

## Note

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### Analysis of mixtures of the common aldoses by gas chromatography-mass spectrometry of their *O*-isopropylidene derivatives

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The usefulness of *O*-isopropylidene derivatives for identification of monosaccharides by mass spectrometry was first shown by DeJongh and Biemann<sup>1</sup>. The advantage of these compounds over acetates and trimethylsilyl ethers is due to the formation from monosaccharides of derivatives that frequently are structural, and not merely configurational, isomers. *O*-Isopropylidene derivatives of most of the common monosaccharides thus give rise to clearly different mass spectra. Despite this advantage and the fact that *O*-isopropylidene derivatives are amenable<sup>1,2</sup> to *glc*, they have not been found suitable for the analysis of complex mixtures of monosaccharides by *glc* or *glc-ms*. This has been explained by the long reaction-time needed for preparation of these derivatives<sup>3</sup> and the dependence of product-mixture composition on reaction conditions for some monosaccharides<sup>1,4,5</sup>. Most of the common aldoses when treated with acetone in the presence of sufficient sulphuric acid give one, or mainly one, product<sup>5</sup>, an exception is ribose<sup>6</sup>. In our experience<sup>7,8</sup>, complete acetonation of aldoses in syrupy mixtures occurs within considerably shorter reaction-times than those usually applied<sup>5,9</sup>.

Thus, treatment of the syrupy mixtures of aldoses with acetone containing 1% of sulphuric acid for 2 h was sufficient for conversion into their *O*-isopropylidene derivatives. Complete separation of the derivatives of fucose, arabinose, xylose, rhamnose, galactose, glucose, and mannose was obtained in 35-40 min by *glc* on XE 60 (Fig. 1) and OV 225. For glucose and rhamnose, a small peak, in addition to the main peak, was observed, and glucose also gave traces of a third product. Some sugars gave small, additional peaks having retention times longer than that of 2,3,5,6-di-*O*-isopropylidene-D-mannofuranose, which is the most strongly retained, main *O*-isopropylidene derivative of the common aldoses. These small peaks do not interfere in the identification of these sugars as *O*-isopropylidene derivatives on the two stationary phases used in the present work. Relative retention times and molar responses are shown in Table I, the retention times of 2,3-*O*-isopropylidene-D-ribose and 1,5-anhydro-2,3-*O*-isopropylidene- $\beta$ -D-ribofuranose are also included as these

derivatives are obtained from D-ribose under conditions similar to those applied in this work<sup>6</sup>.

TABLE I

CHROMATOGRAPHIC DATA FOR THE *O*-ISOPROPYLIDENE-ALDOSES

| Aldose      | Main acetal                                                        | T values <sup>a</sup>                     |                                           | Molar response <sup>b</sup> |
|-------------|--------------------------------------------------------------------|-------------------------------------------|-------------------------------------------|-----------------------------|
|             |                                                                    | OV 225                                    | XE 60                                     |                             |
| L-Fucose    | 1,2 3,4( $\alpha$ )                                                | 0 20                                      | 0 32                                      | 1 24                        |
| L-Arabinose | 1,2 3,4( $\beta$ )                                                 | 0 24                                      | 0 38                                      | 1 07                        |
| D-Xylose    | 1,2 3,5( $\alpha$ )                                                | 0 39                                      | 0 49                                      | 1 01                        |
| L-Rhamnose  | 2,3                                                                | 0 72                                      | 0 82,0 78 <sup>a</sup>                    | 0 66 <sup>c</sup>           |
| D-Galactose | 1,2 3,4( $\alpha$ )                                                | 0 86                                      | 0 90                                      | 0 89                        |
| D-Glucose   | 1,2 5,6( $\alpha$ )                                                | 0 90,0 82 <sup>c</sup> ,0 59 <sup>d</sup> | 0 94,0 87 <sup>c</sup> ,0 60 <sup>d</sup> | 0 90 <sup>e</sup>           |
| D-Mannose   | 2,3 5,6                                                            | 1 00                                      | 1 00                                      | 1 00                        |
| D-Ribose    | 1,5-anhydro-2,3- <i>O</i> -isopropylidene- $\beta$ -D-ribofuranose | 0 097                                     | 0 17                                      |                             |
|             | 2,3                                                                | 0 33                                      | 0 45                                      |                             |

