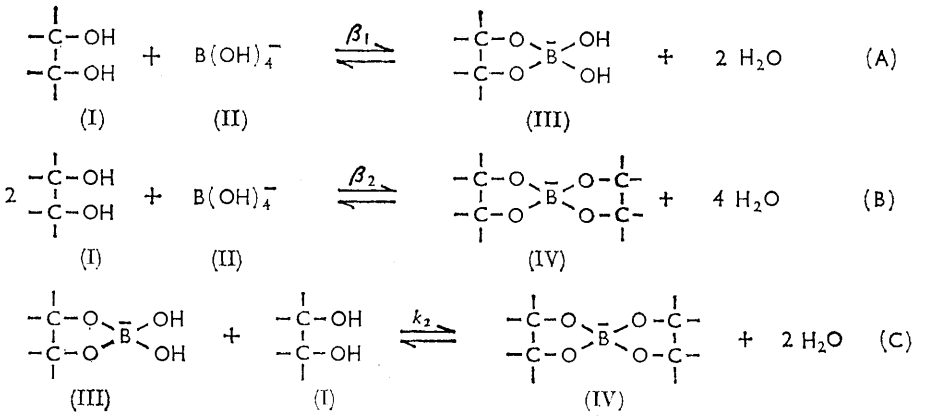
903. *A Study of the Borate–Carbohydrate Complex.*

EARL W. MALCOLM, JOHN W. GREEN, and HAROLD A. SWENSON.

The partially methylated galactosides and the mannosides employed in this study formed the borate complex preferentially with adjacent *cis*-hydroxyl groups, resulting in two types of borate complex, *i.e.*, a monocomplex and a dicomplex. This agrees with previous work in the field. The predominant type of complex at equilibrium was determined from the initial ratio of the concentrations of glycoside and borate. The stability of the monocomplex appeared to be directly related to increasing hydrogen-bonding opportunities between the glycoside molecule and the borate hydroxyl groups, whilst steric-hindrance factors may explain the relative stabilities of the dicomplexes. These results are applied to the galactomannan polymer of guar gum. Whilst the galactose side-units of this polymer are the most likely points of monocomplex formation, the dicomplex is of equal likelihood both on the mannose backbone and on the galactose side-unit. The monocomplex formation on the galactose side-unit thus offers an explanation for the greater solubility of galactomannans than mannans in borate solutions.

THE formation of a complex between the borate anion and carbohydrate molecules is well established,[1-6] but the stability of these complexes has received little attention. Edwards and his co-workers [7,8] determined the stability constants of several sugar–borate complexes, using the change in pH that occurs on complex-formation. However, the existence of polyborate anions in the pH ranges that must be employed to get adequate pH changes on complex-formation complicates the interpretation of these results.[9,10] The present study was made at sufficiently high pH levels (>11·5) to avoid these complications. Glycosides were also used, to avoid the difficulties that arise from the several equilibrium forms of the free sugar and from the reactions of sugars under alkaline conditions. Complex-formation



proceeds by the pathways (A)—(C). Compound (I) represents a polyhydroxy-compound containing an adjacent *cis*-hydroxyl group, (II) is the borate anion, (III) the monocomplex, and (IV) the dicomplex.

1 Dale, *J.*, 1961, 922.
2 Lenz and Heeschen, *J. Polymer Sci.*, 1961, **51**, 247.
3 Antikainen, *Ann. Acad. Sci. Fennicae*, Ser. AII, 1954, p. 61.
4 Foster, *Adv. Carbohydrate Chem.*, 1957, **12**, 81.
5 Böeseken, *Adv. Carbohydrate Chem.*, 1949, **4**, 189.
6 Isbell, Brewster, Holt, and Frush, *J. Res. Nat. Bur. Stand.*, 1948, **40**, 129.
7 Lorand and Edwards, *J. Org. Chem.*, 1959, **24**, 769.
8 Roy, Laferriere, and Edwards, *J. Inorg. Nuclear Chem.*, 1957, **4**, 106.
9 Ingri, *Acta Chem. Scand.*, 1962, **16**, 439.
10 Ingri, Lagerström, Fryman, and Sillén, *Acta Chem. Scand.*, 1957, **11**, 1034.

