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A METHOD FOR THE IDENTIFICATION OF THE MONO-*O*-METHYLGLUCOSES[1]

By R. U. Lemieux and H. F. Bauer[2]

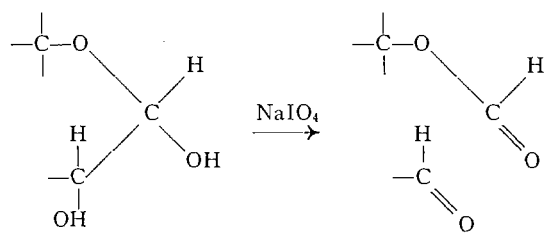
ABSTRACT

Periodate oxidation of any one mono-*O*-methylglucopyranose and alkaline hydrolysis of the product yields a substance which can be positively distinguished by paper chromatography from the products formed under the same conditions from the other mono-*O*-methylglucopyranoses. Thus, the components of a mixture of the mono-*O*-methylglucopyranoses can be readily identified. The method appears useful for the identification of di-*O*-methylglucoses. Reduction with sodium borohydride prior to periodate oxidation renders the method useful for the identification of tri-*O*-methylglucoses. The potential value of the method, which can be used on a microscale, is illustrated by an application to the characterization of the *O*-methylglucoses derived from a water-soluble *O*-methylcellulose.

A method for the identification of the components of mixtures of mono-*O*-methylglucoses was required as an aid in determining the extent and nature of acetyl group migrations during methylation with silver oxide and methyl iodide. This communication describes a method suitable for this purpose which may find useful application in studies on the composition of *O*-methylated polysaccharides.

The position-isomers for a partially *O*-methylated sugar are not usually separable by unidimensional partition paper chromatography. For example, although a mono-*O*-methylglucose fraction which contained three different mono-*O*-methylglucoses was examined (chromatogram 1, Fig. 1), no information on the chemical heterogeneity of the fraction was obtained. Although the solution to the problem could have been approached by application of two-dimensional paper chromatography, it was felt that the following method would yield more decisive information and be of more general application. The method developed involves periodate oxidation of the mixture of *O*-methylglucoses, alkaline hydrolysis of the formyl esters formed, and characterization of the products by unidimensional paper chromatography.

It is now firmly established (1, 4, 5, 12, 16) that the periodate oxidation of reducing sugars may be attended by the formation of formyl esters. The *O*-formyl groups are derived from the lactol carbon atom, as shown below, and are sufficiently stable in slightly acid media to serve as blocking groups toward further periodate oxidation. Recently, the periodate oxidation of



