Preliminary communication

Gas–liquid chromatography of butaneboronates of carbohydrates and their trimethylsilyl ethers*

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The conversion of carbohydrates into suitably volatile derivatives, such as methyl ethers[1], acetates[2,3], and trimethylsilyl (TMS) ethers[4,5], allows analysis by g.l.c. Because free sugars may exist in various structural and anomeric forms, they seldom form single derivatives. Consequently, multiple peaks for a single sugar are generally recorded on g.l.c., and this may complicate the assignment of individual peaks to the parent sugar. Nevertheless, a number of useful systems have been devised[6]. Sweeley *et al.*[5] could find no easily prepared sugar derivative which gave single peaks and which could be completely separated from other configurational isomers. However, good g.l.c. analyses of glycoses after conversion to the alditol acetates[2,3] or the TMS ethers of the aldonolactones[7], have been described.

Cyclic boronic esters of 1,2- and 1,3-diols and other compounds have been used for g.l.c. and mass-spectrometric analysis[8,9], and these derivatives appear to be stable during esterification[10] and silylation[8]. We now report on the g.l.c. behaviour of these mixed derivatives**.

G.l.c. analyses were performed on a Pye 104 dual-column chromatograph with flame-ionisation detection, glass columns (9 ft. × 0.25 in.) of ECNSS-M on Gas Chrom Q (Applied Science Laboratories Inc.) [prepared by soaking Gas Chrom Q (97 parts) with ECNSS-M (3 parts) in chloroform, followed by filtration and drying], a nitrogen flow-rate of 60 ml/min, and injection at 100° with programming at 2°/min from the solvent front.

The effects of different reaction conditions were first studied. D-Glucose (5 mg) was dissolved in pyridine (1 ml) at 60° and butaneboronic acid (25 mg) was added. The solution was stored for various times at room temperature and, at intervals, hexamethyldisilazane (0.2 ml) and chlorotrimethylsilane (0.1 ml) were added and the reaction mixture (0.5 μl) was injected. The chromatograms, after reaction times of ~ 1 min, 0.5 h, and 4 h prior to silylation, are shown in Fig.1. Fig.1*c* was also obtained by heating the above solution to 100° for 10 min. The effect of varying the butaneboronic acid–D-glucose ratio from zero to 5:1 is shown in Fig.1*c* and Fig.2. When a solution of a mixture of various pentoses and hexoses (~ 2 mg each, dried *in vacuo* at 58°) in pyridine (2 ml) was treated

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***Editorial Note.* Since the receipt of this paper, Eisenberg (*Carbohyd. Res.*, 19 (1971) 135) has described the use of cyclic boronic esters in the g.l.c. analysis of carbohydrates.

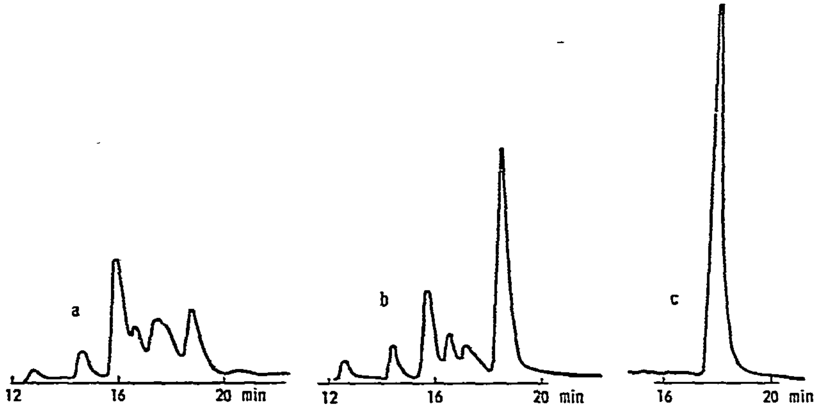

Fig.1. Effect of varying reaction time with butaneboronic acid prior to silylation: (*a*) immediate silylation; (*b*) 0.5-h reaction; (*c*) 4-h reaction.

