the final conclusion on this problem.

The same is true for the case of the radical in benzene solution.

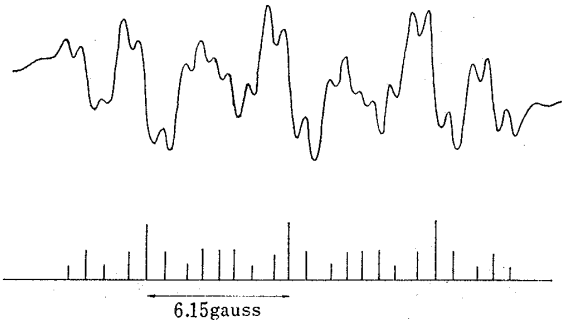

Fig. 3. The Electron Spin Resonance Spectrum of Radical produced by Photoirradiation of 4-Nitroquinoline 1-Oxide in Dioxane and the Reconstruction based on the Coupling Constants, A_N=6.15 gauss, A_{Ha}=2.33 gauss, and A_{Hb} =0.67 gauss, respectively.

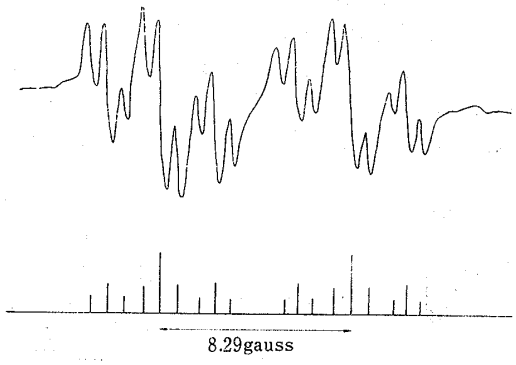

Fig. 4. The Electron Spin Resonance Spectrum of Radical produced by Photoirradiation of 4-Nitroquinoline 1-Oxide (^{15}N) in Dioxane and the Reconstruction based on the Coupling Constants, $A_{^{15}N}$=8.29 gauss, A_{Ha}=2.33 gauss, and A_{Hb} =0.67 gauss, respectively.

The authors wish to thank Dr. Waro Nakahara, Director of this institute, for his continued interest and encouragement in this work.

Summary

UV-irradiation of 4-nitroquinoline 1-oxide in solutions, that is, in dioxane, in benzene, and in hexane, induced stable radicals which were investigated by ESR spectroscopy. Hyperfine structures of spectra thus obtained are different from each other depending on the sort of the solvents used. Determination of these radical structures were carried out by help of isotope-containing 4-nitroquinoline 1-oxides and, in particular, the structure of dioxane type radical was proposed, based on spectral changes by isotope replacement.

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122. **Shigeharu Inouye** : Syntheses of Methyl 3,6-Diamino-3,6-dideoxy-α-D-glucopyranoside and Methyl 3,6-Diamino-3,6-dideoxy-α-D-mannopyranoside.

(*Central Research Laboratories, Meiji Seika Kaisha, Ltd.*[*1])

The synthesis of a diamino-sugar is one of the subjects of current research in the amino-sugar chemistry. Among the 3,6-diamino-3,6-dideoxy-hexose family, the syntheses of methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside[1] and 3,6-diamino-3,6-dideoxy-D-idose[2] were reported.

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*1 Morooka, Kohoku-ku, Yokohama-shi (井上重治).

1) *a*) M. I. Wolfrom, Yen-Lung Hung, D. Horton : J. Org. Chem., **30**, 3394 (1965). *b*) S. Inouye : This Bulletin, in press.

2) S. Hanessian, T. H. Haskell : J. Org. Chem., **30**, 1080 (1965).

Recent work on the chemical modification of the antibiotic kanamycin A in this laboratory, has led to the syntheses of a number of the amino-deoxy derivatives.[3] Introduction of an amino group was made either to C_6 of the 3-amino-3-deoxy-α-D-glucopyranose moiety or to C_3 of the 6-amino-6-deoxy-α-D-glucopyranose moiety, with or without the configurational inversion at C_2 in the respective diamino-hexoses. Thus, the kanamycin derivatives containing 3,6-diamino-3,6-dideoxy-α-D-glucopyranose or 3,6-diamino-3,6-dideoxy-α-D-mannopyranose as the component were synthesized. In order to confirm the structure of these derivatives, it was necessary to prepare the new components, the hitherto unknown diamino-hexoses in the literature. The present syntheses of methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside (VII) and methyl 3,6-diamino-3,6-dideoxy-α-D-mannopyranoside (XV) were therefore undertaken.

The starting materials for the syntheses of the title compounds were the corresponding methyl 3-amino-3- deoxy-α-D-hexopyranosides, which were available from methyl α-D-glucopyranoside utilizing the nitromethane synthesis[4]. These two 3-amino-sugars were isolated in good yield by the improved procedure using the resin chromatography of Dowex 1×2 (OH^- form).[5] In addition to them, a new compound, methyl 3-amino-3-deoxy-β-L-glucopyranoside, was obtained from the mother liquor with 1.2% yield. This indicated, in contrast to the previous assumption,[6] that the two asymmetric centers derived from C_1 and C_5 of methyl α-D-glucopyranoside were involved in partial epimerization, probably before the nitromethane condensation.

