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.

(10) R. A. B. Bannard, A. A. Casselman, W. F. Cockburn, and G. M. Brown, *Can. J. Chem.*, **36**, 1541 (1958).

(11) T. C. McIlvaine, *J. Biol. Chem.*, **49**, 183 (1921).

(12) W. Schneider and K. Eisfeld, *Ber.*, **61**, 1260 (1928); M. Černý, J. Vrkoč, and J. Staněk, *Chem. Listy*, **52**, 311 (1958); *Collection Czech. Chem. Commun.*, **24**, 64 (1959).

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

JAMES E. CHRISTENSEN AND L. GOODMAN

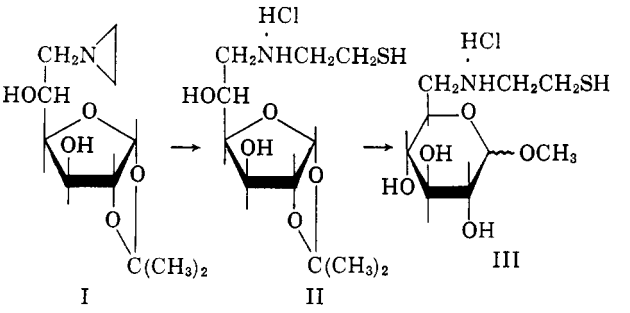
Life Sciences Research, Stanford Research Institute, Menlo Park, California

Received August 13, 1962

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.



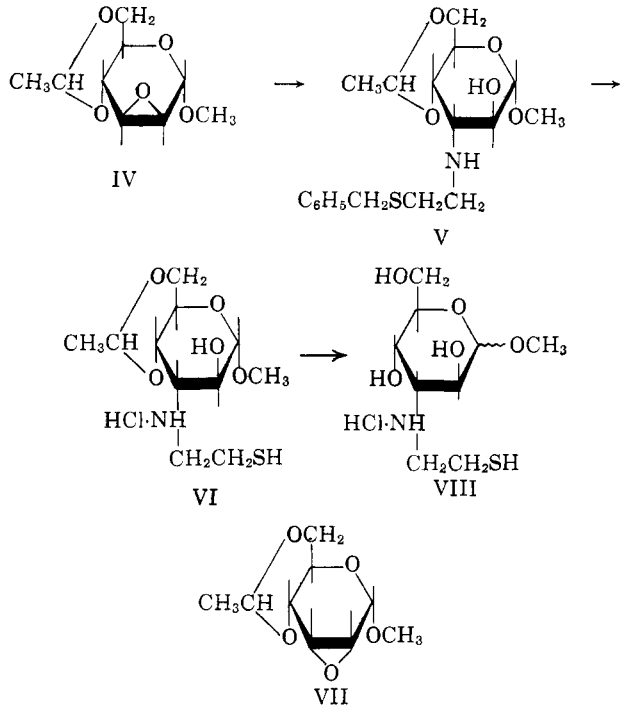
Opening of the epoxide ring of IV[2c] with excess *S*-benzyl-β-mercaptoethylamine[4] gave the blocked derivative (V) in crude form. Opening of IV, predominantly at C-3 is assumed on the basis of other experience with these blocked anhydromannosides.[2] Cleavage of V with sodium in liquid ammonia afforded, 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

(1) The work reported in this paper (no. 5 of the series) was carried out under the joint auspices of the Medical Research and Development Command, Office of the Surgeon General, under contract no. DA-49-193-MD-2068, and of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, Public Health Service, under contract no. SA-43-ph-1892. The opinions expressed in this article are those of the authors and not necessarily those of either sponsoring agency.

(2) (a) L. Goodman and J. E. Christensen, *J. Am. Chem. Soc.*, **83**, 3823 (1961); (b) J. E. Christensen and L. Goodman, *ibid.*, **83**, 3827 (1961); (c) L. Goodman and J. E. Christensen, *J. Org. Chem.*, **28**, 158 (1963).

