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# Oxidation of Carbohydrate Derivatives with Silver Carbonate on Celite. XIII. Oxidation of Methyl Ethers of D-Fructose

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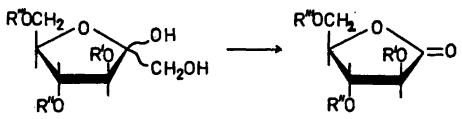
3,4,6-Tri-, 3,4-di- and 3-*O*-methyl-D-fructose are cleaved by silver carbonate on Celite in methanol between C-1 and C-2 to give methyl ethers of D-arabinolactone. 1,4,6-Tri-, 1,4,5-tri- and 1-*O*-methyl-D-fructose are cleaved between C-2 and C-3 by the oxidant whereas the 1,3,4,6-tetra- and 1,3,4-tri-*O*-methyl derivatives resist oxidation. Cleavage between C-2 and C-3 is found to occur more readily than between C-1 and C-2. The oxidant is suggested as a reagent for identification of *O*-methylfructoses in combination with chromatographic methods, and its applicability is demonstrated on the *O*-methylsugars from permethylated inulin and levan.

The oxidation of 2-ketoses with silver carbonate on Celite in methanol causes initial cleavage of the C-2/C-3 bond, leading to glycolic esters of aldoses with two carbon atoms less than the parent ketose.[1] No significant cleavage of the bond between C-1 and C-2 has been observed. Since 1,3-dihydroxy-2-propanone has been found to undergo oxidation to methyl glycolate in methanol with this reagent,[2] it seemed reasonable that an analogous cleavage between C-1 and C-2 would occur in higher 2-ketoses with a 3-*O*-substituent to prevent C-2/C-3 bond cleavage. It was considered of interest to examine the effect of varying *O*-methyl substitution in fructose on the reactivity towards silver carbonate on Celite, and also to investigate the applicability of the oxidant in the identification of fructose methyl ethers. Several *O*-methylfructoses were therefore subjected to treatment with silver carbonate on Celite in methanol, and the results are reported in this paper.

## RESULTS AND DISCUSSION

1,3,4,6-Tetra- (*1*) and 1,3,4-tri-*O*-methyl-D-fructose (*2*), both lacking a free vicinal glycol function involving the anomeric hydroxyl group, were found to resist oxidation. 3,4,6-Tri- (*3*),



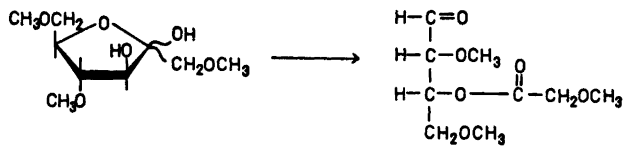
| | | | |
|---|---|---|---|
| *3* | $R' = R'' = R''' = CH_3$ | | *6* |
| *4* | $R' = R'' = CH_3$, | $R''' = H$ | *7* |
| *5* | $R' = CH_3$, | $R'' = R''' = H$ | *8* |

3,4-di- (*4*) and 3-*O*-methyl-D-fructose (*5*) were oxidized to methyl ethers of D-arabinolactone (*6*, *7* and *8*, respectively) by cleavage of the C-1/C-2 bond. These compounds needed a higher reaction temperature, 55 – 65 °C, than the unsubstituted ketose, which is slowly oxidized at room temperature in methanol.

*Table 1.* Time required for 50 % oxidation of *O*-methylfructoses in methanol.

| Compound | Time/min |
|---|---|
| D-Fructose [a] | 1.5 |
| 1-*O*-Methyl-D-fructose (*9*) [a] | 4 |
| 3-*O*-Methyl-D-fructose (*5*) [a] | 10 |
| 1,4,6-Tri-*O*-methyl-D-fructose (*10*) [b] | 4 |
| 1,4,5-Tri-*O*-methyl-D-fructose (*11*) [b] | 27 |

[a] At 60 °C. [b] At 55 °C.



