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Distribution of Methoxyl Groups in the Methylation of Starch Alkoxides Obtained by Metallation with Alkali Metal Naphthalene

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Distribution of Methoxyl Groups in the Methylation of Starch Alkoxides Obtained by Metallation with Alkali Metal Naphthalene

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SUMMARY

The distribution of the alkoxide groups obtained in the metallation of starch in dimethyl sulfoxide solution by alkali metal naphthalenes was studied. The starch alkoxide was reacted with methyl iodide, and the methylated starch was hydrolyzed and analyzed for glucose and O-methyl glucose derivatives. The metallation reaction was found to be random, as seen from the fact that at low alkoxide concentration (D.S = 0.6), 2,3,6-tri-O-methyl glucose was formed, while at relatively high alkoxide concentration (D.S. = 1.5) unreacted glucose was still present. At low alkoxide concentration (D.S. $\simeq$ 0.6) there was, to a certain extent, preferential metallation at the C_2 hydroxyl groups, and to a lesser extent at the C_6 hydroxyl groups, as seen from the relative molar ratios of about 10:4:1 of the 2-, 6-, and 3-O-methyl glucose derivatives obtained, respectively. An increase in the metallation at the C_3 hydroxyl occurred with increasing alkoxide concentration. The distribution of the methoxyl groups with the three alkali metals potassium, sodium, and lithium was similar; there were differences only in the ease of metallation of the starch by the various alkali metal naphthalenes and in the efficiency of the coupling reaction with methyl iodide.

INTRODUCTION

It was recently reported from this laboratory that the alkali metal alkoxide derivatives of polyhydroxy polymers such as starch and other carbohydrates are readily obtained by reaction of the polymers with alkali metal naphthalenes [1-3].

It was the object of the present work to evaluate further this method of metallation in the case of starch, expecially as regards the selectivity of the reaction of the alkali metal naphthalenes with the various hydroxyl groups on the starch, and to compare the metallation reactions with lithium, sodium, and potassium naphthalenes.

To determine the selectivity of the metallation reaction, the alkoxide derivatives were reacted with excess methyl iodide, and the distribution of the methoxyl groups on the polysaccharide backbone was determined by hydrolysis and quantitative chromatography techniques.

EXPERIMENTAL

Lithium, sodium, and potassium naphthalenes were prepared in dry tetrahydrofuran, and their concentration in solution was determined by titration with acid [4]. Soluble starch (Analar, B.D.H.) containing 15% moisture was used. Dimethyl sulfoxide (DMSO) pure grade (Fluka) was used. Dry stock solutions of soluble starch (2 g/50 ml of DMSO) were prepared by dissolving the starch in DMSO and distilling about 15% of the solvent in vacuo at a temperature not more than 60°C to ensure distillation of water present.

2-O-Methyl-D-glucose was prepared according to Hodge and Rist [5], 6-O-methyl-D-glucose was prepared according to Freudenberg and Hull [6], and 3-O-methyl-D-glucose was obtained from Pierce chemicals. 2,3-Di-O-methyl-D-glucose was obtained by hydrolysis of the methyl glucoside (Pierce chemicals) according to Irvine and Scott [7]. The 2,3,6-tri-O-methyl-D-glucose was a sample.

Methylation of Starch Alkoxide

The methyl derivatives of starch were obtained by the following route. Alkali metal naphthalene in THF was added to a starch solution in DMSO under argon with strong stirring to form the alkoxide derivative. The end of reaction was easily seen from the disappearance of the green color of the reagent. Redistilled methyl iodide (4 eq) was added, the temperature of the reaction mixture increased, and the gelatinous alkoxide derivative dissolved. The reaction mixture was left overnight in the dark to minimize decomposition of the iodide, and the methylated starch was precipitated by a mixture of ether-acetone (6:1). It was dissolved in water and purified from alkali metal iodide by dialysis. The methylated starch was recovered by lyophilization in 90-95% yield.

