DETECTION AND QUANTITATIVE DETERMINATION OF ANHYDROGLYCOSES BY GAS CHROMATOGRAPHY*

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(Received May 7th, 1965)

Nonreducing sugar anhydrides, in which the carbonyl carbon is included in the anhydro ring, generally are referred to as anhydroglycoses and are products of pyrolysis of simple sugars or glycosides. The most common class of anhydroglycoses has the 1,6-anhydrohexopyranose structure. Small amounts of anhydroglycoses possessing the 1,6-anhydrohexofuranose structure have also been reported in pyrolyzates of galactose and starch[1,2].

Levoglucosan, 1,6-anhydro-β-D-glucopyranose, is believed to be an important intermediate in the thermal decomposition of starch and cellulose, forming by scission of the 1,4-glycosidic linkage followed by intramolecular rearrangement[3–5]. This product has created interest as a possible intermediate in the synthesis of other carbohydrate derivatives because of the availability of its three hydroxyl groups for further reactions, its ability to polymerize and its stability in alkaline solution. However, methods have been lacking for the quantitative determination of levoglucosan. Infrared analysis[4] and paper chromatography[6] have been used for semiquantitative analysis. A simple, rapid and quantitative procedure for determining levoglucosan and other anhydroglycoses would aid in understanding the mechanism of pyrolytic degradation and in improving the yields of these products.

Although gas chromatography has been used to identify qualitatively the more volatile products of pyrolysis[7–10], gas chromatography of free levoglucosan (m.p. 178°) has had only limited success. High temperatures are necessary, and decomposition of the compound prevents quantitation[10]. Formation of the trimethylsilyl (TMS) ether derivative now affords a product with the desired stability and volatility to be analyzed by gas chromatography. Furthermore, this derivative is readily separated from TMS ether derivatives of 1,6-anhydro-β-D-glucofuranose and D-glucose anomers. Our study deals with the qualitative and quantitative analysis of anhydroglycoses by gas chromatography.

EXPERIMENTAL

Apparatus and material

The gas chromatograph used was model 810R from F & M Scientific Corpo-

* Presented before the Division of Carbohydrate Chemistry, 149th Meeting, American Chemical Society, Detroit, Mich., April 4–9, 1965.

** This is a laboratory of the Northern Utilization Research and Development Division, Agricultural Research Service, U.S. Department of Agriculture.

ration*, Avondale, Penn., equipped with a dual-flame detector system, a Y-153 Minneapolis-Honeywell recorder and 201-B Disc integrator.

Chromosorb W, 80–100 mesh (HMDS treated), and all the stationary phases were from Wilkens Instrument and Research, Inc., Walnut Creek, Calif; hexamethyldisilazane from Peninsular Chemical Research, Inc., Gainesville, Fla., and trimethylchlorosilane from General Electric Co., Waterford, N.Y.

Four columns of 0.25 in. O.D. copper tubing were used, each containing different packing: 8 ft. with 5 % XE-60, 8 ft. with 15 % ethylene glycol succinate (EGS), 8 ft. with 3 % SE-52 and 4 ft. with 10 % Carbowax 20 *M* terminated with terephthalic acid.

The anhydroglycoses used in this investigation were: 1,6-anhydro-β-D-galactopyranose, m.p. 221°; 1,6-anhydro-α-D-galactofuranose, m.p. 103–104°; 1,6-anhydro-β-D-glucopyranose, m.p. 178°; and 1,6-anhydro-β-D-glucofuranose, m.p. 110–111°.

Method

The column packings were prepared by the solution coating technique[11] which was modified slightly. The stationary phases were dissolved in chloroform at a concentration (w/v) of approximately half of that desired for the coated packing (wt. phase/wt. packing). Four milliliters of solution were used for each gram of support. The support was gently stirred into the solution, and after 2 min, the mixture was vacuum-filtered and dried at 100° for 2 h. The phase-coated support was packed into the columns under 40 lb. of nitrogen pressure by using a Column Pac Model G-3 from Illinois Instrument Group, Des Plaines, Ill. The columns were conditioned at their maximum temperatures for 16 h.

Pyrolysis

Five grams of polysaccharide were placed in a 100 ml round-bottomed flask. The flask was connected to a 50 ml receiver by means of a glass tube large in diameter, and the receiver cooled in a solid carbon dioxide trap. The system was evacuated slowly to a pressure of 1 mm. The temperature of the heating mantle under the round-bottomed flask was raised to 360° in 3 to 4 min and maintained until the end of the distillation (about 1 h). Initiation of the reaction was marked by a rise in the pressure to 5 mm. Near the end of the distillation, the pressure rose to 10 mm and then dropped again to 1 mm. Distillate that condensed in the connector tube was washed into the receiver by adding pyridine (3 × 20 ml) to the round-bottomed flask and distilling the pyridine into the receiver. The total volume was made up to 100 ml in a volumetric flask. Internal standard was added to an aliquot of the pyridine solution, and TMS ether derivatives were prepared as described previously[12].

