ASSOCIATION OF CARBOHYDRATES WITH AMINES

PART I. PROTON DONOR–ACCEPTOR COMPLEXES OF D-GLUCOSE, D-MANNOSE, D-GALACTOSE, 2-AMINO-2-DEOXY-D-GLUCOSE, AND MALTOSE WITH ETHYLENEDIAMINE*

S. P. MOULIK AND A. K. MITRA

Carbohydrate Research Unit, Department of Food Technology and Biochemical Engineering, Jadavpur University, Calcutta-32 (India)

(Received November 30th, 1971; accepted in revised form, January 13th, 1972)

ABSTRACT

Ethylenediamine has been observed to activate D-glucose, D-mannose, D-galactose, 2-amino-2-deoxy-D-glucose, and maltose, forming hydrogen-bonded complexes. This has been established from physicochemical studies, such as paper chromatography, pH titration, and u.v. spectroscopy. Spectral measurements confirm a 1:1 complex in each case. Stability-constant values of the complexes in water, as well as in water–alcohol, are reported. Probable amine-binding sites in the carbohydrate moieties are located.

INTRODUCTION

Ethylenediamine has been presumed to activate the D-glucose molecule[1,2] and to form an association complex with it[3]. In a recent Note[3], the probability of binding of D-glucose, D-mannose, D-galactose, and maltose with ethylenediamine was reported, and the carbohydrate moiety was regarded as united with the amine through hydrogen bonding. In the present paper, results of a detailed study on the aforementioned sugars and on 2-amino-2-deoxy-D-glucose, methyl α- and β-D-glucopyranoside, and methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside are given. From these results, probable amine-binding sites are located in the carbohydrates. Some of the physicochemical characteristics of the complexes are also reported.

EXPERIMENTAL

The quality of the materials used, and the experimental methods employed, are described in our previous Note[3]. 2-Amino-2-deoxy-D-glucose hydrochloride was prepared by the hydrolysis of chitin from lobster shells; its purity was checked by paper chromatography and by its optical rotation. Methyl α-D-glucopyranoside

---

*Part of the work described herein was reported in a paper presented at the Convention of Chemists, University of Bombay, India, in November, 1971.

(Cemapol, Prague, by courtesy of Prof. L. Havan, Consulate General, The Czechoslovak Socialist Republic, at Calcutta) and methyl β-D-glucopyranoside (by courtesy of Dr. Hewitt G. Fletcher, Jr., N. I. A. M. D., Bethesda, Maryland 20014, U. S. A.) were gift samples. All other chemical compounds used were of analytical grade (E. Merck). pH titrations were performed with an ELICO pH meter constructed by M/s. Electronic and Industrial Instruments Co. (P) Ltd., Hyderabad, India.

RESULTS

Experimental conditions. — For all of the carbohydrates that associate with the amine, the optical absorbance was found to increase on addition of the base. At room temperature (~30°), this increase was very slow, and alkaline transformation and degradation of the carbohydrates by the prolonged action of the base[4–6] occurred. However, it was discovered that heating of the mixtures for 2 h at ~55° afforded maximum absorbances that were stable for a few hours. Treatment for 2 h at temperatures below or above ~55° gave lower optical absorbances. This optimal temperature of formation is seen in Fig. 1. In all the experiments, therefore, the samples were preheated for 2 h at ~55°, unless otherwise mentioned.

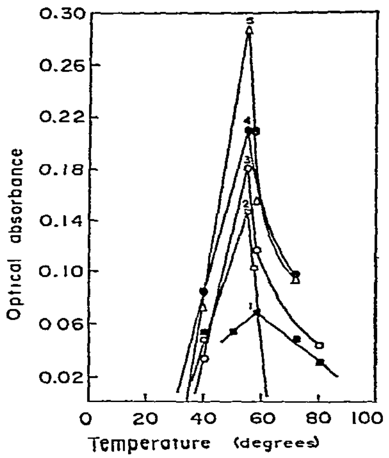


Fig. 1. Temperature dependence of the carbohydrate–ethylenediamine complexes: 1, 2-amino-2-deoxy-D-glucose; 2, 2,6-di-O-(methylsulfonyl)-α-D-glucopyranoside; 3, D-galactose; 4, D-glucose; 5, D-mannose.

