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]

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

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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

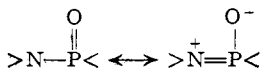
(1) (a) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Department of Health, Education and Welfare, and (in part) by a research grant from Mrs. Albert B. Lasker. (b) School of Science, Brandeis University, Waltham, Mass.

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

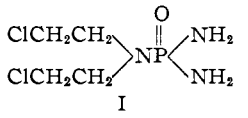
(3) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **69**, 407 (1948).

(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).



would inhibit intramolecular cyclization to the ethyleneimmonium ion necessary for high chemical activity. On this assumption N,N-di-(2-chloroethyl)-phosphorotriamidate (I)[4,5] was prepared and was indeed found to have only a fraction of the toxicity of the parent bis-(β-chloroethyl)-amine in mice.[6] Phosphamidated mustard would be ex-

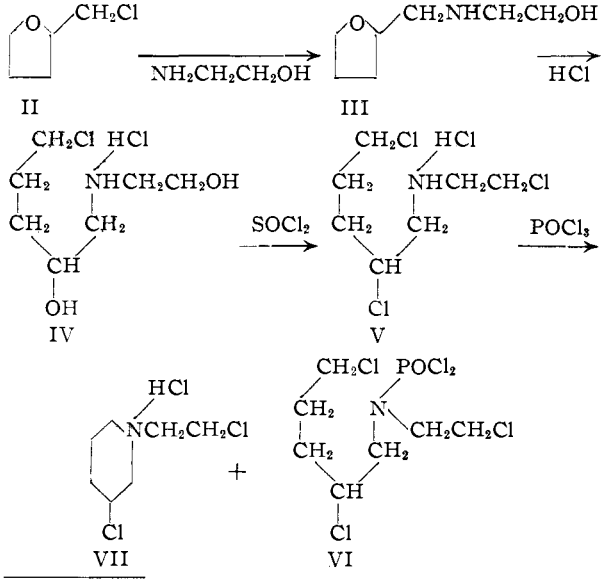


I

pected to yield the parent mustard in cells of tumors if the enzyme, phosphamidase, were present.

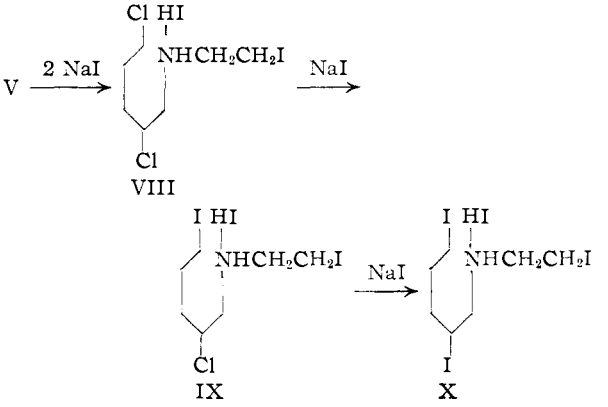
Since secondary amine mustards have only slight cytotoxic activity,[6,7] tumor inhibitory phosphamides should be derived from an amine mustard comparable in cytotoxic activity to the potent tertiary amine mustards. We have now developed a type of secondary nitrogen mustard capable of intramolecular cyclization to the biologically potent tertiary amino state.

When 2-chloromethyltetrahydrofuran[8] (II) was heated with ethanolamine, condensation gave N-tetrahydrofurfurylethanolamine (III), in good yield. Treatment of the product III with hydrogen chloride to open the oxide ring gave a viscous sirupy product which was presumably mainly 2,2′-dihydroxy-5-chloro-N-ethylpentylamine hydrochloride (IV). The crude amine hydrochloride IV treated with thionyl chloride without further purification, gave 2′,2,5-trichloro-N-ethylpentylamine hydrochloride (V) as a crystalline product in good over-all yield. The corresponding cyclic tertiary amine, N-2-chloroethyl-3-chloropiperidine hydrochloride (VII) was obtained inadvertently as



a by-product in the preparation of 2′-chloroethyl-2,5-dichloropentylphosphoramidic dichloride (VI) by phosphorylation of the acyclic secondary amine V with phosphorus oxychloride.