Whilst examples of weak complex-formation with other than the adjacent *cis*-hydroxyl grouping have been noted,[1,4,5] the preferred site of strong complex-formation with sugar molecules in alkaline borate appears to be the *cis*-hydroxyl group. Mazurek and Perlin [11] have shown that the dihedral angle of the hydroxyl pair of 0—40° does not appear to be a controlling factor in the stability of the sugar–borate complex. Hence, some other factor must be involved. The reaction may be similar to the type proposed for the periodate oxidation of sugars where adjacent *cis*-hydroxyl groups are also the preferred site of reaction.[12] Here the crossing action of the *cis*-hydroxyl groups as the molecule shifts in conformation brings the OH groups closer together, whilst the opposite effect occurs with the *trans*-hydroxyl pair.

For comparing the complexing abilities of each carbohydrate, the stoicheiometric overall stability constants, defined by equations (1) and (2), are useful. The stoicheiometric step-stability constant, K_2, is used to compare the relative dominance of (III) and (IV), and is defined as

$$\beta_1 = [\mathrm{III}]/[\mathrm{I}][\mathrm{II}] \quad (1)$$

$$\beta_2 = [\mathrm{IV}]/[\mathrm{I}]^2[\mathrm{II}] \quad (2)$$

$$K_2 = [\mathrm{IV}]/[\mathrm{III}][\mathrm{I}] = \beta_2/\beta_1 \quad (3)$$

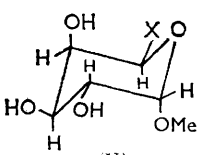
The equilibrium concentrations of the components of the carbohydrate–borate system were determined by solving several simultaneous equations. It can be shown (see Appendix) that the change in a given physical property, Y, of a solution due to complex-formation is given by

$$\Delta Y = G[\mathrm{III}] + H[\mathrm{IV}] \quad (4)$$

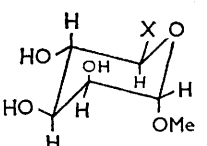
where G and H are experimental constants, and [III] and [IV] represent the molar equilibrium concentrations of (III) and (IV). If two different physical properties are measured on the same solution, two equations in two unknowns are obtained which can be solved simultaneously for the concentrations of (III) and (IV). Once [III] and [IV] are known, the equilibrium concentrations of (I) and (II) are readily calculated from a simple mass-balance. The stability constants can then be calculated directly.

In the present study refractive index and optical rotation were the physical properties employed in equation (4). As the optical rotation increased with decreasing wavelength of the light-source, variation of the wavelength gave additional equations for checking purposes. The refractive index and optical rotation data were plotted, using a method of continuous variations in which the sum of the initial concentrations of (I) and (II) are held constant.

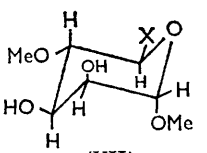
The borate complexes of methyl α-D-galactopyranoside (V), methyl α-D-mannopyranoside (VI), methyl 4-*O*-methyl-α-D-mannopyranoside (VII), and methyl 4-*O*-methyl-β-D-mannopyranoside (VIII) were investigated. These glycosides were chosen to relate



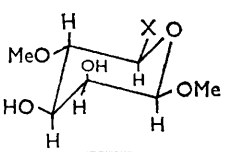
(V)



(VI)



(VII)



(VIII)

(X=CH_2·OH)

to the galactomannan polymer which readily forms a borate complex.[13] The structures of the sugars are shown for the C1 conformation of each glycoside.

