[Chem. Pharm. Bull. 14(11) 1210~1219 (1966)]

UDC 543.422.25 : 547.457.1.03

165. Shigeharu Inouye : Nuclear Magnetic Resonance Spectroscopy of Amino-sugars. III.*1 Substitutional and Configurational Effects of Amino Group and Related Functions on the Chemical Shift and Coupling Constant in Deuterium Oxide.*2

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In recent year, nuclear magnetic resonance studies have shown the utility of this spectroscopic technique in the amino-sugar chemistry. Originating in the classic paper on the acetylated sugars by Lemieux *et al.*,[1] particular attentions have been given for the resonance spectroscopy of the per-acetylated amino-sugars in deuteriochloroform,[2,3] and useful informations for the structure were obtained from the methyl signals as well as the ring proton signals. Despite these successes, comparatively little work has yet been carried out in deuterium oxide solution, and only a few of special cases[4~6] have been reported.

The present paper was concerned with the nuclear magnetic resonance spectroscopy on some methyl glycosides of amino-hexoses in deuterium oxide, which was carried out in connection with the studies on the oligosaccharide amino-sugars, in particular, kanamycin A and its derivatives. Most of the amino-sugars examined were the monosaccharide components of the kanamycin derivatives synthesized in this laboratory.*2

The nuclear magnetic resonance spectra of the amino-hexoses in deuterium oxide showed, in most cases, multiple signals in the narrow region of 6.0~6.5 p.p.m., indicating small differences of chemical shifts between the ring proton signals. As a result, the complete assignment became very difficult. Except for the special cases, the signals assigned with certainty were the anomeric proton signals at lower field deshielded by a ring oxygen and the ring proton signals at higher field weakly deshielded by an amino group. Therefore, the effects of an amino group and the related functions (ammonium and acetamido groups) on the chemical shifts and coupling constants of the above signals were mainly examined, and the results obtained were included in this paper.

Experimental

A sugar to be analyzed (ca. 50 mg.) was dissolved in D_2O (0.5 ml.) and the solution was freeze-dried. After twice deuteration, the compound was dissolved in 0.4 ml. of D_2O or pyridine. NMR spectra were taken at 60 Mc.p.s. with a Varian A-60 spectrometer at 37° or at 100 Mc.p.s. with a Varian HA-100 spectrometer. The decoupling experiments were done with the latter spectrometer using the frequency-sweep technique. Chemical shift values were given on the τ scale by taking DSS (sodium 2,2-dimethyl-2-silapentane-5-sulfonate) peak as an internal standard and corresponded to the midpoint of the each signal.

*1 Part II. S. Inouye : This Bulletin, **14**, 1179 (1966).

*2 Preliminary report. S. Inouye : "Symposium Abstracts, 9th Symposium on the Chemistry of Natural Products," 7 (1965) Osaka.

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1) R. U. Lemieux, R. K. Kulling, H. J. Bernstein, W. G. Schneider : J. Am. Chem. Soc., **80**, 6098 (1958).
2) L. D. Hall : Adv. Carbohydrate Chem., **19**, 51 (1964).
3) H. Agahigian, G. D. Vickers, M. H. von Saltza, J. Reid, A. I. Cohen, H. Gauthier : J. Org. Chem., **30**, 1085 (1965).
4) K. L. Rinehart, W. S. Chilton, M. Hichens, W. von Philipsborn : J. Am. Chem. Soc., **84**, 3217 (1962).
5) P. W. K. Woo, H. W. Dion, L. Durham, H. S. Mosher : Tetrahedron Letters, **1962**, 735.
6) S. Inouye : This Bulletin, **14**, 1112 (1966).

Apparent coupling constants were the directly observed line spacings. The mean errors in chemical shift and apparent coupling constant were ± 0.01 p.p.m. and ± 0.5 c.p.s., respectively. Unless otherwise stated, the resonance data shown were those at 60 Mc.p.s.

Results and Discussion

The results were given in Tables I, II, III and Figs. 1, 2, 3 and summarized into the following three points as a matter of convenience to discussion.

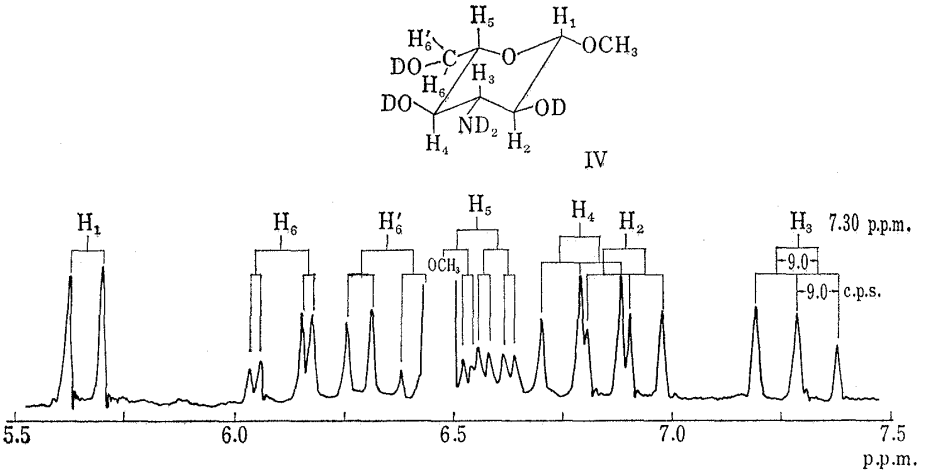

Fig. 1. Nuclear Magnetic Resonance Spectrum of Methyl 3-Amino-3-deoxy-β-L-glucopyranoside (IV) at 100 Mc.p.s. in Deuterium Oxide

