Chemical Reaction between Cellulose and Formaldehyde

II. Structure of Methylene Cellulose[1)]

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荒木幹夫* ：セルロースとホルムアルデヒドとの反応（第2報）
メチレンセルロースの構造

In the previous paper, it has been reported that formaldehyde reacts with cellulose to form methylenedioxy cross-linking by studying the methylation of its reaction products. In the present paper, the structure of methylene cellulose is discussed with the results of quantitative analysis of hydrolyzate obtained by the methanolysis and subsequent hydrolysis of fully methylated methylene celluose. Furthermore, the de-methylation of methylglucoses which seemed to occur as side reactions at methanolysis and hydrolysis of the product is also examined in connection with the use of the present methods.

Results and Discussion

Methanolysis and hydrolysis of fully methylated methylene cellulose—— The sample described in the previous paper[2)] was treated with 1%-methanolic hydrogen chloride in a sealed tube at 130°C for 75 hours by the method of PACSU[3)]. The methyl glucosides thus obtained were hydrolyzed with 4%-hydrochloric acid to methyl glucoses.

Separation of methyl glucoses—— The resulting methyl glucoses were fractionated by cellulose powder chromatography into four fractions, i.e., tri-*O*-methyl, di-*O*-methyl, mon-*O*-methyl D-glucose and D-glucose. Some fractions

Table 1 Composition of the Hydrolyzate from Methylene Cellulose.

Fraction	Constituents	Yield, mg	Mol. %
1	2,3,6-tri-*O*-methyl D-glucose	358	67.5
2	di-*O*-methyl D-glucose	100	20.2
3	mono-*O*-Methyl D-glucose	36	7.8
4	D-glucose	19	4.4

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were obtained in pure state, but others were mixtures and had to be further separated by paper chromatography or paper electrophoresis. The result of cellulose powder chromatography was given in Table 1.

Fractions 1 and 4 were obtained in crystalline state. These fractions consisted of 2, 3, 6-tri-*O*-methyl D-glucose and D-glucose respectively. But it was found that fractions 2 and 3 were mixtures of di-*O*-methyl D-glucoses and mono-*O*-methyl D-glucoses respectively. The former fraction was further fractionated by paper electrophoresis. The components were identified by comparison of their behavior on paper electrophoresis with that of the authentic samples. The proportion of the individuals was estimated by hypoiodite titration. The latter fraction was further fractionated by paper chromatography because of a little difference of MG value between 3-mono-*O*-methyl and 6-mono-*O*-methyl D-glucose in electrophoresis. And the ratio of its constituents was determined by comparison with the area of colored spots on the chromatogram. The results of the analysis were given in Table 2.

Table 2. Composition of Fraction 2 and 3.

Fraction	Constituents	Mol. %	MG*
2	2,3-di-*O*-methyl D-glucose	80	0.14
	2,6-di-*O*-methyl D-glucose	4	0.04
	3,6-di-*O*-methyl D-glucose	16	0.52
3	2-mono-*O*-methyl D-glucose	25**	0.22
	3-mono-*O*-methyl D-glucose	25**	0.78
	6-mono-*O*-methyl D-glucose	50**	0.84

* MG = True distance of migration of the substance/True distance of migration of D-glucose.
** Mol. % of mono-methyl glucoses were determined apporoximately on the paper chromatogram following the method of LENZ.

De-methylation of methyl glucoses in hydrolytic processes—— Some de-methylation of methyl glucoses during the hydrolytic processes is unavoidable. The separated glucose methyl ether previously mentioned may contain de-methylated ethers other than the normal hydrolytic products. In a recent publication, CROON et al.[4] have reported that 2, 3, 6-tri-*O*-methyl D-glucose was de-methylated to give 2, 3-di-*O*-methyl, 2, 6-di-*O*-methyl and 3, 6-di-*O*-methyl D-glucose. But they did not show any selective de-methylation, namely, the methoxyl groups in different position were likely to show comparable reactivities. To investigate the possible products in the condition used, known ethers were subjected

separately to the treatment under the same hydrolytic treatments as in the case of methylene cellulose.

It was observed that glucose methyl ethers were de-methylated to an extent of 2.2 to 8.2% varying with the individuals.

And it was observed that a few re-methylated glucoses were formed from all of the lower degree of methyl ethers used except 2, 3, 6-tri-*O*-methyl D-glucose, under the condition used. Of the de-methylation products of 2, 3, 6-tri-*O*-methyl D-glucose, the analytical results were shown in Table 3.

