## Notes

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# Shigeharu Inouye : Nuclear Magnetic Resonance Spectroscopy of Amino-sugars. II.*1 Structural and Configurational Studies on the Acetyl Derivatives of Methyl 3,6-Diamino-3,6-dideoxy-α-D-glucopyranoside and Methyl 3,6-Diamino-3,6-dideoxy-α-D-mannopyranoside.

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The syntheses of methyl 3,6-diamino-3,6-dideoxy-α-D-glucopyranoside and methyl 3,6-diamino-3,6-dideoxy-α-D-mannopyranoside were reported in the preceding paper.[1] The present paper reported nuclear magnetic resonance spectroscopic evidence that fully supported the structures of the synthesized diamino-sugars.

### Experimental

The spectra were obtained at 60 Mc.p.s. using a Varian A-60 and a JNM-C-60 spectrometers and at 100 Mc.p.s. using a Varian HA-100 and a JNM-4H-100 spectrometers. Sodium 2,2-dimethyl-1,2-silapentane-5-sulfonate was used as an internal standard in $D_2O$ and in pyridine. An internal standard in $CDCl_3$ was tetramethylsilane. Before measurement, the samples were twice deuterated by dissolving in $D_2O$ followed by lyophilization. Unless otherwise stated, the resonance data shown were those obtained at 60 Mc.p.s.

## Results and Discussion

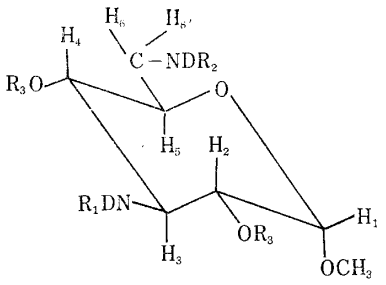
Table I. Resonance Data of the Acetyl Derivatives of Methyl 3,6-Diamino-3,6-dideoxy-α-D-glucopyranoside (I, II), Methyl 3,6-Diamino-3,6-dideoxy-α-D-mannopyranoside (IV, V, VI) and Methyl 3-Amino-3-deoxy-β-L-glucopyranoside (XIII)

| | Solv. | $OCH_3$[a] | $NCOCH_3$ | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ | $H_6$ | $H_{6'}$ | $J_{1,2}$[b] | $J_{2,3}$ | $J_{3,4}$ | $J_{4,5}$ | $J_{5,6}$ | $J_{5,6'}$ | $J_{6,6'}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I[c] | $D_2O$ | 6.60 | 8.00 | 5.22 | 6.45 | 5.98 | 6.74 | 6.38 | 6.98 | 7.33 | 3.5 | 10.0 | 9.2 | 9.2 | 3.1 | 6.7 | 13.5 |
| II | 〃 | 6.63 | 7.99<br>8.01 | 5.25 | 6.45 | 5.98 | 6.76 | | ca. 6.5 | | 3.5 | 10.3 | 9.5 | 9.5 | | | |
| IV | 〃 | 6.59 | 7.97 | 5.29 | 6.16 | 5.88 | 6.48 | | 6.94 | 7.27 | 1.5 | 3.0 | 8.0 | 8.0 | 3.0 | 4.5 | 13.7 |
| V | 〃 | 6.65 | 7.99<br>8.00 | 5.34 | 6.19 | 5.95 | 6.52 | | ca. 6.5 | | 1.6 | 2.9 | 10.0 | | | | |
| V[c] | Pyr. | 6.73 | 7.90<br>7.95 | 5.04 | 5.54 | 5.04 | 5.73 | 5.93 | 5.99 | | 1.3 | 3.5 | 9.0 | 9.0 | 2.0 | 2.0 | |
| VI | $CDCl_3$ | | | 5.39 | 5.10 | 6.16 | 5.32 | | ca. 6.55 | | 1.8 | 2.8 | 9.0 | 9.0 | | | |
| XIII | $D_2O$ | 6.47 | 7.98 | 5.60 | 6.78 | 6.13 | | | 6.07 | 6.31 | 7.6 | 9.5 | 9.5 | | 2.0 | | 11.0 |

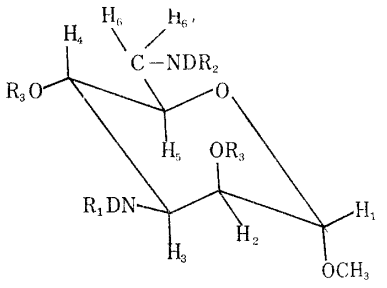
a) Chemical shift in p.p.m. (τ). b) Apparent coupling constant in c.p.s.
c) Analyzed by the 100 Mc.p.s. spectrum.