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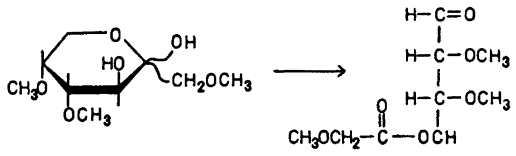
This is not unexpected in light of the earlier finding that C-2/C-3 bond cleavage occurred almost exclusively in the unsubstituted 2-ketoses.

In accordance with these results, it was also found that 1-*O*-methyl-D-fructose (*9*) was oxidized more rapidly at 55 °C in methanol than 3-*O*-methyl-D-fructose (*5*) (Table 1). Chromatography and electrophoresis showed that the product obtained on complete oxidation and hydrolysis of 1-*O*-methyl-D-fructose (*9*) was glyceraldehyde. At the reaction temperature required to oxidize *9*, further degradation occurs of the initially formed erythrose derivative, in contrast to the results obtained on oxidation of the unsubstituted ketose at room temperature.[1]

As expected, 1,4,6-tri-*O*-methyl-D-fructose (*10*) was oxidized more rapidly than the 3-*O*-substituted derivatives, the reaction being completed within 50 min at 45 °C. To obtain information about the difference in the rate of cleavage between C-2 and C-3 in furanose and pyranose derivatives, the rates of oxidation of 1,4,6- (*10*) and 1,4,5-tri-*O*-methyl-D-fructose (*11*) in methanol at 60 °C were determined (Table 1). Since D-fructose is presumably oxidized mainly in the furanose form,[1] it is not unexpected to find that the 1,4,6-tri-*O*-methyl derivative (*10*), which exists in furanose form, is oxidized most rapidly.

The product obtained on oxidation of 1,4,6-tri-*O*-methyl-D-fructose (*10*) at 45 °C was identified as 3-*O*-(methoxyacetyl)-2,4-di-*O*-methyl-*aldehydo*-D-erythrose (*12*). No attempts were made to identify the product from 1,4,5-tri-*O*-methyl-D-fructose (*11*), but a single component



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was apparently present; the infrared spectrum differed only slightly from that of 3-*O*-(methoxyacetyl)-2,4-di-*O*-methyl-*aldehydo*-D-erythrose (*12*), and it is most likely the analogously derived 4-*O*-(methoxyacetyl)-2,3-di-*O*-methyl derivative (*13*).

The lack of reactivity of 1,3-di-*O*-substituted fructoses and the differences in the nature of the products from methyl ethers with the hydroxyl group at C-1 free and that at C-3 substituted and the products from the methyl ethers having their C-3 hydroxyl group free, suggested the possible application of the oxidant in the identification of *O*-methylfructoses. Periodate has been applied earlier in analogous oxidations of 3,4,6-[3] and 1,4,6-tri-*O*-methyl-D-fructose[4] for the identification of these compounds. The silver carbonate oxidation should, because of its simplicity, be well-suited for identification purposes, and TLC, with the application of appropriate spray reagents, makes possible a ready differentiation between unoxidized methyl ethers of fructose, methyl ethers of arabinolactone and esterified methyl ethers of erythrose.

2,3,5-Tri- (*6*) and 2,3-di-*O*-methyl-D-arabinolactone (*7*), as well as unoxidized 1,3,4,6-tetra- (*1*) and 1,3,4-tri-*O*-methyl-D-fructose (*2*), are well-separated by GLC, having retention times of 0.60, 0.72, 0.84 and 0.89, respectively, relative to 2,3,4,6-tetra-*O*-methyl-D-glucose (*14*). The *O*-methylfructoses obtained in methylation analyses of the known types of fructans are 1,3,4,6-tetra- (*1*), 1,3,4-tri- (*2*), 3,4,6-tri- (*3*), and 3,4-di-*O*-methyl-D-fructose (*4*). A common problem in the methylation analysis of fructans consists in distinguishing between *2* and *3*, resulting from levan and inulin type polysaccharides, respectively, on methylation and hydrolysis. To illustrate the application of the silver carbonate oxidation – GLC combination in solving this problem, a levan, isolated from grass (*Poa trivialis* L.), and inulin were methylated, and the *O*-methylsugar mixtures obtained on hydrolysis were subjected to oxida-