Table 3. Composition of Resulting Products of 2, 3, 6-Tri-*O*-Methyl D-Glucose after Hydrolytic Treatments,

Constituents	Yield, Mol. %
2, 3, 6-tri-*O*-methyl D-glucose	90
2, 3-di-*O*-methyl D-glucose	7
3, 6-di*O*-methyl D-glucose	0.5
2, 6-di-*O*-methyl D-glucose	0.5
mono-*O*-methyl D-glucose	2

As shown in the experimental result, the methoxyl group at $C_{(6)}$ was more easily de-methylated than the others. The de-methylation products of other glucose methyl ethers were tabulated in Table 4. And the same was observed at the de-methylation of 6-mono-*O*-methyl and 2-mono-*O*-methyl D-glucose, i.e., 6-mono-*O*-methyl ether was more easily de-methylated than 2-mono-*O*-methyl ether.

Table 4. Composition of Resulting Products of Methyl Glucose after Hydrolytic Treatments.

Treated sugars	Composition of resulting products, Mol. %			
	glucose	mono-methyl-	di-methyl-	tri-methyl-
3-mono-*O*-methyl D-glucose	5.8	92	2.3	trace
6-mono-*O*-methyl D-glucose	8.2	90	2.0	trace
3, 6-di-*O*-methyl D-glucose	none	4.0	93	3.0
2, 3, 6-tri-*O*-methyl D-glucose	none	2.2	8.0	90

From these resuts the constitution of the fraction 2 was estimated as follows.

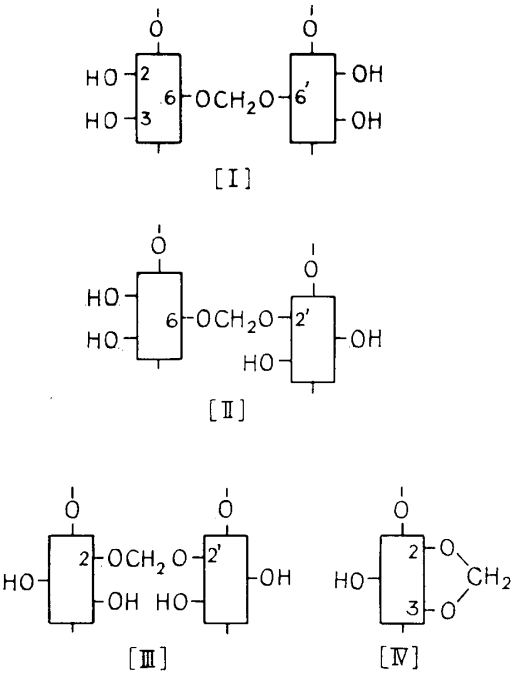
2, 3-di-*O*-methyl D-glucose : 2, 6-di-*O*-methyl D-glucose :
3, 6-di-*O*-methyl D-glucose = 43 : 2 : 14 (in molar ratio)

The position of formaldehyde combined with cellulose—— The molar ratio of

the constituents of di-*O*-methyl D-glucoses shows that of formaldehyde combined with three different hydroxyl groups respectively. Now, the hydroxyl groups at $C_{(2)}$, $C_{(3)}$ and $C_{(6)}$ carbon atoms in the anhydroglucopyranose residue in cellulose molecule are shown by $C_{(2)}$-OH, $C_{(3)}$-OH and $C_{(6)}$-OH, respectively, and so the molar ratio of formaldehyde which reacts with three different hydroxyl groups may be shown as follows;

$$C_{(6)}\text{-OH} : C_{(3)}\text{-OH} : C_{(2)}\text{-OH} = 43 : 2 : 14$$

From the present and previously mentioned experimental results, it was deduced that in the product obtained the anhydroglucopyranose units of neighboring cellulose chain are cross linked together preferentially at $C_{(6)}$ in the form of methylene ether [I]. In the other possible structures (i.e., 6-2'*, 6-3', 2-2' and 3-3'), 6-2' or 2-2' ([II] or [III]) was probably present in a larger extent than the others, but it did not allow to decide the definite order of possible existence of the remaining structures. And the predominant proportion of 6-mono-*O*-methyl glucose over the other two isomeric monomethyl ethers in the hydrolyzate may suggest the presence of 2-3 internal cyclic ether [IV]. The marked change of the chemical and physical properties of treated cellulose resulted from the reaction of a comparatively small proportion of formaldehyde with few corresponding hydroxyl groups were observed[5]. This facts led to the idea of cross-linking of neighboring chain molecules of cellulose, but the possibility of a reaction occurring in intramolecularly has also been undeniable. And the presence of internal cyclic formal (2-3 formal) [IV] has been suggested by ROFF[6] using molecular models.



Partial Structure of Methylene Cellulose Proposed.
(The numbers in the figure show the position of carbon atoms in anhydroglucopyranose residue).