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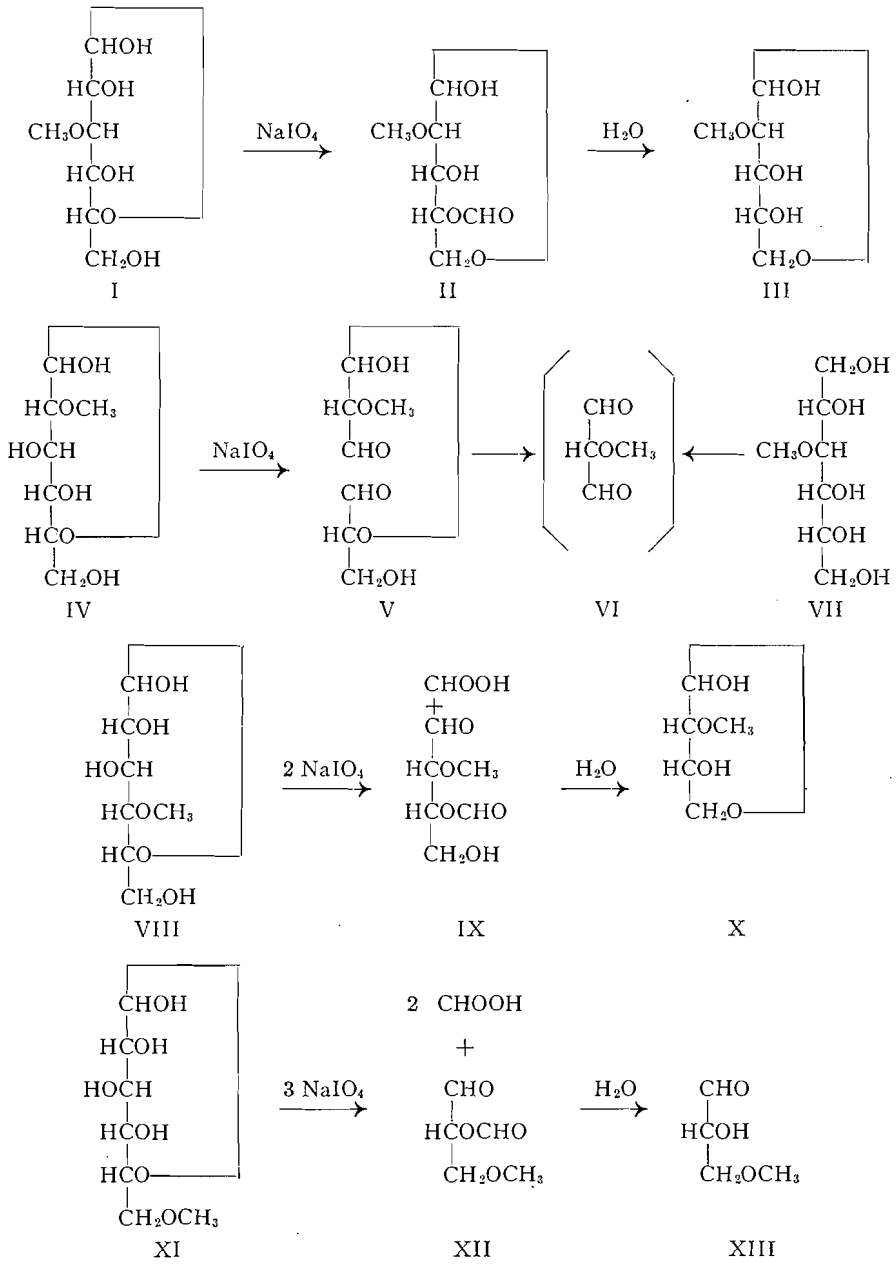
3-*O*-methyl-D-glucopyranose (I) has been shown (1) to yield mono-*O*-formyl-2-*O*-methyl-D-arabinose (II). It is of interest to note that the success met (3) in limiting the consumption of periodate by starch and cellulose, when the oxidation mixture is controlled within the limits of pH 2.05–4.8, to one mole of oxidant per glucose residue is in all probability related to the formation of formyl esters at the reducing ends of the polysaccharide chains.



In the light of the above-described behavior of reducing sugars toward periodate, it was to be expected that the course of oxidation followed on periodate oxidation of an *O*-methylglucose would vary considerably with variation of the position of the methyl group. It could be expected that the first product formed on periodate oxidation of 2-*O*-methyl-D-glucopyranose (IV) would be the trialdehyde (V). However, the course of further oxidation could not be predicted. Oxidation of the 4-*O*-methyl (VIII) and 6-*O*-methyl (XI) derivatives of D-glucopyranose would be expected to yield the mono-*O*-formyl-2-*O*-methyl-D-erythrose (IX) and mono-*O*-formyl-3-*O*-methyl-D-glyceraldehyde (XII), respectively. Identification of the products II, IX, and XII by paper chromatography could then serve as a means for identifying the

