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Note

Determination of carbohydrate composition of soil hydrolysates by high-performance liquid chromatography*

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Carbohydrates represent 5–25% of the organic matter in soils[1]. Their importance in relation to soil aggregation and to other soil processes is well recognized[1]. Carbohydrates are present in soil in many forms, ranging from plant debris and faunal remains to products of chemical, biochemical and microbial synthesis and decomposition.

Paper and gas chromatography, as well as colorimetric procedures, have been used previously to identify and quantitate the monosaccharide components of soil carbohydrates. Gas–liquid chromatography of alditol acetate derivatives has been the most widely used method[1–3]. However, the extensive sample preparation procedures required and the sensitive chromatographic operating parameters are disadvantages of this method. Conversely, high-performance liquid chromatography (HPLC) offers advantages such as speed and minimum sample purification without the need for derivatization.

Six to eight neutral sugars are usually present in soil hydrolysates. The five major ones, representing more than 90% of total neutral sugars, are hexoses (glucose, galactose and mannose) and pentoses (xylose and arabinose). Rhamnose, fucose and ribose are also found but in lesser amounts (<5%). Recently, a resin-based carbohydrate analysis column from Bio-Rad Labs. has been used for the analysis of wood and wood pulp hydrolysates. This column was found appropriate for the separation and quantification of the five sugars (glucose, galactose, mannose, arabinose and xylose) found in this material[4].

In this paper we present the results of the application of HPLC of underivatized sugars to the study of carbohydrates in hydrolysates of five soils differing in their organic matter content.

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MATERIALS AND METHODS

Preparation of samples

Five surface soils from different regions of Québec, Canada were used for the determinations. They cover a range of organic carbon contents from 12 to 86 g kg^{-1}. Between 1.0 and 2.0 g of dry (40°C) finely ground soil was hydrolysed for 24 h at 80°C in 10 ml 1.5 *M* sulfuric acid in sealed tubes. The suspension was then filtered, washed with distilled water and adjusted to a final volume of 25 ml. A 10-ml aliquot was neutralized to pH 7 by adding solid barium carbonate. To remove the barium sulfate precipitate, the suspension was shaken, centrifuged (3000 *g*) and filtered (Whatman 42). The clear sugar solution obtained was stored at 4°C until analysis.

Liquid chromatography

HPLC analyses were performed using a Waters system consisting of a Model 510 pump, Model U6K sample injector, Model 840 system controller and Model 410 refractive index detector. The column was an Aminex HPX-87P carbohydrate analysis column (300 × 7.8 mm) (Bio-Rad Labs.). Cation and anion microguard columns were placed in front of the analytical column. The separation was performed isocratically using demineralized water at a flow-rate of 0.6 ml min^{-1}. The column was maintained at 85°C. The injection volumes depended on the sugar content and were usually 75 or 100 µl. Before injection, all samples were centrifuged (13 000 *g*) to eliminate particles in suspension. Detector response factors were determined from a standard solution of five sugars.

Confirmation of peak identities

Separation of samples was also performed on two other types of columns with different selectivities. A cationic resin in the calcium form (Sugar Pak I, Waters, 300 × 6.5 mm) was eluted with water containing 50 mg l^{-1} EDTA-$Ca^{2+}Na_2^+$ at a flow-rate of 0.5 ml min^{-1}. This column was maintained at 90°C during the separations. The second column was a Carbohydrate Analysis Column (Waters, 300 × 3.9 mm, amino-bonded silica); this column was kept at 25°C during the separations, using acetonitrile–water (80:20) as eluent at a flow-rate of 2.0 ml min^{-1}.

Carbon content of the soil samples was measured by dry combustion using a LECO C determinator and assumed to be total organic carbon because carbonates were absent from the soil samples.

RESULTS AND DISCUSSION

The chromatographic separation of a standard solution of five sugars on the Bio-Rad column Aminex HPX-87P is presented in Fig. 1A. The following elution times were obtained: glucose (13.3), xylose (14.4), galactose (15.1), arabinose (16.4) and mannose (16.9 min). The individual sugars are well resolved and can be quantitated individually. Fig. 1B and C show the separation of sugars obtained from two samples of soil hydrolysates widely different in organic matter content. In both cases, the sugars from the samples had retention times identical to those of the standards.

