solution of dimethyl acetylenedicarboxylate (77.1 g., 0.543 mole) in hexachlorocyclopentadiene (292.1 g., 1.07 mole) was heated to 140°, but without further external heating the temperature rose rapidly to 230° while the light yellow solution became deep carmine red. The exothermic reaction was cooled rapidly to 0°. The solution was then reheated at 147° for 2 hours and finally briefly at 200°. The solution was steam distilled until unreacted hexachlorocyclopentadiene no longer came over in the distillate. The residual aqueous mixture was extracted with ether and the ether extract was treated with charcoal and dried over calcium chloride. The dried ether solution was concentrated by evaporation and methanol was added, causing precipitation of dimethyl 1,4,5,6,7,7-hexachloro-2,5-norbornadiene-2,3-dicarboxylate as white crystals (162.1 g., 0.391 mole, 72%), m.p. 85–86°. The near ultraviolet spectrum in 95% ethanol contained no maxima and only ill-defined inflections at about 288 mμ (log ε 2.42) and 230 (3.58), with rising end absorption (at 220 mμ log ε is 3.80 and at 215, 4.00); $\nu_{C=C}$ ~ 1720 in $CHCl_3$, 1725 in Nujol; $\nu_{C=C}$ 1629, 1596 cm.$^{-1}$ in $CHCl_3$ and in Nujol.

Anal. Calcd. for $C_{10}H_6O_4Cl_6$ (414.90): C, 31.84; H, 1.46. Found: C, 32.06; H, 1.57.

B. By Re-esterification of the Diacid.—A solution of 1,4,5,6,7,7-hexachloro-2,5-norbornadiene-2,3-dicarboxylic acid (2.0 g., 0.0052 mole) and boron fluoride etherate (Baker and Adamson technical grade, 5 cc.) in methanol (50 cc.) was refluxed for 5 hours. Sodium bicarbonate solution (5%, 30 cc.) caused the diester to precipitate. Recrystallization from aqueous 80% methanol gave white crystals (1.2 g., 0.0029 mole, 56%), which gave no depression in mixed melting point with the sample from Part A. The infrared spectra of the two samples in Nujol were identical.

1,4,5,6,7,7-Hexachloro-2,5-norbornadiene-2,3-dicarboxylic Acid (XI).[12]—A mixture of dimethyl 1,4,5,6,7,7-hexachloro-2,5-norbornadiene-2,3-dicarboxylate (25.0 g., 0.0603 mole) and aqueous 5% potassium hydroxide (200 cc.) was refluxed for 6 hours. The resulting dark brown solution was cooled to room temperature, filtered to remove traces of unreacted starting material, and then cooled to 0°. Concentrated sulfuric acid was added slowly, with vigorous shaking, to *p*H 2, causing precipitation of a white solid, assumed to be the monopotassium salt since on ignition it left a residue which gave an orange flame test. Additional concentrated sulfuric acid (20 cc.) was added and the mixture was stirred for 5 minutes. The resulting solid precipitate no longer left a residue upon ignition. The mixture was extracted with ether, which dissolved the precipitate. The ether extract was dried over calcium chloride and treated with charcoal. Most of the ether was evaporated off, light petroleum (b.p. 40–75°) was added, and the solution was set aside overnight. The resulting precipitate was recrystallized from a fairly large amount of water, yielding the hydrate as white crystals (25.5 g.), which dehydrate at ~120–150°, m.p. 190–191.5°. The hydrate shows a great tendency to oil out of concentrated water solutions and invariably oils out of mixtures of water with acetone, methanol, ethanol or dioxane. Ether–light petroleum (b.p. 40–75°) may be used as a recrystallization solvent, but this combination does not produce as pure a product as does water.

Drying of the hydrate at 100° (1 mm.) for 5 hours yielded 1,4,5,6,7,7-hexachloro-2,5-norbornadiene-2,3-dicarboxylic acid as white crystals (18.2 g., 0.0470 mole, 78%) m.p. 192.5–193.5°, reported[8] m.p. 162–163°; ν_{OH} ~2690, 2550; $\nu_{C=O}$ 1715, 1694; $\nu_{C=C}$ 1637, 1606 cm.$^{-1}$ in Nujol; pK_{a1} is 3.75 and pK_{a2} is 6.15 in 50% (by volume) aqueous ethanol at 32°.

Anal. Calcd. for $C_9H_2O_4Cl_6$ (386.85): C, 27.94; H, 0.52. Found: C, 27.97; H, 0.72.

MINNEAPOLIS 14, MINN.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOLOGICAL SCIENCES, STANFORD RESEARCH INSTITUTE]

Potential Anticancer Agents.[1] XXVII. Synthesis of Alkylating Agents Derived from 6-Amino-6-deoxy-D-glucose

BY ELMER J. REIST, ROLAND R. SPENCER AND B. R. BAKER

RECEIVED AUGUST 14, 1959

The synthesis of 6-[bis-(2-chloroethyl)-amino]-6-deoxy-D-glucose hydrochloride (XXIII), 6-[(2-chloroethyl)-ethylamino]-6-deoxy-D-glucose hydrochloride (XXIV) and 6-(2-chloroethylamino)-6-deoxy-D-glucose hydrochloride (XXV) *via* the key intermediate 3,5-O-benzylidene-1,2-O-isopropylidene-6-O-*p*-tolylsulfonyl-D-glucofuranose (XI) is described.

