Preliminary communication

Cyclic butaneboronic acid esters: novel derivatives for the rapid separation of carbohydrates by gas–liquid chromatography

FRANK EISENBERG, Jr.

National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014 (U. S. A.)

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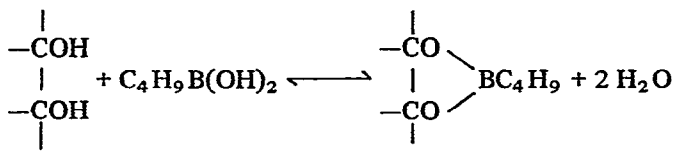
The separation of monosaccharides as acetates and trimethylsilyl ethers by gas–liquid chromatography (g.l.c.) is complicated by the formation of multiple derivatives, representing anomeric forms, with overlapping peaks. For this reason, these sugars are first reduced to glycitols which on acetylation[1] or silylation[2] yield single peaks. Although the introduction of silylation[3] has led to rapid progress in the g.l.c. of carbohydrates, the method fails to separate the common hexitols[2] D-glucitol, D-mannitol, and D-galactitol. While the hexitol acetates have thus remained the only derivatives suitable for the gas–liquid chromatographic separation and quantification of these biologically important sugars[4,5], the procedure is time-consuming.

Recently, the alkaneboronic acids have been introduced as aids in the volatilization of various types of organic compounds[6] for g.l.c., and in this report the application of butaneboronic acid to the g.l.c. of sugars and polyols is described. The general equation for the reaction is as follows:



(1)

diol butaneboronic acid dioldibutaneboronate

A previous application of this type of reaction to carbohydrates was reported by Bourne *et al.*[7] who found increased mobility of sugars chromatographed on paper in the presence of benzeneboronic acid.

The boronates are formed simply by combining the sugar and the reagent in a ratio of 1:5 in pyridine to give a final concentration of 1 mg of sugar per ml. An aliquot is then injected into the gas chromatograph. Boiling may be required to dissolve the sugar; since the reagent is kept moist for preservation, no special attention is necessary to the drying of

sugar or pyridine, although prolonged exposure of the sugar derivative to atmospheric moisture will lead to decomposition. The reagent, a white, crystalline solid, is obtained from Applied Science Laboratories, Inc., State College, Pa. under the name "n-butylboronic acid".

Table I shows retention times for various sugar and polyol butaneboronates. As with other derivatives, the aldohexoses show overlapping peaks, but the cyclitols and alditols invariably give single, sharp, symmetrical peaks.

TABLE I

GAS-LIQUID CHROMATOGRAPHY OF SUGARS AND POLYOLS AS BUTANEBORONATES [a]

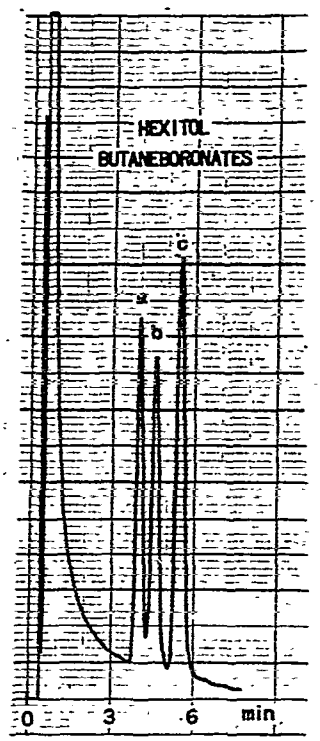
Compound	*Column temp., degrees*	*Retention time, min*
D-Mannose	200	2.5, 4.5
D-Glucose	200	4.0, 4.9 [b], 5.8
D-Galactose	200	5.0
D-Mannitol	200	4.0
D-Glucitol	200	4.5
D-Galactitol	200	5.5
myo-Inositol	200	5.7
muco-Inositol	200	6.0
L-Fucitol	103	5.9
L-Fucose	144	7.1
D-Rhamnose	165	4.1

[a] On a Barber–Colman Model 10 gas–liquid chromatograph equipped with an argon ionization detector at 225°, flash heater 225°, and (1.8 m × 5 mm ID) glass U-tube packed with 3% OV-17 on 100–120 mesh GasChrom Q. [b] Minor peak.

