white gelatinous precipitate was filtered, treated with dilute acid and extracted with ether. The residue from distillation of the ether was crystallized from toluene to give 0.3 g. of oxanilic acid (m.p. 152–154°) identical with an authentic sample. The white solid, from evaporation of the original filtrate, upon recrystallization from alcohol gave 0.18 g. of phenylacetamide.

1,4-Diphenyl-3-phenylaminomaleimide.—1,4-Diphenylhydroxymaleimide (10 g., 0.0378 mole) mixed with 45 cc. of aniline was heated at 185° for 0.5 hr. The cold, dark brown solution was poured with stirring into 500 cc. of water containing a large excess of hydrochloric acid. The bright yellow precipitate was filtered and crystallized from hot alcohol to give fine yellow needles, 12.1 g. (94%), m.p. 209–210°.

Anal. Calcd. for $C_{22}H_{16}O_2N_2$: N, 8.24. Found: N, 8.14.

Ozonolysis.—A standard procedure[6a–c] using a Welsbach-T-25 ozonator was adopted for our purpose. 2-Diethylmethylene-3-ethyloxazolidinedione (IX) (19.7 g., 0.10 mole) dissolved in 80 cc. of glacial acetic acid was treated in the gas dispersion tube with a current of O_3–O_2 until ozone was detected at the outlet. The ozonide was carefully added dropwise to a stirred, boiling mixture of 250 cc. of water, 30 g. of zinc dust, 0.10 g. of silver nitrate and 0.055 g. of hydroquinone over a period of 2 hr. and 20 min. The refluxing was continued for one hr. The Dry Ice trap contained 1.5 g. of diethyl ketone. The reaction mixture was filtered by gravity and the filtrate was distilled with steam. From the steam distillate there was obtained 3 g. of diethyl ketone by extraction with ether and 1.0 g. by precipitation as the 2,4-dinitrophenylhydrazone (m.p. 156–157°); this accounts for 64% of the theoretical amount. The zinc was removed by precipitation as zinc sulfide. Distillation of the filtrate under diminished pressure left 6.2 g. of a greasy solid, b.p. 95–105° (0.65 mm.), m.p. 108–112°. It resisted purification.

A solution of 10 g. (0.0342 mole) of 2-diphenylmethylene-3-ethyloxazolidinedione (VI) in 60 cc. of glacial acetic acid was subjected to ozonolysis. A considerable amount of pale yellow solid (5 g.) was filtered with the zinc dust; this material dissolved in ether. Recrystallization from carbon tetrachloride gave 4.2 g. of benzpinacol, m.p. 192–194° (rapid heating) equivalent to 0.023 mole (67%) of benzophenone; admixture with an authentic sample caused no depression of the m.p.; when it was added to a concentrated solution of sodium ethoxide, a bright blue color was obtained. The above filtrate after removal of the zinc with hydrogen sulfide, yielded 2.2 g. (50%) of calcium oxalate upon treatment with calcium hydroxide. This treatment caused the evolution of ethylamine.

Under similar conditions 1,4,4-triethylpyrrolidinetrione did not react with ozone.

A solution of the ozonide from 10.0 g. (0.0377 mole) of 1,4-diphenylhydroxymaleimide (XV) in 100 cc. of glacial acetic acid was added dropwise with stirring to 200 cc. of water at 5° in the course of 1 hr. After 2 hr. at room temperature the mixture was concentrated under diminished pressure to 16 g. The residue was dissolved in ether and extracted repeatedly with water. The residue from the distillation of the ether was dissolved in alcohol and treated with Darco. The filtrate was diluted with an equal volume of water to give 6.0 g. (70%) of phenylglyoxylanilide, m.p. 62–63°. This was identical with an authentic sample prepared from mandelic acid. The yield of calcium oxalate from the above aqueous extract was 2.9 g. (60%).

A solution of the ozonide from 10.0 g. of 1,4-diphenyl-3-phenylaminomaleimide was similarly decomposed to give, after recrystallization from toluene, 5.2 g. (72%) of oxanilide; m.p. and mixed m.p. 249–250°. The aqueous acetic acid solution was concentrated under diminished pressure and toluene was added until the distillate consisted only of toluene. The water extract from the dark residue was extracted with ether. Evaporation of the ether left 2 g. of gummy material which resisted purification. Extraction of the toluene layer with sodium bicarbonate solution led to the isolation of 1.2 g. of benzoic acid. No phenylglyoxylic acid could be detected.