(3) L. Vargha, L. Toldy, Ö. Fehér, and S. Lendvai, *J. Chem. Soc.*, 805 (1957).

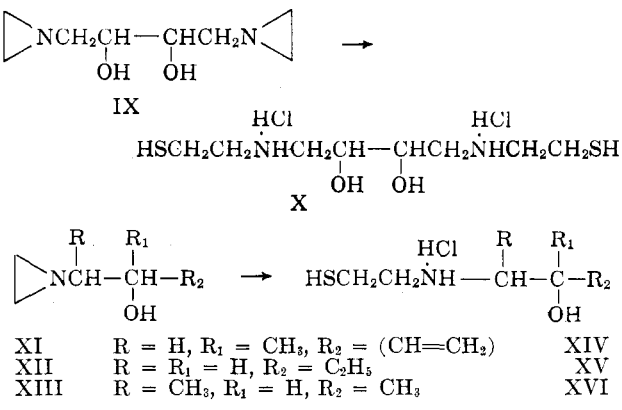
(4) J. G. Moffatt and H. G. Khorana, *J. Am. Chem. Soc.*, **83**, 663 (1961).



during hydrolysis. When the anhydroalloside (VII) was treated with *S*-benzyl-β-mercaptoethylamine, much more severe conditions were required to cause complete reaction than in the case of IV. No single product could be isolated from the reaction, possibly because of appreciable ring opening at C-3 as well as at C-2.[5]

Several attempts were made to prepare VIII via the reaction of ethylene monothiolcarbonate[6] with a blocked derivative of methyl 3-amino-α-D-altropyranoside[2a] but little or no reaction could be realized, even in refluxing *p*-xylene.

A number of ethylenimines, prepared as potential anticancer drugs, were treated with hydrogen sulfide to produce certain other hydroxyl-containing β-mercaptoethylamines. The bisethylenimine (IX)[7] under these conditions gave a good yield of a crystalline solid, which readily polymerized by oxidation to a polydisulfide and was best preserved as the dihydrochloride (X). In a



similar fashion, the monoethylenimines (XI–XIII)[8] were opened with hydrogen sulfide; the free bases were distillable liquids that were oxidized to disulfides very rapidly on air exposure and were stored as the hydrochlorides (XIV–XVI).

Experimental[9]

6-Deoxy-6-(β-mercaptoethyl)-amino-1,2-*O*-isopropylidene-D-glucofuranose Hydrochloride (II).—A stirred, chilled (0°) solution of 1.14 g. (4.65 mmoles) of 6-deoxy-6-ethylenimino-1,2-*O*-isopropylidene-D-glucofuranose (I)[3] in 25 ml. of absolute methanol was treated with a stream of hydrogen sulfide for 2 hr., while the temperature was maintained below 5°. The solution was evaporated *in vacuo* to give 1.34 g. (103%) of a colorless, viscous sirup that slowly crystallized to a white, nitroprusside-positive solid, m.p. 100–103°. The solid was recrystallized from 60 ml. of hot benzene with the addition of petroleum ether (b.p. 30–60°) to turbidity, affording 0.85 g. (65%) of solid, m.p. 98–104°; $\lambda_{max(\mu)}^{Nujol}$ 2.95, 3.26 (NH, OH), 3.83–3.92 (SH), 3.72, 4.09, 4.19, and 6.32 (unassigned bands probably due to hydrogen-bonding between SH, NH, and OH groups).

Anal. Calcd. for $C_{11}H_{21}NO_5S$: C, 47.3; H, 7.58; S, 11.5. Found: C, 46.8, 46.8; H, 7.29, 7.48; S, 10.7, 11.0.

A second recrystallization of the analytical sample from benzene–petroleum ether gave a solid with the increased melting range of 96–111°, suggesting that the product was unstable to such treatment.

