[CONTRIBUTION FROM THE CANCER RESEARCH LABORATORY, DEPARTMENT OF PHARMACEUTICAL CHEMISTRY, UNIVERSITY OF FLORIDA]

Methyl 3,4-Anhydro-β-D-galactopyranoside. II. Reaction with Methanethiol[1,2]

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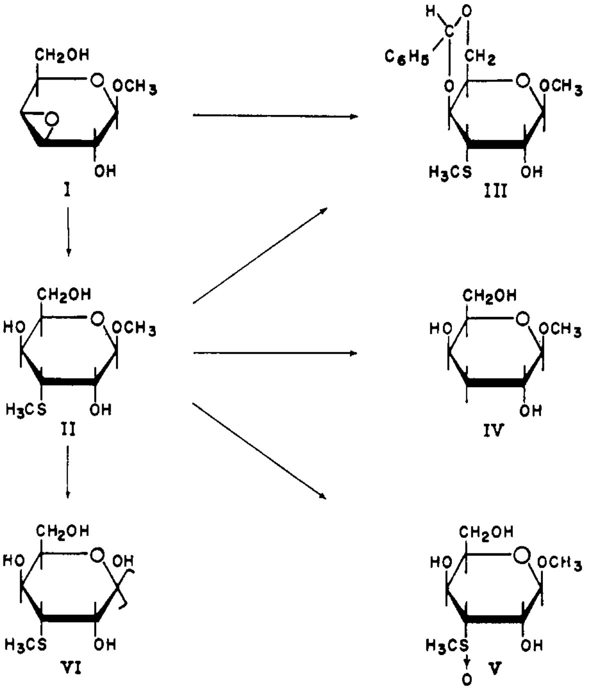
Reaction of methyl 3,4-anhydro-β-D-galactopyranoside with methanethiol gave as the sole isolable product methyl 3-deoxy-3-methylthio-β-D-gulopyranoside as an oil which formed a crystalline benzylidene derivative. Methyl 3-deoxy-3-methylthio-β-D-gulopyranoside desulfurated to methyl 3-deoxy-β-D-galactopyranoside; it reacted with periodate ion to give a crystalline sulfoxide. Hydrolysis gave 3-deoxy-3-methylthio-D-gulose as an oil, purified by paper chromatography.

In a search for possible antimetabolites of the pentose phosphate pathway of carbohydrate metabolism for use as potential anticancer agents,[4] it was of interest to prepare hexose analogs containing sulfur in place of oxygen.

One of the more convenient ways to introduce sulfur into a carbohydrate is to react a sugar epoxide with an alkylthiol. Methanethiol has been used frequently by others, especially with 2,3-anhydroglycosides, as upon reductive desulfuration the methylthio derivatives give deoxyglycosides.[5] We decided to investigate the reaction of methanethiol on methyl 3,4-anhydro-β-D-galactopyranoside in hopes of obtaining both 3-methylthio-D-gulose and 4-methylthio-D-glucose derivatives.

When methyl 3,4-anhydro-β-D-galactopyranoside (I) was treated with methanethiol and sodium methoxide, a theoretical yield of crude methyl 3-deoxy-3-methylthio-β-D-gulopyranoside (II) was obtained as an oil with $[\alpha]^{26}\text{D}$ −69.1°. Hydrolysis of a small sample of II followed by chromatography on Whatman No. 1MM paper showed one main spot with R_{xylose} 1.72 (*n*-butyl alcohol–acetic acid–water; 4:1:1). Neither II nor its acetate could be crystallized, but upon benzylidenation II gave methyl 4,6-benzylidene-3-deoxy-3-methylthio-β-D-gulopyranoside (III), which was obtained as crystalline material from benzene-hexane. When III was hydrolyzed back to II, the oil had $[\alpha]^{25}\text{D}$ −64.3°.