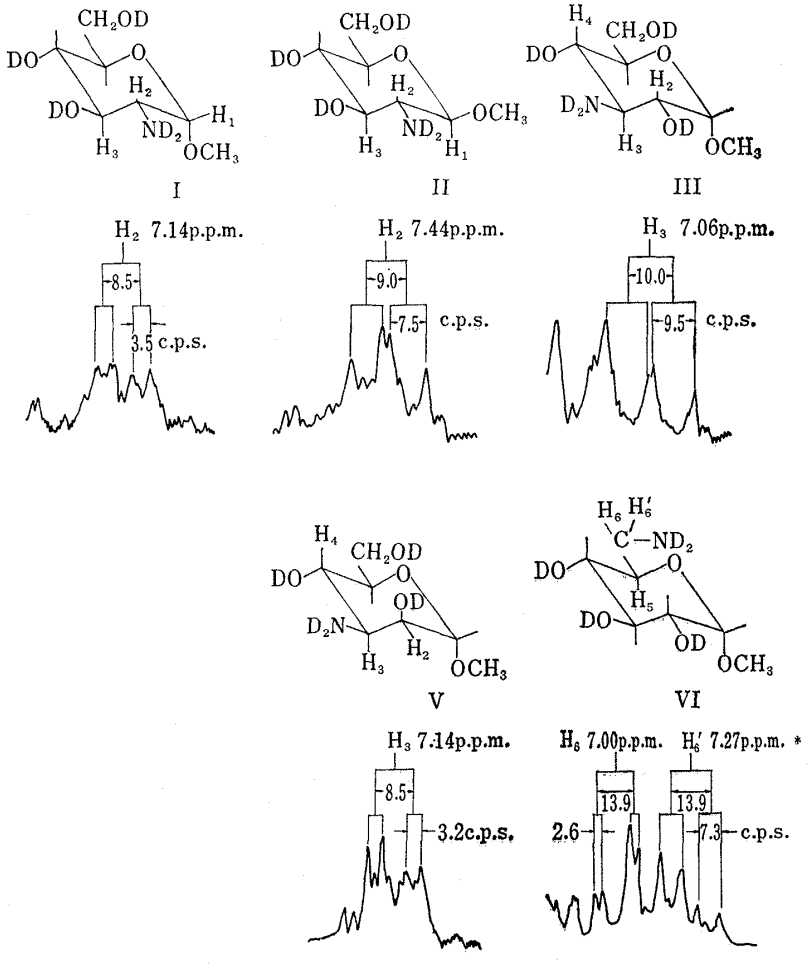

Fig. 2. Proton Signals Weakly Deshielded by an Amino Group

* 100 Mc.p.s. spectrum

1) Weakly Deshielding Effect of an Amino Group upon the Proton Attached to the Same Carbon

The spectra of the amino-hexopyranosides examined in the form of free base showed a multiplet at the higher field than 7 p.p.m., separated from the main signals. Six examples of the multiplet were presented in Figs. 1 and 2. The peak intensity of these signals was approximated to that of an anomeric proton (H_1) signal in the cases of methyl 2-amino-2-deoxy-α-D-glucopyranoside (I), methyl 2-amino-2-deoxy-β-D-glucopyranoside (II), methyl 3-amino-3-deoxy-α-D-glucopyranoside (III), methyl 3-amino-3-deoxy-β-L-glucopyranoside (IV) and methyl 3-amino-3-deoxy-α-D-mannopyranoside (V), whereas the peak area in the case of methyl 6-amino-6-deoxy-α-D-glucopyranoside (VI) was roughly corresponded to the doubled area of the H_1. As already mentioned in the preceeding papers, the aminomethine (>CH-ND_2) proton resonated at higher field than the hydroxylmethine (>CH-OD) proton, the difference arising from the different electronegativities of a nitrogen and an oxygen atoms.[7] The difference of the chemical shifts found for some open-chain amino-alcohols (XII and XIII in Table I) in deuterium oxide was about 0.95 p.p.m. Thus, the multiplet signals mentioned above were assigned to the protons weakly deshielded by an amino group attached to the same carbon atom. This assignment was confirmed by the large paramagnetic shifts of these signals accompanied with N-protonation and N-acetylation as will be shown later.