NEWARK, DELAWARE

(6) (a) C. R. Noller and Roger Adams, THIS JOURNAL, **48**, 1074 (1926); (b) F. C. Whitmore and J. M. Church, *ibid.*, **54**, 3710 (1932); (c) J. M. Church, F. C. Whitmore and R. V. McGrew, *ibid.*, **56**, 176 (1934).

[CONTRIBUTION FROM THE SCHOOL OF SCIENCE, BRANDEIS UNIVERSITY]

New Nitrogen Mustards for "Toxagenic" Anti-cancer Agents[1a,b]

BY ORRIE M. FRIEDMAN AND ELIAHU BOGER

RECEIVED JANUARY 21, 1956

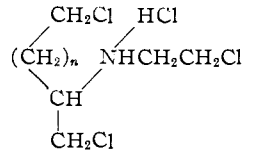
Two new secondary amine mustards, 5-chloro-1-(chloromethyl)-*n*-pentyl-2-chloroethylamine hydrochloride (V) and 6-chloro-1-(chloromethyl)-*n*-hexyl-2-chloroethylamine hydrochloride (VI), capable of intramolecular cyclization to potent tertiary amine mustards VII and VIII, respectively, have been synthesized. While both were phosphorylated with phosphorus oxychloride the latter VI, the more toxic of the two, appears ideal for the preparation of toxagenic phosphamides for use against tumors in which phosphamidase or functionally related enzymes may be found. The former V appears insufficiently toxic.

One of the promising approaches to cancer chemotherapy is based upon the use of toxagenic substrates—substances from which highly cytotoxic agents would be liberated by the action of enzymes.[2a–f] Advantage of a favorable distribution of an enzyme in a tumor might be taken, for example, by the use of a toxagenic substrate that would be acted upon by the enzyme to liberate a cytotoxic agent intracellularly. In this way a lethal dose of a cytotoxic agent might be delivered to the cells of the tumor whereas a relatively smaller, non-lethal dose would reach the cells of normal tissues in which less of the enzyme was present. This report describes the synthesis of two new nitrogen mustards of interest for the synthesis of toxagenic agents for use against malignant tumors in which the enzyme ahosphamidase[3a–g] or functionally related enzymes may be found.[2b]

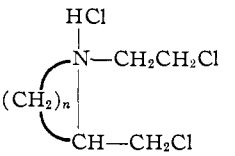
(1) (a) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Department of Health, Education and Welfare, U. S. Public Health Service. (b) Presented in part at the 128th meeting of the American Chemical Society, Minneapolis, September, 1955.

(2) (a) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **70**, 3082 (1948); (b) **76**, 655 (1954); (c) **76**, 658 (1954); (d) A. M. Seligman, M. M. Nachlas, L. H. Manheimer, O. M. Friedman and G. Wolf, *Ann. Surg.*, **130**, 333 (1949); (e) A. M. Seligman, M. Milden and O. M. Friedman, *Cancer*, **2**, 701 (1949); (f) A. M. Rutenberg, L. Persky, O. M. Friedman and A. M. Seligman, *J. Pharm. Exp. Therap.*, **III**, 483 (1954)

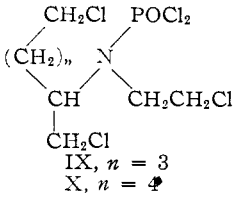
(3) (a) M. Ichihara, *J. Biochem. (Japan)*, **18**, 87 (1933); (b) E. Waldschmidt-Leitz, *Biochem. Z.*, **258**, 360 (1933); (c) H. Bredereck and E. Ceyer, *Z. physiol. Chem.*, **254**, 223 (1938); (d) G. Gomori, *Proc. Soc. Exp. Biol. Med.*, **69**, 407 (1948); (e) E. H. Strecker, *Dent. Med. Wochschr.*, **74**, 1268 (1951); (f) L. A. Tseitlin, *Biokhimiya*, **17**, 208 (1952); (g) J. Meyer and J. Weinman, *J. Dent. Res.*, **32**, 669 (1953).



