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COMPLEXATION OF D-GLUCOSE WITH BORATE

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ABSTRACT

Matrix analysis of a polarimetric titration of D-glucose by tetraborate ($B_4O_7^{2-}$) indicates that only two species are involved, namely D-glucose·$B_4O_7^{2-}$ and free D-glucose. No detectable amount of a bis(bidentate) species (D-glucose)$_2$·$B_4O_7^{2-}$ was present.

INTRODUCTION

When tetraborate $(B_4O_7)^{2-} = (B^-)$ is added to D-glucose (Glc), a reaction occurs that may be monitored by physical changes: in pH (with respect to $Na_2B_4O_7$), optical rotation of polarized light, a difference of the summed refractive indices, or shifts in the magnetic resonances of protons and of other pertinent atoms (^{13}C, ^{11}B). Boronates of D-glucose have been isolated only as derivatized products[1], and even so, the 2,4-benzeneboronate of *N*-(*p*-bromophenyl)-α-D-ribopyranosylamine[2] appears to be the only such ester whose absolute structure, as determined by X-ray analysis, is known. Structures have been inferred as involving dehydration at O-4,6 of D-glucopyranose to form a six-membered ring, including the boron atom, or with O-3,4 to give a five-membered ring. Similarly, Foster[3] and Foster and Stacey[4] concluded, on the basis of electrophoretic transport-coefficients of D-glucose and homologous derivatives in borate buffer, that borate complexes with D-glucose as bidentate and bis(bidentate) structures, specifically with (*a*) O-2,4 or O-4,6 in the open-chain form, or (*b*) O-1,2 or O-4,6 in the pyranoid form, or (*c*) O-1,2 (or the tridentate O-3,5,6 structure) in the furanoid form.

We have recently proposed[5], on the basis of p.m.r. studies, that D-glucose reacts with borate to form a D-glucofuranose 1,2-borate complex, whereas D-fructose forms a tridentate D-fructofuranose 1,3,6-borate complex. The purpose of this communication is to provide additional rationale for the structure of the D-glucose derivative beyond that previously given[5], and (to the extent permitted by matrix analysis) for the apparent absence of changes (or the transparency) in optical rotatory dispersion of a D-glucose solution upon successive additions of sodium tetraborate.

RESULTS AND DISCUSSION

P.m.r. studies of borate complexation with D-*glucose*

The addition of tetraborate (or aryl- or alkyl-boronate) to a solution of D-glucose in D_2O causes a drastic change of its p.m.r. spectrum (Fig. 1). The reaction does not occur with orthoboric acid, HBO_3, or GeO_2. Equally drastic changes may

TABLE I

COMPLEXATION OF D-GLUCOSE DERIVATIVES WITH TETRABORATE

Compound	*Complexation*	
	Yes	*No*
Methyl α-D-glucopyranoside		×
α-D-Glucopyranosyl phosphate		×
α-D-Glucopyranose 6-phosphate	×	
2-Deoxy-D-*arabino*-hexose[a]		×
3-*O*-Methyl-α-D-glucopyranose	×	
Sucrose		×
Cellobiose		×
Maltose		×
Gentiobiose	×	
Raffinose		×

[a]This commercial product behaved anomalously. On the addition of tetraborate, the doublet furthest downfield (5.445, 5.405), presumably that of H-1α, disappeared and no new band appeared and there were no gross changes in the rest of the spectrum.

TABLE II

P.M.R. TITRATION OF D-GLUCOSE WITH BORATE[a]

Concentrations (M)						*Pyranose/furanose*	
Glc_0	B_0	*Glc*	$[B^-]$	$[GlcB^-]$	$[Glc_2B^-]$	*Calc.*	*Found*
0.2	0.01	0.185	0.000180	0.000448	0.00534	18.8	3.0
0.2	0.03	0.156	0.000694	0.0146	0.0147	5.3	1.1
0.2	0.05	0.129	0.00153	0.0264	0.0221	2.7	0.62
0.2	0.075	0.100	0.00323	0.0436	0.0281	1.4	0.43
0.2	0.10	0.75	0.00619	0.0631	0.0307	0.80	0.20
0.2	0.15	0.041	0.0187	0.104	0.0275	0.31	0.17
0.2	0.20	0.023	0.0435	0.136	0.0203	0.15	0.11

