^{13}C-NMR SPECTROMETRIC IDENTIFICATION OF ACID HYDROLYSED HYDROXYPROPYL STARCH COMPONENTS

By :

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Abstract

The products of acidic hydrolysis of hydroxypropyl starch (molar substitution 0.28) were isolated by HPLC using an amine treated μ Poracil coloumn. Based on the ^{13}C-NMR spectral data the individual fractions were assigned 1,2-0-[1,2-(S)-propylene]-α-D-glucofuranose, 1,2-O-[1,2-(R) and (S)-propylene]-α-D-glucopyranose, 2-O-[2-hydroxypyl-(R) and (S)]-α,B-D-glucopyranose, and 6-0-[2-(R) and (S)-hydroxypropyl]-α,B-D-glucopyranose, respectively.

Introduction

Starch is widely used in the paper, textile and food industries. Increasing knowledge of the structure of starch and its effect on physical properties has enabled chemists to modify native starch to meet special needs of food and other industries. Hydroxypropylation is one important type of such modification.

The increased use of hydroxypropyl starches in food industry makes it necessary to have quick, sensitive and relatively simple analytical techniques for the quantification of hydroxypropyl groups. The spectrophotometric method (Johnson, 1969) and the NMR method (Stahl and McNaught, 1970) are the most commonly used techniques currently available. Yet, they do not furnish any information on substitution pattern, data of potential interest and importance because of its probable effects on starch properties.

HPLC allows for the separation of individual components of hydroxypropyl starch hydrolysates (Wootton *et al.*, 1985). Thus more detailed study of their structure can be carried out. The use of ^{13}C-NMR spectroscopy in the structure elucidation of hydroxypropyl cellulose hydrolysates has been previously reported (Lee *et al.*, 1982). This technique applied to compounds isolated by HPLC should allow for the structural elucidation of individual starch ether hydrolysate components.

Experimental

Preparation of Hydrolisate

Hydroxypropyl starch (50 g, molar substitution 0.28) was transferred into 1000 mL flask with 500 mL of 0.75 M H_2SO_4. The mixture was heated on a boiling water bath for 4 hr. After cooling the mixture was neutralised using $Ba(OH)_2$, and filtered. Glucose was removed by fermentation with baker's yeast as described by Leegwater *et al.* (1973).

After seven days the mixture was neutralised by adding powdered $Ba(OH)_2$, and was then filtered. The filtrate was evaporated at 50°C, leaving behind approximately 50 mL of syrup. The syrup

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was filtered through an ultrafiltration membrane.

Fractionation Using HPLC

The instrument used was manufactured by Waters Associates and comprised a liquid chromatography equipped with a refractive index detector. The coloumn used was a stainless steel semi-preparative μ Poracil coloumn (7.5 mm x 30 cm). Solvent used was acetonitrile/water 85 : 15, added with SAM I 0.1%.

Sample injection volumes of 100 μ l were employed and each peak was collected. The isolated fractions were concentrated by vacuum evaporation, treated with a small amount of Bio-Rad Dowex 50W-X8 (H^+) resin, ultrafiltered and then freeze dried. The individual fractions were purified by repeated chromatography using the same method for fractionation. Each purified fraction was then concentrated, and freezed dried.

NMR Analisis

A Bruker CXP-300 spectrometer was used to record ^{13}-NMR spectra of the individual fractions of acid hydrolysed hydroxypropyl starch. The following conditions were applied.

Solvent	: D_2O
Diameter of sample tube	: 10 mm
Concentration of sample	: 100 — 200 mg/ml
Internal standard	: Acetone (30.5 ppm)
Frequnecy	: 75 MHz
Pulse width	: 5 μ s (30°)
Total acquisition time	: 1 hr
Temperature of sample	: 27°C

The ^{13}C-chemical shift values were obtained from the computer print-out.

Results and Discussion

A number of general rules relating to the assignment of signals in ^{13}C-NMR spectra of monosaccharides has been summarised by Perlin *et al.* (1970) as follows. (a) The anomeric carbon atoms in pyranoses and furanoses, and in their derivatives, resonate at lowest field (90 — 110 ppm); (b) Carbon atoms carrying primary hydroxyl groups are found at 60 — 64 ppm; (c) Carbon atom bearing secondary hydroxyl groups, in pyranoses and furanoses, give signals at 65 — 85 ppm. Signals of hydroxyl substituted carbon atoms are shifted to lower field when compared with the corresponding unsubstituted hydroxyl carbon atoms.