A rationale for the design of specific irreversible enzyme inhibitors was presented in paper XVII of this series.[2] This rationale proposed that substrates, properly substituted by an alkylating group, could fit the specific enzyme site for the substrate, then replace a nearby active hydrogen by alkylation, thus resulting in specific irreversible inactivation of the enzyme.

Recent work devoted to the synthesis of nitrogen mustards in which the carrier is a metabolite, or resembles a metabolite, has resulted in several promising anticancer compounds, such as phenylalanine mustard (sarcolysin),[3] *m*-phenylalanine mustard,[2,4] chlorambucil,[5] uracil mustard,[6] and benzimidazole mustard.[7] As part of the rationale,[2] it was proposed that a nitrogen mustard, attached to a substrate as a carrier, might be a type of compound that could operate as a specific irreversible enzyme inhibitor. If such is the case, then a "one-armed" mustard should be as good as, or possibly even better than, the corresponding "two-armed" mustard as an anticancer agent.

Vargha and co-workers[8,9] have synthesized several nitrogen mustards in the sugar series which have some interesting biological activities. N,N-Bis-(2-chloroethyl)-D-glucosamine hydrochloride (I)

(1) This work was carried out under the auspices of the Cancer Chemotherapy National Service Center, National Cancer Institute, Contract No. SA-43-ph-1892. For the preceding paper in this series, *cf.* E. J. Reist, P. A. Hart and B. R. Baker, *J. Org. Chem.*, **24**, 1040 (1959).

(2) H. F. Gram, C. W. Mosher and B. R. Baker, THIS JOURNAL, **81**, 3103 (1959).

(3) F. Bergel, V. C. E. Burnop and J. A. Stock, *J. Chem. Soc.*, 1223 (1955); L. F. Larinov, A. S. Khokhlov, E. N. Shkodinskaia, O. S. Vasina, V. I. Trusheikina and M. A. Novikova, *Lancet*, **269**, 169 (1955).

(4) T. S. Osdene, D. N. Ward, W. H. Chapman and H. Rakoff, THIS JOURNAL, **81**, 3100 (1959).

(5) J. L. Everett, J. J. Roberts and W. C. J. Ross, *J. Chem. Soc.*, 2386 (1953).

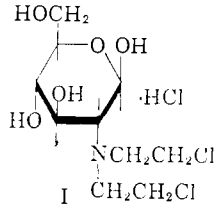
(6) D. A. Lyttle and H. G. Petering, THIS JOURNAL, **80**, 6459 (1958).

(7) E. Hirschberg, A. Gellhorn and W. S. Gump, *Cancer Research*, **17**, 904 (1957).

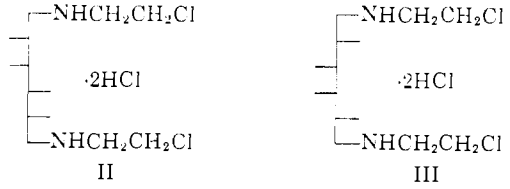
(8) L. Vargha, L. Toldy, Ö. Fehér and S. Lendvai, *J. Chem. Soc.*, 805 (1959).

(9) L. Vargha, Ö. Fehér and S. Lendvai, *ibid.*, 810 (1959).

was found to be extremely toxic, with some antitumor activity.[9] On the other hand, 1,6-bis-(2-chloroethylamino)-1,6-dideoxy-D-mannitol dihydrochloride (II) was found to be strongly cytoactive and to have tumor-inhibiting activity.[8] It may be



I

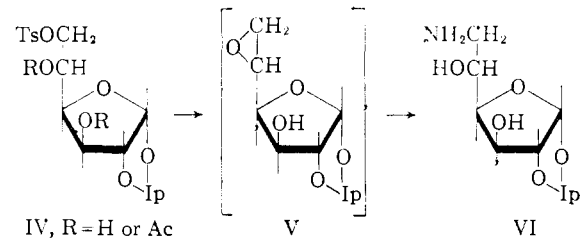


II III

argued that the mannitol mustard (II) meets the requirement of difunctionality which has seemed necessary for activity but it is certainly obvious that II is not a bis-nitrogen mustard in the normally accepted sense, in that the two arms of the mustard of II are not connected to the same nitrogen. It is interesting to note that 1,6-bis-(2-chloroethylamino)-1,6-dideoxydulcitol (III)[10] and N,N'-bis-(2-chloroethyl)-1,6-hexanediamine[8] were found to be inactive.

