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Nuclear Magnetic Resonance Spectra of α-D-Glucans in the Presence of 1,1,3,3-Tetramethylurea

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1,1,3,3-Tetramethylurea (Me_4U) promotes the alkylation of α-D-glucans and prevents caramelization and side reactions in acetolysis and acid hydrolysis of α-D-glucans even at high reaction temperatures. This might be caused by strong intermolecular hydrogen bondings between hydroxyls of carbohydrates and the carbonyl group of Me_4U, which prevail over intramolecular hydrogen bondings between the alcoholic hydroxyls. The hydrogen bondings as such induce deformation of the rigid higher structure of polyglucans to promote the alkylation, while they protect hydroxyls from side reactions during the course of modified acetolysis and acid hydrolysis even at high temperature to produce oligosaccharide fragments in good yields.

In the present study the interaction between Me_4U and hydroxyls of glucans has been demonstrated by means of proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectroscopy.

The ^{1}H- and ^{13}C-NMR spectra of carbohydrates remain unchanged even after heating for 30 min at 160 °C in the presence of d_{12}-Me_4U in d_6-dimethyl sulfoxide, whereas without d_{12}-Me_4U in the medium, heating the solution causes remarkable changes.

Keywords——1,1,3,3-tetramethylurea; α-D-glucan; ^{1}H-NMR spectrum; ^{13}C-NMR spectrum; alkylation; acetolysis; acid hydrolysis

As reported previously, we found that addition of 1,1,3,3-tetramethylurea(Me_4U) to the reaction medium remarkably promoted the permethylation[2)] and carboxymethylation[3)] of polysaccharides. In connection with these findings, we utilized Me_4U in the acetolysis[4)] and partial acid hydrolysis[5)] of α-glucans to prevent side reactions even at the high reaction temperature, and to increase the production of oligosaccharide fragments.

The effect of urea on proteins (inducing their denaturation) has well been recognized, but the interaction of urea or its derivatives with carbohydrates has not been so extensively studied.

Although Hirano[6–8)] reported the modification of higher structure of complex carbohydrates by strong hydrogen bonding with urea, stereochemical conversion of neutral polysaccharides in the presence of urea or its derivatives has never been elucidated. On the other hand, it is well-known that stereochemical conversions of carbohydrate compounds in solution are induced readily by heating. On heating, the proton nuclear magnetic resonance (^{1}H-NMR) singnals of the hydroxyls of mono- and oligosaccharides in d_6-dimethyl sulfoxide (DMSO) are shifted upfield, indicating the release of inter- and intramolecular hydrogen bonding.[9,10)] Heating of sugar solution also induces dehydration, etherification and other side reactions to result ultimately in caramelization of the solution. Therefore, carbohydrate compounds are usually subjected to chemical reactions at low temperature to avoid such side reactions.

As the presence of Me_4U prevented the caramelization of sugar solution even at higher temperature, Me_4U might interact strongly with the hydroxyls of carbohydrates, protecting

them. In the present paper the interaction between Me_4U and glucans has been demonstrated by means of ^1H- and ^{13}C-NMR spectroscopy.

Experimental

^1H-NMR (400 MHz) and ^{13}C-NMR (100 MHz) spectra were measured at 20 °C using a JEOL GX400 instrument, with digital resolution of 0.000775 ppm (0.31 Hz) and 0.00756 ppm (0.76 Hz), respectively.

Samples——1) Maltose, β-cyclodextrin, amylose A (mol. wt. 2900) and dextran A (mol. wt. 177000) (100 mg each) were each dissolved in d_6-DMSO (1 ml) and placed in NMR tubes (5 mm i.d.).