Moisture-free polysaccharide was prepared by evacuating the sample in the presence of phosphorous pentoxide for 18 h at room temperature and then heating the sample for 3 to 4 h at 100°.

Polysaccharides used were waxy maize corn starch and B-512 dextran obtained from *Leuconostoc mesenteroides* NRRL B-512[13].

* Reference to commercial products or companies is for identification only and does not imply endorsement by USDA.

Calibration with internal standard

Xylitol was chosen as the internal standard because of its desirable retention time and linear detector response as related to glycosans. Varying amounts of each anhydroglycose were chromatographed with constant amounts of internal standard. The ratio of the area of each anhydroglycose to the internal standard, when plotted against the ratio of the weight of solute to the internal standard, resulted in the desired calibration curve (Fig. 1). The analysis of unknown samples was then carried out by adding the same amount of internal standard to unknown mixtures and by determining directly the solute concentration in the original mixture from comparison with the calibration curve.

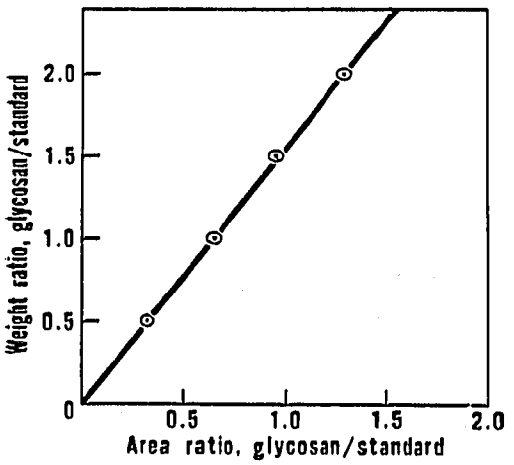

Fig. 1. Calibration curve for anhydroglycose derivatives with an internal standard (xylitol).

RESULTS AND DISCUSSION

TMS ethers of the anhydroglycoses are readily resolved by gas chromatography as evident in Fig. 2. Retention times for four anhydroglycoses on four different liquid phases are listed in Table I. Only the EGS column showed slight adsorption effects which resulted in asymmetrical peaks. With the SE-52 column, the pyranose and furanose peaks of the anhydroglucoses and the anhydrogalactoses were well resolved, but 1,6-anhydro-α-D-galactofuranose and 1,6-anhydro-β-D-glucopyranose peaks overlapped slightly.

Quantitative analysis of synthetic mixtures of anhydroglycoses was achieved with high degree of accuracy (Table II) on the XE-60 column.

The applicability of the method was tested on levoglucosan formed by pyrolysis of waxy maize starch and B-512 dextran (Table III). Although yields of levoglucosan by our pyrolysis method were not as high as the maximum reported yields (up to 40 %) from starch[14], analysis of duplicate pyrolysis samples of polysaccharides showed reproducible results. A substantial decrease in the yield of levoglucosan was observed when the starch was not dried before pyrolysis, and 1,6-anhydro-β-D-glucofuranose was not detected (Table III, materials 1 and 2).

Also, the formation of both levoglucosan and 1,6-anhydro-β-D-glucofuranose appears to depend on the type of glycosidic linkage in the polysaccharide. Yields were approximately 40 % less in pyrolyzates of the α-1,6-linked polymer, B-512 dextran,

TABLE I

RETENTION TIMES FOR ANHYDROGLYCOSES AND HEXOSES AS TRIMETHYLSILYL ETHER DERIVATIVES

Compound	*Retention times* (min)*			
	XE-60	*EGS*	*Carbowax-20M terminated with terephthalic acid*	*SE-52*
	145°	*168°*	*160°*	*145°*
1,6-Anhydro-β-D-galactopyranose	10.2	5.8	6.5	16.2
1,6-Anhydro-α-D-galactofuranose	12.2	7.4	8.5	18.8
1,6-Anhydro-β-D-glucopyranose	14.2	9.0	10.2	20.1
1,6-Anhydro-β-D-glucofuranose	19.8	11.4	12.0	24.1
α-D-Galactose	15.6			
β-D-Galactose	21.4			
α-D-Galactose**	13.0			
α-D-Glucose	16.1			
β-D-Glucose	30.5			

* Retention times were measured from the pyridine peak.
** Galactofuranose derivative.

TABLE II

ANALYSIS OF ANHYDROGLYCOSE MIXTURES

Mixture	*Compound*	*Amount present (mg)*	*Amount recovered (%)*
1	1,6-Anhydro-β-D-galactopyranose	2.00	100
	1,6-Anhydro-α-D-galactofuranose	2.00	101
	1,6-Anhydro-β-D-glucopyranose	1.00	102
	1,6-Anhydro-β-D-glucofuranose	1.00	102
2	1,6-Anhydro-β-D-galactopyranose	1.00	99
	1,6-Anhydro-α-D-galactofuranose	1.00	99
	1,6-Anhydro-β-D-glucopyranose	2.00	102
	1,6-Anhydro-β-D-glucofuranose	1.00	99.5

TABLE III

ANALYSIS OF ANHYDROGLYCOSES IN PYROLYZATES OF STARCH AND DEXTRA.