Then, the multiplet constituted the X part (methine proton) or the AB part (non-equivalent methylene protons) of an ABX system depending upon whether an amino group attached to a secondary ring carbon or to a primary terminal carbon. First-order analyses of the X part signals were illustrated in Figs. 1 and 2. Although the apparent coupling constants (J_{AX}, J_{BX}) obtained from the analysis of the X part alone did not necessarily represent the true coupling constants,[8] they gave reasonable values in this case, as far as the C1 (1C) conformation was assumed for the respective D (L) sugars.*4

The splitting patterns in III (Fig. 2) and IV (Fig. 1) indicated large values for $J_{2,3}$ and $J_{3,4}$ (9.5, 10.0 c.p.s. in III; 9.0, 9.0 c.p.s. in IV), both of which were compatible with all the axial orientation of H_2, H_3 and H_4. The quartet ($J_{2,3}$=3.2 c.p.s., $J_{3,4}$=8.5 c.p.s.) in V corresponded to the axial-equatorial and axial-axial splittings. The H_2 signal in I gave more complex fine structure arising from the second-order effect, but was possible to analyze as the axial-axial splitting of 8.5 c.p.s. ($J_{2,3}$) and the axial-equatorial splitting of 3.5 c.p.s. ($J_{1,2}$). The splittings (7.5, 9.0 c.p.s.) in II corresponded with all the axial orientations of H_1, H_2 and H_3. First-order analysis of the AB part signal in VI (Fig. 2) gave the chemical shifts of the two methylene protons (H_6, 7.00 p.p.m., H_6', 7.27 p.p.m.) and the coupling constants ($J_{5,6}$ = 2.6 c.p.s., $J_{5,6'}$ = 7.3 c.p.s., $J_{6,6'}$ = 13.9 c.p.s.). The assignment was further supported by the double resonance experiment. By irradiating the H_5 signal (6.54 p.p.m.), the asymmetric multiplet changed to a symmetrical AB quartet, thereby proving the terminal methylene protons. The H_2 signal lay accidentially in the irradiating field, and this decoupling experiment was accompanied with the collapse of the H_1 doublet into a single line.

*4 The assignment of the C1 (1C) conformation was supported by the chemical shifts of the methoxyl groups at C_1 (ca. 6.6 p.p.m. for an axial methoxyl; ca. 6.45 p.p.m. for an equatorial methoxyl) and the chemical shifts and coupling constants of the anomeric proton signals given in Table I.

7) L. M. Jackman: "Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," (Japanese Language Edition), 82 (1962). Tokyo Kagaku Dojin, Tokyo.

8) N. S. Bhacca, D. H. Williams: "Application of NMR Spectroscopy in Organic Chemistry" 135 (1964). Holden-Day, Inc., San Francisco.

It was noted that, among the axial protons weakly deshielded, the H_2 and H_3 of the α-isomers (I, III) resonated at much lower field (0.24~0.3 p.p.m.) than those of the β-isomers (II, IV). The deshielding effect of the *trans* or *syn*-diaxial methoxyl group at C_1 was probably responsible for the paramagnetic shifts.

In the cases of 2-amino-2-deoxy-D-glucopyranoses (a mixture of α (IX) and β (X)) and methyl 3,6-diamino-3,6-dideoxy-α-D-hexopyranosides (VII, VIII) as well, the proton signals weakly deshielded by an amino group could be recognized in the field around 7 p.p.m., and gave the expected peak intensity in the respective cases. But the analysis on the first-order basis was difficult owing to the overlapping of the two signals.

It was obvious from the above results that the spectral analysis was greatly facilitated by the use of the spectrum taken in the form of free base, particularly when the ring protons showed similar chemical shifts in the spectrum of hydrochloride or acetates. The proton signals appeared at the higher field than 7 p.p.m. could offer structural informations in the vicinity of an amino group and give a clue for the analysis. An example of the latter situation was provided by the spectra of IV in the forms of free base and hydrochloride. The spectrum of the free base in deuterium oxide showed well-resolved signals (Fig. 1) which permitted a complete first-order analysis as illustrated. The analysis gave further support for the assignment of the H_3 signal mentioned above and the 1C conformation. In the spectrum of the hydrochloride, on the other hand, the resolved signals were the H_1 and H_6, and the other protons in the molecule resonated in a small region of the spectrum (6.3~6.7 p.p.m.), seriously overlapped.

2) Deshielding Effects of Ammonium and Acetamido Groups upon the Anomeric Proton

TABLE I. Chemical Shifts and Coupling Constants of Anomeric Protons and Methoxyl Protons of Amino-sugars and Amino-alcohols in Deuterium Oxide

