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Note

High-resolution gas chromatographic separation of alditol acetates on fused-silica wall-coated open-tubular columns

RYUICHI OSHIMA*, AKIRA YOSHIKAWA and JU KUMANOTANI
Institute of Industrial Science, University of Tokyo, 7-22-1, Roppongi, Minatoku, Tokyo 106 (Japan)
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Alditol acetates have been analyzed by gas–liquid chromatography (GLC) on packed columns with polar phases[1,2]. If the maximum operating temperature of capillary columns with polar phases is high enough to analyze alditol acetates, they are to be preferred because of their possibly higher performance. Holzer *et al.*[3] first reported the successful separation of thirteen alditol acetates on a glass capillary column with a chiral liquid phase, though some pairs of alditol acetates, *i.e.*, ribitol/arabinitol, rhamnitol/fucitol and mannitol/4-O-methylglucitol, could not be completely separated. This disadvantage was overcome using a longer column and a rather complicated temperature program[4]. As an alternative, a glass capillary column coated with OV-275 was used by Klok *et al.*[5]. Although, unfortunately, the separation between glucitol and galactitol was not described in their presentation, baseline separation of ten alditol acetates was achieved using a simple temperature-programmed operation. However, glass capillaries are of poor mechanical strength and troublesome in handling.

Recently, flexible fused-silica wall-coated open-tubular (WCOT) columns have become available, which are either of higher thermal stability or of improved efficiency compared to glass capillary columns. This note shows the superior capability of fused-silica WCOT columns to that of other GLC columns already in use for the separation of alditol acetates.

EXPERIMENTAL

Three different types of columns were examined; a fused-silica WCOT column with a non-polar liquid phase (SP-2100, 50 m × 0.2 mm; maximum operating temperature, 280°C; Hewlett-Packard, Avondale, PA, U.S.A.) and those with polar phases (FFAP and PEG-20M; 25 m × 0.25 mm; maximum operating temperature, 220 and 200°C, respectively; Gasukuro Kogyo, Tokyo, Japan). Columns were sealed into fittings through graphite ferrules or silicone septa. GLC was performed using a Hitachi 063 gas chromatograph with a flame ionization detector using helium as carrier gas. The carrier gas flow-rate was 1 ml/min, and the scavenger flow-rate was 20 ml/min. A 0.1–0.2-μl volume of a 1% (w/v) sample solution in chloroform was injected with a splitting ratio of *ca.* 1/100. GLC–mass spectrometry (MS) was carried out with a Hitachi M-80 instrument operated at 70 eV, and the spectra were processed on a Hitachi M-003 on-line computer system.

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Most alditol acetates were prepared conventionally[6] from commercial aldoses. Iditol acetate was obtained from L-sorbose as a mixture with glucitol acetate. 4-O-Methylglucitol acetate was prepared from 4-O-methylglucose which was a gift from Dr. A. Ishizu, University of Tokyo, Tokyo, Japan.

RESULTS AND DISCUSSION

On the SP-2100 column, hexitol acetates were well separated within 20 min under isothermal operation at 190°C; however, pentitol acetates and 6-deoxy-hexitol acetates exhibited close retention times. They could not be completely separated even with a temperature program of 130–190°C at a rate of 1°C/min (see Fig. 1). The FFAP column provided a more efficient and rapid separation of seventeen alditols acetates under isothermal operation at 205°C as may be seen in Fig. 2, which is advantageous for the reproducibility of retention times. A similar result was also obtained on the PEG-20M column at 195°C; however, the life-time of the column was unsatisfactory as the required operating temperature was too close to the maximum allowable temperature, *i.e.*, 200°C.

