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Note

Fused-silica capillary gas chromatographic separation of alditol acetates of neutral and amino sugars

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In a previous paper[1] we demonstrated that fused-silica wall-coated open tubular (WCOT) columns provide a convenient and efficient tool for the analysis of alditol acetates of neutral monosaccharides, which are the most commonly used derivatives for analyses of polysaccharides and glycoconjugates[2]. Those coated with polar liquid phases such as FFAP and PEG 20M gave excellent separations of either alditol acetates or partially methylated substances under isothermal operations.

In carbohydrate chemistry, fused-silica WCOT columns have recently been employed in separations of partially methylated alditol acetates derived from the extracellular polysaccharide of the bacterium *Rhizobium japonicum*[3] and in analysis of the methanolysate of lipopolysaccharides[4] on a column with methylsilicone (SE-30) stationary phase.

Here we extend the use of such columns to the analysis of amino sugars as alditol acetates. Separation of the alditol acetates of glucosamine and galacosamine by gas–liquid chromatography (GLC) was first reported on a packed column with 1% ECNSS-M on Gas-Chrom A[5], and was followed by some improvements in packed column GLC[6–8]. Recently, Doctor and co-workers[9] reported that a glass capillary SCOT column coated with chiral polysiloxane liquid phase is able to separate the alditol acetates of glucosamine, galactosamine and mannosamine and thirteen neutral monosaccharides.

In the present study, the separation of alditol acetates of neutral and amino sugars has been examined on fused-silica WCOT columns with non-polar (silicone OV-1), slightly polar (silicone SE-54) and polar stationary phases (Carbowax 20M). Glucosamine, galacosamine and mannosamine were readily separated as alditol acetates by any column employed, whereas the Carbowax 20M column was superior for the separation of the neutral alditol axetates.

EXPERIMENTAL

GLC was carried out with a Hewlett-Packard 5880A instrument equipped with

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a flame ionization detector at linear carrier gas (helium of hydrogen) velocities of 39–53 cm/sec. A sample solution (1 % w/v) in methylene chloride (0.2 μl) was applied to a column in split mode (splitting ratio 100/1).

The following fused-silica WCOT columns (Hewlett-Packard, Avondale, PA, U.S.A.) were used: silicone OV-1 (dimethylsilicone gum), 50 m × 0.2 mm, D_f (thick-

