Both products were assayed by conversion to their 1-naphthylamine derivatives,[9] 1-acetamido- (4 mg.) and 1-propionamido- (6 mg.) naphthalene.

The authors are indebted to Prof. S. Shibata, University of Tokyo, for his encouragements through this work, to Prof. J. Haginiwa, University of Chiba, Dr. M. Yamazaki, and Dr. T. Hino, This Institute, for their kind advices and to the members of the Tokyo University Forestry Experimental Station in Chiba Pref., for the collection of plant materials.

Summary

Skimmianine isolated from *Skimmia* plants fed with anthranilic acid (T), acetate (1-^{14}C), acetate (2-^{14}C) and DL-tryptophan (3-^{14}C) was shown to be radioactive. Degradation of the labeled skimmianine showed that the radioactivity was located in the benzene ring when anthranilic acid (T) was administered, while it was located at the position 3 of quinoline nucleus when acetate (2-^{14}C) or DL-tryptophan (3-^{14}C) was used as the labeled precursors.

On the basis of these results, a scheme of the biosythesis of skimmianine was proposed in which skimmianine was shown to be derived from anthranilic acid and acetate.

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10) E. P. Kennedy, H. A. Barker: Anal. Chem., **23**, 1033 (1951).

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UDC 543.422.25 : 547.457

153. Shigeharu Inouye: Nuclear Magnetic Resonance Spectroscopy of Amino-sugars. I. Conformation of Methyl 3,6-Diamino-3,6-dideoxy-α-D-altropyranoside and Its Derivatives.

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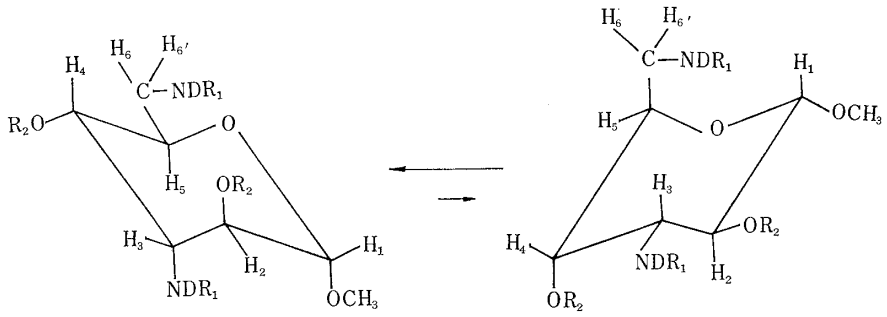
The synthesis of methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside was recently reported by wolfrom, *et al.*[1] and the structure of this compound was confirmed by a sequence of degradation reactions to the known α-amino acids. The synthesis of this diamino-sugar *via* essentially the same route as that reported was independently carried out in this laboratory, as a modeled reaction relating to the synthesis of the aminated kanamycin derivatives.[2] The diamino-sugar was characterized by the crystalline free base, di-N-acetate, and disalicylidene derivative, and the structure was determined mainly on the basis of the nuclear magnetic resonance data.

Conformational analysis based on the instability factors presented by Reeves,[3] indicated that α-D-altropyranose existed in a C1⇆1C conformational equilibrium of about 1:1. It would be of interest, therefore, to see if this diamino-sugar and its derivatives are actually in an equal proportion of the C1 and 1C conformers.

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

1) M. L. Wolfrom, Yen-Lung Hung, D. Horton: J. Org. Chem., **30**, 3394 (1965).
2) S. Inouye: "Symposium Abstracts, 9th Symposium on the Chemistry of Natural Products," 7 (1965), Osaka.
3) E. L. Eliel, N. L. Allinger, S. J. Angyal, G. A. Morrison: "Conformational Analysis," 360 (1965), Interscience Publishers, New York.



C1 Conformer 1C Conformer

I : $R_1=R_2=D$
II : $R_1=Ac$, $R_2=D$
III : $R_1=R_2=Ac$

Nuclear magnetic resonance spectroscopy is ideally suited as a method for studying such a conformational equilibrium in solution[4] : the time-averaged spectrum that is usually obtained represents the weighted mean of all the conformers in solution. Hence, the relative proportion of the individual species may be determined from the spectrum. The nuclear magnetic resonance spectra of the 3,6-disubstituted altrose derivatives were, therefore, examined for this purpose and the results were described in this paper.

Conformation of Methyl 3,6-Diamino-3,6-dideoxy-α-D-altropyranoside (I) and Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-altropyranoside (II)

The resonance data of I and II in deuterium oxide, pyridine and deuteriodimethylsulfoxide were listed in Table I, and illustrated in Fig. 1, 2 and 3.

TABLE I. Resonance Data of Methyl 3,6-Diamino-3,6-dideoxy-α-D-altropyranoside (I) and Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-altropyranoside (II)

	Solvent	OCH_3[a]	H_1	H_2	H_3	H_4	H_5	H_6	$H_{6'}$	$J_{1,2}$[b]	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
I[c]	D_2O	6.59	5.40	6.26	7.01	ca. 6.22	ca. 6.21	7.04	7.22	3.0	5.3	2.5		2.0	5.0	13.0
I	DSO[d]	6.71	5.56	6.43	7.05	ca. 6.52	ca. 6.52	ca. 7.75		1.8	3.8					
I·HCl	D_2O	6.52	5.15	5.91	6.29	ca. 5.9	ca. 5.9	ca. 6.6		1.5	3.8	3.8				
II[c]	〃	6.58	5.35	6.25	5.74	6.10	ca. 6.10	ca. 6.47		3.2	5.5	2.5		3.9	3.9	
II[c]	Pyrid.	6.58	4.92	5.52	4.75	5.31	5.60	5.82	6.05	1.9	4.1	4.1	9.1	3.1	5.5	13.9

a) Chemical shift in p.p.m. (τ).
b) Apparent coupling constant in c.p.s.
c) Analyzed from the 100 Mc.p.s. spectrum.
d) Deuteriodimethylsulfoxide.

