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# Gas Chromatographic Analysis of Aldoses

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Gas chromatography is an ideally suited method for the identification and quantification of carbohydrates in a mixture, although each carbohydrate gives several peaks resulting from anomeric and ring isomerization and often accurate quantification can not be achieved. To prevent multiplicity of peaks, the reduction of aldoses to corresponding alditols followed by the preparation of their volatile derivatives has been studied by many investigators.[2-5] Gas chromatographic separation of alditols as their trimethylsilyl (TMS) derivatives was found to be unsatisfactory,[6] while Sawardeker, *et al.*[3] succeeded in improved separation of acetylated alditols. However, rather long time has been required for preparation and separation of the acetyl derivatives. In the previous paper,[7] we described the gas chromatographic separation of alditols as their trifluoroacetyl (TFA) derivatives. In this work, we established the procedure for quantitative analysis of aldoses, which involved reduction of aldoses, trifluoroacetylation of the alditols produced and gas chromatography of the TFA derivatives.

## Experimental

**Reagents**——Ethyl acetate (GR; Kanto Chemical Co., Ltd.), trifluoroacetic anhydride (GR; Tokyo Kasei Kogyo Co., Ltd.) and sodium borohydride (GR; E. Merck AG) were used directly. Most of aldoses and alditols used in this study were also commercially available.

**Gas Chromatography**——Gas chromatography was performed on a Shimadzu GC-1C gas chromatograph equipped with a hydrogen flame ionization detector. The glass tube (1.8 m × 4 mm *i.d.*) was packed with 2% XF-1105, 2% QF-1 or 7% DC-1107 on a support of Gas-Chrom P (80—100 mesh). Gas chromatographic conditions were described in Figure and Table.

**Preparation of Derivatives**——To 0.5 ml of aqueous solution (containing 100—500 $\mu$g of a mixture of aldoses), 0.5 ml of 1% $NaBH_4$ in water was added. The solution was stand for 30 min at room temperature and the excess borohydride was destroyed by addition of 0.5 ml of Amberlite CG-120 ($H^+$). The resin removed by filtration, and the filtrate was evaporated to dryness. To the residue 1 ml of MeOH was added and evaporated to dryness to remove the borate as methyl borate. After the repetition of the final treatment two or three times, the residue was dried *in vacuo*. The sample was treated with 0.1 ml of AcOEt and 0.1 ml of trifluoroacetic anhydride (TFAA) for 30 min at room temperature and 1—2 $\mu$l of the reaction mixture was injected directly to the gas chromatograph.

## Results and Discussion

Although the reduction of aldoses to alditols was carried out according to the usual method, only 30 min was required for completion of the reaction in this case. Trifluoroacetylation of alditols with trifluoroacetic anhydride in tetrahydrofuran, acetonitrile or ethyl

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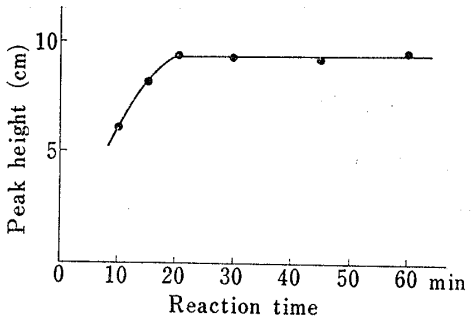


Fig. 1. Trifluoroacetylation of Mannitol

Mannitol (500 $\mu$g) was treated with 0.1 ml of AcOEt and 0.1 ml of TFAA at room temperature (20°).

acetate proceeded without catalyst at room temperature and the reaction was accomplished in 20 min as illustrated in Fig. 1, where the fine crystals of mannitol was used as an example. Among the solvent tested, ethyl acetate was found most suitable, because tetrahydrofuran usually contained the unknown substance, which was not removed by distillation and gave a peak overlapping with fucitol trifluoroacetate under the gas chromatographic condition described below and acetonitrile gave long tailing. TFA derivatives of alditols are very volatile and stable in the reaction mixture in which the excess of TFAA is present, but the purified derivatives are easily hydrolyzed even with the moisture and furthermore they are prone to decompose on catalytic surfaces during gas chromatography. Consequently, it is necessary to use glass tubes for gas chromatographic separation to prevent thermal decomposition.