To prove that the methylthio group was attached to carbon 3, II was subjected to reductive desulfuration with Raney nickel to give methyl 3-deoxy-β-D-galactopyranoside (IV), a known compound. Upon oxidation of II with sodium metaperiodate by the spectrophotometric method of Aspinall and Ferrier,[6] 0.99 mole of periodate ion was consumed. As there are no adjacent hydroxyl groups present in II, the consumption of periodate ion indicated that the sulfur atom had been oxidized to the sulfoxide (V); it is known that sulfur-containing compounds can undergo such an oxidation.[7] II was therefore treated with one mole of sodium metaperiodate and the sulfoxide (V) was isolated as a white crystalline compound m. 139–140°.



Hydrolysis of II with 0.2*N* sulfuric acid gave 3-deoxy-3-methylthio-D-gulose (VI) as an oil. VI was purified by chromatography on Whatman 3-*MM* filter paper to give an oil with $[\alpha]^{25}\text{D}$ −27.1°.

Huber and Schier[8] have made an extensive survey of the literature dealing with pyranosides which do not contain fused rings. They predicted from conformational analysis that of the two possible chair conformations, pyranosides exist in that form having the majority of the larger groups

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[1] This work was supported by Grant No. T-68, American Cancer Society and Grant No. CY-3885, National Cancer Institute, National Institutes of Health, U.S.P.H.S.

[2] For Part I see M. Dahlgard, B. H. Chastain, and R. L. Han, *J. Org. Chem.*, 27, 929 (1962).

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[4] M. Dahlgard and E. Kaufmann, *J. Org. Chem.*, 25, 781 (1960).

[5] See for example R. Jeanloz, D. A. Prins, and T. Reichstein, *Helv. Chim. Acta*, 29, 371 (1946).

[6] G. O. Aspinall and R. J. Ferrier, *Chem. & Ind.*, 1216 (1957).

[7] B. H. Nicolet and L. A. Shinn, *J. Biol. Chem.*, 61, 1615 (1939).

[8] G. Huber and O. Schier, *Helv. Chim. Acta*, 43, 129 (1960).

in the equatorial position; in nucleophilic attack of methyl 3,4-anhydro-β-D-galactopyranoside, the ring will open to give the new groups in the diaxial conformation (rule of Fürst and Plattner); attack should, therefore, occur predominantly at position 3. Our results have confirmed this not only in the case of methanethiol but also with lithium aluminum hydride reduction,[2] and reaction with hydrogen sulfide,[9] where the main or only product isolated resulted from attack at C-3. Huber and Schier observed that free radical reactions do not follow this prediction and this was confirmed in the case of catalytic reduction at high pressure, where a larger percentage of the product arising from attack at C-4[2] was obtained. It is apparent that 4-substituted glucose derivatives cannot conveniently be prepared from this oxide.

EXPERIMENTAL[10]

Methyl 3-deoxy-3-methylthio-β-D-gulopyranoside (II). The procedure used was essentially that described by Jeanloz *et al.*[11] To a solution of sodium methoxide prepared from 9.0 g.(0.391 mole) of sodium metal and 180 ml. of absolute methanol was added at 0° 25 g. (0.52 mole) of methanethiol, followed by 16.6 g. (0.094 mole) of methyl 3,4-anhydro-β-D-galactopyranoside.[2] Most of the oxide dissolved immediately. The reaction mixture was heated at reflux for two hours, cooled, distilled to half volume *in vacuo* at <40°, treated with 30 ml. of water, and then distilled free of methanol *in vacuo*. The yellow aqueous solution was treated with Amberlite IR-120 (H^+) ion exchange resin to remove the sodium ions, methanethiol being evolved. Upon evaporation to dryness *in vacuo*, there was obtained a theoretical yield of crude methyl 3-deoxy-3-methylthio-β-D-gulopyranoside (II) as a viscous yellow oil with $[\alpha]^{26}_D$ −69.1° (c 1.793; water). Attempts to prepare the triacetate of II with acetic anhydride in pyridine gave an oil which could not be crystallized. A sample of the crude product was hydrolyzed with dilute sulfuric acid. Chromatography on Whatmann No. 1*MM* filter paper (*n*-butyl alcohol–acetic acid–water, 4:1:1; 16 hr.) showed only one main spot with R_{xylose} 1.72 when sprayed with aniline hydrogen phthalate in *n*-butyl alcohol and heated at 125° for 5 min. A faint spot showed R_{xylose} 1.33, very near a control spot of 3-*O*-methyl-D-glucose, R_{xylose} 1.37, which may be due to the presence of 3-*O*-methyl-D-gulose, an expected by-product of the reaction.