[11] Mazurek and Perlin, *Canad. J. Chem.*, 1963, **41**, 2403.
[12] Bobbitt, *Adv. Carbohydrate Chem.*, 1956, **11**, 7.
[13] Smith and Montgomery, " The Chemistry of Plant Gums and Mucilages," Reinhold, New York, 1959, pp. 59—61.

Recent work with nuclear magnetic resonance (n.m.r.) by Lenz and Adrian [14] has shown the C1 conformation to be the most probable for aqueous solutions of (VI). As the present work was done under conditions that did not cause changes in the optical rotation values from those obtained in water, it can be assumed that C1 conformation is of main importance in these studies. Compounds (VII) and (VIII) are substituted in equatorial positions in the C1 conformation, and it can be assumed that these compounds also favour the C1 conformation. No similar n.m.r. data are available for compound (V), and it must be assumed from classical theory that, since it has the same number of axial and equatorial groups as

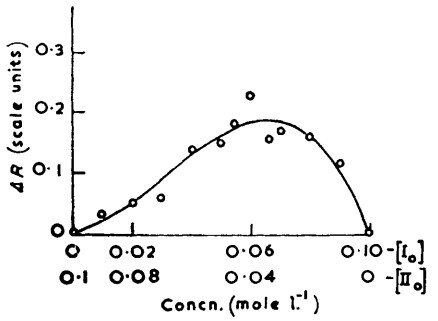


FIG. 1. Variation of ΔR with molar ratio of methyl α-D-galactopyranoside to borate.

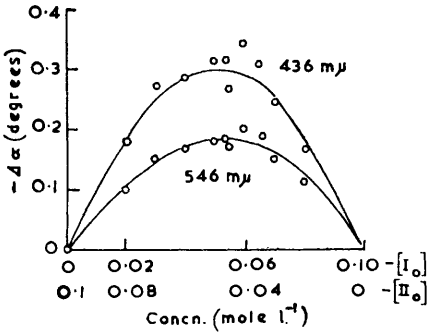


FIG. 2. Variation of $\Delta\alpha$ with molar ratio of methyl α-D-galactopyranoside to borate.

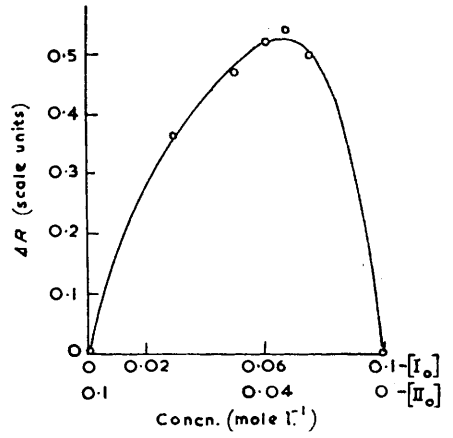


FIG. 3. Variation of ΔR with the molar ratio of methyl 4-*O*-methyl-α-D-mannopyranoside to borate.

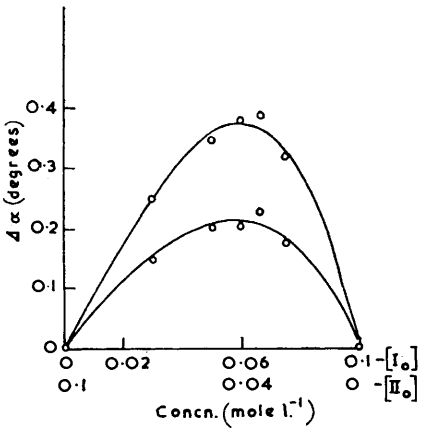


FIG. 4. Variation of $\Delta\alpha$ with the molar ratio of methyl 4-*O*-methyl-α-D-mannopyranoside to borate.

(VI), it too will favour the C1 conformation. As will be discussed, it appears that the C1 conformation favours the formation of the borate complex. However, this does not necessarily fix the conformation of the complex itself.