In the light of these results, it is apparent that chloroethylamines other than the bis-chloroethylamines can have antitumor activity and that more work is necessary to correlate the activity of the bis-nitrogen mustards with monofunctional nitrogen mustards. Since the sugar nitrogen mustards represent a relatively unexplored territory and fit nicely into the carrier–antimetabolite concept,[2,11] a program was instituted toward the synthesis of nitrogen mustards of a variety of amino sugars. The synthesis of three nitrogen mustards derived from 6-amino-6-deoxy-D-glucose, namely, 6-[bis-(2-chloroethyl)-amino]-6-deoxy-D-glucose hydrochloride (XXIII), 6-[(2-chloroethyl)-ethylamino]-6-deoxy-D-glucose hydrochloride (XXIV) and 6-(2-chloroethylamino)-6-deoxy-D-glucose hydrochloride (XXV), is the subject of this paper.

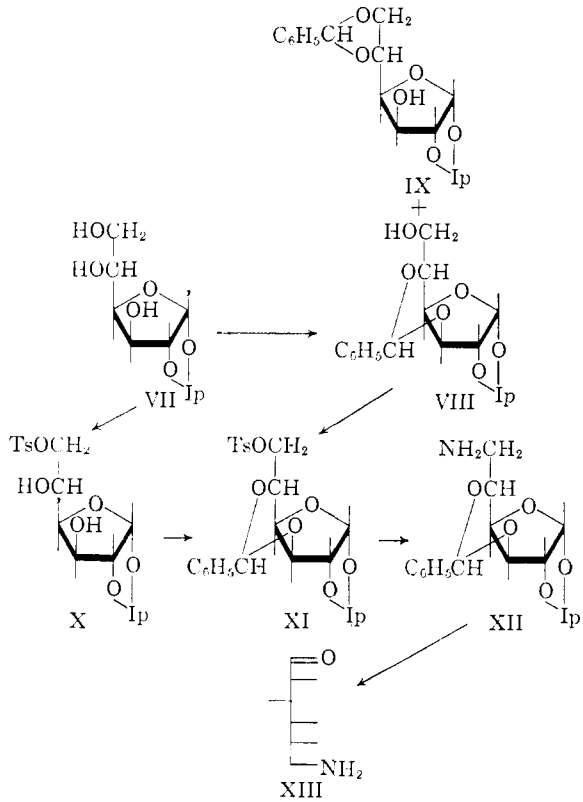
The synthesis of 6-amino-6-deoxy-D-glucose and several of its derivatives has been accomplished by a variety of methods,[12,13] one of the smoothest of which utilizes the treatment of a 5,6-anhydro-D-glucose or a potential 5,6-epoxide with ammonia, as illustrated in the sequence IV → VI. This approach suffers the disadvantage of yielding a 6-aminoglucose derivative which possesses unblocked hydroxyls; these could easily cause undesired side reactions in a subsequent step involving the chlorination of a 2-hydroxyethylamine with thionyl



IV, R = H or Ac V VI

chloride. Thus, it is desirable to have all the sugar hydroxyls blocked by groups which can withstand the basic conditions involved in the displacement of the 6-tosylate by amines and which can be removed by acid hydrolysis. A starting material such as 1,2:3,5-di-O-isopropylidene-6-O-*p*-tolylsulfonyl-D-glucofuranose,[13a] 3,5-O-benzylidene-1,2,O-isopropylidene-6-O-methylsulfonyl-D-glucofuranose[13b] or 3,5-O-benzylidene-1,2-O-isopropylidene-6-*p*-tolylsulfonyl-D-glucofuranose (XI)[14] meets these qualifications. The latter was chosen as the most convenient starting material.

The synthesis of XI has been reported in several laboratories[14] starting from 1,2-O-isopropylidene-D-glucofuranose (VII), usually *via* 3,5-O-benzylidene-1,2-O-isopropylidene-D-glucofuranose (VIII). When this sequence was used in these laboratories,



the product was nearly always contaminated with varying amounts of by-product, probably the isomeric 5,6-O-benzylidene derivative IX[14a] which hindered crystallization of the desired VIII. Selective tosylation of VII to give 1,2-O-isopropylidene-

(10) L. Vargha, *Am. N. Y. Acad. Sci.*, **68**, 875 (1958).

(11) F. Bergel, *ibid.*, **68**, 1238 (1958).

(12) (a) E. Fischer and K. Zach, *Ber.*, **44**, 132 (1911); (b) H. Ohle and L. Vargha, *ibid.*, **61**, 79 (1928); (c) J. M. Grosheintz and H. O. L. Fischer, THIS JOURNAL, **70**, 1476 (1948); (d) F. Cramer, H. Otterbach and H. Springmann, *Ber.*, **92**, 384 (1959).

(13) (a) H. Ohle and L. Vargha, *ibid.*, **62**, 2425 (1929); (b) B. Helferich and R. Mittag, *ibid.*, **71B**, 1585 (1938).