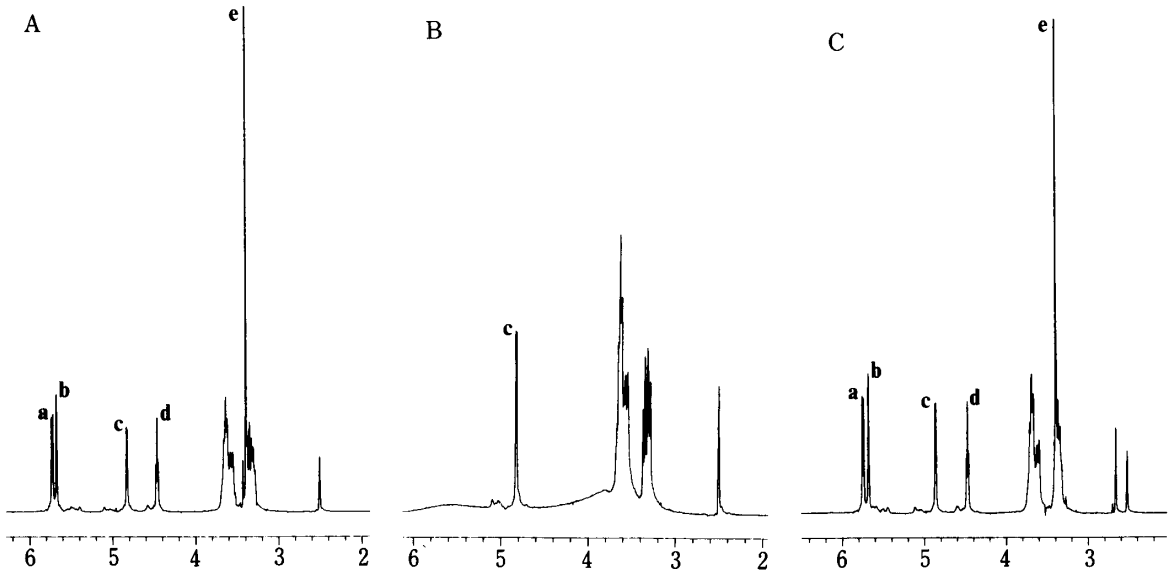

Fig. 1. ^1H-NMR Spectral Profile of the Hydroxyl Region of β-Cyclodextrin in d_6-DMSO

(A) Measured at room temperature. (B) Measured after heating for 30 min at 160 °C. (C) Measured after heating for 30 min at 160 °C in the presence of Me_4U.
a, $C_{(2)}$-OH; b, $C_{(3)}$-OH; c, $C_{(1)}$-H; d, $C_{(6)}$-OH; e, DOH.

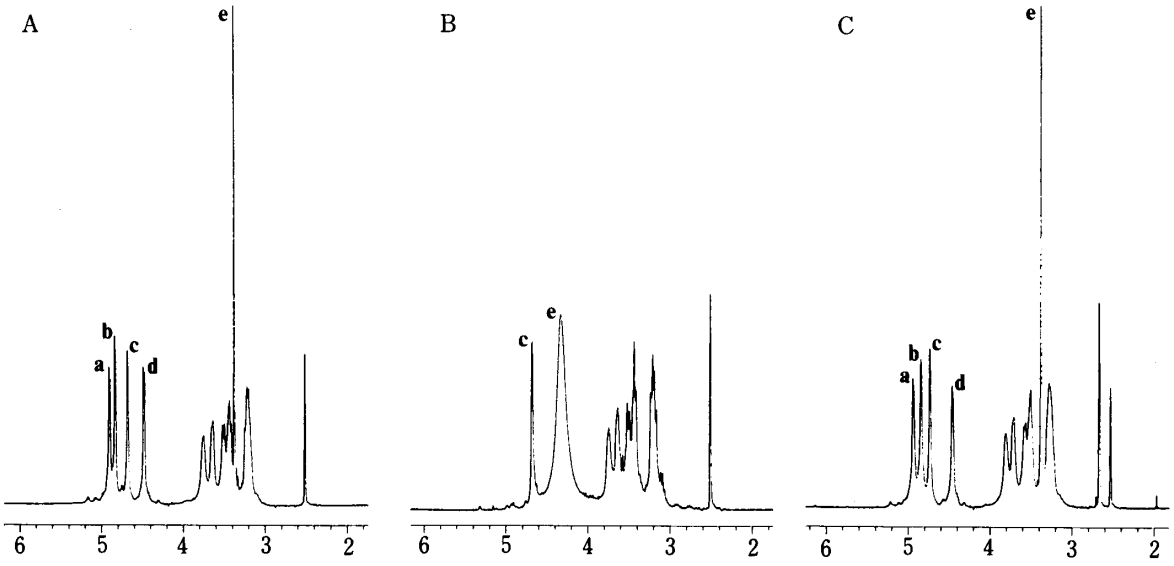

Fig. 2. ^1H-NMR Spectral Profile of the Hydroxyl Region of Dextran in d_6-DMSO

(A) Measured at room temperature. (B) Measured after heating for 30 min at 160 °C. (C) Measured after heating for 30 min at 160 °C in the presence of Me_4U.
a, $C_{(2)}$-OH; b, $C_{(3)}$-OH; c, $C_{(1)}$-H; d, $C_{(4)}$-OH; e, DOH.