The difference between the present observation and that of WAGNER and PACSU[3] is probably due to the difference of the catalyst used. The catalytic effect of boric acid on the reaction may be attributable to its complex forming

* 6-2' indicates bridging from number 6 carbon atom of one anhydroglucopyranose residue to number 2 carbon of other anhydroglucopyranose residue in cellulose chain.

ability (e.g. to form a cellulose-boric acid complex*) other than as an acid. So, the reaction that proceeds through the complex as an intermediate, may from the product with different from that obtained by the usual acid catalyst.

The structure of the product obtained by the reaction between cellulose and formaldehyde in the presence of hydrogen chloride in vapor phase has been studied. Although much more studies will be necessary before the detailed structure for these products can be given, certain characteristics can be deduced from the above observations.

Experimental

In this experiment, the methyl methylene cellulose containing 4.2% combined formaldehyde (calculated as methoxyl-free) and 38.6% methoxyl group was used. (See part I. of this series)

Hydrolytic procedure—— (A) Treatment with 72%-sulphuric acid : One gram of methylated methylene cellulose was dissolved in 10 g of 72% sulphuric acid at 0° and the solution kept for one day at the same temperature. The solution was then diluted with water to 500 ml. and kept under nitrogen at 60°C for 12 hours. The acid was neutralized with barium carbonate and barium salts were removed by filtration and washed with water. The combined filtrate and washings were concentrated to dryness under a reduced pressure, dissolved in ethanol to remove traces of inorganic salts. Concentration of ethanol extract gave 0.3 g. of hydrolyzate. Because of a poor yield of this treatment, the following procedure was used.

(B) Methanolysis and hydrolysis : In a preliminary experiment, it was observed that the methanolysis of the sample did not proceed satisfactorily under the condition using 1%-methanolic hydrogen chloride in a sealed tube at 100°C for 70 hours. Therefore, the procedure of WAGNER and PACSU[3] was used ; the dried sample, 1.0 g., was treated with 1%-methanolic hydrogen chloride in a sealed tube at 130°C for 70 hours. After cooling the resulting solution was filtered and concentrated to 5 ml. The methyl glucosides thus obtained were hydrolyzed by refluxing, with 95 ml. of 4%-hydrochloric acid for 12 hours. The acid was removed by filtration through a column of Amberlite IR4B. The combined filtrate and washings were evaporated to dryness under a reduced pressure at 40°C ; yield 0.8 g. The methyl glucose mixture thus obtained was used for the following experiments.

* The possible complex may be not the internal cyclic complex relating to $C_{(2)}$-OH and $C_{(3)}$-OH of a glucopyranose residue of cellulose molecule, but some other intermolecular complex according to the observation of FOSTER (J. Chem. Soc. : 982 (1953)).

a) Paper chromatography— Toyo filter paper No. 50 was used for analytical separation and quantitative determinations.

Solvent system (A) n-buthanol-ethanol-water, 5 : 4 : 1[7], (B) 2, 3, 6-trimethyl pyridine-ethyl acetate-water, 2 : 5 : 5[8].

Spraying reagent; aniline hydrogen phthalate[9].

b) Paper electrophoresis— The technique used was essentially the same as described by FOSTER[10] with a 0.2M borate buffer of pH 10, and the spraying reagent for analytical separation and guiding spot for quantitative determination, p-anisidine trichloroacetate[11] was used.

Fractionation of hydrolyzate—— The hydrolyzate (0.5 g.) dissolved in a minimum quantity of water was added to the top of cellulose powder column (3.8× 50 cm.), the same as described by HOUGH, JONES and WADMAN[12], which was eluted with n-buthanol saturated with water. The eluate was fractioned into circa 8 ml. portion at 10 min. intervals, and divided into 170 fractions. After investigation of each fraction by paper chromatography (solvent system A) fractions containing similar component were combined and evaporated to dryness. The amount of sugars in fractions was determined by weight. The result was shown in Table 1.

Fraction 1 was obtained in a crystalline state, m.p. 117~118°C. Mixed m.p. with authentic 2, 3, 6-tri-*O*-methyl D-glucose showed no difference. Fraction 2 was obtained as a syrup. It proved to be a mixture of 2, 3-di-*O*-methyl, 2, 6-di-*O*-methyl and 3, 6-di-*O*-methyl D-glucose by paper electrophoresis. The proportion of the constituents were determined after fractionation on a filter paper (Toyo filter paper No. 50, 4×40 cm.) by paper electrophoresis and the areas containing the sugar were cut off by the aid of guide spots and were extracted with water. The amounts were determined by hypoiodite oxidation, following the procedure of HIRST, HOUGH and JONES[7]. CROON and LINDEBERG[13] have shown that the determination of methyl sugars by the hypoiodite oxidation is useful even in the presence of borate. Fraction 3 was obtained as a syrup. It showed that the fraction was a mixture of 2-mono-*O*-methyl, 3-mono-*O*-methyl and 6-mono-*O*-methyl D-glucose by the paper chromatography (solvent system B). These mono-methyl glucose were identified by comparison of their Rf values with those of authentic samples.