<sup>a</sup>Retention time relative to that of 2,3 5,6-di-*O*-isopropylidene-D-mannose <sup>b</sup>Based on the parent aldose relative to that of mannose <sup>c</sup>Minor products. <sup>d</sup>Traces <sup>e</sup>Only the main peak is included

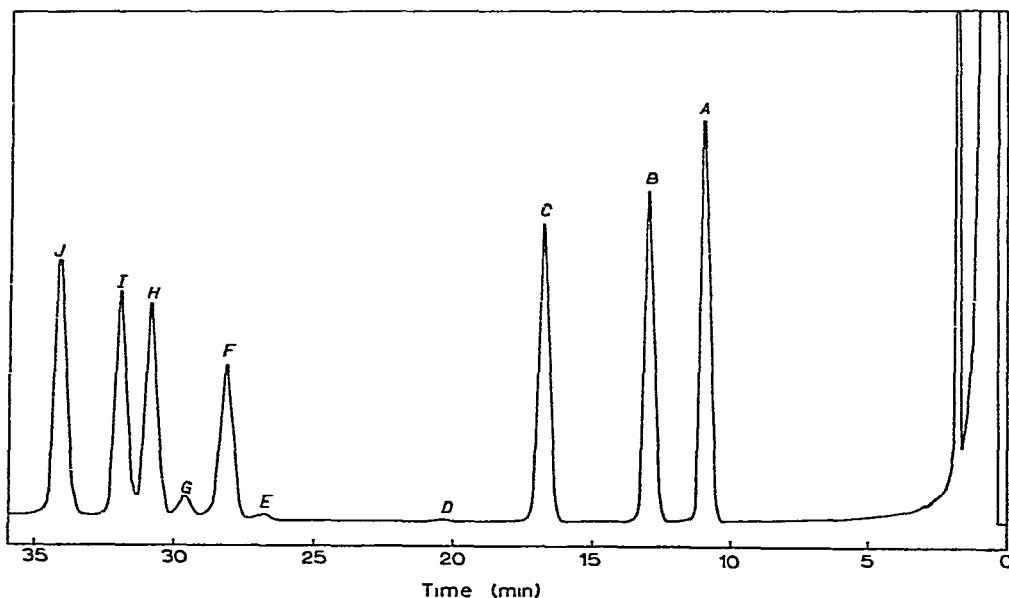


Fig 1 Separation of the *O*-isopropylidene-aldoses on XE 60 A, 1,2 3,4-di-*O*-isopropylidene- $\alpha$ -L-fucopyranose, B, 1,2 3,4-di-*O*-isopropylidene- $\beta$ -L-arabinopyranose, C, 1,2 3,5-di-*O*-isopropylidene- $\alpha$ -D-xylofuranose, D, product from D-glucose, E, product from L-rhamnose, F, 2,3-*O*-isopropylidene-L-rhamnose, G, product from D-glucose, H, 1,2 3,4-di-*O*-isopropylidene- $\alpha$ -D-galactopyranose, I, 1,2 5,6-di-*O*-isopropylidene- $\alpha$ -D-glucufuranose, J, 2,3 5,6-di-*O*-isopropylidene-D-mannofuranose

The formation of small amounts of three di-*O*-isopropylidene derivatives in addition to the main product, 1,2:5,6-di-*O*-isopropylidene- $\alpha$ -D-glucofuranose, has been reported on acetonation of D-glucose in the presence of 4% of sulphuric acid<sup>10</sup>.

The mass spectrum of 2,3-*O*-isopropylidene-L-rhamnose does not contain a molecular ion, but contains a fragment at  $m/e$  189 ( $M - Me$ )<sup>+</sup> typical of *O*-isopropylidene derivatives<sup>1</sup>. The peak at  $m/e$  187 is presumably ( $M - OH$ )<sup>+</sup>, and is characteristic of monosaccharide derivatives in which the anomeric hydroxyl group is unsubstituted. Elimination of water from the  $M - 15$  fragment is usually observed in analogous compounds<sup>1</sup>, and a peak with  $m/e$  171 is also seen in the spectrum of the rhamnose derivative. A fragment with  $m/e$  159, always formed for compounds in which the C-4-C-5 bond may be cleaved to give an *O*-isopropylidene furanoid-ion, is also present in this spectrum, indicating the presence of a furanoid form. 2,3-*O*-Isopropylidene-D-ribose gives<sup>1</sup> two different fragments of  $m/e$  129, one presumably containing C-1, C-2, and C-3, the second, C-2, C-3, C-4, and C-5. Two peaks at  $m/e$  129 and 143 in the spectrum of the rhamnose derivative may be analogues of the  $m/e$  129 peak in the ribose derivative, a shift of 14 mass-units will occur in the C-5-containing fragment due to methyl substitution.