Fig. 1 showed the low-field spectrum of I taken at 100 Mc.p.s. in deuterium oxide. In addition to the methoxyl signal on C_1 (6.59 p.p.m.) which was indicated, the anomeric proton (H_1) signal was observed at the lowest field (5.40 p.p.m.) as a doublet, with an apparent coupling constant of 3.0 c.p.s. ($J_{1,2}$). The C_2 methine (H_2) signal was recognized as a sharp quartet at 6.26 p.p.m., partly overlapped with the H_4 and H_5 signals, and the magnitude of a coupling

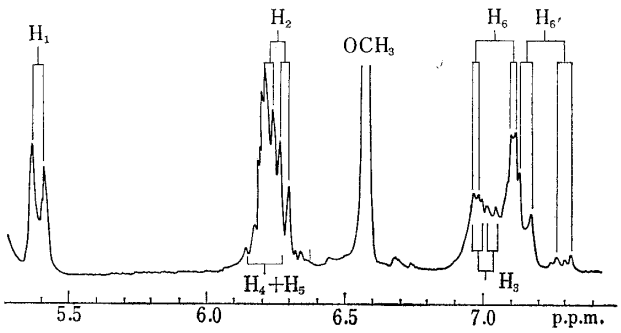


Fig. 1. Nuclear Magnetic Resonance Spectrum of Methyl 3,6-Diamino-3,6-dideoxy-α-D-altropyranoside (I) at 100 Mc.p.s. in Deuterium Oxide

4) L. D. Hall : Advan. Carbohydrate Chem., **19**, 51 (1964).

constant, $J_{2,3}=5.3$ c.p.s., was estimated by the first-order analysis. The broad multiplets, equivalent to the three proton signals, were observed in the high field region above 6.9 p.p.m., and assigned to H_3 and H_6 weakly deshielded by the amino groups attached to the same carbons. This assignment was further supported by the fact that those signals shifted largely to lower field in the spectra of the hydrochloride and di-N-acetate as described below. The H_3 signal appeared as a broad quartet owing to coupling with H_2 and H_4, while the H_6 signal formed an eight-line pattern, typical of the AB portion of an ABX system and the two non-equivalent protons showed a geminal coupling of 13.0 c.p.s. The H_4 signal appeared at about 6.2 p.p.m., seriously overlapped with the H_5 signal. When measured in the form of hydrochloride, the H_1 signal shifted much lower field (5.15 p.p.m.) with the change of a coupling constant ($J_{1,2}=1.5$ c.p.s.). The down-field shift (0.25 p.p.m.) accompanied with the protonation was largely attributable to the electrostatic deshielding of the ammonium cations at C_3 and C_6. The change of the coupling constant suggested the conformational change as shown later. The H_2 signal in the hydrochloride appeared at 5.91 p.p.m. with $J_{2,3}=3.8$ c.p.s. The H_3 signal appeared as a triplet at 6.29 p.p.m., 0.72 p.p.m. to lower field than that in the form of free base. This was due to the increased polar deshielding of the ammonium group. The $J_{3,4}$ value estimated from the line spacing was 3.8 c.p.s. The H_4, H_5 and H_6 signals gave the incomplete resolution.

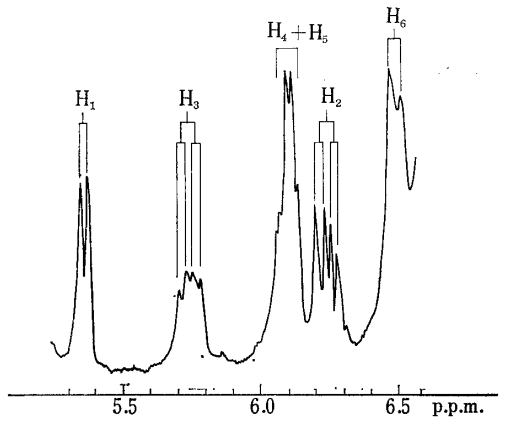


Fig. 2. Nuclear Magnetic Resonance Spectrum of Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-altropyranoside (II) at 100 Mc.p.s. in Deuterium Oxide

The spectrum of II at 100 Mc.p.s. in deuterium oxide showed much resolved signals which was analyzed by the first-order basis as illustrated in Fig. 2. The chemical shift (5.35 p.p.m.) and coupling constant ($J_{1,2}=3.2$ c.p.s.) of the H_1 signal were very similar to those in I. The H_2 signal (a sharp quartet) was observed at 6.25 p.p.m., well separated from the H_4 and H_5 signals at about 6.10 p.p.m. The H_3 signal appeared at relatively low field, 5.74 p.p.m., as a broad quartet. The broadness of the H_3 signal, which was frequently observed in the spectra of 3-acetamido-sugars, would be partly attributable to the quadrupole relaxation of the nitrogen atom. The assignment of the H_2 and H_3 signals was further confirmed by the decoupling experiment at 60 Mc.p.s. The doublet assigned to H_1 was collapsed into a singlet by irradiating 55 c.p.s. to higher field (the center of the H_2 quartet), accompanied with the considerable deformation of the H_3 signal. The H_4 and H_5 signals were found to be fully overlapped, and the analysis on the first-order basis became difficult.