Type of Complex Present.—Although the presence of more than one complex invalidates strict use of the continuous-variations procedure, the method did indicate the existence of only two types of borate complex, with peaks near a 1 : 1 and 2 : 1 ratio of (I) to (II). The plots for (V) and (VII) are given in Figs. 1—4, and serve as typical examples of the results obtained with the glycosides. The actual curves represent third-order polynomials fitted by the least-squares method to the experimental data.

In the case of glycoside (V) (Figs. 1 and 2) the refractive index data indicate a 2 : 1

[14] Lenz and Adrian, *J. Polymer Sci.*, 1964, in the press.

glycoside–borate complex, while the optical rotation curves indicate a 1 : 1 complex. When equation (4) is solved for each physical measurement, equations (5) and (6) are obtained for (V).

$$\Delta R = 4{\cdot}9[\mathrm{III}] + 15{\cdot}0[\mathrm{IV}] \quad (5)$$

$$\Delta\alpha = 6{\cdot}4[\mathrm{IV}] \quad (6)$$

Here ΔR and $\Delta\alpha$ represent the respective change in the refractometer reading and the optical rotation reading, due to complex-formation. From the above equations it is seen

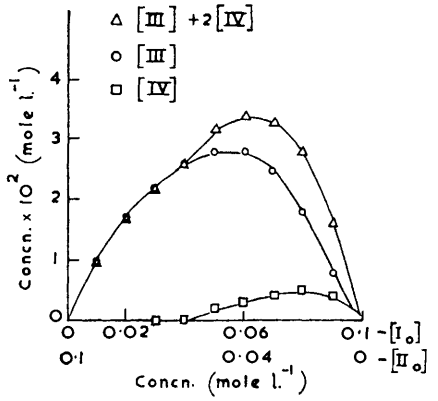


FIG. 5. Concentration of the borate complexes as a function of the molar ratio of methyl α-D-galactopyranoside to borate.

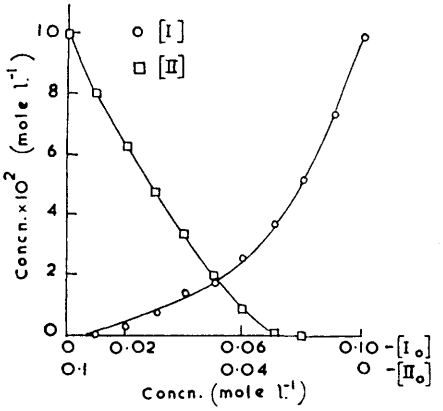


FIG. 6. Concentration of (I) and (II) as a function of the molar ratio of methyl α-D-galactopyranoside to borate.

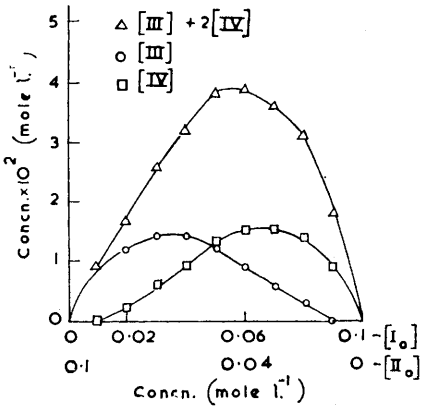


FIG. 7. Concentration of the borate complex as a function of the molar ratio of methyl 4-*O*-methyl-α-D-mannopyranoside to borate.

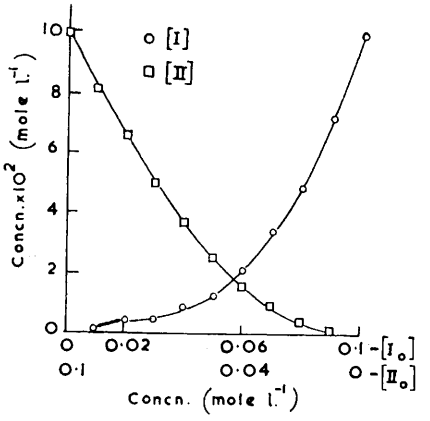


FIG. 8. Concentration of (I) and (II) as a function of the molar ratio of methyl 4-*O*-methyl-α-D-mannopyranoside to borate.

that the optical-rotation points follow the monocomplex and there is a peak near a 1 : 1 ratio, while the refractive index results are dominated by the dicomplex and show a peak near a 2 : 1 ratio. These results point out the caution that must be exercised when the continuous-variations method is applied to systems of more than one complex.

