GAS CHROMATOGRAPHIC SEPARATIONS OF SUGARS AND RELATED COMPOUNDS AS ACETYL DERIVATIVES

W. J. A. VandenHeuvel and E. C. Horning

Laboratory of Chemistry of Natural Products, National Heart Institute, Bethesda 14, Md.

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The identification and estimation of sugars is a matter of considerable importance in many biological and biochemical problems. The presence of high intermolecular forces, leading to low volatility, makes it unlikely that pentoses, hexoses and higher sugars will ever be amenable to direct separation by gas chromatography, but derivatives including ethers and esters have long been recognized as relatively volatile materials. The superb resolving power of current gas chromatographic techniques and the ability to deal with micro samples suggests that these methods would have great potential usefulness for work with polyhydroxy compounds if a suitable combination of phases and derivatives could be found.

This problem was investigated by Bishop (1960) and Gunner et al. (1961). With Apiezon and polyester phases in the usual thick-film columns it was demonstrated that methyl ethers and acetyl derivatives of simple sugars could be separated by gas chromatography. These results are valuable, but conventional gas chromatographic methods are of limited use in work with many natural products. Excessively high temperatures or excessively long column residence times are needed to separate compounds of moderately high molecular weight when conventional columns are used. However, the use of thin-film columns and phases with increased thermal stability has resulted in the extension of gas chromatographic methods to the separation of steroids and many other substances of comparable molecular size and complexity (VandenHeuvel, Haahti and Horning, 1961).

Acetates of sugars and related polyhydroxy compounds have many advantages for gas chromatographic work, and the present investigation was limited to these esters. The derivatives are easily prepared and almost all known polyhydroxy compounds have been characterized as acetates. The Table contains retention time data obtained for a variety of compounds containing from four to eight acetyl groups. All compounds gave single peaks with no evidence of specific thermal change or decomposition. A sample of α-cellobiose octaacetate recovered after chromatography was found to be unchanged (infrared comparisons) and repeated chromatography of the sample showed no indication of thermal alterations. In agreement with earlier experiences, an SE-30 phase was particularly useful in separations in order of molecular size. The increase in retention times with an increasing number of acetyl groups was so marked that different column temperatures were used for the separations. However, this phase has very little selective action, and most stereoisomers were separated slightly or not at all. This is an advantage when separations are desired on the basis of molecular size alone. At present the most useful selective phase for work with polyfunctional naturally occurring compounds is QF-1 (VandenHeuvel, Haahti and Horning, 1961), a fluoroalkyl silicone polymer (Dow-Corning Corp.). The Table contains retention time data for a variety of compounds obtained with a QF-1 column. Examples of stereoselective separations with this phase are shown in Figure 1. The diastereoisomers α-D-glucose pentaacetate and β-D-glucose pentaacetate are separated, and α-methyl-D-glucoside tetraacetate and α-methyl-D-mannoside tetraacetate are also separated. The lack of trailing, and the speed and effectiveness of the separations is characteristic of polyacetates chromatographed with a QF-1 phase.

The separation of a pair of disaccharide acetates was carried out for sucrose and α-cellobiose octaacetates; this indicates that disaccharides may be included in the present scope of gas chromatography.

These results indicate that sugars and related polyhydroxy compounds up

Table 1

Relative Retention Time for Acetates of Polyhydroxy Compounds[a]

Compound	SE-30[b] 152°	QF-1[c] 152°
i-Erythritol tetraacetate	0.19	2.31
L-Arabitol pentaacetate	0.64	7.32
Androstane	1.00[d]	1.00[e]
		170°
D-Mannitol hexaacetate	1.99	1.14
Dulcitol hexaacetate	2.10	1.19
D-Sorbitol hexaacetate	2.06	1.51
Cholestane		1.00[f]
α-Methyl-D-mannoside tetraacetate	0.83	0.57
α-Methyl-D-glucoside tetraacetate	0.93	0.65
α-D-Glucose pentaacetate	1.41	1.29
β-D-Glucose pentaacetate	1.41	1.10
	220°	229°
α-Cellobiose octaacetate	3.04	52.3
Sucrose octaacetate	1.95	17.8
Cholestane	1.00[g]	1.00[h]

[a] Relative to androstane or cholestane, as indicated.

[b] Argon ionization detector; 0.75% SE-30 silicone polymer on 100-140 mesh Gas-Chrom P; 6 ft. x 4 mm. I.D. column; 40 ml./min. flow at indicated temperatures.

[c] Argon ionization detector; 1% QF-1 (10,000 CS.) on 100-140 mesh Gas-Chrom P; 6 ft. x 5 mm. I.D. column; 40 ml./min. flow at indicated temperatures.

