PAPER CHROMATOGRAPHY OF CARBOHYDRATES AND RELATED COMPOUNDS IN THE PRESENCE OF BENZENEBORONIC ACID

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The well-known reaction of polyhydroxy-compounds with borate ions to form anionic complexes has been used extensively for the separation of carbohydrates and related compounds by paper electrophoresis in borate solution[1] and chromatography on columns of anion exchange resins[2] and charcoal[3]. The presence of boric acid has also been shown to affect the paper chromatographic behaviour of carbohydrates[4], the increase or decrease in R_F value being dependent on the pH of the solvent[5]. We now report the paper chromatographic behaviour of carbohydrates and related compounds in the presence of benzeneboronic acid.

EXPERIMENTAL

Solvents

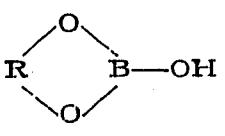
The solvents used for descending chromatography on Whatman No. 1 paper were (a) ethyl acetate–acetic acid–water (9:2:2 v/v) and (b) 0.55 % solution of benzeneboronic acid in ethyl acetate–acetic acid–water (9:2:2 v/v). The solvent front moves about 30 cm in 4–5 h.

Spray reagent

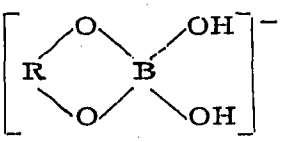
The compounds were detected on paper chromatograms with potassium periodatocuprate and rosaniline[6].

RESULTS AND DISCUSSION

The products of the reactions between boric acid or borate ions and polyhydroxy-compounds have structures of the types I–IV.



(I)



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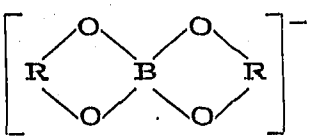

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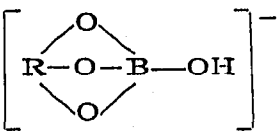
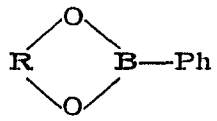


(IV)

Since boric acid, $B(OH)_3$, does not act as a proton donor but a Lewis acid[7], accepting the electron pair of the base, *e.g.* OH^-, to form the anion $B(OH)_4^-$, the compounds formed at acidic pH values are neutral esters (I) whereas those formed under alkaline conditions are anionic complexes (II–IV). Compounds with structure I should have higher R_F values in solvents with a stationary aqueous phase than those with structures II–IV. This is indeed confirmed by the chromatographic behaviour of D-glucitol in solvents containing (i) boric acid and acetic acid (R_G 2.2; movement with respect to glucose) and (ii) boric acid and pyridine (R_G 0.3)[5].

Benzeneboronic acid, $Ph \cdot B(OH)_2$, is known to react with several polyhydroxy-compounds to give esters with structure V[8–10]. The detailed structures of some of these have been elucidated[11,12].



(V)

The replacement of the hydroxyl group of I by a phenyl group should increase the affinity of the ester for the organic solvent and thus result in an increase in R_F value. The results (Table I) show that this is indeed the case; in solvent (b), in which the boric acid has been replaced by benzeneboronic acid, D-glucitol moves with an R_G value of 5.6.

TABLE I

R_F VALUES OF CARBOHYDRATES AND RELATED COMPOUNDS IN SOLVENTS (a) AND (b)

Compound	*R_F value*	
	Solvent (a)	*Solvent (b)*
Glycerol	0.32	0.35
Erythritol	0.23	0.31
D-Arabitol	0.14	0.50
1-deoxy-	0.45	0.71
5-deoxy-	0.46	0.85
Ribitol	0.14	0.48
2-deoxy-D-	0.32	0.46
Xylitol	0.14	0.45
Allitol	0.17	0.49
D-Altritol	0.16	0.51
1-deoxy-	0.36	0.85
1,6-dideoxy-	0.57	0.97
Galactitol	0.07	0.47
1-deoxy-D-	0.31	0.68
1,6-dideoxy-	0.58	0.85
D-Glucitol	0.08	0.45
2-deoxy-	0.22	0.60
3-O-methyl-	0.19	0.44
4-O-methyl-	0.30	0.40
D-Mannitol	0.08	0.43
1,6-dideoxy-	0.58	0.96
2-O-methyl-	0.22	0.70
1,2-di-O-methyl-	0.46	0.82

(continued on p. 255)

TABLE I (*continued*)