[a]The pyranose/furanose ratio was calculated by assuming that both H-1α*p* and H-1β*p* were converted into H-1α*f*. Knowing the concentrations of B_0 and Glc_0, then $[Glc]/([GlcB^-]+[Glc_2B^-])$ may be calculated by using Connor and Bulgrin's equilibrium constants[10] and comparing those with the graphically integrated values found. The consistently higher calculated values of [Glc] reflect closer agreement with the data of Nazarenko and Ermak[12]. However, even with the latter's value for K_{21} and with the interpolated value for $K_1 = 37.2$, then at $Glc_0 = 0.2$M, $B_0 = 0.01$M, the pyranose/furanose ratio is calculated to be 12.3, a value appreciably higher than that found.

be observed in some, but not all, derivatives or homologs of D-glucose, including selected disaccharides (Table I). Conspicuous among the changes for D-glucose is the disappearance of the H-1αp and H-1βp doublets and the appearance downfield of a doublet at approximately the position expected[7] for H-1αf. The equilibrium for the reaction with tetraborate is attained rapidly, and field-positions of H-1αf and H-1αp may be monitored titrimetrically (Fig. 2, Table II).

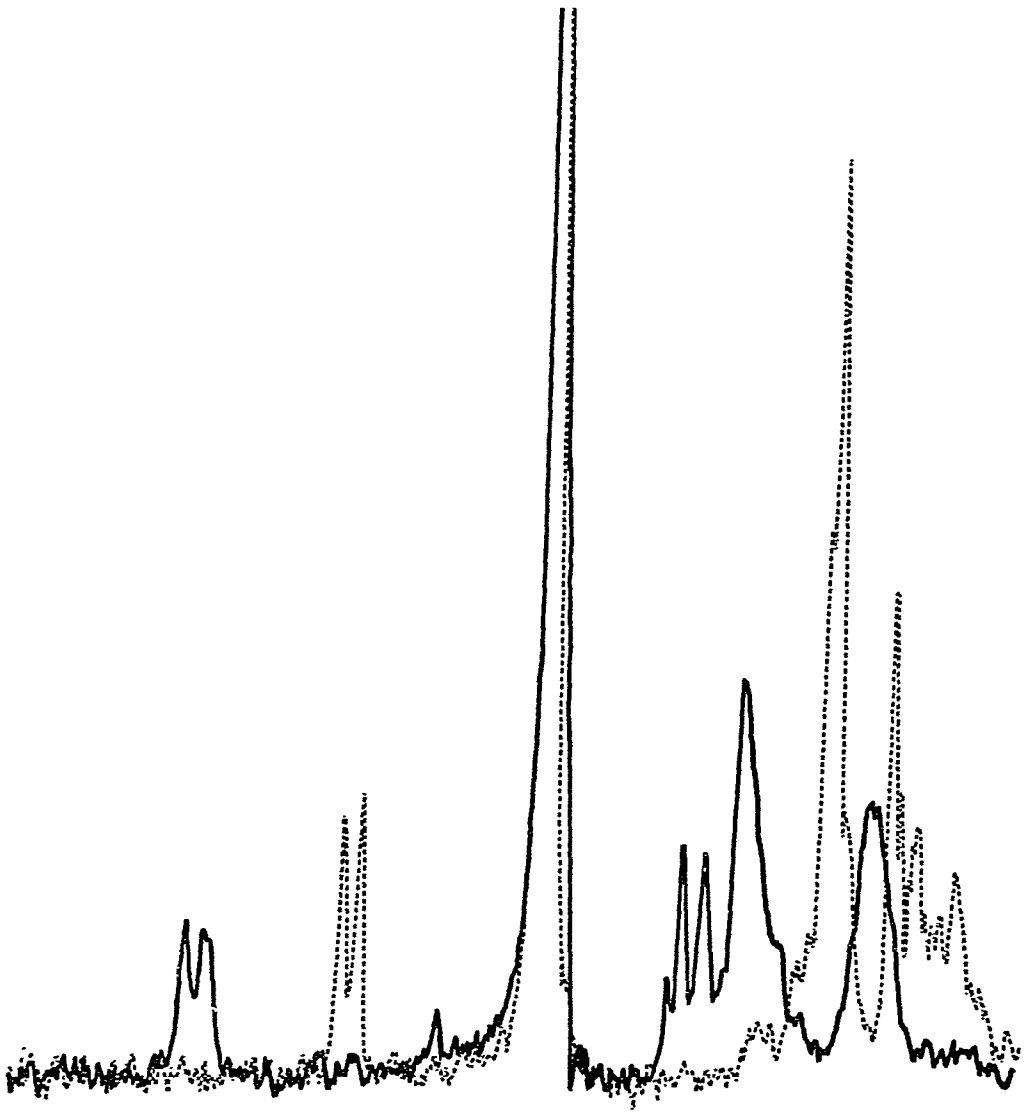

Fig. 1. P.m.r. curves (60 MHz, single scan, room temperature) for D-glucose (0.2M) (dotted) and D-glucose (0.2M) (solid) in D_2O.

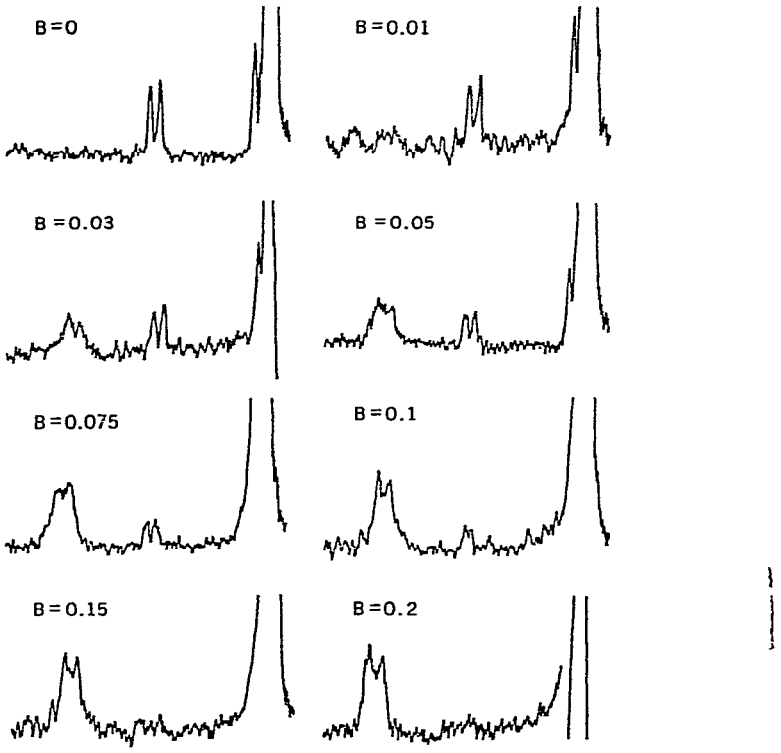

