cc. absolute alcohol over 0.6 g. of platinum oxide took up 345 cc. of hydrogen in 65 minutes. After another 0.6 g. of platinum oxide was added the mixture took up an additional 385 cc. of hydrogen in the next hour. Cyclohexylamine, 0.8 cc., was added to the mixture from which the solvent was removed by distillation at reduced pressure. The solid residue crystallized from a small volume of methanol on dilution with ethyl acetate; 0.7 g., m.p. 160–161°. A second crop, 0.5 g., m.p. 145°, was obtained from the mother liquor by further dilution with ethyl acetate. The first crop recrystallized from acetone as fine white needles, m.p. 160–161°.

Anal. Calcd. for $C_{15}H_{33}N_2PO_3Cl_2$: C, 46.04; H, 8.50; N, 7.16. Found: C, 46.46; H, 8.84; N, 7.55.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY, AND THE DEPARTMENT OF SURGERY, BETH-ISRAEL HOSPITAL, AND HARVARD MEDICAL SCHOOL]

Preparation of Secondary Amine Mustards with High Toxicity[1a]

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RECEIVED JULY 6, 1953

A type of secondary nitrogen mustard capable of intramolecular cyclization to the potent tertiary state at *p*H 7.4 was developed for possible use against tumors in which the enzyme phosphamidase is found. The mustard, 2',2,5-trichloro-N-ethylpentylamine (V), was found to be significantly more toxic than bis-(β-chloroethyl)-amine in mice and could be N-phosphorylated. Successive replacement of chlorine by iodine in V gave mustards (VIII, IX and X) which were increasingly toxic. Both the isomeric N-2'-chloroethyl-1,5-dichloro-2-aminopentane (XIV) and cyclic tertiary amine XV, derived from XIV, were highly toxic in mice. When phosphorylation was attempted, however, XIV was spontaneously transformed to XV.

The activity of phosphamidase in mammalian tissues has been studied quantitatively[2] and histochemically[3] by the use of N-*p*-chlorophenyl phosphorodiamidic acid[4] as a substrate. Although the ease with which this substrate undergoes hydrolysis at *p*H 5 casts some doubt on the reliability of the histochemical method, it was found that enzymatic activity was higher in a number of malignant tumors than in normal tissues.[2] A re-examination of the question of the abundance of phosphamidase activity in malignant tissues as compared to normal tissues has been undertaken[5] with substrates of possible use as chemotherapeutic agents should these results[2] represent the true state of affairs.

Since the chemical activity and presumably the biological potency of the nitrogen mustards is known to depend on basicity of the nitrogen atom, N-phosphorylation might be expected to give products significantly less toxic than the parent mustard. Loss of basicity of the nitrogen atom in the phosphamide link owing to the inductive effect of the PO_3 or PON_2 group and possibly to resonance in the sense

(1) (a) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Department of Health, Education and Welfare, and (in part) by a research grant from Mrs. Albert B. Lasker. (b) School of Science, Brandeis University, Waltham, Mass.

(2) M. Ichihara, *J. Biochem.* (*Japan*), **18**, 87 (1933).

(3) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **69**, 407 (1948).

(4) (a) K. Rorig, THIS JOURNAL, **71**, 3561 (1949); P. Otto, *Ber.*, **28**, 617 (1895). (b) The system of nomenclature is in accord with the recommendations of the American Chemical Society Committee on Nomenclature, A.C.S. Official Reports, *Chem. Eng. News*, October (1952).

(5) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **76**, 655 (1954).