the nearest 0.1 mg. and adding the requisite amount of acetic acid at 25.0° with an automatic pipet. Then the desired amount of perchloric acid solution was added to the magnetically stirred solution of lead alkyl, using the same automatic pipet. The reaction mixture was immediately immersed in a constant temperature bath at 25.0°. Aliquot samples were removed periodically and titrated for acid content using a 0.0100 *M* solution of sodium acetate in acetic acid. The reactions were followed to at least 50% completion. Rate constants were calculated using an integrated form of the second-order rate equation, assuming the reaction to be first order in perchloric acid and first order in tetraalkyllead compound. The form assumed for this reaction was verified by the constancy and freedom from drift of the rate constants obtained.

Thiosugars. II. Rearrangement of 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea[1a,b]

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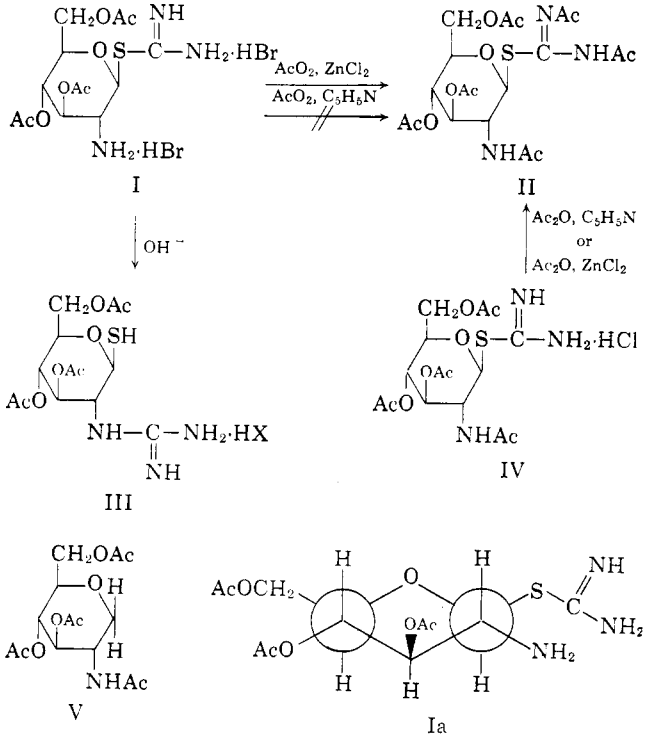
In neutral solution 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea dihydrobromide (I) undergoes rearrangement to form 3,4,6-tri-*O*-acetyl-2-deoxy-2-guanidino-1-thio-D-glucose (III). A product from the acetylation of I in acidic solution was identical with 1,3-di-*N*-acetyl-2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea (II) which had been prepared by acetylation of 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea hydrochloride (IV). The behavior of I and related derivatives on desulfurization is discussed.

The condensation of 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranosyl bromide hydrobromide with thiourea was described in part I,[1a] and the product was formulated as 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea dihydrobromide (I) by analogy with the products formed by condensation of other glycosyl halide derivatives with thiourea. Optical rotatory data supported a β-D anomeric configuration. In the case of the *N*-acetyl hydrochloride analog of I, 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea hydrochloride (IV), the C-1 to S linkage was established by reductive desulfurization.[1a] The present work establishes a direct correlation between I and IV by acetylation to the same derivative, and firmly establishes the existence of a C-1 to S bond in I. Acetylation of IV in pyridine with acetic anhydride gave a crystalline product, containing two more acetyl groups, formulated as 1,3-di-*N*-acetyl-2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea (II). The possibility of anomerization in this reaction is very improbable. Under the same conditions, acetylation of I gave no trace of II, although the thin layer chromatographic procedure used would have revealed this product in very low concentration in the reaction mixture. This is to be expected in view of the readiness with which I rearranges in neutral or basic media, as described below. Acetylation with acidic catalysts permits reversible anomerization,[3] and the thermodynamically more stable anomer preponderates in the product at equilibrium. Acetylation of either I or IV with acetic anhydride and zinc chloride under the same conditions gave a mixture of two principal products in each case, with identical R_f values and intensities on thin layer chromatograms. The product formed in lesser amount was isolated from each reaction mixture and was shown to be identical with II; the other product was probably the α-D anomer of II.



Compound I was synthesized as a carbohydrate analog of 2-(2-aminoethyl)-2-thiopseudourea (AET) hydrobromide, which is one of the best known agents for protection of mammals against ionizing radiation.[4] It was hoped that a carbohydrate analog might retain this protective ability while exhibiting reduced toxicity.