Fig. 2. N.m.r. titration (60 MHz, single scan, room temperature) of α-D-glucose (0.2M) with tetraborate, showing disappearance of H-1α*p* and appearance of new doublet at low field, postulated as H-1α*f*

Borate is not a general shift-reagent. In those instances (Table I) where no reaction occurs, as with sucrose or D-glucosyl phosphate, there is no qualitative spectral change or shift upon the addition of tetraborate. However, when reactions do occur, the entire spectrum changes, including the H-1 signals, and the final spectra of diverse compounds may be remarkably similar, for example D-glucose *vs.* gentiobiose (Fig. 3). Inspection of Table I shows that no reaction occurs in those (1→4)-linked disaccharides (sucrose, maltose, and cellobiose) where the glucofuranose ring cannot be formed, nor does it occur when the pyranoid ring is fixed and C-1 is involved in ring closure, as in α-D-glucosyl phosphate. On the other hand, a reaction does occur when the pyranose→furanose transformation can occur, as in the (1→6)-linked disaccharide gentibiose, or in 3-*O*-methyl-α-D-glucopyranose. The failure to observe a definitive reaction with 2-deoxy-D-*arabino*-hexose (however, see the footnote to Table I) suggests that the reaction of D-glucose with borate gives a *cis*-1,2-complex, in agreement with the ^{11}B-n.m.r. studies of Kennedy and How[6].