Methyl 4,6-benzylidene-3-deoxy-3-methylthio-β-D-gulopyranoside (III).The crude product II (5.0 g.; 0.02 mole) was shaken for 1 hr. with 15 ml. of freshly distilled benzaldehyde and 5.0 g. of pulverized fused zinc chloride. After standing overnight at room temperature, the reaction mixture was worked up using the procedure of Bollinger and Prins[12] as follows: The reaction mixture was diluted with methanol (100 ml.) and treated with 2*N* sodium carbonate solution to *p*H >8. After warming on the steam bath for a few minutes the ppt. of zinc carbonate was removed by filtration and washed with methanol (4 × 50 ml.). The combined methanol filtrates were evaporated to about 25 ml., then treated with water and distilled several times to remove the unreacted benzaldehyde, finally being taken to dryness. The residue was extracted with chloroform (4 × 50 ml.), the chloroform extract was washed with water (4 × 50 ml.) and then dried over sodium sulfate. Filtration and evaporation to dryness below 50° gave a theoretical yield (6.9 g.) of crude methyl 4,6-benzylidene-3-deoxy-3-methylthio-β-D-gulopyranoside (III) as a viscous oil. Upon recrystallization from benzene-hexane, there was obtained 4.1 g. (68%) of crystalline material. The analytical sample was purified by chromatography of the crude product (0.5 g.) on a column of Alumina (2.3 × 25 cm.), the column being eluted with 500 ml. each of benzene, ether, and chloroform. The residue from the chloroform eluate was recrystallized from benzene-hexane as fluffy white crystals with a double melting point: melted 60°, cleared 89–90°; $[\alpha]^{25}_D$ −69.6° (*c* 0.78; $CHCl_3$).

Anal. Calcd. for $C_{15}H_{20}O_5S$: C, 57.69; H, 6.41; S, 10.26. Found: C, 57.86; H, 6.84; S, 10.24.

Methyl 3-deoxy-3-methylthio-β-D-gulopyranoside II *from methyl 4,6-benzylidene-3-deoxy-3-methylthio-β-D-gulopyranoside* (III). Hydrolysis of III was effected with 0.025 *N* hydrochloric acid in acetone by heating on the steam bath to constant rotation (4 hr.) followed by neutralization with barium carbonate. After removal of acetone on the evaporator, the residue was taken up in water and extracted with pentane to remove benzaldehyde. After evaporation of the aqueous layer to dryness the residue was taken up in ethanol, treated with Norit, and filtered through a mat of Celite No. 535. The ethanol was removed and the residue was dried by evaporating three times with benzene, giving a theoretical yield of methyl 3-deoxy-3-methylthio-β-D-gulopyranoside as a light yellow oil with $[\alpha]^{25}_D$ −64.3° (c 1.2; water).