Amino-sugars (Amino-alcohols)	H_1[a]			OCH_3		
	Free base	HCl	N-acetate	Free base	HCl	N-acetate
Methyl 2-amino-α-D-glucoside (I)	5.18 (3.5)	4.97 (3.5)		6.59	6.55	
Methyl 2-amino-β-D-glucoside (II)	5.72 (8.0)	5.33 (8.5)	5.54 (7.5)	6.44	6.41	6.49
Methyl 3-amino-α-D-glucoside (III)	5.22 (3.5)	5.13 (3.5)	5.19 (3.5)[b]	6.58	6.54	6.56
Methyl 3-amino-β-L-glucoside (IV)	5.67 (8.0)	5.55 (7.5)	5.60 (8.0)	6.47	6.42	6.47
Methyl 3-amino-α-D-mannoside (V)	5.27 (1.7)	5.20 (1.6)	5.27 (1.5)[e]	6.58	6.57	6.56
Methyl 6-amino-α-D-glucoside (VI)	5.19 (3.1)*,[d]	5.14 (3.2)*	5.23 (3.2)*	6.59	6.56	6.62
Methyl 3,6-diamino-α-D-glucoside (VII)	5.28 (3.5)	5.11 (3.5)	5.25 (3.5)	6.61	6.53	6.63
Methyl 3,6-diamino-α-D-mannoside (VIII)	5.29 (1.5)	5.16 (1.5)	5.34 (1.6)	6.59	6.55	6.65
2-Amino-α-D-glucose (IX_a)	4.78 (3.5)	4.53 (3.5)	4.81 (2.5)*			
2-Amino-β-D-glucose (IX_b)	5.40 (7.5)	5.03 (8.3)	5.30 (7.0)*			
2-Amino-α-D-galactose (X_a)	4.73 (4.0)*	4.52 (3.5)	4.79 (3.5)			
2-Amino-β-D-galactose (X_b)	5.47 (7.5)	5.10 (8.0)	5.37 (7.5)			
Axial H_2 of 2-deoxy-streptamine (XI)	8.90(11.7)[e]	8.16(11.7)[e]	8.64(12.0)[e]			
Equatorial H_2 of XI	8.05 (4.0)[e]	7.50 (4.0)[e]	8.00 (3.8)[e]			
Axial H_2 of II	7.44 (9.0)[f]	6.98 (9.0)[f]				
Equatorial H_2 of V	6.16 (3.2)[f]	5.89 (3.0)[f]				
Equatorial H_2 of VIII	6.17 (3.2)[f]	5.85 (2.8)[f]				
3-Hydroxyl-*n*-propylamine (XII) (H_1)	7.34	6.87	6.75			
(H_2)	8.37	8.12	8.28			
(H_3)	6.37	6.27	6.36			
2-Hydroxyl-ethylamine (XIII) (H_1)	7.30	6.88	6.68			
(H_2)	6.41	6.17	6.34			

* an asymmetric multiplet

a) chemical shift in p.p.m. (τ). Number in parentheses was an apparent coupling constant ($J_{1,2}$) in c.p.s.

b) H_1, 5.01 (3.2), OCH_3, 6.63 in pyridine *c*) H_1, 4.97 (1.5), OCH_3, 6.64 in pyridine

d) H_1, 5.19 (3.2) at 100 Mc.p.s. H_1, 4.96 (3.1), OCH_3, 6.58 in pyridine *e*) $J_{1,2}=J_{2,3}$ values *f*) $J_{2,3}$ value

TABLE II. Chemical Shifts and Coupling Constants of Anomeric Protons of Sugars in Deuterium Oxide

Sugars	H_1 $(J_{1,2})^{a)}$	Sugars	H_1 $(J_{1,2})^{a)}$
Methyl α-D-glucoside	5.19(3.0)*,b)	α-D-Mannose	4.83(1.4)
Methyl β-D-glucoside	5.62(7.0)	β-D-Mannose	5.11(1.0)
Methyl α-D-mannoside	5.24(1.6)	α-D-Galactose	4.73(2.0)*
Methyl α-D-galactoside	5.16(2.5)*,c)	β-D-Galactose	5.41(7.8)*
Methyl 6-deoxy-α-D-glucoside	5.26(3.1)*	2-Deoxy-α-D-glucose	4.23(1.4, 3.5)
Methyl 6-iodo-α-D-glucoside	5.21(3.2)*	2-Deoxy-β-D-glucose	5.08(2.1, 9.7)
Methyl 3-acetamido-6-tosyl-α-D-glucoside	5.00(3.5)d)	2-Deoxy-α-D-galactose	4.58(2.5, 2.5)
Methyl 3-acetamido-6-tosyl-α-D-mannoside	4.96(1.5)	2-Deoxy-β-D-galactose	5.14(2.7, 9.6)
Methyl 3-acetamido-6-azido-α-D-glucoside	5.24(3.5)	6-Deoxy-α-L-galactose	4.77(2.8)*
Methyl 3-acetamido-6-azido-α-D-mannoside	5.32(1.5)	6-Deoxy-β-L-galactose	5.43(8.0)*
α-D-Glucose	4.77(3.3)	2-Propionamido-α-D-glucose	4.80(2.9)*
β-D-Glucose	5.36(7.3)	2-Propionamido-β-D-glucose	5.38(7.0)*

* an asymmetric multiplet
a) chemical shift in p.p.m. (τ). Number in parentheses was apparent coupling constant in c.p.s.
b) H_1, 5.22 (3.3) at 100 Mc.p.s. *c*) H_1, 4.85 (3.4) in pyridine *d*) pyridine

TABLE III. Deshielding Effects ($\Delta=\tau_{\text{free base}}-\tau$) of Ammonium and Acetamido Groups upon Ring Protons in Deuterium Oxide