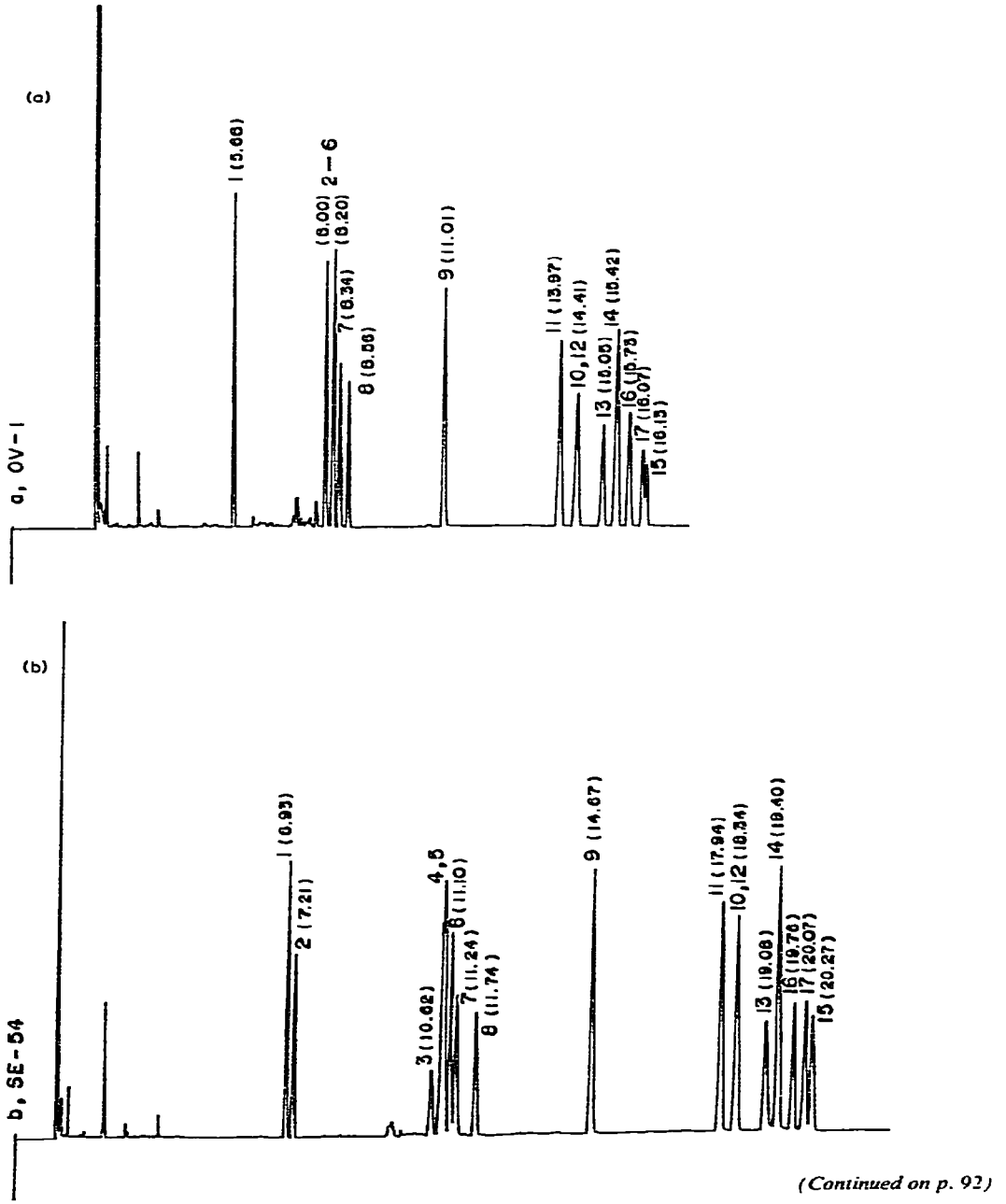

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Fig. 1.

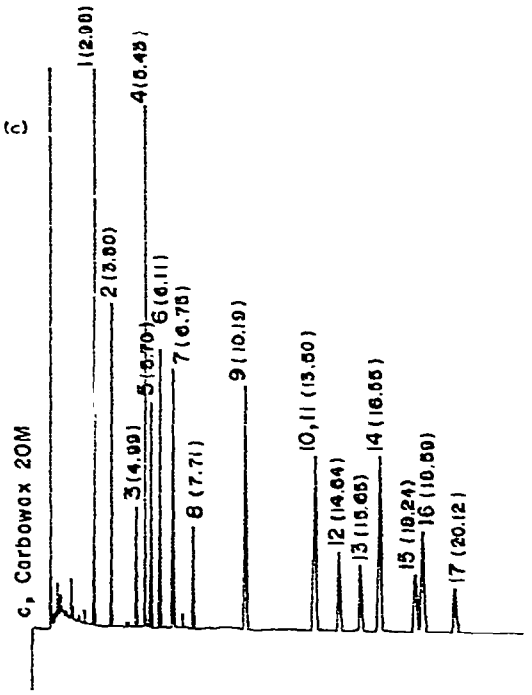

Fig. 1. Chromatograms of alditol acetates of neutral sugars on fused-silica WCOT columns using helium as the carrier gas: a, OV-1 (50 m × 0.2 mm, D_f = 0.17 μm), linear velocity ($\bar{u}$) 39 cm/sec, temperature 170–190°C at 1°C/min; b, SE-54 (25 m × 0.3 mm, D_f = 0.52 μm, $\bar{u}$ = 40 cm/sec, temperature 140–200°C at 2 C min; c, Carbowax 20M (25 m × 0.2 mm), $\bar{u}$ = 50 cm/sec, temperature 20°C. Peaks correspond to acetates of: 1 = D-digitoxitol; 2 = 2-deoxy-D-ribitol; 3 = L-rhamnitol; 4 = D-fucitol; 5 = 6-deoxy-D-glucitol; 6 = D-ribitol; 7 = L-arabinitol; 8 = D-xylitol; 9 = 2-deoxy-D-galactitol; 10 = D-allitol; 11 = 3-O-methyl-D-glucitol; 12 = 4-O-methyl-D-glucitol; 13 = D-altritol; 14 = D-mannitol; 15 = L-glucitol; 16 = D-galactitol; 17 = L-iditol. The retention times (min) are also shown.

ness of liquid phase film) = 0.17 μm; silicone SE-54 (1% vinyl, 5% phenyl), 25 m × 0.3 mm, D_f = 0.52 μm; Carbowax 20M, 25 or 12 m × 0.2 mm.