The question of pyranoid *vs.* furanoid structure of the complexes may be argued as follows. D-Glucose and D-mannose are conspicuous among the aldoses in displaying no detectable p.m.r. signals for the furanoid forms. However, after

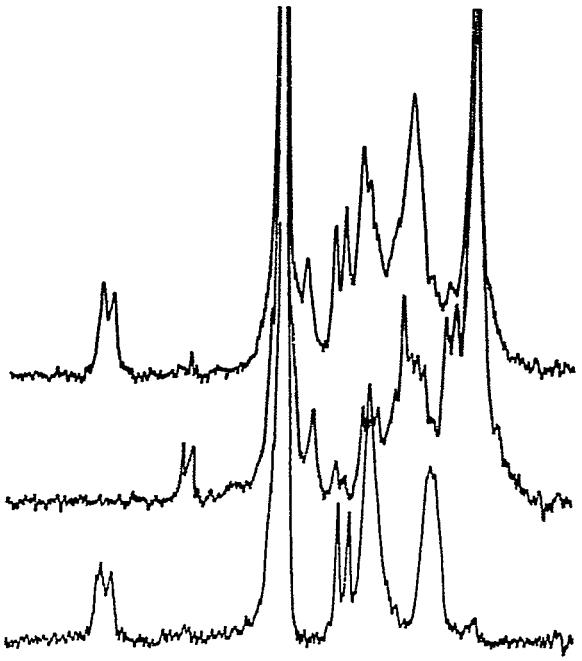

Fig. 3. Complexation of gentiobiose with tetraborate. Upper, gentiobiose and borate; middle, gentiobiose; lower, D-glucose and borate. All compounds, 0.2M (60 MHz, single scan, room temperature).

derivatizing the 5-position, signals for such forms become discernible[9] and give additional low-field signals in the anomeric-proton region of the spectrum. In those α-aldoses we have examined wherein the 1,2-hydroxyl groups are *cis*, the addition of borate results in disappearance of the H-1α*p* signal and the appearance downfield of a new, broader doublet. These changes are not restricted to the anomeric protons; indeed the gross changes upfield in the spectrum also imply gross structural-changes, although this does not readily permit distinction between conformations and tautomers. Conformational effects are presumed to apply also in the normal spectra and, consequently, gross changes are more probably tautomeric.

In their ^{13}C n.m.r. studies of borate addition to 1,2-*O*-isopropylidene-α-D-glucofuranose, Gorin and Mazurek[9] postulated either a 3,5-bidentate or, possibly, a 3,5,6-tridentate structure, in agreement with the earlier proposal of Foster[3] for the free sugar. That these borate ligands can form in the absence of the C-1,2 hydroxyl groups indicates the complexity of the problem. Specifically, it does not exclude the possibility that other structures may be favored with free D-glucose.

A thermodynamic argument may be proposed for the preponderance of the less-stable glucofuranose over the glucopyranose form in the borate complex. Conner and Bulgrin[10] have evaluated the association constants for both *cis*-1,2-cyclohexanediol ($K_1^{25} = 1$, $K_{21}^{25} = 0.7$), and for *cis*-1,2-cyclopentanediol ($K_1^{25} = 26$; $K_{21}^{25} = 142$),

which may be compared with those for D-glucose: $K_{25}^{1} = 135$; $K_{25}^{21} = 870$. An obvious inference is that the criterion for complexation is the *lifetime* of a complex, which may not equate with the overall formal energy when comparing two different compounds. It may be argued that, for isomeric structures whose geometry will permit complexation with borate, that isomer will preponderate which has the longest existence as a complex, despite its conformational variations. Although the furanoid ring undergoes conformational changes between envelope and twist forms, the angles presented to the borate ion by the 1α- and 2-hydroxyl groups are appreciably less affected than those resulting from changes between the various pyranose conformations. An assumption in this argument is that the rate of esterification by borate is lower than the rate of conformational transformation.

This argument accords with the thermodynamic data of Conner and Bulgrin[10] showing that K_1 for complexation of D-glucose *increases* with *decreasing* temperature ($K_1^{35} = 125$; $K_1^{0} = 215$). Corresponding $\Delta G°$ values are -2956 cal and -2914 cal, respectively, for 35 and 0°. Furthermore, the $\Delta G°$ (25°) value calculated for the cyclopentanediol was negative (-1930 cal), whereas that of the cyclohexandiol was zero. A similar relationship may be expected for the complexation of D-glucofuranose *vs.* D-glucopyranose.