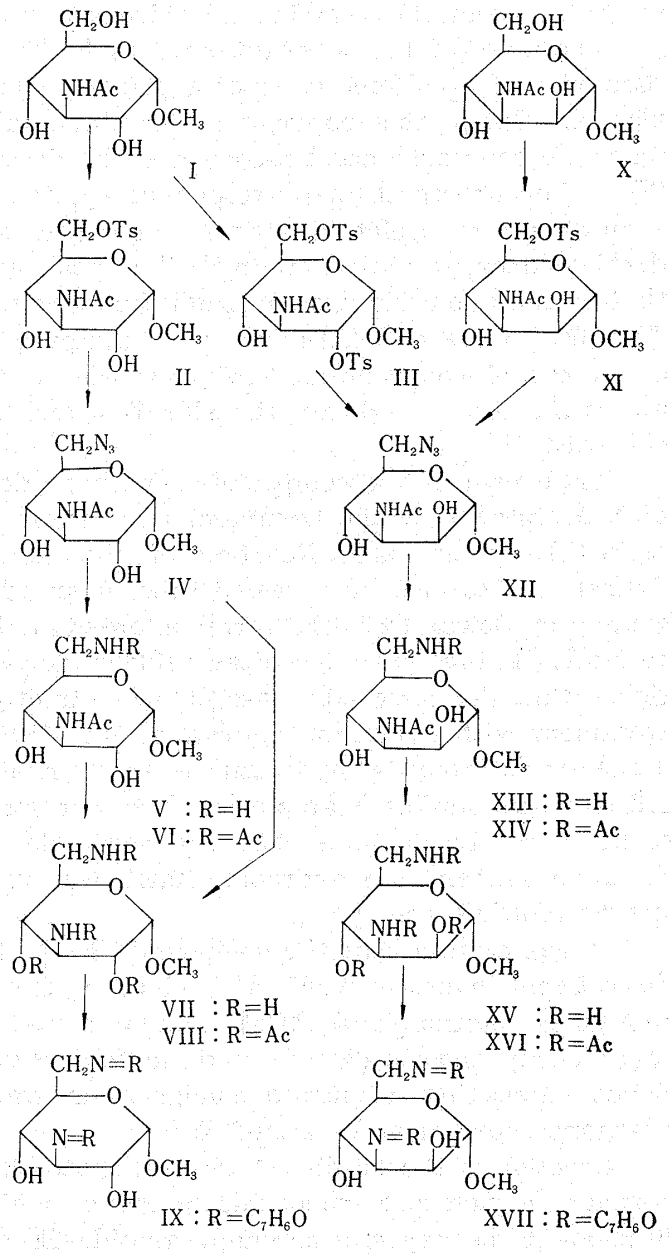

Chart 1.

Reaction of methyl 3-acetamido-3-deoxy-α-D-glucopyranoside (I),[7] which was prepared from the parent 3-amino-sugar by the treatment with acetic anhydride in methanol, with 1.2 mol. of *p*-tolylsulfonyl chloride in pyridine effected a selective sulfonylation of the terminal hydroxyl function to give methyl 3-acetamido-3-deoxy-6-O-*p*-tolylsulfonyl-α-D-glucopyranoside (II) in 45.5% yield.

3) S. Inouye : "Symposium Abstracts, 9th Symposium on the Chemistry of Natural Products," 7 (1965), Osaka.
4) F. W. Lichtenthaler : Angew. Chem., **76**, 84 (1964).
5) S. Inouye, H. Ogawa : J. Chromatog., **13**, 536 (1964).
6) H. H. Baer, H. O. L. Fischer : J. Am. Chem. Soc., **82**, 3709 (1960).
7) H. Ogawa, T. Ito, S. Kondo, S. Inouye : Bull. Agr. Chem. Soc. Japan, **23**, 289 (1959).

Thin-layer chromatography of the mother liquor, however, showed, in addition to the 6-O-sulfonate (II), the presence of the 2,6-di-O-sulfonate (III), which was isolated by the use of a column chromatography of silicic acid. Treatment of the 6-O-sulfonate (II) in refluxing dimethylformamide with an excess of sodium azide gave methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-glucopyranoside (IV), which was purified by the resin chromatography on a column of Dowex 50W×2 (H^+ form). Reduction of this compound with a large amount of Raney nickel for 2 hours afforded quantitatively methyl 3-acetamido-6-amino-3,6-dideoxy-α-D-glucopyranoside (V).