Alditol acetates were prepared as described previously[1] from corresponding neutral and amino sugars.

RESULTS AND DISCUSSION

Separations of alditol acetates of neutral and amino sugars are compared on three different types of columns in Figs. 1 and 2, respectively.

Hexitol acetates are well separated on each of the present columns; it may be noticed that glucitol acetate emerges after galactitol acetate on the OV-1 or SE-54 column, but the elution order is the opposite on the Carbowax 20M column. Resolution of acetates of pentitols and deoxyhexitols was improved on the SE-54 column compared to the OV-1 column, and complete separation was achieved on the Carbowax 20M column as on the FFAP column described previously[1].

Amino sugars are completely separated as alditol acetates in 8–12 min (Fig. 2);

the shorter Carbowax 20M column (12 m) was used, since it was required to operate near the maximum operating temperature for the separation of these compounds. The elution order of galactosaminitol and mannosaminitol acetates on the Carbowax 20M column is opposite to that on the OV-1 or SE-54 column.

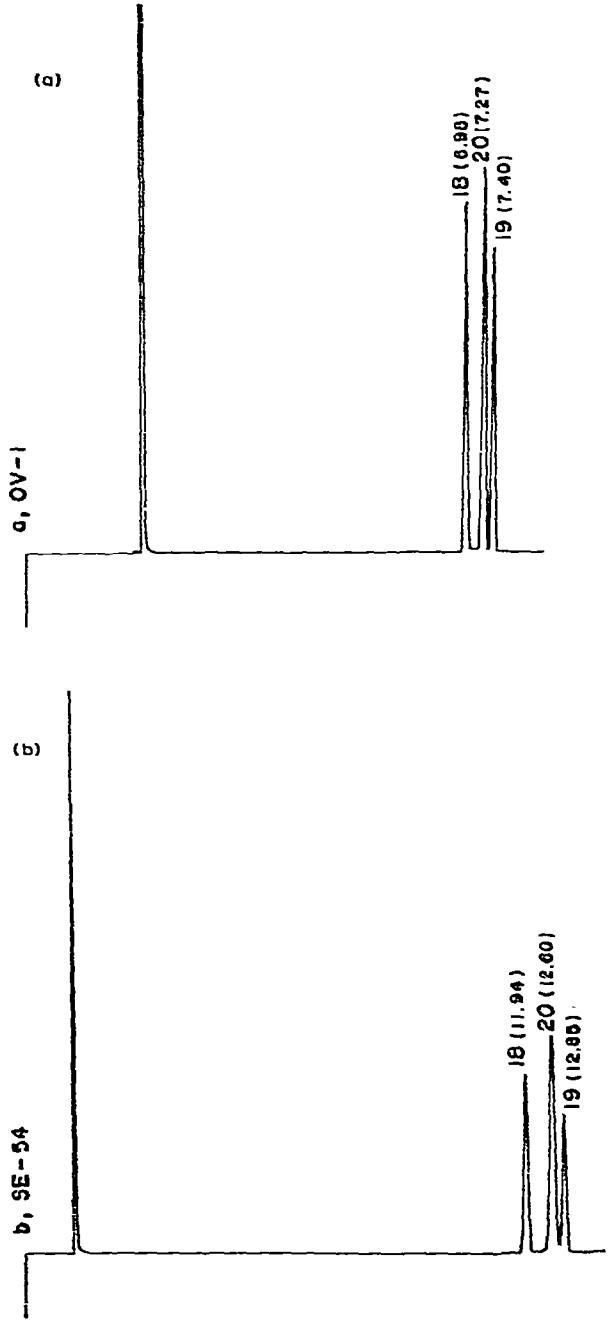

Fig. 2.