Matrix isolation and the number of observable complexes

Given a chiral polybasic acid such as BH_2, experience has shown that each of the microspecies present in solution (BH_2, BH^-, and B^{2-}) will have its own, characteristic, optical rotatory dispersion (o.r.d.) curve. If the curves are sufficiently distinct, they may be distinguished titrimetrically at some convenient wavelength or combination of wavelengths. In the case of D-glucose (Glc), the addition of tetraborate (B^-) results in a profound change in the o.r.d. curve. Assuming that only a 1α,2-complex is formed, and that both bidentate and bis(bidentate) complexes result, according to the Glc/B^- ratio, there are then five possible compounds contributing to the o.r.d. curve: Glc, and the four microspecies of the complex, GlcB^-, Glc$B$H, Glc$_2B^-$, and Glc$_2B$H, where the protonated compounds are the undissociated complexes[11,12]:

$$Glc_2BH + H_2O \rightleftarrows Glc_2B^- + H_3O^+, \qquad K_a^{25} = 4.1 \times 10^{-7}$$

$$B(OH)_3 + 2H_2O \rightleftarrows B(OH)_4 + H_3O, \qquad K_a^{25} = 5.8 \times 10^{-10}$$

The K_a value for the complex resulting from the matrix: Glc$B$H + $H_2O \rightleftarrows$ GlcB^- + H_3^+O, is not yet known. Under the conditions used[11,12] to determine the bis(bidentate acid dissociation-constant (high Glc, low pH), it was believed that only the Glc$_2B$ species existed. We assumed that, at the pH values of the experiment[8,5–10], there was appreciable undissociated microspecies present. This was obviously valid for Glc$_2B$H, but for large proportions of tetraborate (yielding GlcB^-) and for the presumably weaker, but unknown Glc$B$H, this assumption is arguable.

Matrix analysis and the number of observable complexes

Matrix analysis has been used to determine of the number of species in a homogeneous solution by using absorption spectrophotometry or fluorimetry. It is obviously adaptable to any analysis in which the property being measured is the linear sum of the active components of the system. In polarimetry, the optical rotatory dispersion [α] at some wavelength (i) is the sum, for each of the species, of the product of the specific optical rotation $[\alpha_c]_i$ of a specific species c_i at concentration $[c_i]$. Consequently, for the three species in our example,

$$\alpha = [\alpha_{Glc}]_i[Glc]_j + [\alpha_{GlcB^-}]_j + [\alpha_{Glc_2B^-}]_i[Glc_2B]_j^- ;$$

the specific α values having been determined for a constant path-length, with the reasonable assumption that Glc_2BH and $GlcBH$ can be neglected, and that $[\alpha_{Glc_2B}]_i \neq [\alpha_{Glc_2B}]_i$.

It has been shown[13] that, in unrestricted stoichiometry, the rank (R) of the matrix is that of the number of independent species. Consequently all matrices A_{ij} of rank $(R+1)$ or greater will vanish; for example, if only a single species is present, the matrix of rank 2:

$$\begin{vmatrix} A_{11} & A_{12} \\ A_{21} & A_{22} \end{vmatrix} = 0.$$

By convention, the columns are solute parameters (such as concentration) and the rows are optical parameters (such as wavelength). Thus A_{12} is the optical reading at wavelength *1* of a solution containing a concentration *2* of the solute.

A solution having two absorbing components will possess a matrix that vanishes at $R = 3$, namely,

$$\begin{vmatrix} A_{11} & A_{12} & A_{13} \\ A_{21} & A_{22} & A_{23} \\ A_{31} & A_{32} & A_{33} \end{vmatrix} = \begin{vmatrix} A_{11}/A_{13} & A_{12}/A_{13} & 1 \\ A_{21}/A_{23} & A_{22}/A_{23} & 1 \\ A_{31}/A_{33} & A_{32}/A_{33} & 1 \end{vmatrix} = 0.$$

The right-hand determinant is then of the form:

$$\begin{vmatrix} x & y & 1 \\ x_1 & y_1 & 1 \\ x_2 & y_2 & 1 \end{vmatrix} = 0.$$

Expansion of this determinant, coupled with simultaneous addition and subtraction of x_1y_1, demonstrates that this determinant is the point–slope equation of a straight line, namely,

$$y - y_1 = \frac{(y_2 - y_1)}{(x_2 - x_1)}(x - x_1).$$

Consequently, all three (x,y) points:

$$(A_{11}/A_{13},\ A_{12}/A_{13}),\quad (A_{21}/A_{23},\ A_{22}\ A_{23}),\quad \text{and}\quad A(_{31}/A_{33},\ A_{32}/A_{33})$$

will lie on a straight line and a plot of A_{mj}/A_{nj} *vs.* A_{ij}/A_{nj} gives a straight line. Similarly, it may be shown that, in a solution containing three active components, a straight line is obtained by plotting

$$(A_{mx}A_{iy}-A_{my}A_{ix})/(A_{mx}A_{iy}-A_{mz}A_{ix})\ \textit{vs.}\ (A_{mx}A_{ij}-A_{mj}A_{ix})/(A_{mx}A_{iz}-A_{mz}A_{iz}),$$

$i \neq x,\ y,$ or z.

