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Note

Assignment of absolute configuration of sugars by g.l.c. of their acetylated glycosides formed from chiral alcohols

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Some sugars occur both as D and L forms in Nature, whereas others only occur in one form. The D and L forms of a sugar may even occur together in biological material. In studies of natural carbohydrates, the component sugars should therefore be fully characterized. The absolute configuration is generally assigned from the optical rotation of the isolated sugar or by enzymic methods. Sometimes, however, a natural carbohydrate is available only in small amounts, and suitable enzymes may not be obtainable. There is therefore a need for a rapid and reliable method for determining the absolute configuration of sugars, using only small amounts of material.

Glycosides of a D and an L sugar with a chiral alcohol are diastereomeric and it should be possible to separate them, *e.g.*, by g.l.c. of suitable derivatives. Pilot experiments indicated that the differences in g.l.c. of acetylated glycosides of chiral primary alcohols, *e.g.*, 2-methyl-1-butanol and 2-methoxy-1-propanol, often were too small to be useful in practice. However, glycosides of chiral secondary alcohols, *e.g.*, 2-butanol and 2-octanol, in which the glycosidic oxygen is linked to the chiral centre, were well separated. Further studies were performed with (+)-2-octanol, which is commercially available in pure form.

A solution of the sugar and a catalytic amount of trifluoroacetic acid in (+)-2-octanol was kept at 130° overnight and then concentrated, and the resulting mixture of glycosides was acetylated. The product was characterized by g.l.c. on an SP-1000 W.C.O.T. glass column. For most sugars, four peaks are obtained, *i.e.*, the two pyranosides and the two furanosides. The presence of fewer peaks may be due to overlap. Relative retention times (within ±3%) and intensities are reproducible, but the relative intensities of the peaks obtained from a D and an L sugar differ. Relative retention times and approximate intensities of the peaks are given in Table I. For seven of the eight pairs of sugars, the results were checked by performing the glycosidation of one of the forms with (±)-2-octanol. As expected, the chromatograms so obtained were the sum of the chromatograms obtained from the D and L forms when treated with (+)-2-octanol. This offers a means of determining the

TABLE I

RETENTION TIMES[a] OF ACETYLATED (+)-2-OCTYL GLYCOSIDES

Sugar	D *Form*[b]		L *Form*	
Rhamnose	1.69 L	2.39 M	1.69 L	2.21 S
	2.21 S		1.96 S	2.44 S
Fucose	2.05 L	2.40 M	2.15 L[c]	
	2.25 L	2.96 M	2.61 M[c]	
Ribose	2.83 M	3.26 S	3.02 S	3.36 L
	3.00 S	3.47 L	3.13 M	
Arabinose	2.82 L	3.31 S	2.75 S	3.41 S
	3.21 M		2.93 L	
Xylose	2.41 M	3.11 M	2.48 M	3.02 M
	2.63 L	3.28 L	2.97 M	3.28 M
Mannose	6.22 L	8.40 S	6.86 L	7.91 S
	7.88 M	8.50 S	7.13 S	8.90 M
Galactose	6.56 S	10.47 S	7.29 L	8.81 S
	8.08 L		7.78 M	9.01 M
Glucose	7.24 L	10.30 M	7.24 M	8.46 M
	8.03 M		8.22 M	8.94 M

[a]Relative to that (~7 min) of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol. [b]Key: L = large, M = medium, and S = small, relative intensities. [c]The shape of the peak revealed that it did not contain a single component.

chromatograms expected for both forms of a sugar when only one form is available. L-Rhamnose was the only form of this sugar which we had at hand, and the values for D-rhamnose are determined by this method. For some sugars, a peak given by one of the forms overlaps with a peak given by the other form. This should not cause any problems in determining the absolute configuration of a pure sugar, even if only one form is available as reference material.