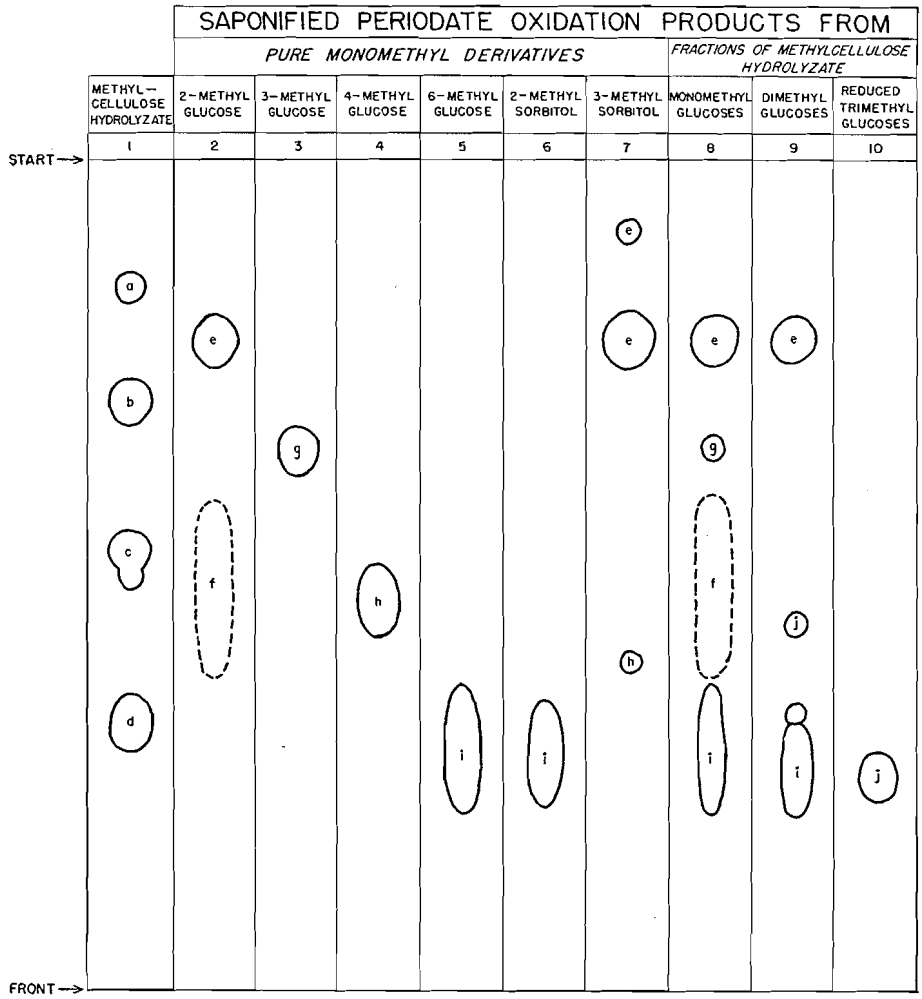
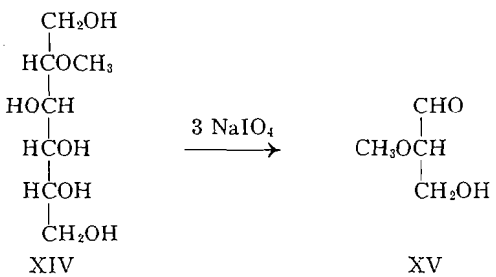


FIG. 1. Paper chromatograms developed with butanol–ethanol–water (6) and sprayed with aniline phthalate solution (15). *a*—glucose, olive brown. *b*—mono-*O*-methylglucoses, plum. *c*—di-*O*-methylglucoses, plum. *d*—tri-*O*-methylglucoses, plum. *e*—lemon yellow. *f*—faint canary yellow. *g*—2-*O*-methylarabinose, plum. *h*—citrine. *i*—canary yellow. *j*—olive brown.

mono-*O*-methylglucose oxidized. This appeared to be the case. However, since the formyl esters underwent hydrolysis during the chromatography, the resulting chromatograms were ill-defined. When the *O*-formyl groups were saponified prior to chromatography, the well-defined chromatograms shown in Fig. 1 were obtained.

The chromatograms shown in Fig. 1 were developed with the butanol–ethanol–water system (6) and the reducing compounds were detected with the aniline phthalate reagent (15). The periodate oxidations were carried out for one hour at 0°C. with an excess of reagent. Ethylene glycol was added to destroy the excess periodate and the solution was neutralized to phenolphthalein before application to the chromatogram. The dark spots formed at the starting point through reaction of the aniline spray reagent with the sodium iodate were not included in Fig. 1.