Identification of the complex. — *(a) By paper chromatography.* Low and high concentrations of ethylenediamine were added to solutions of a carbohydrate, the mixtures were heated, and then paper chromatography was performed as described earlier[3]. On successively spraying the paper with ninhydrin and ammoniacal silver nitrate solutions, spots for the amine and the carbohydrates were located. Such paper chromatography was performed for D-glucose, D-mannose, D-galactose, and maltose. In Fig. 2, the results of a typical experiment employing D-glucose and the amine are shown. It is clear that an excess of the amine either lessens the mobility of the carbohydrate or causes an amine-bound entity to migrate between these two components. A low concentration of the amine has practically no effect on the carbohydrate, except for a little tailing of the carbohydrate front. On the other hand, 2-aminoethanol has no influence on the movement of the carbohydrate; presumably, therefore, binding does not occur. It may be noted that, in the chromatogram, the spots for the amine and the amine-bound carbohydrate are elongated and large, as compared to the spots for the pure carbohydrate.

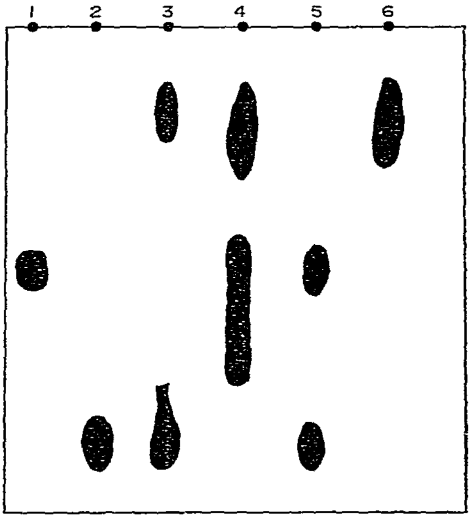


Fig. 2. Paper chromatography of D-glucose–ethylenediamine and D-glucose–2-aminoethanol mixtures: 1, 2-aminoethanol; 2, D-glucose; 3, D-glucose+ethylenediamine (1:2 ratio); 4, D-glucose+ethylenediamine (1:10 ratio); 5, D-glucose+2-aminoethanol (1:10 ratio); 6, ethylenediamine. Solvent, 8:2:1 ethyl acetate–pyridine–water; time, *18* h; spray reagents, ninhydrin and ammoniacal silver nitrate solutions.

(*b*) *By pH titration.* It seemed likely that, if there is an interaction between a sugar and the base, ethylenediamine titrated with standard hydrochloric acid in the absence and presence of a carbohydrate should produce different patterns of the titration curves. In Fig. 3, such titration curves for systems containing no carbohydrate or containing D-glucose, D-mannose, D-galactose, or maltose are given. Binding of the carbohydrates with the amine is evident. This conclusion is further supported by the observation that the curve obtained for the amine titrated within 30 min of mixing with a carbohydrate is identical with the titration curve of the amine alone. Moreover, when sufficient time (16 h) was allowed for the reaction, although the pattern was different, the total volume of acid consumed was the same. It seems that the hydrochloric acid removes the amine from the bound state with a carbohydrate, and itself associates with the amine, forming the dihydrochloride. On the other hand, if a comparatively strong acid (potassium hydrogen phthalate) is first reacted with the amine, and then the mixture is titrated with hydrochloric acid, no such reaction occurs (see curve 8, Fig. 3B). For a parallel study, acid titration of the amine in the presence and absence of phenol (where complex formation with the amine has been established[7,8]) was also performed (curve 6). A difference in behavior of the first neutralization of the base was observed, although the consumption of acid was identical with that required for the free amine. An analogous, proton donor–acceptor complex in the case of carbohydrates is thus not unreal. A strongly complexing acid such as violuric acid is retained by the amine, and the binding remains until the end of the titration (see curve 7).