Iodo derivatives, presumably VIII, IX and X, in which the three chlorine atoms are successively replaced by iodine were prepared by treatment of 2′,2,5-trichloro-N-ethylpentylamine (V) with sodium iodide in acetone under increasingly severe conditions.



Structures VIII and IX are suggested for the mono- and diiodo derivatives, respectively, on the basis that primary alkyl halides are known to undergo displacement more readily than do secondary alkyl halides; and of the two primary sites, C-2′ would be favored over C-5 as the preferred point of nucleophilic attack because of the proximity of the former to the positively charged nitrogen. The choice of C-2′ iodide VIII as the structure of the monoiodo derivative is consistent with the results obtained in an analogous case with bis-(β-chloroethyl)-amine hydrochloride which underwent reaction with sodium iodide to give the corresponding bis-(β-iodoethyl)-amine hydroiodide under conditions essentially the same as those under which the monoiodo derivative VIII was formed.[9]

Ethyl glutamate (XI), prepared by an improved procedure, when acylated with acetylglycolyl chloride in benzene in the presence of pyridine gave N-acetylglycolyldiethylglutamate (XII) obtained pure as an oil by distillation. When this triester amide XII was treated with lithium aluminum hydride in tetrahydrofuran the four functional groups were reduced simultaneously and 1,2′,5-trihydroxy-N-ethyl-2-aminopentane (XIII) was obtained. The crude triolamine XIII on treatment with thionyl chloride afforded 1,2′,5-trichloro-N-ethyl-2-aminopentane hydrochloride (XIV) as a crystalline product. The trichloroamine hydrochloride XIV was transformed almost quantitatively into the cyclic tertiary amine, N-2-chloroethyl-2-chloromethylpyrrolidene hydrochloride (XV) either by passage in methanol over acid-washed alumina or by treatment with phosphorus oxychloride heated under reflux.

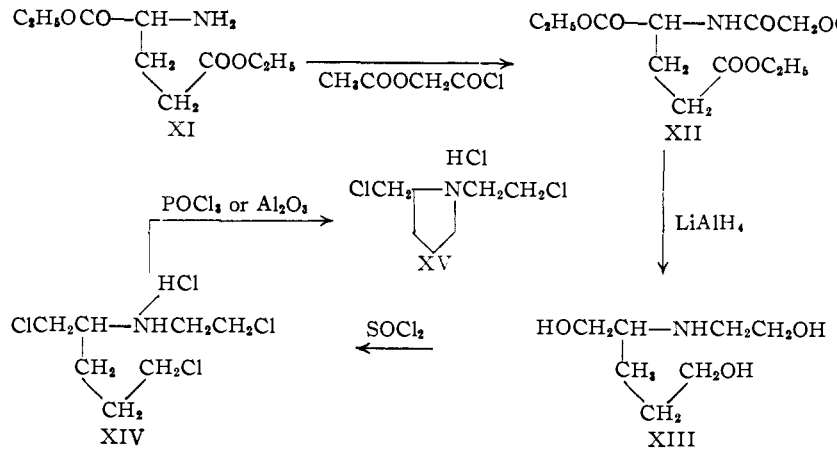
Although the toxicity in mice of the acyclic trichloroamine V was four times that of bis-(β-chloroethyl)-amine, it was only one-twentieth as

(6) A. M. Rutenberg, O. M. Friedman, L. Persky and A. M. Seligman, to be published.

(7) W. P. Anslow, Jr., D. A. Karnofsky, B. V. Jager and H. W. Smith, *J. Pharm. Exp. Therap.*, **91**, 224 (1947).

(8) L. A. Brooks and H. R. Snyder, *Org. Syntheses*, **25**, 84 (1945).

