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Studies of Aminosugars. XI. Configurational Studies of Aminosugar Glycosides and Aminocyclitols by a Copper Complex Method*1

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An extensive study has been made of the determination of the rotational shift of aminosugars in three kinds of cuprammonium solutions, i. e., in solutions of tetramminecopper(II) sulfate, ammoniacal cuprous chloride and Cupra B, with special consideration of the spacial relationships between adjacent amino and hydroxyl groups in six-membered chair molecules. This paper is an extension of the work of Reeves which provided a tool for the determination of the spacial relationships between adjacent two-hydroxyl groups. Some significant generalizations have been obtained. About ten derivatives of glucosamine have been prepared, and their structures, as indicated by their rotational shifts, have been discussed. The absolute configurations of *d*- and *l-trans*-2-aminocyclohexanol have been successfully determined.

The optical rotatory behavior of glucopyranosides in a cuprammonium solution was first explained by Reeves[1a] by means of conformational considerations, and his experimental evidence confirmed the predicted favored conformational structures.

The findings of Reeves are concerned with the structural relationship between hydroxyl groups on adjacent carbon atoms. It has been concluded that, in the case of a substance with a six-membered chair form, a pair of adjacent hydroxyl groups in the molecule can form a copper complex when dissolved in "Cupra B,"[1b] and if the pair is located sterically so as to give near the $\pm 60°$ projected angle,[2a] the difference in the molecular rotations of the substance in Cupra B and in water, $\Delta[M]_{\text{Cupra B}}$,[3] is approximately ± 2000.

The present investigation was undertaken to see if a practical method could be worked out for the determination of a configurational relationship between amino and hydroxyl groups on adjacent carbon atoms in a six-membered chair molecule. The need for such a method is keenly felt for the elucidation of the absolute structures of a variety of aminosugar glycosides recently found in the field of antibiotics.

Optical rotatory studies have been carried out with a series of aminosugar derivatives and aminocyclitols in a tetramminecopper(II) sulfate[4] solution (TACu), a solution of cuprous chloride in concentrated aqueous ammonia (CuAm), and a

*1 This constitutes Part XXIV of a series entitled "Studies of Antibiotics and Related Substances," by Sumio Umezawa. A portion of this paper was presented at the 18th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1965. Previous reports on aminosugars: S. Umezawa and Y. Ito, This Bulletin, **34**, 69 (1961); Y. Ito, S. Koto and S. Umezawa, ibid., **35**, 1618 (1962); S. Umezawa, S. Koto and Y. Ito, ibid., **36**, 183 (1963); T. Suami, S. Ogawa and S. Umezawa, ibid., **36**, 459 (1963); T. Tsuchiya and S. Umezawa, ibid., **38**, 1181 (1965); S. Fukatsu and S. Umezawa, ibid., **38**, 1443 (1965); S. Koto, Y. Ito and S. Umezawa, ibid., **38**, 1447 (1965); S. Umezawa and T. Tsuchiya, *J. Antibiotics*, **A15**, 51 (1962); T. Tsuchiya, H. Fujita and S. Umezawa, ibid., **A17**, 181 (1964); S. Umezawa and S. Koto, ibid., **A17**, 186 (1964).

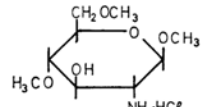
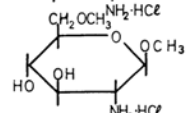
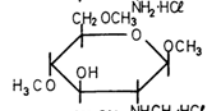
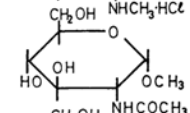
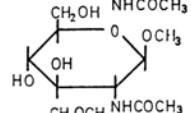
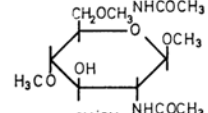
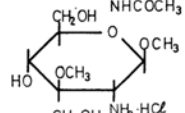
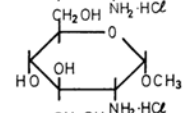
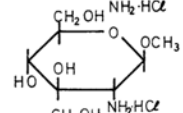
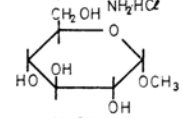
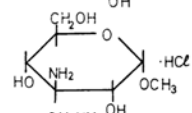
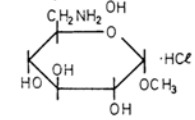
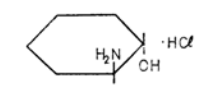
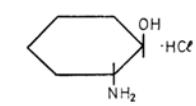
1) a) R. E. Reeves, "Advances in Carbohydrate Chemistry," Vol. 6, Academic Press, New York (1951), p. 107; b) Cupra B is a standard cuprammonium solution containing 15 g. of copper, 240 g. of ammonia and 1 g. of glycerol per liter: R. E. Reeves, *J. Am. Chem. Soc.*, **71**, 2116 (1949).

2) a) Reeves called the projection of the angle made by the two valence bonds onto a plane perpendicular to the carbon-carbon bond the projected angle between the groups under consideration. By convention this angle is negative if measured in a clockwise direction from the nearer group, and positive if measured counterclockwise, (Ref. 1a, p. 110). b) Ref. 1a, p. 127.

3) $\Delta[M]_{\text{Cupra B}} = ([\alpha]_{436}\ \text{Cupra B} - [\alpha]_{436}\ \text{water}) \times \frac{\text{Mol. wt.}}{100}$

4) Tetramminecopper(II) sulfate can easily be prepared from cupric sulfate pentahydrate dissolved in concentrated aqueous ammonia by adding ethanol and drying the resulting precipitate (D. W. Horn and E. E. Taylor, *Am. Chem. J.*, **32**, 253 (1904)).

TABLE. I.