It was reasonably predicted that the equatorial anomeric proton in the C1 conformation should have lower chemical shift with smaller $J_{1,2}$ value of equatorial-equatorial coupling than the axial counterpart in the 1C conformation, where a large $J_{1,2}$ value arising from axial-axial coupling was expected. In view of the fact that the chemical shift and coupling constant of the H_1 signal in deuterium oxide depended largely upon the configuration at C_2[5,6], these values of I and II were compared with those of methyl α-mannopyranosides having an axial hydroxyl group at C_2 (models for a C1 conformer) and those of methyl β-glucopyranosides having an equatorial

5) L. D. Hall: Tetrahedron Letters, **1964**, 1457.
6) S. Inouye: This Bulletin, in press.

hydroxyl group at C_2 (models for a 1C conformer). As a result, the chemical shifts of I and II were found to be 0.1 p.p.m. higher than those of the former and 0.3 p.p.m. lower than those of the latter. The magnitudes of the $J_{1,2}$ values also were found to be intermediate (1.5 c.p.s. larger than the former and 5.0 c.p.s. smaller than the latter). It was further noted that the $J_{2,3}$ values (5.3 and 5.5 c.p.s.) were a little larger than the reported values for an equatorial-equatorial coupling (2~4 c.p.s.) in sugars, as required by the C1 conformation, but smaller than those of an axial-axial coupling (8~10 c.p.s.) in the 1C conformation. The $J_{3,4}$ values which arised from an axial-equatorial coupling in both of the conformations, were found to be in the expected range (both 2.5 c.p.s.). The above considerations suggested a mixed conformation of C1 and 1C for I and II in deuterium oxide.

Supporting evidence was obtained from the nuclear magnetic resonance spectra in pyridine and in deuteriodimethylsulfoxide. The spectrum of II in pyridine (Fig. 3) gave well resolved signals and permitted a complete first-order analysis, which was fully compatible with the C1 conformation of high degree of purity. It showed the H_1 signal at 4.92 p.p.m., as a doublet with a small $J_{1,2}$ value (1.9 c.p.s.). The H_1 chemical shift and $J_{1,2}$ value were found to be very close to those of the α-D-mannose isomer.*2 The H_2 signal appeared at relatively high field, 5.52 p.p.m., as a sharp quartet. A triplet of the H_3 signal appeared at the lower field than the doublet of the H_1 signal. The remarkable down-field shift in pyridine was shown to be characteristic of the H_3 signal.[7] The small values of $J_{2,3}$ and $J_{3,4}$ (both 4.1 c.p.s.) were consistent with the equatorial-equatorial coupling of H_2 and H_3 and the equatorial-axial coupling of H_3 and H_4 in the C1 conformation. The H_4 signal appeared at 5.31 p.p.m., well separated from the H_2 and H_5 signals. The large $J_{4,5}$ value was indicative of the diaxial relationship between H_4 and H_5, supporting the C1 conformation. The H_5 signal, partially overlapped with the H_2 signal, formed a eight-line pattern through coupling with the three different protons (H_4, H_6 and H_6'). The H_6 signal appeared at the highest field (5.82, 6.05 p.p.m.) as the AB part of an ABX system and showed normal geminal coupling.

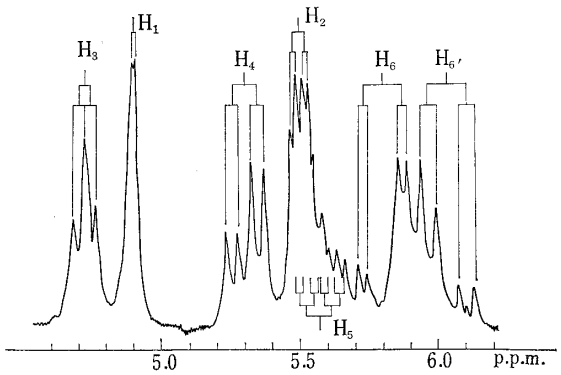


Fig. 3. Nuclear Magnetic Resonance Spectrum of Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-altropyranoside (II) at 100 Mc.p.s. in Pyridine

The spectrum of I in a deuteriodimethylsulfoxide solution showed similar J values as those found for II in a pyridine solution. The H_1 signal appeared as a narrow doublet at 5.56 p.p.m. The H_2 signal was recognized as a sharp quartet at 6.43 p.p.m., showing an equatorial-equatorial coupling with H_1 ($J_{1,2}$=1.8 c.p.s.) and an equatorial-equatorial coupling with H_3 ($J_{2,3}$=3.8 c.p.s.). The H_3 and H_6 signals were observed as very broad multiplets at a high field, attributable to the weakly deshielding effect of the nitrogen atoms. The fully overlapped signals of H_4 and H_5 appeared at about 5.9 p.p.m.