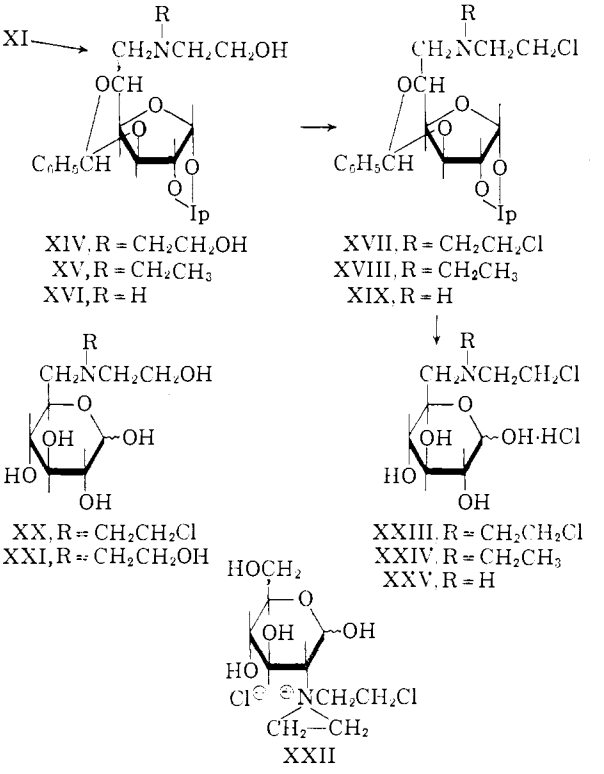
(14) (a) P. A. Levene and A. L. Raymond, *ibid.*, **66B**, 384 (1933); (b) D. J. Bell, E. Friedmann and S. Williamson, *J. Chem. Soc.*, 252 (1937); (c) A. S. Meyer and T. Reichstein, *Helv. Chim. Acta*, **29**, 139 (1946); (d) P. Karrer and A. Boettcher, *ibid.*, **36**, 570 (1953).

6-O-*p*-tolylsulfonyl-D-glucofuranose (X) followed by treatment of the 6-tosylate X with benzaldehyde, a sequence described by Meyer and Reichstein,[14c] obviated this difficulty and gave a product (XI) which was easily purified by recrystallization. It was found that the crude tosylate X was satisfactory for the reaction with benzaldehyde to give XI; the over-all yield (30%) of XI from VII was higher from the crude tosylate X than from recrystallized X (14%).

There has been no mention in the literature concerning the displacement of the *p*-tolylsulfonyl group of XI with ammonia or amines. However, the 6-amine XII has been synthesized by treatment of the 6-O-mesylate of VIII with liquid ammonia for three weeks[13b]; displacement of the tosyl from 1,2:3,5-di-O-isopropylidene-6-O-*p*-tolylsulfonyl-D-glucofuranose by methanolic ammonia at 100° for 4 hours has also been reported.[13a] Ammonolysis of the 3,5-benzylidene-6-tosylate XI with methanolic ammonia at 100° gave a 67% yield of the crude *p*-toluenesulfonic acid salt of 6-amino-3,5-O-benzylidene-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XII). Conversion to the free base and recrystallization from ethanol gave pure XII in 52% yield; melting point and rotation were in satisfactory agreement with those reported by Helferich and Mittag.[13b] Removal of the blocking groups with 3 *N* hydrochloric acid gave a quantitative yield of crude 6-amino-6-deoxy-D-glucose (XIII) hydrochloride, which was recrystallized in 41% yield, m.p. 163–165° dec.[15]

Treatment of the tosylate XI with the various ethanolamines at 140–160° for 3 hours was adequate to bring about the displacement of the 6-tosylate. Thus, 2,2′-iminodiethanol gave an 81% yield of 3,5-O-benzylidene-6-deoxy-6-[bis-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (XIV), 2-ethylaminoethanol gave 64% of 3,5-O-benzylidene-6-deoxy-6-[ethyl-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (XV), and 2-aminoethanol gave 81% of 3,5-O-benzylidene-6-deoxy-6-(2-hydroxyethylamino)-1,2-O-isopropylidene-D-glucofuranose (XVI), all as crystalline solids.

Chlorination of the bis-(hydroxyethyl)-amine XIV with thionyl chloride in dichloromethane[16] gave crystalline 3,5-O-benzylidene-6-[bis-(2-chloroethyl)-amino]-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XVII) in 70% yield. Hydrolysis of the blocked bis-mustard XVII with 6 *N* hydrochloric acid gave 6-[bis-(2-chloroethyl)-amino]-6-deoxy-D-glucose hydrochloride (XXIII) as an uncrystallizable sirup which was homogeneous, as shown by paper chromatography,[17] with R_g 1.28, and was analytically pure. The use of 6 *N* hydrochloric acid is a critical point. More concentrated acid causes excessive decomposition,



whereas more dilute acid gives the mustard contaminated with a second material, as shown by paper chromatography. This second material, with R_g 0.95, is thought to be the partially hydrolyzed N-hydroxyethyl-N-chloroethylamine (XX), since the dihydroxyamine XXI is an even slower moving material in this paper chromatography system, having R_g 0.58.