		$[\alpha]_{589}^{15}$	$[\alpha]_{436}^{15}$	$\Delta[M]_{TACu}$	$\Delta[M]_{CuAm}$	$\Delta[M]_{Cupra\ B}$
I		− 2	− 18	− 930	− 850	− 980
II		− 4	− 8	− 1010	+ 1580	+ 1570
III		− 1	− 15	− 980	− 880	—
IV		+ 103	+ 180	+ 52	+ 2210	+ 1930
V		− 48	− 86	+ 50	+ 2180	+ 2040
VI		− 23	− 51	− 20	+ 50	+ 43
VII		+ 10	+ 17	− 60	− 80	− 40
VIII		+ 153	+ 242	− 840	+ 1710	+ 1710
IX		− 20	− 34	− 820	+ 1830	+ 1820
X		+ 161	+ 309	+ 30	+ 430	+ 260
XI		+ 110	+ 193	− 70	+ 460	+ 270
XII		+ 135	+ 249	+ 80	+ 570	+ 390
XIII		+ 42	+ 71	− 920	− 960	− 950
XIV		− 40	− 67	+ 930	+ 970	+ 920

Cupra B solution. The results obtained by experiments on a number of substances of known configurations have led to some general conclusions, by which the absolute configurations of *d*- and *l*-*trans*-2-aminocyclohexanol[5] have been successfully determined.

As an example of a configurationally-known substance with a pair of adjacent amino and hydroxyl groups, methyl 2-amino-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (I) has been measured for the $\Delta[M]$ values in the above-mentioned three kinds of copper solutions; it gave $\Delta[M]_{TACu}$ −930, $\Delta[M]_{CuAm}$ −850 and $\Delta[M]_{Cupra\ B}$ −980 respectively. The projected angle between the adjacent amino and hydroxyl groups of the substance is about −60°.

An *N*-methyl derivative (III) of I, methyl 2-deoxy-4, 6-di-*O*-methyl-2-methylamino-β-D-glucopyranoside, showed nearly the same $\Delta[M]$ values as did I (Table I).

Methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranosides (IV, V), which have a pair of adjacent hydorxyl groups, showed the values of $\Delta[M]_{TACu}\sim 0$, $\Delta[M]_{CuAm}\sim +2200$ and $\Delta[M]_{Cupra\ B}\sim +2200$ respectively. The projected angles between the adjacent hydroxyl groups of both substances are about +60°.

Methyl 2-acetamido-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (VI), in which no hydroxyl or amino groups are free except for a hydroxyl group of C-3, showed nearly zero $\Delta[M]$ values in the above-mentioned three kinds of copper solutions. Methyl 2-amino-2-deoxy-3-*O*-methyl-β-D-glucopyranoside (VII), which has isolated hydroxyl and amino groups, also showed nearly zero $\Delta[M]$ values.

It has been further found that none of the compounds mentioned above showed any change in optical rotations when determined in an aqueous solution of cupric sulfate.

Methyl 2-amino-2-deoxy-α- and -β-D-glucopyranoside (VIII, IX) were also examined. These glucosides contain two kinds of pairs of adjacent groups, a pair of adjacent amino and hydroxyl groups and a pair of adjacent hydroxyl groups; they correspond to the case in which a competitive formation of a copper complex is to be expected between a pair of amino and hydroxyl groups and a pair of hydroxyl groups because these glucosides contain the –C(OH)–C(OH)–C(NH_2)– grouping. It has been found that the corresponding $\Delta[M]$ values of VIII and IX are essentially equal, being $\Delta[M]_{TACu}\sim -830$, $\Delta[M]_{CuAm}\sim +1770$ and $\Delta[M]_{Cupra\ B}\sim +1770$ respectively. The negative sign and the magnitude of $\Delta[M]_{TACu}$ indicate that a formation of copper complex occurs with a pair of adjacent amino and hydroxyl groups at C-2 and C-3, not with a pair of hydroxyl groups at C-3 and C-4, and that the projected angle between the amino and hydroxyl groups is −60°. On the contrary, the positive sign and the magnitude of $\Delta[M]_{CuAm}$ and $\Delta[M]_{Cupra\ B}$ indicate that a complex formation occurs with a pair of adjacent hydroxyl groups at C-3 and C-4, and that the projected angle between them is +60° (Chart 1).

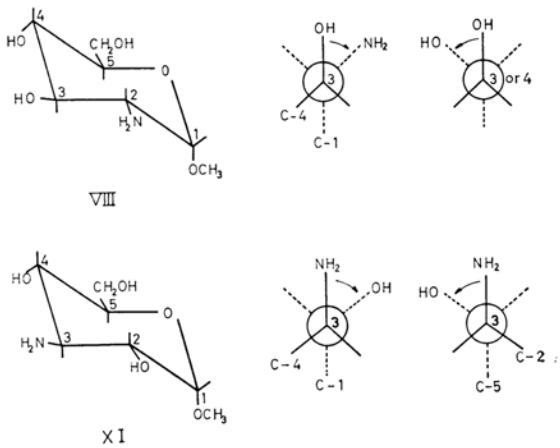


Chart 1.

Methyl α-D-glucopyranoside (X), methyl 3-amino-3-deoxy-α-D-glucopyranoside[6] (XI) and methyl 6-amino-6-deoxy-α-D-glucopyranoside[6] (XII) were further measured for $\Delta[M]$. These glucosides correspond to the cases in which we can pick up two pairs of adjacent hydroxyl groups or adjacent amino and hydroxyl groups, and the projected angles of the two pairs are equal, though opposite in sign. For example, in XI, the projected angular relationship between the amino group at C-3 and the hydroxyl group at C-2 is clockwise and that between the amino group at C-3 and the hydroxyl group at C-4 is counterclockwise. As expected, the experimental values of $\Delta[M]$ were all small (Table I); however, $\Delta[M]_{CuAm}$ and $\Delta[M]_{Cupra\ B}$ showed values which, though slight, could not be deemed nearly zero or negligible, suggesting that the complex formation is slightly one-sided. A similar tendency had been observed by Reeves.[2b]

The above observation led to the following generalizations:

(1) TACu shows a significant $\Delta[M]_{TACu}$ value in compounds which have a pair of adjacent amino (or methylamino) and hydroxyl groups in a six-membered chair molecule, but in compounds which have a pair of adjacent hydroxyl groups or isolated hydroxyl or amino groups, $\Delta[M]_{TACu}$ is nearly zero. The sign of $\Delta[M]_{TACu}$ is in accord with the sign conventionally expected from the projected angle

5) R. M. Godchot and M. Mousseron, *Compt. rend.*, **194**, 981 (1932). The separation of the optical antipodes was improved by T. Suami, S. Ogawa and S. Umezawa, This Bulletin, **36**, 459 (1963).