The free base, prepared as above, was immediately added to 100 ml. of a 4% ethereal solution of hydrogen chloride. A gummy solid formed, which was separated and washed by decantation with several portions of ether, affording after drying a quantitative yield of a white, nitroprusside-positive powder that yellowed on being heated at 100°; $\lambda_{max(\mu)}^{Nujol}$ 3.05 (OH), 6.30 (NH_2^+); $[\alpha]^{25}D$ −2° (1% in methanol).

Anal. Calcd. for $C_{11}H_{22}ClNO_5S$: C, 41.8; H, 7.02; Cl, 11.2; S, 10.2. Found: C, 41.4; H, 7.26; Cl, 11.2; S, 10.4.

Methyl 6-Deoxy-6-(β-mercaptoethyl)-amino-D-glucoside Hydrochloride (III).—A sample of the blocked free base of II was prepared from 12.25 g. (50.00 mmoles) of 6-deoxy-6-ethylenimino-1,2-*O*-isopropylidene-D-glucofuranose (I) as described above. The sirupy product immediately was dissolved in 300 ml. of 2% methanolic hydrogen chloride and the solution, protected from moisture, was heated at reflux for 1.5 hr., then evaporated *in vacuo*. The residue was washed with several portions of dry ether and vacuum-dried to afford 14.2 g. (98%) of a yellow nitroprusside-positive solid; $\lambda_{max(\mu)}^{Nujol}$ 2.93 (OH), 3.94 (SH), 6.22 ($NH_2^{\oplus}$).

Anal. Calcd. for $C_9H_{20}ClNO_5S \cdot 0.1$ HCl: C, 36.8; H, 6.91; Cl, 13.3; S, 10.9. Found: C, 36.7; H, 7.03; Cl, 13.3; S, 10.9.

Methyl 3-(β-Benzylthioethyl)-amino-3-deoxy-4,6-*O*-ethylidene-α-D-altropyranoside (V).—A mixture of 12.0 g. (59.0 mmoles) of *S*-benzyl-β-mercaptoethylamine hydrochloride[4] in 120 ml. of 1 *M* aqueous sodium hydroxide was stirred for 10 min., then extracted with two 80-ml. portions of dichloromethane. The combined extracts were washed with 80 ml. of water, dried over potassium carbonate, filtered, and evaporated *in vacuo* to afford 9.86 g. (59.0 mmoles) of *S*-benzyl-β-mercaptoethylamine as a sirup, which was added to 40 ml. of dimethyl sulfoxide. This solution was added to a solution of 4.00 g. (19.8 mmoles) of the anhydromannoside (IV)[2c] in 40 ml. of dimethyl sulfoxide and the reaction mixture was stirred at 110–115° for 18 hr. in a nitrogen atmosphere, then poured, with stirring, into 500 ml. of ice-water. The mixture was extracted with three 100-ml. portions of chloroform, the combined extracts were washed with two 100-ml. portions of water, dried over potassium carbonate, and evaporated *in vacuo*, giving a yellow liquid. The residue was triturated with five 30-ml. portions of petroleum ether (b.p. 62–70°), decanting each time, affording finally 5.48 g. (75%) of a yellow sirup whose nitrogen analysis suggested that it contained about 10% of *S*-benzyl-β-mercaptoethylamine.

(5) See A. B. Foster, M. Stacey, and S. V. Vardheim, *Acta Chem. Scand.*, **12**, 1605 (1958), for the ammonolysis of such an anhydroalloside; and N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **63**, 1727 (1941), for the cleavage with potassium hydroxide.

(6) D. D. Reynolds, M. K. Massad, D. L. Fields, and D. L. Johnson, *J. Org. Chem.*, **26**, 5109 (1961).

(7) L. Vargha, L. Toldy, and E. Kasztreiner, *Acta Chem. Hung.*, **19**, 295 (1959); E. J. Reist, I. G. Junga, M. E. Wain, O. P. Crews, L. Goodman, and B. R. Baker, *J. Org. Chem.*, **26**, 2139 (1961).

(8) E. J. Reist I. G. Junga, and B. R. Baker, *J. Org. Chem.*, **25**, 1673 (1960).