Compound No.	Deshielded proton	Deshielded Type[a)]	Δ_{NCOCH_3}	Δ_{HCl}
I	H_1	β_{ee}		0.21
II	H_2	α	ca. 1.1	0.54
II	H_1	β_{ea}	0.18	0.39
III	H_3	α	1.14	
III	H_1	γ	0.03	0.09
IV	H_2	β_{ea}	0.11	0.31
IV	H_1	γ	0.07	0.12
V	H_3	α	0.99	ca. 0.6
V	H_2	β_{ee}	0.02	0.28
V	H_1	γ	0.00	0.07
VI	H_1	γ	−0.04	0.05
VII	H_1	$\gamma+\gamma$	0.03	0.17
VIII	H_2	$\beta_{ee}+\gamma$	−0.02	0.32
VIII	H_1	$\gamma+\gamma$	−0.05	0.13
XIV[b)]	H_3	$\alpha+\gamma$	1.27	0.72
XIV	H_2	$\beta_{ae}+\gamma$	0.01	0.35
XIV	H_1	$\gamma+\gamma$	0.05	0.25
IX	H_1	β_{ee}	0.36	0.25
IX	H_1	β_{ea}	0.42	0.37
XI	H_2	$\beta_{ee}+\beta_{ee}$	0.05	0.55
XI	H_2	$\beta_{ea}+\beta_{ea}$	0.26	0.74
XII	H_1	α	0.59	0.47
XII	H_2	β	0.09	0.25
XII	H_3	γ	0.01	0.10
XIII	H_1	α	0.62	0.42
XIII	H_2	β	0.07	0.24

a) Type α: shift of a proton attached to the same carbon atom as substituents
Type β: shift of a proton due to substituents attached to the adjacent carbon atom
Type β_{ee}: shift of an equatorial proton due to equatorial substituents attached to the adjacent carbon atom
Type β_{ea}: shift of an axial proton due to equatorial substituents attached to the adjacent carbon atom
Type β_{ae}: shift of an equatorial proton due to axial substituents attached to the adjacent carbon atom
Type γ: shift of a proton due to substituents attached to the carbon atom separated from more than two C-C bonds
b) methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside[6)]

The chemical shifts of the anomeric protons (H_1) in the form of free base, hydrochloride and N-acetate were given in Table I. A comparison of the H_1 chemical shifts of the amino-substituted sugars with those of the parent sugars shown in Table II indicated that the replacement of a hydroxyl group by an amino group effected essentially no significant shift of the H_1 signal, including the reducing sugars (IX and X), which showed slight up-field shifts (ca. 0.03 p.p.m.). This suggested the similar long-range shielding effects of a hydroxyl and an amino groups. However, it was not the case with an ammonium group. The H_1 signals of the hydrochlorides showed down-field shifts in all the cases examined. This was clearly seen by Table III, which indicated the signal shifts of H_1 as well as other ring protons induced by N-protonation and N-acetylation. The Δ values shown were the difference of the chemical shifts between free base and hydrochloride (or N-acetate). In a series of the amino-substituted methyl glucopyranosides, the magnitude of the paramagnetic shift of H_1 varied from 0.05 to 0.39 p.p.m. The largest shift occurred when an ammonium group was on C_2. Similar shifts (about 0.2~0.4 p.p.m.) were observed in the case of the H_1 of the reducing sugars (IX, X). The averaged Δ values obtained for the ring protons including H_1, H_2 and H_3, were 0.5~0.6 p.p.m. for the α-protons (attached to the same carbon atom as an ammonium group), 0.3~0.4 p.p.m. for the β-protons (attached to the adjacent carbon atom), ca. 0.1 p.p.m. for the γ-protons (attached to the carbon atom separated through two C-C bonds). These values were considerably larger than those of open-chain amino-alcohols (XII, XIII) simultaneously determined for a comparison (Table III).

It was of considerable interest that the magnitude of the paramagnetic shifts depended not only on the location of an ammonium group in the molecule but also on the relative configuration between an ammonium group and the deshielded proton. The latter was clearly seen by comparing the Δ_{HCl} values of the H_1 pair of methyl 2-amino-glucosides (I, II), and of the two methylene protons of 2-deoxystreptamine (XI). In both cases, the paramagnetic shifts were more remarkable for the neighboring axial protons than the equatorial counterparts.

Recently, Lemieux *et al.* reported the unusual case of down-field shift of the ring protons in the presence of tetramethylammonium chloride in acetonitril.[9] They ascribed the shift to the complex formation of chloride ion to the hydrogen atoms.

An ammonium cation undoubtedly formed ion-pair with a counter anion in solution. In order to see the deshielding effect owing to a counter anion, the H_1 chemical shifts of 2-amino-2-deoxy-D-glucopyranose (an equilibrium mixture of IXa and IXb) in the forms of sulfate, trifluoroacetate and *p*-tolylsulfonate as well as hydrochloride were compared. As a result, no significant shift was observed either on the H_1 of α-epimer or on the H_1 of β-epimer. It might be concluded from this that the effect of a counter anion was not so strongly reflected on the chemical shifts of the ring protons.*[5] This was probably due to the strong hydration which formed a shell of solvent molecules around the ions.