6) H. Ogawa, T. Ito and S. Kondo, *J. Antibiotics*, **11A**, 70, 72 (1958).

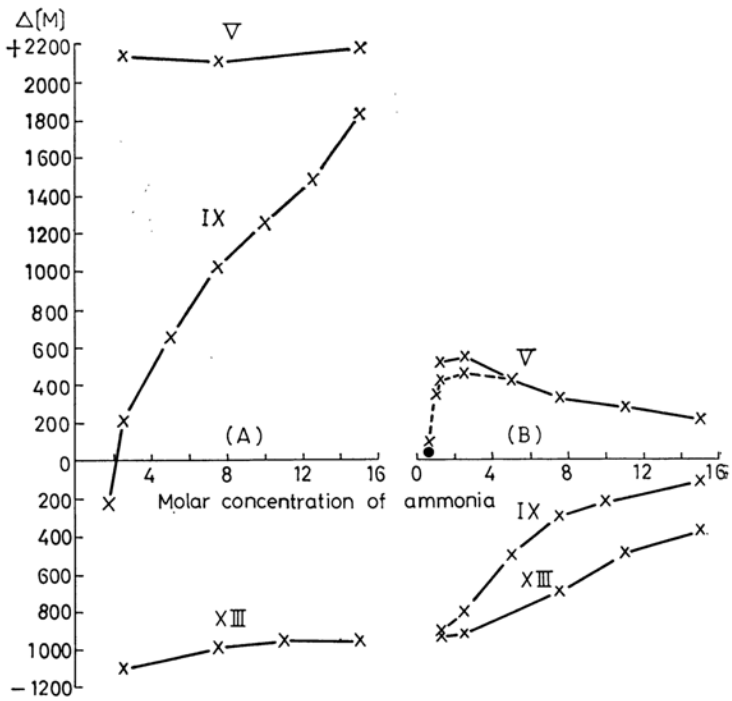


Fig. 1. Effect of the concentration of ammonia on Δ[M] of compounds V, IX and XIII (0.02 mmol.) in solutions (2 ml.) containing cuprous chloride (0.32 mmol.) (A), and cupric chloride (0.32 mmol.) (B). Since the cupric chloride solutions of low concentration of ammonia (<1.5 M ammonia) were not clear, cupric sulfate (0.32 mmol.) was used instead of cupric chloride; this range is represented by a dotted line.
● in B designates $\Delta[M]_{TACu}$ value of V.

made by adjacent hydroxyl groups.[2a] However, the magnitude of the $\Delta[M]_{TACu}$ value of the compounds with a pair of adjacent amino (or methylamino) and hydroxyl groups is about a half of the $\Delta[M]_{CuAm}$ or $\Delta[M]]_{Cupra\ B}$ value of the compounds with a pair of adjacent hydroxyl groups.

(2) Both CuAm and Cupra B show significant $\Delta[M]_{CuAm}$ and $\Delta[M]_{Cupra\ B}$ values in both cases, i. e., in cases of compounds which have a pair of adjacent amino (or methylamino) and hydroxyl groups and in cases of compounds which have a pair of adjacent hydroxyl groups. The sign of these Δ[M] values is again in accord with that conventionally expected from the projected angle. However, the magnitudes of the Δ[M] values in the former cases are about a half of those in the latter cases.

(3) In compounds with pairs both of adjacent amino and hydroxyl groups and of adjacent hydroxyl groups, TACu shows Δ[M] values exclusively corresponding to the pair of the former groups, while CuAm and Cupra B show Δ[M] values exclusively corresponding to the pair of the latter groups.

The above-mentioned generalizations were then successfully applied to an elucidation of the absolute configurations of *d*- and *l-trans*-amino-cyclohexanol.[5] The high negative increment ($\Delta[M]_{TACu}-920$) observed with the former was similar to that observed with I ($\Delta[M]_{TACu}-930$), but of a sign opposite to that of the latter ($\Delta[M]_{TACu}$ +930). Thus, (1s : 2s)-2-aminocyclohexanol (XIII) and (1R : 2R)-2-aminocyclohexanol (XIV) have been assigned to *d*- and *l-trans*-2-aminocyclohexanol respectively.

Discussion

As can be seen from the Table I, TACu has a significant effect in Δ[M], one quite different from that of CuAm and Cupra B, which are essentially similar. It may be considered that the significant difference is due to the formation of a copper(II) complex in the case of TACu and of a Copper(I) complex in the case of CuAm. On the other hand, however, it became of special interest to investigate how the concentration of ammonia affects the Δ[M] value, because either CuAm or Cupra B contains a far larger amount of ammonia than TACu.

Three compounds (V, IX and XIII) were chosen for the investigation. Each of them was dissolved in mixtures of aqueous ammonia of a series of concentrations, and of cuprous or cupric chloride. The Δ[M] values of the resulting solutions are