Of the six kinds of the derivatives examined, 3-N-acetates (I, IV), 3,6-di-N-acetates (II, V) and 2,3,4,6-tetra-O,N-acetates (III, VI) gave the spectra with the sufficiently resolved signals for an analysis. The spectra of the diamino-sugars themselves, on the other hand, gave the unsatisfactory resolution. Therefore, the analysis was carried out mainly on the spectra of the former six, and the results obtained were summarized in Table I, II, III, and IV.

*1 Part I. S. Inouye : This Bulletin, **14**, 1112 (1966).
*2 Morooka, Kohoku-ku, Yokohama-shi (井上重治).
1) S. Inouye : This Bulletin, **14**, 902 (1966).



I : $R_1$=Ac, $R_2$=$R_3$=D
II : $R_1$=$R_2$=Ac, $R_3$=D
III : $R_1$=$R_2$=$R_3$=Ac



IV : $R_1$=Ac, $R_2$=$R_3$=D
V : $R_1$=$R_2$=Ac, $R_3$=D
VI : $R_1$=$R_2$=$R_3$=Ac

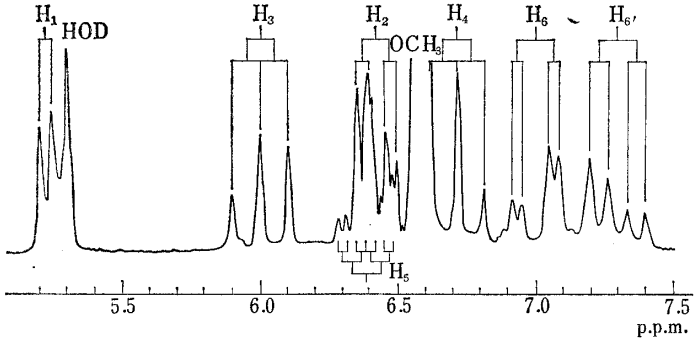



Fig. 1. Nuclear Magnetic Resonance Spectrum of Methyl 3-Acetamido-6-amino-3,6-dideoxy-$\alpha$-D-glucopyranoside (I) at 100 Mc.p.s. in Deuterium Oxide

**Methyl 3-Acetamido-6-amino-3,6-dideoxy-$\alpha$-D-glucopyranoside (I)**

The resonance spectrum of this compound in deuterium oxide gave well resolved signals, which permitted a complete first-order analysis. The low field portion of the 100 Mc.p.s. spectrum was illustrated in Fig. 1. In common with all of the compounds studied in deuterium oxide, the anomeric proton ($H_1$) signal appeared as a well-defined doublet at the lowest field (5.22 p.p.m.), largely separated from the other ring proton signals. The apparent $J_{1,2}$ value estimated from the line spacing was 3.5 c.p.s., indicating an approximate dihedral angle between $H_1$ and $H_2$ of 60°. The $H_2$ signal appeared at 6.45 p.p.m. as a quartet, partly overlapped with the $H_5$ signal. The low field triplet at 5.98 p.p.m. was assigned to $H_3$. The large spacings ($J_{2,3}$=10.0 c.p.s., $J_{3,4}$=9.2 c.p.s.) indicated the diaxial couplings of $H_2$ and $H_3$ and of $H_3$ and $H_4$. The $H_4$ signal appeared at high field, 6.74 p.p.m., partly obscured by the intense signal of the methoxyl group on $C_1$. The large $J_{4,5}$ value (9.2 c.p.s.) indicated the *trans*-diaxial arrangement of $H_4$ and $H_5$. Thus, all the *trans*-axial arrangement of $H_2$, $H_3$, $H_4$ and $H_5$ was established, indicating the glucose configuration in the C1 conformation as shown in I. The small $J_{1,2}$ value was consistent with the $\alpha$-configuration.