It has been shown[5] that the protective ability of 2-(2-aminoethyl)-2-thiopseudourea is retained in the propyl analog 2-(3-aminopropyl)-2-thiopseudourea (APT) hydrobromide, but that activity falls off rapidly as the length of the alkyl chain is extended further. The protective activity of these two thiopseudourea derivatives

(1)(a) Part I of this series: D. Horton and M. L. Wolfrom, *J. Org. Chem.*, **27**, 1794 (1962); (b) Supported by contract no. DA-49-193-MD-2143 (R. F. Proj. 1187) from the Walter Reed Army Institute of Research, Washington, D. C. The opinions expressed in this article are those of the authors, and not necessarily those of the sponsoring agency.

(2) D. H. Hutson gratefully acknowledges a travel grant from the Wellcome Trust, 52 Queen Anne Street, London, W. 1.

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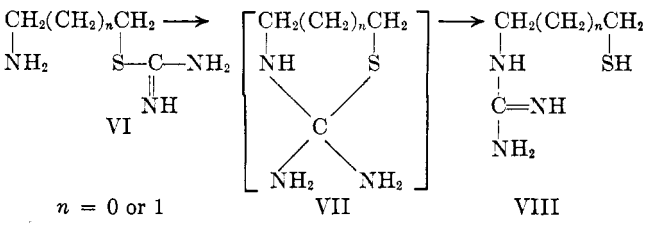
(VI) parallels their ability to undergo an irreversible intramolecular rearrangement at or near neutral pH with formation of guanidinoalkylthiols (VIII), by way of a cyclic intermediate (VII).[6]



It was of interest to determine whether such a rearrangement occurs with compound I at physiological pH. The two functional groups are formally *trans* in the ring system, but it can be seen from a Newman projection formula (Ia) of I in the favored *C1* conformation that the groups are staggered only 60° from the true *cis* orientation, and rearrangement should be sterically possible. It is known[7] that 3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-amino-2-deoxy-1-thio-D-glucose very readily undergoes acetyl migration to give 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-β-D-glucose, a related rearrangement which has the same steric requirements. It was found that an aqueous solution of I gave a negative thiol reaction, but when the solution was brought to about pH 7, with sodium hydroxide solution or a phosphate buffer, a strong thiol reaction was obtained and the solution exhibited a strong Sakaguchi reaction, indicative of the guanidino group. Analogs of I with an acetoxy or acetamido group at C-2 gave no guanidine reaction after similar treatment, nor did they give the thiol reaction; this indicates that the pseudothiourea group does not give the Sakaguchi reaction, and the amidino group is not cleaved by the reagent. It therefore is evident that I undergoes rearrangement to the guanidinothiol (III). Subsequent O → N acetyl migration might be possible but apparently did not occur. Reductive desulfurization of the reaction mixture gave an amorphous nonreducing sulfur-free product which gave a strong Sakaguchi reaction; this product was presumably a salt of 3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-2-guanidino-D-glucitol.

The possibility exists that anomerization might take place at some stage of the rearrangement I → III. It is known that stable 1,2-fused rings on the D-glucose molecule have the α-D configuration. Since, however, the rearrangement occurs without significant change in specific rotation it would appear that the amidino group migrates directly without anomerization.

The reductive desulfurization of a number of derivatives of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-1-thio-D-glucose to give 2-acetamido-3,4,6-tri-*O*-acetyl-1,5-anhydro-2-deoxy-D-glucitol (V) has been described.[1a] Application of the reaction to I, with acetylation of the product, gave a sirupy product which did not contain V. Under similar conditions 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl ethylxanthate hydrochloride[1a] likewise gave a sirup which did not contain V. It would appear that the course of the reaction when a free amino group is present at C-2 differs from that occurring when this group is substituted.

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(7) W. Meyer zu Reckendorf and W. A. Bonner, *J. Org. Chem.*, **26**, 4596 (1961).

Experimental[8]

1,3-Di-*N*-acetyl-2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea (II).—(a) Acetylation of 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea hydrochloride (IV).—A suspension of IV[1a] (2 g.) in pyridine (20 ml.) was treated at room temperature with acetic anhydride (10 ml.), and after 3 hr. the solution was poured on ice (200 g.). After 3 hr. the clear solution was extracted with chloroform, the extract was washed with water, dried (magnesium sulfate), and evaporated to give the crude crystalline product; yield 1.9 g. (86%). Recrystallization from ethanol–petroleum ether gave dendritic needles; m.p. 156–158°, $[\alpha]^{24}D$ +25.6 ± 0.4° (*c* 0.7, chloroform), $\lambda_{max(\mu)}^{KBr}$ 2.97 (NH), 5.74 (OAc), 6.02, 6.49 (NHAc), 6.13 (C=N), X-ray powder diffraction data[9]: 11.33 vs (2), 9.96 vs (1), 8.63 w, 7.53 w, 6.37 m, 5.52 w, 4.89 s (3), 4.60 m, 4.27 w, 4.09 w, 3.08 w, 3.54 m, 3.28 w.