A comparison of J values in deuterium oxide and in pyridine (or dimethylsulfoxide) clearly indicated that the high values of $J_{1,2}$ in deuterium oxide was accompanied with the high values of $J_{2,3}$ and *vice versa*. These discrepancy of J values in both of the solvents could be easily interpreted if we assumed an equilibrated mixture of C1 and 1C in

*2 The low $J_{1,2}$ value was widely recognized in α-hexopyranoses having an axial substituent at C_2.[6]

7) S. Inouye: This Bulletin, **14**, 1171 (1966).

deuterium oxide and the predominant C1 conformation in pyridine (or dimethylsulfoxide). The equilibrium proportion of a C1 and a 1C conformers was roughly estimated from the magnitudes of the coupling constants. Assuming that the $J_{1,2}$ and $J_{2,3}$ values in the 1C conformation were 7.6 and 9.3 c.p.s. (J values of methyl 3-acetamido-3-deoxy-β-L-glucopyranoside), the observed J values in deuterium oxide were compatible with a mixture of C1 and 1C in an approximate ratio of 3:1.*3

In this connection it was interesting to note that the low values of $J_{1,2}$ and $J_{2,3}$ in the conjugate acid form of I suggested a slightly different conformation from that of I, probably higher percentage of the C1 conformation. This was not the unexpected result, since the free energy of the conjugate acid was known to be sensitive to the steric factors around a cationic center as well as the electrostatic repulsion of the two cationic centers.

Conformation of Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-altropyranoside (III)

TABLE II. Resonance Data of Methyl 3,6-Diamino-3,6-dideoxy-2,4-di-O-acetyl-α-D-altropyranoside (III) and Reference Compounds (V~IX) in Deuteriochloroform[a]

	CONH ($J_{NH, CH}$)	H_1 Equatorial ($J_{1,2}$)	OCH_3 Axial	$OCOCH_3$ Axial	$OCOCH_3$ Equatorial		$NCOCH_3$ Axial	$NCOCH_3$ Equatorial	
					Second.	Primary		Second.	Primary
III[b]	3.49[d] (9.0), 3.91[t] (4.5)	5.33 (1.2)	6.55	7.87	8.00		8.00		8.00
V	4.22[d] (8.0)	5.20 (4.0)	6.56		7.92, 7.92	7.95		8.10	
VI	4.11[d] (9.0)	5.27 (2.0)	6.57	7.83	7.92	7.94		8.08	
VII		5.23 (3.5)	6.57		7.92, 7.94, 7.98	8.01			
VIII		5.27 (1.5)	6.58	7.85	7.90, 7.96	8.01			
IX		5.01 (2.5)	6.58	7.86	7.92, 7.96	8.02			

t: triplet d: doublet

a) Chemical shift in p.p.m. (τ). Number in parentheses was the apparent coupling constant in c.p.s.

b) Other ring proton signals: H_2, 5.20; H_3, 5.99; H_4, 5.08; H_5, ca. 5.38. $J_{2,3}$=3.0, $J_{3,4}$=4.2, $J_{4,5}$=10.0 (100 Mc.p.s. spectrum).

The resonance datum of III in deuteriochloroform was shown in Table II, which fully supported the C1 conformation of high degree of purity. As the previous studies have demonstrated, the methyl chemical shifts of acetoxy and acetamido groups attached to a pyranose ring depended upon the configuration of the substituents. The averaged values obtained from the studies on the acetylated products of aminocyclitols,[8] methyl amino-hexopyranosides,[9] and methyl hexopyranosides,[4] were 8.05 p.p.m. for an equatorial acetamido, 7.95 p.p.m. for an axial acetamido, 8.0 p.p.m. for an equatorial acetoxymethyl, 7.95 p.p.m. for an equatorial acetoxy, and 7.85 p.p.m. for an axial acetoxy groups.

*3 The possibility of the distortion of the C1 chair form in stead of ring inversion in deuterium oxide seemed to be ruled out by considering the effect of such a distortion on J values. The distortion of the pyranose ring that could be expected was the movement of the three axial substituents at C_1, C_2 and C_3 away from the axis of the ring. This change would reduce any 1,3 interaction with the axial groups, and would have the effect of increasing the dihedral angles between H_1 and H_2 and between H_2 and H_3 to above the nominal 60° and decreasing the angle between H_3 and H_4. Such a change would, according to the Karplus equation, result in decreases of $J_{1,2}$ and $J_{2,3}$ and an increase of $J_{3,4}$. But the J values actually found showed increases of $J_{1,2}$ and $J_{2,3}$ and a decrease of $J_{3,4}$.

8) F. W. Lichtenthaler: Chem. Ber., **96**, 2047 (1963).

9) H. Agahigian, G. D. Vickek, M. H. von Saltza, J. Reid, A. I. Cohen, H. Gauthin: J. Org. Chem., **30**, 1085 (1965).

Since the difference in the chemical shifts of an axial and an equatorial substituents was rather small, the chemical shifts of III were compared with those of the reference compounds determined under the same condition. The reference compounds examined included methyl 3-acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-glucopyranoside (V), methyl 3-acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-mannopyranoside (VI), methyl 2,3,4,6-tetra-O-acetyl-α-D-glucopyranoside (VII), methyl 2,3,4,6-tetra-O-acetyl-α-D-mannopyranoside (VIII), and methyl 2,3,4,6-tetra-O-acetyl-α-D-galactopyranoside (IX). All of them gave the spectra consistent with the anticipated C1 conformation as shown in Table II.

