

Separation of Methylated Sugars on Carbon Columns

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The fractionation of mixtures of methylated sugars is a problem of great importance in structural polysaccharide chemistry. The distillation techniques, developed and successfully employed by the Haworth school, have been replaced in recent years by chromatographic methods. Partition chromatography on silicagel columns¹, paper strips and cellulose columns² has proved to be of great value in the separation of methylated sugars, but often fails to separate isomeric substances.

Following the observation that mannitol monoacetate moves at about the same rate as a disaccharide on a carbon column³, we have investigated the possibility of separating methylated sugars by this means**.

Initially, the separation of glucose, 3-*O*-methyl-glucose, 2,3-di-*O*-methyl glucose, 2,3,6-tri-*O*-methyl-glucose and 2,3,4,6-tetra-*O*-methylglucose was investigated. A mixture of these sugars, 0.5 g of each, was added to the top of a carbon-Celite column (20 × 3 cm) and subjected to linear gradient elution with aqueous ethanol. The optical rotation-volume curve (Fig. 1 a) indicated a good separation of the substances, which were recovered chromatographically pure and with the exception of the tetramethyl ether, in good yields, by combining and concentrating suitable fractions. Between the maxima, corresponding to the di- and trimethyl ethers, there is a small peak obviously due to an impurity in the dimethyl ether, as our sample was known to be not pure. The optical rotation of the dimethyl ether used, agrees fairly well with that of the sum of the dimethyl ether and the impurity fractions. Thus no material appears to have been lost or modified during the separation. The gradient chosen seemed suitable for the first four substances but the tetramethyl-glucose was spread over a rather large volume, which is inconvenient. In order to elute the tetra-ether in a smaller volume, a steeper gradient was chosen for this concentration range (Fig. 1 b).

A disadvantage of aqueous ethanol as eluant is the increase in viscosity with concentration causing a retardation in the rate of flow through the

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** During the course of this investigation we learnt from Dr. W. J. Whelan of Bangor that he and Mr. Morgan are pursuing similar independent studies. Dr. Whelan kindly sent us the manuscript of their publication⁴, and where the investigations overlap, the results agree.

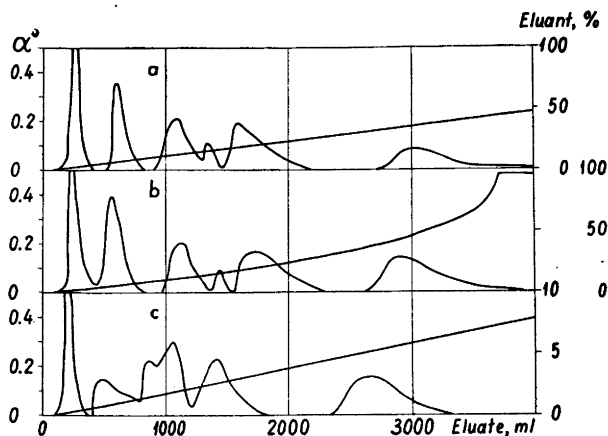


Fig. 1. Chromatographic separations of glucose and some of its methyl ethers. The peaks, from left to right, correspond to glucose, 3-*O*-methyl-glucose, 2,3-di-*O*-methyl-glucose, an unknown impurity, 2,3,6-tri-*O*-methyl-glucose and 2,3,4,6-tetra-*O*-methyl-glucose.

column. Alm⁵ has suggested the use of ethyl methyl ketone, which he found to be about five times more efficient than ethanol as an eluant. A run with this solvent showed that the flow was almost independent of the concentration in the range selected. The tri- and tetra-ethers were separated well, but for the lower members the picture was complicated (Fig. 1 c). This may be due to strong adsorption of the ketone itself upon the column, so that its concentration in the initially weak solution is reduced considerably, but when the column is almost saturated it breaks through as a steep front.

The next step was to investigate whether isomeric, methylated sugars could be separated in the same way, and for this, the pair 2,3,6- and 2,3,4-tri-*O*-methyl-glucose were chosen. For the second substance, which is a non-crystalline syrup, different values for the specific rotation between 43—67° (water), are recorded in the literature. According to Irvine and Oldham⁶, the substance cannot be purified by distillation. We prepared the substance by acid hydrolysis of 2,3,4-tri-*O*-methyl-levoglucosan, and obtained a product with the specific rotation of +86° in water. This high value and a satisfactory analysis suggest that our sample has a higher degree of purity than previous preparations. For the separation of the two trimethyl ethers (0.4 g of each) a larger column (43 × 3.5 cm) was selected and ethyl methyl ketone used as eluant. A complete separation was achieved (Fig. 2) and the substances were recovered pure, in almost quantitative yields.