Although the bis-mustard hydrochloride XXIII, as an anhydrous sirup, appears to be relatively stable, an aqueous solution of XXIII underwent alteration over a period of days at room temperature. Paper chromatography[17] of this solution showed the conversion of XXIII to be a material with R_g 0.95 which was presumably the same as the second component of the dilute acid hydrolysis, and was assumed to be the partially hydrolyzed mustard XX. It should be noted that Vargha[9] observed a similar behavior by aqueous solutions of glucosamine mustard (IV), in that about half of the chlorine of IV was converted to chloride ions. He attributed this change to the formation of the aziridinium cation XXII. Although it seems likely that solutions of IV and XXIII suffer the same type of decomposition, no further work has been done to determine the product of this decomposition.

Treatment of the N-hydroxyethylamine XV with thionyl chloride in dichloromethane gave crystalline 3,5-O-benzylidene-6-[(2-chloroethyl)-ethylamino]-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XVIII) in 60% yield. Hydrolysis of the blocked mustard XVIII with 6 *N* hydrochloric acid gave a quantitative yield of 6-[(2-chloroethyl)-ethylamino]-6-deoxy-D-glucose hydrochloride (XXIV) as an uncrystallizable sirup which, however, was homogeneous as

(15) M. J. Cron, O. B. Fardig, D. L. Johnson, H. Schmitz, D. F. Whitehead, I. R. Hooper and R. U. Lemieux, THIS JOURNAL, **80**, 2342 (1958).

(16) The use of dichloromethane in place of chloroform as solvent gave much less darkening during the chlorination; *cf.* ref. 4.

(17) Paper chromatograms were run by the descending technique on Whatman No. 1 paper with *n*-butyl alcohol–acetic acid–water (4:1:5). Spots were detected by an aniline citrate spray. Glucose was used as a standard and spot locations were expressed as R_g units, with glucose at R_g 1.00.

shown by paper chromatography,[17] with R_g 0.91, and was analytically pure.

It is interesting to note that whereas an aqueous solution of the bis-mustard XXIII had decomposed in three to four days, as shown by paper chromatography,[17] an aqueous solution of the "one-armed" mustard XXIV showed no evidence of decomposition and, even after several weeks at room temperature, the solution maintained its homogeneity, as shown by paper chromatography.

Treatment of the blocked monohydroxyethylamine XVI with thionyl chloride in dichloromethane gave an uncrystallizable oil which was presumably the blocked chloroethylamine XIX. Hydrolysis of XIX with 6 *N* hydrochloric acid gave the sirup 6-(2-chloroethylamino)-6-deoxy-D-glucose hydrochloride (XXV), which was essentially homogeneous on paper chromatography,[17] with R_g 1.00, and was analytically pure.

Experimental[17,18]

3,5-O-Benzylidene-1,2-O-isopropylidene-6-O-p-tolylsulfonyl-D-glucofuranose (XI) was prepared by a slight modification of the procedure described by Meyer and Reichstein[14c] in which 45 g. of 1,2-O-isopropylidene-D-glucofuranose[19] was converted to 47.5 g. (61%) of the crude chloroform extracted tosylate as a tan-colored sirup. Treatment of the crude tosylate X with 150 ml. of freshly distilled benzaldehyde and 40.0 g. of zinc chloride gave after recrystallization from methanol, 28.5 g. (30% based on VII) of XI, m.p. 110–112°, $[\alpha]D$ +15° (1% in chloroform); λ_{max}^{KBr} 7.28 (CH_3, OSO_2), 8.50 (OSO_2), 9.20, 9.82 (C-O-C), 13.20, 14.28 μ (monosubstituted phenyl). Bell, *et al.*,[14b] report m.p. 118° and $[\alpha]D$ +14.2° (chloroform).

6-Amino-3,5-O-benzylidene-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XII).—A solution of 6.0 g. (13.0 mmoles) of 3,5-O-benzylidene-1,2-O-isopropylidene-6-*O*-*p*-tolylsulfonyl-D-glucofuranose (XI) in 50 ml. of absolute methanol, which had been previously saturated with anhydrous ammonia at 0°, was heated in a Parr bomb at 100° for 24 hours. At the end of this time, the reaction mixture was cooled, then poured into 100 ml. of a 1:1 mixture of ice and saturated aqueous sodium bicarbonate and extracted with three 50-ml. portions of chloroform. The chloroform extracts were washed with two 25-ml. portions of water, then combined, dried over magnesium sulfate and evaporated to dryness *in vacuo* to yield 2.68 g. (67%) of a pale yellow, gummy solid. Recrystallization from 6 ml. of absolute ethanol gave 2.07 g. (52%) of white crystals, m.p. 120–125°, $[\alpha^{30}]D$ +24.5° (0.66% in chloroform). Helferich and Mittag[13b] reported m.p. 127° and $[\alpha]^{21}D$ +25.4° (chloroform).