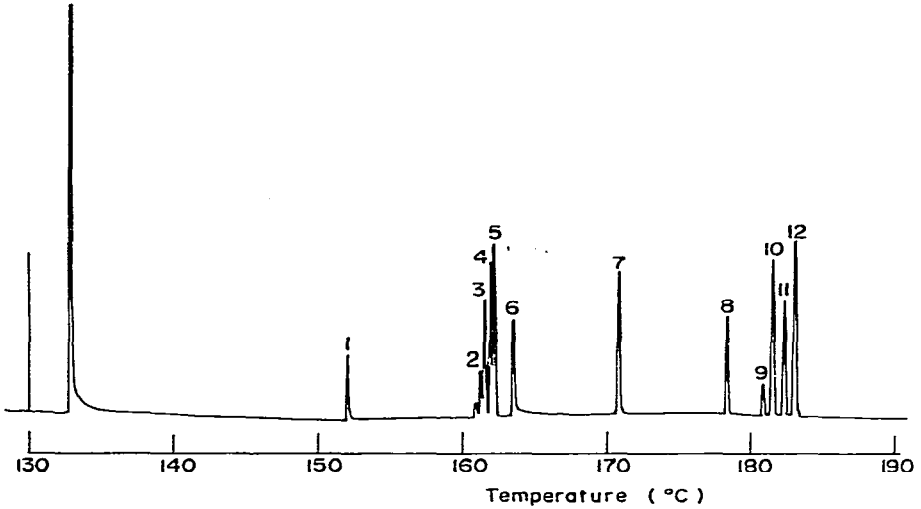

Fig. 1. Chromatogram of standard alditol acetates on the fused-silica WCOT column, SP-2100 (50 m × 0.2 mm). The column temperature was programmed from 130–190 C at a rate of 1 C/min, the injection-port and detector temperatures were 230 and 210 C, respectively. Peaks correspond to the acetates of: 1 = 2-deoxy-D-ribitol; 2 = D-ribitol; 3 = L-rhamnitol; 4 = D-fucitol; 5 = L-arabinitol; 6 = D-xylitol; 7 = 2-deoxy-D-galactitol; 8 = 3-O-methyl-D-glucitol; 9 = D-altritol; 10 = D-mannitol; 11 = D-galactitol; and 12 = D-glucitol.

Usage of the FFAP column was exemplified by the determination of the monosaccharide composition of the acidic polysaccharide separated from the sap of lac trees (*Rhus vernicifera*). After reduction and acetylation, the acid hydrolyzate of the carboxy-reduced polysaccharide derived using sodium borodeuteride and water-soluble carbodiimide[7] was applied to the FFAP column (see Fig. 3). Comparing chromatograms for standards and the hydrolyzate, the peaks shown in Fig. 3 were readily identified as the respective acetates of rhamnitol, arabinitol, 4-O-methylglucitol-6-d_2*, glucitol-6-d_2* and galactitol.

* Deuterium-substituted positions were determined by GLC–MS analyses[8].

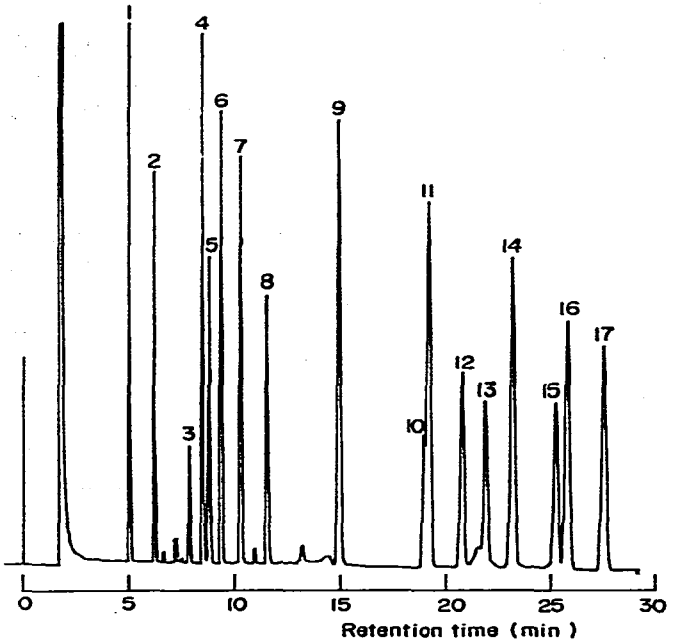

Fig. 2. Chromatogram of standard alditol acetates on the fused-silica WCOT column, FFAP (25 m × 0.25 mm). The column temperature was maintained at 205°C and the injection-port and the detecter temperatures were 230 and 210°C, respectively. Peaks correspond to the acetates of: 1 = D-digitoxitol; 2 = 2-deoxy-D-ribitol; 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; and 17 = L-iditol.