Methyl 3-deoxy-β-D-galactopyranoside IV *from methyl 3-deoxy-3-methylthio-β-D-gulopyranoside* (II). Using the procedure of Jeanloz *et al.*[11] II was subjected to reductive desulfuration. Raney nickel catalyst was prepared from 15 g. of nickel aluminum alloy by the method of Mozingo *et al.*,[13] final heating being carried out for 1 hr. at 50°. To the catalyst, freed from excess alcohol by decantation, was added a solution of methyl 3-deoxy-3-methylthio-β-D-gulopyranoside (0.5 g.) in 20 ml. of ethanol. Water (7 ml.) was added and the mixture was heated under reflux on the steam bath for 2 hr. The mixture was cooled, filtered free of catalyst, the catalyst was washed well with ethanol, and the combined ethanol filtrates were evaporated to dryness. Recrystallization of the residue from isopropyl alcohol gave 216 mg. (54%) of methyl 3-deoxy-β-D-galactopyranoside (IV) m.p. 173–174.5° and $[\alpha]^{25}_D$ −69.6° (*c*, 1.2; water), in good agreement with the values m.p. 173–174°, $[\alpha]^{25}_D$ −69.4° (*c*, 2.12; water) previously reported by us[2] for compound IV. Hydrolysis of IV to the free sugar and chromatography on Whatman No. 1 MM paper (*n*-butyl alcohol–acetic acid–water; 4:1:1) gave a spot with silver nitrate:sodium hydroxide having R_f 1.36 in close agreement with R_f 1.33 reported previously by us for 3-deoxy-D-galactose.[2]

Methyl 3-deoxy-3-methylsulfoxy-β-D-gulopyranoside (V). Compound II purified through its benzylidene derivative consumed 0.99 mole of periodate ion when oxidized with sodium metaperiodate by the spectrophotometric method of Aspinall and Ferrier.[6] To isolate the sulfoxide derivative, 2.0 g. (0.009 mole) of II in 30 ml. of water was treated with 1.9 g. (0.009 mole) of sodium metaperiodate in 20 ml. of water. After shaking for 5 min., the mixture was allowed to react in the dark at room temperature overnight. Iodate ion was then precipitated with barium hydroxide to pH 8, Amberlite IR-120 (H^+) ion exchange resin was added until acidic, then the reaction mixture was shaken with Duolite A-4 ion exchange resin until neutral. Evaporation of the

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[9] M. Dahlgard, B. H. Chastain, and R. L. Han, unpublished results.

[10] Melting points are uncorrected. Microanalyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Rotations were determined with a Keston standard polarimeter. Descending technique was used for all chromatograms.

[11] R. Jeanloz, D. A. Prins, and T. Reichstein, *Helv. Chim. Acta*, **29**, 371 (1946).

[12] H. R. Bollinger and D. A. Prins, *Helv. Chim. Acta*, **29**, 1061 (1946).

[13] R. Mozingo, D. E. Wolf, S. A. Harris, and K. Folkers, *J. Am. Chem. Soc.*, **65**, 1013 (1943).

water and treatment of the residue with ethanol precipitated a white solid which was removed by filtration. The ethanol was evaporated from the filtrate and the residue was dissolved in acetone with heating. Upon standing, a white crystalline compound precipitated m. 138–139°, (0.83 g.; 38%). The analytical sample was recrystallized from acetone, m.p. 139–140°, $[\alpha]^{28}$D $-109.9°$ (*c* 1.18; H_2O).

Anal. Calcd. for $C_8H_{16}O_6S$: C, 40.00; H, 6.67; S, 13.33. Found: C, 40.68; H, 6.69; S, 13.39.

*3-Deoxy-3-methylthio-*D*-gulose* (VI). Methyl 3-deoxy-3-methylthio-β-D-gulopyranoside (II) which had been purified through its benzylidene compound (1.0 g.) was hydrolyzed by heating with 0.2*N* sulfuric acid for 4 hr. on the steam bath. After neutralization with barium carbonate, the filtrate was taken to dryness. The residue was dissolved in ethanol, filtered through a mat of Celite No. 535, and evaporated to constant weight to give a theoretical yield of VI as an oil with $[\alpha]^{25}$D $-25.2°$ (*c* 2.0; water), showing no mutarotation. A sample purified by chromatography on Whatman No. 3MM filter paper (*n*-butanol-acetic acid-water; 4:1:1) had $[\alpha]^{25}$D $-27.1°$ (*c*, 1.5; water). A chromatogram on Whatman No. 1MM paper with the same solvent system (16 hr., aniline hydrogen phthalate as developer) gave an R_{xylose} value of 1.72.