6-Amino-6-deoxy-D-glucose (XIII) Hydrochloride.—A solution of 300 mg. (3.3 mmoles) of 6-amino-3,5-O-benzylidene-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XII) in 1 ml. of methanol was heated on a steam-bath with 2 ml. of 3 *N* hydrochloric acid for 15 minutes. After being cooled, the aqueous solution was extracted with two 5-ml. portions of chloroform. The aqueous layer was lyophilized to give 210 mg. of a white foam which gave a positive test with Benedict reagent for reducing sugars and traveled as a single spot with R_f 0.07 on paper chromatography.[17]

From a similar run, recrystallization of 700 mg. of the foam from 4 ml. of methanol gave 290 mg. (41%) of a white solid, m.p. 172–176°. After two more recrystallizations from methanol, it had m.p. 163–165° dec., $[\alpha]D$ +18° → +33° (0.36% in water); λ_{max}^{KBr} 2.98 (OH), 6.20, 6.62 (NH_3^+),[1] 9.07, 9.45, 9.82 μ (C-O-C and C-OH). Cron, *et al.*,[15] reported m.p. 161–162° dec., $[\alpha]^{25}D$ + 23.0° → + 50.1° 1.0% in water), R_f 0.06.[17]

Anal. Calcd. for $C_6H_{13}NO_5 \cdot HCl$: C, 33.4; H, 6.54; Cl, 16.4; N, 6.49. Found: C, 33.4; H, 6.65; Cl, 16.1; N, 6.39.

(18) Melting points were taken on a Fisher–Johns apparatus and are uncorrected. Optical rotations were measured with a Standard Polarometer model D attachment to the Beckman DU spectrophotometer calibrated with standard sucrose solutions.

(19) S. C. Laland, *Acta Chem. Scand.*, **8**, 866 (1954).

3,5-O-Benzylidene-6-deoxy-6-[bis-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (XIV).—A solution of 0.50 g. (1.1 mmoles) of 3,5-O-benzylidene-1,2-O-isopropylidene-6-O-*p*-tolylsulfonyl-D-glucofuranose (XI) in 5 ml. of 2,2′-iminodiethanol was heated at 150–160° for 3 hours. The solution was cooled to room temperature, then diluted with 15 ml. of chloroform. The chloroform solution was washed with two 5-ml. portions of water. The water washes were back extracted with 3 ml. of chloroform. The combined chloroform layers were dried over magnesium sulfate, then concentrated to dryness *in vacuo* to give 0.39 g. (93%) of a white solid, m.p. 120–123°. Recrystallization from ethanol gave 0.21 g. (50%) of white crystals, m.p. 127.5–128.5°, $[\alpha]^{33}D$ +19.2° (0.5% in water); λ_{max}^{KBr} 2.90 (OH), 7.25 (CH_3), 9.25, 9.80 (C-O-C and C-OH), 13.14, 14.28 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{20}H_{29}NO_7$: C, 60.7; H, 7.39; N, 3.54. Found: C, 60.9; H, 7.18; N, 3.59.

On a large scale, 50 g. of tosylate XI gave 33 g. (81%) of crystalline product, m.p. 127.5–128.5°, that was suitable for the next step.

3,5-O-Benzylidene-6-deoxy-6-[ethyl-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (XV) was prepared in the manner described for XIV from 2.5 g. of 3,5-O-benzylidene-1,2-O-isopropylidene-6-O-*p*-tolylsulfonyl-D-glucofuranose (XI) and 5 ml. of 2-(ethylamino)-ethanol to give, after two recrystallizations from Skellysolve C, 1.31 g. (64%) of crystalline material, m.p. 101–102°.

A similar preparation had m.p. 105.5–106.5° $[\alpha]^{24}D$ +14.4° (0.5% in ethanol); λ_{max}^{KBr} 2.90 (OH), 7.22 (CH_3), 8.88, 9.24, 9.82 (C-O-C, C-OH), 13.24, 14.32 μ (monosubstituted phenyl) and was analyzed.

Anal. Calcd. for $C_{20}H_{29}NO_6$: C, 63.3; H, 7.70; N, 3.69. Found: C, 63.3; H, 7.29; N, 3.76.

On a large scale, 60 g. of tosylate XI gave 42.0 g. (76%) of product, m.p. 104–105°, that was suitable for the next step.

3,5-O-Benzylidene-6-deoxy-6-(2-hydroxyethylamino)-1,2-O-isopropylidene-D-glucofuranose (XVI) was prepared from 60 g. of 3,5-O-benzylidene-1,2-O-isopropylidene-6-O-*p*-tolylsulfonyl-D-glucofuranose (XI) and 900 ml. of 2-aminoethanol in the same manner as XIV to give, after two recrystallizations from Skellysolve C, 33.9 g. (81%) of white crystals, m.p. 137–139°.

A similar preparation formed white needles upon recrystallization from ethanol-Skellysolve C, m.p. 138.5–139.5° $[\alpha]^{24}D$ +26° (0.5% in ethanol); λ_{max}^{KBr} 2.91 (OH), 7.25 (CH_3), 9.30, 9.80 (C-O-C and C-OH), 13.19, 14.28 μ (monosubstituted phenyl) and was analyzed.