Confirmation of the identities of sugars from the samples was obtained by using

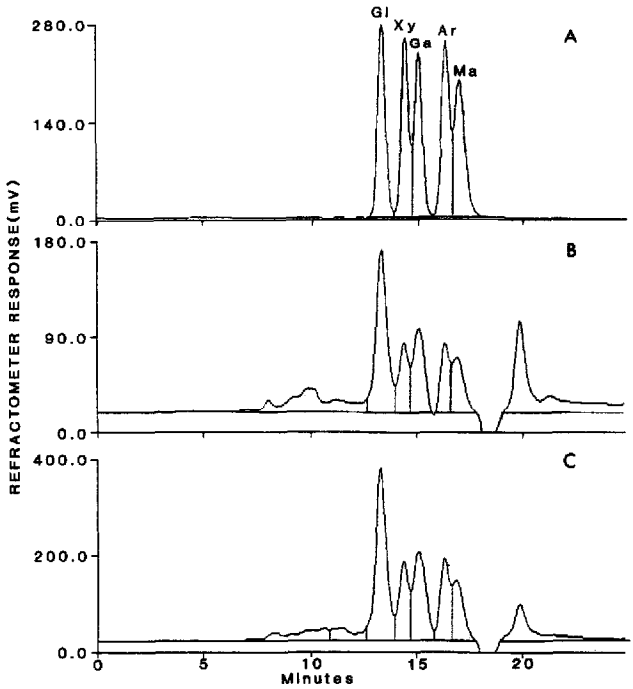

Fig. 1. Chromatographic separation on HPX-87P column (Bio-Rad Labs.) of the sugars glucose (Gl), xylose (Xy), galactose (Ga), arabinose (Ar) and mannose (Ma). (A) Separation of a standard solution containing 10 μg of each of the standard sugars. (B) Separation of sugars from a soil hydrolysate from Yamaska loam. (C) Separation of sugars from a soil hydrolysate from Normandin clay.

two other chromatographic systems offering different selectivities. Contrary to the HPX-87P column, sugars could not be individually resolved on the Sugar Pak column (Fig. 2). Only three peaks were obtained from the five component sugars (Fig. 2A). Glucose eluted in the first peak; galactose, mannose and xylose co-eluted in the second peak, and arabinose in the last peak. When soil samples were chromatographed on the same column (Fig. 2C), peaks corresponding directly to the elution times of the standard sugars were present, thus suggesting the presence of these sugars in the samples. With the Carbohydrate Analysis Column, the retention times for glucose and mannose were too close to each other (Fig. 3A) for them to appear as individual peaks. When soil samples were analyzed on this column (Fig. 3B and C), peaks corresponding to the elution times of the sugars of the standard were present. As a direct correspondence between the elution times of sugars from soil samples and those from the calibration solution was obtained with all three chromatographic systems, we are confident with the results of the identification of the sugars in our samples.

The method presented in this paper was intended first to separate five major soil sugars. Small amounts of rhamnose (<6%), fucose (<3%) and ribose (<2%) can also be detected in soil hydrolysates. Chromatography of standards on the HPX-87P column containing these minor sugars showed that rhamnose co-eluted with galactose and that fucose co-eluted with arabinose. Separation of these minor constituents was therefore not possible.

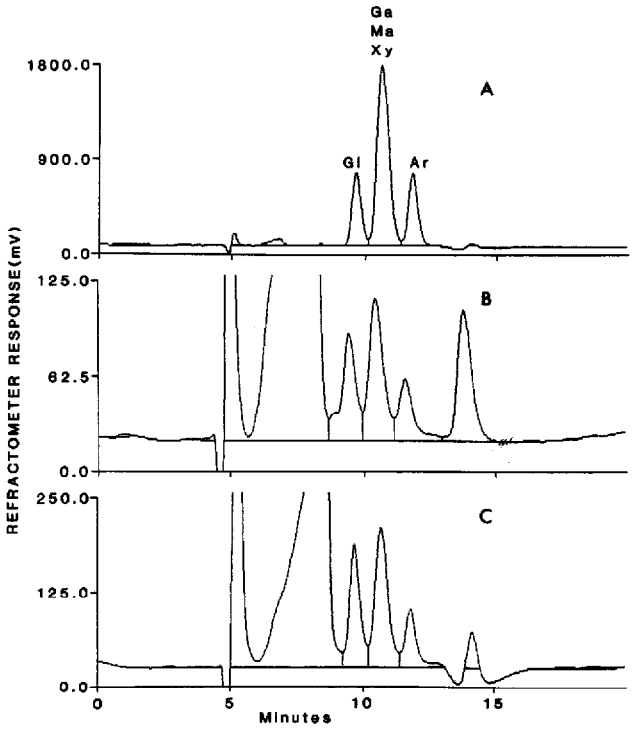

Fig. 2. Chromatographic separation of sugars on Sugar Pak column (Waters). (A) Separation of a standard solution containing 10 μg of each of the standard sugars. (B) Separation of sugars from a soil hydrolysate from Yamaska loam. (C) Separation of sugars from a soil hydrolysate from Normandin clay.