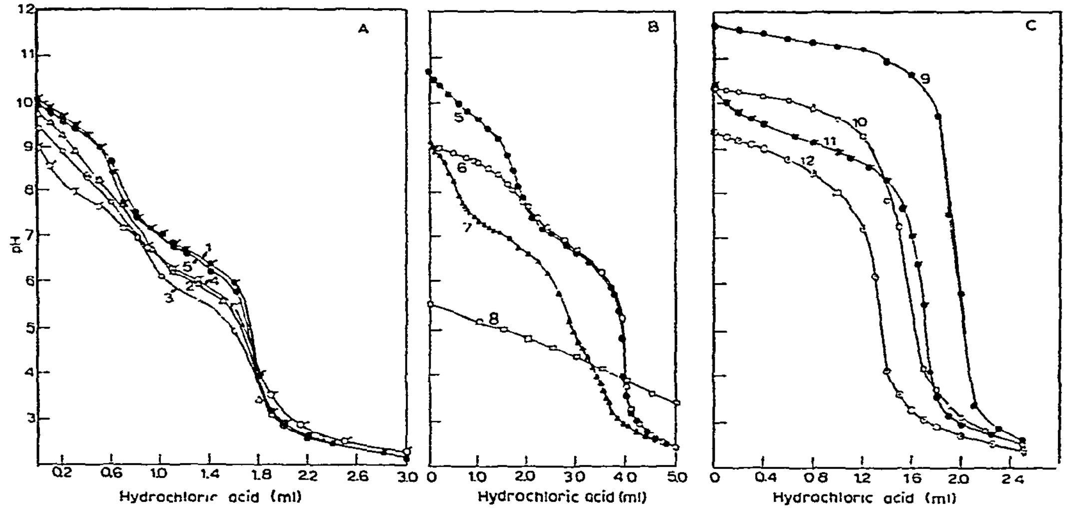


Fig. 3. pH titrations of 0.1M solutions of different bases by 0.1M HCl in the presence and absence of carbohydrates at 25°: 1, ethylenediamine (dashed points indicate similar titration, but in presence of 0.5M D-glucose, done within 30 min of mixing); 2, ethylenediamine in 0.5M D-galactose; 3, ethylenediamine in 0.5M D-glucose; 4, ethylenediamine in 0.5M D-mannose; 5, ethylenediamine; 6, ethylenediamine in 0.1M phenol; 7, ethylenediamine in 0.1M violuric acid; 8, ethylenediamine in 0.1M potassium hydrogen phthalate; 9, NaOH; 10, NaOH in 0.5M D-glucose; 11, NH_4OH; 12, NH_4OH in 0.5M D-glucose.

In addition, the action of sodium hydroxide and of ammonium hydroxide was studied (see Fig. 3C). It was observed that, in these cases, D-glucose used up part of the base, since the volume of standard acid required for complete neutralization was low; presumably, the action of ethylenediamine differs from that of sodium hydroxide or ammonium hydroxide on the carbohydrates. Otherwise, removal of the last two bases from the bound states (the carbohydrate–base binding, if it exists, should be unstable and weak) should have been achieved by the addition of a strong acid such as hydrochloric acid.

(c) *By spectrophotometry*. Measurement of the u.v. spectrum of a mixture of a carbohydrate with ethylenediamine can be a potential method for identification of the complex[3]. Both of the components were mixed at concentrations where neither of them absorbed. To a fixed concentration of the amine (*e.g.*, 0.2M), increasing amounts of a carbohydrate at a concentration of the order of 10^{-4}M were added. The mixtures were heated for 2 h at ~55°, and cooled to room temperature, and the optical absorbance was measured within 30 min. A family of curves was then drawn, for each of the carbohydrates, by plotting the optical absorbances (see Fig. 4). It may be seen that the complexes of D-glucose, D-mannose, D-galactose, and D-fructose have maximum absorption at 324 nm, the complex of 2-amino-2-deoxy-D-glucose hydrochloride absorbs at 320 nm, and the maltose complex absorbs in the range of 335 to 340 nm. Methyl α- and β-D-glucopyranoside produced no absorption band when treated in the same way. The curves depicted in Fig. 4 suggest that interaction takes place in all of the other cases. Binding of the amine with D-fructose and with methyl 2,6-di-*O*-(methylsulfonyl)-D-glucopyranoside was also studied; a band at 324 and 292 nm, respectively, was observed.