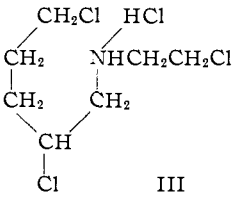
I, $n = 2$
V, $n = 3$
VI, $n = 4$



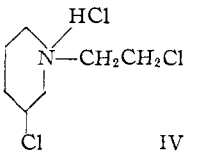
II, $n = 3$
VII, $n = 4$
VIII, $n = 5$



IX, $n = 3$
X, $n = 4$



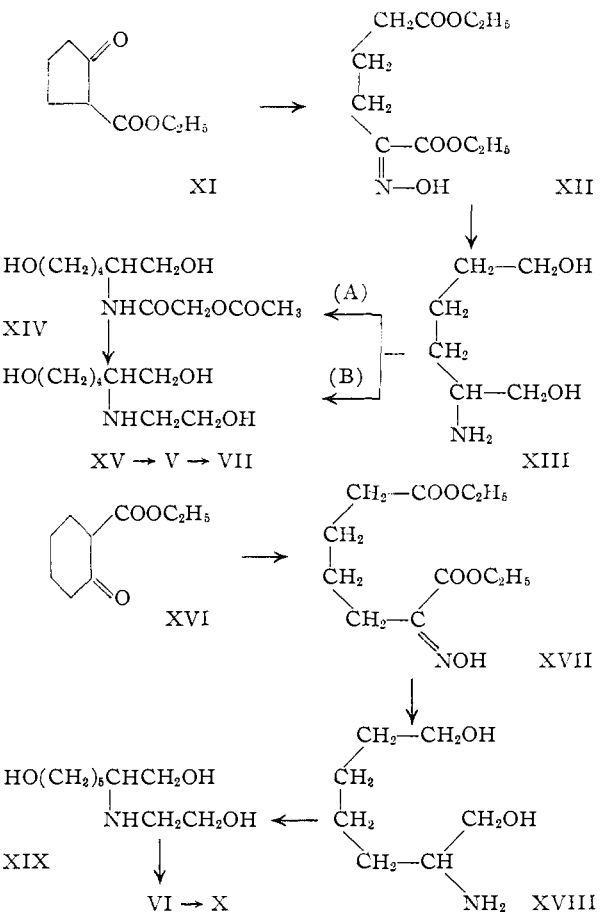
III



IV

N-Phosphorylated nitrogen mustards[2b] would serve ideally as toxagenic agents in this respect since they are biologically inactive *per se*,[2f] but would liberate a biologically potent nitrogen mustard on hydrolysis. Secondary amine mustards capable of intramolecular cyclization to potent tertiary amine mustards are required for the preparation of potentially chemotherapeutic phosphamides.[2c] Nitrogen mustards of this type previously prepared[2c] were found to be unsatisfactory. Although 4-chloro-1-(chloromethyl)-*n*-butyl-2-chloroethylamine hydrochloride (I) was highly toxic in mice,[2f] it spontaneously cyclized to the pyrrolidine II when phosphorylation with phosphorus oxychloride was attempted. The isomeric nitrogen mustard 2,5-dichloro-*n*-pentyl-2-chloroethylamine hydrochloride (III), on the other hand, was readily phosphorylated but proved to be significantly less toxic than I.[2f] The dichloro-piperidine derivative IV, however, to which III cyclizes, is appreciably more toxic than III, in contrast to the dichloropyrrolidine derivative II, which has essentially the identical high toxicity of I from which it is derived. These results strongly suggest that cyclization *in vivo* takes place very rapidly in the latter case and relatively slowly in the former. The low toxicity of III apparently derives at least in part from this fact.