The carbon content and concentration of each sugar component for the five soil types are presented in Table I. As expected, large differences in sugar content were found for soils differing in carbon content. Total sugars accounted for nearly 20% of organic carbon, in accordance with published results[1]. The relative proportions of each sugar relative to total sugars are also in agreement with previously published data[1]. However, galactose was present in slightly larger concentration than is usually reported. This can be explained by the co-elution of rhamnose with galactose.

The chromatographic separations were made on samples obtained by using a partial hydrolysis of soil. A "total hydrolysis" procedure[1] was also assayed for comparison. This method involves a pretreatment in concentrated (72%) sulfuric acid prior to the hot dilute (0.5 *M*) acid hydrolysis. Total hydrolysis of the five soil samples resulted in an increase in total monosaccharides (Table II). Most of the effect could be attributed to a large increase in the concentration of glucose (2–3 fold) and xylose (1.3-fold) with little or no consistent changes in the content of the other sugars. This confirms that the pretreatment with concentrated sulfuric acid resulted in the hydrolysis of cellulose and the consequent release of large amounts of glucose[1].

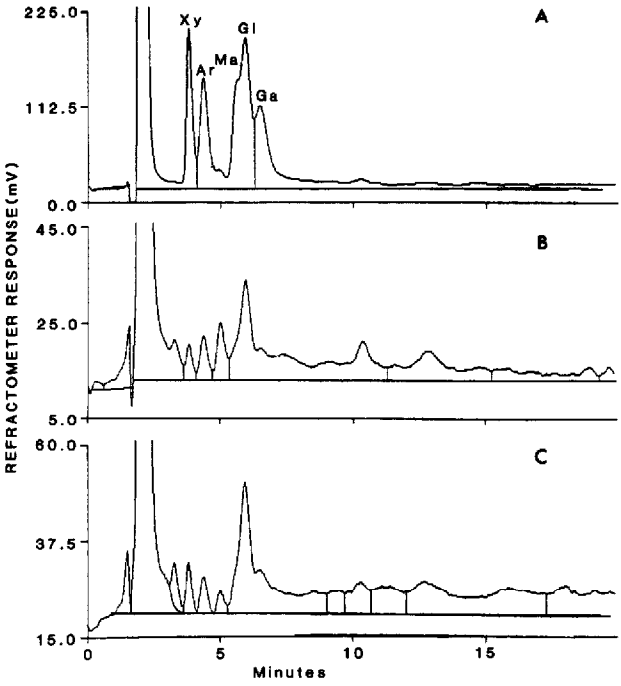

Fig. 3. Chromatographic separation of sugars on Carbohydrate Analysis Column (Waters). (A) Separation of a standard solution containing 10 μg of each of the standard sugars. (B) Separation of sugars from a soil hydrolysate from Yamaska loam. (C) Separation of sugars from a soil hydrolysate from Normandin clay.

CONCLUSION

Our results clearly demonstrate the applicability of the HPLC separation techniques to the analyses of sugars from soil hydrolysates. This method combines rapid analysis times and ease of sample preparation. No derivatization is necessary and sample prepurification is brief. The Bio-Rad HPX-87P column permits appropriate

TABLE I

CARBON CONTENT AND SUGAR CONCENTRATION IN HYDROLYSATES OF FIVE SOIL SAMPLES

Coefficient of variation varied between 2 and 10% with an average of 5%.

Soil	*Carbon* ($g\ kg^{-1}$)	*Sugars* ($mg\ kg^{-1}$)					
		Glucose	*Galactose*	*Mannose*	*Arabinose*	*Xylose*	*Total*
St-Nicolas clay loam	12	1037	645	299	222	367	2570
Yamaska loam	21	1507	1077	599	574	580	4337
Kamouraska clay	24	1651	1244	593	660	703	4851
Soulanges sandy loam	41	1807	1399	630	722	1005	5563
Normandin clay	86	3417	2744	1435	1615	1448	10659

TABLE II
SUGAR CONCENTRATION IN TOTAL HYDROLYSATES OF FIVE SOIL SAMPLES
Coefficient of variation varied between 2 and 10% with an average of 5%.

Soil	*Sugars* ($\overline{mg\ kg^{-1}}$)					
	Glucose	*Galactose*	*Mannose*	*Arabinose*	*Xylose*	*Total*
St-Nicolas loam	2695	614	127	425	543	4404
Yamaska loam	3763	978	703	360	815	6619
Kamouraska clay	3946	1207	360	736	973	7222
Soulanges sandy loam	5697	1339	268	872	1442	9638
Normandin clay	8863	3115	1253	2145	1933	17309

separation and quantitation of the most important sugars present in soil samples. However, analysis of minor sugars (rhamnose and fucose particularly) is not easily achieved due to their co-elution with the major sugars. Nevertheless, this method has a considerable potential for simplifying comparison studies of soils containing various amounts of carbohydrates.

ACKNOWLEDGEMENTS

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