Distribution of the Methoxyl Groups

The methylated starch was hydrolyzed in boiling 1 N hydrochloric acid for 3 hr. Under these conditions soluble starch was found to undergo complete hydrolysis, as seen from paper chromatography [8], and determination of the glucose by iodometric titration [9]. The acid hydrolysate was neutralized by a weakly basic ion-exchange resin (Amberlite IR-45) and lyophilized to give the crystalline methyl glucose derivatives. Only 3-5% degradation of the methoxyl groups occurred during the hydrolysis.

To find qualitatively the presence of D-glucose, mono-, di-, and tri-O-methyl-D-glucose in the hydrolysate of the methylated starch, the descending method of paper chromatography was employed. The eluent was n-butanol saturated with water (top layer) [8]. The chromatograms were developed using p-anisidine hydrochloride 0.3% in n-butanol followed by heating at 110°C for 10-15 min [10]. All the above compounds were separated clearly one from the other, as seen from authentic markers. The R_f values were: glucose, 0.06; mono-O-methyl-D-glucose derivatives, 0.21; di-O-methyl-D-glucose derivatives, 0.4; and tri-O-methyl-D-glucose, 0.65. However, no separation of the 2-, 3-, or 6-O-methyl-D-glucose derivatives could be achieved by this method. To determine quantitatively the amount of the O-methyl-D-glucose derivatives, gas chromatography of their trimethylsilyl derivatives was employed [11]. The crystalline methyl glucose derivatives obtained from the hydrolysate were dissolved in dry pyridine (10 mg/1 ml of solvent), and hexamethyl disilazane (0.2 ml) and trimethylchlorosilane (0.1 ml) were added. The reaction mixture was shaken for 2 min and left to stand for about 10 min before injection into the gas chromatograph. The precipitated ammonium chloride did not interfere. The gas chromatograph used was Perkin-Elmer Model 820. Helium was used as carrier gas, and 2.0 m of 0.25-in. tubing was used to contain the packing of SE 30 (5%) on Chromosorb P60/80. The carrier gas flow was set at about 33 ml/min, the column temperature at 185°C, the injector at 280°C, and the detector at 290°C. Authentic samples of D-glucose, 2-O-methyl-,

Table 1. Methylation of Starch Alkoxide[a]

Expt. no.	Alkoxide % of total hydroxyl groups	Metal	OCH_3, %		D. S. of methyl[c]		Efficiency of methylation,[d] %
			Calculated[b]	Found	Calculated	Found	
265	20	K	10.9	10.0	0.60	0.55	92.0
266	20	K	10.9	10.2	0.60	0.56	93.5
267	30	K	16.0	15.2	0.90	0.85	95.2
268	30	K	16.0	15.6	0.90	0.88	97.5
269	40	K	20.8	20.5	1.20	1.19	98.5
270	40	K	20.8	20.8	1.20	1.20	100.0
271	50	K	25.4	25.6	1.50	1.51	100.5
272	50	K	25.4	25.8	1.50	1.53	101.5
273	20	Na	10.9	8.7	0.60	0.47	79.8
274	20	Na	10.9	8.5	0.60	0.46	78.0
275	20	Li	10.9	7.5	0.60	0.42	68.8
276	20	Li	10.9	8.0	0.60	0.44	73.4
279	80	Na	38.0	21.2	2.40	1.22	55.6
278	90	Li	41.8	18.8	2.70	1.07	45.0

[a] The starch alkoxide derivative, prepared in DMSO, by addition of alkali metal naphthalene, was methylated by excess of methyl iodide.

[b] Assuming quantitative reaction of the methyl iodide with the starch alkoxide.

[c] Calculated from the equation: $\% OCH_3 = (D.S. \times 31 \times 100)/(162 + D.S. \times 14)$, where 162 is molecular weight of anhydrous glucose unit and 14 is molecular weight of CH_2 group, added for every methoxyl group (molecular weight, 31) introduced.

[d] Calculated from the equation: $\text{Efficiency} = (\% OCH_3 \text{ found} \times 100)/(\% OCH_3 \text{ calculated})$.