The proof of the substitution at C_6 has been based, in many cases, on the greater reactivity of a sulfonic ester of a primary alcohol compared to that of a secondary alcohol. But in this paper, the terminal substitution was proved by the nuclear magnetic resonance spectroscopy and the dissociation constant of the amino derivative (V). The nuclear magnetic resonance spectrum of V in deuterium oxide showed an asymmetric multiplete around 7.1 p.p.m., which was assigned to the protons weakly deshielded by an amino group.*2 The peak area of this signal was about double of that of the anomeric proton signal, indicating the aminomethylene structure ($-CH_2-N<$). The pKa' value of V (8.84) in water compared well with that of methyl 6-amino-6-deoxy-α-D-glucopyranoside (8.86), but was widely different from those of methyl 3-amino-3-deoxy-α-D-glucopyranoside (7.82) and methyl 2-amino-2-deoxy-α-D-glucopyranoside (7.54).*3

Treatment of the compound (V) with acetic anhydride in methanol gave the 3,6-di-N-acetate (VI), while treatment with acetic anhydride in pyridine gave the 2,3,4,6-tetra-O,N-acetate (VIII). Reaction of the compound (V) with an excess of hydrazine hydrate at 145~150° in a sealed tube, followed by purification with the resin chromatography of Dowex 1×2 (OH^- form), afforded methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside (VII) that gave 3,6-disalicylidene derivative (IX). It was worthy to be mentioned that the same free base (VII) was directly obtained from the 6-azide (IV) by the treatment with hydrazine hydrate at 145~150°. In this case, de-N-acetylation of the 3-acetamido group by hydrazine was accompanied with reduction of the 6-azido group. Although no similar example has been reported, reduction of an azido group seemed to be caused by di-imide that was derivable from hydrazine, since in many cases, di-imide, rather than hydrazine itself, was responsible for the reduction of a symmetric multiple bond.[8]

It was certain that the compounds (II~IX) had a glucose configuration, as any configurational inversion was not involved in this synthesis. In accordance with this, the molecular rotation of VII (29,200) compared well with those of methyl 3-amino-3-deoxy-α-D-glucopyranoside (30,000) and methyl α-D-glucopyranoside (30,900). Further evidence supporting a glucose configuration was obtained from the nuclear magnetic resonance spectra of V, VI and VIII.[9]

Reaction of the 2,6-di-O-*p*-tolylsulfonate (III) with sodium azide in refluxing dimethylformamide yielded a monoazide compound which was identified as methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-mannopyranoside (XII) through a comparison with the sample derived from methyl 3-acetamido-3-deoxy-α-D-mannopyranoside (X).[10] The identification was further corroborated by reduction of this compound to methyl 3-acetamido-6-amino-3,6-dideoxy-α-D-mannopyranoside (XIII). The above result indicated that

*2 Further discussion on this assignment will be made in a subsequent paper.[9]

*3 More detailed information on the structure and dissociation constant of amino-sugars will be presented in a separate paper.

8) S. Hünig, H. R. Müller, W. Thier : Angew. Chem., **77**, 368 (1965).
9) S. Inouye : This Bulletin, in press.
10) A. C. Richardson : J. Chem. Soc., **1962**, 373.

dimolar *p*-tolylsulfonylation of I occurred preferably at C_2 and C_6, rather than at C_4 and C_6. In the latter case, the formation of methyl 3-acetamido-6-azido-3,6-dideoxy-*α*-D-galactopyranoside would be expected. The inversion of the *p*-tolylsulfonyloxy group at C_2 without replacement by a powerful nucleophilic reagent (azide ion) was undoubtedly due to the neighboring group participation of the 3-acetamido group, situated *trans* to the vicinal substituent.[11]

Treatment of methyl 3-acetamido-3-deoxy-*α*-D-mannopyranoside (X),[10] which was prepared from the tetra-O,N-acetate by the de-O-acetylation, with a slight excess of *p*-tolylsulfonyl chloride in pyridine afforded the 6-O-sulfonate (XI). Treatment of the latter with sodium azide in a manner similar to that described for the glucose derivative, gave the 6-azide (XII), which was then reduced with Raney nickel to give the 6-amine (XIII). Evidence for the amination at C_6 was presented by the nuclear magnetic resonance spectrum and the dissociation constant (pKa' 8.80) of XIII. The methylene signal weakly deshielded by the primary amino group in XIII appeared at ca. 7.1 p.p.m. in deuterium oxide.

De-N-acetylation of the compound (XIII) with hydrazine hydrate afforded a sirupy methyl 3,6-diamino-3,6-dideoxy-*α*-D-mannopyranoside (XV) that gave an amorphous dihydrochloride. XV was homogeneous in paper chromatography and characterized by conversion to the crystalline disalicylidene derivative (XVII). Since no configurational inversion was involved in this synthesis, it was apparent that the compounds (XI～XVII) had a mannose configuration. The nuclear magnetic resonance spectra of XIII, XIV (di-N-acetate) and XVI (tetra-O,N-acetate) were also compatible with a mannose configuration[9]. The synthesis of XV through the alternative route, namely, from methyl 3-acetamido-3-deoxy-*α*-D-glucopyranoside (I) *via* the 2,6-di-O-sulfonate, was already described.