Compound	R_F value	
	Solvent (a)	Solvent (b)
DL-Glycerose	0.38	0.40
D-Erythrose	0.31	0.84
L-Threose	0.31	0.53
D-Arabinose	0.12	0.11
D-Lyxose	0.18	0.18
D-Ribose	0.25	0.50
2-deoxy-	0.40	0.41
D-Xylose	0.15	0.15
D-Altrose		
1,6-anhydro-β-pyranose	0.20	0.19
D-Galactose	0.06	0.08
6-deoxy-	0.19	0.18
1,6-anhydro-β-pyranose	0.33	0.38
D-Glucose	0.08	0.08
3-O-methyl-	0.21	0.23
5-deoxy-	0.28	0.27
methyl α-pyranoside	0.20	0.21
1,6-anhydro-β-pyranose	0.33	0.31
D-Gulose	0.13	0.27
1,6-anhydro-β-pyranose	0.31	0.30
L-Idose	0.09	0.16
D-Mannose	0.08	0.09
6-deoxy-	0.22	0.25
3,4-di-O-methyl-	0.55	0.59
methyl α-pyranoside	0.42	0.42
1,6-anhydro-β-pyranose	0.33	0.39
D-Fructose	0.11	0.12
L-Sorbose	0.10	0.16
allo-Inositol	0.04	0.11
dextro-Inositol		
3-O-methyl-	0.09	0.08
epi-Inositol	0.01	0.04
levo-Inositol	0.03	0.02
2-O-methyl-	0.07	0.07
muco-Inositol	0.05	0.05
1-deoxy-	0.08	0.08
myo-Inositol	0.02	0.02
1-deoxy-	0.07	0.06
scyllo-Inositol	0	0

Table I shows the R_F values of some carbohydrates and related compounds in the solvent containing the benzeneboronic acid [solvent (b)]. In all cases comparison was made with a solvent from which benzeneboronic acid was omitted [solvent (a)]. It can be seen that a number of useful separations are obtained, *e.g.* most aldoses and ketoses are well separated from their reduction products within 4 to 5 hours.

The isolated benzeneboronates of many polyhydroxy-compounds are easily hydrolysed, even during chromatography, with a solvent containing water, *e.g.* solvent (a). In this solvent the benzeneboronic acid, which can be detected under U.V. light, moves almost with the solvent front and hence is easily separated from the polyhydroxy-compounds. Thus, solvent (b) offers an advantage for separations on a preparative scale. Normally, boric acid is removed from an eluate by repeated distillation with methanol. However, the benzeneboronic acid can be separated from the polyhydroxy-

compounds by re-chromatography of the eluate in solvent (a), avoiding any destruction of the polyhydroxy-compounds which might occur when boric acid is removed by repeated distillation with methanol.

It seems reasonable to assume that only compounds which have at least two hydroxyl groups in an appropriate spatial arrangement to react with benzeneboronic acid will have significantly higher R_F values in solvent (b) than in solvent (a). However, comparison of the R_F values in the two solvents cannot be regarded as a satisfactory method to detect such an arrangement in a compound, since *e.g.*, the R_F values of glycerol and D-glucose are not appreciably altered by the presence of benzeneboronic acid, although crystalline benzeneboronates of these have been obtained[11,12]. It is likely that, under the conditions of the chromatography, the equilibrium does not favour the formation of certain benzeneboronates, which will of course have differing relative stabilities according to ring size, substituents, etc. On the other hand, Table I shows that the aldoses and cyclitols, the R_F values of which are markedly affected by the presence of benzeneboronic acid, have in their more stable conformation a 1 (*ax*), 3 (*ax*)-diol grouping. *muco*-Inositol and 1,6-anhydro-β-D-glucopyranose also possess such a diol grouping, but as mentioned earlier the conditions of the chromatography might not favour the formation of their benzeneboronates. 2-Deoxy-D-ribose, the R_F value of which is the same in both solvents, possesses such a diol grouping only in the C1 conformation (REEVES' nomenclature)[13] of its α-anomer. It is not possible to decide which anomer and conformation of D-ribose reacts with benzeneboronic acid.

During the course of this work GAREGG AND LINDBERG[14] reported the paper electrophoretic behaviour of carbohydrates in solutions of sulphonated benzeneboronic acid. Presumably, under the conditions used, the esters formed migrate due to the ionisation of the sulphonic acid group.

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SUMMARY

The R_F values of a number of polyhydroxy-compounds are markedly increased by the addition of benzeneboronic acid to the solvent. The increase is due to the formation of esters between benzeneboronic acid and the polyhydroxy-compounds. For certain carbohydrates and cyclitols the increase has been related to their structures. Acyclic polyhydroxy-compounds have, in general, much higher R_F values in the solvent containing benzeneboronic acid than the aldoses or ketoses from which they derive. This provides a rapid method for the separation of pairs of such compounds.

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