The results obtained with (VII) can be explained in a similar manner, using equation (4) to interpret the continuous-variations method. Here, both the refractive index and optical

rotation results are dominated by the dicomplex, with the resulting maxima occurring near a 2 : 1 ratio (Figs. 3 and 4).

Stability Constants.—The concentrations of the components of the glycoside–borate system were calculated, using the simultaneous-equations method. Figs. 5—8 show the results of the concentration determinations for (V) and (VII) which again serve as typical examples of the results obtained with the glycosides. It is apparent that the type of glycoside affects both the amount and kind of borate complex. Also, the relative dominance of each complex can be controlled by appropriate choice of the initial glycoside-to-borate ratio. The relative pattern of the results is similar to that predicted.[6,15]

Once the concentrations were known, the stability constants were calculated (Table). These represent an average of values calculated in the 0·03—0·07 mole $l.^{-1}$ range of (I_0). Calculations were not made where any value was less than the 0·005 mole $l.^{-1}$ range of estimated experimental error. Because of the limited amount of base sugars, sufficient points, *e.g.*, 10—20, were not obtained for accurate application of statistical methods. However, work with compound (V) showed that the 95% confidence limits for the concentration values were similar to those predicted from an analysis of experimental error. All the calculated constants fell within the experimental-error limits and were within 25% of the final average value. No definite trend occurred in the individual stability constants, showing the present analysis of the borate–carbohydrate system to be accurate within the limits of the experimental methods employed.

Stability constants for the glycoside–borate system.

	β_1 (l. mole^{-1})	β_2 (l.^{2} mole^{-2})	K_2 (l. mole^{-1})
Methyl α-D-galactopyranoside (V)	100	400	4
Methyl α-D-mannopyranoside (VI)	50	600	10
Methyl 4-*O*-methyl-α-D-mannopyranoside (VII)	30	3000	100
Methyl 4-*O*-methyl-β-D-mannopyranoside (VIII) ...	2	400	200

Steric Hindrance.—The total amounts of borate complex formed with (V) and (VI) were almost equal, but (VI) formed the dicomplex more readily. This can be explained, since with (V) the complex forms across the 3- and 4-hydroxyl groups where the 5-hydroxymethyl group can easily interfere with dicomplex formation, while with (VI) the complex forms across the 2- and 3-hydroxyl groups without major interference. In both glycosides the 1-methoxyl group is in an axial position in the preferred C1 chair conformation, and is relatively remote from the site of complex-formation.

The substitution of the 4-hydroxyl group on (VI) to give (VII) favoured complex-formation. Not only was the largest amount of total complex obtained with (VII), but the stability of the dicomplex increased greatly. This is reasonable, as the addition of the 4-methoxyl group stabilizes the C1 chair conformation, in which the 4-methoxyl group is equatorial. From a consideration of molecular models, the C1 chair form gives the complex with only minor interference from adjacent groups, whilst the 1C chair form brings both the 1- and the 4-methoxyl groups into interfering positions. Therefore, groups which stabilize the C1 chair conformation will allow ready formation of the complex. This will result in an equilibrium shift towards the complex, on the assumption that destabilization of the final complex molecule by the added group is relatively small. The increase in the amount of complexing which occurs with (VII) also shows that the 2- and 3-*cis*-hydroxyl groups are the major site of complex-formation in the mannose derivatives, for if complexing occurred across other adjacent hydroxyl groups (VII) would have complexed less than (VI).

