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# SEPARATION OF METHYL(METHYL O-METHYL-α-D-GLUCOPYRANOSID)URONATES BY GAS CHROMATOGRAPHY

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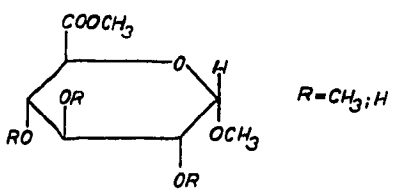
## SUMMARY

Methyl derivatives of methyl(methyl-α-D-glucopyranosid)uronate have been separated without derivatization as well as corresponding per-O-trimethylsilyl, trifluoroacetyl and per-O-acetyl derivatives on columns of different polarity. Good separation was obtained with both of the compounds bearing hydroxyl groups and their per-O-acetates. For the quantitative analysis derivatives are preferred.

## INTRODUCTION

Methylation analysis is an extremely powerful technique for the elucidation of the structures of complex polysaccharides and other carbohydrate-containing substances. The method comprises exhaustive methylation of the unsubstituted alcoholic groups, disruption of the molecule by hydrolysis or methanolysis and identification of the resulting fragments. Among the products of the last step of such a treatment of a glucuronic acid-containing substance, compounds of the type




where R is a hydrogen atom or a methyl group, may be found. The identification of compounds of this class by conventional methods is very laborious and sometimes, owing to the small amount of the material available, almost impossible.

To make the use of the combined gas chromatography–mass spectrometry (GC–MS) technique for the identification of these substances possible, we have attempted to separate methyl(methyl O-methyl-α-D-glucopyranosid)uronates as such, as well as in the form of derivatives commonly used in the gas chromatography of carbohydrates (per-O-trimethylsilyl (TMS), trifluoroacetyl (TFA) and per-O-acetyl (Ac) derivatives). To our knowledge, the separation of these compounds or their derivatives has not been described in the literature so far.

## EXPERIMENTAL

*Derivatives*

Methyl(methyl 2,3,4-tri-O-methyl-α-D-glucopyranosid)uronate (1) was prepared by methylation of the crystalline methyl(methyl 3,4-di-O-methyl-α-D-glucopyranosid)uronate with methyl iodide and silver oxide. The preparation of methyl(methyl 2,3-di-O-, 2,4-di-O-, 3,4-di-O-, 2-O-, 3-O- and 4-O-methyl-α-D-glucopyranosid)uronates (2–7, respectively), was carried out as described elsewhere[1]. Methyl(methyl-α-D-glucopyranosid)uronate (8) was prepared in a similar manner. The details of this synthesis will be published elsewhere.

Silylation of the compounds was carried out as recommended by Sweeley *et al.*[2] and trifluoroacetylation by treatment of compounds 2–8 with trifluoroacetic anhydride[3] without the addition of pyridine to the reaction mixture. The acetates were obtained by an overnight treatment of compounds 2–8 with an excess of acetic anhydride in pyridine followed by concentration of the reaction mixture on a rotary evaporator. These derivatives were injected as solutions in chloroform.

*Apparatus*

The instrument used was a Hewlett-Packard Model 5754 G chromatograph, with a dual column system and a dual ionization detector. The temperature in the injection port and that of the detector were 50° higher than that of the column. Nitrogen was used as the carrier gas.