The mass spectra of the other *O*-isopropylidene-aldoses did not differ importantly from those reported by DeJongh and Biemann<sup>1</sup>, and need not be discussed.

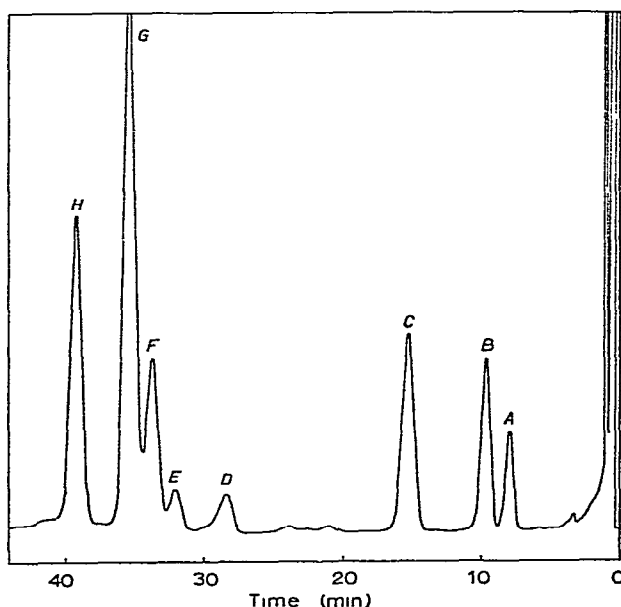


Fig 2 Gas chromatogram of the *O*-isopropylidene-aldoses obtained from an acid hydrolysate of raw humus, on OV 225. The derivatives are those of A, fucose, B, arabinose, C, xylose, D, rhamnose, E, glucose (minor product), F, galactose, G, glucose, and H, mannose.

*O*-Isopropylidene derivatives may be used for quantitative analyses of aldose mixtures, since the peak areas in g l c are proportional to the molar concentrations of the parent aldoses. The use of two injections allowed the determination of amounts as small as 4% of one aldose, relative to the most abundant one, with an accuracy of  $\pm 5\%$ .

The chromatogram of an acetonated, acid hydrolysate of dried, hexane- and ether-washed, raw humus is shown in Fig 2. Fucose, arabinose, xylose, rhamnose, galactose, glucose, and mannose are present, but not ribose (*cf* ref 11). Acetonation of an acid hydrolysate of a purified carbohydrate fraction from humus gave a chromatogram almost identical to that shown in Fig 2. Thus, information about the monosaccharide composition of soil and other crude materials may be obtained, without laborious separation and purification work, by the application of the procedure reported herein.

#### EXPERIMENTAL

G l c was performed on a Perkin-Elmer F 11 gas chromatograph, equipped with a flame-ionisation detector, using a glass column (6 ft  $\times$  1.5 mm i d) with 3% of XE 60 on Chromosorb G AW-DMCS (80-100 mesh) and a stainless-steel column (2 m  $\times$  2.2 mm i d) with 3% of OV 225 on Gas Chrom Q (80-100 mesh), and a nitrogen flow-rate of 20 ml/min. The operating temperatures were (1) XE 60 column, 120° for 5 min, and then a temperature programme of 2°/min, (2) OV 225 column, 110° for 12 min, and then programmed at 1.5°/min.

G l c -m s was performed on OV 225 with helium as the carrier gas, using a Perkin-Elmer model 900 gas chromatograph, connected by a Biemann-Watson separator to a Hitachi-Perkin-Elmer model RMU-6L mass spectrometer operating at 70 eV.

*Preparation of the O-isopropylidene derivatives* — The residues (1-2 mg) obtained on concentration of aldose solutions were shaken with 1% (v/v) sulphuric acid in acetone (1.5 ml) for 2 h. The solutions were neutralised with solid sodium hydrogen carbonate and injected into the gas chromatograph. The crude mixture obtained by hydrolysis of raw humus in M sulphuric acid for 18 h at 100° was acetonated, the acetone was removed under diminished pressure, and the *O*-isopropylidene-aldoses were dissolved in chloroform prior to injection.

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