Foster,[4] from ionophoretic studies, proposed that the β-glycoside of mannose does not form the borate complex as readily as the α-glycoside owing to the interference of the glycosidic methoxyl group. The present results support this supposition, as the total amount of complex dropped considerably on changing from (VII) to (VIII). From a

[15] Böeseken and Vermaas, *Rec. Trav. chim.*, 1935, **54**, 833.

consideration of molecular models, it is apparent that the 1-methoxyl group does interfere with complex-formation in (VIII). The unstabilizing effect of the C(2) axial oxygen will also increase with complex-formation across the 2- and 3- hydroxyl groups. This effect is also present in (VII), but in (VIII) the C(2)–O bond bisects the tetrahedral angle of the two C(1)–O bonds, giving an enhanced unstabilizing effect.[16]

Steric considerations do not explain why (VIII) formed the dicomplex in marked preference to the monocomplex (K_2 value). It may be that repulsive forces between sugar units keep the interference of the 1-methoxyl group lower in the dicomplex than in the monocomplex, but, as will be seen, differences in hydrogen bonding offer a more likely explanation for the small amount of monocomplex formed.

Hydrogen Bonding.—In the case of the borate complex, the boron atom carries a negative charge, and the oxygen atoms bonded to the boron can be expected to have increased electronegative character. Hence, the hydrogen bonds should form between the hydroxyl hydrogens of the sugar molecule that are not involved in complex-formation and the oxygen atoms of the boron atom. These hydrogen bonds will decentralize the negative charge and increase the stability of the complex. This stabilization is especially important in the monocomplex where the boron hydroxyl groups are relatively free to move and can readily come into position for hydrogen bonding.

Compound (V), which has hydroxyl groups available on either side of the point of complex-formation, has the best opportunity of the model compounds to form hydrogen bonds. Compound (VI) has hydrogen-bonding opportunities with only one neighbouring hydroxyl group and across the ring to the 6-hydroxyl group, while (VII) has no adjacent hydroxyl group and can only form hydrogen bonds across the ring. In the case of (VIII), hydrogen bonding will be even less likely, as the glycosidic methyl group interferes with bonding across the ring. Because hydrogen bonding stabilizes the monocomplex, (V) should form the most stable monocomplex, with (VI), (VII), and (VIII) decreasing in order. As can be seen from the values for β_1, this was the order experimentally obtained, supporting the hydrogen-bonding hypothesis.

The relatively small β_1 value for (VIII) can then be explained as being due to the relative lack of hydrogen-bonding to stabilize the monocomplex. It appears that the 6-hydroxyl group has an important stabilizing effect, since a large change in β_1 did not occur until hydrogen-bonding across the ring was hindered by the glycosidic methyl group of the β-glycoside. A consideration of molecular models also indicates that the 6-hydroxyl group is the most likely point of hydrogen bonding. However, steric hindrance of the actual complex-formation also enters in, and, as the relative magnitude of each factor is not known, it cannot be said for certain if the 6-hydroxyl group is the dominant point of hydrogen bonding. Taken together, the postulated effects of hydrogen bonding and steric hindrance reasonably account for the observed differences in stability constants.

Extension to the Galactomannan Polymer.—Compounds (V) and (VIII) are directly related to the galactomannan polymer, with the galactoside representing the side-unit of the polymer and the 4-*O*-methyl-β-mannoside representing the mannose backbone. It appears that the borate complex is formed more readily on the galactose side-units than on the mannose backbone. However, the crosslinking site appears to be of equal likelihood on either the mannose or galactose units (see β_2 values). The ability of the galactose unit to form the monocomplex should aid the solubility of the polymer in borate solutions, offering an explanation for the decreased solubility of straight-chain mannans in borate systems in comparison to the galactomannans.