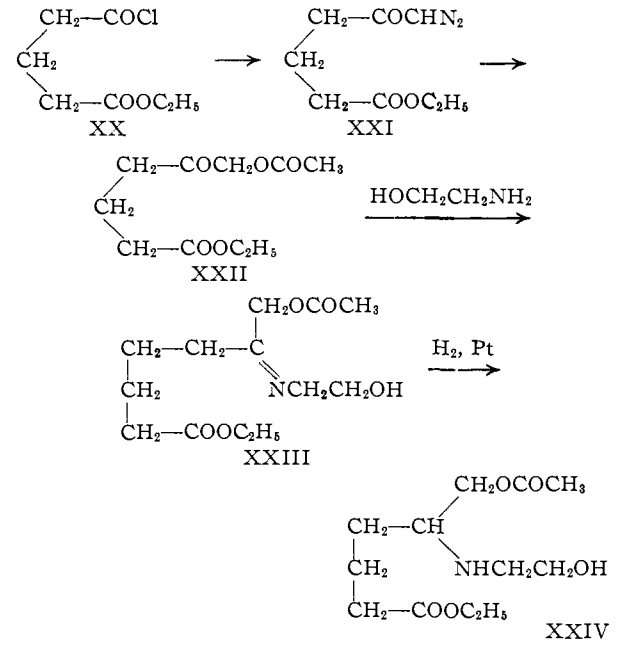
On the basis of these results the two nitrogen mustards of this new type that are reported here, 5-chloro-1-(chloromethyl)-*n*-pentyl-2-chloroethylamine hydrochloride (V) and 6-chloro-1-(chloromethyl)-*n*-hexyl-2-chloroethylamine hydrochloride (VI), were prepared. Both are homologs of I and resemble the highly potent nitrogen mustard, methyl-bis-(β-chloroethyl)-amine, to the extent that the functional chlorides are primary. The former V cyclized to a piperidine derivative VII and in this way resembles III, while the latter VI cyclized to a hexamethylene imine VIII. The lower homolog readily gave a phosphoramidic dichloride derivative IX with phosphorus oxychloride but showed low toxicity in mice, of the same order as III. The higher homolog VI,



however, appeared three to four times as toxic as V on the basis of preliminary toxicity data, and was also readily converted to the corresponding phosphoramidic dichloride X. The toxicity results are surprising in view of the expected less ready formation of the seven-membered ring by VI as compared to the six-membered ring by V. In fact, 6-bromohexylamine in dilute alkali gives only 5.7% of the cyclized hexamethylene imine[4] while 5-chloropentylamine yields piperidine almost quantitatively.[5] The trichloroamine VI is, nonetheless, well suited for the preparation of toxagenic phosphamides as it is sufficiently toxic to be lethal to tumor cells at low concentrations, and the phosphoramidic dichloride X derived from it is a versatile intermediate from which various desired derivatives may be prepared.[2b]

The two new mustards V and VI were ultimately prepared by essentially the same route. In the former, the cyclic ketoester XI prepared by a Dieckmann cyclization[6] of diethyl adipate, was converted to the α-ketodiethyl adipate oxime XII, which was in turn reduced with lithium aluminum hydride to 1,6-dihydroxy-2-aminohexane (XIII). The diolamine XIII was condensed with ethylene oxide to give the triolamine XV which on treatment with thionyl chloride gave the trichloroamine V as the hydrochloride. In the latter the

(4) A. Miller and P. Krauss, *Monatsh.*, **61**, 219 (1932).
(5) S. Gabriel, *Ber.*, **25**, 421 (1892).
(6) "Organic Syntheses," Col. Vol. II, Reinhold Publ. Corp., New York, N. Y., 1943, p. 116.



starting material employed was 2-carbethoxycyclohexanone XVI, prepared from cyclohexanone with diethyl oxalate.[7] This ketoester XVI was converted to the trichloroamine VI by a series of transformations XVI-XIX analogous to those for the lower homolog, XII to XV.

The triolamine XV was also prepared alternatively by treatment of the diolamine XIII with acetylglycolyl chloride followed by reduction of the amide XIV with lithium aluminum hydride.

An unsuccessful attempt to synthesize V was made starting with monoethyl-glutarate chloride (XX) which was transformed to the acetoxyketone XXII through the diazoketone XXI. The acetoxyketone XXII on condensation with ethanolamine in benzene produced the theoretical one equivalent of water, removed azeotropically, and gave a product with an absorption band at 6.12 μ in the infrared characteristic for the C=N—bond, presumably the Schiff-base XXIII. Reduction of the Schiff-base XXIII catalytically and subsequent reduction of the product XXIV with lithium aluminum hydride was intended to give the required triolamine XV, but neither the Schiff-base nor a mixture of the ketone XXII and ethanolamine in alcohol could be hydrogenated catalytically. The amine XXIV was not obtained even at high temperature and pressure over platinum.

Acknowlegment.—Technical assistance by Jerome E. Sheiffer and Maurice J. Elovitz is gratefully acknowledged.