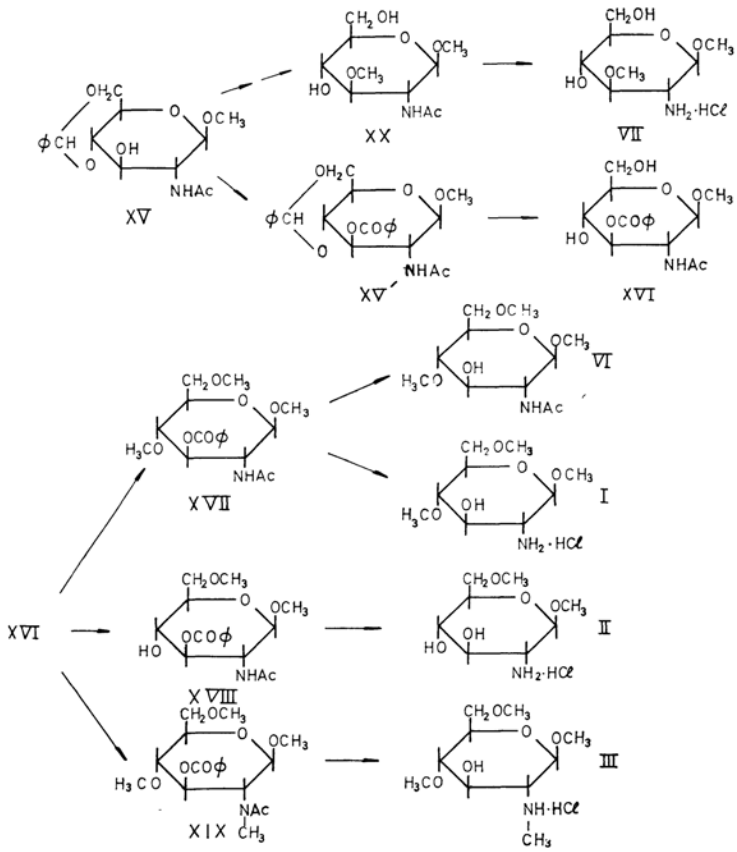


Chart 2. Scheme for the syntheses of derivatives of glucosamine.
Φ: C_6H_5 Ac: $COCH_3$

plotted against the molar concentrations of ammonia in Fig. 1.

As can be seen there, the Δ[M] values of cupric solutions of compounds with a pair of adjacent amino and hydroxyl groups are closely related to the concentration of ammonia. In the cupric chloride - ammonia solutions, the Δ[M] values for IX and XIII are significantly dependent on the concentration of ammonia. The absolute values of IX and XIII increase with the decrease in the concentration of ammonia, reaching a maximum where the concentration of ammonia is nearly equivalent to that in TACu. This indicates that TACu is an excellent reagent for the determination of Δ[M]. The Δ[M] value for V in the cupric solutions showed low values at various concentrations of ammonia.

In the cuprous chloride - ammonia solution, the Δ[M] values for both V and XIII are nearly independent of the concentrations of ammonia between two and fifteen moles, whereas the values for IX increase with the concentration of ammonia, reaching a point where the concentration of ammonia is nearly equivalent to that (14 mol.) in Cupra B. This suggests that, in a region of high concentration of ammonia, a preferential complex-formation occurs with adjacent hydroxyl groups and, as the concentration of ammonia becomes lower, adjacent amino and hydroxyl groups competitively participate in the complex-formation.

Synthesis.—Chart 2 outlines the synthetic steps for the derivatives of glucosamine. Excepting XV and XX, all the derivatives have never been prepared to the authors' knowledge. Methyl 2-amino-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (I) has been prepared by starting with methyl 2-acetamido-4, 6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[7] (XV). The 3-*O*-benzoyl derivative (XV') of XV was debenzylidenated to give methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (XVI). The Purdie methylation of XVI by a general procedure gave methyl 2-acetamido-3-*O*-benzoyl-2-deoxyl-4, 6-di-*O*-methyl-β-D-glucopyranoside (XVII) in a low yield. However, it has been found that the use of a "flash mixer" equipped with an eccentrically-rotating stirrer gave a high yield of XVII, as will be described in the "Experimental" section. The

7) W. Roth and W. Pigman, *J. Am. Chem. Soc.*, **82**, 4608 (1960).

hydrolysis of XVII to I was performed by treatment with hydrazine, a method which was first introduced by Fujinaga and Matsushima[8] for the deacetylation of *N*-acetylated aminosugar glucosides.

In the above methylation, if the reaction period was curtailed, a partially-methylated product, methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-6-*O*-methyl-β-D-glucopyranoside (XVIII), was obtained in addition to XVII, indicating that 6-*O*-methylation occurs easier than 4-*O*-methylation. The evidence for the structure of XVIII was obtained by a study of the infrared and NMR spectra (Fig. 2) and by the determination of three Δ[M] values of the deacetylated product (II) of XVIII (Table I).

Furthermore, it has been found that, if more hours were used in the above methylation, the *N*-methyl derivative of XVII, i. e., methyl 3-*O*-benzoyl-2-deoxy-4,6-di-*O*-methyl-2-*N*-methylacetamido-β-D-glucopyranoside (XIX), was obtained in a low yield. This was then converted to methyl 2-deoxy-4, 6-di-*O*-methyl-2-methylamino-β-D-glucopyranoside (III) by the action of hydrazine.

Methyl 2-acetamido-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (VI) was obtained by curtailing the reaction period of XVII with hydrazine for the preparation of I.

Methyl 2-amino-2-deoxy-3-*O*-methyl-β-D-glucopyranoside (VII) was prepared by starting with XV. Methyl 2-acetamido-2-deoxy-3-*O*-methyl-β-D-glucopyranoside (XX) obtained from XV by the method of Roth et al.[7] was treated with hydrazine to give VII. Fujinaga and Matsushima[9] have recently reported that XX can be converted to VII with hydrazine in a low yield; it has been found that the yield of VII can be successfully increased by raising the reaction temperature to about 130°C.

The mixture of α- and β-anomers (VIII and IX) of methyl 2-amino-2-deoxy-D-glucopyranoside was prepared by the route of Irvine et al.[10] However, as an alternative synthesis, the above mixture has been successfully prepared by a direct Fischer method, only raising the reaction temperature to about 150°C. The anomers could be separated by the method of Austin et al.[11] with resin column chromatography using Dowex 1×2. The separation patterns of anomers in the resin chromatography of the two mixtures were nearly the same, giving two clear-cut peaks. The anomers obtained from the both mixtures were proved to be identical in their physical properties.[12]

8) M. Fujinaga and Y. Matsushima, This Bulletin, **37**, 468 (1964).

9) M. Fujinaga and Y. Matsushima, at the 18th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1965.

10) J. C. Irvine, D. McNicoll and A. Hynd, *J. Chem. Soc.*, **99**, 250 (1911).

11) P. W. Austin, F. E. Hardy, J. G. Buchanan and J. Baddiley, ibid., **1963**, 5350.