Four methyl signals of acetoxy and acetamido groups were observed in the region of 7.83~8.10 p.p.m. in the respective spectra taken in deuteriochloroform. The lowest signal in the spectrum of III (7.87 p.p.m.) was found to be in the region of an axial acetoxy group. This was verified by the chemical shifts of the axial acetoxy groups of the reference compounds (7.83 p.p.m. (VI), 7.85 p.p.m. (VII), 7.86 p.p.m. (IX)). One of the three signals at 8.00 p.p.m. in III was ascribed to an equatorial acetoxy group, since the corresponding signals were observed in the spectra of V (7.92 p.p.m.), VII (7.98 p.p.m.), VIII (7.96 p.p.m.) and IX (7.96 p.p.m.). Then, the remaining two methyl signals at 8.00 p.p.m. were assigned to an axial acetamido and an equatorial acetamidomethyl groups. The methyl signals of the equatorial acetamidomethyl group was observed at 8.00 p.p.m. in the spectrum of methyl 3,6-diacetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-mannopyranoside[7] under the identical condition. Lack of the methyl signal around 8.10 p.p.m. in III probably denied the presence of an equatorial acetamido group at C_3, which was observed at 8.10 p.p.m. in V and at 8.08 p.p.m. in VI. A methoxyl signal, although less sensitive to the conformation than an acetoxy,[4] appeared at 6.55 p.p.m. in III, being very close to those of the axially oriented ones (Table II). An equatorial methoxyl signal would be expected to appear around 6.45 p.p.m.

The 100 Mc.p.s. spectrum of III gave the well resolved signals for the ring protons. The H_1 signals appeared at 5.33 p.p.m. as a narrow doublet ($J_{1,2}$=1.2 c.p.s.), partially overlapped with the H_5 signal. The chemical shift and coupling constant were found to be close to those of VI and VIII (5.27 p.p.m., $J_{1,2}$=1.5~2.0 c.p.s.), suggesting the equatorial anomeric proton coupled to the equatorial H_2. The H_2 and H_4 signals appeared, both as a quartet, at lower field than the H_1 owing to the marked local deshielding of the acetoxy groups, while the H_3 and H_6 signals appeared at relatively high field, reflecting the weaker deshielding of the acetamido groups. The signal patterns of the latter were very complex owing to coupling with the three or four different protons. The H_3 signal formed a broad eight-line pattern with the total width of 17.5 c.p.s., which was approximately equal to the sum of the first-order coupling constants $J_{2,3}+J_{3,4}+J_{NH,H_3}$.

The small values of $J_{2,3}$ (3.0 c.p.s.) and $J_{3,4}$ (4.2 c.p.s.) were consistent with an equatorial-equatorial and an equatorial-axial couplings, while the large $J_{4,5}$ value (10.0 c.p.s.) indicated *trans*-diaxial arrangement of H_4 and H_5, supporting the C1 conformation. In the 1C conformation, large values for $J_{1,2}$ and $J_{2,3}$ and a small value for $J_{4,5}$ would be expected. In addition to the above signals, the spectrum of III showed, in a lower field, a triplet and a doublet, both of which were broadened by the quadrupole relaxation of the nitrogen atoms. They were ascribed to the amide protons coupled with the two protons on C_6 and a proton on C_3, respectively (Table II).

The above assignment indicated that the conformation of III in deuteriochloroform was represented by the C1 conformation of high degree of purity.

Conformation of Methyl 3,6-Diazido-3,6-dideoxy-α-D-altropyranoside (IV)

In contrast to I and II, this compound was soluble in non-polar solvents as well as polar solvents, and the spectrum was taken in deuterium oxide, pyridine, acetone,

benzene and deuteriochloroform. Unfortunately, the resolution of the ring proton signals was unsatisfactory in all the cases, and hence the detailed analysis on the conformation became difficult. The signals assigned with certainty were the anomeric proton signals at a low field deshielded by a ring oxygen and the methyl signals of a methoxyl group. These were listed in Table Ⅲ and three examples were shown in Fig. 4, in parallel with the infrared spectra in the hydroxyl stretching region taken in the same solvents.

Table Ⅲ. Resonance Data of Methyl 3,6-Diazido-3,6-dideoxy-α-D-altropyranoside (Ⅳ)[a]

Solvent	OCH_3	H_1 ($J_{1,2}$)
D_2O	6.59	5.38(1.0)
Pyridine	6.43	4.87(1.2)
Acetone	6.62	5.43(1.2)
Benzene	6.84	5.67(1.5)
$CDCl_3$	6.51, 6.53, 6.61	5.10(<1), 5.19(4.4), 5.38(1.2)
$CDCl_3$[b]	6.54	5.37(1.2)

a) Chemical shift in p.p.m (τ). Number in parentheses was the apparent coupling constant in c.p.s.
b) Sample containing water.