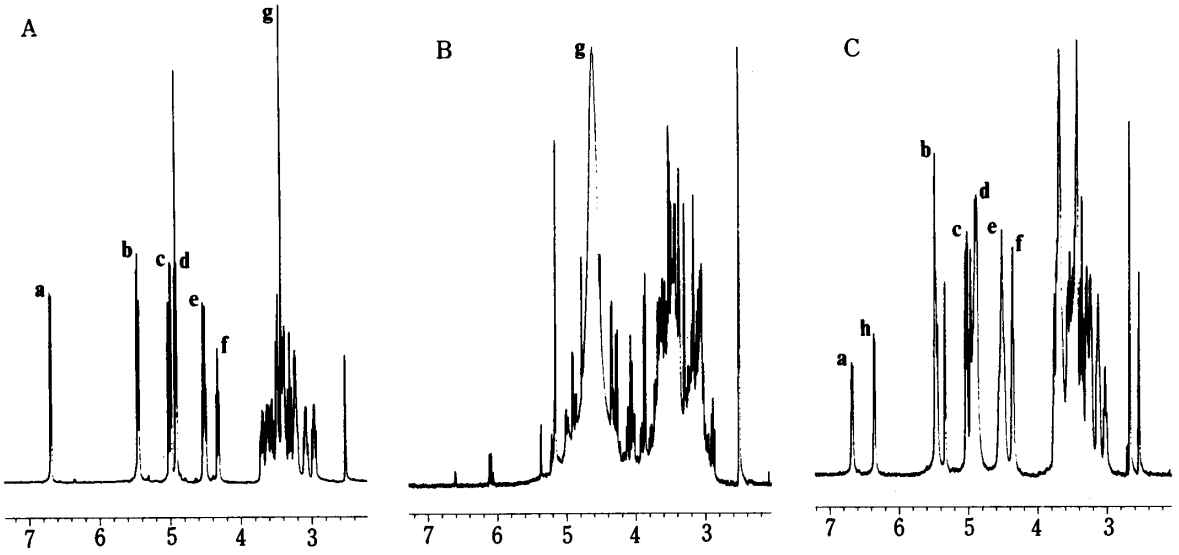

Fig. 3. ^{1}H-NMR Spectral Profile of the Hydroxyl Region of Maltose in d_6-DMSO

(A) Measured at room temperature. (B) Measured after heating for 30 min at 160 °C. (C) Measured after heating for 30 min at 160 °C in the presence of Me_4U.
a, $C_{(1)}$-OH (β); b, $C_{(2,3)}$-OH (internal); c, $C_{(1)}$-H (α); d, $C_{(2,3,4)}$-OH (external); e, $C_{(6)}$-OH; f, $C_{(1)}$-H (β); g, DOH; h, $C_{(1)}$-OH (α).