Experimental*4

Improved Isolation of Methyl 3-Amino-3-deoxy-*α*-D-mannopyranoside and Methyl 3-Amino-3-deoxy-*α*-D-glucopyranoside from Nitromethane Condensation Products——Periodate oxidation of methyl *α*-D-glucopyranoside (100 g.), followed by cyclization with nitromethane and subsequent reduction with Raney Ni (W-4) were carried out according to the procedure reported by Richardson.[10] To the mother liquor remaining afrer the collection of the crystalline methyl 3-amino-3-deoxy-*α*-D-mannopyranoside·HCl (16.8 g.), was added Amberlite IRA-400 (OH, 400 ml.) suspended in H_2O. The neutralized solution was evaporated to a black-red sirup, which was dissolved in H_2O (100 ml.) and applied to a column (3.3×74 cm.) of Dowex 1×2 (OH) resin (200～400 mesh). Elution was carried out with H_2O and the effluents were collected in 12.3 ml. fractions. Evaporation of the fractions 43～71 gave a sirup, from which was crystallized an amino-hexoside (21.5 g., m.p. 169～171°)*5 upon addition of EtOH. This was proved to be identical with the authentic sample of methyl 3-amino-3-deoxy-*α*-D-glucopyranoside.[7] Addition of conc. HCl to the mother liquor resulted in further recovery of the crystals (11.7 g.) of methyl 3-amino-mannoside·HCl (total yield, 28.5 g., 24% based on the starting material). The above mother liquor, after concentration and neutralization, was again chromatographed on a column of Dowex 1×2 (OH) and from the H_2O eluates was recovered 7.5 g. of methyl 3-amino-glucoside (total yield, 29.0 g., 29%).

Isolation of Methyl 3-Amino-3-deoxy-*β*-L-glucopyranoside——The mother liquor that remained after removal of the above two amino-hexosides, was kept for 5 days in a refrigerator. The crystals (1.21 g.) that was deposited was collected and recrystallized from MeOH to give a pure compound. Rf Value in paper

*4 Melting points were uncorrected. UV Spectra were taken in MeOH with a Hitachi Recording Spectrometer EPS-2, and IR spectra in Nujol mull with a Koken Infrared Spectrometer 401. NMR Spectra were determined at 60 Mc.p.s. using a JNM-3-H-60 Spectrometer. Tetramethylsilane and sodium 2,2-dimethyl-1,2-silapentane-5-sulfonate were used as an internal standard in $CDCl_3$ and D_2O, respectively. Paper chromatography was carried out on Toyo Roshi No. 50 filter paper by the descending method with a solvent of *n*-BuOH-pyridine-AcOH-H_2O (6:4:1:3). Spots were detected by dipping in 0.5% ninhydrin in acetone-pyridine (10:1).

*5 Previously this compound was isolated as its tetra-O,N-acetate (H. H. Baer : J. Am. Chem. Soc., **84**, 83 (1962)).

11) B. R. Barker : "Methods in Carbohydrate Chemistry" edited by R. I. Whistler amd M. I. Wolfrom, Vol. II, 444 (1963). Academic Press, New York.

chromatography was indistinguishable from methyl 3-amino-3-deoxy-α-D-glucopyranoside (Table I), but IR spectrum was definitely different. IR cm^{-1} : $\nu_{O-H, N-H}$ 3500, 3368, 3308; δ_{N-H} 1566; ν_{C-O} 1016; δ_{C-H} 892, 869, 723, 704. *Anal.* Calcd. for $C_7H_{15}O_5N$: C, 43.5; H, 7.8; N, 7.3. Found : C, 43.8; H, 7.8; N, 7.5. N-Acetate and tetra-O,N-acetate were prepared by the usual way. *Anal.* Calcd. for $C_9H_{17}O_6N$ (N-acetate) : C, 46.0; H, 7.3; N, 6.0. Found : C, 46.2; H, 7.3; N, 6.5. Calcd. for $C_{15}H_{23}O_9N$ (tetra-O, N-acetate) : C, 49.9; H, 6.4; N, 3.9. Found : C, 50.2; H, 6.4; N, 4.2. N-Acetate consumed no periodate. NMR Spectrum of the free base in D_2O showed an axial anomeric proton (5.70 p.p.m.) with diaxial coupling ($J_{1,2}=8.0$ c.p.s.), and an equatorial OCH_3 (6.50 p.p.m.). NMR Spectrum of the tetraacetate in $CDCl_3$ showed an equatorial CH_3CON (8.11 p.p.m.), three equatorial CH_3COO (7.94, 7.94, 7.96 p.p.m.), an equatorial OCH_3 (6.50 p.p.m.) and a broad NHCO (3.89 p.p.m., $J_{N-H, C-H}=9.0$ c.p.s.). Under the same condition, the spectrum of methyl 2,3,4,6-tetra-O-acetyl-β-D-glucopyranoside showed four equatorial CH_3COO (7.92, 7.96, 7.98, 8.01 p.p.m.) and an equatorial OCH_3 (6.49 p.p.m.). Thus, the β-glucopyranose configuration was assigned. $[\alpha]_D$ of the free base and its acetates were identical with those reported for methyl 3-amino-3-deoxy-β-D-glucopyranoside and its acetates,[12] except for the sign of rotation. Melting points were also very close to those reported (Table II), indicating the β-L-isomer.