In general, the results obtained with the model compounds substantiate the view that the behaviour of the galactomannan–borate system can be explained on a basis of two types of complex being formed, that is, the dicomplex, which gives crosslinking, and the monocomplex, which gives increased aqueous solubility. Not only the amount of complexing,

[16] Pigman, " The Carbohydrates," Academic Press, New York, 1957, 902 pp.

but, more importantly, the type of complex present can be influenced by experimental conditions, such as changes in the ratio of carbohydrate to borate. By suitable changes in the glycoside-to-borate ratio, the borate system can be made to give widely different results, *i.e.*, gelation or solvation.

Experimental

Preparation of Glycoside–Borate Solutions.—The sum of the initial concentrations of glycoside and borate was set at 0·100M. To avoid polyborate-anion formation, the pH of all solutions was maintained above 11·0 by the addition of potassium hydroxide to a constant initial concentration of 0·105M, thereby fixing the ionic strength of the solutions at 0·105. The desired amounts of stock solutions of each compound were added volumetrically to a 10-ml. volumetric flask, and made up to the correct volume with carbon dioxide-free distilled water. Care was taken at all stages to avoid absorption of carbon dioxide.

Measurement of Refractive Index and Optical Rotation.—The refractive index measurements were made with a Bausch and Lomb dipping refractometer at 30·00 ± 0·02°. The actual scale reading, R, of the refractometer was adequate for this study, and no conversion into refractive-index values was made. The instrument was standardized to read 11·70 with distilled water at 30·00 ± 0·02°.

The optical rotations were measured on a Zeiss Winkel polarimeter, using the mercury lines of 436 and 546 mμ. Jacketed 2-dm. polarimeter tubes were employed, with the temperature held at 30·00 ± 0·02°.

Preparation of Glycosides.—The procedures used to prepare the glycosides of galactose [17] and mannose [18] are well established. The 4-*O*-methyl-D-mannose necessary for the production of (VII) and (VIII) was prepared by the procedure of Haskins, Hann, and Hudson.[19] Because the usual methods of glycoside production yield predominantly the α-glycosides of mannose,[16,18] direct alkylation with dimethyl sulphate [20] was used to obtain the β-glycoside of 4-*O*-methyl-D-mannose. As long as the temperature of the reaction was held below 5°, and the pH was maintained near 10·5, the methyl glycosides were the dominant products, with only traces of methylation of non-glycosidic hydroxyl groups. While the α-glycoside still predominated, a sufficient amount of the β-glycoside was obtained.

The reaction products were separated by column chromatography on cellulose, with butanol–ethanol–water (5 : 1 : 4, top layer) as developer. The α-glycoside of 4-*O*-methyl-D-mannose readily crystallized from butan-2-one, with properties that agreed closely with literature values.[19] The β-glycoside of 4-*O*-methyl-D-mannose, which has not been reported in the literature, was obtained as a chromatographically pure syrup that resisted attempts at crystallization. It was tentatively identified by paper chromatography, having a rate of migration between that of the free sugar and the α-glycoside (VII), as is the case with the methyl glycosides of D-mannose. It was non-reducing, and on acid hydrolysis gave only 4-*O*-methyl-D-mannose. An $[\alpha]_{20}^{589}$ value of −58·3° was obtained for the β-glycoside, which agreed closely with the value predicted by Hudson's isorotation rules.[16] The concentration of the β-glycoside was determined spectrophotometrically, using acidic orcinol [21] as the colour reagent and the α-glycoside of 4-*O*-methyl-D-mannose as the reference compound.

Appendix

If a physical property, Y, of a solution is a linear function of the respective concentrations of the components in solution, and an additive relation can be assumed, the value of Y is given by

$$Y = a[\mathrm{A}] + b[\mathrm{B}] + \ldots . + z[\mathrm{Z}] + Y_0 \qquad (7)$$

where a, b, ... z are the appropriate constants corresponding to compounds A, B, ... Z, and Y_0 is the Y value of the pure solvent. On applying this relation to the alkaline borate system, Y is given by

$$Y = a[\mathrm{I}] + b[\mathrm{II}] + c[\mathrm{III}] + d[\mathrm{IV}] + e[\mathrm{KOH}] + Y_0 \qquad (8)$$