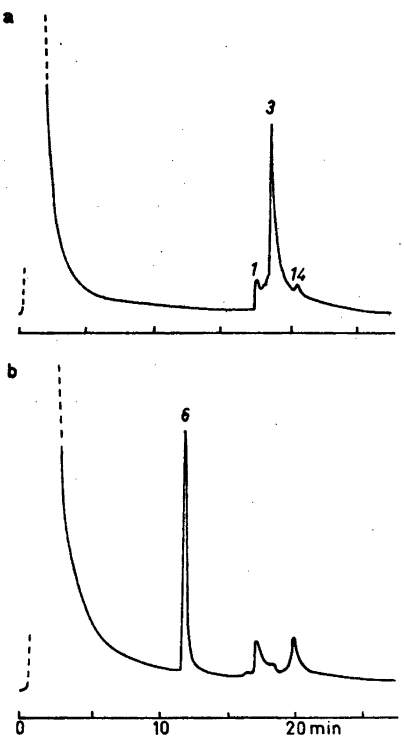


*Fig. 1.* GLC of the *O*-methylsugars from methylated inulin. a, before oxidation; b, after oxidation. *1*, 1,3,4,6-Tetra-*O*-methyl-D-fructose; *3*, 3,4,6-tri-*O*-methyl-D-fructose; *14*, 2,3,4,6-tetra-*O*-methyl-D-glucose; *6*, 2,3,5-tri-*O*-methyl-D-arabino-1,4-lactone. For conditions, see experimental.

tion with silver carbonate on Celite in boiling methanol for 20 min, and direct GLC analysis of the solution. The gas chromatograms obtained before and after oxidation of the *O*-methylsugars from inulin are shown in Fig. 1. The gas chromatograms from the hydrolysate of methylated levan were identical before and after oxidation because of the resistance of 1,3,4-tri-*O*-methyl-D-fructose towards the oxidant.

GLC separation of the acetates of unreduced 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose has been reported.[5] Borohydride reduction and subsequent trifluoroacetylation also give separable derivatives.[6] GLC-MS analysis of the acetates of borodeuteride reduced 1,3,4- and 3,4,6-tri-*O*-methyl-D-fructose also allows differentiation between these compounds.[5] Silver carbonate oxidation in combination with GLC, is rapid and simple compared to these methods. More work is, however, necessary to establish its possible application in analysis of fructans, in particular with regard to the determination of relative amounts of the *O*-methylsugars.

## EXPERIMENTAL

Paper electrophoresis was performed on Whatman No. 1 paper in borate buffer at pH 10 and TLC on Silica gel G in (A) benzene–ethanol 5:1 and (B) benzene–ethanol 3:1 (v/v). Reducing sugars were detected with diphenylamine–aniline–phosphoric acid,[7] esters and lactones with hydroxylamine–ferric chloride.[8] A Perkin-Elmer F-11 gas chromatograph, equipped with a flame ionization detector and a glass column packed with SE-30 (3 %) on Chromosorb G AW-DMCS was used for GLC. The nitrogen flow rate was 20 ml/min, and the column temperature was programmed from 90 to 160 °C at 3 °C/min.

*Oxidation of 3,4,6-tri-*O*-methyl-*D*-fructose (3).* 3,4,6-Tri-*O*-methyl-D-fructose (*3*, 50 mg) in methanol (30 ml) was stirred at reflux temperature with silver carbonate on Celite (1.5 g) for 1 h. The solution was filtered and the solvent evaporated under reduced pressure to give syrupy 2,3,5-tri-*O*-methyl-D-arabino-1,4-lactone (*6*) (35 mg, 81 %), indistinguishable from an authentic sample [3] by GLC and TLC (solvent A). IR ($CHCl_3$): 1785 $cm^{-1}$ (s) (C=O, 1,4-lactone), identical with the spectrum of the authentic specimen, except for a small additional shoulder on the carbonyl stretching band (1740 $cm^{-1}$), $[\alpha]_D$ +20° (*c* 1, water) (lit.[3] +24°). TLC in addition showed the presence of traces of a second, unidentified product.