Table I. Rf Values of Methyl 3,6-Diamino-3,6-dideoxy-α-D-glucopyranoside, Methyl 3,6-Diamino-3,6-dideoxy-α-D-mannopyranoside and Related Compounds in Paper Chromatography

	$R_{deoxyst.}$[a]
Methyl 3-amino-α-D-glucoside	4.38
Methyl 3-amino-β-L-glucoside	4.38
Methyl 3-amino-α-D-mannoside	4.96
Methyl 3-acetamido-6-amino-α-D-glucoside (V)	3.00
Methyl 3-acetamido-6-amino-α-D-mannoside (XIII)	3.60
Methyl 3,6-diamino-α-D-glucoside (VII)	1.65
Methyl 3,6-diamino-α-D-mannoside (XV)	2.02
3,6-Diamino-D-glucose	1.20
3,6-Diamino-D-mannose	1.39

a) Ratio of the migration distance of a sample to that of 2-deoxystreptamine (1.00).

Table II. Comparison of Melting Point and $[\alpha]_D$ of Methyl 3-Amino-3-deoxy-β-D-glucopyranoside and Its β-L-Isomer

	β-D-Isomer[10]		β-L-Isomer		
	m.p. (°C)	$[\alpha]_D^{20}$	m.p. (°C)	$[\alpha]_D^{22}$	
Free base	207~208	−34°	205~206	+34°	(H_2O)
N-Acetate	214~215	−21°	211~212	+22°	(H_2O)
Tetra-O,N-acetate	159	−22°	151~152	+23°	($CHCl_3$)

Methyl 3-Acetamido-3-deoxy-6-O-*p*-tolylsulfonyl-α-D-glucopyranoside (II)——To a stirred solution of methyl 3-acetamido-3-deoxy-α-D-glucopyranoside (I) (10.0 g.) in pyridine (150 ml.) was added, dropwise, *p*-tolylsulfonyl chloride (11.0 g.) in pyridine (35 ml.) at 5°. The mixture was cooled in ice-H_2O for 3 hr., then left at room temperature (30°) overnight. Addition of a little MeOH decomposed the excess of acid chloride, and subsequent concentration yielded a sirup which crystallized upon addition of a mixture of H_2O and $CHCl_3$. Recrystallization from EtOH (250 ml.) gave II (4.7 g,). The mother liquor upon concentration and enriching with $CH_3C_2H_5CO$ gave further crystals (2.3 g.) : total yield, 7.0 g., 42.5%, m.p. 190~191° (decomp.), $[\alpha]_D^{22}$ +122° (c=0.91, MeOH). UV Spectrum of this compound was almost the same as that of methyl 6-O-*p*-tolylsulfonyl-α-D-glucopyranoside : UV λ_{max} mμ (ε) : 225 (12,200), 256 (470), 263 (580), 268 (570), 273 (520). IR cm^{-1} : $\nu_{S=O}$ 1172. *Anal.* Calcd. for $C_{16}H_{23}O_8NS$: C, 49.4; H, 6.0; N, 3.6; S, 8.2. Found : C, 49.6; H, 6.2; N, 4.1; S, 8.6.

Methyl 3-Acetamido-3-deoxy-2,6-di-O-*p*-tolylsulfonyl-α-D-glucopyranoside (III)——The $CHCl_3$ layer and the mother liquor described above were combined and brought to dryness. The residual sirup was taken up in $CH_3C_2H_5CO$ and the remaining 6-O-sulfonate (II) was removed by the addition of half a volume of

12) H. H. Baer : Chem. Ber., **93**, 2865 (1960).

ether. Thin-layer chromatographic examination*6 of the filtrate revealed the presence of disulfonate (a faster moving spot), together with a small amount of monosulfonate (a slower moving spot). A portion of this solution was evaporated to a sirup (2.5 g.), which was dissolved in benzene–AcOEt (1:1) and chromatographed on a column (2×60 cm.) of silicic acid (100~200 mesh, Mallinckrodt Chemical Works), the elution being performed with benzene–AcOEt (1:1), followed by AcOEt. The di-O-sulfonate (910 mg.) was recovered from the AcOEt eluate, and crystallized from AcOEt–ether. Recrystallization from the same solvent mixture gave Ⅲ (600 mg.). Yield from Ⅰ was ca. 8%, m.p. 130~131°, $[\alpha]_D^{21}$ +71°(c=0.91, MeOH). UV λ_{max} mμ (ε): 226 (20,700), 256 (950), 263 (1,240), 268 (1,140), 274 (1,050). NMR (p.p.m.): 8.11 (CH_3CON), 7.56 ($2CH_3$–Ts), 6.75 (OCH_3) ($CDCl_3$). *Anal.* Calcd. for $C_{23}H_{29}O_{10}NS_2 \cdot H_2O$: C, 49.2; H, 5.6; N, 2.5. Found: C, 49.1: H, 5.6; N, 2.4.