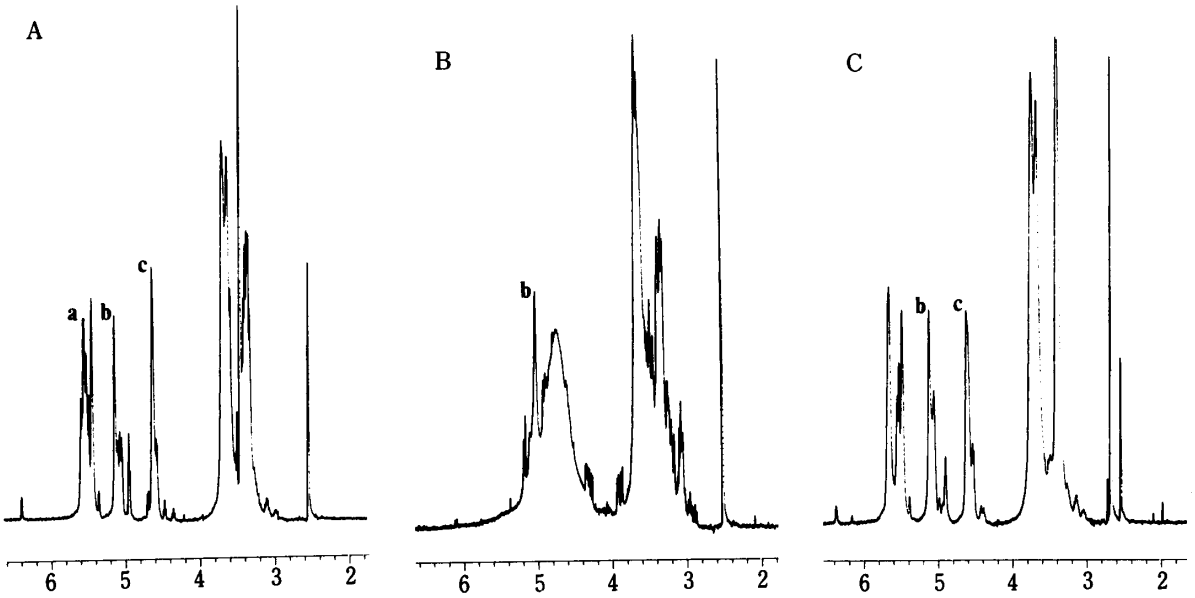

Fig. 4. ^{1}H-NMR Spectral Profile of the Hydroxyl Region of Amylose in d_6-DMSO

(A) Measured at room temperature. (B) Measured after heating for 30 min at 160 °C. (C) Measured after heating for 30 min at 160 °C in the presence of Me_4U.
a, $C_{(2,3)}$-OH; b, $C_{(1)}$-H; c, $C_{(6)}$-OH.

2) The same samples (100 mg each) were dissolved in a mixture of d_6-DMSO (0.7 ml) and d_{12}-Me_4U (0.3 ml) and used for ^{1}H- and ^{13}C-NMR spectral measurements.

3) The above eight kinds of samples were heated, separately, in an oil bath for 30 min at 160 °C. After cooling, their ^{1}H- and ^{13}C-NMR spectra were measured.

Results and Discussion

The ^{1}H-NMR spectra of oligo- and polysaccharides measured in d_6-DMSO at room temperature gave well-defined proton signals (Figs. 1A, 2A, 3A and 4A). In these cases, at

room temperature the addition of d_{12}-Me_4U caused no remarkable change in the spectral profiles. On heating for 30 min at 160 °C, without d_{12}-Me_4U, all the signals of hydroxyl protons disappeared, while a large signal of H_2O appeared (Figs. 1B, 2B, 3B and 4B). However, in the presence of d_{12}-Me_4U, all the proton signals of sugar hydroxyls remained unchanged on heating as above (Figs. 1C, 2C, 3C and 4C). On the other hand, the ^{13}C-NMR spectra of several kinds of oligo- and polysaccharides showed some differences between those measured in d_6-DMSO at room temperature (Figs. 5A and 6A) and those after heating for

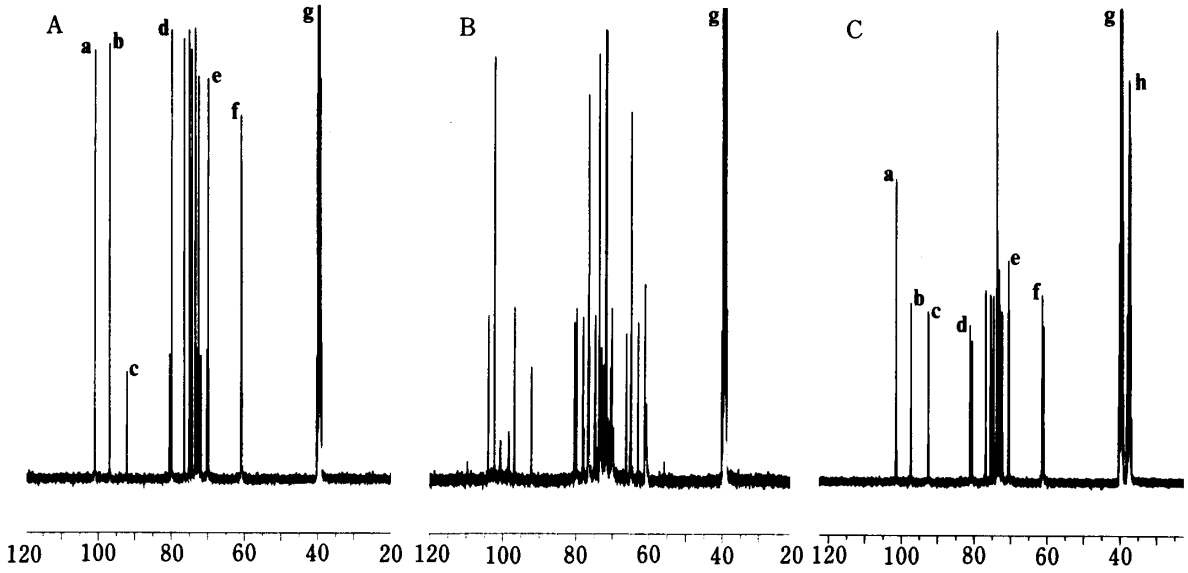

Fig. 5. ^{13}C-NMR Spectral Profile of the Skeletal Carbon Region of Maltose in d_6-DMSO

(A) Measured at room temperature. (B) Measured after heating for 30 min at 160 °C. (C) Measured after heating for 30 min at 160 °C in the presence of Me_4U.
a, $C_{(1)}$ (linked); b, $C_{(1)}$ (β); c, $C_{(1)}$ (α); d, $C_{(4)}$ (linked); e, $C_{(4)}$ (external); f, $C_{(6)}$; g, CD_3 (d_6-DMSO); h, CD_3 (d_{12}-Me_4U).