The isolated fractions, designed A, B, C, E dan F in deuterium oxide (D_2O) solution were analysed by ^{13}C-NMR spectrometry. Acetone (^{13}C-chemical shift 30.5 ppm) was used as an internal reference compound to enable assignments of chemical shifts of the samples.

In this experiment structural assignment of the fractions of starch hydrolysate relied on comparison with ^{13}C-NMR spectra of model compounds described by Lee and Perlin (1982). They used deuterated methanol (CH_3OD) as a reference compound and assumed a chemical shift of at 50.4 ppm. However the chemical shift of methanol was reported 49.6 ppm by Bock and Pederson, 1983. Hence it was necessary to subtract 0.8 ppm from the chemical shifts of model compounds as reported by Lee and Perlin (1982).

The ^{13}C-NMR spectra of fractions A, B, C, E and F showed the carbon signals at 15.5 — 18.4 ppm (the methyl carbon of hydroxypropyl substituent) and the signals from 60.6 — 100.5 ppm (the six

carbon atoms of glucose, together with the methylene and methyne carbons of hydroxypropyl substituent), indicating that the compounds were hydroxypropyl derivatives of glucoses.

Fractions A, B and C

The ¹³C-NMR spectra of components A and B were as shown in Figure 1 and Figure 2. The spectrum of B indicated the presence of minor fraction designated B′.

It has been established that five-membered rings have ¹³C-chemical shifts downfield from those of the configurationally related six-membered rings (Perlin *et al.*, 1970). Since the ¹³C-chemical shifts of A appeared at lower field than those of B, it was certain that A was a glucofuranose and B was a mixture of glucopyranose derivatives.

The anomeric carbon atom resonating at very low field (99.7 ppm) is a typical of 1,2-0-[2,1-(S)-propylene]-α-D-glucofuranose (1) (Lee and Perlin, 1982). The spectrum of A showed the presence of similar signal at 99.2 ppm.

The assignment of ¹³C-chemical shift in A, B and B′ was based on the close agreement with those of the related glucose derivatives (Table 1) after subtracting by a factor of 0.8 ppm. The ¹³C-chemical shifts for A, B and B′ agreed within 0.5 ppm with those for the related compounds (Table 1), and thus A was indetified as 1,2-0-[2,1-(S)-propylene]-α-D-glucofuranose (1), B was 1,2-O-[2,1-(R)-propylene]-α-D-glu-copyranose (2), and B′ was 1,2-O-[2,1-(S)-propylene]-α-D-glucopyranose.

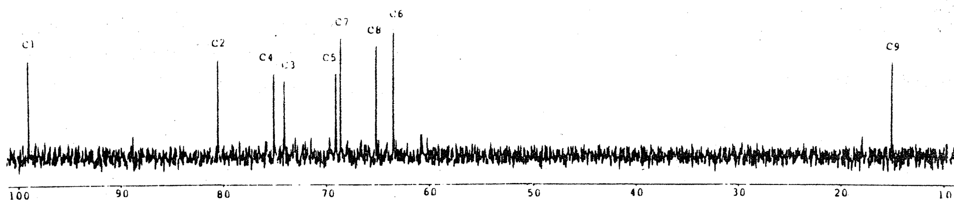


Figure 1. **¹³C-NMR spectrum of component A in D20 at 27°C, acquired at 75 MHz.**