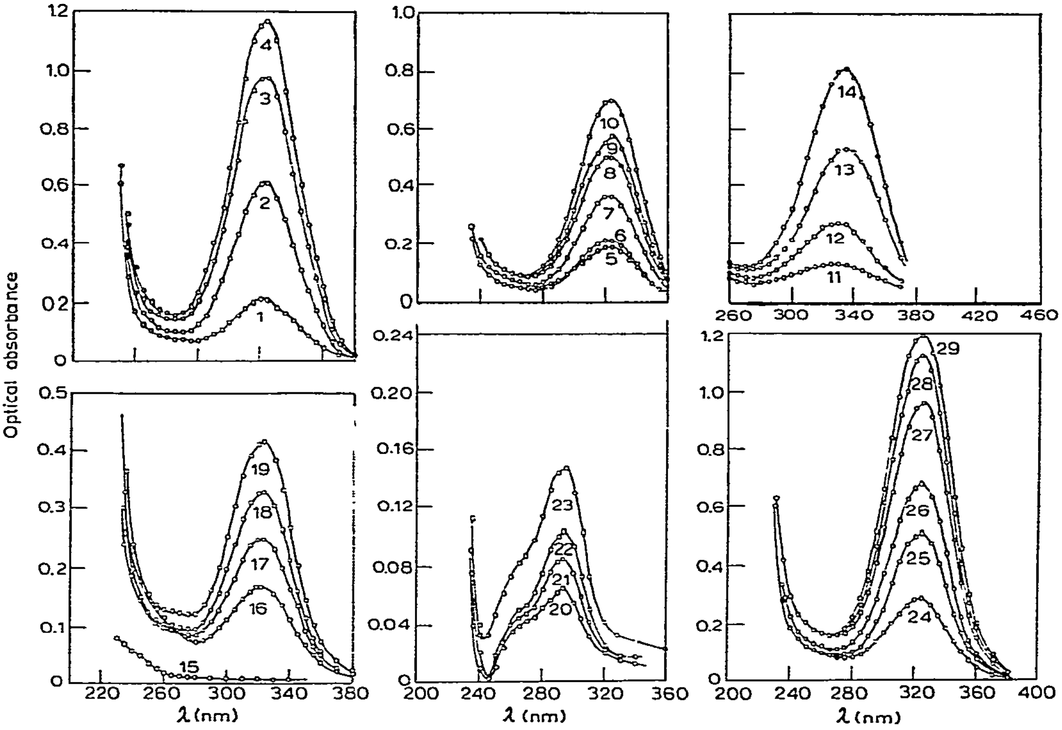


Fig. 4. U.v. spectra of carbohydrates (10^{-4}M) in presence of ethylenediamine (0.2M): 1–4, 2.0, 4.0, 6.0, and 8.0 units of D-glucose; 5–10, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 units of D-mannose; 11–14, 1.0, 2.0, 2.6, and 3.0 units of maltose; 16–19, 4.0, 6.0, 8.0, and 10.0 units of 2-amino-2-deoxy-D-glucose hydrochloride; 15, 10.0 units of 2-amino-2-deoxy-D-glucose hydrochloride in absence of the amine; 20–23, 4.0, 6.0, 8.0, and 10.0 units of 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside; 24–29, 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 units of D-galactose.

The absorption maxima are not those that would be observed for the aldehyde group of the carbohydrates if it were liberated by ring opening, or for α–β transformation, or pyranose–furanose conversion[4]. Had the maxima been due to a free aldehyde group at C-1, a band should appear between 270 and 310 nm (found for saturated aldehydes[4]). That this conclusion was correct was confirmed by following the reverse procedure, wherein the amine was first treated with a carbohydrate, and then amounts of standard hydrochloric acid requisite to neutralize the amine were added, and the u.v. spectra were recorded. For D-glucose, D-mannose, and D-galactose, the 324-nm band disappeared, and a band at ~270–280 nm appeared. Similar observations were made for 2-amino-2-deoxy-D-glucose and maltose. It is presumed that the complex is broken on addition of hydrochloric acid, and that this is followed by ring opening of the carbohydrates, which then absorb at 270–280 nm for the free aldehyde group (see Fig. 5). Another important feature was observed when (*a*) half-neutralized ethylenediamine reacted with the carbohydrates (except the D-glucosides), and (*b*) amine-treated carbohydrates were half-neutralized by adding half an equivalent of