From the results of the present investigation it is evident that chromatography on carbon columns offers a good method for the separation of monosaccharides with different degrees of methylation. One advantage over other methods is the high capacity of the carbon columns, thus permitting the quantitative isolation of a minor component of a mixture in reasonable amounts, e.g. a low percentage of a dimethyl hexose from the hydrolysate of a

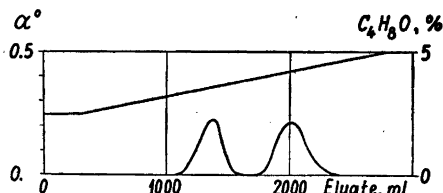


Fig. 2. Separation of 2,3,6-tri-O-methylglucose (left peak) and 2,3,4-tri-O-methylglucose (right peak) on a carbon column.

methylated polysaccharide. It is also evident from our experiments with the two trimethyl ethers that the method may be useful in the separation of isomeric methylated sugars.

EXPERIMENTAL

Substances (All melting points uncorrected)

Glucose. Merck & Co. analytical reagent $[\alpha]_D^{20} + 52.5^\circ$ (water, $c = 2$).

3-O-Methylglucose was prepared according to Glen, Myers and Grant⁷, except that the methylation of diisopropylidene glucose was performed in dry dioxane as described below for 2,3,4-tri-O-methyllevoglucosan. Yields were somewhat lower than those obtained by Glen *et al.* M. p. 159–161°, $[\alpha]_D^{20} + 55.5^\circ$ (water, $c = 2$).

2,3-Di-O-methylglucose was prepared according to Evans *et al.*⁸ but methylation of 4,6-benzylidene- α -methyl glucoside was performed with methyl sulphate and powdered sodium hydroxide in dry dioxane (yield of methylated derivative 80 % of theory), and further the hydrolysis to 2,3-di-O-methylglucose of the product so obtained was made in one step, omitting the isolation of the corresponding methyl glucoside. The product, however, was noncrystalline and was shown by hypiodite oxidation⁹ to contain 83 % reducing sugar calculated as dimethyl ether. The existence of one impurity with high optical rotation is revealed by the chromatographic experiments. $[\alpha]_D^{20} + 60.8^\circ$ (water, $c = 2$).

2,3,6-Tri-O-methyl glucose. A sample prepared from methylated starch was purified by crystallisation from isopropyl ether-chloroform. M. p. 112–116°, $[\alpha]_D^{20} + 67^\circ$ (water, $c = 2$).

2,3,4-Tri-O-methyl glucose was prepared by hydrolysis of levoglucosan trimethyl ether⁸ using a slight modification of previous methods. In a flask fitted with a reflux condenser and an effective stirrer, containing levoglucosan (8 g), powdered sodium hydroxide (24 g), and dry peroxide-free dioxane (150 ml), methyl sulphate (29 ml) was slowly added with vigorous stirring. The temperature was raised to 60° and kept there during the addition of the methyl sulphate (1 hour) and for the following 5 hours. After standing at room temperature overnight the mixture was filtered, care being taken not to let the filter cake crack. The residue was washed with dry benzene and the dioxane-benzene solution concentrated. The residue was distilled *in vacuo*. B. p. 8 mm 127–128° (7.5 g) and 127–132° (1.7 g), mobile oils. After a few hours the remainder in the distillation flask crystallised, and after inoculation the distillates also were obtained crystalline. M. p. 58–60° (first fraction) and 55–58° (second fraction). Total yield was 9.2 g or 91 % of theory.

For the preparation of 2,3,4-tri-O-methylglucose trimethyl levoglucosan (2.5 g) was dissolved in 1 *N* sulphuric acid (50 ml) and the solution heated on a boiling water bath for 17 hours. The aqueous solution obtained by neutralisation of the hydrolysate with BaCO₃ and filtration (90 ml) was extracted with chloroform in 10 ml portions until a drop of the chloroform phase gave a negligible residue on evaporation (9 extractions). From the combined chloroform solutions trimethyl levoglucosan (0.65 g), m. p. 58–60°, was recovered.

The extracted aqueous solution was concentrated under reduced pressure, the residue extracted with chloroform and the chloroform solution after filtration concentrated to dryness *in vacuo*, yielding 2,3,4-tri-O-methyl glucose (1.76 g) as a faintly yellow syrup. $[\alpha]_D^{20} + 86^\circ$ (water, $c = 2.3$).