The main product from 2-*O*-methyl-D-glucopyranose (IV) gave a strong lemon yellow spot in the cold. Since a material was formed on periodate oxidation of 3-*O*-methylsorbitol (VII) which possessed the same R_f value (compare chromatograms 2 and 7, Fig. 1) and which gave the same color reaction, it seems likely that the substance is derived from methoxymalonaldehyde (VI). This conclusion is supported by the appearance of this spot on the chromatogram (Fig. 1, 9) for the oxidation products from the di-*O*-methylglucose fraction of the *O*-methylcellulose hydrolyzate. 2,6-Di-*O*-methyl-D-glucopyranose would also be expected to yield methoxymalonaldehyde (VI) on periodate oxidation. Attempts are being made to characterize the substance actually responsible for the color reaction. A diffuse band of greater R_f value also appeared on the chromatogram of the product from 2-*O*-methylglucose. This complication may be serious should it be desired to identify 4-*O*-methylglucose (see Fig. 1, 4) in the presence of large amounts of 2-*O*-methylglucose. 3-*O*-Methyl-D-glucopyranose (I) gave only 2-*O*-methyl-D-arabinose (III) (1). The R_f value for the product obtained from 4-*O*-methyl-D-glucopyranose is of the order expected for 2-*O*-methyl-D-erythrose (X). The product from 6-*O*-methyl-D-glucopyranose (XI) appeared to be 3-*O*-methyl-D-glyceraldehyde (XIII) since a substance of similar R_f value was obtained from 2-*O*-methylsorbitol (XIV) (compare chromatograms 5 and 6 in Fig. 1). This substance should yield 2-*O*-methyl-L-glyceraldehyde (XV). 5-*O*-Methylglucose was not tested.



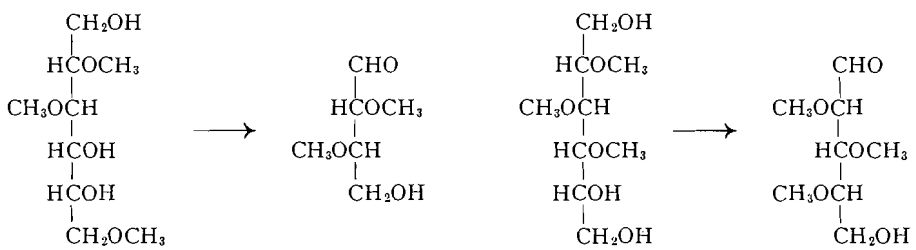
XIV XV

Inspection of chromatograms 2 to 5 in Fig. 1 shows that the method allows a ready and definite identification of a pure mono-*O*-methylglucose which

possesses the methyl group at positions 2, 3, 4, or 6. Furthermore, the method allows the identification of these substances in the presence of one another except, as noted above, for some interference between the 2- and 4-substituted compounds.

The potential value of the method is illustrated by the following application to the methylglucoses derived from a technical cold-water-soluble *O*-methylcellulose. The *O*-methylcellulose was completely hydrolyzed (see Fig. 1, 1) and the hydrolyzate was fractionated by means of preparative paper chromatography to yield the fractions of mono-, di-, and tri-*O*-methylglucoses. The tri-*O*-methylglucose fraction crystallized immediately to yield 2,3,6-tri-*O*-methyl-D-glucose. Application of the procedure to the mono-*O*-methylglucose fraction showed clearly the presence of the 2-, 3-, and 6-substituted glucoses. Although it could undoubtedly be accomplished, no attempt was made to put the method on a quantitative basis for analysis. Nevertheless, a comparison of chromatogram 7 with chromatograms which contained varying amounts of the products from the individual mono-*O*-methylglucoses indicated that the ratio of mono-*O*-methylglucoses was approximately 10:1:5 for the glucoses substituted at positions 2, 3, and 6, respectively. This conclusion is in agreement with the conclusions reached by several investigators (18) that, at least under certain conditions of methylation, the 2-position of cellulose is considerably more reactive than the primary position and that the 3-position is quite unreactive (11). On the basis that the 2- and 6-positions are much more reactive toward methylation than the 3-position, it must be expected that the main di-*O*-methylglucose in the *O*-methylcellulose hydrolyzate would be the 2,6-di-*O*-methylglucose. Inspection of chromatogram 9 of Fig. 1 leaves little doubt that this was the case and that only relatively small amounts of the 2,3- and 3,6-di-*O*-methylglucoses were present. Timell (19) has established analytical methods for determining the distribution of the methyl groups in a partially methylated cellulose (20).

The method should be of value as an aid in the identification of methylated sugars. For example, it could be shown in a very simple manner that the tri-*O*-methylglucose derived from the *O*-methylcellulose hydrolyzate was 2,3,6-tri-*O*-methylglucose. Only a few milligrams of material were required. The substance was unchanged by the periodate treatment (4). This result obviously eliminated 3,4,6-tri-*O*-methylglucose. The substance was reduced with sodium borohydride and the tri-*O*-methylsorbitol was subjected to the procedure. The reducing substance formed (see Fig. 1, 10) must be 2,3-di-*O*-



XVI XVII

methyl-L-threose (XVI). 2,3,4-Tri-*O*-methyl-D-glucose would have yielded 2,3,4-tri-*O*-methyl-L-xylose (XVII) of higher R_f value and 2,4,6-tri-*O*-methyl-D-glucose would have resulted in the periodate resistant 2,4,6-tri-*O*-methylsorbitol.