Experimental[8]

1,6-Dihydroxy-2-aminohexane (XIII).—To a boiling mixture of 30 g. of lithium aluminum hydride and 200 ml. of tetrahydrofuran, a solution of 40 g. of 2-oximinoadipic acid ethyl ester XII[9] in 130 ml. of tetrahydrofuran was gradually added, and the mixture was refluxed for six hours. It was subsequently decomposed by careful dropwise addition of water, and the product was isolated by extraction with alcohol of the precipitated aluminum hydroxide. The alcoholic extract was concentrated to a small volume and diluted with tetrahydrofuran, and the precipitate filtered. The combined filtrates were concentrated, and the residual oil distilled.[10] The fraction, b.p. 135–145° (0.1 mm.), was collected, 14 g. (60% yield).

The diolamine XIII was characterized as its neutral oxalate, prepared with 0.5 molar equivalent of oxalic acid in alcoholic solution. The crude oxalate, m.p. 139°, was recrystallized from glycol monoethyl ether, m.p. 140.5°.

Anal. Calcd. for $C_{14}H_{32}N_2O_8$: C, 47.19; H, 8.98; N, 7.87. Found: C, 46.84; H, 8.95; N, 7.46.

N-2'-Hydroxyethyl-1,6-dihydroxy-2-aminohexane (XV). A. With Acetylglycolyl Chloride.—To a solution of 9 g. of the diolamine XIII and 10 ml. of triethylamine in 25 ml. of dimethylformamide, a solution of 8 ml. of acetylglycolyl chloride[11] in 8 ml. of dimethylformamide was added dropwise with cooling and stirring. The mixture was left overnight, heated one hour on a water-bath, then cooled and filtered to remove the crystalline precipitate of triethylamine hydrochloride. The filtrate was concentrated under reduced pressure and the residual acetylglycolyl amide XIV was dissolved in 100 ml. of dry tetrahydrofuran. This solution was added to a boiling solution of lithium aluminum hydride, 10 g., in 100 ml. of tetrahydrofuran, and the reaction was kept under reflux for six hours. It was then decomposed with water in the usual way and the product extracted from the precipitate with tetrahydrofuran and with alcohol successively. The residual oil obtained after elimination of the solvents was distilled[10] at 175–182° (0.1 mm.). The yield of the triolamine XV thus obtained was 1.8 g. (15%).

B. With Ethylene Oxide.—Into a cooled solution of the diolamine XIII (10 g.) in 10 ml. of alcohol, 8.25 ml. of a cooled solution of ethylene oxide in alcohol (1:1 by weight) was added. The mixture was kept in a refrigerator for three days, with occasional shaking, then allowed to stand for six hours at room temperature. It was then concentrated, and the residual oil distilled.[10] The triolamine XV fraction distilled at 175–183° (0.1 mm.). The yield was 5 g. (38%).

Anal. Calcd. for $C_8H_{19}NO_3$: C, 54.23; H, 10.73; N, 7.91. Found: C, 54.07; H, 10.81; N, 7.75.

5-Chloro-1-(chloromethyl-*n*-pentyl)-2-chloroethylamine Hydrochloride (V).—Dry hydrochloric acid gas was bubbled into a mixture of the triolamine XV (4 g.) and 8 ml. of chloroform. The mixture was then cooled and 6 ml. of thionyl chloride in 10 ml. of chloroform was added dropwise, with stirring. A drop of pyridine was then added, and the whole was left overnight. The mixture was subsequently diluted with 200 ml. of ether, in two portions, and the ether layer in each case decanted. Ethyl acetate, 5 ml., was then added and the solution allowed to evaporate for several hours. The residue was triturated and washed with cold ethyl acetate, and then recrystallized from the same solvent. The trichloroamine hydrochloride V, m.p. 101–102°, 1.8 g. (30% yield), was isolated as a crystalline white solid. More of the product remained in the mother liquor.

Anal. Calcd. for $C_8H_{17}NCl_4$: C, 35.72; H, 6.32; N, 5.21; Cl, 52.80. Found: C, 35.61; H, 6.32; N, 5.17, Cl, 52.80.