When the stoichometry is *restricted*, such as when the sum of the concentrations of the active microspecies is constant, the rank of the vanishing matrix corresponding to the number of active components is decreased by one; that is, the matrix of rank two vanishes for a 2-component solution, and one of rank three similarly equals zero for a 3-component solution. This result arises from the possibility of describing the system by reference to one component, so that if $Glc_0 = Glc + GlcB^- + Glc_2B^-$, it may be stated that $Glc = Glc_0 - GlcB^- - Glc_2B^-$, and so on. With restricted stoichiometry in a 2-component system, a plot of $(A_{ij}-A_{ij'})$ *vs.* $(A_{i'j}-A_{i'j'})$ gives a straight line. Similarly, in a 3-component system, $(A_{nj}-A_{ij'})/(A_{mj}-A_{mj'})$ *vs.* $(A_{pj}-A_{pj'})/(A_{mj}-A_{mj'})$ yields a straight line.

The o.r.d. titration of D-glucose (0.01M) by various concentrations of tetraborate (up to 0.1M) is shown in Fig. 4. In the initial portions of the titration it might be presumed that, where $[Glc] \gg [B^-]$, the major complex is Glc_2B^-. At the end of

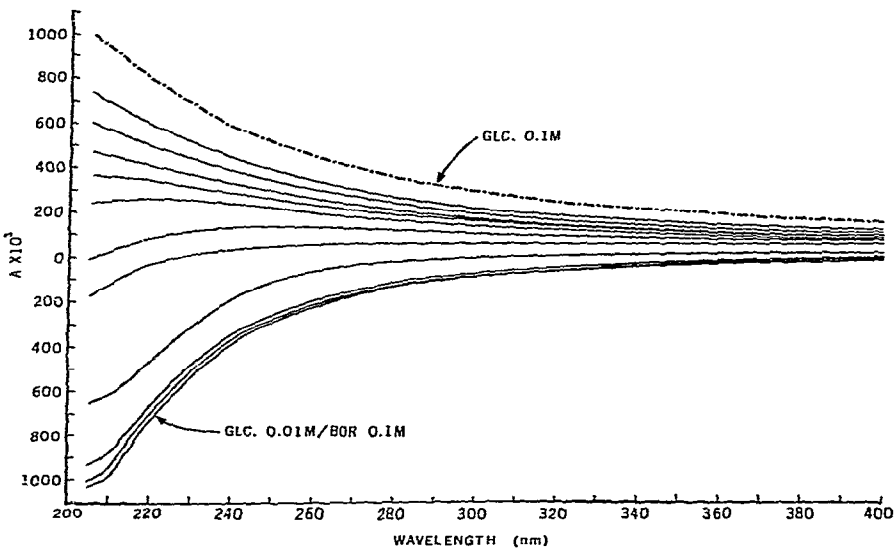

Fig. 4. Spectropolarimetric titration of α-D-glucose (0.1M) added to sodium tetraborate (0.01→0.1M), at room temperature; constant volume.

the titration, when $[B^-] \gg [Glc]$, the primary complex should be $GlcB^-$. Borate itself is transparent to o.r.d.; consequently, at the pH used, (that is, neglecting the undissociated Glc_2BH and $GlcBH$), three species are expected. These were sought by matrix isolation.

Table III provides the o.r.d. matrix for the D-glucose–tetraborate system and Fig. 5 shows the data applied to a two-component system. The results clearly demonstrate linearity, despite the presumption of three species. However the following rationale and calculations show that, under the conditions of the experiment, the results are within the range of expectation.