Material		*Yield of 1,6-anhydro-β-D-gluco-pyranose (%)*	*Yield of 1,6-anhydro-β-D-gluco-furanose (%)*
1a	Waxy corn starch (contg. 11.81% moisture)	12.0	—
1b	Waxy corn starch (contg. 11.81% moisture)	11.0	—
2a	Waxy corn starch (dried)	19.6	0.9
2b	Waxy corn starch (dried)	18.7	0.9
3a	Dextran (dried)	11.0	0.6
3b	Dextran (dried)	12.0	0.8

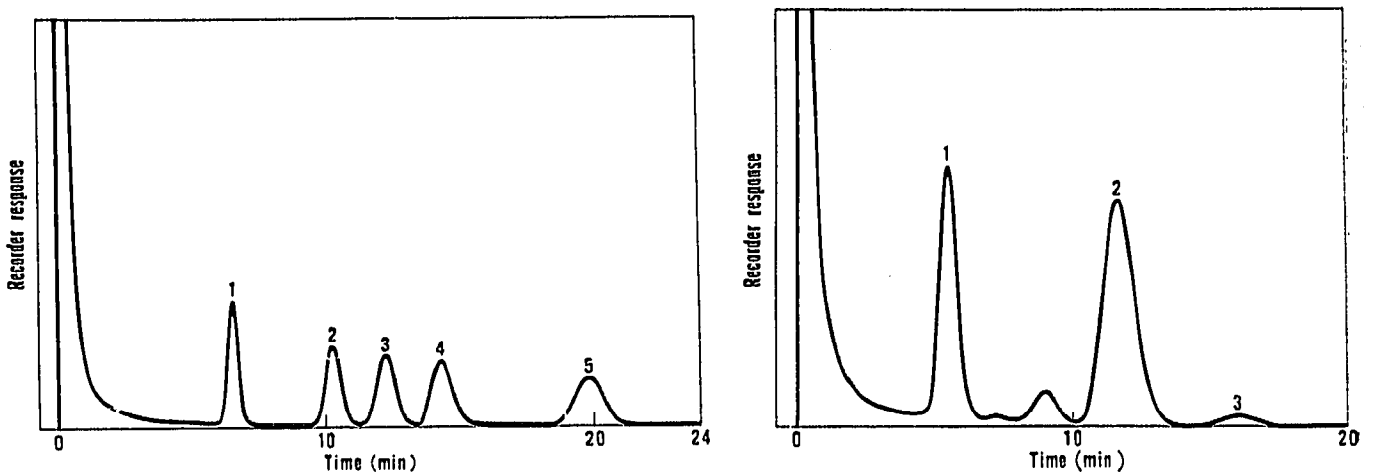

Fig. 2. Gas chromatogram of anhydroglycoses as trimethylsilyl ether derivatives on an 8 ft., 5% XE-60 column. Sensitivity: Range 100; attn. 8 ×; column temp. 145°; injection port temp. 300°; detector temp. 270°; helium flow 70 ml/min; sample size 1 μl of 0.5% soln. 1 = Internal standard (xylitol); 2 = 1,6-anhydro-β-D-galactopyranose; 3 = 1,6-anhydro-α-D-galactofuranose; 4 = 1,6-anhydro-β-D-glucopyranose; 5 = 1,6-anhydro-β-D-glucofuranose.

Fig. 3. Gas chromatogram of trimethylsilyl ether derivatives of pyrolysis products from starch. Column and conditions same as for Fig. 2 except the column temp. was 155°. 1 = Internal standard (xylitol); 2 = 1,6-anhydro-β-D-glucopyranose; 3 = 1,6-anhydro-β-D-glucofuranose.

as compared to the pyrolyzate of the predominantly α-1,4-linked waxy maize starch.

A typical chromatogram of a starch pyrolyzate is shown in Fig. 3.

1,6-Anhydro-β-D-glucofuranose is clearly visible following the large levoglucosan peak and amounts to 1% or less in quantity (Table III). Free glucose could not be detected in the products formed. However, small amounts (about 4%) of several unidentified materials having volatility close to that of levoglucosan were present in the pyrolyzates of both starch and dextran. Identification of these products and elucidation of the mechanism of formation of glycosans must await further investigation.

SUMMARY

A rapid method for detection and quantitative determination of several anhydroglycoses has been achieved by gas chromatography on an 8 ft. column packed with 5% XE-60. Quantitative determination of anhydroglycoses formed during vacuum pyrolysis of starch and of dextran was made directly on pyridine solutions of the pyrolysis products. The method is sensitive enough to detect less than 1% of 1,6-anhydro-β-D glucofuranose in pyrolysis products.

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