(9) Boiling points and melting points are uncorrected; the latter were obtained with the Fisher–Johns apparatus. Paper chromatography was done by the descending technique on Whatman no. 1 paper, using *n*-butyl alcohol–acetic acid–water (5/2/3) as the solvent system and detecting the spots with sodium nitroprusside and sodium cyanide sprays. The spots were located relative to adenine, *i.e.*, R_f adenine = 1.00.

Methyl 3-(β-Mercaptoethyl)-amino-3-deoxy-4,6-*O*-ethylidene-α-D-altropyranoside Hydrochloride (VI).—A solution of 2.89 g. (7.05 mmoles, calculated as 90% pure) of crude V in 17 ml. of 1,2-dimethoxyethane was added dropwise and with stirring to a solution of 0.90 g. (0.039 g.-atom) of sodium in 40 ml. of liquid ammonia. The mixture was stirred for 30 min. under reflux, then the blue color was discharged by the addition of excess solid ammonium chloride. The ammonia was evaporated under nitrogen, 30 ml. of water was added to the residue, and the aqueous solution was adjusted to pH 7 with glacial acetic acid. The aqueous solution was extracted with three 20-ml. portions of dichloromethane, the combined extracts were washed with 10 ml. of water, dried over magnesium sulfate while maintaining a nitrogen atmosphere, then filtered and evaporated *in vacuo* to give 1.58 g. (80%) of a pale yellow, crystalline solid whose infrared spectrum showed an –SH absorption band at 3.9 μ.

The solid was immediately dissolved in 10 ml. of 1,2-dimethoxyethane and to this solution was added, with cooling and stirring, 20 g. of a 2.2% solution of hydrogen chloride in 1,2-dimethoxyethane. The crystalline solid, 1.44 g. (64%), m.p. 183–191° dec., was collected and recrystallized by dissolving in warm methanol, then adding enough ether to initiate crystallization. The analytical sample had m.p. 178–186° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.00 (OH), 3.59, 3.69, 3.72, 6.27 ($NH_2^{\oplus}$); $[\alpha]^{30}D$ +89° (1% in methanol); it traveled as a single spot with R_{Ad} 1.16 on paper chromatography.

Anal. Calcd. for $C_{11}H_{22}ClNO_5S$: C, 41.8; H, 7.02; Cl, 11.2; N, 4.44, S, 10.2. Found: C, 41.9; H, 6.64; Cl, 11.2; N, 4.41; S, 10.3.

Some anomerization evidently accompanied the recrystallization, since another sample taken directly from the hydrogen chloride treatment had m.p. 184–190° dec. and $[\alpha]^{27}D$ +96° (1% in methanol).

Methyl 3-(β-Mercaptoethyl)-amino-3-deoxy-D-altropyranoside Hydrochloride (VIII).—A solution of 5.0 g. of VI in 300 ml. of 1% hydrogen chloride in methanol was heated at reflux for 3 hr., then evaporated *in vacuo*. The residue was triturated several times with ether and dried, yielding 4.23 g. (92%) of a yellow, hygroscopic, nitroprusside-positive solid, m.p. 50–70°; $\lambda_{max(\mu)}^{Nujol}$ 3.05 (OH), 6.32 ($NH_2^{\oplus}$); it moved as a single spot on paper chromatography with R_{Ad} 0.70.

Anal. Calcd. for $C_9H_{20}ClNO_5S$: C, 37.3; H, 6.96; Cl, 12.2; N, 4.83; S, 11.1. Found: C, 37.0; H, 6.98; Cl, 13.0, 12.8; N, 4.73, S, 11.4.