12) Recently we learned of another method for the preparation of VIII and IX using Amberlite IR-120 (Y. Matsushima and T. Miyazaki, This Bulletin, **38**, 1325 (1965)).

Experimental

The Preparation of Copper Solutions.—Cupra B: This was prepared by the method of Reeves.[1b] CuAm (cuprous chloride in aqueous ammonia): Cuprous chloride (1.60 g.) was dissolved in 15 N ammonia (100 ml.) at 15°C. TACu (tetramminecopper-(II) sulfate solution): Tetramminecopper(II) sulfate ($[Cu(NH_3)_4]SO_4 \cdot H_2O$) (3.95 g.) was dissolved in fresh distilled water (100 ml.).

The Determination of Optical Rotations.—A sample (0.1 mmol.) was dissolved in each of the above-mentioned three copper solutions (10 ml.), placed in a usual optical cell (10 cm. in length, 1—2 ml.), and the optical rotation at 436 mμ was measured at 15°C by the method of symmetrical angles using a Hitachi EPU-2 spectrophotometer coupled with a Hitachi A-2 polarimeter. An aqueous solution of the same sample was prepared, and the optical rotations at 436 and 589 mμ were also measured.

Thin Layer Chromatography and Silica Gel Column Chromatography.—Thin layer chromatography was conducted by the use of silica gel (Daiichi Pure Chemicals Co.); the prepared plate was activated at 110°C and then stored in a desiccator. Coloration was performed with concentrated sulfuric acid or 0.25% ninhydrin in pyridine. Solvent systems used: benzene-ethyl acetate-acetone (1 : 1 : 1) (Solvent I) and benzene-methanol-acetone (1 : 1 : 8) (Solvent II). Silica gel column chromatography was carried out by the use of silica gel (Kanto Chemical Co.) and was activated at 110°C before use.

Methyl 2-Acetamido-4, 6-*O*-benzylidene-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (XV′).—According to the procedure used for the preparation of the corresponding α-anomer by Jeanloz,[13] XV′ was prepared from XV in a 60% yield; m. p. 292—294°C, $[\alpha]_{589}^{15}$ −97° (*c* 0.5, chloroform).

Found: C, 64.66; H, 5.89; N, 3.14. Cacld. for $C_{23}H_{25}O_7N$: C, 64.62; H, 5.90; N, 3.28%.

Methyl 2-Acetamido-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (XVI).—A mixture of XV′ (380 mg.) and glacial acetic acid (14 ml.) was heated to 80°C, and to this mixture water (9.7 ml.) was added portion by portion. After 30 min. of heating at the same temperature, the mixture was cooled and evaporated to dryness under reduced pressure. To the residue water was again added, and it was again evaporated to dryness; this treatment was thrice repeated. To the residue toluene (3 ml.) was then added and the mixture was evaporated; this treatment was thrice repeated. Finally, the toluene solution was filtered and, after the removal of the solvent, petroleum ether was added to afford crystals of XVI; m. p. 100—102°C (decomp.), $[\alpha]_{589}^{15}$ −47° (*c* 0.5, chloroform).

Found: C, 56.30; H, 6.39. Calcd. for $C_{16}H_{21}O_7N$: C, 56.63; H, 6.24%.

Methyl 2-Acetamido-3-*O*-benzoyl-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (XVII), Methyl 2-Acetamido-3-*O*-benzoyl-2-deoxy-6-*O*-methyl-β-D-glucopyranoside (XVIII) and Methyl 3-*O*-Benzoyl-2-

13) The derivative XV′ had been isolated as a minor product from a reaction mixture obtained from methyl 2-acetamido-4, 6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (R. W. Jeanloz, *J. Am. Chem. Soc.*, **76**, 555 (1954)); reported m. p. 293—295°C, $[\alpha]_D$ −102±2° (*c* 0.73, chloroform).

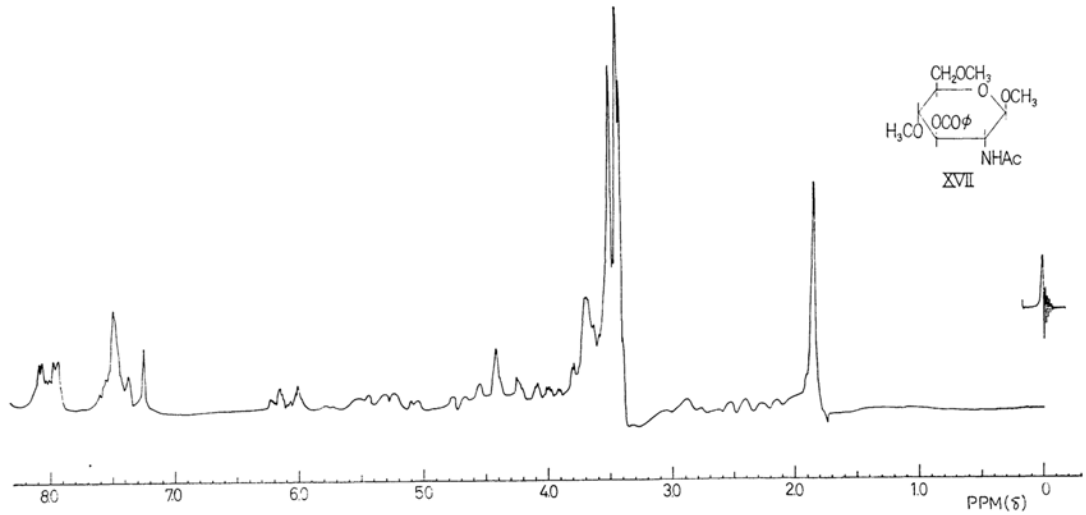


Fig. 2-A. The NMR spectrum at 60 Mc.p.s. of methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (XVII) in deuteriochloroform.