The $H_5$ signal appeared at 6.38 p.p.m. as a multiplet, through coupling with the three protons on $C_4$ and $C_6$. The $H_6$ signal was observed at the highest field, forming an eight lines, typical pattern of the AB portion of an ABX system, and the two protons showed a geminal coupling of 13.5 c.p.s. The $H_6$ signal at 6.98 p.p.m. showed small coupling with $H_5$ ($J_{5,6}$=3.1 c.p.s.), while the $H_{6'}$ at 7.33 p.p.m. showed larger coupling with $H_5$ ($J_{5,6'}$=6.7 c.p.s.). The chemical shifts of the two $H_6$ signals were found to be about 0.95 p.p.m. to higher field than those of methyl 3-acetamido-3-deoxy-$\beta$-L-glucopyranoside (XIII), simultaneously examined for a comparison. Since the deshielding effect of an amino group on the proton attached to the same carbon atom was known to be weaker than that of a hydroxyl group,[2] the up-field shift of the $H_6$

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

signals supported that the amine function was on $C_6$. This was further confirmed by the remarkable down-field shift of the $H_6$ signals (ca. 0.6 p.p.m.) accompanied with N-acetylation as described later. Thus, all of the data fully supported the structure (I) depicted.

**Methyl 3-Acetamido-6-amino-3,6-dideoxy-*α*-D-mannopyranoside (IV)**

The resonance spectrum of this compound in deuterium oxide was fully compatible with the structure shown in Ⅳ. The spectrum (Table I) showed the $H_1$ doublet at the lowest field, 5.29 p.p.m. The small $J_{1,2}$ value (1.5 c.p.s.) was typical for the *α*-mannopyranose derivatives*[3]. The $H_2$ signal appeared as a narrow quartet at 6.16 p.p.m. The small $J_{2,3}$ value (3.0 c.p.s.) was consistent with the equatorial-axial arrangement of $H_2$ and $H_3$. The $H_3$ signal was observed as a broad quartet at relatively low field (5.88 p.p.m.), while the $H_4$ appeared as a triplet at relatively high field, 6.48 p.p.m., incompletely resolved with the $H_5$. The large values of $J_{3,4}$ and $J_{4,5}$ (both 8.0 c.p.s.) indicated 3,4 and 4,5-*trans*-diaxial arrangements. The $H_6$ signal was observed at the highest field, separated from the other ring proton signals. The multiplet constituted the AB portion of an ABX system, whose first-order analysis gave the $J_{5,6}$ (3.0 c.p.s.), $J_{5,6'}$ (4.5 c.p.s.) and $J_{6,6'}$ (13.7 c.p.s.). The chemical shifts of the $H_6$ and $H_{6'}$ (6.94, 7.27 p.p.m.) were found to be very close to those of I, and attributable to the weaker deshielding of the amino group on $C_6$. The assignment was verified by the resonance spectrum of the di-N-acetate (V) described below.

**Methyl 3,6-Diacetamido-3,6-dideoxy-*α*-D-glucopyranoside (II) and Methyl 3,6-Diacetamido-3,6-dideoxy-*α*-D-mannopyranoside (V)**

The spectra of Ⅱ and Ⅴ were consistent with the proposed structures (Ⅱ) and (V) in each case. The $H_1$ doublet in the spectrum of Ⅱ in deuterium oxide appeared at 5.25 p.p.m. with the apparent $J_{1,2}$ value of 3.5 c.p.s. The $H_2$ quartet was observed at 6.45 p.p.m. showing the equatorial-axial coupling with $H_1$ and the axial-axial coupling with $H_3$ ($J_{2,3}$=10.3 c.p.s.). The $H_3$ signal was observed at low field, 5.98 p.p.m., and the large $J_{3,4}$ value (9.5 c.p.s.) was indicative of the *trans*-diaxial arrangement of $H_3$ and $H_4$. The $H_4$ triplet appeared at 6.76 p.p.m. incompletely resolved with the $H_5$ and $H_6$ signals. The $H_6$ was recognized around 6.5 p.p.m.

Compound (V) in deuterium oxide gave the spectrum similar to that of Ⅱ. The narrow doublet assigned to $H_1$ appeared at the lowest field, 5.34 p.p.m., with a small $J_{1,2}$ value (1.6 c.p.s.), typical for the *α*-mannose derivatives. The $H_2$ quartet at 6.19 p.p.m. showed the equatorial-equatorial coupling with $H_1$ and the equatorial-axial coupling with $H_2$ ($J_{2,3}$=2.9 c.p.s.). A broad quartet of $H_3$ at low field, 5.95 p.p.m. showed the large value of $J_{3,4}$ (10.0 c.p.s.), consistent with the 3,4-diaxial orientation. The $H_6$ signal was observed around 6.5 p.p.m., seriously overlapped with the $H_4$ and $H_5$.