The signal positions in the spectrum of the hydrochloride might be considered as the weighted average of those of the ammonium cation and the nonprotonated free base equilibrated in solution, the relative proportion being determined by the basic strength. As will be reported in a separate paper, the basicity of methyl amino-deoxy-D-glucopyranosides decreased in the following order: 6-amino (α) > 3-amino (α) > 2-amino (α) > 2-amino (β). This order was just the reverse of the Δ_{HCl} values of H_1. Accordingly, the influence of the non-protonated form was assumed to be unimportant

*5 This was not true in the case of a weak base. Indeed, the H_1 signals of kanamycin A showed slight dependency upon the acidity of the counter anion. Details will be reported in a subsequent paper.

9) R. U. Lemieux, J. S. Martin, J. Hayami: "Preliminary Report, International Symposium on Nuclear Magnetic Resonance," M-2-17 (1965), Tokyo.

in the present case.

The shift due to the conformational distortion was also eliminated, since no significant alteration of the coupling constants was induced by N-protonation (or by N-acetylation). Thus, the down-field shift should be ascribed to the deshielding effect of the quaternary ammonium group.

N-Acetylation also caused the paramagnetic shifts of the ring protons in many cases, but the Δ_{NCOCH_3} values on the β-protons were much reduced (about 0.1 p.p.m.), although they were maniplied on the α-protons (about 1 p.p.m.). The different degree of the deshielding effect on the α- and β-protons by the two groups (ammonium and acetamido) suggested different mechanisms for the local and long-range deshielding.

The local paramagnetic shift of the α-proton was largely attributable to the σ-inductive effect of an ammonium group, which was known to be larger than an amino group. The Δ_{HCl} values, however, were smaller than those caused by acetamido function. For the long-range deshielding effect of an ammonium group, which was greater than the other groupings, the inductive effect was probably unimportant, because, it was suggested that the effect was reduced markedly at the positions separated by more than one single bond from a substituent.[10]

Of the remaining possible effects which could contribute to the long-range down-field shift, the most significant in this case appeared to be the electrostatic field effect[11~13] of the electric charge of an ammonium group. The electric field produced by a polar substituent in the molecule was known, through a solvent or the low-dielectric cavity provided by the organic solute, to induce the polarization of the C-H bonding electrons, resulting in the deshielding of the protons. Evidently, the field effect on proton was correlated with the distance from the polar substituent in space. The observation that the Δ_{HCl} values of *cis*-ammonium methine (–C(H) – C(ND$_3^+$)H–) were larger than those of *trans*-methine (–C(H) – C(ND$_3^+$)–) and open-chain compounds was consistent with the above view. A similar difference was observed in the *cis*- and *trans*-acetamidomethines, though the Δ_{NCOCH_3} values were rather small.

3) Configurational Effect of Polar Substituent at C_2 upon the Chemical Shift and Coupling Constant of Anomeric Proton Signal

An anomeric proton (H_1) signal usually appeared as a well-defined doublet coupled to H_2. But it was found out that some of the common glucose and galactose derivatives showed, at 60 Mc.p.s., unusual multiplets as the H_1, which were indicated by asterisk in Tables I and II. That these multiplicities arised from the virtual long-range coupling of H_1, H_2, H_3——system,*6 but not from the presence of minor isomers*7

*6 Virtual coupling observed in the H_1 signal was due to the strongly coupled system of H_1, H_2, H_3——, where H_2 signal was not appreciably shifted from those of H_3, H_4 ——, as compared with $J_{1,2}$. Under these conditions, line spacing of the H_1 should be smaller than the true value of the $J_{1,2}$ (J. I. Musher, E. J. Corey : Tetrahedron, **18**, 791 (1962)). Many examples of virtual coupling were recently reported in the acetylated sugars in deuteriochloroform.[14]

*7 Rudrum and Shaw reported that the spectrum of galactose showed a small additional doublet incompletely separated from the main doublet. They assigned the minor doublet to the anomeric proton of a furanose form (M. Rudrum, D. F. Shaw : J. Chem. Soc., **1965**, 52).

10) M. J. S. Dewar, P. J. Grisdale : J. Am. Chem. Soc., **84**, 3548 (1962).
11) J. W. Marshall, J. A. Pople : Mol. Phys., **1**, 199 (1958).
12) A. D. Buckingham : Can. J. Chem., **38**, 300 (1960).
13) J. I. Musher : J. Chem. Phys., **37**, 34 (1962).
14) R. U. Lemieux, J. D. Stevens : Can. J. Chem., **43**, 2059 (1965).

was shown by the following observations. The H_1 signals of methyl α-D-glucopyranoside and methyl 6-amino-6-deoxy-α-D-glucopyranoside (Ⅵ) were triplets at 60 Mc.p.s., but normal doublets at 100 Mc.p.s., which collapsed into the singlets by the decoupling of the H_2 signals. No signal due to minor isomer was detected in the 100 Mc.p.s. spectra. The spectrum of the deuterated methyl α-D-galactopyranoside, which gave an asymmetric triplet in deuterium oxide, showed a doublet when measured in a pyridine solution accompanied with the considerable spreading of the main multiplet. The lack of the extra signal in the latter solvent was explained by the solvent-induced shifts of the ring proton signals, thereby the condition of virtual coupling being unsatisfied. These results required the careful comparison of the $J_{1,2}$ values obtained from the line spacings, in particular, when the multiplicity was observed.