The ratio of the costituents was determined approximately by the comparison of the area of coloured spots on the paper.

Fraction 4 was obtained in a crystalline state, m.p. 144~146°C. Mixed m.p. with authentic D-glucose showed no difference.

De-methylation reaction of methyl glucose ether in the hydrolytic processes—— 3-mono-*O*-methyl, 6-mono-*O*-methyl, 3, 6-di-*O*-methyl and 2, 3, 6-tri-*O*-methyl

D-glucose were respectively prepared by the conventional methods. Each sample of the glucose methyl ethers (0.15 g.) was subjected to hydrolytic treatments under the same conditions as previously mentioned. The acid was removed by filtering through a column of Amberlite IR4B, and the aqueous solution was evaporated to dryness. The recovery was approximate 85% in weight and no appreciable difference in recovery among these methyl sugars was observed. The reaction products of each methyl glucose were fractionated by paper chromatography (solvent system A) and the amounts of the constituents were determined by the methods of FLOOD et al.[14] and HIRST et al.[7] that was mentioned previousely. For the reaction products of 2, 3, 6-tri-*O*-methyl D-glucose, the analysis was carried out by a paper electrophoresis followed by hypoiodite oxidation as previously mentioned.

Summary

(1) The structure of methylene cellulose obtained by the reaction between cellulose and formaldehyde in the presence of hydrogen chloride as catalyst has been investigated. It was demonstrated that formaldehyde reacts preferentially at $C_{(6)}$ hydroxyl group in anhydroglcopyranose residue to form methylenecross-linking. The ratio of the three hydroxyl groups in the glucopyranose residue in cellulose reacting with formaldehyde was shown as follows;

$$C_{(6)}\text{-OH} : C_{(3)}\text{-OH} : C_{(2)}\text{-OH} = 43 : 2 : 14$$

(2) The magnitude of de-methylation of some methyl glucose ethers during the hydrolytic processes has been studied. It was shown that de-methylation reaction occurred more easily at $C_{(6)}$ methoxyl group than at $C_{(2)}$ and $C_{(3)}$.

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摘　　要

さきに得られた完全メチル化メチレンセルロースをメタノリシスしてメチルグルコシドを，さらにそれを4%塩酸で加水分解してメチルグルコースをそれぞれ得た。この場合本生成物は通常の多糖類と比較して，メタノリシスあるいは加水分解され難いことを見出した。したがつてある程度の糖の分解，あるいは脱メチル反応はさけ難いものとなつた。生成メチルグルコースはセルロース粉末によるカラムクロマトグラフ法，ろ紙電気泳動法，ペーパークロマトグラフ法で分析定量した（表1，2）。

このようにして分離されたメチルグルコースには，正常な加水分解生成物のほかに，脱メチル化反応生成物も含有していることが考えられるので3-モノ-*O*-メチル，6-モノ-*O*-メチル，3,6-ジ-*O*-メチル，2,3,6-トリ-*O*-メチルグルコースをそれぞれ合成し，前述メタノリシスおよび加水分解に用いたと同条件でそれらのメチル化グルコースを処理して脱メチル化反応を検討した（表3および4）。その結果2～8％（モル％）のメチルグルコースが脱メチル反応をうけ，とくに第6炭素に結合するメトキシル基が脱離され易いことを見出した。

以上の分析結果ならびに脱メチル反応からの結果を綜合して，ホルムアルデヒドのセルロース鎖中のグルコピラノース残基中の3つの水酸基に対する結合モル比は次のように示される。

$$C_{(6)}\text{-OH} : C_{(3)}\text{-OH} : C_{(2)}\text{-OH} = 43 : 2 : 14$$

（グルコピラノース残基中の3つの炭素原子 $C_{(2)}$，$C_{(3)}$ および $C_{(6)}$ に結合する水酸基をそれぞれ $C_{(2)}$-OH, $C_{(3)}$-OH, $C_{(6)}$-OH で示した。）

すなわちホルムアルデヒドは第1水酸基にもつとも多く反応し，つぎに $C_{(2)}$-OH, $C_{(3)}$-OH の順で小となる。

この実験結果を第1報で得られた結果と総合して次の結論を得た。

(1) ホルムアルデヒドとセルロースとの反応生成物は6—6′〔I〕，6—2′〔II〕あるいは2—2′〔III〕を主とする部分構造（たとえば6—6′は一つのグルコピラノース残基の $C_{(6)}$ と他のセルロース鎖のグルコピラノース残基の $C_{(6)}$ と架橋結合していることを示す）を主としてとり，3—3′などの構造はほとんどとらない。

(2) 6-モノ-*O*-メチルグルコースが他のモノメチルグルコースに比較して多量に存在することから，分子内環状ホルマール（IV）の存在は否定することが出来ない。

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