N-(2-Chloroethyl)-N-(1-chloromethyl-5-chloropentyl)-phosphoramidic Dichloride (IX).—The trichloroamine hydrochloride, V, 0.9 g., and 5 ml. of phosphorus oxychloride (freshly distilled, b.p. 106–107°) were heated for 20 hours under reflux. The excess of phosphorus oxychloride was eliminated by distillation under reduced pressure, and the residue was distilled.[16] A small amount of colorless crystals, probably **N-2-chloroethyl-2-chloromethylpiperidine hydrochloride (VII)**, sublimed at 133–140° (0.1 mm.). The phosphoramidic dichloride IX distilled at 175–180° (0.1 mm.) and was obtained as a colorless oil. The yield was 0.6 g. (52%).

Anal. Calcd. for $C_8H_{15}NOCl_5P$: C, 27.45; H, 4.28; N, 4.01; Cl, 50.80. Found: C, 27.29; H, 4.48; N, 3.98; Cl, 51.40.

(7) Ref. 6, p. 531.

(8) Microanalysis by S. M. Nagy and Associates, Microchemical Laboratory, M.I.T. All melting points are uncorrected.

(9) W. Dieckmann, *Ber.*, **33**, 586 (1900).

(10) A type of short-path distillation employing a series of glass bulbs heated in an air-bath was used.

(11) O. M. Friedman and A. M. Seligman, THIS JOURNAL, **76**, 661 (1954).

N-2'-Hydroxyethyl-1,7-dihydroxy-2-aminoheptane (XIX).—Ethyl 2-oximinopimelate (XVII) was prepared according to Dieckmann[12] from 2-carbethoxycyclohexanone (XVI). The oximinoester (25 g.) was reduced to 1,7-dihydroxy-2-aminoheptane (XVIII) by the procedure described for the preparation of XIII. The diolamine XVIII was distilled[10] at 150–155° (0.1 mm.); yield 9 g. (60%). It was then treated with ethylene oxide according to procedure B for the preparation of XV. The triolamine XIX thus formed was distilled[10] at 195–210° (0.1 mm.); yield 4.5 g. (36%).

Anal. Calcd. for $C_9H_{21}NO_3$: C, 56.60; H, 10.98; N, 7.33. Found: C, 57.02; H, 11.04; N, 7.61.

6-Chloro-1-(chloromethyl)-*n*-hexyl-2-chloroethylamine Hydrochloride (VI).—The triolamine XIX, 5 g., suspended in 60 ml. of chloroform and stirred vigorously, was treated with 10 ml. of thionyl chloride followed by three drops of pyridine. The mixture was stirred for 3 hours during which period a clear solution resulted, then allowed to stand at room temperature for 2 days. Solvent and thionyl chloride were distilled under reduced pressure, benzene was added and distilled to remove traces of thionyl chloride. The residue was treated with 10 ml. of ethyl acetate and left overnight in an open flask where it solidified. The product was triturated and washed with cold ethyl acetate, then recrystallized from the same solvent; m.p. 85–87°, yield 3.5 g. (47%).

Anal. Calcd. for $C_9H_{19}NCl_4$: C, 38.20; H, 6.72; N, 4.95; Cl, 50.13. Found: C, 38.14; H, 6.91; N, 4.87; Cl, 49.76.

N-(2-Chloroethyl)-N-(1-chloromethyl-6-chlorohexyl)-phosphoramidic Dichloride (X).—A mixture of the trichloroamine VI (1.0 g.) and freshly distilled phosphorus oxychloride (5 ml., b.p. 105–107°) was heated under reflux for 24 hours. The phosphorus oxychloride was eliminated by distillation and the residue distilled.[10] The fraction, b.p. 180–190° (0.1 mm.), was collected, yield 0.2 g.

Anal. Calcd. for $C_9H_{17}NOCl_5P$: C, 29.71; H, 4.68; N, 3.86; Cl, 48.83. Found: C, 30.07; H, 4.77; N, 4.19; Cl, 48.14.

5-Keto-6-acetoxycaproic Acid Ethyl Ester (XXII).—Monoethylglutarate chloride (XX) was prepared from monoethyl glutarate[13] and thionyl chloride by refluxing for 3–4 hours, the thionyl chloride in excess was then distilled and the acid chloride distilled under reduced pressure, b.p. 125° (35 mm.). From 14 g. of the monoester, 15 g. of the acid chloride was obtained.