EXPERIMENTAL

Materials

The 2-*O*-methyl-β-D-glucopyranose (10, 2), 3-*O*-methyl-α-D-glucopyranose (7, 8), and 6-*O*-methyl-α-D-glucopyranose (9) used in this work were pure crystalline compounds. Although the 4-*O*-methyl-D-glucopyranose was a sirup (14), it was prepared from pure crystalline 4-*O*-methyl-D-glucose dibenzyl mercaptal (17).

Periodate Oxidations

The *O*-methylglucose or *O*-methylglucose mixture, 1 mgm., was dissolved at 0°C. in 0.120 ml. of 0.5 *N* sodium metaperiodate solution. After one hour at 0°C., 2–3 mgm. of ethylene glycol was added and the solution was warmed to room temperature. After five minutes the solution was made alkaline to phenolphthalein by titration with 0.5 *N* sodium hydroxide solution to a stable pink end point. After five minutes, the solution, 2–30 cu.mm., was applied by means of a microburette to Whatman No. 1 paper for chromatography. The spots were kept to about 6 mm. in diameter. The same procedure was used for the *O*-methylsorbitols.

Chromatography

A descending chromatographic procedure was used with the butanol–ethanol–water system (6). Aniline phthalate solution was used as spraying reagent (15). The results obtained are summarized in Fig. 1. Table I lists the sensitivity of the color test for the products formed from the mono-*O*-methylglucoses.

TABLE I

SENSITIVITY OF THE ANILINE PHTHALATE REAGENT IN DETECTING THE PERIODATE OXIDATION PRODUCTS FROM THE MONO-*O*-METHYLGLUCOSES

Mono-*O*-methylglucose oxidized	Color of spot	Approximate R_f	Sensitivity in μgm. of *O*-methylglucose
2-Methyl	Lemon yellow	0.22	2–3
3-Methyl	Plum	0.35	2–3
4-Methyl	Citrine	0.53	12–15
6-Methyl	Canary yellow	0.71	5–7

O-Methylsorbitols

The *O*-methylglucose, 25 mgm., and 10 mgm. of sodium borohydride were dissolved in 0.2 ml. of water. After one hour at room temperature, the excess sodium borohydride was destroyed with acetic acid and the solution was deionized in the usual manner (21). The products from the 2- and 3-mono-*O*-

methyl-D-glucoses and from the 2,3,6-tri-*O*-methyl-D-glucose were nonreducing sirups.

Hydrolysis of O-Methylcellulose

A technical *O*-methylcellulose, soluble in cold water, 26.3% methoxyl, 1.56 methyl groups per glucose residue, 1.0 gm., was dissolved in 25 ml. of concentrated hydrochloric acid, and the solution was left at room temperature for 24 hr. Water, 100 ml., was added and the clear solution was heated on the steam bath for 1.5 hr. The nearly colorless solution was evaporated *in vacuo* below 40°C. to a sirup which was dissolved in 25 ml. of water. The solution was left at room temperature overnight before the remaining hydrochloric acid was removed by passage through a column of Amberlite IR4B. Evaporation gave 1.0 gm. of colorless sirup. Chromatogram 1 of Fig. 1 shows that hydrolysis was complete.

Preparative Paper Chromatography

The *O*-methylglucoses in the above *O*-methylcellulose hydrolyzate were separated into the mono-*O*-methyl-, di-*O*-methyl-, and tri-*O*-methylglucose fractions by preparative partition chromatography (13). A 20% aqueous solution of the sirup was streaked across 47 × 57 cm. sheets of Whatman No. 3 MM paper using a mechanical device which ensured a uniform distribution over the whole length of the streak. In this way 100–120 mgm. of the sirup was applied to each sheet. The chromatograms were developed as described above. The positions of the bands were located by spraying strips cut out from each end of the sheets. The *O*-methylglucoses were extracted from the paper strips simply by stirring in water.

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