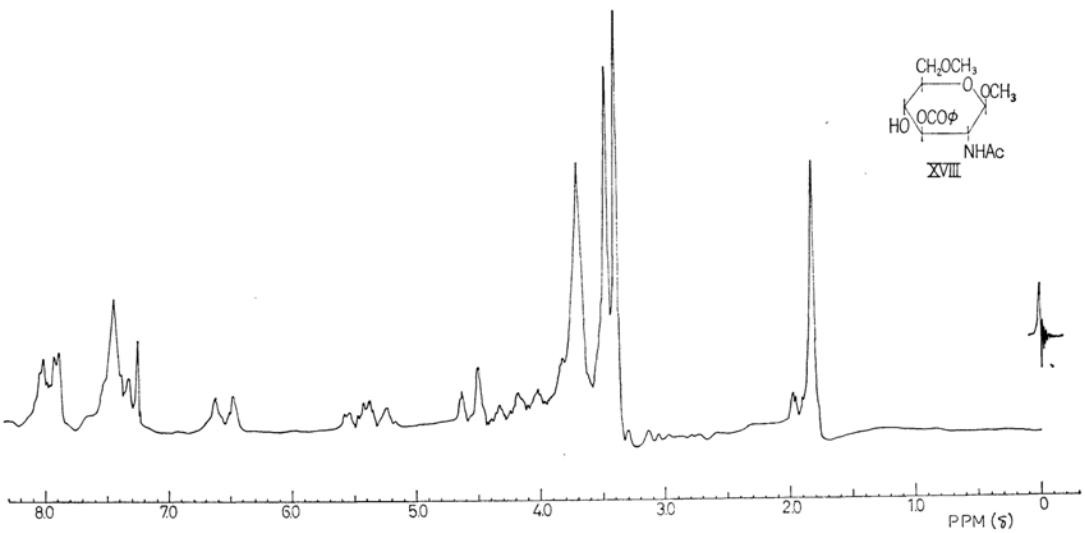


Fig. 2-B. The NMR spectrum at 60 Mc.p.s. of methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-6-*O*-methyl-β-D-glucopyranoside (XVIII) in deuteriochloroform.

deoxy-4, 6-di-*O*-methyl-2-(*N*-methylacetamido)-β-D-glucopyranoside (XIX).—A mixture of methyl 2-acetamido-3-*O*-benzoyl-2-deoxy-β-D-glucopyranoside (XVI) (300 mg.), silver oxide (1.2 g.), methyl iodide (0.9 g.), Drierite (anhydrous calcium sulfate) (0.9 g.) and dry acetone (3 ml.) was placed in a thick, tightly-capped glass tube and agitated vigorously for 6 hr. at room temperature with an eccentrically-rotating stirrer.*2 After filtration, the precipitate was washed with acetone, and the combination of the filtrate and the washings was evaporated to dryness, giving 330 mg. of a solid. In thin layer chromatography with solvent I, the solid was proved to be composed of three components with R_f values of 0.42, 0.58 and 0.90 (minor spot). The solid was dissolved in a solvent mixture of benzene-ethyl acetate-acetone (1 : 1 : 1) and chromatographed on a silica gel column (48×130 mm.) with the same solvent. Every fraction (11 g.) was tested by thin layer chromatography. Substances with R_f values of 0.58 and 0.42 appeared in the fractions of tubes Nos. 16—19 and 22—27 respectively. The former fractions were collected and evaporated to give a residue which crystallized from chloroform, thus affording XVII, 130 mg.; m. p. 167—169°C, $[\alpha]_{589}^{15}$ −11.1° (*c* 0.9, methanol); IR spectrum (KBr disk): 3305 (νNH, sharp), 2940, 2850, 1735 (ester), 1665 (Amide I), 1565 (Amide II), 1455, 1378 cm^{-1}.

Found: C, 58.92; H, 6.85; N, 3.82. Calcd. for $C_{18}H_{25}O_7N$: C, 58.84; H, 6.86; N, 3.81%.

*2 A "Flash Mixer" (Mitamura Riken Kogyo Co.) with an eccentric radius of 1 mm. was used at 2000 r. p. m.

From the latter fractions, XVIII was obtained in the way described above; yield, 115 mg. (hygroscopic); m. p. 179—181°C (sintered once at 75—85°C), $[\alpha]_{589}^{15}$ +17.7° (*c* 1.2, methanol); IR spectrum (KBr disk): 3340, 2920, 2830, 1725, 1660, 1550, 1450, 1375 cm^{-1}.

Found: C, 54.89; H, 6.72; N, 3.74. Calcd. for $C_{17}H_{23}O_7N \cdot H_2O$: C, 54.98; H, 6.79; N, 3.77%.

When the above agitaion with an eccentrically-rotating stirrer was continued for 20 hr., the resulting crude product was composed of two components, R_f 0.59 and 0.90 (minor component). On a silica gel chromatography on the scale described above, the eluted fractions of tubes Nos. 16—21 and 10—12 gave 180 mg. of XVII and 30 mg. of XIX respectively.

When the above agitation was continued for 45 hr., the yield of XIX was increased to 67 mg., ($[\alpha]_{585}^{15}$ −10.5° (*c* 1, methanol); IR spectrum (KBr disk): 2930, 2900, 2830, 1730 (ester), 1690 (Amide I), 1455, 1380 cm^{-1}). No absorption bands corresponding to νNH (~3300) and δNH (~1550, Amide II) were observed, indicating that XIX is a *N*-methylated product.

Found: C, 59.60; H, 6.88; N, 3.54. Calcd. for $C_{19}H_{27}O_7N$: C, 59.83; H, 7.13; N, 3.67%.

The NMR spectra of compounds XVII and XVIII were determined in deuteriochloroform at a frequency of 60 Mc. p. s. with a Varian A60 spectrometer (Fig. 2). Tetramethylsilane was used as an internal reference. The comparison of the relative strength of the peaks at ~6.5 τ (methyl protons of methoxyl groups) with the other peaks [acetoxyl protons at ~8.2 τ, anomeric protons (3.91 τ for XVII and 3.45 τ for XVIII), and aromatic protons] revealed that XVII and XVIII have three and two methoxyl groups respectively.