A solution of 15 g. of the acid chloride XX in 50 ml. of ether was added, with cooling and stirring, to 450 ml. of a cooled and dried ethereal solution of diazomethane, prepared from 45 g. of nitrosomethylurea.[14] Evolution of nitrogen gas was observed. The solution was stirred in the cold for one-half hour and at room temperature for another two hours. The ether and excess of diazomethane were then eliminated under reduced pressure and the residual diazoketone XXI[15] transferred to a 100-ml. flask, to which was added 35 ml. of glacial acetic acid. The mixture was first stirred at room temperature until the evolution of nitrogen ceased and then heated gradually from 40 to 90°, until evolution of gas was complete. Potassium acetate, 3 g., was added and the whole was heated under reflux in an oil bath at 150–160° for one hour. The mixture was then cooled, poured into water (250 ml.) and extracted with benzene. The benzene layer was washed with water, sodium carbonate solution, and again with water, and finally dried over sodium sulfate. After the benzene was distilled, the ketone was distilled in a high vacuum, b.p. 129–135° (0.1 mm.); 148–151° at 9 mm. The yield was 12.5 g. (69%).

Anal. Calcd. for $C_{16}H_{16}O_5$: C, 55.55; H, 7.41. Found: C, 55.77; H, 7.46.

(12) W. Dieckmann, *Ber.*, **33**, 593 (1900).

(13) Markownikow, *J. Russ. Phys. Chem. Soc.*, **9**, 283; "Beilstein," Vol. II, H 633 (fourth edition, 1920).

(14) Ref. 6, p. 461.

(15) See "Organic Reactions," ed. R. Adams, Vol. I, 1942, p. 43.

WALTHAM 54, MASSACHUSETTS

[CONTRIBUTION FROM THE BIOLOGICAL LABORATORIES OF HARVARD UNIVERSITY]

Geometrical Isomerization of Vitamin A, Retinene and Retinene Oxime

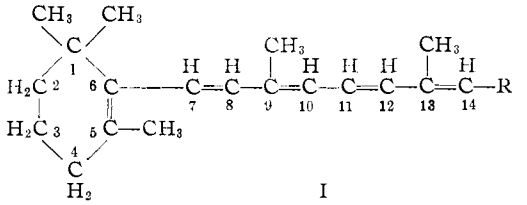
BY RUTH HUBBARD[1]

RECEIVED FEBRUARY 13, 1956

Stereoisomerization experiments have been performed with geometrical isomers of vitamin A, retinene and retinene oxime. These permit comparisons of the isomerization of mono-*cis* and di-*cis* forms, of unhindered and hindered *cis* linkages, and of the effects of terminal $-CH_2OH$, $-\overset{|}{C}=O$ and $-\overset{|}{C}=NOH$ groups.

Introduction

Vitamin A, retinene and retinene oxime possess the same carbon skeleton (I) containing five conjugated double bonds, to which vitamin A (R = $-CH_2OH$) adds an alcohol group, retinene (R = $-C{=}O$) a conjugated carbonyl group, and retinene oxime (R = $-C{=}NOH$) a conjugated C=N double bond. The terminal C=O and C=N linkages exert different effects on the conjugated system. The carbonyl group receives a large resonance contribution from the dipole, $\overset{+}{C}-\overset{-}{O}$, thereby enhancing the resonance of the entire conjugated system. The C=N linkage, on the other hand, is hardly more polar than a C=C double bond, so that the conjugated system of retinene oxime is comparable to that of a vitamin A analog containing one additional double bond. This family of compounds therefore presents the opportunity to study the effects of the intramolecular environment on the ease of isomerization of the various double bonds.



I

Six geometrical isomers of vitamin A and retinene have been identified.[2-5a,b] These are the all-

(1) This research was supported in part by grants to Prof. George Wald from the U. S. Public Health Service (Grant Number B-568C), and the Rockefeller Foundation. I should like to thank Prof. Wald for advice and criticism. My particular thanks are due to Mr. P. K. Brown for supplies of retinene isomers, and for a continuous interchange of ideas and observations.

(2) R. Hubbard, R. I. Gregerman and G. Wald, *J. Gen. Physiol.*, **36**, 415 (1952–53).

(3) C. D. Robeson, J. D. Cawley, L. Weisler, M. H. Stern, C. C. Eddinger and A. J. Chechak, THIS JOURNAL, **77**, 4111 (1955).