(9) O. M. Friedman and A. M. Seligman (unpublished results).



toxic as N-methyl-bis-(β-chloroethyl)-amine.[6] The relative lack of toxicity of this compound V may derive in part from the steric factor introduced by the six-membered ring structure into which it is presumably transformed at *p*H 7.3 or more importantly, perhaps, from the fact that one chlorine atom is secondary and, thereby, less susceptible to displacement. This hypothesis is borne out by the toxicity in mice of the compounds in which iodine is substituted for chlorine (VIII, IX and X), since secondary alkyl iodides are known to undergo displacement by nucleophilic reagents more rapidly than corresponding chlorides. Although the monoiodo compound VIII, for example, was over twice as toxic as the parent substance V, the triiodo derivative X was more toxic than methyl-bis-(β-chloroethyl)-amine and over twenty times as toxic as V.[6]

Consideration of these factors suggested the synthesis of 1,2′,5-trichloro-N-ethyl-2-aminopentane (XIV) in which the three chlorine atoms are primary. This compound XIV was highly toxic in mice,[6] having the same order of toxicity as methyl-bis-(β-chloroethyl)-amine. The cyclic intermediate XV into which the trichloroamine (XIV) was presumed to have been transformed at physiological *p*H gave toxicity in mice essentially identical to that of the parent mustard XIV.

Amine mustards of the type described appeared sufficiently toxic to make worthwhile attempts to prepare N-phosphorylated derivatives. It is noteworthy, however, that when XIV was treated with phosphorus oxychloride cyclization to the pyrrolidene XV took place exclusively, whereas with V cyclization to the corresponding piperidine VII took place to a minor extent and the dichlorophosphamide VI was the major product. This derivative VI has been used analogously to the dichlorophosphamide of bis-(β-chloroethyl)-amine[5] as an intermediate for preparation of other phosphorylated derivatives that will be described subsequently.

Experimental[10]

N-Tetrahydrofurfurylethanolamine (III).—Tetrahydrofurfuryl chloride (II) was prepared from tetrahydrofurfuryl alcohol with thionyl chloride according to reference 8. The chloride II, 50 cc., in 200 cc. of ethanolamine in which 60 g. of anhydrous sodium carbonate was suspended, was heated under reflux for 24 hours. A solid residue that separated was removed by filtration and excess ethanolamine was distilled from the filtrate at reduced pressure, b.p. 55–60° (1–2 mm.), until a solid mass remained. The solid was transferred to a filter, washed thoroughly with chloroform and then discarded. The filtrate was distilled to remove chloroform and the oil that remained was distilled at reduced pressure. The product was collected as a colorless fraction, b.p. 103–105° (3 mm.), 51 g. (85%), $n^{24}D$ 1.4730.

Anal. Calcd. for $C_7H_{15}NO_2$: C, 57.90; H, 10.41. Found: C, 57.66; H, 9.90.

2′,2,5-Trichloro-N-ethylpentylamine Hydrochloride (V).—Hydrogen chloride was bubbled for eight hours through 20 g. (20 cc.) of N-tetrahydrofurfurylethanolamine (III) heated on the steam-bath. The crude product, presumably **2′,2-dihydroxy-5-chloro-N-ethylpentylamine hydrochloride (IV)**, with 40 cc. of chloroform as a two-layer mixture, was added dropwise over a period of 30 minutes to 45 cc. of distilled thionyl chloride and 0.2 cc. of pyridine, heated under reflux. The mixture was heated over steam for an additional three hours and then distilled at reduced pressure to remove the chloroform and most of the thionyl chloride. The mixture was dissolved in 50 cc. of methanol from which 18 g. of product crystallized on cooling and was separated by filtration. Dilution of the filtrate with ether gave an additional 14 g. of crude material. The combined product was recrystallized from methanol (Norit)–ether as a white granular solid, 26 g., m.p. 143–145°.

Anal. Calcd. for $C_7H_{15}NCl_4$: C, 32.97; H, 5.93; Cl, 55.63. Found: C, 33.23; H, 5.89; Cl, 55.49.

N-2-Chloroethyl-3-chloropiperidine Hydrochloride (VII).—The dichloropiperidine (VII) was obtained as a minor product when 25 g. of the trichloroamine hydrochloride (V) in 65 cc. of redistilled phosphorus oxychloride was heated under reflux for 20 hours. After removal of excess phosphorus oxychloride by distillation at reduced pressure and on distillation of the sirupy residue there was obtained 21.4 g. of crude **2′-chloroethyl-2,5-dichloropentylphosphoramidic dichloride (VI)** as a colorless oil, b.p. 170–175° (0.1 mm.). The by-product VII sublimed onto the walls of the condenser in the forerun and was obtained after two recrystallizations from methanol–ether as a granular white crystalline solid, 0.8 g., m.p. 211–212°.