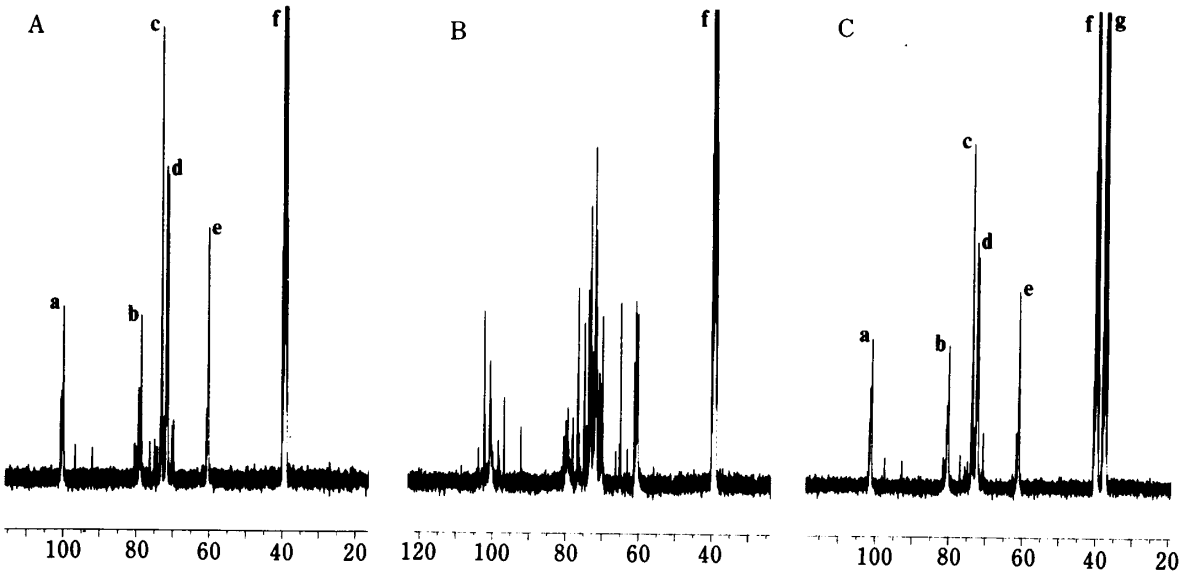

Fig. 6. ^{13}C-NMR Spectral Profile of the Skeletal Carbon Region of Amylose in d_6-DMSO

(A) Measured at room temperature. (B) Measured after heating for 30 min at 160 °C. (C) Measured after heating for 30 min at 160 °C in the presence of Me_4U.
a, $C_{(1)}$; b, $C_{(4)}$; c, $C_{(3)}$; d, $C_{(2),(5)}$; e, $C_{(6)}$; f, CD_3 (d_6-DMSO); g, CD_3 (d_{12}-Me_4U).

30 min at 160 °C. Some small additional unidentified signals were observed in the latter cases (Figs. 5B and 6B). In the presence of Me_4U, the ^{13}C-NMR spectral profile showed no change even after heating under the same conditions as above (Figs. 5C and 6C).

Me_4U, as a strong hydrogen bond-acceptor, would dissociate the intramolecular hydrogen bonds of sugar hydroxyls to relax the rigid higher structure, pulling out the hydroxyls and making them reactive. Moreover, the solvent effect of Me_4U dissolving alkali metal ion would also participate to promote alkylation.[11,12] The iodine color reaction in aqueous solution of amylose is strongly, inhibited by the addition of Me_4U at room temperature. This also indicates the relaxation of α-helix structure of amylose[13] in the presence of Me_4U. Thus the Hakomori permethylation and carboxymethylation of polysaccharides are remarkably promoted by the addition of Me_4U.

By the interaction with Me_4U, the hydroxyls of polysaccharides are protected even at higher reaction temperatures against side reactions, and the desired oligosaccharide fragments are formed in acetolysis and partial acid hydrolysis without accompanying caramelization. Further, the addition of Me_4U remarkably shortens the reaction time required to complete acetolysis, as it can be performed at a higher temperature.

References and Notes

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