Anal. Calcd. for $C_{18}H_{25}NO_6 \cdot 1/2C_2H_5OH$: C, 61.0; H, 7.48; N, 3.75. Found: C, 60.9; H, 7.12; N, 4.12.

3,5-O-Benzylidene-6-[bis-(2-chloroethyl)-amino]-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XVII).—A solution of 1.0 g. (2.52 mmoles) of 3,5-O-benzylidene-6-deoxy-6-[bis-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (XIV) in 20 ml. of dry dichloromethane was treated with 3.0 ml. (26.2 mmoles) of thionyl chloride, then heated under reflux for 1 hour. The reaction mixture was cooled, then poured onto 50 g. of ice. The resulting mixture was adjusted to *p*H 6 with saturated aqueous sodium carbonate (about 40 ml. was required); then the dichloromethane was separated, dried over magnesium sulfate and evaporated to dryness *in vacuo* to yield 1.04 g. (95%) of a yellow sirup, which crystallized slowly. Recrystallization from 4 ml. of absolute ethanol gave 0.76 g. (70%) of white needles, m.p. 108–109°, $[\alpha]^{25}D$ +8.9° (0.9% in chloroform); λ_{max}^{KBr} no OH at 2.9, 7.25 (CH_3), 9.24, 9.85 μ (C-O-C), 13.25, 14.33 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{20}H_{27}Cl_2NO_5$: C, 55.5; H, 6.25; Cl, 16.4; N, 3.24. Found: C, 55.5; H, 6.38; Cl, 16.2; N, 3.22.

6-[Bis-(2-chloroethyl)-amino]-6-deoxy-D-glucose Hydrochloride (XXIII).—A solution of 610 mg. (1.4 mmoles) of 3,5-O-benzylidene-6-[bis-(2-chloroethyl)-amino]-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XVII) in 6 ml. of 6 *N* hydrochloric acid was heated on a steam-bath for 30 minutes. The reaction mixture was cooled, then extracted with 4 ml. of chloroform. Lyophilization of the aqueous layer after treatment with Norit gave 320 mg. (67%) of a white foam. Paper chromatography[17] showed a strong spot at Rg 1.35.

Anal. Calcd. for $C_9H_{19}Cl_2NO_5 \cdot HCl$: C, 35.3; H, 5.92; Cl, 31.2; N, 4.12. Found: C, 35.2; H, 5.8; Cl, 31.4; N, 4.27.

3,5-O-Benzylidene-6-[(2-chloroethyl)-ethylamino]-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XVIII).—A solution of 2.0 g. (5.26 mmoles) of 3,5-O-benzylidene-6-deoxy-6-[ethyl-(2-hydroxyethyl)-amino]-1,2-O-isopropylidene-D-glucofuranose (XV) in 50 ml. of dry dichloromethane was treated with 2.0 ml. (27.7 mmoles) of thionyl chloride at reflux temperature for 1.5 hours. The solution was cooled, diluted with 20 ml. of dry dichloromethane, then added dropwise with stirring to 200 ml. of saturated aqueous carbonate. The dichloromethane layer was separated, washed with 25 ml. of water, dried over magnesium sulfate, then evaporated to dryness *in vacuo* to yield 1.82 g. (86%) of a tan sirup. Recrystallization from absolute ethanol gave 1.26 g. (60%) of white crystals, m.p. 65–67°, $[\alpha]^{28}D$ +4.8° (0.5% in ethanol); λ_{max}^{KBr} no OH at 2.9, 7.25 (CH_3), 9.12, 9.26, 9.84 (C-O-C), 13.20, 14.30 μ (monosubstituted phenyl).

Anal. Calcd. for $C_{20}H_{28}ClNO_5$: C, 60.3; H, 7.04; Cl, 8.92; N, 3.52. Found: C, 60.3; H, 7.30; Cl, 8.86; N, 3.51.

6-[(2-Chloroethyl)-ethylamino]-6-deoxy-D-glucose hydrochloride (XXIV) was prepared from 3,5-O-benzylidene-6-[(2-chloroethyl)-ethylamino]-6-deoxy-1,2-O-isopropylidene-D-glucofuranose (XVIII) by the procedure used for the preparation of 6-[bis-(2-chloroethyl)-amino]-6-deoxy-D-glucose hydrochloride (XXIII). The "one-armed" mustard was obtained in quantitative yield as a pale brown sirup which was homogeneous on paper chromatography[17], with R_g 0.91.

Anal. Calcd. for $C_{10}H_{20}ClNO_5 \cdot HCl \cdot 1/3H_2O$: C, 38.5; H, 6.94; Cl, 22.8. Found: C, 38.7; H, 7.22; Cl, 22.3.