Anal. Calcd. for $C_7H_{14}NCl_3$: C, 38.47; H, 6.46; N, 6.42. Found: C, 38.32; H, 6.37; N, 6.42.

N-2′-Iodoethyl-2,5-dichloropentylamine Hydroiodide (VIII).—A solution of 1.0 g. of the trichloroamine hydrochloride V and 1.4 g. of sodium iodide in 30 cc. of reagent acetone was heated under reflux for one hour. Sodium chloride was removed by filtration. After concentration of the filtrate a crude product precipitated on addition of ether. After several recrystallizations from acetone–benzene this material was obtained as a white crystalline solid, 1.0 g., m.p. 151–152°.

Anal. Calcd. for $C_7H_{15}NCl_2I_2$: C, 19.24; H, 3.23. Found: C, 18.97; H, 3.20.

N-2′-Iodoethyl-2-chloro-5-iodopentylamine Hydroiodide (IX).—A solution of 0.5 g. of the trichloroamine hydrochloride (V) and 0.9 g. of sodium iodide in 15 cc. of acetone was heated in a sealed tube at 100° for 1.5 hr. The precipitate formed was collected on a filter and the product was separated from sodium chloride with boiling acetone. The acetone extract was concentrated and after dilution with benzene to cloudiness gave silky white needles, 0.1 g., m.p. 192–194°.

Anal. Calcd. for $C_7H_{15}NClI_3$: C, 15.85; H, 2.84; N, 2.65. Found: C, 15.68; H, 2.82; N, 2.39.

The acetone filtrate from the reaction mixture diluted with ether gave 0.4 g. of a gummy yellow product from which a small amount of the monoiodo compound VIII was obtained by recrystallization from acetone–benzene.

(10) Microanalysis by S. M. Nagy and Associates, Microchemical Laboratory, M.I.T.; all melting points are corrected.

N-2'-Iodoethyl-2,5-diiodopentylamine Hydroiodide (X).—A solution of 0.75 g. of the trichloroamine hydrochloride (V) and 2.7 g. of sodium iodide in 21 cc. of acetone was heated in a sealed tube to 130–135° for 45 minutes. There was obtained by filtration of the reaction mixture 1.4 g. of crystalline solid which was extracted with a boiling mixture of two parts of acetone to one part of methanol. The extract which was concentrated gave after dilution with benzene 0.5 g. of crystalline product X m.p. 202–204°. Further dilution of the mother liquor with benzene gave 0.3 g. of yellowish product, m.p. 130–160°.

When the acetone filtrate from the reaction mixture was concentrated and diluted with benzene sodium iodide precipitated and on further concentration 0.5 g. of crude discolored material was obtained. On recrystallization from acetone the combined crop of crude material gave an additional 0.3 g. of crystalline product X, m.p. 202–204°.

Anal. Calcd. for $C_7H_{15}NI_4$: C, 13.54; H, 2.43. Found: C, 13.86; H, 2.41.

L-Diethylglutamate Hydrochloride (XI).—The ester XI was prepared from L-glutamic acid by a modification of the method of Chiles and Noyes.[11] Partially esterified contaminants were removed by extraction of the product with cold dilute aqueous potassium carbonate. The chloroform solution was dried by azeotropic distillation and treated with hydrogen chloride. The hydrochloride XI was precipitated from the mixture with ether as a solid mass and was obtained in 60% yield as a white flaky crystalline solid m.p. 114–115°.

Acetylglycolyl chloride[12] was prepared in almost quantitative yield from glycolic acid by treatment with acetyl chloride and thionyl chloride successively without isolation of the intermediate product as follows: A mixture of 50 g. of glycolic acid and 125 cc. of acetyl chloride in a flask fitted with a reflux condenser reacted very vigorously on addition of a few drops of pyridine and was cooled externally with water. When the exothermic reaction subsided the mixture was warmed on the steam-bath for 15 minutes. After removal of excess acetyl chloride by distillation the residue was heated under reflux for 30 minutes with 60 cc. of thionyl chloride containing two drops of pyridine. Excess thionyl chloride was removed by distillation under reduced pressure and the residue distilled. There was obtained 82 g. (94%) of water-white product, b.p. 55° (12 mm.).