*Columns and operating conditions*

(1) Stainless steel, 180 × 3.1 mm O.D., packed with 3% OV-17 (Applied

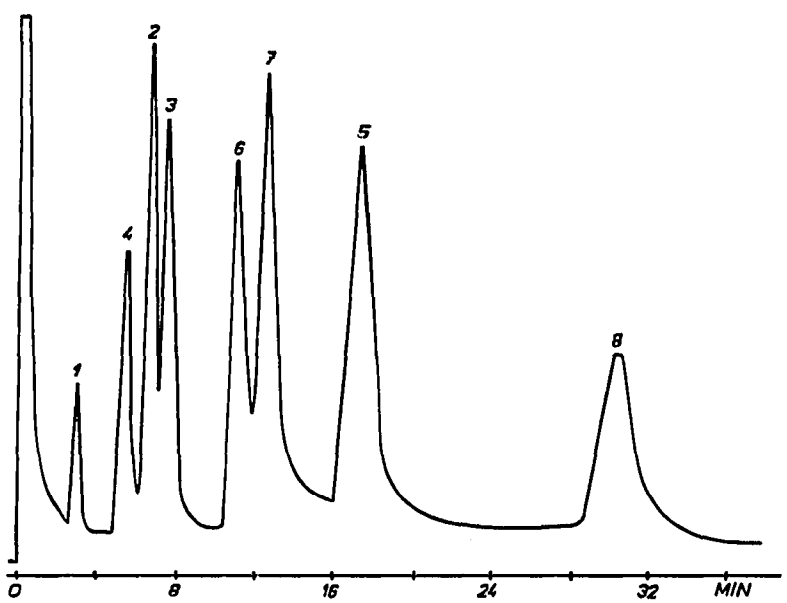



Fig. 1. Separation of methyl(methyl O-methyl-α-D-glucopyranosid)uronates on column 2 at 175°.

Science Labs., State College, Pa., U.S.A.) on Gas-Chrom P Silanizzato, 100–120 mesh (Carlo Erba, Milan, Italy); flow-rate 14 ml/min.

(2) Brass, 120 cm × 3.1 mm O.D., packed with 4% GE-XE-60 (Hewlett-Packard, Avondale, Pa., U.S.A.) on Gas-Chrom Z, 80–100 mesh (Applied Science Labs.); flow-rate 14.1 ml/min.

(3) Aluminium, 180 cm × 3.1 mm O.D., packed with 5% butanediol succinate (BDS) (Lachema, Brno, Czechoslovakia) on Gas-Chrom Z (Applied Science Labs.); flow-rate 13.3 ml/min.

(4) Aluminium, 180 cm × 3.1 mm O.D., packed with 3% ECNSS-M (Applied Science Labs.) on Gas-Chrom Z, 80–100 mesh; flow-rate 14.0 ml/min.

Isothermal conditions were used throughout in order to assure constant bleeding of the columns and therefore a constant background of the mass spectra.

## RESULTS AND DISCUSSION

Conditions were found for the satisfactory separation of compounds 1–8. Fig. 1 shows an example of a separation of this type. Except for the fact that variable amounts of compounds bearing hydroxyl groups are irreversibly retained by the column material, the separation obtained (Fig. 1, Table I) would be satisfactory for

TABLE I

RELATIVE RETENTION TIMES OF METHYL(METHYL O-METHYL-α-D-GLUCOPYRANOSID)URONATES AND THEIR PER-O-ACETYL DERIVATIVES

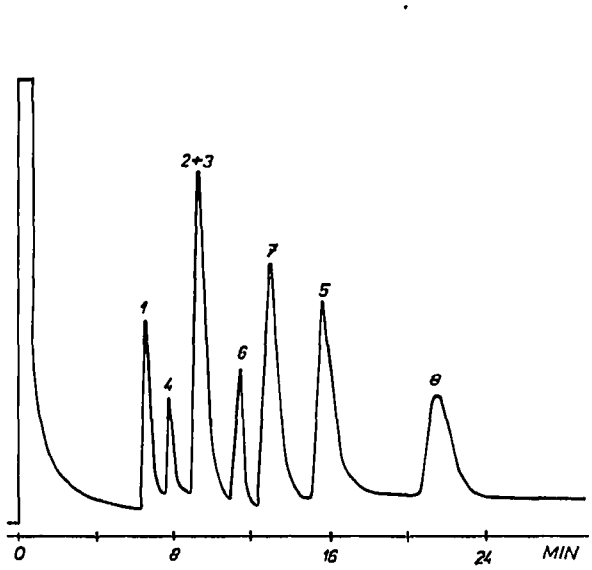
Retention times relative to compound 1. Adjusted retention times of compound 1 (min): a = 2.95; b = 3.07; c = 3.83; d = 1.57; e = 2.96; f = 1.81; g = 3.23; h = 1.57.