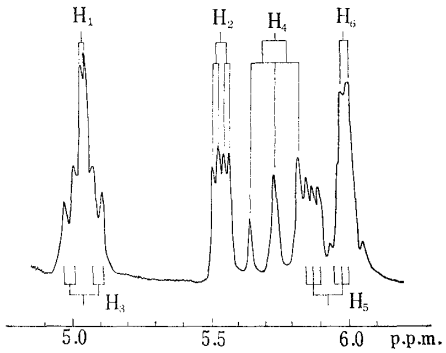



Fig. 2. Nuclear Magnetic Resonance Spectrum of Methyl 3,6-Diacetamido-3,6-dideoxy-*α*-D-mannopyranoside(V) at 100 Mc.p.s. in Pyridine

A comparison of the chemical shifts of Ⅱ and Ⅴ with those of I and Ⅳ in the respective cases, revealed the remarkable down-field shift of the $H_6$ in the di-N-acetates(Ⅱ, Ⅴ), whereas the chemical shifts of $H_1$, $H_2$, $H_3$ and $H_4$ remained essentially unchanged by the N-acetylation. The down-field shift was attributable to the increased deshielding

*[3] The low value of $J_{1,2}$ seemed to be characteristic of an equatorial-equatorial coupling in the *α*-hexopyranosides having an axial substituent at $C_2$(S. Inouye : This Bulletin, in press). See also reference 4.

effect of an acetamido function, as compared with an amino function[2], and gave further confirmation on the amination at $C_6$.

TABLE II. Solvent Shifts ($\Delta=\tau_{D_2O}-\tau_{pyridine}$) of Methoxyl and Ring Proton Resonances for Some Sugar Derivatives

| Compound | $\Delta$ ($\tau_{D_2O}-\tau_{pyridine}$) | | | | | |
|---|---|---|---|---|---|---|
| | $OCH_3$ | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ |
| V | −0.08 | 0.30 | 0.65 | 0.91 | 0.79 | |
| VII | 0.00 | 0.43 | 0.73 | 0.99 | 0.79 | 0.50 |
| VIII | 0.10 | 0.36 | 0.62 | 0.85 | 0.73 | |
| X | −0.04 | 0.28 | 0.40 | 0.63 | | |
| X[a] | −0.01 | 0.10 | 0.22 | 0.46 | | |
| XI | −0.12 | 0.15 | 0.28 | 0.51 | 0.16 | |
| XI[a] | 0.05 | 0.14 | 0.24 | 0.48 | 0.22 | |

a) $\Delta(\tau_{D_2O}-\tau_{pyridine-D_2O\ (1:1)})$

The spectrum of V taken in a pyridine solution afforded much resolved signal pattern, whose low-field portion was illustrated in Fig. 2. The two overlapping signals (a doublet and a quartet) at the lowest field, 5.04 p.p.m., were assigned to $H_1$ and $H_3$. It was noted that, of the five ring proton signals, the $H_3$ showed the largest pyridine-induced shift. This was clearly seen by comparing the chemical shifts determined in deuterium oxide and pyridine as shown in Table II. The large $\Delta$ value ($\Delta=\tau_{D_2O}-\tau_{pry.}$) of the $H_3$ was further shown in the spectra of methyl 3,6-diacetamido-3,6-dideoxy-$\alpha$-D-altropyranoside (VII),*1 methyl 3-acetamido-6-azido-3,6-dideoxy-$\alpha$-D-glucopyranoside (VIII), methyl 6-azido-6-deoxy-2-O-methanesulfonyl-$\alpha$-D-glucopyranoside (X) and methyl 2,6-di-O-methanesulfonyl-$\alpha$-D-glucopyranoside (XI) (Table II).

The narrow quartet at 5.54 p.p.m. was assigned to the $H_2$ signal, which gave the anticipated small values for $J_{1,2}$ and $J_{2,3}$. The $H_4$ signal was recognized at 5.73 p.p.m., well separated from the $H_5$ and $H_6$. The large values of $J_{3,4}$ and $J_{4,5}$ were consistent with the *trans*-axial arrangement of $H_3$, $H_4$ and $H_5$. The $H_5$ signal formed a six-line pattern, through coupling with $H_4$ and nearly equivalent $H_6$ pair. Thus, all of the data provided firm support for the structure (V) predicted.

**Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-$\alpha$-D-glucopyranoside (III) and Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-$\alpha$-D-mannopyranoside (VI)**

Independent verification of the structures (III) and (VI) was provided by the resonance data of the tetra-O,N-acetates in deuteriochloroform given in Table III. As

TABLE III. Chemical Shifts of Methyl and Amide Protons of Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-$\alpha$-D-glucopyranoside (III) and Methyl 3,6-Diacetamido-3,6-dideoxy-2,4-di-O-acetyl-$\alpha$-D-mannopyranoside (VI) in Deuteriochloroform

| | CONH[a] ($J_{NH,CH}$) | | $OCH_3$ Axial | $OCOCH_3$ Axial Second. | $OCOCH_3$ Equatorial Second. | $NCOCH_3$ Equatorial Second. | $NCOCH_3$ Equatorial Primar. |
|---|---|---|---|---|---|---|---|
| III | 4.03 t (5.0) | 4.33 d (8.0) | 6.60 | | 7.94, 7.94 | 8.11 | 8.01 |
| VI | 3.98 t (5.5) | 4.11 d (8.5) | 6.61 | 7.84 | 7.91 | 8.09 | 8.00 |

t: triplet d: doublet

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

the previous studies have demonstrated, the acetoxy and acetamido groups oriented axially resonated at lower field than the corresponding equatorial groups. Each of the spectra of Ⅲ and Ⅵ showed four signals in the acetyl region. The methyl signals at the highest field (8.11 p.p.m. in Ⅲ, 8.09 p.p.m. in Ⅵ) were ascribed to the equatorial acetamido groups at $C_3$, since the corresponding signals were found in the spectra of the sugars containing the equatorial acetamido groups*[1]. The signal observed in the lowest field in Ⅵ (7.84 p.p.m.) was found to be in the range of the axial acetoxy group. The signals at 7.94 p.p.m. in Ⅲ and at 7.91 p.p.m. in Ⅵ fell in the equatorial region. Then the remaining signals at 8.01 p.p.m. in Ⅲ and at 8.00 p.p.m. in Ⅵ could be attributable to the equatorial acetamidomethyl groups at $C_6$. The chemical shifts of the methoxyl groups on $C_1$ (6.60 p.p.m. in Ⅲ and 6.61 p.p.m. in Ⅵ) were close to those of the axially oriented ones*[1]. The above assignment supported the $\alpha$-glucose configuration for Ⅲ and the $\alpha$-mannose or $\alpha$-galactose configuration to Ⅵ. But, the analysis of the ring proton signals shown in Table Ⅰ supported the $\alpha$-mannose configuration.

The $H_1$ doublet in Ⅵ appeared at 5.39 p.p.m. with the small coupling to $H_2$ ($J_{1,2}$= 1.8 c.p.s.), characteristic of the $\alpha$-mannose derivatives. The $H_2$ was recognized at the lower field than the $H_1$, 5.10 p.p.m., as a narrow quartet. The remarkable down-field shift indicated the presence of an acetoxy group on $C_2$. The low values of $J_{1,2}$ and $J_{2,3}$ (2.8 c.p.s.) showed the equatorial orientation of $H_2$, and hence supported the $\alpha$-mannose configuration. The $H_3$ signal appeared at higher field (6.16 p.p.m.), indicating the substitution of an acetamido group, whose deshielding effect was known to be weaker than an acetoxy group[2]. The $H_3$ was complex, owing to coupling with the three different protons ($H_2$, $H_4$ and amide NH), and the spacing of the principal outer lines was about 20 c.p.s., approximately equal to $J_{2,3}+J_{3,4}+J_{NH,CH}$. Reflecting the substitution of an acetoxy group on $C_4$, the $H_4$ appeared at lower field (5.32 p.p.m.), and the large values of $J_{3,4}$ and $J_{4,5}$ (both 9.0 c.p.s.) established the *trans*-axial orientations of $H_3$, $H_4$ and $H_5$. The $H_6$ was found at relatively high field (ca. 6.55 p.p.m.) as a multiplet, suggesting the acetamido group on $C_6$. This was further confirmed by a broad triplet, which appeared at 3.98 p.p.m. partly overlapped with a broad doublet at 4.11 p.p.m. The former could be ascribed to the amide proton coupled with the methylene protons on $C_6$ ($J_{NH,H_6}$=ca. 5.5 c.p.s.), while the latter was assigned to the NH proton coupled with $H_3$ ($J_{NH,H_3}$=8.5 c.p.s.). A similar triplet and a doublet were also recognized in the spectrum of Ⅲ (Table Ⅲ).