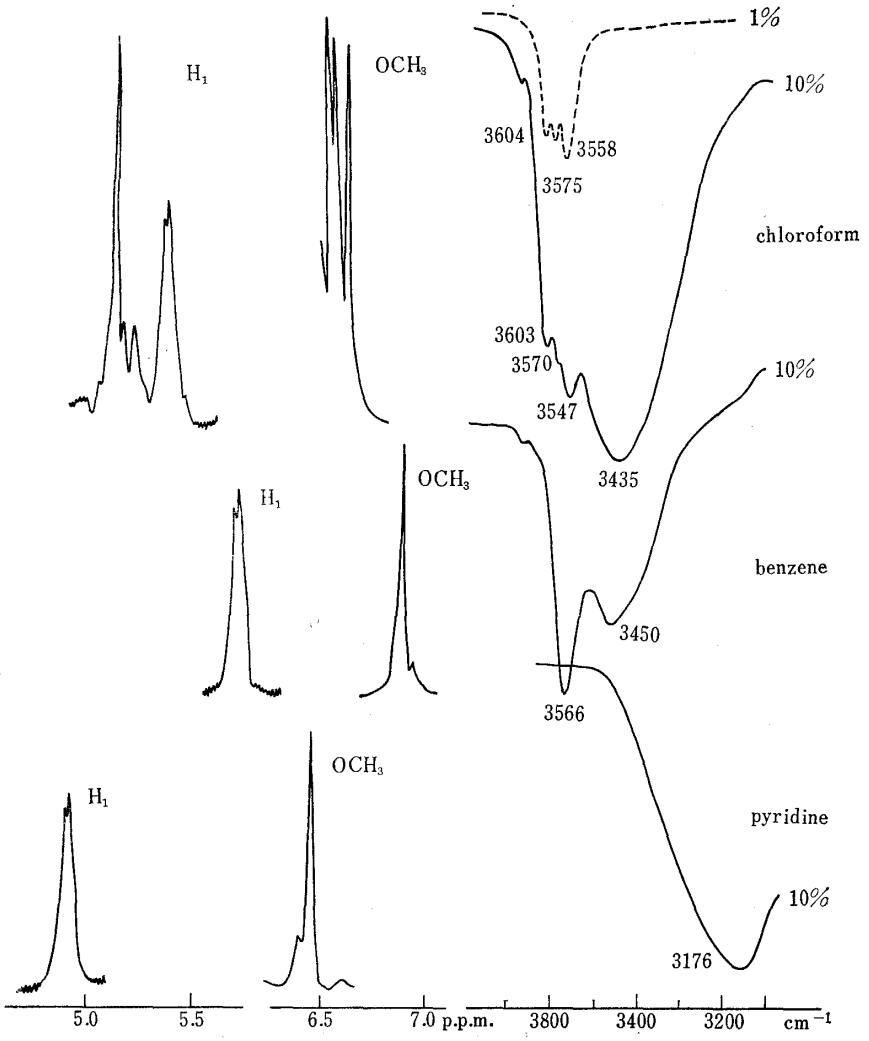
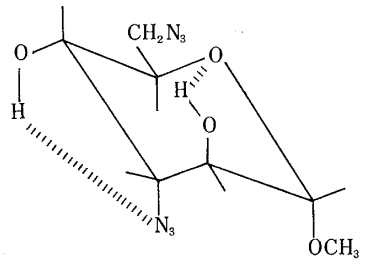


Fig. 4. Nuclear Magnetic Resonance Spectra and Infrared Absorption Spectra of Methyl 3,6-Diazido-3,6-dideoxy-α-D-altropyranoside (Ⅳ) in Chloroform, Benzene and Pyridine

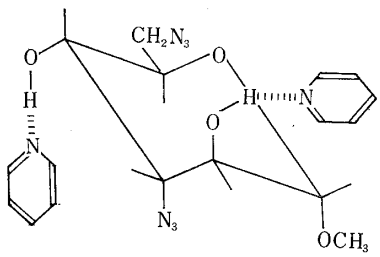
The anomeric proton signal in the solutions of deuterium oxide, pyridine, acetone and benzene appeared as a sharp doublet with a singlet of a methoxyl group. But, it was recognized in deuteriochloroform three sets of doublet for the H_1 signal accompanied with three singlets of a methoxyl group. The multiplicity of the signals in the latter solvent remained essentially unaltered in high temperature (65°), though a slight change of the relative intensity was observed. Since the free rotation of a methyl and an azido groups was expected to be sufficiently fast, the three sets of signals were reasonably ascribed to the individuals of the three conformers equilibrated in solution. Multiplicity disappeared when the hydrated sample was measured in deuteriochloroform.

From the close similarity of the $J_{1,2}$ values (1.0~1.5 c.p.s.) in deuterium oxide, pyridine, acetone and benzene, with those of I and II in pyridine and dimethylsulfoxide, the C1 conformation was assigned to IV in these solvents. In the same way, the H_1 signal with $J_{1,2}=1.2$ c.p.s. in deuteriochloroform might be assignable to C1. A narrow line at 6.51 p.p.m. with $J_{1,2}<1$ c.p.s. suggested the approximate dihedral angle between H_1 and H_2 of 90°, but no definite conformation could be derivable from the data presented in this paper.

It was recently shown that intramolecular hydrogen bonding played an important role in determining the conformation in an inert solvent.[3] Accordingly, the infrared spectrum of IV was taken, using essentially the same solvents and the same concentration as those employed for the measurement of the nuclear magnetic resonance spectra. As shown in Fig. 4, the spectrum in the 10% chloroform solution gave three sharp hydroxyl bands at 3547 cm^{-1}, 3570 cm^{-1}, and 3603 cm^{-1}, together with a broad hydroxyl band at 3435 cm^{-1}. The last band disappeared in the 1% concentration, indicating that it was due to intermolecular hydrogen bonding. Since the former three were observed as such in the dilute solution of carbon tetrachloride (0.00065 mol.), they were assigned to the intramolecularly hydrogen bonded hydroxyl groups. Absence of the free hydroxyl band indicated that the two hydroxyl groups in IV were almost internally bonded. The spectrum in the 10% benzene solution gave a sharp hydroxyl band of intramolecular or solvent bonding nature at 3566 cm^{-1}, together with a broad band of intramolecular bonding nature at 3450 cm^{-1}.