[17] Dale and Hudson, *J. Amer. Chem. Soc.*, 1940, **52**, 2534.
[18] Mowery, Abstracts of papers 130th meeting, Amer. Chem. Soc., 1956, p. 9D.
[19] Haskins, Hann, and Hudson, *J. Amer. Chem. Soc.*, 1943, **65**, 70.
[20] Isbell and Frush, *J. Res. Nat. Bur. Stand.*, 1940, **24**, 125.
[21] Brückner, *Biochem. J.*, 1955, **60**, 200—205.

where a, b, c, d, and e are the appropriate constants for each component and KOH is used to control the alkalinity. Because the cation takes no part in the complexing reaction, for the sake of brevity, it is omitted.

From a general mass balance,

$$[\mathrm{I}] = [\mathrm{I}_0] - [\mathrm{III}] - 2[\mathrm{IV}] \quad (9)$$

$$[\mathrm{II}] = [\mathrm{II}_0] - [\mathrm{III}] - [\mathrm{IV}] \quad (10)$$

$$[\mathrm{KOH}] = [\mathrm{KOH}_0] - [\mathrm{II}_0] \quad (11)$$

The subscript zero refers to the initial concentration of material added to the solution. Since the initial concentration of KOH was held constant, $[\mathrm{KOH}_0]$ represents a constant value. Also, the use of excess of alkali enables $[\mathrm{II}_0]$ to be defined as the initial amount of boric acid added to a solution.

Combination of equations (8)—(11) yields

$$Y = (c - a - b)[\mathrm{III}] + (d - 2a - b)[\mathrm{IV}] + a[\mathrm{I}_0] + (b - e)[\mathrm{II}_0] + e[\mathrm{KOH}_0] + Y_0 \quad (12)$$

The continuous-variations method used in this study fixes the sum of $[\mathrm{I}_0]$ and $[\mathrm{II}_0]$ at a constant value, M, or

$$[\mathrm{I}_0] + [\mathrm{II}_0] = M \quad (13)$$

Substitution of equation (13) into equation (12) gives

$$Y = (c - a - b)[\mathrm{III}] + (d - 2a - b)[\mathrm{IV}] + (a + e - b)[\mathrm{I}_0] + (b - e)M + e[\mathrm{KOH}_0] + Y_0 \quad (14)$$

which defines the variation of Y during use of the continuous-variations method of studying the borate complex.

If no complex forms, equation (14) becomes

$$Y_{nc} = (a + e - b)[\mathrm{I}_0] + (b - e)M + e[\mathrm{KOH}_0] + Y_0 \quad (15)$$

Therefore, the change in Y due to complex formation becomes

$$\Delta Y = Y_{nc} - Y \quad (16)$$

$$\Delta Y = (c - a - b)[\mathrm{III}] + (d - 2a - b)[\mathrm{IV}] \quad (17)$$

Because a, b, c, and d are constants, equation (17) has the form of equation (4), containing two unknown quantities, [III] and [IV], and one experimental value, Y.

Before equation (17) can be used, the values of the constants must be determined. The values of a, b, and e can be determined directly from the individual compounds, and the values of c and d can be determined indirectly. For example, if a large excess of (II) is present and the initial concentration of (II) is held constant, the concentration of (IV) will be negligible and the concentration of (III) will equal the initial concentration of (I). In such a case,

$$Y = (c - b)[\mathrm{I}_0] + (b - e)[\mathrm{II}_0] + e[\mathrm{KOH}_0] + Y_0 \quad (18)$$

Because $[\mathrm{II}_0]$ is a constant, equation (18) is linear with a slope of $(c - b)$. As b is known, c can be determined from a plot of Y against $[\mathrm{I}_0]$. In a similar manner, d can be calculated if an excess of (I) is used and $[\mathrm{I}_0]$ is held constant.

The Institute of Paper Chemistry,
Appleton, Wisconsin, U.S.A.

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