N-Acetylglycolyldiethyl-L-glutamate (XII).—To a stirred mixture of 26.2 g. of ethylglutamate hydrochloride (XI) and 19 cc. of acetylglycolyl chloride in 40 cc. of dry benzene, 19 cc. of dry pyridine was added dropwise over a period of 20 minutes. The mixture was heated under reflux for an additional 10 minutes to complete the reaction. After the reaction mixture was washed successively with cold dilute hydrochloric acid and cold dilute sodium bicarbonate, the benzene was distilled. The crude residue (31.3 g.) on distillation gave 17 g. of colorless oil, b.p. 193° (0.4 mm.), $n^{25}D$ 1.4598.

(11) H. M. Chiles and W. A. Noyes, THIS JOURNAL, **44**, 1798 (1922).

(12) R. Anschütz and W. Bertram, *Ber.*, **36**, 467 (1903).

Anal. Calcd. for $C_{13}H_{21}O_7N$: C, 51.48; H, 6.98. Found: C, 51.35; H, 6.86.

N-2'-Chloroethyl-1,5-dichloro-2-aminopentane Hydrochloride (XIV).—A solution of 17 g. of the triester monoamide XI in 100 cc. of dry tetrahydrofuran was added slowly over a period of 30 minutes to a vigorously stirred solution of 18 g. of lithium aluminum hydride in 200 cc. of dry tetrahydrofuran. The mixture was then heated under reflux for 10 hours to complete the reaction. The reaction was decomposed by careful dropwise addition of 50 cc. of water. After filtration of the reaction mixture and distillation of the solvent, a slightly discolored oil remained. The oil on distillation gave a predominant fraction, b.p. 93° (0.05 mm.), which was light and colorless and a small residue of higher boiling material. The aluminum oxide residue was extracted with 250 cc. of boiling ethanol which after filtration was concentrated to small volume and diluted to excess with tetrahydrofuran. The mixture was filtered to remove the precipitate of lithium hydroxide and distilled to an oily residue. On distillation of the residue there was obtained a forerun of colorless liquid, b.p. 93° (0.05 mm.), and a main fraction of crude **N-2'-hydroxyethyl-1,5-dihydroxy-2-aminopentane** (XIII), as a colorless heavy sirup, b.p. 160–180° (0.05 mm.), 5.3 g. The crude trihydroxyamine (XIII) gave uncrystallizable oils when treated with hydrochloric or oxalic acid. The lower boiling material, 3.4 g., was not characterized.

A mixture of 2.4 g. of the triolamine XIII, 5 cc. of thionyl chloride and 5 cc. of chloroform containing a drop of pyridine was heated under reflux for two hours. Removal of excess thionyl chloride and chloroform by distillation at reduced pressure left a heavy brown oil most of which dissolved in 100 cc. of dry ether. The ether solution left an oily residue when distilled to dryness. The oily residue precipitated from ethyl acetate on cooling as a granular crystalline product, 1.4 g., m.p. 70°. Two additional crops, 0.7 g., m.p. 75°, and 0.2 g., m.p. 70°, were obtained where the mother liquors were stored in the cold after dilution with ether. The combined product after three recrystallizations from ethyl acetate and one from chloroform–benzene was obtained as a white granular crystalline material, m.p. 77–78°.

Anal. Calcd. for $C_7H_{15}NCl_4$: C, 32.97; H, 5.93; N, 5.49. Found: C, 33.22; H, 6.11; N, 5.53.

N-2-Chloroethyl-2-chloromethylpyrrolidene Hydrochloride (XIV).—When a sample of the trichloroamine hydrochloride (XIV) in chloroform was put on a column of acid-washed alumina and eluted with acetone there was obtained in good yield a white crystalline product, m.p. 232–233°. The same product was obtained when the trichloroamine hydrochloride XIV was heated in phosphorus oxychloride under reflux for two hours. The product deposited from the mixture during the reaction in almost quantitative yield.

Anal. Calcd. for $C_7H_{14}NCl_3$: C, 38.47; H, 6.46; N, 6.42; Cl, 48.75. Found: C, 38.31; H, 6.23; N, 6.48; Cl, 48.86.

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