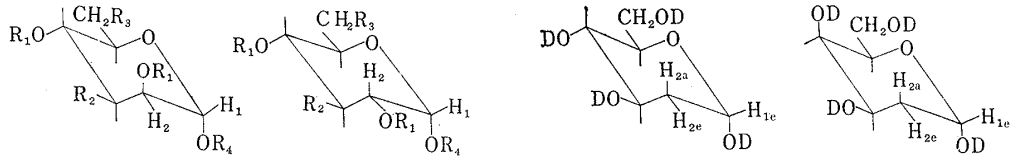

Fig. 3. Dependence of $J_{1,2}$ Values on the Configuration at C_2
R_1=D, CH_3, $COCH_3$ R_2=OD, ND_2, ND_3^+, $NDCOCH_3$
R_3=OD, OTs, ND_2, ND_3^+, $NDCOCH_3$, N_3, R_4=D, CH_3, $COCH_3$, Br, Cl

A comparison of the H_1 chemical shifts and $J_{1,2}$ values in α-glucopyranose and α-mannopyranose derivatives given in Tables I and Ⅱ immediately revealed that the H_1 of α-glucose derivatives showed always higher chemical shift with greater $J_{1,2}$ value than the H_1 of α-mannose derivatives. This was true, in the solutions of deuterium oxide and pyridine, of reducing sugars, methyl glycopyranosides and their derivatives, in which the hydroxyl groups at C_3 and C_6 were replaced by amino, ammonium, acetamido, azido, and *p*-tolylsulfonyloxy groups. The consistency of the same relation was further shown by many members of methyl tetra-O-acetyl-α-D-glycopyranosides,[6] and tetra-O-acetyl-α-D-glycopyranosyl halides[15] in deuteriochloroform.

The large dependency of the H_1 chemical shifts on the configuration at C_2 has been first pointed out by Hall.[16] Lemieux and Stevens demonstrated a greater shielding effect on an equatorial proton by a neighboring axial acetoxyl group than by an equatorial acetoxyl group in chloroform.[14] As shown in Fig. 3, the structural difference in the two isomers lay in the configuration at C_2. Thus, the difference of the equatorial H_1 (Δ = 0.04~0.08 p.p.m.) should be attributable to the different degree of long-range shielding between the axial and equatorial substituents at C_2. The chemical shifts of H_1 in α-galactose and its derivatives shown in Tables I and Ⅱ were close to those of α-glucoses, but definitely lower than those of α-mannoses, in consistence with the anticipated shifting.

Much interested was the consistent difference of the coupling constants between the glucose and mannose derivatives. The $J_{1,2}$ value (1.5 ± 0.5 c.p.s.) in α-mannopyranoses came from the coupling between the equatorial H_1 and the equatorial H_2, while the $J_{1,2}$ (3.5 ± 0.5 c.p.s.) in α-glucopyranoses arised from the coupling between the equatorial H_1 and the axial H_2.

The consistent observation of the relation $J_{ee} < J_{ea}$ in a wide variety of the derivatives, where H_2 was considerably chemically shifted from H_3, excluded the possibility that the small values of the apparent coupling constants of the α-mannoses might be due to the departure from the true values owing to the second-order effect.

15) D. Horton, W.N. Turner : J. Org. Chem., **30**, 3387 (1965).
16) L.D. Hall : Tetrahedron Letters, **23**, 1457 (1964).

Recently, Williams and Bhacca reported that, for certain hydroxyl and acetoxyl derivatives of steroids, the vicinal coupling constants depended on the configuration of the polar substituent, in addition to the dihedral angle, bond-length and the nature of polar group.[17] Booth indicated that the maximum electronegativity effect (decrease of $J_{1,2}$) coincided with *trans*-coplanarity of R and H_2 in the system $R-\underset{H_1}{C}-\underset{H_2}{C}$.[18] But the substituent effect of this type seemed to be not the dominant factor in this case, because, the same relation as $J_{ee} < J_{ea}$ with little change of $J_{1,2}$ values was observed in 2-deoxy-α-D-glucopyranose (2-deoxy-α-D-mannopyranose) (XV)[19] and its methyl glycopyranoside,*8,[20] where C_2 was not substituted by a polar group. The electronegativity effect, even though important, would be largely cancelled out, since the axial H_2 and the equatorial H_2 in XV were in the *trans*-coplanarity relation with the hydroxyl group at C_1 and the ring oxygen atom, respectively.

Thus, the high value of J_{ea} and in particular low value of J_{ee} observed in the α-glucose and α-mannose derivatives were favorable for the distorted chair conformation of a pyranose ring. Calculation of the dihedral angle (θ) as a function of coupling constant by Karplus equation[21] suggested the relation $\theta_{1e2a} < \theta_{1e2e}$, which required some flattening of the ring. Such a distortion might relieve the non-bonded interactions between the axial oxygens at C_1 and C_2 and the *syn*-diaxial hydrogens. The pyranose ring would be more readily distorted than the cyclohexane ring, provided that the non-bonded interaction with electron pairs on the ring oxygen was less than with hydrogen atom.[22,23] In this connection, it was interesting to see that 2-deoxy-α-D-galactopyranose (2-deoxy-α-D-talopyranose) (XVI) gave a triplet as the H_1, which indicated the equal axial-equatorial and equatorial-equatorial couplings to the two neighbors at C_2. The result might be anticipated to be, since the equatorial H_1 bisected the angle between the two methylene protons at C_2 in the idealized cyclohexane geometry. The $J_{1,2}$ values of XVI were found to fall in the intermediate (2.5, 2.5 c.p.s.) of the two $J_{1,2}$ values of XV (1.4, 3.5 c.p.s.). It was possible that an axial hydroxyl group at C_4 (XVI) might inhibit the distortion of the ring mentioned above.