The purity of the trimethyl glucose was estimated at 99 % by hypiodite oxidation. *2,3,4,6-Tetra-O-methyl glucose*. A sample, m. p. 76–90°, was recrystallised twice from light petroleum (b.p. 90–100°), but no change in the melting point could be observed. However, according to hypiodite oxidation data the product was 100 % pure. $[\alpha]_D^{20} + 82.5^\circ$ (water, $c = 2$).

Procedure

Columns. Equal parts of animal charcoal "zur Analyse" (J. D. Riedel-E. de Haen AG) and Celite No 535 (Johns-Manville) were mixed and treated with conc. hydrochloric acid. The mixture was then washed with large quantities of water and ethanol and then poured into the columns as a thick slurry. Two columns, with the dimensions 20 × 3 cm and 43 × 3.5 cm, were used in this investigation. The adsorbent in the first column was treated with cold, that in the second with boiling hydrochloric acid. This treatment probably involves a desirable deactivation of the carbon, in addition to the removal of inorganic material.

Gradient elution. Two open, cylindrical flasks, connected by a siphon, were filled to hydrostatical equilibrium with the required initial and final concentrations of the eluant. The content of the flask containing the lower concentration was slowly stirred and siphoned directly on to the column, three meters below the flasks. All connections were made of glass or polyethene tubings. When the two flasks have the same diameter a linear gradient is obtained, and by altering the diameter ratio, gradients of different types may be obtained. A more detailed treatment of gradient elutions will be published elsewhere.

Separation experiment. In the first three runs, the small column (20 × 3 cm) was used. The substances to be separated, in 10 % aqueous solutions, were added to the top of the column, washed down with a small amount of water and the elution started. Fractions of equal volume (26.5 ml) were collected, and the optical rotation of the fractions determined (2 dm tube). Appropriate fractions were combined and concentrated to dryness under reduced pressure. The amount of sugars present was determined by hypiodite titration^a and by determination of the optical rotation. The purity was checked by paper chromatography. The optical rotation-volume curves are given in Fig. 1 and the actual data for the separations in Table 1.

Table 1. Separation of glucose, 3-mono, 2,3-di, 2,3,6-tri and 2,3,4,6-tetra-O-methylglucose.

| | | Glucose | Mono- | Di- ^a | Tri- | Tetra- | |
|---|------------|--------------|-------|------------------|------|--------|-----|
| Flask A 2 500 ml 1% EtOH ^b | Added mg | 500 | 443 | 492 | 511 | 500 | |
| Flask B 2 500 ml 60% EtOH Diameter ratio A : B = 1 | Recovered% | By rotation | 97 | 100 | 95 | 86 | |
| | | By titration | 96 | 96 | 100 | 99 | 89 |
| Flask A 2 620 ml 1% EtOH | Added mg | 499 | 497 | 456 | 501 | 497 | |
| Flask B 940 ml 96% EtOH Diameter ratio A : B = 1.8 | Recovered% | By rotation | 98 | 100 | 95 | 102 | 98 |
| | | By titration | 97 | 96 | 95 | 98 | 98 |
| Flask A 2 500 ml water | Added mg | 499 | 493 | 490 | 503 | 494 | |
| Flask B 2 500 ml 10% C ₄ H ₈ O Diameter ratio A : B = 1 | Recovered% | By rotation | 98 | — | — | 96 | 96 |
| | | By titration | 93 | — | — | 100 | 100 |

^a) As mentioned above this substance was not pure. The figures given in this column are based upon rotation and titration determinations on the impure starting material, and include the almost completely separated impurity.

^b By volume.

In the last run, 2,3,6- and 2,3,4-tri-*O*-methyl-glucose, 410 mg and 392 mg respectively, were dissolved in 2.5 % aqueous ethyl methyl ketone (10 ml), added to the top of the larger column (43 × 3.5 cm), prewashed with 2.5 % ethyl methyl ketone and the sugars washed down with the same solvent mixture. The eluant was taken from two flasks with the same diameter, containing 1 500 ml of 2.5 % and 5.5 % aqueous ethyl methyl ketone and the eluate worked up as described above. The recovery of chromatographically pure starting materials were, by rotation 97 % and 90 % and by titration 96 % and 95 %, respectively.

SUMMARY

Glucose and a mono-, di, tri and tetra-methyl ether of this sugar have been separated on carbon columns, using the gradient elution method with aqueous ethanol or ethyl methyl ketone as eluents. The isomeric pair 2,3,6- and 2,3,4-tri-*O*-methyl glucose was also separated by the same method. The separations are complete and the recoveries quantitative, hence the method may be of value in structural carbohydrate chemistry.

An improved method for the preparation of 2,3,4-tri-*O*-methylglucose is reported.

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