IVa



IVb

In view of the fact that intramolecular bonding was well competed with intermolecular bonding even in a high concentration, the intramolecularly hydrogen bonded structure such as IVa, might be responsible for the stabilization of the conformation in an inert solvent,*4 and various combinations of the hydrogen donor groups and the receptor groups in the molecule, as suggested by the multiple bands

*4 Intramolecular hydrogen bonding between an axial hydroxyl group and a ring oxygen atom was shown to effect strongly on conformational stability in carbon tetrachloride (A.B. Foster, R. Harrison, J. Lehman, J. M. Webber : J. Chem. Soc., **1963**, 4471).

in chloroform, probably were responsible for the presence of various conformers having differences of free energy. As expected, intramolecular hydrogen bonding was not recognized in pyridine, which was known to strongly solvate to a hydroxyl group. The solvated hydroxyl band appeared at 3176 cm^{-1}. In this case, therefore, the solvation to the hydroxyl groups at C_2 and C_4 might play an important role for the stabilization of C1 (Ⅳb).

Experimental

Nuclear magnetic resonance spectra were taken at 60 Mc.p.s. using a Varian A-60 and JNM-C-60 spectrometers and at 100 Mc.p.s. using a Varian HA-100 and JNM-4H-100 spectrometers. Tetramethylsilane and sodium 2,2-dimethyl-1,2-silapentane-5-sulfonate were used as an internal standard in organic solvents and D_2O, respectively. Mean errors were ±0.01 p.p.m. for chemical shifts and ±0.5 c.p.s. for apparent coupling constants. In order to minimize the intensity of a OH signal, the samples were twice deuterated by dissolving in D_2O followed by lyophilization, and measured in the 10% concentration. Unless otherwise stated, the resonance data shown were those at 60 Mc.p.s.

Infrared spectra in the OH stretching region were obtained with a Koken 401 grating spectrometer combined with the KBr cells in 0.1 and 50 mm. layers. Frequencies were checked by the bands of a polystyrene film.

Methyl 3,6-Diamino-3,6-dideoxy-α-D-altropyranoside (Ⅰ)——a) By hydrazine replacement. A solution of methyl 2,6-di-O-methanesulfonyl-α-D-glucopyranoside (Ⅺ)*5,10) (15 g.) in anhydrous hydrazine (35 ml.) was heated in a sealed tube at 140° for 3 days. The cooled solution was kept in a desiccator containing H_2SO_4 to remove excess of hydrazine, and the residue was treated with Raney Ni (W-4, 10 g.) at room temperature overnight. The reduction product was purified by the repeated resin chromatography on a column of Dowex 1X2 (OH^-, 200~400 mesh) developed with H_2O,[11] and crystallized from EtOH. Recrystallization from EtOH–ether gave Ⅰ, 2.54 g., m.p. 142~143°, $[\alpha]_D^{25}$ +108°(c=1.08, H_2O). Rf Value in paper chromatography*6 was 1.96. *Anal.* Calcd. for $C_7H_{16}O_4N_2$: C, 43.7; H, 8.4; N, 14.6. Found: C, 43.4; H, 8.0; N, 14.6.

Methyl 3,6-disalicylideneimino-3,6-dideoxy-α-D-altropyranoside was prepared by the usual procedure and crystallized from benzene. m.p., 118~120°. *Anal.* Calcd. for $C_{21}H_{24}O_6N_2$: C, 63.1; H, 6.0; N, 7.0. Found: C, 64.0; H, 6.3; N, 6.7.

b) By azide replacement. To a solution of Ⅺ (12 g.) in dimethylsulfoxide (150 ml.) was added sodium azide (20 g.), and the mixture was kept at 100° for 20 hr. The reaction product was isolated by means of the resin chromatography of Dowex 50WX2 (H^+, 200~400 mesh), developing with H_2O, and crystallized from H_2O. Recrystallization from the same solvent gave methyl 6-azido-6-deoxy-2-O-methanesulfonyl-α-D-glucopyranoside (X) (6.5 g.), m.p., 137~138°, $[\alpha]_D^{21}$ +116°(c=0.94, H_2O). *Anal.* Calcd. for $C_8H_{15}O_7N_3S$: C, 32.5; H, 5.1; N, 14.1; S, 10.8. Found: C, 33.0; H, 5.4; N, 13.8; S, 10.75.

Table Ⅳ. Resonance Data of Methyl 6-Azido-6-deoxy-2-O-methanesulfonyl-α-D-glucopyranoside (X) and Methyl 2,6-Di-O-methanesulfonyl-α-D-glucopyranoside (Ⅺ) in a mixture of Pyridine and Deuterium Oxide (1:1)

	OCH_3[a]	SO_2CH_3	H_1	H_2	H_3	H_4	H_5	H_6	$H_{6'}$	$J_{1,2}$[b]	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$
X[c]	6.53	6.50	4.83	5.21	5.67	6.15		6.32	5.97	3.5	9.1	8.5	8.5	3.7	5.4	13.0
Ⅺ	6.49	6.46, 6.54	4.78	5.21	5.62	5.91	ca. 5.85	ca. 5.12		3.5	9.0	8.0	9.0	2.7		

a) Chemical shift in p.p.m. (τ). b) Apparent coupling constant in c.p.s.
c) Analyzed from the 100 Mc.p.s. spectrum.

