

## Note

### Gas chromatography of silylated oxime derivatives of peat monosaccharides\*

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Gas chromatography of pentoses and hexoses have been performed extensively with the aid of the acetate or trimethylsilyl derivatives. The latter derivatives have the disadvantage that more than one derivative can be formed from a single sugar. Use of the acetates, on the other hand, does not allow the differentiation of glucose from fructose. In this latter regard, the butane boronates have been proposed as an alternative<sup>1</sup>. Here again, certain sugars afford more than one volatile derivative.

As an alternative to the aforementioned derivatives the use of silylated oxime derivatives have been proposed<sup>2-4</sup>. These derivatives have been reported to afford distinctive mass spectra readily suited for structural analysis. A limitation of the oxime derivatives, however, is the possibility of forming the *syn*- and *anti*-isomers. Nevertheless the derivatives are attractive because they are readily prepared (in one reaction vial) and the reagents are now commercially available.

We now wish to report the experimental conditions that permit the use of the silylated oxime derivatives for the gas chromatographic analyses of mixtures of xylose, arabinose, ribose, rhamnose, galactose and glucose. Moreover, analyses can be performed with submicrogram quantities of the monosaccharides. Heretofore, the chromatographic pattern of a mixture of the six silylated oxime derivatives has not been described. The monosaccharides cited here are useful for the characterization of peat carbohydrates.

#### MATERIALS AND METHODS

Analyses were performed with a Perkin-Elmer Model 3920 gas chromatograph fitted with two 6 ft.  $\times$   $\frac{1}{8}$  in. stainless-steel columns packed with 2% OV-17 on Chromosorb W HP (80-100 mesh). With the flame-ionization detector, the operating conditions were: injection port temperature, 200°; detector temperature, 300°; carrier gas (helium) flow-rate, 30 ml/min; oven temperature, 120° for 8 min and then increased to 8°/min to 300°.

Analyses based on the nitrogenous function were accomplished with the aid of a Pye three-electrode alkali flame-ionization detector fitted with a rubidium chloride tip. The attenuation was  $50 \times 1$ . The detector temperature was 270°. Air flow-rate through the detector was 300 ml/min while the hydrogen flow-rate was 30 ml/min.

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### Preparation of derivatives

To a solution containing a milligram of each monosaccharide in 50  $\mu\text{l}$  of pyridine (gas chromatographic grade, Pierce, Rockford, Ill., U.S.A.), contained in a 1-ml screw-cap septum vial, was added 100  $\mu\text{l}$  of "STOX" reagent (Pierce). The mixture was heated for 10 min at 70–75°. Then, the reaction mixture was cooled to room temperature and 100  $\mu\text{l}$  of hexamethyldisilazane was added, followed by 10  $\mu\text{l}$  of trifluoroacetic acid. The reaction vial was tightly sealed, shaken, and allowed to stand for 5 min. Aliquots (0.2 to 0.5  $\mu\text{l}$ ) were injected into the gas chromatograph.

Gas chromatography with the nitrogen detector required the removal of pyridine from the reaction mixture. This was readily accomplished by placing a 5- $\mu\text{l}$  aliquot of the reaction mixture into a tapered reaction vial (e.g., a 0.3-ml "Reactive-Vial", Pierce) and evaporating the mixture to dryness in a vacuum dessicator. The residue was subsequently dissolved in an appropriate volume of hexane (pesticide grade). Submicroliter volumes of the hexane solution were then used for analyses. The analytical solutions were stable for several days.

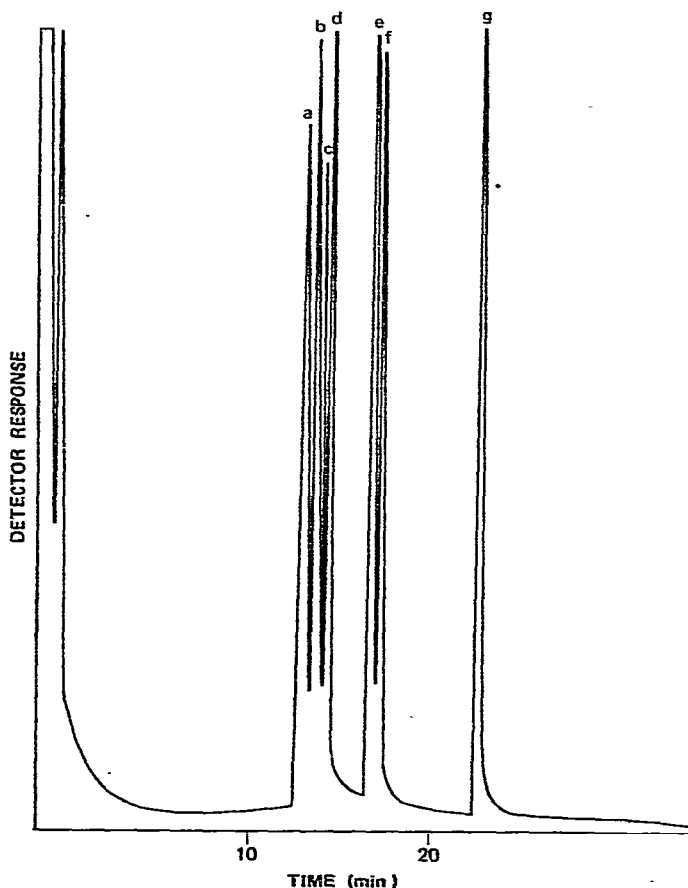


Fig. 1. Gas chromatogram of silylated oxime derivatives of peat monosaccharides (50 ng each). Retention times are: (a) xylose, 14.2; (b) arabinose, 14.3; (c) ribose, 14.7; (d) rhamnose, 15.1; (e) galactose, 17.3; (f) glucose, 17.5 and (g) phenyl glycoside (internal standard) 22.7 min.

## RESULTS AND DISCUSSION

Under the experimental conditions described here, the monosaccharides of interest, namely xylose, arabinose, ribose, rhamnose, galactose and glucose, were clearly distinguishable on the chromatogram (Fig. 1). Moreover the chromatograms were not complicated by the presence of the *syn*- and *anti*-isomers of the oximes so that quantitative analysis of the individual monosaccharides was relatively simple. The least detectable amount of the sugars with the flame-ionization detector was 10 ng.

The advantages of the silylated oxime derivatives for the analyses of the monosaccharides was most apparent when the nitrogen detector was used. The least detectable amount of the pentoses was 300 pg and for hexoses, 1600 pg. When gas chromatographic grade pyridine was used, the chromatograms were devoid of spurious peaks due to nitrogen-containing impurities, which could interfere with the quantitative analyses. This finding is significant inasmuch as micro gas chromatography of sugar alditol acetates can be complicated by interferences arising from the carbonaceous impurities in the solvent or the reagents<sup>5</sup>. The selectivity of the nitrogen detector is most appropriate in this regard.

As a practical application of the method described here, a surface peat sample (derived mainly from *Typha latifolia*) taken at Keswick (Ontario, Canada) was subjected to acid hydrolysis. Work-up of the hydrolyzate afforded the following monosaccharide composition: rhamnose, 6.0; ribose, 2.3; arabinose, 31.3; xylose, 45.8; galactose, 17.8; and glucose, 61.4 mg per g of freeze-dried peat.

The results described here demonstrate that under appropriate experimental conditions, silylated oximes are useful derivatives for the gas chromatographic analyses of monosaccharides particularly when used in conjunction with the selective and sensitive alkaline flame-ionization detector.

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