It was well established that the equatorial ring proton attached to a pyranose ring resonated at lower field than the axial counterpart, owing to the increased anisotropic deshielding of a pyranose ring. In addition, the detailed studies in the steroid field indicated that the electronegative substituent at the *trans*-diaxial or *syn*-diaxial relation caused the down-field shift of the axial ring proton signal.[3] In accordance with this, the deshielding effect of the *syn*-diaxial bromine atom was demonstrated in the acetylated aldopyranosyl bromides.[4]

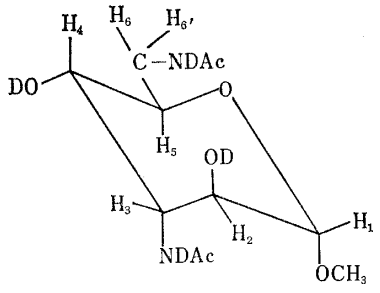
Table Ⅳ indicated the relative chemical shifts of the ring proton signals of some 3-acetamido-sugars with or without the replacement of the hydroxyl group on $C_6$. The parameters shown were the differences between the chemical shifts of Ⅴ (assumed arbitrarily zero) and those of the compound to be compared in deuterium oxide or in pyridine. The derivation of parameters among the sugars having the same configurations were found to be within 0.15 p.p.m. Therefore, the difference above

3) N. S. Bhacca, D. H. Williams : "Applications of NMR Spectroscopy in Organic Chemistry," 183 (1964). Holden-Day, Inc., San Francisco.
4) D. Horton, W. N. Turner : J. Org. Chem., **30**, 3387 (1965).

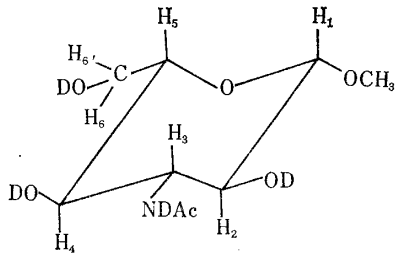
TABLE IV. Relative Chemical Shifts ($\Delta=\tau_{HV}-\tau_H$) in the Methyl 3-Acetamido-3-deoxy-hexopyranosides with Mannose, Glucose and Altrose Configurations

| Compound | Solvent | $\Delta(\tau_{HV}-\tau_H)$ | | | | |
|---|---|---|---|---|---|---|
| | | $H_1$ | $H_2$ | $H_3$ | $H_4$ | $H_5$ |
| V | $D_2O$ (Pyr.) | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| IV | $D_2O$ | −0.05 | −0.03 | −0.07 | −0.04 | |
| IX | 〃 | −0.02 | −0.01 | −0.02 | −0.10 | |
| XII | Pyr. | −0.08 | 0.04 | 0.11 | | |
| I | $D_2O$ | −0.12 | 0.26 | 0.03 | 0.22 | |
| II | 〃 | −0.09 | 0.26 | 0.03 | 0.24 | |
| VIII | Pyr. | −0.15 | 0.29 | 0.09 | 0.30 | |
| VII | $D_2O$ | 0.01 | 0.06 | −0.21 | −0.42 | |
| VII | Pyr. | −0.12 | −0.02 | −0.29 | −0.42 | −0.33 |
| VIII | $D_2O$ | 0.26 | 0.59 | 0.18 | | |

VII : Methyl 3,6-diacetamido-3,6-dideoxy-α-D-altropyranoside.
VIII : Methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-glucopyranoside.
IX : Methyl 3-acetamido-6-azido-3,6-dideoxy-α-D-mannopyranoside.
XII : Methyl 3-acetamido-3-deoxy-6-O-*p*-tolylsulfonyl-α-D-mannopyranoside.
XIII : Methyl 3-acetamido-3-deoxy-β-L-glucopyranoside.




VII




XIII

0.15 p.p.m. might be considered to be significant and attributable to the configurational effects of a polar substituent and a pyranose ring.