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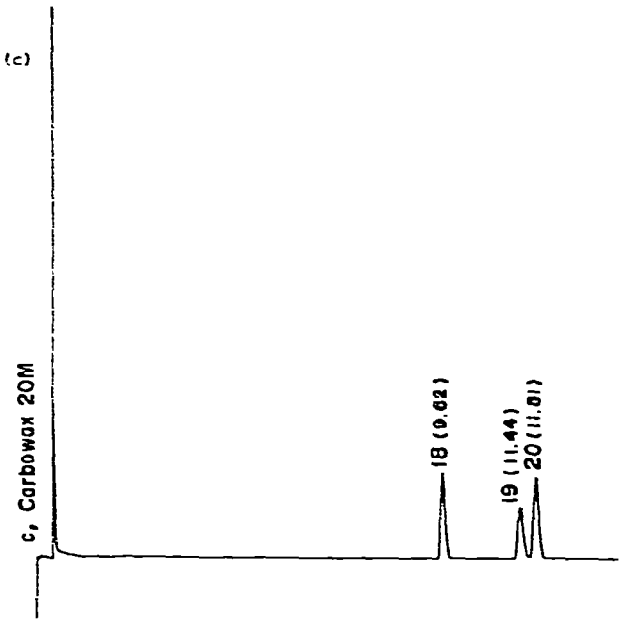

Fig. 2. Chromatograms of alditol acetates of amino sugars on fused-silica WCOT columns using helium as the carrier gas: a, OV-1, $\bar{u}$ = 45 cm/sec, temperature 210°C; b, SE-54, $\bar{u}$ = 35 cm/sec, temperature 210°C; c, Carbowax 20M (12 m × 0.2 mm), $\bar{u}$ = 53 cm/sec, temperature 220°C; characteristics of the OV-1 and SE-54 columns as in Fig. 1. Peaks correspond to acetates of: 18 = D-glucosaminitol; 19 = D-galactosaminitol; 20 = D-mannosaminitol.

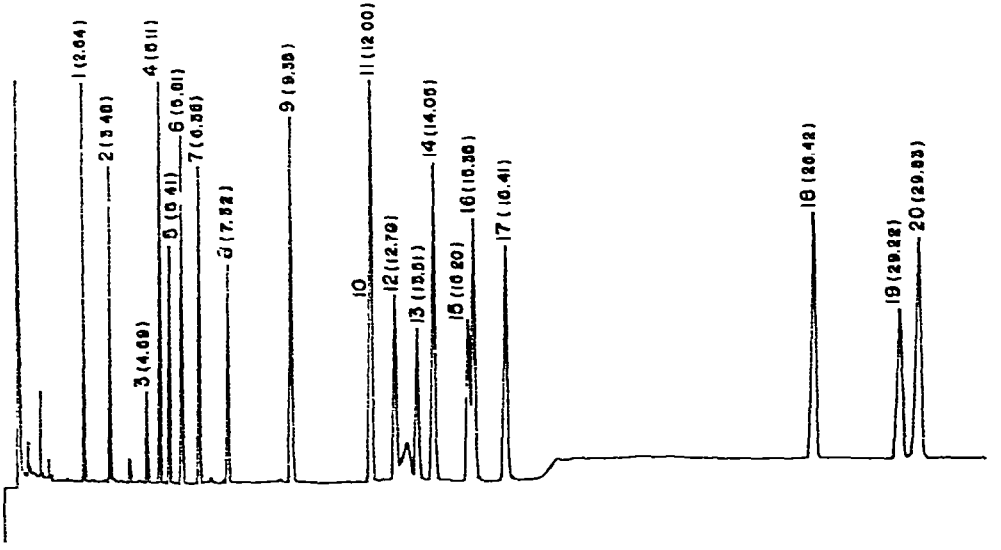

Fig. 3. Chromatogram of alditol acetates on the Carbowax 20M (12 m × 0.2 mm) fused-silica WCOT column using hydrogen as the carrier gas. Temperature, 185–200°C at 1°C/min, maintained at 200°C for 2 min and increased to 220°C at 20°C/min. Peak identities as in Figs. 1 and 2.

Simultaneous separation of alditol acetates of both neutral and amino sugars was tried on the 12-m Carbowax 20M column, giving the chromatogram of Fig. 3. Although the resolution of the acetates of glucitol and galactitol was not satisfactory, almost all monosaccharides could be rapidly separated as alditol acetates by this GLC system.

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