Methyl 3-Acetamido-6-azido-3,6-dideoxy-α-D-glucopyranoside (Ⅳ)——A solution of Ⅱ (2.9 g.) in dimethylformamide (60 ml.) and sodium azide (2.6 g.) in H_2O (6 ml.) was refluxed for 7 hr. and then evaporated to a sirup. This was twice extracted with hot EtOH, the insoluble salts were removed, and the extracts were concentrated and added to ether when the product precipitated as a pale brown solid (3.98 g.). This was dissolved in H_2O (20 ml.) and chromatographed on a column of Dowex 50W×2 (H^+, 200~400 mesh, 85 ml.) using H_2O as a developer. After the first acidic fractions were discarded, the next neutral fractions were pooled and evaporated to give a crystalline residue (2.29 g.). Recrystallization from AcOEt (80 ml.) afforded Ⅳ (1.07 g.). Further crystals (0.41 g.) were recovered from the mother liquor. Total yield, 1.48 g., 77%, m.p. 204°. IR cm^{-1}: $\nu_{N=N=N}$ 2100. *Anal.* Calcd. for $C_9H_{16}O_5N_4$: C, 41.5; H, 6.2; N, 21.5. Found: C, 41.5; H, 6.4; N, 21.4.

Methyl 3-Acetamido-6-amino-3,6-dideoxy-α-D-glucopyranoside (Ⅴ)——A solution of Ⅳ (1.23 g.) in H_2O (50 ml.) was treated with Raney Ni (W-4, 5 ml.) at room temperature for 2 hr. The filtered solution was evaporated to a crystalline mass (1.10 g.), which was recrystallized from MeOH–EtOH. Yield, quantitative, m.p. 225~227°(decomp.), $[\alpha]_D^{22}$ +151°(c=0.91, H_2O). Rf Value in paper chromatography was given in Table Ⅰ. *Anal.* Calcd. for $C_9H_{18}O_5N_2$: C, 46.1; H, 7.7; N, 11.9. Found: C, 45.6; H, 7.8; N, 11.6.

Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-glucopyranoside (Ⅵ)——A solution of Ⅴ (230 mg.) and Ac_2O (0.4 ml.) in MeOH was left at room temperature overnight. Concentration afforded crystals which were recrystallized from MeOH–EtOH with quantitative yield. m.p. 223~224°, $[\alpha]_D^{22}$ +126°(c=0.81, H_2O). *Anal.* Calcd. for $C_{11}H_{20}O_6N_2$: C, 47.8; H, 7.3; N, 10.1. Found: C, 47.7; H, 6.8; N, 9.4.

Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-glucopyranoside (Ⅷ)——A mixture of Ⅴ (200 mg.), pyridine (10 ml.) and Ac_2O (2 ml.) was kept at room temperature overnight. Dilution with MeOH and concentration gave crystals which were washed with ether. Recrystallization from acetone (50 ml.) gave Ⅷ (295 mg., 96%), m.p. 238~239°, $[\alpha]_D^{23}$ +93°(c=0.91, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{24}O_8N_2$: C, 50.0; H, 6.7; N, 7.8. Found: C, 49.7; H, 6.6; N, 7.6. In contrast to the common sugar polyacetates, this compound was more soluble in H_2O than in $CHCl_3$, and the most part of it remained in H_2O layer after extraction with $CHCl_3$.

Methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside (Ⅶ)——a) A solution containing Ⅴ (490 mg.) and 80% hydrazine hydrate (8 ml.) was heated in a sealed tube at 145~150° for 46 hr. Excess hydrazine was removed by keeping in a desiccator containing H_2SO_4 for 2 days. The residue was dissolved in EtOH, insoluble materials were filtered, and the filtrate was concentrated to give crystals. Recrystallization from EtOH afforded the pure product (Ⅶ) (160 mg.). The mother liquor was evaporated to dryness. The residue was dissolved in H_2O and chromatographed on a column of Dowex 1×2 (OH) resin (100 ml.) developed with H_2O. The effluents were collected in 3.3 ml. fractions. The alkaline eluates (tube nos. 24~30) were pooled and evaporated to a crystalline mass (293 mg.). Recrystallization from EtOH afforded further Ⅶ (200 mg.). Total yield, 360 mg., 85%, m.p. 162~163°, $[\alpha]_D^{22}$ +152°(c=0.93, H_2O). *Anal.* Calcd. for $C_7H_{16}O_4N_2$: C, 43.7; H, 8.4; N, 14.6. Found: C, 43.5; H, 8.4; N, 13.8. Hydrolysis of a sample in 4*N* HCl for 3 hr. at 100° gave 3,6-diamino-3,6-dideoxy-D-glucose as the only reducing sugar detectable by paper chromatography. Rf Values of Ⅶ and its demethyl compound were shown in Table Ⅰ.

b) A solution of Ⅳ (280 mg.) in 80% hydrazine hydrate (4 ml.) was heated in a sealed tube at 145~150° for 46 hr. The cooled solution was treated in a similar manner as described in the part a). Purification by the Dowex 1×2 (OH) resin chromatography gave a crystalline mass (135 mg.), which was recrystallized from EtOH. 95 mg. (58%), m.p. 160~161°. This substance was identified as Ⅶ by mixed melting point and IR spectrum. Paper chromatographic examination of the above mother liquor showed that this compound was only the ninhydrin-positive product obtained.

Methyl 3,6-Disalicylideneamino-3,6-dideoxy-α-D-glucopyranoside (Ⅸ)——To a suspension of Ⅶ (130 mg.) in EtOH (2 ml.) was added salicylaldehyde (200 mg.) in EtOH (2ml.). From the resulting clear solution, was immediately deposited yellow crystals which were recrystallized from EtOH to give Ⅸ (230 mg.,85%), m.p. 243~244°. *Anal.* Calcd. for $C_{21}H_{24}O_6N_2$: C, 63.1; H, 6.1; N, 7.0. Found: C, 62.4; H, 5.9; N,7.0.