Methyl 2-Amino-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside Hydrochloride (I).—A mixture of XVII (60 mg.) and hydrazine hydrate*3 (0.9 ml.) in a sealed tube was heated in a boiling water bath for 17 hr. After the hydrazine had been removed in vacuo, the residue was dissolved in a mixture of benzene-methanol-acetone (1 : 1 : 8) and chromatographed on a silica gel column (27×70 mm.) with the same solvent. A ninhydrin active product was eluted between 40—120 ml. This fraction was evaporated to dryness; the aqueous solution of the residue was passed through a small column of Dowex 1×2 (OH form), and the ninhydrin-active portion was again collected and evaporated to dryness. The residue was dissolved in chloroform and dried over sodium sulfate, and, after filtration, the filtrate was evaporated to dryness. The residue was again dissolved in water, neutralized with hydrochloric acid, treated with active charcoal, and evaporated to give a solid which was crystallized from methanol by adding acetone; yield, 23 mg.; m. p. 203—204°C (decomp.), $[\alpha]_{589}^{15}$ −2.0° (*c* 0.3, water); IR spectrum (KBr disk): 3320, 2900, 1600 ($\delta_{as}NH_3^+$), 1500 (δ_s NH_3^+), 1450, 1400—1370 (3 bands) cm^{-1}.

Found: C, 41.71; H, 7.61; N, 5.41. Calcd. for $C_9H_{19}O_5N \cdot HCl$: C, 41.94; H, 7.81; N, 5.44%.

On thin layer chromatography with solvent II, I showed an R_f of 0.54. To an aqueous solution (2.6 ml.) of the hydrochloride (I) (2.6 mg.) periodic acid (9.1 mg.), was added, and the mixture was allowed to stand at room temperature for 20 hr. A drop of the solution was then subjected to thin layer chromatography. No spot corresponding to I was discerned.

Methyl 2-Amino-2-deoxy-6-*O*-methyl-β-D-glucopyranoside Hydrochloride (II).—A mixture of XVIII (110 mg.) and hydrazine hydrate (1.6 ml.) was treated for 60 hr. as above. The thin layer chromatography of the crude product showed an R_f value of 0.32 with solvent II. Isolation and purification was carried out in the same way as described in the above synthesis of I; yield, 39 mg. (hygroscopic), $[\alpha]_{589}^{15}$ −4.0° (*c* 0.5, water); IR spectrum (KBr disk): ~3300, 2900, 1600, 1510, 1450, 1400—1380 (2 bands) cm^{-1}.

Found: C, 39.62; H, 7.41. Calcd. for $C_8H_{17}O_5N \cdot HCl$: C, 39.43; H, 7.45%.

Methyl 2-Acetamido-2-deoxy-4, 6-di-*O*-methyl-β-D-glucopyranoside (VI).—A mixture of XVII (150 mg.) and hydrazine hydrate (2.3 ml.) in a sealed tube was heated in a boiling water bath for 1.5 hr. The removal of the solvent in vacuo left a solid which, on thin layer chromatography with solvent II, showed a single spot (R_f 0.7). The solid was then dissolved in the same solvent system and chromatographed on a silica gel column (27×200 mm.) with the same solvent. An eluate between 50—80 ml. was evaporated to dryness, and the residue was dissolved in ethanol. The solution was treated with active charcoal and concentrated to a small volume, from which crystals of VI was obtained by the addition of ether; yield, 75 mg.; m. p. 177—179°C (decomp.), $[\alpha]_{589}^{15}$ −23.3° (*c* 0.9, water); IR spectrum (KBr disk): 3500—3400, 3270, 2920, 2820, 1650 (Amide I), 1565 (Amide II), 1450, 1375 cm^{-1}.

Found: C, 50.44; H, 7.74; N, 5.20. Calcd. for $C_{11}H_{21}O_6N$: C, 50.18; H, 8.04; N, 5.32%.

Methyl 2-Deoxy-4, 6-di-*O*-methyl-2-methylamino-β-D-glucopyranoside Hydrochloride (III).—A mixture of XIX (90 mg.) and hydrazine hydrate (1.4 ml.) in a sealed tube was heated in a boiling water bath for 22 hr. The removal of the solvent in vacuo left a solid which, on thin layer chromatography with solvent II, showed a single spot (R_f 0.52).

The solid was then dissolved in the same solvent system and chromatographed on a silica gel column (27×90 mm.) with the same solvent. An eluate between 50—110 ml. was evaporated, and the residue was treated in the way described in the synthesis of I; yield, 39 mg. (hygroscopic); $[\alpha]_{589}^{15}$ −1.1° (*c* 0.5, water); IR spectrum (KBr disk): 3350, 2900, 1600 (δ_{as} NH_3^+), 1505 (δ_s NH_3^+), 1455, 1405 cm^{-1}.

Found: C, 43.81; H, 8.03. Calcd. for $C_{10}H_{21}O_5N \cdot HCl$: C, 44.19; H, 8.16%.

Methyl 2-Amino-2-deoxy-3-*O*-methyl-β-D-glucopyranoside Hydrochloride (VII).—A mixture of methyl 2-acetamido-2-deoxy-3-*O*-methyl-β-D-glucopyranoside*4 (XX) (83 mg.) and hydrazine hydrate (2 ml.) in a sealed tube was heated at 130°C for 40 hr. The removal of the solvent in vacuo left an solid which, on thin layer chromatography with solvent II, showed a single spot (R_f 0.38). (XX showed an R_f of 0.54 with solvent II.) The solid was then dissolved in a mixture of benzene, methanol and acetone (1 : 1 : 8); the mixture was chromatographed on a sillica gel column (27×88 mm.) with the same solvent. An eluate (between 40—100 ml.) was evaporated, and the residue was treated

*3 Commercial hydrazine hydrate (80%) was fractionally distilled; the fraction constant at 119°C was taken for use.

