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Note

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[1]. 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[1,2] to g l c, they have not been found suitable for the analysis of complex mixtures of monosaccharides by g l c or g l c–m s This has been explained by the long reaction-time needed for preparation of these derivatives[3] and the dependence of product-mixture composition on reaction conditions for some monosaccharides[1,4,5] Most of the common aldoses when treated with acetone in the presence of sufficient sulphuric acid give one, or mainly one, product[5], an exception is ribose[6] In our experience[7,8], complete acetonation of aldoses in syrupy mixtures occurs within considerably shorter reaction-times than those usually applied[5,9]

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 g l c 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-β-D-ribofuranose are also included as these

derivatives are obtained from D-ribose under conditions similar to those applied in this work[6].

TABLE I

CHROMATOGRAPHIC DATA FOR THE *O*-ISOPROPYLIDENE-ALDOSES

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

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

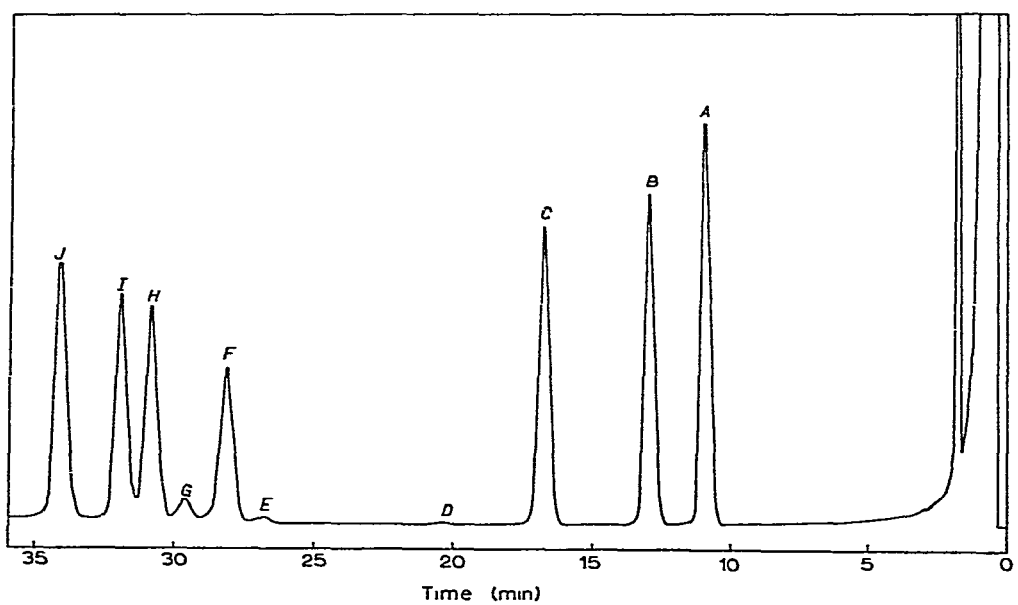

Fig 1 Separation of the *O*-isopropylidene-aldoses on XE 60 *A*, 1,2 3,4-di-*O*-isopropylidene-α-L-fucopyranose, *B*, 1,2 3,4-di-*O*-isopropylidene-β-L-arabinopyranose, *C*, 1,2 3,5-di-*O*-isopropylidene-α-D-xylofuranose, *D*, product from D-glucose, *E*, product from L-rhamnose, *F*, 2,3-*O*-isopropylidene-L-rhamnose, *G*, product from D-glucose, *H*, 1,2 3,4-di-*O*-isopropylidene-α-D-galactopyranose, *I*, 1,2 5,6-di-*O*-isopropylidene-α-D-glucofuranose, *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-α-D-glucofuranose, has been reported on acetonation of D-glucose in the presence of 4% of sulphuric acid[10].

The mass spectrum of 2,3-*O*-isopropylidene-L-rhamnose does not contain a molecular ion, but contains a fragment at *m/e* 189 $(M-Me)^+$ typical of *O*-isopropylidene derivatives[1] The peak at *m/e* 187 is presumably $(M-OH)^+$, 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[1], 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[1] 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[1], and need not be discussed

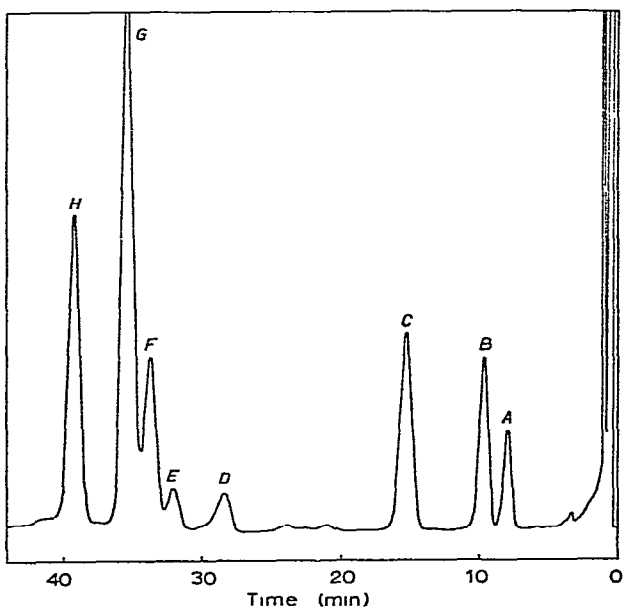

Fig 2 Gas chromatogram of the *O*-isopropylidene-aldoses obtained from an acid hydrolysate of raw humus, on OV 225 The derivatives are those of *A*, fucose, *B*, arabinose, *C*, xylose, *D*, rhamnose, *E*, glucose (minor product), *F*, galactose, *G*, glucose, and *H*, mannose

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 ±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 × 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 × 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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