*6 It was carried out on silica gel (M. Woelm–Eschwege), with benzene–EtOH (10:2), *n*-BuOH–AcOH–H_2O (4:1:5) and AcOEt saturated with H_2O. Spots were detected by spraying 1% anthrone in H_2SO_4.

Methyl 3-Acetamido-3-deoxy-6-O-*p*-tolylsulfonyl-α-D-mannopyranoside (XI)——A suspension of methyl 3-acetamido-3-deoxy-α-D-mannopyranoside (X) (5.4 g.) in pyridine (100 ml.) was treated with *p*-tolylsulfonyl chloride (5.3 g.) in pyridine (20 ml.) at 5°. The mixture was stirred at room temperature overnight, and then filtered to remove the insoluble materials. To the filtrate was added a little H_2O and the solution was brought to dryness. The residue was dissolved in $CHCl_3$ and the crystals were immediately deposited upon addition of H_2O. Recrystallization from EtOH (40 ml.) afforded XI (3.8 g., 43%), m.p. 136~137°, $[\alpha]_D^{22}$ +53° (c=0.91, MeOH). UV λ_{max} mμ (ε): 225 (11,400), 257 (420), 263 (550), 268 (540), 273 (470). IR cm^{-1}: $\nu_{S=O}$ 1170. *Anal.* Calcd. for $C_{16}H_{23}O_8NS \cdot H_2O$: C, 47.2; H, 6.2; N, 3.4; S, 7.9. Found: C, 47.4; H, 6.2; N, 4.0; S, 8.4.

Methyl 3-Acetamido-6-azido-3,6-dideoxy-α-D-mannopyranoside (XII)——a) A solution of the 6-O-sulfonate (XI) (3.0 g.) and sodium azide (2.6 g.) in dimethylformamide (60 ml.) containing enough H_2O (6 ml.) to give a homogeneous solution, was refluxed for 8 hr. The cooled solution was evaporated to dryness, the residue was extracted with EtOH, and the extracts were again brought to dryness. After washing with ether, the residue was dissolved in H_2O and chromatographed on a column of Dowex 50W×2 (H, 75 ml.). Elution with H_2O gave XII (1.925 g.), which crystallized from AcOEt to give the pure product (1.79 g., 90%), m.p. 120~122°. IR cm^{-1}: $\nu_{N=N=N}$ 2100. *Anal.* Calcd. for $C_9H_{16}O_5N_4$: C, 41.5; H, 6.2; N, 21.5. Found: C, 41.5; H, 6.4; N, 21.4. Elution with 50% EtOH gave the starting material (XI, 80 mg.).

b) A mixture of methyl 3-acetamido-3-deoxy-2,6-di-O-*p*-tolylsulfonyl-α-D-glucopyranoside (III) (3.0 g.) in dimethylformamide (60 ml.) and sodium azide (2.6 g.) in H_2O (6 ml.) was refluxed for 8 hr., and the cooled solution was treated in the similar manner as described in the part a). The crystalline residue (1.36 g.) recovered from a resin column was recrystallized from AcOEt to give the pure product (1.11 g., 78%), m.p. 119~120°. IR Spectrum of this compound was identical with that of the preparation (XII) obtained under the part a). Hydrogenation of this compound gave XIII. The identity was confirmed by melting point, IR and Rf value in paper chromatography.

Methyl 3-Acetamido-6-amino-3,6-dideoxy-α-D-mannopyranoside (XIII)——A mixture of XII (1.4 g.) and Raney Ni (W-4, 5 ml.) in H_2O (100 ml.) was stirred for 2 hr. at room temperature and left overnight. The filtered solution was evaporated to a crystalline residue, which was washed with MeOH. Recrystallization was effected from H_2O-MeOH-EtOH to give XIII (yield, quantitative), m.p. 227°(decomp.), $[\alpha]_D^{22}$ +47°(c=0.90, H_2O). *Anal.* Calcd. for $C_9H_{18}O_5N_2$: C, 46.1; H, 7.7; N, 11.9. Found: C, 45.5; H, 7.8; N, 11.5.

Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-mannopyranoside (XIV)——A solution of XIII (200 mg.) and Ac_2O (0.3 ml.) in MeOH (20 ml.) was left at room temperature overnight, and then evaporated to a solid, which was crystallized from iso-PrOH and recrystallized from EtOH. 210 mg., 89%, m.p. 211°(decomp.), $[\alpha]_D^{22}$ +46°(c=0.91, H_2O). *Anal.* Calcd. for $C_{11}H_{20}O_6N_2$: C, 47.8; H, 7.3; N, 10.1. Found: C, 47.8; H, 7.0; N, 10.7.

Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-mannopyranoside (XVI)——A mixture of XIV (250 mg.), Ac_2O (2.5 ml.) and pyridine (10 ml.) was left at room temperature overnight, and the solution was concentrated. Addition of ether to the concentrate yielded a crystalline product, which was dissolved in H_2O and extracted with $CHCl_3$. From the $CHCl_3$ extracts was obtained 210 mg. of XVI, while 230 mg. was recovered from H_2O layer. Both were combined and recrystallized from AcOEt to give the pure product. Yield, 98%, m.p. 189~190°, $[\alpha]_D^{22}$ 0.0°(c=0.91, $CHCl_3$). *Anal.* Calcd. for $C_{15}H_{24}O_8N_2$: C, 50.0; H, 6.7; N, 7.8. Found: C, 49.8; H, 6.7; N, 7.5.

Methyl 3,6-Diamino-3,6-dideoxy-α-D-mannopyranoside (XV)——A solution of XIII (650 mg.) in 80% hydrazine hydrate (8 ml.) was heated in a sealed tube at 145~150° for 46 hr., and then brought to dryness by keeping in a desiccator for 2 days. The residue was dissolved in H_2O (5 ml.) and purified by the use of the resin chromatography of Dowex 1×2 (OH). The ninhydrin-positive, alkaline fractions were pooled and evaporated to a pale yellow sirup (480 mg.), which was soluble in acetone and ether. This material, although it failed to crystallize, was homogeneous in paper chromatography (Table I) and its NMR spectrum showed one signal assignable to an anomeric proton. Acid hydrolysis of this material gave 3,6-diamino-3,6-dideoxy-D-mannose as the only reducing sugar detectable by paper chromatography (Table I). A portion of this sirup was dissolved in EtOH and neutralized with HCl in MeOH. Upon addition of acetone, amorphous dihydrochloride was precipitated and re-precipitated from the same solvent combination. $[\alpha]_D^{22}$ +43°(c=1.07, H_2O). *Anal.* Calcd. for $C_7H_{18}O_4N_2Cl_2$: Cl, 26.8. Found: Cl, 25.5.

Methyl 3,6-disalicylideneamino-3,6-dideoxy-α-D-mannopyranoside (XVII)——To a solution of the sirupy free base (XV) (210 mg.) in EtOH (2 ml.) was added salicylaldehyde (250 mg.) in EtOH (2 ml.). After standing in a refrigerator overnight, the yellow solution was concentrated to a sirup, which was crystallized from benzene. Recrystallization from EtOH gave XVII (300 mg., 69%), m.p. 183~184°. *Anal.* Calcd. for $C_{21}H_{24}O_6N_2$: C, 63.1; H, 6.1; N, 7.0. Found: C, 62.5; H, 5.9; N, 7.2.

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Summary

The preparation of the title compounds from the respective methyl 3-acetamido-3-deoxy-α-D-hexopyranosides was described. Introduction of an amino group at C_6 was confirmed by nuclear magnetic resonance spectroscopy and dissociation constants. De-N-acetylation of methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-glucopyranoside by hydrazine was accompanied with reduction of the azido group to give directly methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside. Treatment of methyl 3-acetamido-3-deoxy-2,6-di-O-*p*-tolylsulfonyl-α-D-glucopyranoside with sodium azide afforded, *via* an 2,3-oxazolinium intermediate, methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-mannopyranoside. The isolation of a new compound, methyl 3-amino-3-deoxy-β-L-glucopyranoside from nitromethane condensation products was added.

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123. Kenji Suzuki: Synthesis of Peptides Related to the C-Therminus of B-Peptide of Ox Co-fibrin (Positions 16~21, 15~21, and 13~21).

(*Tohoku College of Pharmacy*[*1])

B-Peptide of ox co-fibrin is one of the peptides which is released from ox fibrinogen by the proteolytic enzyme thromin.[1] The amino acid sequence of B-peptide of ox co-fibrin was elucidated by Folk, *et al.*[2] as a linear peptide, N-acetyl-L-threonyl-L-glutamyl-L-phenylalanyl-L-prolyl-L-asparatyl-O-sulfonyl-L-tyrosyl-L-aspartyl-L-glutamyl-glycyl-L-glutamyl-L-aspartyl-L-arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine. The marked potentiating effect of B-peptide and hydrolysate by trypsin which splitted the bond between the lysine and the valine of B-peptide upon the bradykinin-induced contraction of isolated rat uterus was observed by Gladner,[3] *et al.*

In this paper, synthesis and biological activities of the nonapeptide, L-arginyl-L-prolyl-L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (Ⅸ) corresponding to the C-terminal portion of B-peptide of ox-cofibrin were described. In addition, the results of biological assay of the two synthetic intermediates, L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (Ⅵ), and L-lysyl-L-valylglycyl-L-leucylglycyl-L-alanyl-L-arginine (Ⅸ) were reported. The second hexapeptide (Ⅵ) is one of the products of trypsin hydrolysis of B-peptide and the first nonapeptide (Ⅺ) has a partially similar amino acid sequence to bradykinin.[4] The method of peptide synthesis used here was virtually similar with a previous report on the synthesis of bradykinin and its analogs.[5] The synthetic route for the nonapeptide (Ⅺ) is illustrated in Fig. 1.

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*1 Nankozawa, Sendai (鈴木謙次).

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