Anal. Calcd. for $C_{19}H_{27}N_3O_{10}S$: C, 46.62; H, 5.57; N, 8.58; S, 6.55. Found: C, 46.90; H, 5.63; N, 8.52; S, 6.68.

This product (II) could be recrystallized without change after heating for 10 min. at 80° in either water or ethanol.

(b) Acetylation of 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea dihydrobromide (I).—A suspension of I[1a] (200 mg.) in a mixture of acetic anhydride (5 ml.) and powdered fused zinc chloride (300 mg.) was shaken for 1 hr. at 10°. The resultant solution, after standing overnight at room temperature, was poured into ice and water containing sodium acetate, and after 7 hr. the product was extracted with chloroform, the extract washed with water, dried (magnesium sulfate), and evaporated. Thin layer chromatography of the resultant sirup with 4:1 ethyl acetate–acetone developer revealed two principal zones, R_f 0.35 and 0.45 in approximate intensity ratios 1:4, together with four minor components migrating faster or slower than the principal zones. The component R_f 0.35 was identical in chromatographic properties with a reference sample of compound II prepared in (a) above. Preparative thin layer chromatography of the reaction product on two 8 × 8 in. plates with 1.5 mm. Silica Gel G adsorbent[8] thickness gave the pure component of R_f 0.35, which was obtained crystalline from ethanol–petroleum ether; 20 mg. (11%), m.p. 156–158°, m.p. 155–158° on admixture with II from (a) above, X-ray powder diffraction pattern identical with that recorded above for II.

Acetylation of IV with acetic anhydride–zinc chloride, as described for I, gave a sirupy product which on thin layer chromatography (4:1 ethyl acetate–acetone eluent) gave a mixture of components closely similar to that formed from I, with components R_f 0.35 and 0.45 in the approximate intensity ratio 1:4. Isolation of the component R_f 0.35 gave crystalline II, which gave a single discrete zone on a chromatogram.

Acetylation of I with acetic anhydride–pyridine, under the conditions used for IV in (a) above, gave a sirupy product which on thin layer chromatography (4:1 ethyl acetate–acetone eluent) gave a streak R_f 0.00–0.20 and no other component. There was no component present with the mobility of a reference sample of authentic II.

Rearrangement of 2-(3,4,6-Tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea Dihydrobromide (I) to 3,4,6-Tri-*O*-acetyl-2-deoxy-2-guanidino-1-thio-D-glucose (III).—A solution of I (0.5 g.) in water (13 ml.) had a specific rotation of −13 ± 1° and gave a negative sodium nitroprusside test for free thiol.[9b] The solution was brought to pH 7.0 by addition of sodium hydroxide solution. There was no observable change in specific

(8) Melting points were taken on a Fisher–Johns apparatus and correspond to corrected melting points. Infrared spectra were determined with a Baird-Atomic Model B infrared spectrophotometer. Thin layer chromatography was performed on Silica Gel G (E. Merck, Darmstadt, West Germany) activated at 100°, with ascending solvent; zones were detected, unless otherwise stated, by spraying the developed plates with silver nitrate in aqueous acetone.

(9)(a) Interplanar spacing, Å CuKα radiation. Relative intensity, estimated visually: s, strong; m, medium; w, weak; v, very. Three strongest lines are numbered (1, strongest); double numbers indicate approximately equal intensities. (b) Further experiments (with W. A. Cramp) have shown that 2,6-dichloroindophenol thiol assay procedure, as used by Doherty and coworkers,[6] gives quantitatively reproducible values and is the preferred method for identification of free thiol. The thiol reactions reported in the present work were confirmed by this procedure.

rotation, but aliquots of the resultant solution gave a strongly positive thiol reaction, which reached a maximum after *ca.* 15 min., and did not start to diminish in intensity until the reaction mixture had been exposed to the air for several hours. The solution gave at all times a strongly positive Sakaguchi reaction, as described by Brown and co-workers,[10] for a guanidino derivative. Identical behavior in the color reactions was observed when a 0.1% solution of I was prepared in a phosphate buffer,[11] pH 7.2. No thiol or guanidine reactions were detectable when either 2-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-2-thiopseudourea hydrobromide[12] or 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl) - 2 - thiopseudourea hydrochloride (IV)[1a] was treated with sodium hydroxide solution or pH 7.2 phosphate buffer.