The choice of chiral secondary alcohol, mode of substitution of hydroxyl groups, and liquid phase for the g.l.c. are probably not critical. However, an unexpected observation was that the separations, although equally good, differed on the SP-1000 capillary columns manufactured by Varian Ass. and LKB-products, respectively. Indeed, the relative positions of some peaks were reversed when going from one column to the other.

The method was exemplified with two natural polysaccharides, larch arabinogalactan[1] and *Helix pomatia* galactan[2]. The presence of L-arabinose and D-galactose in the former and of both D- and L-galactose (in the ratio ~6:1) in the latter (Fig. 1)

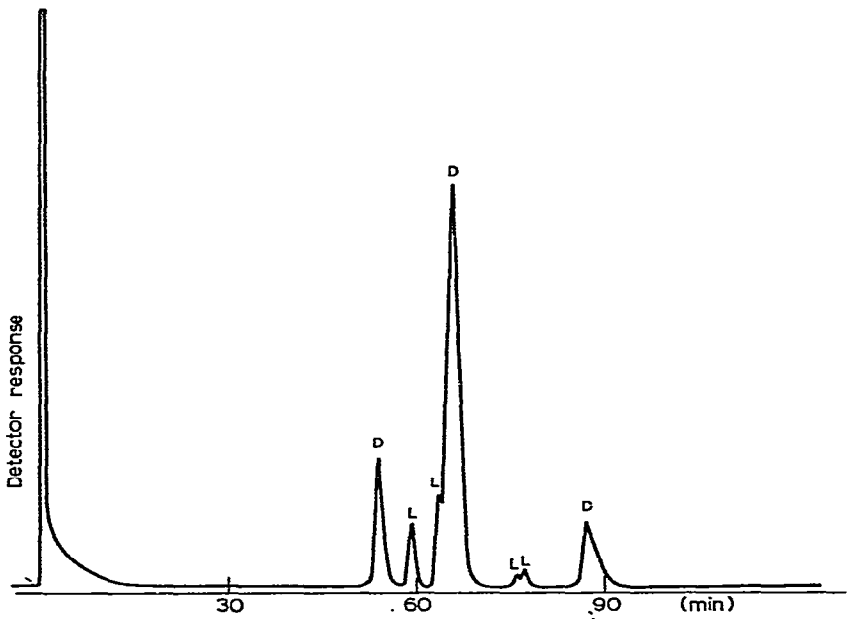

Fig. 1. G.l.c. separation of acetylated (+)-2-octyl galactosides obtained from the hydrolysate of *Helix pomatia* galactan.

was confirmed. As the peaks given by arabinose and galactose do not overlap, there was no need to separate these sugars in the hydrolysate of the arabinogalactan before determining their absolute configurations. However, this method is not recommended for sugar analysis, as there are other derivatives more suitable for quantitative work[3].

EXPERIMENTAL

General methods. — G.l.c. was performed on a Hewlett–Packard 5830 A instrument with FID, using an SP-1000 W.C.O.T. glass column (25 m × 0.25 mm, Varian Ass.) at 230°. Approximately 0.2 μl of a 10% chloroform solution of the sample was injected, and the split ratio was 1:20. The carrier-gas flow was 0.4 ml per min.

Preparation of acetylated 2-octyl glycosides. — The sugar (0.3–3 mg), one drop of trifluoroacetic acid, (+)-2-octanol (Fluka AG) or (±)-2-octanol (0.5 ml), and a small magnetic rod (coated with glass) were transferred to an ampoule. The ampoule was sealed and heated, with stirring, in an oil bath at 130° overnight. The solution was then concentrated to dryness in a vacuum rotator, connected to an oil pump, at a bath temperature of 55°.

A solution of the product in acetic anhydride–pyridine (1:1, 1 ml) was kept at 100° for 20 min and then concentrated. Excess of reagent was removed by co-distillation with ethanol.

Each polysaccharide was hydrolysed by treatment with 0.25M sulfuric acid at

100° overnight, the acid neutralized with barium carbonate, the solution freeze–dried, and the product derivatised as above.

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