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The Structures of Diethylaminoethylated Glucose and Oligosaccharides Derived from Cationic Starch†

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The cationic starch prepared by alkylamination of starch is produced in large quantity for the paper making industry. However, it is not well characterized which of the OH groups of the glucose residue is substituted by the functional group. Therefore, we isolated diethylaminoethylated glucose (DEAE-glucose) from the hydrolyzate of cationic starch and examined its structure by means of NMR spectrometry.

To a suspension of corn starch (100 g) in 160 ml of water was added 3.0 g of diethylaminoethylchloride at pH 10.5, and the mixture was kept for 16 hours at 40°C. After filtration of slurry at pH 5.0, the filtered cake was washed with water and dried in hot air. The degree of substitution was determined as 0.025 by the Kjeldahl method.

The cationic starch was gelatinized with 1 N HCl for 30 min at 100°C and further hydrolyzed completely for 72 hours at 70°C. After being adjusted to pH 7.0, the insoluble material was removed by centrifugation and the supernatant applied to a column of Dowex 50W-X2 (H^+ form). The column was washed with water, then eluted with 0.5 M NH_4OH and the eluate was evaporated *in vacuo* to yield a crude fraction.

The silics gel TLC ($CHCl_3$–MeOH–conc. NH_3 2 : 3 : 1) of the crude fraction gave two components, a main spot at *Rf* 0.77 and a minor one at *Rf* 0.66. In agreement with this, the ^{13}C-NMR spectrum of the crude fraction (in D_2O) showed 16 carbon signals as well as some very weak signals. Of the former signals, 12 were assignable to a pair of α,β-anomers of the glucose residue. These findings suggest that substitution occurred mainly at the single OH group of glucose together with a slight side reaction at other OH groups. The crude fraction was further purified by Dowex 50W-X2 column chromatography using a 0.5 M pyridine-AcOH buffer, pH 6.4. The eluate was monitored by TLC and appropriate fractions were combined to give purified DEAE-glucose (**1**, *Rf* 0.77).

In order to confirm the substitution pattern, **1** was peracetylated with acetic anhydride/pyridine to afford tetraacetyl DEAE-glucose, **2**, (EI mass M^+ 447.2029, $C_{20}H_{33}NO_{10}$ Calcd. 447.2102). In the 400 MHz ^1H-NMR spectrum of **2** in $CDCl_3$, the signals due to H-1β, 3β and 4β were observed at δ 5.59, 5.17 and 5.02 ppm, respectively. On the other hand, the H-2β proton signal appeared at δ 3.47 ppm which resonated at upper field than H-1β, 3β and 4β, clearly indicating that derivation took place at C2-OH. This established the structures of **1** and **2** as shown below.

The assignment of the carbon signals for **1** could be made by calculation of the

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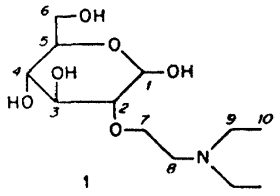

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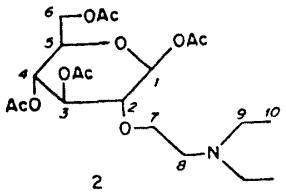

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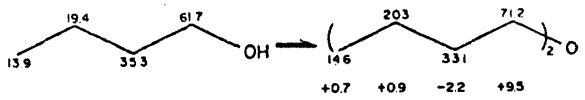
Table I. ^{13}C-NMR Data of **1** and **2**

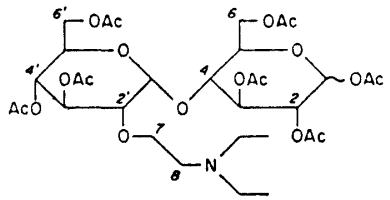
	1	2	3	4	5	6	7	8	9	10
α-Glucose	95.10	75.75	74.35	72.05	74.35	63.55				
	−2.2	+9.5	−2.2	+0.9	+0.7					
1-α Calcd.	92.90	85.25	72.15	72.95	75.05	63.55				
Obsd.	92.16	81.97	73.75	72.16	74.58	63.03	65.94	54.04	49.87	10.52
β-Glucose	98.95	77.10	78.70	72.05	78.85	63.70				
	−2.2	+9.5	−2.2	+0.9	+0.7					
1-β Calcd.	96.75	86.60	76.50	72.95	79.55	63.70				
Obsd.	98.13	85.10	77.67	72.16	78.39	63.23	68.06	54.04	49.87	10.52
2-β	93.43	78.76	74.01	68.20	72.36	61.51	70.66	51.79	46.90	10.62
-CO-	170.43	169.82	169.56	168.73						
-CH_3	20.86	20.65	20.57	20.49						



substituent effects on an aliphatic alcohol by bulky ether (see Table I).[1] In this case, C-2, located at an α-position of the ether bond, was shifted downfield by 9.5 ppm and both C-1 and C-3, at β-positions, were shifted upfield by 2.2 ppm from the parent sugar. All the carbon signals assigned to the glucose residue were well in accord with those of the calculated chemical shift. The ^{13}C-NMR data of **2** (in $CDCl_3$), assigned by a selective proton decoupling experiment, are also listed in Table I. The minor signals of crude DEAE-glucose were assigned as 3-*O*-DEAE and 6-*O*-DEAE derivatives in the same manner (data not shown). The minor spots on TLC (*Rf* 0.66) might be attributed to these two derivatives.