3,4,6-Tri-*O*-acetyl-1,5-anhydro-2-deoxy-2-guanidino-D-glucitol Hydrobromide.—A solution of I (200 mg.) in water was brought to pH 7.0 with sodium hydroxide, the solution was evaporated to a small volume, and the product was refluxed in ethanol with Raney nickel (2 g.) for 3 hr. The catalyst was filtered, the solution was evaporated, and ether was added. An amorphous solid precipitated overnight; yield 70 mg. This product was nonreducing, contained no sulfur, but gave a strong positive Sakaguchi reaction.

Anal. Calcd. for $C_{13}H_{22}BrN_3O_7$: N, 10.21. Found: N, 9.55.

Desulfurization Experiments.—Derivatives of 2-amino-2-deoxy-1-thio-D-glucose were desulfurized with Raney nickel, with subsequent acetic anhydride–sodium acetate acetylation as previously described,[1a] and the products were examined by thin layer chromatography with 4:1 ethyl acetate–acetone developer. The zones were revealed by spraying with concentrated sulfuric acid. A reference sample of 2-acetamido-1,5-anhydro-2-deoxy-D-glucitol (V) migrated as a discrete zone of R_f 0.80. Three control experiments, with the derivatives 2-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea hydrochloride, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl ethylxanthate, and 2-acetamido-3,4,6-tri-*O*-acetyl-1-*S*-acetyl-2-deoxy-1-thio-β-D-glucopyranose, all gave V as a zone of R_f 0.80 with only traces of side products. Under identical conditions 2-(3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl)-2-thiopseudourea dihydrobromide (I) and 3,4,6-tri-*O*-acetyl-2-amino-2-deoxy-β-D-glucopyranosyl ethylxanthate hydrobromide[1a] gave no detectable product with R_f 0.80, and no crystalline product could be isolated from either reaction mixture.

Acknowledgment.—The technical assistance of W. N. Rond is gratefully acknowledged.

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Preparation of Sugars and Carbohydrate-like Compounds Carrying a β-Mercaptoethylamine Moiety[1]

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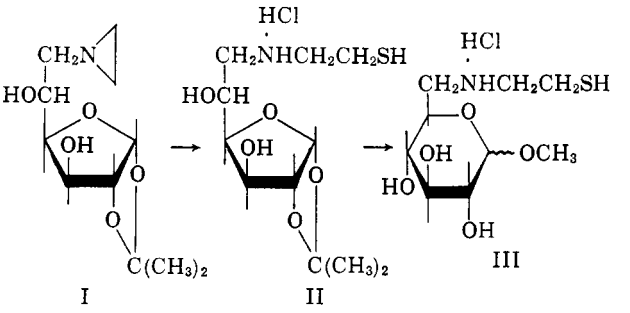
The preparation of two sugar glycosides carrying "pendant" β-mercaptoethylamine moieties is described, as is the synthesis of some related analogs of β-mercaptoethylamine.

Previous papers[2] in this series have described the preparation of sugars that have the β-mercaptoethylamine (MEA) moiety incorporated into the sugar ring and that are potential radiation protective chemicals. Another variation of the β-mercaptoethylamino sugar is that class of compounds in which one of the sugar hydroxyls is replaced by the MEA group. The preparation of the latter type of compound is the subject of this paper.

The 6-ethylenimino sugar (I)[3] when treated with hydrogen sulfide gave an excellent yield of a β-mercaptoethylamine which could be isolated as a crystalline solid but which was best stored as the hydrochloride (II) to minimize oxidation to the disulfide. The reaction of II with methanolic hydrogen chloride afforded the glycoside (III), which is written as the pyranoside although no rigorous structure proof of the ring size was carried out. The presence of the thiol function rendered impractical the standard periodate method of determining ring size for III. Aqueous hydrolysis of II in an effort to prepare the free sugar gave much darkening and decomposition and no discrete product.



Opening of the epoxide ring of IV[2c] with excess *S*-benzyl-β-mercaptoethylamine[4] gave the blocked derivative (V) in crude form. Opening of IV, predominantly at C-3 is assumed on the basis of other experience with these blocked anhydromannosides.[2] Cleavage of V with sodium in liquid ammonia afforded, in good yield, a crystalline thiol that was converted to the hydrochloride salt (VI) without loss of the ethylidene blocking group. Treatment of VI with methanolic hydrogen chloride gave the glycoside salt (VIII). Aqueous hydrolysis of VI in an effort to prepare the free sugar corresponding to VIII did not give a clean product. Aqueous acid hydrolysis of VI could lead to formation of a 1,6-anhydride or to thioacetal formation by attack of the pendant β-mercaptoethylamine group at C-1. The fact that the acetylation of the hydrolysis product gave no infrared *S*-acetyl carbonyl absorption suggests that thioacetal formation might have occurred

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