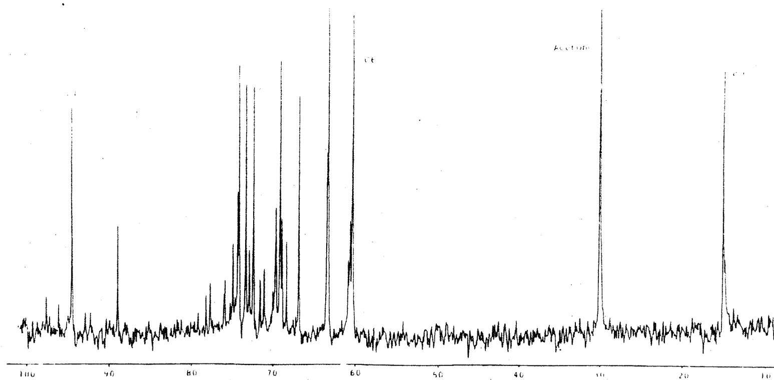


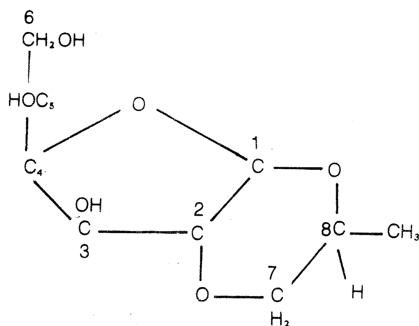
Figure 2. **¹³C-NMR spectrum of component B in D20 at 27°C, acquired at 75 MHz. Signal of internal standard (acetone) is at 30.5 ppm.**

A was identified by ^{1}H-NMR analysis as 1,2-O-[2,1-(R)-propylene]-α-D-glucofuranose mixed with assumed (S) diastereoisomer (Haryadi, 1987), whereas the ^{13}C-NMR assignment gave (S) form. Perhaps the procedure of acetylation caused this difference. The ^{13}C-NMR identification of B and B′ coincided with those by ^{1}H-NMR method.

Table 1. **^{13}C-chemical shifts of the (S) furanose (1), (R) and (S) pyranose (2 and 3) forms of 1,2-O-propylene-D-glucose[a] and fraction A and B of hydroxypropyl starch hydrolysate[b]**

	1	2	3	A	B	B′
Glucose moiety						
C1	100.5	95.5	90.2	99.2	94.6	89.1
C2	81.9	75.2	76.0	80.8	74.5	75.1
C3	75.5	73.6	71.2	74.4	72.6	69.9
C4	76.6	70.5	70.3	75.4	69.3	69.1
C5	70.4	74.4	75.5	69.3	73.5	74.5
C6	64.9	61.7	62.0	63.8	60.6	61.0
Propylene moiety						
C7	70.1	64.6	69.4	69.3	63.6	68.6
C8	66.7	68.2	65.4	65.5	67.1	63.7
C9	16.7	16.6	16.7	15.5	15.5	15.5

a Lee and Perlin (1984b); internal standard CH_3OD at 50.4 ppm.
b Internal standar acetone at 30.5 ppm.



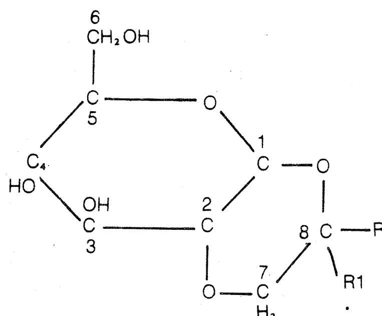
(1)

The ^{13}C-NMR spectrum of C was complicated. However, it was assumed that the signals in the region 61.0 — 96.1 ppm were attributable to the resonances of carbon atoms of glucose moiety together with the methylene and methyne carbon atoms of the propylene moiety. The signal at 18.3 ppm must arise from the methyl group. Therefore, C was likely a mixture of hydroxypropyl glucose derivatives.

The ^{1}H-NMR spectrum of acetylated fraction C was also complicated. However C was identified chemically and chromatographically by comparison with reference compounds as 1,2-0-propylene-D-glucose (Haryadi, 1987).

Fractions F and E

The ^{13}C-NMR spectrum of fraction F showed abundant signals in the region 70.0 — 70.6 ppm (Figure 3). Predominating chemical signals at 70.6 ppm (after correction by a factor of 0.8 ppm), due to C4 and C6 resonances are typical of 6-O-hydroxypropyl-D-glucose. The ^{13}C-chemical shifts of F (Table 2) agreed within 0.4 ppm to those of the related model compound assigned by Lee and



(2) R = H, R1 = CH_3
(3) R = CH_3 R1 = H

Perlin (1982). Hence, F was identified as a mixture of (R) and (S) diastereoisomers of 6-O-(2-hydroxypropyl)-D-glucose anomers (4a and 4b). F was also identified by ^{1}H-NMR analysis as a diastereoisomeric mixture of 4a and 4b (Haryadi, 1987).