***meso*-3,8-Diaza-5,6-dihydroxydecanedithiol-1,10 Dihydrochloride (X).**—A stirred suspension of 0.40 g. (2.32 mmoles) of *meso*-1,4-diaziridinyl-2,3-dihydroxybutane (IX)[7] in 15 ml. of 2-methoxyethanol was cooled to 5° and treated with a slow stream of hydrogen sulfide. The solid slowly dissolved and a complete solution resulted after 1 hr. A precipitate then formed rapidly after allowing the hydrogen sulfide flow to continue for 10 min. more. The white precipitate was collected and washed with 2-methoxyethanol and hexane to afford 0.28 g. (50%) of a white solid, m.p. 110–115°, which gave a strong nitroprusside test. The product was recrystallized by extracting the powder with 10 ml. of boiling absolute ethanol and filtering the hot solution. The filtrate deposited 0.21 g. (38%) of white, water-soluble crystals, m.p. 111–114°; $\lambda_{max(\mu)}^{Nujol}$ 3.07 and 3.22 (OH, NH), 3.94 (SH), 9.32 and 9.45 (C–OH).

Anal. Calcd. for $C_8H_{20}N_2O_2S$: C, 40.0; H, 8.39; S, 26.7. Found: C, 40.5; H, 8.58; S, 26.5.

When the reaction period was extended to 2 hr., the yield of recrystallized product was 64%.

A mixture of 0.30 g. of the free base of X in 20 ml. of methanol was filtered to remove polymer that had formed by air oxidation. To the filtrate was added 10 ml. of methanol that had been saturated with hydrogen chloride at room temperature. The solution was stirred at room temperature for 5 min., then evaporated *in vacuo*, leaving 0.29 g. of a white, nitroprusside-positive solid; m.p. 206–212°; $\lambda_{max(\mu)}^{Nujol}$ 2.98 (OH), 3.95 (SH), 6.26 ($NH_2^{\oplus}$).

Anal. Calcd. for $C_8H_{22}Cl_2N_2O_2S_2$: C, 30.7; H, 7.08; Cl, 22.6; S, 20.5. Found: C, 30.6; H, 7.23; Cl, 22.6; S, 20.3.

The other β-mercaptoethylamine hydrochlorides (XIV, XV, and XVI) were prepared essentially as described for X, except that the ethylenimine was opened with hydrogen sulfide in methanol.

3-Aza-5-hydroxy-1-mercapto-5-methylheptene-6 hydrochloride (XIV) was obtained in 82% yield from XI as a hygroscopic, white solid; $\lambda_{max(\mu)}^{Nujol}$ 3.02 (OH), 6.08 (C=C), 3.62, 6.31 ($NH_2^{\oplus}$).

Anal. Calcd. for $C_7H_{16}ClNOS$: C, 42.5; H, 8.16; Cl, 17.9; S, 16.2. Found: C, 42.8; H, 8.13; Cl, 18.0; S, 16.0.

3-Aza-5-hydroxyheptanethiol-1 hydrochloride (XV) was obtained in 96% yield from XII as a hygroscopic, white solid; $\lambda_{max(\mu)}^{Nujol}$ 2.8–3.1 (OH), 3.90–4.00 (SH), 6.1–6.3 ($NH_2^{\oplus}$).

Anal. Calcd. for $C_6H_{16}ClNOS$: C, 38.8; H, 8.69; Cl, 19.1; S, 17.3. Found: C, 38.5; H, 8.60; Cl, 19.4; S, 17.1.

3-Aza-5-hydroxy-4-methylhexanethiol-1 hydrochloride (XVI) was obtained in 97% yield from XIII as a hygroscopic, white solid; $\lambda_{max(\mu)}^{Nujol}$ 3.04 (OH), 3.60 and 6.30–6.42 ($NH_2^{\oplus}$), 3.98–4.05 (SH).

Anal. Calcd. for $C_6H_{16}ClNOS$: C, 38.8; H, 8.69; Cl, 19.1; S, 17.3. Found: C, 39.0; H, 8.60; Cl, 18.8; S, 17.2.

Acknowledgment.—The authors wish to thank Dr. Peter Lim and his group for the infrared spectral data, optical rotations, and paper chromatography, and Mr. O. P. Crews and staff for the large-scale preparation of intermediates.