Fig.1 shows the separation of a mixture of the butaneboronates of D-mannitol, D-glucitol, and D-galactitol. Identical results were obtained after boronation of a sodium borohydride-reduced mixture of the parent aldohexoses. The separation is complete in 6 min compared to 30 to 80 min for the acetates[1,4] of the same three hexitols. The rapidity of boronation compared to acetylation brings the total time required for derivatization and separation of the hexitols, as boronates, to less than one-tenth that of the acetates. As expected, retention times of the individual hexitol boronates are the same when assayed singly or mixed. Excess reagent is innocuous since it emerges with the solvent.

In addition to rapid and facile separation, these compounds can be condensed from the effluent gas for further study and characterization. For this purpose, *myo*-inositol was treated with butaneboronic acid in pyridine and chromatographed as shown in Fig.2. The effluent material was collected between 5.25 and 6 min, in 50% yield, in an open capillary attached to the exit port of the gas chromatograph. The derivative, a clear, colorless liquid, on rechromatography in chloroform gave results identical with those shown in Fig.2. Addition of 3M hydrochloric acid to a methanolic solution of the collected derivative caused immediate crystallization of a compound melting at 224°, in agreement with the literature value for *myo*-inositol[8]; the m.p. of a mixture of this compound with authentic *myo*-inositol was not depressed.

The mass spectrum of the condensate, Fig.3, shows the molecular ion at *m/e* 378,



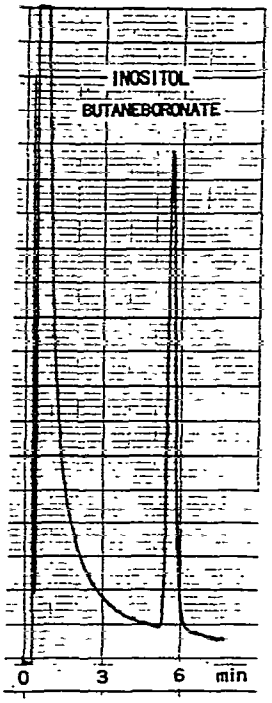


Fig.1. Gas–liquid chromatogram (detector response versus retention time in min) of a mixture of butaneboronates of: (a) D-mannitol, (b) D-glucitol, and (c) D-galactitol; 1 μg of each hexitol. Column temp. 200°.

Fig.2. Gas–liquid chromatogram of *myo*-inositol tris(dibutaneboronate); 1 μg *myo*-inositol. Column temp. 200°.

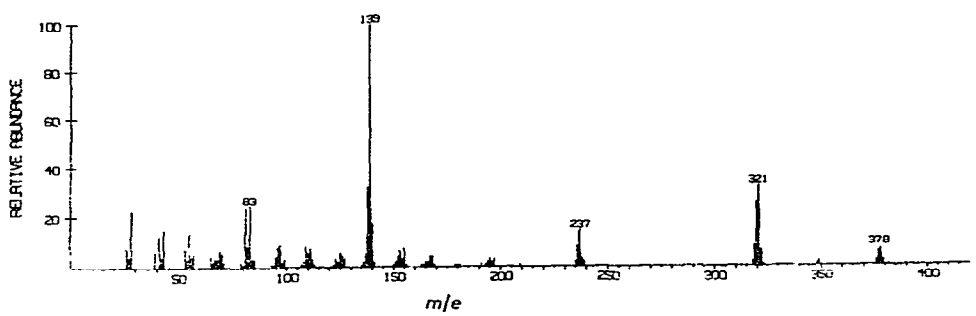


Fig.3. Mass spectrum of *myo*-inositol tris(dibutaneboronate), determined with a LKB GC–MS mass spectrometer at 70 eV ionization voltage.

theoretical for $C_6H_6O_6B_3(C_4H_9)_3$, a major fragment at *m/e* 321, representing the loss of a butyl group, and the principal fragment at *m/e* 139, theoretical for $C_3H_3O_2BC_4H_9$, indicating rupture of the carbon ring and loss of two boronate residues. These results unequivocally support equation (1) and the identification of the derivative as *myo*-inositol tris(dibutaneboronate).

Studies are in progress on the application of this reaction to the quantitative analysis of the carbohydrate components of glycoproteins.

Note added in proof: (Received June 14th, 1971)

Butaneboronic acid may be contaminated with isobutaneboronic acid which likewise reacts with diols to form derivatives with slightly shorter retention times than the normal isomers. With all pure polyols tested one batch of reagent gave single peaks as shown above, but another batch gave an additional minor peak (10% of the major) 0.5 min earlier. The minor forepeaks seen in other studies (see Ref. 6) may also be the result of this contamination. Reagent received from the supplier can be tested for purity by g.l.c. in pyridine on the column above at 92°; butaneboronic acid emerges at 10 min and the contaminant at 7 min.

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