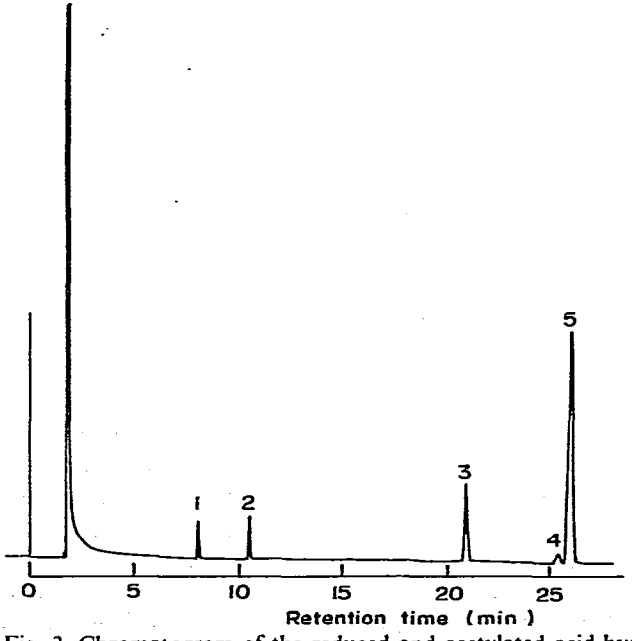

Fig. 3. Chromatogram of the reduced and acetylated acid-hydrolyzate of the carboxy-reduced polysaccharide from the sap of lac trees (*Rhus vernicifera*). Chromatographic conditions were the same as those for Fig. 2. Peaks correspond to the acetates of: 1 = rhamnitol; 2 = arabinitol; 3 = 4-O-methylglucitol-6-d_2; 4 = glucitol-6-d_2; 5 = galactitol.

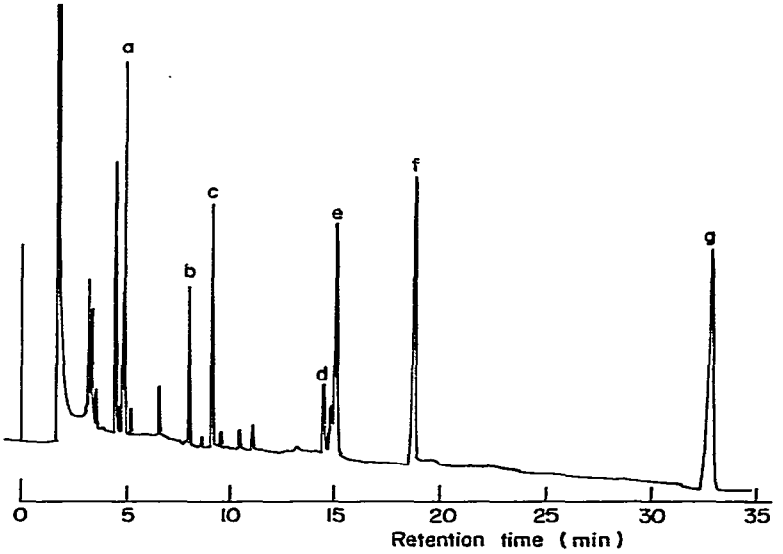

Fig. 4. Chromatogram of partially methylated alditol acetates obtained from the fully methylated carboxy-reduced polysaccharide found in the sap of lac trees (*Rhus vernicifera*). Temperatures: column, 190°C; injection port, 230°C; detector, 210°C. Column: FFAP. Peaks: a = 1,4-diacetyl-2,3,5-tri-O-methylarabinitol; b = 1,5-diacetyl-2,3,4,6-tetra-O-methylglucitol-6-d_2; c = 1,5-diacetyl-2,3,4,6-tetra-O-methylgalactitol; d = 1,4,5-triacetyl-2,3,6-tri-O-methylgalactitol; e = 1,3,5-triacetyl-2,4,6-tri-O-methylgalactitol; f = 1,5,6-triacetyl-2,3,4-tri-O-methylgalactitol; and g = 1,3,5,6-tetraacetyl-2,4-di-O-methylgalactitol.

The FFAP column is also effective for the separation of partially methylated alditol acetates from the hydrolyzate of the fully methylated[9] carboxy-reduced polysaccharide as illustrated in Fig. 4*; each peak in the chromatogram was identified by mass spectral analyses[8].

ACKNOWLEDGEMENTS

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* Partially methylated alditol acetates have recently[10] been separated on a glass support-coated open-tubular column with Silar-10C.