The structure of this compound was established by the nuclear magnetic resonance spectrum shown in Table Ⅳ. The spectrum of X in a mixture of pyridine-D_2O (1:1) showed a sharp quartet at relatively low field (5.21 p.p.m.). Since the similar quartet was observed at 5.21 p.p.m. in Ⅺ, it was assigned to the H_2 signal, shifted to down-field by the marked deshielding effect of the methanesulfonyl group on C_2. Similarly,

*5 Purification of the sirupy 2,6-disulfonate by passing through a column of Dowex 1X2 (OH^-) resin yielded crystals of Ⅺ in 65% yield.

*6 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). Relative rate of flow was expressed by the ratio of the migration distance of a sample to that of 2-deoxystreptamine (1.00).

10) A. K. Mitra, D. H. Ball, L. Long: J. Org. Chem., **27**, 161 (1962).
11) S. Inouye, H. Ogawa: J. Chromatog., **13**, 536 (1964).

the down-field shift of the H_6 signal was observed in XI but not in X, indicating the replacement of the sulfonyl group on C_6 by an azide in the latter case. The H_6 signals in X were observed at a high field, 5.97 and 6.32 p.p.m. Furthermore, the large values of $J_{2,3}$, $J_{3,4}$ and $J_{4,5}$ revealed all *trans*-axial relationship of H_2, H_3, H_4 and H_5, supporting the glucose configuration in the C1 conformation.

A solution of X (2.0 g.) in dimethylformamide (40 ml.) and sodium azide (2.0 g.) in H_2O (4 ml.) was refluxed for 18 hr. After purification by the resin chromatography of Dowex 50WX2 (H^+), was obtained a sirup (1.66 g.), from which the unreacted material (X) (400 mg.) was crystallized. By using preparative thin-layer chromatography (silica gel) developed with $CHCl_3$–AcOEt(3:1), was isolated 400 mg. of the sirupy methyl 3,6-diazido-3,6-dideoxy-α-D-altropyranoside (IV) (a faster moving fraction), together with 250 mg. of X (a slower moving fraction). This sirup (IV), although it failed to crystallize, was homogeneous on silica gel plates in benzene–acetone (3:1), benzene–AcOEt (1:1), benzene–EtOH (10:2) and $CHCl_3$–AcOEt (3:1). IR cm^{-1} : $\nu_{N=N=N}$ 2100 (CCl_4, $CHCl_3$, benzene). $[\alpha]_D^{19}$ +58°(c=0.97, H_2O). The structure of IV was established by conversion to I as follows.

A solution of IV (200 mg.) in H_2O (20 ml.) was treated with Raney Ni (4 ml.) at room temperature for 2 hr. Evaporation of a solution gave a crystalline mass, which was recrystallized from EtOH–ether. 100 mg., m.p. 140~141°. Infrared spectrum and Rf value in the paper chromatography were identical with those of the sample prepared *via* route a.

Methyl 3,6-Diacetamido-3,6-dideoxy-α-D-altropyranoside (II)——A solution of crude I (1.2 g.) in MeOH (50 ml.) and Ac_2O (2.5 ml.) was left at room temperature overnight, and evaporated to a sirup. This was purified by the resin chromatography on a column of Dowex 1X2 (OH^-). From the H_2O eluate were obtained crystals, which were twice recrystallized from *n*-PrOH; yield. 1.48 g. m.p. 167~169°. $[\alpha]_D^{20}$ +50°(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, 47.6; H, 7.1; N, 9.9. II consumed no periodate when subjected to quantitative periodate oxidation.

Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-α-D-altropyranoside (III)——A mixture of crystalline II (500 mg.), Ac_2O (2.0 ml.) and pyridine (10 ml.) was left at room temperature overnight. The solution was evaporated to a sirup, which was chromatographed on a column of silica gel (100 ml.), developing with benzene–MeOH (5:1). The eluates which contained III were pooled and evaporated to a sirup. It was dissolved in H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was brought to dryness to give a white powder. Reprecipitation from AcOEt–ether gave III, 400 mg., $[\alpha]_D^{20}$ +40° (c=0.94, $CHCl_3$). Rf Values in thin-layer chromatography (silica gel) with benzene–MeOH (5:1) were 0.39(III), 0.24 (II), 0.23 (methyl 3-acetamido-3-deoxy-α-D-mannopyranoside) and 0.74 (methyl 3-acetamido-3-deoxy-2,4,6-tri-O-acetyl-α-D-mannopyranoside). *Anal.* Calcd. for $C_{15}H_{24}O_8N_2$: C, 50.0; H, 6.7; N, 7.8. Found : C, 49.0; H, 6.7; N, 8.1.

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Summary

Conformations of four 3,6-disubstituted derivatives of methyl α-D-altropyranoside in various solvents were studied by nuclear magnetic resonance spectroscopy. The derivatives examined included methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside (I), its di-N-acetate (II), tetra-O,N-acetate (III) and methyl 3,6-diazido-3,6-dideoxy-α-D-altropyranoside (IV). Analysis of the spectra revealed that the predominant conformation in all the cases was C1, but I and II in deuterium oxide were shown to exist partly in the 1C conformation. IV in deuteriochloroform existed probably in a mixture of three conformers. Infrared spectroscopy indicated that the conformers of IV in inert solvents existed in the intramolecularly hydrogen bonded forms. The resonance data of methyl 6-azido-6-deoxy-2-O-methanesulfonyl-α-D-glucopyranoside (X) and methyl 2,6-di-O-methanesulfonyl-α-D-glucopyranoside (XI) were added.

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