| *No.* | *Compound** | *-OMe-OH* | | | | *-OMe-O-Ac* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *OV-17, 170°* | *XE-60, 175°* | *BDS, 190°* | *ECNSS-M, 180°* | *OV-17, 170°* | *XE-60, 170°* | *BDS, 190°* | *ECNSS-M, 180°* |
| 1 | 2,3,4 | 1.00 (a) | 1.00 (b) | 1.00 (c) | 1.00 (d) | 1.00 (e) | 1.00 (f) | 1.00 (g) | 1.00 (h) |
| 2 | 2,3 | 1.46 | 2.25 | 3.03 | 3.21 | 2.47 | 3.30 | 3.10 | 3.04 |
| 3 | 2,4 | 1.46 | 2.37 | 3.46 | 3.61 | 2.87 | 5.04 | 4.22 | 4.92 |
| 4 | 3,4 | 1.11 | 1.81 | 2.24 | 2.36 | 1.72 | 2.00 | 1.90 | 1.90 |
| 5 | 2 | 1.98 | 5.72 | 10.28 | 12.04 | 4.66 | 8.01 | 6.61 | 8.13 |
| 6 | 3 | 1.42 | 3.67 | 6.35 | 7.02 | 4.16 | 6.52 | 5.63 | 6.60 |
| 7 | 4 | 1.54 | 4.21 | 7.19 | 7.82 | 3.50 | 5.92 | 4.71 | 5.60 |
| 8 | 0 | — | 9.41 | — | 27.24 | 5.69 | 10.31 | 7.85 | 10.8 |

* The numbers refer to the positions of the methoxyl groups in methyl(methyl α-D-glucopyranosid)uronate.

quantitative work. It can be seen that compound 8, which contains three hydroxyl groups, was not eluted from two of the four columns used. No measurable peak corresponding to compound 8 appeared within 120 min, although the amount of the mixture injected was close to that of the column capacity with respect to the other components present.

In order to minimize the unwanted irreversible adsorption, the next approach was the use of the less polar and/or much more volatile TMS and TFA derivatives.



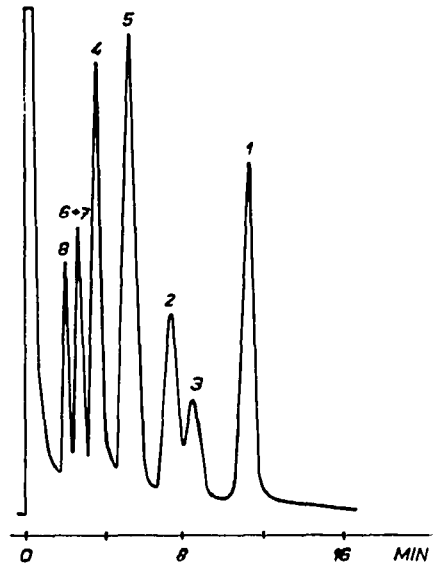




Fig. 2. Separation of methyl(methyl O-methyl-per-O-TMS-α-D-glucopyranosid)uronates on column 1 at 150°.

Fig. 3. Separation of methyl(methyl O-methyl-per-O-TFA-α-D-glucopyranosid)uronates on column 3 at 160°.