6-(2-Chloroethylamino)-6-deoxy-D-glucose Hydrochloride (XXV).—A solution of 2.53 g. (7.2 mmoles) of 3,5-O-benzylidene-6-deoxy-6-(2-hydroxyethyl-amino)-1,2-O-isopropylidene-D-glucofuranose (XVI) and 2.53 ml. (35.2 mmoles) of thionyl chloride in 20 ml. of dry dichloromethane was heated at reflux for 1 hour. The reaction mixture was allowed to cool to room temperature, then it was diluted with 10 ml. of dry dichloromethane and added dropwise with stirring to 75 ml. of saturated aqueous sodium carbonate. The dichloromethane layer was separated and the aqueous layer was extracted with 20 ml. of dichloromethane. The combined dichloromethane layers were dried over magnesium sulfate, then evaporated to dryness *in vacuo* to yield the blocked mustard XIX as an oil.

The crude blocked mustard XIX was heated on a steam-bath with 10 ml. of 6 *N* aqueous hydrochloric acid for 30 minutes. After being cooled to room temperature, the acid solution was extracted with two 10-ml. portions of chloroform, then the aqueous layer was lyophilized to give 0.96 g. of a brown foam which contained a major spot on paper chromatography,[17] with R_g 1.02 plus a trace component with R_g 0.60.

Anal. Calcd. for $C_8H_{16}ClNO_5 \cdot HCl$: C, 34.6; H, 6.12; Cl, 25.5; N, 5.03. Found: C, 35.2; H, 6.21; Cl, 25.2; N, 5.04.

Acknowledgments.—The authors are indebted to Dr. Peter Lim and staff for the chromatograms and optical rotations, as well as the interpretation of the infrared spectra; and to Mr. O. P. Crews, Jr., and staff for large-scale preparations of intermediates.

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Action of Grignard Reagents. XVII.[1] Action of Organomagnesium Compounds on 5-Arylidene Derivatives of 3-Arylrhodanines, of 3-*p*-Tolyl-2,4-thiazolidinedione and on 2-Arylidene-3(2H)-4,5-benzthianaphthenone-1,1-dioxides

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RECEIVED AUGUST 14, 1959

Treatment of 5-arylidene derivatives of 3-arylrhodanines and of 3-*p*-tolyl-2,4-thiazolidinedione with Grignard reagents does not effect hetero-ring opening and only the double bond of the lateral chain of II and IV enters into reaction, yielding colorless products, believed to have structures III and V, respectively. 3-Phenylrhodanine and 3-*p*-tolyl-2,4-thiazolidinedione were proved to be stable when treated with phenylmagnesium bromide under similar conditions. When IIIa was treated with aqueous sodium hydroxide solution, α-mercapto-β,β-diphenylpropionic acid was obtained. Conjugate addition, without any indication of cleavage, now has been observed when the newly prepared 2-arylidene-3(2H)-4,5-benzthianaphthenone-1,1-dioxides (Xa-b) are allowed to react with organomagnesium halides, yielding colorless reaction products, believed to have structures XI.

Recently,[2] in conjunction with a study of the pharmacological and toxicological properties of rhodanine derivatives,[3] Mustafa and co-workers have prepared derivatives of 5-methylrhodanine and 5-methyl-3-phenyl-2,4-thiazolidinedione, through the action of Grignard reagents on 5-aralkylidene rhodanines and 5-aralkylidene-3-phenyl-2,4-thiazolidinediones, respectively.[4]

We now would like to report our extension of this investigation for the aim of preparation of a number of new derivatives needed for the pharmacological studies.

In this investigation, the action of Grignard reagents on 5-arylidene-3-arylrhodanines (IIa-h) and on 5-arylidene-3-*p*-tolyl-2,4-thiazolidinediones (IVa-e) has now been undertaken with the formation of the reaction products IIIa-l and Va-f, respectively (*cf.* Scheme A and B).

The Grignard reagents do not effect the hetero-ring opening and only addition of organomagnesium compounds to the conjugation created by attachment of an exocyclic double bond in the 5-position of a heterocyclic ring having a carbonyl function II and IV takes place.

The structure of IIIa, which is taken as an

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(2) S. Tawab, A. Mustafa and A. F. A. Shalaby, *Nature*, **183**, 607 (1959).

(3) In view of the marked interest in many derivatives of thiazolidone which proved to be useful as anesthetics (A. R. Surrey, THIS JOURNAL, **71**, 3354 (1949)), anti-convulsants (H. D. Troutman and L. M. Long, *ibid.*, **70**, 3436 (1948)) and amebacidal agents (A. R. Surrey and R. A. Cutler, *ibid.*, **76**, 578 (1954)), the presence of a thiazolidine moiety in penicillin, the fungi-toxic or bacteria-toxic activity shown by many derivatives of rhodanines (H. K. Pujari and M. K. Rout, *J. Sci. Ind. Res. (India)*, **14B**, 398 (1955)); F. C. Brown and C. K. Bradsher, *Nature*, **168**, 171 (1951); F. C. Brown, C. K. Bradsher, E. C. Morgan, M. Tetenbaum and P. Wilder, THIS JOURNAL, **78**, 384 (1956).

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