TABLE III

PARTIAL MATRIX FOR TITRATION OF D-GLUCOSE BY TETRABORATE

O.r.d.								[*Glc*]/[B]
λ_{nm}	205	210	220	240	250	280	300	
j	1	2	3	4	5	6	7	
1	1001	935	790	575	443	344	285	1/0
2	725	688	586	433	333	260	215	1/0.1
3	600	570	498	378	296	236	193	1/0.15
4	470	450	404	320	254	206	167	1/0.25
5	363	360	340	280	222	185	154	1/0.35
6	238	250	256	232	192	159	135	1/0.5
7	−5	24	87	134	130	115	100	1/0.75
8	−168	−128	−33	36	60	66	60	1/1
9	−650	−630	−440	−170	−68	−24	−7	1/2
10	−1028	−1000	−720	−374	−216	−128	−87	1/10

The association constants are defined as:

$$K_1 = [GlcB]^-/[B^-]\,[Glc] \quad (1),$$

and $$K_2 = [Glc_2B]^-/[GlcB]^-\,[Glc]^2 \quad (2),$$

and therefore $$K_{21} = [GlcB^-]/[B^-]\,[Glc]^2 = K_2 \cdot K_1.$$

The conservation of Glc requires that:

$$Glc_0 = Glc + GlcB^- + 2Glc_2B^- \quad (3),$$

whereas that of B requires that:

$$B_0^- = B^- + GlcB^- + GlcB^- \quad (4).$$

From these four relationships it may be shown that:

$$(K_{21})Glc^3 + (K_1 - K_{21}Glc_0 + 2K_{21}B_0)Glc + (1 - K_1Glc_0 = K_1B_0)Glc - Glc_0 = 0. \quad (5).$$

Values of B_0 and Glc_0 are known from the protocol for the experiment. Values of K_1 and K_{21} have previously been calculated by various investigators. We have used the most-recent data of Conner and Bulgrin[10] who gave $K_1^{25} = 135$, and $K_{21}^{25} = 870$. The latter value is in poor agreement with that of Nazarenko and Ermak[12] ($K_{21} = 238$) under conditions where only Glc_2B was presumed to exist, but is in reasonable agreement with earlier work by Roy *et al.*[14] ($K_1^{25} = 70$, and $K_{21}^{25} = 770$). Knowing Glc, B may be calculated from Eq. 6:

$$B_0^- = (1 + K_1 Glc + K_{21} Glc^2) B^- \qquad (6),$$

and consequently $GlcB^-$ and Glc_2B^- may be calculated from the complexation relations.

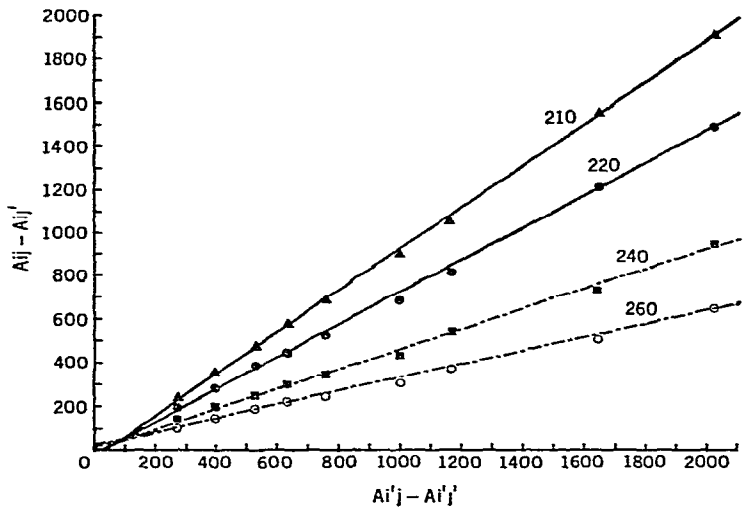

Fig. 5. Matrix-rank test of the o.r.d. curves (see text), showing the presence of only two detectable species.