A comparison of the parameters in the α-glucose (I, II) and α-mannose (IV, V) isomers revealed the increased down-field shifts of the $H_2$ and $H_4$ in the latter, whereas the parameters of $H_1$ and $H_3$ were found to be very similar in both of the isomers. The marked deshielding of $H_2$ was probably due to the anisotropy of a pyranose ring, since $H_2$ in the mannoses was equatorial, in contrast to the axial orientation in the glucoses. The low value of the $H_4$ signal was accounted for by the deshielding effect of the *syn*-diaxial hydroxyl group at $C_2$. Similar differences of the $H_2$ and $H_4$ were consistently observed in pyridine solution. A comparison in the mannose (V) and altrose (VII) derivatives presented further examples of the down-field shift caused by an axial substituent. While the parameters of $H_1$ and $H_2$ were very similar in both the isomers, the altrose isomer showed low values for $H_3$, $H_4$ and $H_5$, both in deuterium oxide and in pyridine. The low value of $H_3$ was readily understandable since $H_3$ in VII was equatorial in the C1 conformation,*4 in contrast to the axial $H_3$ in V. More marked deshieldings of $H_4$ and $H_5$ in VII seemed to be associated with the axial acetamido group at $C_3$, since they were in the *trans*-diaxial and *syn*-diaxial relationship, respectively. Finally, the chemical shifts were compared between α-mannose (V) and β-glucose (XIII) derivatives. As expected, the axial $H_1$ and $H_2$ in XIII resonated at higher

*4 Although VII in deuterium oxide was shown to be present partly in 1C, it might be represented by C1 as the first-order approximation.

field than the equatorial counterparts in V. In addition, the $H_3$ signal of XIII showed higher value, since the *syn*-diaxial relation was not present in this case.

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## Masayuki Onda,*1 Masuko Akagawa,*2 Motoko Komamiya, and Fumi Nishiuchi*1 : Concerning the Stereoisomers of Cholest-4-en-3-one 2,4-Dinitrophenylhydrazone.

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Cholest-4-en-3-one 2,4-dinitrophenylhydrazone (2,4-DNPH), which was synthesized from cholest-4-en-3-one[1] under a mild condition to obtain the pure sample for identification, exhibited a wide range of melting point and two colored spots on carefully thin-layer chromatography (TLC). We isolated two compounds by means of preparative TLC; *e.g.* dark red crystal of m.p. 233~234° (decomp.) (D-I) from upper band and red crystal of m.p. 196~197° (decomp.) (D-II) from lower band were respectively obtained. Both compounds were formulated as $C_{33}H_{48}O_4N_4$ by analytical data.

D-I and D-II were converted to cholest-4-en-3-one by hydrolysis,[2] from which a mixture of D-I and D-II was again obtained with 2,4-dinitrophenylhydrazine. On boiling in alcohol or chloroform or benzene for several hours D-I and D-II did not change, but D-II changed to D-I in the presence of a catalytic amount of acetic acid. D-I corresponds to cholest-4-en-3-one 2,4-DNPH, which is known in the literatures,[3,4] from melting point and visible absorption spectra. A possibility that D-II might be cholest-5-en-3-one 2,4-DNPH is discarded from purity of starting material cholest-4-en-3-one[1,4] and visible absorption spectra (Table I). Accordingly, D-I and D-II must be the stereoisomers of cholest-4-en-3-one 2,4-DNPH.

Table I. Visible Absorption Spectra of D-I and D-II

| Substance | $\lambda_{max}^{DMF}$ mμ (ε) | $\lambda_{max}^{CHCl_3}$ mμ (ε) |
|---|---|---|
| D-I | 404(24,000) | 396(32,500) |
| D-II | 396(19,900) | 390(31,100) |
| Cholest-4-en-3-one-2,4-DNPH | 405(24,300)a) | |
| Cholest-5-en-3-one-2,4-DNPH | 380(20,000)a) | |

a) lit. 4).

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1) L. F. Fieser : J. Am. Chem. Soc., **75**, 5421 (1953).
2) C. H. DePuy, B. W. Ponder : *Ibid.*, **81**, 4629 (1959).
3) J. Willkison : J. Chem. Soc., **1942**, 391.
4) T. Nishina, M. Kimura : Yakugaku Zasshi, **84**, 390 (1964).