The resolution of TFA derivatives of alditols was examined on several columns and relative retention times are given in Table I. The satisfactory separation was achieved on

Table I. Relative Retention Times for Alditol Trifluoroacetates

| Parent aldose | 2% XE–1105 140° | 2% QF–1 140° | 7% DC–1107 80° |
|---|---|---|---|
| Ribose | 0.70 | 0.86 | 0.82 |
| Arabinose | 0.85 | 0.93 | 0.92 |
| Xylose | 1.00 (6.91 min) | 1.00 (5.31 min) | 1.00 (6.57 min) |
| Mannose | 1.44 | 1.32 | 1.38 |
| Glucose | 1.92 | 1.43 | 1.62 |
| Galactose | 2.12 | 1.57 | 1.82 |
| Fucose | 0.57 | 0.71 | 0.85 |
| Rhamnose | 0.42 | | |
| 2-Deoxyribose | 0.55 | | |
| 2-Deoxyglucose | 1.23 | | |
| 2-Deoxygalactose | 1.57 | | |

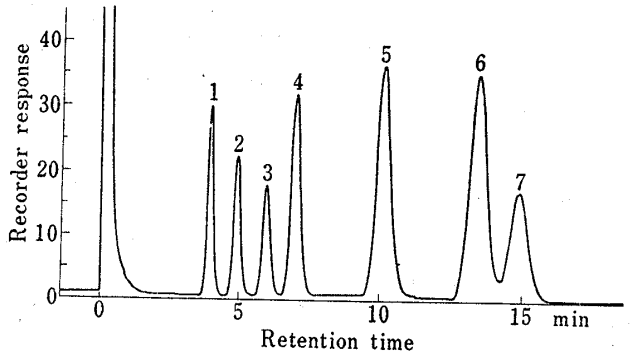


Fig. 2. Gas Chromatogram of Alditol Trifluoroacetates produced from a Standard Mixture of the Parent Aldoses

peak: 1. fucose 2. ribose 3. arabinose 4. xylose 5. mannose 6. glucose 7. galactose
column: 2% XF–1105 (on Gas-Chrom P) 1.8 m×4 mm i.d.
temperature: column 140°, HFID 200°
carrier gas: $N_2$ 70 ml/min
sens. 100
range 0.4 V

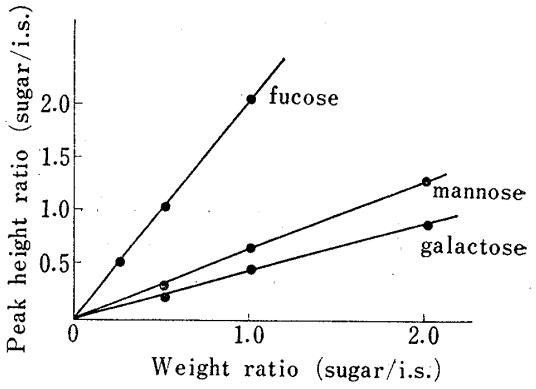


Fig. 3. Calibration Curves for Aldoses Using Xylose as an Internal Standard (i.s.)

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2% XF–1105 column in 20 min under isothermal condition (Fig. 2). This column is currently the most efficient chromatographic system, because tailing and broad peaks often appear with the columns of SE–30, DC–1107 and SE–52, and other polar columns can not be used owing to the adsorption and decomposition of the derivatives on them. When a ketose is submitted to this method, it usually gives two peaks owing to the isomers produced by the reduction. Accordingly, the presence of ketoses often complicates the analysis of aldoses.

The calibration curves for fucose, mannose and galactose using xylose as an internal standard are shown in Fig. 3. The standard samples were analyzed and the variation of the data obtained are illustrated in Table II. This indicates that the method is accurate enough

Table II. Analysis of a Standard Sample

| Compound | Added (μg) | Found (μg) 1 | 2 | 3 | 4 | 5 | Average μg | Recovery (%) |
|---|---|---|---|---|---|---|---|---|
| Fucose | 50 | 50.2 | 49.8 | 51.3 | 52.5 | 50.0 | 50.8 | 101.6 |
| Mannose | 150 | 148.2 | 150.9 | 150.0 | 153.6 | 152.1 | 151.2 | 100.8 |
| Galactose | 200 | 197.8 | 200.0 | 198.0 | 200.0 | 202.0 | 199.6 | 99.8 |

for quantitative analysis of aldoses. The application of this method to analysis of glycoproteins will be published in this Bulletin.

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