3-O-methyl-, and 6-O-methyl-D-glucose, 2,3-di-O-methyl-D-glucose, and 2,3,6-tri-O-methyl-D-glucose were injected under the same conditions. Calibration curves for all these markers were prepared, from which the amount of glucose and the O-methyl-D-glucose derivatives was determined. It was observed that under the conditions used, only glucose, and 2-O-methyl-D-glucose were separated into the α- and β-anomers, so that there was a relatively smaller number of peaks in the chromatogram and the determination of the O-methyl-D-glucose derivatives was not difficult.

RESULTS

The metallation of starch in DMSO solution by alkali metal naphthalenes was found to depend on the type of alkali metal. While with potassium naphthalene only up to about 60% of the hydroxyl groups were easily converted to alkoxide groups, with sodium naphthalene about 80% (maximum) of the hydroxyl groups could be metallated, and with the corresponding lithium compound all the hydroxyl groups were easily metallated. Another significant difference between the starch alkoxides was found in the efficiency of the methylation reaction with methyl iodide, which was in the order potassium > sodium > lithium. As seen from Table 1, the potassium alkoxide gave, in all the concentrations investigated, an almost quantitative reaction with methyl iodide (92% efficiency for low and 100% for high alkoxide concentrations), whereas sodium and lithium alkoxides did not. For this reason the distribution of the methoxyl groups in methylated starch obtained from methylation of the potassium starch alkoxides was investigated in more detail than that with the corresponding sodium or lithium alkoxides.

The results show that the metallation of the starch in DMSO by potassium naphthalene was random, and even at low starch alkoxide, D.S. = 0.6 (equivalent to conversion of 20% of the hydroxyl groups to alkoxide groups), di-O-methyl-D-glucose derivatives and 2,3,6-tri-O-methyl glucose were found in the hydrolysate of the methylated starch. With increase of the per cent starch alkoxide, there was a corresponding increase in the di- and tri-O-methyl-D-glucose. At 50% alkoxide (D.S. of alkoxide = 1.5), there were still free glucose units in the methylated starch. These facts show clearly that the metallation of the starch by potassium naphthalene is random, although there is preference to metallation at C_2, and to a smaller extent at C_6.

Only 2,3-di-O-methyl-D-glucose was available, and the chromatograms

Table 2. Distribution of Methylation Products[a]

Expt. no.	Alkoxide % of total hydroxyl groups	Metal	Glucose, mole %	2-O-methyl-D-glucose, mole %	6-O-methyl-D-glucose, mole %	3-O-methyl-D-glucose, mole %	2,3-di-O-methyl-D-glucose, mole %	2,3,6-tri--O-methyl-D-glucose, mole %	Total,[b] mole %	2,6-,[c] 3,6-di-O-methyl and 2,3,4,6-tetra-O-methyl-D-glucose, mole %
265	20	K	57.0	24.0	9.8	2.6	2.9	$\ll$1.0	96.3	3.7
266	20	K	55.0	25.2	9.7	2.3	3.0	$\ll$1.0	95.2	4.8
267	30	K	36.5	29.5	12.3	4.5	5.4	1.5	89.7	10.3
268	30	K	35.0	29.4	12.1	4.8	6.1	1.1	88.5	11.5
269	40	K	23.5	30.4	13.0	5.6	7.4	4.0	83.9	16.1
270	40	K	22.0	31.5	13.2	5.8	7.9	3.8	84.2	15.8
271	50	K	12.0	27.5	12.8	8.0	8.9	7.8	77.0	23.0

272	50	K	10.6	29.0	12.5	8.3	9.2	8.2	77.2	22.8
273	20	Na	62.0	22.2	8.7	2.0	2.4	⩽1.0	97.3	2.7
274	20	Na	63.0	21.4	8.5	1.8	2.2	⩽1.0	96.9	3.1
275	20	Li	65.0	20.2	7.5	1.8	2.0	⩽1.0	96.5	3.5
276	20	Li	64.0	21.0	8.0	1.9	2.0	⩽1.0	96.9	3.1
279	80	Na	18.0	30.2	13.0	7.2	8.5	6.8	83.7	16.3
278	90	Li	25.0	25.2	12.0	5.0	7.2	4.5	78.9	21.1

[a] The experiment numbers are those of Table 1. The methylated starch was subjected to acid hydrolysis, and the methyl glucose derivatives were determined by gas chromatography via their trimethylsilyl derivatives.