TABLE IV

CALCULATED DISTRIBUTION OF MICROSPECIES IN THE TITRATION OF D-GLUCOSE (Glc_0) BY TETRABORATE (B_0^-)

	Curve 3 $Glc_0 = 0.01$M $B_0^- = 0.0025$M		*Curve 4* $Glc_0 = 0.01$M $B_0^- = 0.0035$M		*Curve 7* $Glc_0 = 0.01$M $B_0^- = 0.01$M		*Curve 10* $Glc_0 = 0.01$M $B_0^- = 0.1$M	
	Conc. (M)	%	Conc. (M)	%	Conc. (M)	%	Conc. (M)	%
Glc	0.00856	86.2	0.00804	81.2	0.00550	55.9	0.00748	44.8
B^-	0.00113		0.00163		0.00565		0.00908	
$GlcB^-$	0.00130	13.1	0.00177	17.9	0.00420	42.6	0.00916	54.9
Glc_2B^-	0.0000718	0.7	0.0000919	0.9	0.000149	1.5	0.0000442	0.3

From Table IV it is apparent that the magnitude of Glc_2B^- is never adequate to be determined polarimetrically under the conditions used. In the experiments of Nazarenko and Ermak[12], $B(OH)_3 = 0.01$M, and $Glc_0 = 0.12$M at the highest concentration, resulting in a pH value of 5.45. Without considering the effects of ionic strength at this pH, $B_0^- = 1.6 \times 10^{-4}$M; $GlcB^- = 8.8 \times 10^{-5}$M; $Glc_2B^- = 6.8 \times 10^{-5}$M; and $B^- = 5.5 \times 10^{-6}$M. Consequently, despite a Glc_0/B_0^- ratio of 740, $GlcB^-$ is not only a prominent species, but its concentration exceeds that of Glc_2B^-.

In summary, matrix analysis of a polarimetric titration of D-glucose by tetraborate under the conditions given is most compatible with the existence of only two species. Calculation shows them to be Glc and $GlcB^-$. The species Glc_2B^- is quite minor, unless it is indistinguishable from $GlcB^-$ both in dispersion (λ) and in intensity (α).

Fieser models suggest the most probable structure for the D-glucofuranose–borate complex is a 1,2-substituted species. A bis(bidentate) structure is to be expected, but, under the conditions employed, it does not exist in detectable amounts. Similar models for D-fructose plus borate, with complexation-constants two orders of magnitude larger, suggest a tridentate (O-1,3,6) D-fructofuranose–borate complex. It appears plausible that all carbohydrate–borate complexes whose association constants are in the thousands or greater, such as those of D-glucitol and D-mannitol, are tridentate.

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REFERENCES

1 J. M. SUGIHARA AND J. C. PETERSEN, *J. Amer. Chem. Soc.*, 78 (1956) 1760–1763.
2 H. SHIMANOUCHI, N. SAITO, AND Y. SASADA, *Bull. Chem. Soc. Jap.*, 42 (1969) 1239–1247.
3 A. B. FOSTER, *J. Chem. Soc.*, (1953) 982–986.
4 A. B. FOSTER AND M. STACEY, *J. Chem. Soc.*, (1955) 1778–1781.
5 Annual Meeting of the Canadian Federation of Biological Societies, 1973, University of Saskatchewan, Saskatoon; Abstract No. 524.
6 G. R. KENNEDY AND M. J. HOW, *Carbohyd. Res.*, 28 (1973) 13–19.
7 S. J. ANGYAL AND V. A. PICKLES, *Aust. J. Chem.*, 28 (1972) 1692–1710.
8 P. A. J. GORIN AND M. MAZUREK, *Carbohyd. Res.*, 27 (1973) 325–339.
9 S. J. ANGYAL AND V. A. PICKLES, *Aust. J. Chem.*, (1972) 1711–1718.
10 J. M. CONNER AND V. C. BULGRIN, *J. Inorg. Nucl. Chem.*, 29 (1967) 1953–1961.
11 V. M. POTAPOV AND O. G. YABLOKOVA, *Akad. Nauk U.S.S.R.*, 183 (1968) 838.
12 V. A. NAZARENKO AND L. D. ERMAK, *Russian J. Inorg. Chem.*, 12 (1967) 1304–1306 (Engl. Transl.); *ibid.*, 12 (1967) 335–339.
13 R. M. WALLACE AND S. M. KATZ, *J. Phys. Chem.*, 68 (1964) 3890–3892, R. M. WALLACE, *ibid.* 64 (1960) 899–901; J. S. COLEMAN, L. VARGA, AND S. H. MASTIN, *Inorg. Chem.*, 9 (1970) 1015–1020; B. W. BUDEGINSKY, *Anal. Chim. Acta*, 62 (1972) 95–101.
14 G. L. ROY, A. L. LAFERIERRE, AND J. O. EDWARDS, *Anal. Chim. Acta*, 4 (1957) 106–114.