*Oxidation of 3,4-di-*O*-methyl-*D*-fructose (4).* 3,4-Di-*O*-methyl-D-fructose (*4*, 35 mg) in methanol (15 ml) was stirred with silver carbonate on Celite (1 g) at reflux temperature for 45 min. The solution was filtered, and the solvent was evaporated at reduced pressure affording 2,3-di-*O*-methyl-D-arabinolactone (*4*) (22 mg, 74 %). The product was homogeneous by GLC and TLC (solvent A), and indistinguishable from an authentic sample, prepared by bromine oxidation of 2,3-di-*O*-methyl-D-arabinose. IR ($CHCl_3$): 1785 $cm^{-1}$ (s) (C=O, 1,4-lactone), 1740 $cm^{-1}$ (w), identical with the spectrum of authentic 2,3-di-*O*-methyl-D-arabinolactone.

*Oxidation of 3-*O*-methyl-*D*-fructose (5).* 3-*O*-Methyl-D-fructose (*5*, 115 mg) in methanol (45 ml) was stirred at 55 °C with silver carbonate on Celite (3 g) for 30 min. The solution was filtered and the solvent removed under reduced pressure. The residue (88 mg) was treated with small amounts of acetone to give crystalline 2-*O*-methyl-D-arabino-1,4-lactone (*8*) (69 mg, 70 %), m.p. 84–85 °C (lit.[9] 87°) $[\alpha]_D$ +49° (*c* 1, water) (lit.$_0$ +52.7°).

*Oxidation of 1-O-methyl-D-fructose (9).* 1-*O*-Methyl-D-fructose (*9*, 42 mg) in methanol (17 ml) was stirred at 55 °C with silver carbonate on Celite (1.7 g) for 15 min. The solution was filtered, the solvent evaporated under reduced pressure and the residue hydrolyzed in 0.2 M sulfuric acid for 48 h at room temperature. The solution was neutralized with Dowex 1 ($HCO_3^-$) ion exchanger and the solvent removed under diminished pressure, yielding a syrup (16 mg), which contained as major component a compound indistinguishable from glyceraldehyde by electrophoresis ($M_{Glc}$ 0.75) and TLC (solvent B). As a minor component, the syrup contained some unoxidized 1-*O*-methyl-D-fructose (*9*).

*Oxidation of 1,4,6-tri-O-methyl-D-fructose (10).* 1,4,6-Tri-*O*-methyl-D-fructose (*10*, 36 mg) in methanol (15 ml) was stirred at 45 °C for 50 min with silver carbonate on Celite (1.5 g). TLC (solvent A) indicated the presence of a single component, indistinguishable from 3-*O*-(methoxyacetyl)-2,4-di-*O*-methyl-*aldehydo*-D-erythrose (*12*),[4] detectable on the TLC plate with both spray reagents. Filtration of the solution and evaporation of the solvent under diminished pressure gave a syrup (32 mg, 90 %). IR ($CHCl_3$): 1755 $cm^{-1}$ (s), 1735 $cm^{-1}$ (s), identical with the spectrum of the authentic sample, $[\alpha]_D$ +21° (*c* 1, methanol) (lit.[4] +18°).

*Oxidation of 1,4,5-tri-O-methyl-D-fructose (11).* 1,4,5-Tri-*O*-methyl-D-fructose (*11*, 25 mg) in methanol (10 ml) was stirred with silver carbonate on Celite (1 g) for 1 h at 55 °C. TLC (solvent A) indicated the presence of a single product, detectable with both spray reagents. Filtration of the solution and evaporation of the solvent gave a syrup. IR ($CHCl_3$): 1755 $cm^{-1}$ (s), 1735 $cm^{-1}$ (s), the spectrum differed only slightly from that of *12*.