These derivatives, however, could not be separated satisfactorily. Typical examples of these separations are shown in Figs. 2 and 3. The respective retention times of compounds 2–8 relative to the adjusted retention time of compound 1 are summarized in Table II. The per-O-acetates, on the other hand, gave excellent separations,

TABLE II

RELATIVE RETENTION TIMES OF METHYL(METHYL O-METHYL-PER-O-TMS- AND PER O-TFA-α-D-GLUCOPYRANOSID)URONATES

Retention times relative to compound 1. Adjusted retention times of compound 1 (min): a = 6.61; b = 9.76; c = 11.1; d = 5.04; e = 14.73; f = 9.61; g = 11.10; h = 12.29.

| *No.* | *Compound** | *-OMe-O-TMS* | | | | *-OMe-O-TFA* | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | *OV-17, 150°* | *XE-60, 130°* | *BDS, 160°* | *ECNSS-M, 150°* | *OV-17, 140°* | *XE-60, 140°* | *BDS, 160°* | *ECNSS-M, 130°* |
| 1 | 2,3,4 | 1.00 (a) | 1.00 (b) | 1.00 (c) | 1.00 (d) | 1.00 (e) | 1.00 (f) | 1.00 (g) | 1.00 (h) |
| 2 | 2,3 | 1.45 | 1.25 | 0.88 | 0.80 | 0.57 | 0.99 | 0.69 | 1.01 |
| 3 | 2,4 | 1.42 | 1.24 | 0.79 | 0.75 | 0.60 | 1.29 | 0.76 | 1.22 |
| 4 | 3,4 | 1.19 | 1.02 | 0.65 | 0.61 | 0.32 | 0.51 | 0.34 | 0.50 |
| 5 | 2 | 2.39 | 1.86 | 0.88 | 0.78 | 0.35 | 1.18 | 0.49 | 0.57 |
| 6 | 3 | 1.73 | 1.31 | 0.59 | 0.59 | 0.12 | 0.57 | 0.25 | 0.44 |
| 7 | 4 | 1.98 | 1.57 | 0.67 | 0.64 | 0.12 | 0.60 | 0.25 | 0.41 |
| 8 | 0 | 3.27 | 2.36 | 0.31 | 0.73 | 0.11 | 0.64 | 0.20 | 0.47 |

* The numbers refer to the positions of the methoxyl groups in methyl(methyl α-D-glucopyranosid)uronate.

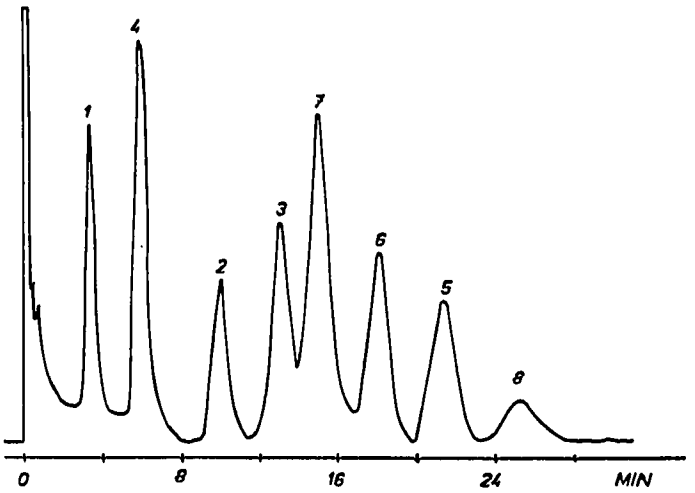



Fig. 4. Separation of methyl(methyl per-O-Ac-methyl-α-D-glucopyranosid)uronates on column 3 at 190°.

apparently with no disadvantages involved in the gas chromatography of the parent compounds 2–8 (Table I), and it is recommended that these derivatives should be used for the identification of methyl(methyl O-methyl-α-D-glucopyranosid)uronates by GC–MS. Fig. 4 shows the record of this analysis.

## REFERENCES

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2 C. C. Sweeley, R. Bentley, M. Makita and W. W. Wells, *J. Amer. Chem. Soc.*, 85 (1963) 2497.
3 J. Shapira, *Nature (London)*, 222 (1969) 792.