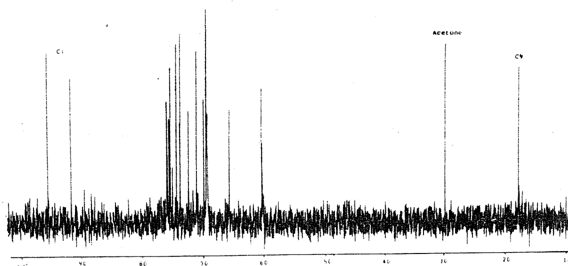


Figure 3. **^{13}C-NMR spectrum of component F in D2O at 27°C, acquired at 75 MHz. Signal of internal standard (acetone) is at 30.5 ppm.**

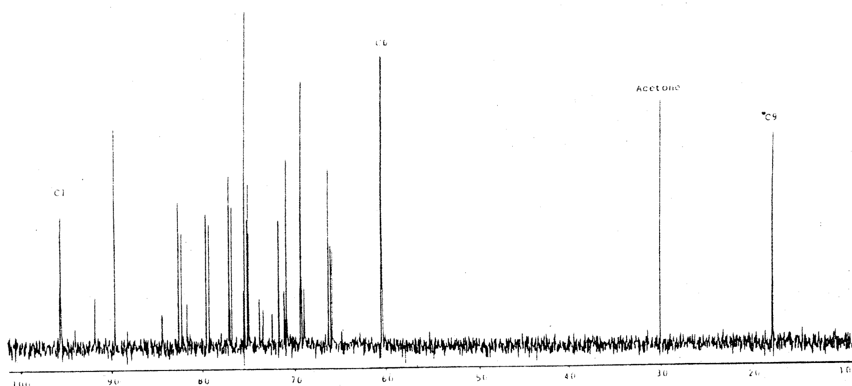
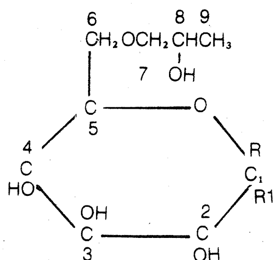
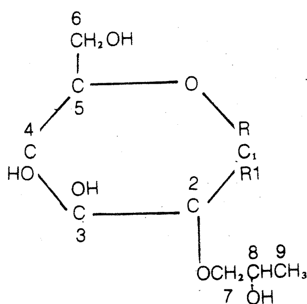


Figure 4. **^{13}C-NMR spectrum of component E in D2O at 27°C, acquired at 75 MHz. Signal of internal standard (acetone) is at 30.5 ppm.**



(4a) R = H
R1 = OH

(4b) R = OH
R1 = H



(5a) R = H
R1 = OH

(5b) R = OH
R1 = H

The presence of signals in the regions 80.4 — 80.7 ppm and 83.4 — 83.7 ppm in the ^{13}C-NMR spectra due to C2 is typical of 2-O-hydroxypropyl-D-glucose (Lee and Perlin, 1982; after correction the figures by a factor of 0.8 ppm). The spectrum of E (Figure 4) showed the carbon signals in the regions 80.0 — 80.4 ppm and 83.0 — 83.4 ppm.

Whereas the assigned ^{13}C-chemical shifts of E (Table 3) agreed within 0.5 ppm to those corrected figures of related model compound as reported by Lee and Perlin (1982). Therefore E was identified as an anomeric mixture of (R) and (S) diastereoisomers of 2-O-hydroxypropyl-D-glucose (5a and 5b). This ^{13}C-NMR identification of fraction E was consistent with that using ^{1}H-NMR spectrometry (Haryadi, 1987).

Table 2. **^{13}C-NMR chemical shifts of 6-O-hydroxypropyl-D-glucose (4a and 4b)[a] and fraction F[b] of hydroxypropyl starch hydrolysate**

	4a	4b	Assigned F	
			a	b
Glucose moiety				
C1	93.6	97.4	92.3	96.2
C2	72.9	75.6	71.7	74.5
C3	74.2	77.2	73.0	76.2
C4	71.4	71.4	70.1	70.2
C5	71.8	76.2	70.6	76.1
C6	71.4	71.4	70.0	70.1
Propylene moiety				
C7	77.8	77.8	76.5	76.6
C8	67.6 67.5	67.6 67.5[c]	66.4 66.3	66.4 66.3
C9	19.6	19.6	18.4	18.4

a Lee and Perlin (1982); internal standard CH_3OD at 50.4 ppm

b Internal standard acetone at 30.5 ppm.