*Comparison of reactivity of the mono-O-methyl-D-fructoses and D-fructose.* The sugars (25 mg) in methanol (10 ml) were stirred at 55 °C with silver carbonate on Celite (1 g). Aliquots (25 µl) were withdrawn at intervals, and unoxidized sugar present determined colorimetrically with the thiobarbituric acid reagent,[10] the results are shown in Table 1.

*Comparison of reactivity of 1,4,6- (10) and 1,4,5-tri-O-methyl-D-fructose (11).* The tri-*O*-methylfructoses (25 mg) in methanol (10 ml) were stirred at 60 °C with silver carbonate on Celite (1 g). Aliquots (1 ml) were withdrawn at intervals, and the amount of carboxylic ester formed was determined by the hydroxamic acid reaction,[11] the results are shown in Table 1.

*Treatment of 1,3,4,6-tetra- (1) and 1,3,4-tri-O-methyl-D-fructose (2) with silver carbonate on Celite.* The methyl fructoses (*1* and *2*) were stirred with silver carbonate on Celite at 65 °C in methanol for 45 min as described for the other *O*-methylfructoses. Starting material exclusively could be observed by TLC (solvent A).

*Methylation of the fructans.* The water-soluble levan from *Poa trivialis* L.,[12] and a commercial sample of inulin were methylated according to Hakomori.[13] The methylated fructans were hydrolyzed in 60 % aqueous formic acid at 90 °C for 1 h and subsequently, after removal of the formic acid and water under reduced pressure, the hydrolysis was completed in 0.01 M sulfuric acid for 45 min at 90 °C. The solutions were neutralized with Dowex 1 ($HCO_3^-$) ion exchanger, and the solvent was removed at diminished pressure.

*Oxidation of O-methylsugars from the methylated fructans.* The *O*-methylsugars (about 2 mg), resulting from hydrolysis of the methylated fructans, were stirred in boiling methanol (1 ml) with silver carbonate on Celite (0.2 g) for 20 min, and samples were injected on the gas chromatograph directly from the solutions. Immediate injection was found to be of importance, since a secondary, unidentified product gradually appeared when the solution containing the *O*-methylsugars from inulin was kept for some time at room temperature, possibly due to methanolysis of the lactone.

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## REFERENCES

1. Morgenlie, S. *Acta Chem. Scand. 27* (1973) 1557.
2. Morgenlie, S. *Acta Chem. Scand. 27* (1973) 3009.
3. Hirst, E. L., Mitchell, W. E. A., Percival, E. E. and Percival, E. G. V. *J. Chem. Soc.* (1953) 3170.
4. Wickström, A., Courtois, J. E., Le Dizet, P. and Archambault, A. *Bull. Soc. Chim. Fr.* (1959) 871.
5. Lindberg, B., Lönngren, J. and Thompson, J. L. *Acta Chem. Scand. 27* (1973) 1819.
6. Kubberød, G. *Acta Chem. Scand. 26* (1972) 2544.
7. Schwimmer, S. and Bevenue, A. *Science 123* (1956) 543.
8. Abdel-Akher, M. and Smith, F. *J. Am. Chem. Soc. 73* (1951) 5859.
9. Schmidt, O. T. and Simon, A. *J. Prakt. Chem. 152* (1939) 190.
10. Percheron, F. *C. R. Acad. Sci. Ser. C 255* (1962) 2521.
11. Goddu, R. F., LeBlanc, N. F. and Wright, C. M. *Anal. Chem. 27* (1955) 125.
12. Challinor, S. W., Haworth, W. N. and Hirst, E. L. *J. Chem. Soc.* (1934) 1560.
13. Hakomori, S. *J. Biochem. (Tokyo) 55* (1964) 205.

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