*4 M. p. 221—222°C (decomp.) (lit.[7] m. p. 229°C); $[\alpha]_{589}^{15}$ −46.2°, $[\alpha]_{436}^{15}$ −85.3° (*c* 0.6, water) (lit.,[7] $[\alpha]_D$ −45.9°); Found: C, 48.04; H, 7.57. Calcd. for $C_{10}H_{19}O_6N$: C, 48.17; H, 7.70%.

in the way described in the synthesis of I; yield, 55 mg. (70%). A sample (11 mg.) was recrystallized from ethanol to yield 9 mg. of an analytically-pure sample of VII; m. p. 139—140°C (decomp.). $[\alpha]_{589}^{15}$ +10.3° (*c* 0.3, water); IR spectrum (KBr disk): 3370, 2920, 1600 (δ_{as} NH_3^+), 1500 (δ_s NH_3^+), 1450, 1395 cm^{-1}.

Found: C, 39.54; H, 7.39; N, 5.68. Calcd. for $C_8H_{17}O_5N \cdot HCl$: C, 39.43; H, 7.45; N, 5.75%.

Methyl 2-Amino-2-deoxy-α- and -β-D-glucopyranoside Hydrochloride (VIII and IX).—A mixture of 2-amino-2-deoxy-D-glucose hydrochloride (215 mg.) and 2 N hydrochloric acid in methanol (5 ml.) was placed in a sealed glass tube and heated at 150°C for 4 hr. The resulting solution was evaporated to give a brown syrup, which was then dissolved in water and passed through a small column of Dowex 1×2 (OH form). This solution, after having been treated with active charcoal, was then evaporated to give a syrup; this syrup was then applied to a column (18×270 mm.) of Dowex 1×2 (OH form), and elution was carried out with water. Methyl 2-amino-2-deoxy-α-D-glucopyranoside and methyl 2-amino-2-deoxy-β-D-glucopyranoside emerged in the eluates between 63—69 ml. and between 75—84 ml. respectively. The former fraction was collected, concentrated to a small volume, and neutralized with hydrochloric acid. After evaporation to dryness, the solid obtained was recrystallized from ethanol to give 34 mg. of VIII, m. p. 189°C (decomp.)*5 $[\alpha]_{589}^{15}$ +153° (*c* 0.23, water)*5.

Found: C, 36.78; H, 7.23; N, 5.95. Calcd. for $C_7H_{15}O_5N \cdot HCl$: C, 36.61; H, 7.02; N, 6.10%.

From the latter fraction, by the same procedure as above, IX was obtained, 36 mg., m. p. 188°C (decomp.)*6 $[\alpha]_{589}^{15}$ −20° (*c* 0.23, water)*6.

Found: C, 36.72; H, 7.01; N, 6.14. Calcd. for $C_7H_{15}O_5N \cdot HCl$: C, 36.61; H, 7.02; N, 6.10%.

Methyl 2-Acetamido-2-deoxy-α- and -β-D-glucopyranoside (IV and V).—To an aqueous ethanol (7 : 3) solution of the hydrochloride VIII (1.0 g.), acetic anhydride and powdered barium hydroxide octahydrate were added alternately until the solution became negative to a ninhydrin test; then a little excess of sulfuric acid was added and the mixture was filtered. The filtrate was treated with Dowex 2×8 (OH form) and, after the solvent had been removed, the residue was recrystallized from ethanol to give 680 mg. of IV, m. p. 187—188°C*7 $[\alpha]_{589}^{15}$ +103° (*c* 0.3, water); IR spectrum (KBr disk): 3370, 3300, 2920, 2860, 1655 (Amide I), 1550 (Amide II), 1445, 1375, 900 (Type 1 of pyranose ring), 853 (Type 2a) cm^{-1}.

Found: C, 46.18; H, 7.23; N, 5.87. Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.28; N, 5.95%.

The β-anomer (V) was also prepared from IX (1.0 g.) in the same way as above; yield, 610 mg.; m. p. 199—200°C,*8 $[\alpha]_{589}^{15}$ −48° (*c* 0.3, water);*8 IR spectrum (KBr disk): 3400, 3300, 2930, 2880, 1650 (Amide I), 1555 (Amide II), 1450, 1380, 940 (Type 1 of pyranose ring), 859 (Type 2b) cm^{-1}.

Found: C, 45.94; H, 7.24; N, 5.90. Calcd. for $C_9H_{17}O_6N$: C, 45.95; H, 7.28; N, 5.95%.

***d*- and *l*-*trans*-2-Aminocyclohexanol Hydrochloride (XIII and XIV).**—To an aqueous solution of *d*-*trans*-2-aminocyclohexanol L-(+)-tartrate (200 mg.), 10 ml. of Amberlite IRA-400 (OH form) was added. After filtration, the filtrate was concentrated to a small volume and neutralized with hydrochloric acid. The addition of acetone gave crystals of XIII; yield, 80 mg.; m. p. 157.5—158°C, $[\alpha]_{589}^{15}$ +41.7° (*c* 0.5 water); IR spectrum (KBr disk): 3435, 3330, 2990, 2920, 2840, 1975 (νNH_3^+), 1595 (δ_{as} NH_3^+), 1505 (δ_s NH_3^+), 1465, 1385, 1358 cm^{-1}.

Found: C, 47.63; H, 9.02; N, 9.08. Calcd. for $C_6H_{13}ON \cdot HCl$: C, 47.52; H, 9.31; N, 9.24%.

The hydrochloride (XIV) of the *l*-isomer was prepared from *l*-*trans*-2-aminocyclohexanol L-(+)-tartrate (200 mg.); yield, 77 mg.; m. p. 157.5 −158°C, $[\alpha]_{589}^{15}$ −40.3° (*c* 0.5, water).

Found: C, 47.67; H, 9.17; N, 9.10. Calcd. for $C_6H_{13}ON \cdot HCl$: C, 47.52; H, 9.31; N, 9.24%.

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*5 Reported m. p. 190—192°C, $[\alpha]_D$ +145° (Ref. 11).

*6 Reported m. p. 191—192°C, $[\alpha]_D$ −26° (Ref. 11).

*7 Reported m. p. 187—188°C (R. Kuhn, F. Zilliken and A. Gauhe, *Ber.*, **86**, 466 (1953)).

*8 Reported m. p. 190—191°C, $[\alpha]_D$ −42° (Ref. 7).