[d] Time, 11.8 min.

[e] Time, 2.4 min.

[f] Time, 15.0 min.

[g] Time, 5.8 min.

[h] Time, 1.3 min.

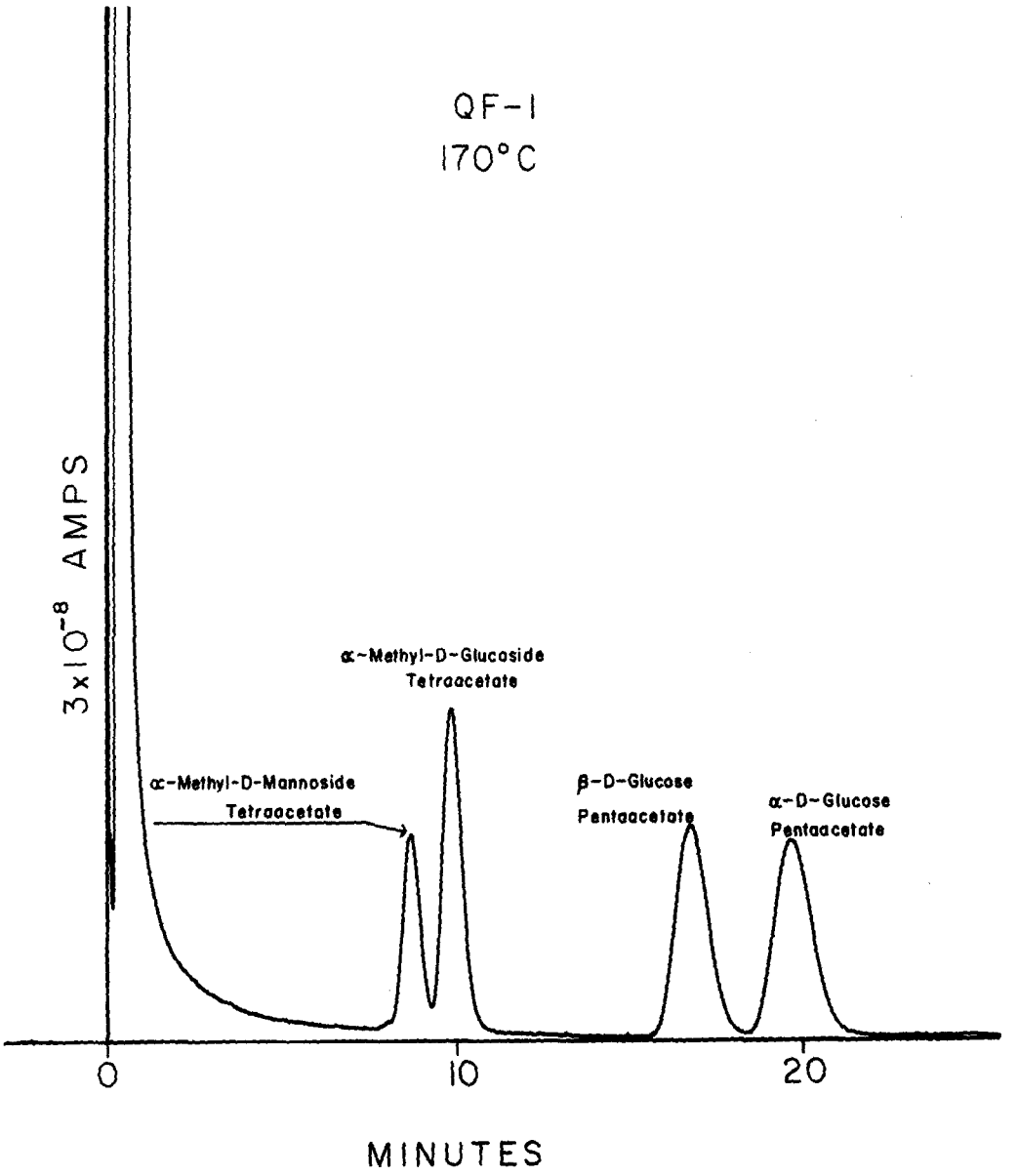

Figure 1. Separation of two pairs of epimers on a QF-1 (fluoroalkyl silicone polymer) column. The conditions are those described in the Table.

to the approximate size of a disaccharide may be separated effectively in the form of acetyl derivatives. Methyl ethers and trifluoracetates are more volatile and may offer advantages in special instances, but the use of acetates with a selective phase (QF-1) and a non-selective phase (SE-30) in

thin-film columns opens the way to general use of gas chromatographic methods for these compounds.

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