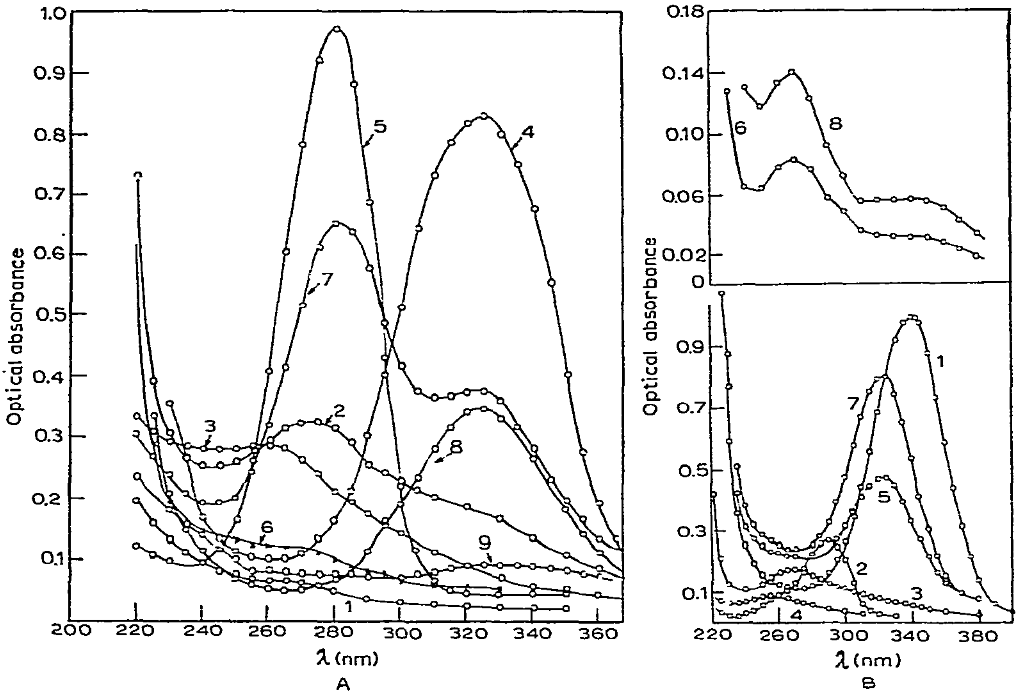


Fig. 5. Effect of HCl on the u.v. spectra of carbohydrate–base adducts at 10^{-4}M. A: 1, D-glucose in 0.1M NH_4OH; 2, D-glucose in 0.1M NaOH; 3, mixture 2 neutralized with HCl; 4, D-fructose in 0.1M ethylenediamine; 5, mixture 4 neutralized with HCl; 6, D-fructose in 0.1M NaOH; 7, mixture 8 neutralized with HCl (spectrum recorded within 30 min of mixing); 8, D-glucose in 0.2M ethylenediamine; 9, spectrum of solution 7 recorded 24 h after mixing.
B: 1, maltose in 0.1M ethylenediamine; 2, mixture 1 neutralized with HCl; 3, maltose in 0.05M NaOH; 4, mixture 3 neutralized with HCl; 5, D-mannose in 0.2M ethylenediamine; 6, mixture 5 neutralized with HCl; 7, D-galactose in 0.2M ethylenediamine; 8, mixture 7 neutralized with HCl.

hydrochloric acid. In both cases, bands appeared in the range of ~270–280 nm. These observations suggest that half-neutralized ethylenediamine does not form a complex, but that it may open the ring of the carbohydrates by the action of its basic property. *p*-Nitrophenol, also, cannot complex with ethylenediamine under similar conditions[15].

Validity of Beer's law. — Except for maltose, the carbohydrates were found to obey Beer's law. In establishing this fact, increasing amounts of a carbohydrate were added to an excess of the amine (assuming that all of the carbohydrate forms the complex). A plot of optical absorbance *versus* concentration of the carbohydrate (equivalent to the concentration of the 1:1 complex) provided a straight line in each case. The limits of concentration for which this was studied were 1 to 10×10^{-4}M. The millimolar extinction values are given in Table I.

Composition of the complexes, and their stability. — The Job method of continued variation[9] was employed for this study. For each carbohydrate, formation of a 1:1 complex was observed. The values of optical absorbance (A) at 1:1 molar ratio were used for calculating the stability, according to the general optical absorbance–extinction concentration scheme[10]. The stability values (K_s) for the complexes were calculated by using equation *2*, and are given in Table I. For a mixture:

$$A_{\text{solution}} = A_{\text{complex}} + A_{\text{free carbohydrate}} + A_{\text{free base}}$$

Since the carbohydrate and the base do not absorb,

$$A_{\text{solution}} = A_{\text{complex}} = \varepsilon x,$$

where ε and x are the molar extinction and the concentration of the complex, or,

$$x = A_{\text{solution}}/\varepsilon.$$

Now, for the reaction,

$$\begin{array}{ccc} \text{Carbohydrate} + \text{Base} & \overset{K_s}{\rightleftharpoons} & \text{Complex} \\ (a-b) \quad (b-x) & & x \end{array}$$

$$K_s = \frac{[x]}{[a-x]\,[b-x]} = \frac{A/\varepsilon}{(a-A/\varepsilon)\,(b-A/\varepsilon)} \quad \ldots \tag{2}$$

