GAS CHROMATOGRAPHY OF SUGARS AND OTHER POLYHYDROXY COMPOUNDS

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Gas-liquid chromatography of polyhydroxy compounds has been hindered by the difficulty of preparing suitably volatile derivatives of these materials. Acetyl and methyl derivatives of carbohydrates have, however, been separated and have demonstrated the potential of the technique (1). VandenHeuvel and Horning have extended the range of application of gas chromatography to the separation of cellobiose octaacetate and sucrose octaacetate (molecular weights, 678) with columns containing low concentrations of the liquid phases, SE-30 and QF-1 (a fluorinated silicone). Several fully methylated disaccharides have been resolved by Bishop and Cooper (3).

We have investigated the preparation and analysis of trimethylsilyl (TMS) derivatives. This development stemmed logically from a convenient method for trimethylsilylation of bile acids described by Makita and Wells (4). The compounds, dissolved or suspended in pyridine, are treated with hexamethyldisilazane and trimethylchlorosilane (as catalyst) at room temperature. Such a procedure was particularly attractive in view of the solubility of many carbohydrates in pyridine. When this method was applied to nearly 100 carbohydrate compounds trimethylsilylation took place rapidly and within a few minutes injections could be made directly onto a column without further manipulation.

TMS derivatives were prepared as follows. 10 mg portions of carbohydrate in a 1 dram vial (plastic stopper) were treated with 1 ml of dry pyridine, 0.1 ml of hexamethyldisilazane, and 0.1 ml of trimethylchlorosilane. After shaking vigorously for about 30 seconds, then standing at room temperature for about five minutes, 0.1 to 0.5 μl portions were injected directly into the instrument. The small precipitate of ammonium chloride which formed in the solution did not affect the analysis. Materials not readily soluble at room temperature were warmed gently with pyridine to effect solution.

In studying the gas chromatography of these TMS derivatives we used principally a polar liquid phase, polyethylene glycol-succinate*, and a non-polar phase, SE-52, but other liquid phases such as HiEff-8B, XE-60 (a nitrile silicone) and Carbowax 1540 have also proved satisfactory. With many carbohydrates we have obtained single, symmetrical peaks; anomeric pairs such as α- and β-glucose and anomeric glycosides are generally well separated (5). The peak obtained by applying this trimethylsilylation procedure to methyl α-D-glucopyranoside is indistinguishable from that of an authentic sample of methyl (tetra-O-trimethylsilyl)-α-D-glucopyranoside prepared as described by Hedgley and Overend (6).

A major development of our work has been to extend the useful range of gas chromatography of carbohydrates from the present upper limit of disaccharides to tetrasaccharides and to complex glycosides such as phloridzin. In the case of the tetrasaccharide, stachyose, the original carbohydrate has 14 hydroxyl groups and the molecular weight of the TMS derivative is 1676. That a substance of this molecular

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* 15% polyethylene glycol-succinate coated on 80 - 100 mesh Chromosorb W and SE-52, HiEff-8B and XE-60 were obtained from Applied Science Labs, State College, Pa. We prepared 3% SE-52, 4% XE-60, 5% HiEff-8B and 10% Carbowax 1540 packings on Chromosorb W by the solution coating technique (7).

weight can be volatilized is indeed remarkable; to our knowledge this is the highest molecular weight yet recorded for a substance which has yielded to analysis by gas chromatography. For the separation of di-, tri-, and tetra-saccharides, the most useful method has been with SE-52 columns operated at 250°, as shown by the results in Table I. Alternatively, by carrying out programmed runs from 125 to 250°, separation

Table I

Gas Chromatography of Trimethylsilyl Derivatives of Sugars and Plant Glycosides

Compound[a]	No. of C atoms	No. of OH groups	3% SE-52 Column 140°	210°	250°
α-D-xylose	5	4	0.41		
α-D-glucose	6	5	1.00[b]	1.00[c]	
β-D-glucose	6	5	1.55	1.22	
D-*glycero*-D-*gulo*heptose	7	6	2.39	1.62[d]	
sucrose	12	8		10.4	1.00[e]
α-maltose	12	8		11.6	
β-maltose	12	8		13.1	
raffinose	18	11		99.0	6.4
stachyose	24	14			52.4
esculin	15	5			3.2
phloridzin	21	7			7.3

[a] The values given are relative retention times with respect to α-glucose or sucrose as indicated. Analyses were carried out with an F & M Model 609, equipped with a flame ionization detector.

[b] The retention time for α-D-glucose at 140° was 20.0 ± 2 minutes over a period of several months.

[c] The retention time for α-D-glucose at 210° was 1.1 - 1.3 minutes.

[d] A small peak with relative retention time of 2.92 was also observed.

[e] The retention time of sucrose at 250° was 2.3 minutes.

of sugars ranging from erythrose (C_4) through pentoses, hexoses and heptoses to di- and tri-saccharides has been accomplished in the relatively short time of 75 minutes. The range from C_4 to C_{24} could equally well be covered in programmed analyses of somewhat longer duration.

In previous preparations of TMS derivatives of free sugars Hedgley and Overend carried out reactions in pyridine at reflux (1.5 hours) or at room temperature (6 hours). Using Apiezon M (20 - 50%) as stationary phase, and operating temperatures in excess of 220°, multiple peaks were obtained with the free sugars while derivatives of anomeric glycosides were not resolved. These disadvantages have been overcome by our procedure. Further, the useful life of Apiezon columns was short. Columns of SE-52 and polyester have, however, been operated for several months without deterioration.

It may be expected that this method will be effective in the preparative gas chromatography of carbohydrates. Hedgley and Overend have reported that TMS derivatives are quantitatively cleaved to the parent compound by refluxing with 50% aqueous methyl alcohol (6). With the development of an extraordinarily rapid and convenient process for preparation of TMS derivatives, analysis of carbohydrates by gas chromatography is now a practical laboratory procedure with unique resolving power.

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