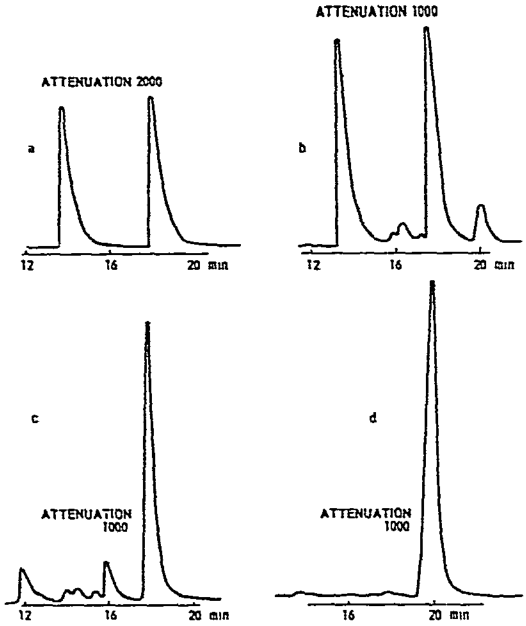

Fig.2. Effect of varying the butaneboronic acid–D-glucose ratio: (*a*) zero; (*b*) 1:5; (*c*) 1:1; (*d*) 2:1.

with butaneboronic acid (80 mg) at 60° for 10 min and then at room temperature for 48 h followed by addition of an aliquot (0.5 ml) of hexamethyldisilazane (0.2 ml) and chlorotrimethylsilane (0.1 ml), the g.l.c. trace in Fig.3 was obtained. In control reactions, each component of the mixture gave essentially a single peak; two or more peaks were obtained when the TMS derivatives were prepared without prior addition of butaneboroni acid. The retention times of the butaneboronates of L-fucose, D-arabinose, and D-xylose were not affected by silylation and therefore, presumably, these derivatives contain no unblocked hydroxyl groups.

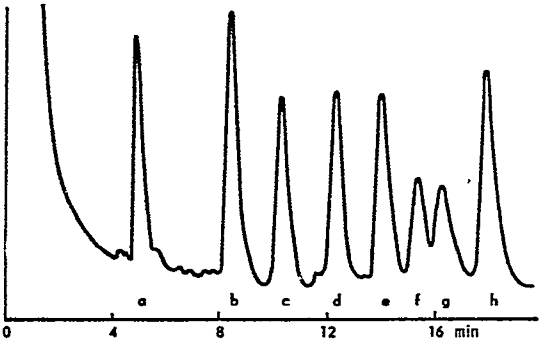

Fig.3. G.l.c. of a mixture of D-lyxose (*a*), L-fucose (*b*), D-arabinose (*c*), D-xylose (*d*), D-fructose (*e*), D-galactose (*f*), D-mannose (*g*), and D-glucose (*h*) after conversion into the butaneboronate–TMS derivatives.

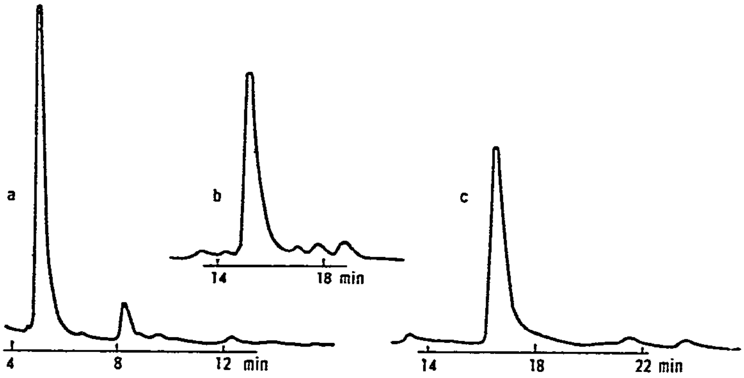

Fig.4. G.l.c. traces of butaneboronate–TMS derivatives of sugars after equilibration in water: (*a*) D-lyxose; (*b*) D-galactose; (*c*) D-mannose.

The anomeric composition of aqueous solutions of sugars differs from that obtained when dry samples are directly dissolved in pyridine. When aqueous solutions of monosaccharides (4 mg/ml) were stored for ≮ 24 h at room temperature and then evaporated and the residues dried and converted into the butaneboronate–TMS derivatives, g.l.c. revealed that minor peaks were more prominent than for unequilibrated sugars, particularly for D-galactose, D-mannose, and D-lyxose (Fig.4). These minor peaks did not complicate the analysis of mixtures, and it is not known at present whether the peaks arise from different anomeric equilibria or the presence of traces of water. Addition of one drop of water to a solution of D-galactose and butaneboronic acid in pyridine, followed by silylation, resulted in the appearance of multiple peaks. Water added after the silylation stage had little effect in the presence of excess of the silylation reagent. However, even with thoroughly dried samples, minor peaks do occur.

Of the other compounds investigated, D-ribose, L-rhamnose, erythritol, D-arabinitol, xylitol, and D-glucitol do not give a single, major peak when treated as described above.

The above procedure allows a simple, rapid, qualitative analysis of some monosaccharide mixtures. Quantitative aspects are under investigation, and preliminary results indicate that, for instance, mixtures of D-glucose and D-fructose may be analysed. Further work on this and structural and synthetic aspects is in progress.

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