[b] Total mole % of glucose and methyl glucose derivatives.

[c] Calculated by difference from the derivatives accounted for.

Table 3. Relative Ratios of the Mono-O-Methyl-D-glucose Derivatives[a] (Metallation of Starch by Potassium Naphthalene)

Expt. no.	D. S. of methoxyl	Mono-O-methyl-D-glucose derivatives, mole %[b]	2-O-methyl-D-glucose, %[c]	3-O-methyl-D-glucose, %[c]	6-O-methyl-D-glucose, %[c]
265	0.55	36.4	66.0	7.15	27.0
266	0.56	37.2	67.7	6.2	26.1
267	0.85	46.3	63.8	9.7	26.6
268	0.88	46.3	63.5	10.3	26.2
269	1.19	49.0	62.0	11.4	26.6
270	1.20	50.5	62.5	11.5	26.2
271	1.51	48.3	57.0	16.6	26.5
272	1.53	49.8	58.3	14.3	25.2

[a] Experiment numbers as in Table 1.

[b] Mole % out of total glucose and methyl glucose derivatives obtained in the methylated starch.

[c] Per cent from total mono-O-methyl-D-glucose.

showed other peaks in the region of the dimethyl glucose derivatives belonging probably to 2,6- and 3,6-di-O-methyl-D-glucose. Furthermore, the presence of 2,3,4,6-tetra-O-methyl-D-glucose could not be ascertained due to unavailability of a suitable marker. The percentage amount of these glucose derivatives was calculated by the difference of the determined methyl glucose derivatives from 100%, and, as such, these figures may not be very accurate. It can be seen that at the high alkoxide concentrations these derivatives were formed in significant amounts.

As seen from Tables 2 and 3, at an alkoxide D.S. of about 0.6, all three isomers of mono-O-methyl-D-glucose were formed, viz., the 2-, 6-, and 3-O-methyl derivatives, the relative molar ratios being about 10:4:1, respectively. These ratios reflect the hydroxyl groups' relative reactivity toward metallation. Although there is some preferential formation of the 2-O-methyl-D-glucose derivative, no high degree of selectivity in the metallation reaction was found. With an increase in the per cent starch alkoxide, there is an increase in the total substitution (including di- and tri-O-methylation) at the 2 position. A significant increase also occurs in the amount of 3-O-methyl-D-glucose and the di- and tri-O-methyl-D-glucose derivatives substituted at the 3 position.

The results obtained with the potassium alkoxides were compared with the respective sodium and lithium alkoxides to find out whether there is an effect on the distribution of the methoxyl groups after methylation of the alkoxides. Experiments carried out under comparable conditions showed that there was no significant difference among the results obtained with the three alkali metals.

DISCUSSION

The order of the ease of metallation of starch by the alkali metal naphthalenes, which was found to be Li > Na > K, may be in accordance with the observation of Rendelman [12, 13] that the ability of a metal alcoholate to accommodate an additional molecule of carbohydrate increases with an increase in the ionic radius of the metal, as was previously indicated from the findings of Brewer [14], that the maximum number of donor molecules that can be oriented about an alkali metal cation is four for lithium and six for sodium and potassium.

The order of efficiency of the alkali metal alkoxides in the methylation reaction with methyl iodide, which was K >Na >Li, may be in accordance with the greater ionic character of the potassium alkoxide, which increases the nucleophilicity of the potassium alkoxide as compared with the others. As known, the lithium-oxygen bond in lithium alkoxide has more of a covalent bond character [15, 16]. Furthermore, the alkali metal alcoholates of the carbohydrates form chelates with adjacent free hydroxyl groups [13]. The stability of the chelates increases with decreasing ionic radius of the alkali metal; thus, the smaller reactivity of the lithium alkoxide is understood. Cram and co-workers [17] have observed large differences in the rate of cleavage in DMSO of various alkali metal alkoxides, viz., potassium, sodium, and lithium alkoxides, the former giving the fastest reaction.