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On Bacitracin F

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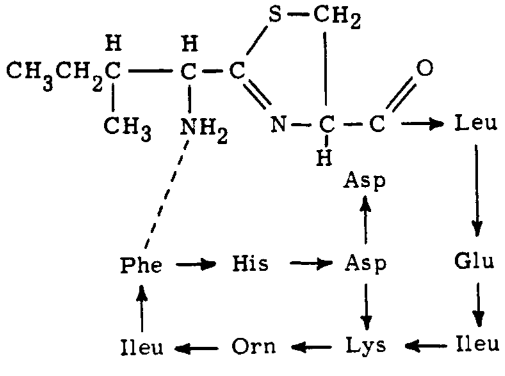
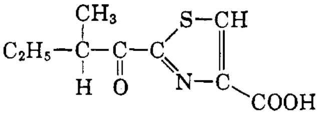
Bacitracin F has been further purified and more precisely characterized by the improved separation and analytical procedures now available. One of the possible structures earlier considered has been shown to be the correct one. Bacitracin F is derived from A by oxidation of the *N*-terminal amino group of the isoleucine residue to form a ketone and by oxidation of the thiazoline ring to a thiazole. Contrary to the earlier view, the amide nitrogen of the aspartic acid residue is retained.

One of the most characteristic features of the bacitracin polypeptides is the limited *p*H range within which the antibiotics retain their activity. In a previous publication,[1] we have described the processes leading to inactivation at low *p*H's. In this paper we will be dealing with the transformation that occurs at *p*H 7 or slightly higher to give the inactive product called bacitracin F.[2,3]



Formula 1

The slow inactivation of bacitracin A (Formula 1) at or above neutral *p*H was first observed rather early in the study.[2,3] Since that time several proposals have been made for the structure of bacitracin F and for the steps involved in its formation from bacitracin A.[4] The structure proposed was based primarily on two lines of evidence. Degradation studies led to the isolation of a ketothiazole acid[5] in 50% yield from an acid hydrolysate of purified bacitracin F. This ketothiazole acid was shown to have Formula 2 by degradation[5] and synthesis.[6] Secondly, the empirical formula for bacitracin F derived from analytical data seemed to require the loss of two nitrogens assuming the Dumas analysis to be reliable. The intermediate was thought to be an amino thiazole.[5]



Formula 2

A more recent proposal postulated the partial structure for bacitracin F[4] shown in Formula 3. Neither this nor the earlier proposal can reasonably account for all the evidence now available. One problem in the earlier work was the liberation of a fractional mole of ammonia during the Conway amide determination. Another problem concerned the electrophoretic behavior of bacitracin F. A third problem centered around the low yields of the

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[1] William Konigsberg and L. C. Craig, *J. Org. Chem.*, **26**, 3867 (1961).

[2] L. C. Craig, J. R. Weisiger, W. Hausmann, and E. Harfenist, *J. Biol. Chem.*, **199**, 259 (1952).

[3] G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **47**, 257 (1950).

[4] L. C. Craig, William Konigsberg, and R. J. Hill, in Ciba Foundation Symposium on Amino Acids and Peptides with Antimetabolic Activity, 1958, p. 226.

[5] J. R. Weisiger, W. Hausmann, and L. C. Craig, *J. Am. Chem. Soc.*, **77**, 3123 (1955).

[6] J. R. Weisiger, unpublished results. This derivative was synthesized by a method similar to that employed by Brookes, Fuller, and Walker [*J. Chem. Soc.*, **137**, 689 (1957)] for the synthesis of 2-isobutyrylthiazole.