Mild acid hydrolysis (0.5 N HCl, 70°C, 72 hours) of the cationic starch after gelatinization gave a DEAE-oligosaccharide mixture, together with DEAE-glucose, and these were separated by Dowex 50W-X2 column chromatography. The DEAE-maltose (TLC *Rf* 0.54) was peracetylated to give heptaacetyl DEAE-maltose, **3**, (EI mass M^+ 735, $C_{32}H_{49}NO_{18}$, Calcd. C 52.24, H 6.67, N 1.09%, Obsd. C 52.32, H 6.71, N 1.93%). In the ^{1}H-NMR spectrum ($CDCl_3$), the signal resonating at 3.48 ppm was assigned to H-2′ by its splitting pattern and coupling constants (dd, 10 Hz, 4 Hz). This fact and the result of spin decoupling experiments afforded the structure for **3** as shown below.

In order to obtain some information about the action mechanism of amylases on



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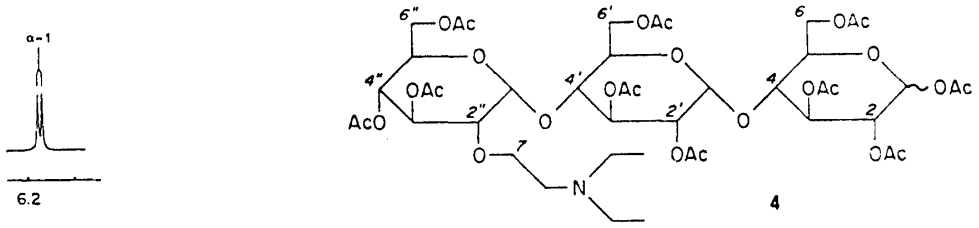

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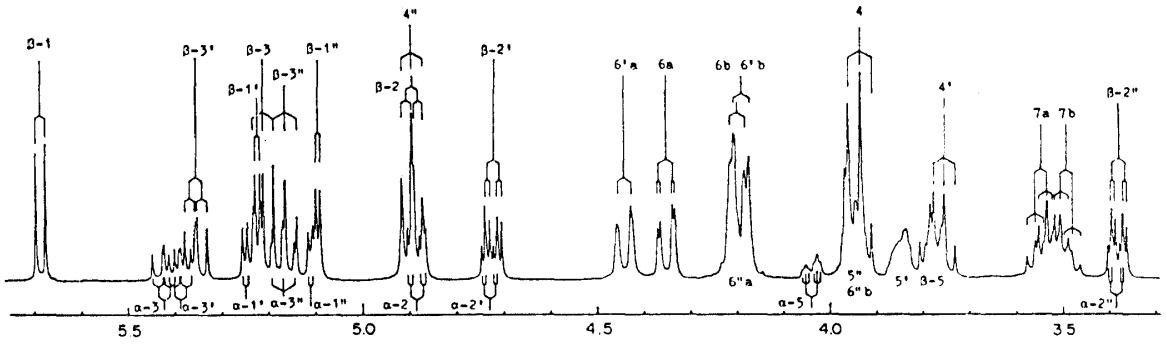
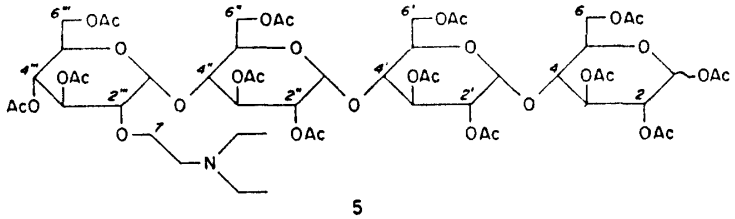


Fig. 1. ^{1}H-NMR Spectrum of 4 in $CDCl_3$.



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oligosaccharides having such a bulky substituent, cationic starch was subjected to hydrolysis using the combination of thermostable α- and β-glucoamylases. The enzymatic hydrolyzate was purified by the same procedure described above. As a result, DEAE-maltotriose and DEAE-maltotetraose were mainly obtained, but no DEAE-maltose (TLC *Rf* 0.33 and 0.19, respectively). Peracetylation of these DEAE-oligosaccharides gave peracetyl DEAE-maltotriose, **4**, (EI mass M^+ 1023, $C_{44}H_{65}NO_{26}$, Calcd. C 51.61, H 6.35, N 1.37%. Obsd. C 51.20, H 6.16, N 1.63%) and peracetyl DEAE-maltotetraose, **5**, (EI mass M^+ 1311, $C_{56}H_{81}NO_{34}$, Calcd. C 51.26, H 6.18, N 1.07%, Obsd. C 51.20, H 6.16, N 1.00%). ^{1}H-NMR spectral analysis of **4** in $CDCl_3$, including exhaustive spin decoupling and PRFT experiments, permitted us to assign all the signals and the structure was determined as shown in Fig. 1. The structure of **5** was also identified by the same NMR technique,

although complete assignment was not successful. The oligosaccharide sequences of **4** and **5** were again confirmed by EI mass spectrometry (data not shown).

Based on these results, it could be concluded, that 1) α-amylase could attack at the glucosidic linkages located at 3 or 4 glucose units away from the barrier in the direction of the reducing end and 2)glucoamylase hydrolyzed the glucosidic linkages of the resultant oligomers just before the alkylaminated glucose residue. Since the results of enzymatic degradation may depend on the origin of the enzymes used, it is suggested that some information concerning the action mechanism of amylases may easily be obtained by using the cationic starch as an enzyme substrate.

Reference

1) J.B. Stothers, "Carbon-13 NMR Spectroscopy," Academic Press, 1972.