Much work has been done on the location of the alkoxide on the carbohydrate backbone by using the method of substitutive methylation of starch and cellulose alkoxides. The method assumes that methylation either by dimethyl sulfate or methyl iodide occurs only at the anionic oxygen atom. Thus, for example, Bines and Whelan [18] methylated the monosodium alcoholate of amylose (prepared by heating amylose with butanolic sodium hydroxide) by reaction with methyl iodide in toluene. Hydrolysis of the product gave a mixture of mono-O-methyl-D-glucose derivatives (36.4%), di-O-methyl-D-glucose derivatives (14.6%), and unsubstituted glucose (43.5%). The 2-,3-, and 6-O-methyl-D-glucose derivatives were present in proportions of 2.6:1:1.2, respectively. Doane and co-workers [19] methylated starch by the method of Hodge et al. [20] (sodium metal-liquid ammonia-methyl iodide) to degrees of substitution of 0.34 and 0.90 methoxyl per D-glucose residue. Hydrolysis of the products gave D-glucose and mono-, di-, and tri-O-methyl-D-glucose derivatives in the approximate molar ratios of 36:6:2:1 and 6:3:2:1, respectively. The mono-O-methyl-D-glucose fraction in the former case was found to be composed of a 3:2 ratio of 2-O-methyl-D-glucose to 6-O-methyl-D-glucose and a 1:1 ratio in the latter case. Significantly there was no 3-O-methyl-D-glucose in either case.

Croon [21] methylated amylose and amylopectine and found preferential methylation at C_2 and C_6.

From these studies carried out on starch it can be concluded that methylation occurs at all three positions, but predominantly at C_2 and C_6.

Comparing our results with those previously found, there is qualitatively the same trend of distribution of the methoxyl groups, but there are quantitative differences. Although there is preference to metallation by the potassium naphthalene at the C_2 and, to a lesser extent, at the C_6 position, nevertheless there is considerable metallation at the C_3 position.

The preferential metallation at the secondary hydroxyl at C_2 has in general been explained as due to its greater acidity due to the inductive effect of the two oxygen atoms at C_1 [22]. The relatively low extent of metallation at the C_3 position may be attributed to the relatively low acidity of this hydroxyl group [23].

It has recently been shown by Casu et al. [24] and by Erlander and Tobin [25] that amylose and amylopectin exist in DMSO solution in a helical conformation. This helix is held together by hydrogen bonds between the C_2 and C_3' hydroxyl groups on adjacent α-1,4-linked glucose units. It was further suggested by Erlander and Tobin [25] that DMSO can also interact with the hydroxyl groups at C_2 or C_3 of the anhydro glucose units of starch. These interactions induce these neighboring hydroxyl groups to produce a greater attraction to each other. These suggestions can further explain in our case the low extent of metallation of starch at the C_3 hydroxyl groups at the low alkoxide concentrations.

On increasing the alkoxide concentration there is significant increase in substitution at the C_3 position, as seen from the increase in the formation of 3-O-methyl-D-glucose, 2,3-O-dimethyl-glucose, and 2,3,6-tri-O-methyl glucose. Increase in the substitution at C_3 with increasing alcoholate concentration was also observed by Croon [21] and by Doane and co-workers [19]. It seems that on increasing the amount of added base to the starch in DMSO, there is increased destruction of hydrogen bonds in the carbohydrate. Thus, further metallation at the C_2 and especially at the C_3 hydroxyl groups occurs.

In conclusion, the results obtained show clearly that the metallation of starch by alkali metal naphthalenes is random, as seen from the formation of tri-O-methyl-D-glucose at low alkoxide concentrations (D.S. = 0.6) and the presence of free glucose even at higher alkoxide concentrations (D.S. = 1.5). In addition, the ratio of the three mono-O-methyl-D-glucose derivatives obtained at low alkoxide concentrations indicates that there is some preferential metallation of the hydroxyl groups at C_2 and to a lesser extent at C_6 as compared with those at C_3.

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