c This pair (and each of the other pairs) of signals represents a pair of diastereoisomers)

^{13}C-NMR identification of the fractions of acid hydrolysed hydroxypropyl starch is simpler than that of ^{1}H-NMR in view of the following reasons. (a) The number of carbon atoms in hydroxypropyl derivatives of glucose is less than that of hydrogen atoms. (b) The hydrolysate fractions can be examined readily by ^{13}C-NMR. Whereas acetylation is necessary for ^{1}H-NMR spectral simplication. (c) The ^{13}C-NMR spectra are well resolved at much lower field (75 MHz) than those of ^{1}H-NMR (300 MHz).

Table 3. **^{13}C-chemical shifts of 2-O-hydroxypropyl-D-glucose (5A and 5b)[a] and fraction E[b] of hydroxypropyl starch hydrolysate**

	5a	5b	Assigned E	
			a	b
R Glucose moiety				
C1	91.4	97.3 97.2[c]	90.3	96.1 96.1
C2	81.5 81.2	84.5 84.2	80.4 80.1	83.4 83.1
C3	73.6	76.8	72.4	75.7
C4	71.0	71.0	70.0	70.0
C5	72.7	77.3	71.6	76.1
C6	62.1	62.2	61.0	61.1
Propylene moiety				
C7	79.1 78.8	77.0 76.8	77.8 77.5	76.1 75.8
C8	68.3 67.8	68.3 68.2	67.9 66.8	67.0 66.6
C9	19.4	19.4	18.2	18.3

a Lee and Perlin (1982); internal standard CH_3OD at 50.4 ppm

b Internal standard acetone at 30.5 ppm

c This pair (and each of the other pairs) of signals represents a pair of diastereoisomers.

Both NMR methods are able to detect the anomeric mixtures of 6-O- and 2-O-hydroxypropyl-D-glucoses. However,

borohydride reduction involving anomeric carbon atom of the reducing sugars (Lee and Perlin, 1982), *i.e.* F and E in this case, prior to NMR examination may reduce the complexity existing in the spectra of the anomeric mixtures of hydroxypropyl glucoses.

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Bibliography

Bock, K. & Pederson, C. Carbon-13 nuclear magnetic resonance spectroscospy of monosaccharides. Adv. in Carbohydr. Chem. and Biochem. 41: 27—66; 1983.

Haryadi. Identification of Hydroxypropyl Starch Hydrolysate components. Ph.D. Thesis, The Uni. of New South Wales, Sydney; 1987.

Johnson, D.P. Spectrophotometric determination of hydroxypropyl group in starch ethers. Anal. Chem. 41(6) : 859-60; 1969.

Lee, D.S. and Perlin, A.S. ^{13}C-NMR-Spectral and related studies on the distribution of substituents in O-(2-hydroxypropyl) cellulose. Carbohydr. Res. 106: 1; 1982.

Lee, D.S. and Pelrin, A.S. Acid catalyzed conversion of 2-O-(2-hydroxypropyl)-glucose derivatives into. 1,2-0-(1-methyl-1,2-ethanediyl)-D-glucose acetals. Studies related to O-(2-hydroxypropyl) cellulose. Carbohydr. Res. 125: 265; 1984a.

Lee, D.S. and Perlin, A.S. Formation, and stereochemistry, of 1,2-O-(1-methyl-1,2-ethanediyl)-D-glucose acetals formed in the acid catalyzed of O-(2-hydroxypropyl) cellulose. Carbohydr. Res. 126: 101; 1984b.

Leegwater, D.C., Marsman, J.W. & Mackor, A. Analysis of an acid hydrolysate of low substituted hydroxypropyl stach. Starch 25(4): 142-5; 1973.

Perlin, A.S., Casu, B. & Koch, H.J. Configurational and conformational influence on the carbon-13 chemical shifts of some carbohydrates. Canad. J. Chem. 48: 2596-606; 1970.

Stahl, H. & McNaught, R.P. A rapid nuclear magnetic resonance method for determining hydroxypropyl group in modified starch. Cereal Chem. 47: 345-50; 1970.