In any event, measurement of the $J_{1,2}$ value as well as chemical shift of an anomeric proton allowed ready distinction between an α-glucopyranose and α-mannopyranose isomers.

The author is grateful to Drs. H. Ogawa and T. Ito of this laboratory for their interests and encouragements. Thanks are also due to Drs. T. Nishida, M. C. Woods and Y. Arata for the NMR measurement and useful suggestions.

Summary

The nuclear magnetic resonance spectra of methyl amino-hexopyranosides in the forms of free base, hydrochloride and N-acetate were measured in deuterium oxide and, in part, in pyridine. The spectrum of the free base showed a multiplet at higher field than 7 p.p.m., caused by weakly deshielding of an amino group. The analysis of this signal was shown to offer information in the vicinity of an amino

*8 The reported values in deuterium oxide were $J_{ee}=1.4$ c.p.s., $J_{ea}=3.8$ c.p.s.[20]

17) D. H. Williams, N. S. Bhacca: J. Am. Chem. Soc., **86**, 2742 (1964).
18) H. Booth: Tetrahedron Letters, **1965**, 411.
19) R. W. Lenz, J. P. Heeschen: J. Polymer Sci., **51**, 247 (1961).
20) R. U. Lemieux, S. Levine: Can. J. Chem., **42**, 1473 (1964).
21) M. Karplus: J. Chem. Phys., **30**, 11 (1959).
22) R. J. Ferrier, W. G. Overend: Quart. Rev., **1964**, 265.
23) S. J. Angyal: "Conformational Analysis," 373 (1965), Interscience Publishers, New York.

group. The spectrum of the hydrochloride showed long-range deshielding of an anomeric proton along with other ring protons, possibly induced by the electric field of an ammonium cation. The long-range deshielding due to an acetamido group was weaker than an ammonium group. It was observed in a variety of the derivatives that H_1 of α-glucopyranoses resonated at lower field with greater $J_{1,2}$ values than those of α-mannopyranoses. The differences presented ready distinction between the two isomers.

(Received March 8, 1966)

[Chem. Pharm. Bull. 14(11)1219~1227(1966)]

UDC 547.785.5.07

166. Shirō Takahashi and Hideo Kanō: Benzimidazole N-Oxides. Ⅶ.*1 The Reactivity of 1,2-Dimethylbenzimidazole 3-Oxide.

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In the previous paper of this series,[1] we have reported that 1-methylbenzimidazole 3-oxide is very reactive at the 2-position for nucleophilic reagents showing similar chemical behavior to six-membered heteroaromatic N-oxides, except a few peculiar reactions.

In connection with the previous investigation, this paper deals with the reactivity of 1,2-dimethylbenzimidazole 3-oxide (I), the 2-position of which has no replaceable hydrogen atom.

Schulenberg and Archer[2] have reported that 2-methyl-1-phenylbenzimidazole 3-oxide is obtained by catalytic reduction of N-phenyl-2′-nitroacetanilide in absolute ethanol in the presence of hydrogen chloride. A modification of this method provided a more convenient synthesis of I from N-methyl-2′-nitroacetanilide than the previous ammonium-hydrogen sulfide method.[3]

The N-oxide I was deoxygenated by phosphorus trichloride, sulfur dioxide, sodium hydrogensulfite, and sodium borohydride besides by catalytic reduction with Raney nickel.[3] Although, it was reported that heteroaromatic N-oxides are indifferent towards sulfur dioxide in contrast to aliphatic N-oxides,[4] more recently the deoxygenation reaction has been discovered on 2-phenylquinoxaline N-oxide.[5]

As an example of deoxygenation of heteroaromatic N-oxide with sodium borohydride, the conversion of 1,2,3,4-tetrahydrophenazine dioxide into cis-1,2,3,4,4a,5,10,10a-octahydrophenazine has been reported recently.[6]

In six-membered nitrogen-heteroaromatic compounds, mobility of the hydrogen atoms of the methyl groups are generally enhanced when the methyl groups are in conjugation with the ring-nitrogen atom by inductive effect of the hetero atom. The mobility was estimated chemically by the capacity for condensing with carbonyl, nitroso

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*2 Fukushima-ku, Osaka (高橋史郎, 加納日出夫).

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3) S. Takahashi, H. Kanō: This Bulletin, **11**, 1375 (1963).
4) B. Umezawa: *Ibid.*, **8**, 698 (1960).
5) E. Hayashi, C. Iijima: Yakugaku Zasshi, **82**, 1093 (1962).
6) M. J. Haddadin, C. H. Issidorides: Tetrahedron Letters, No. 36, 3253 (1965).