For a 1:1 molar composition,

$$K_s = \frac{A/\varepsilon}{(a-A/\varepsilon)^2} \quad \ldots \tag{1}$$

DISCUSSION

The results led to the conclusion that each of the carbohydrates studied (except for methyl α- and β-D-glucopyranoside) interacts with ethylenediamine, forming a complex. The complex is of a hydrogen-bonded or proton-transfer type[11], where an

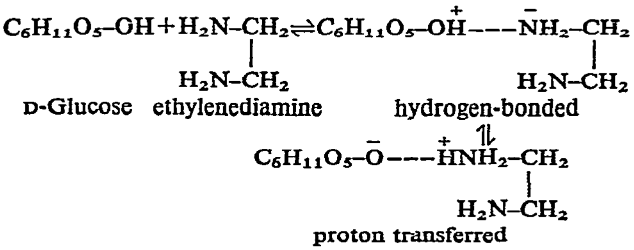
TABLE I

PHYSICAL CHARACTERISTICS OF THE COMPLEXES

Carbohydrate[a]	*pK at 35°*	*Millimolar extinction coefficient at 30°*	λ_{max} *(nm) at 30°*	*Stability constant* $K_s \times 10^{-1}$			$-\Delta G$ *(kcal/mole)*		
				H_2O	*50% alc.*	*75% alc.*	H_2O	*50% alc.*	*75% alc.*
D-Glucose	12.46	1.51	324	1.56	1.24	0.58	1.665	1.526	1.065
D-Mannose	12.08	1.00	324	3.27	2.20	0.25	2.114	1.874	0.556
D-Galactose	12.27	1.76	324	1.30	0.56	0.11	1.555	1.044	0.058
2-Amino-2-deoxy-D-glucose hydrochloride		0.43	320	2.34	1.76	no formation	1.912	1.740	
Methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside		0.15	292	2.57			1.923		

[a] Calculations were not performed for maltose, since Beer's law is not valid for it.

acidic –OH group of a carbohydrate donates, through its H^+, to the basic accceptor group (–NH_2) of the amine[3].



$$C_6H_{11}O_5{-}OH + \begin{matrix} H_2N{-}CH_2 \\ | \\ H_2N{-}CH_2 \end{matrix} \rightleftharpoons C_6H_{11}O_5{-}\overset{+}{O}H{-}{-}{-}\begin{matrix} \overset{-}{N}H_2{-}CH_2 \\ | \\ H_2N{-}CH_2 \end{matrix}$$

D-Glucose ethylenediamine hydrogen-bonded

$$\upharpoonleft\downharpoonright$$

$$C_6H_{11}O_5{-}\overset{-}{O}{-}{-}{-}\begin{matrix} \overset{+}{H}NH_2{-}CH_2 \\ | \\ H_2N{-}CH_2 \end{matrix}$$

proton transferred

It is accepted that the hydroxyl group on C-1 of the cyclic aldohexoses is acidic[12,13]. Therefore, this position is presumed to be the site of amine binding (maltose also binds at C-1 of the reducing D-glucose residue). However, the sites of association for D-fructose and methyl 2,6-di-*O*-(methylsulfonyl)-α-D-glucopyranoside cannot be explained on this basis. It is not unlikely that D-fructose binds through the hydrogen atom of the hydroxyl group on C-2. However, the hydroxyl group available on C-2 of methyl α- and β-D-glucopyranoside is unable to bind the amine. The site of complexation of the dimethanesulfonate molecule is still uncertain; whether this complex-formation is due to the influence of the methylsulfonyl groups on the neighboring free hydroxyl groups is yet to be decided.

The observation that 2-aminoethanol cannot react with a carbohydrate (studied chromatographically and spectrophotometrically) suggests that the reaction with ethylenediamine may be specific. At the same concentration, 2-aminoethanol degrades the carbohydrates far more readily than ethylenediamine.

It was observed that, although the amine has two contributory basic groups (NH_2), the composition of the complex is always 1:1. This is a general characteristic of the proton donor–acceptor complexes[7,11,14]. Even *p*-nitrophenol, a comparatively strong acid, also unites with ethylenediamine in 1:1 ratio. Half-neutralized amine cannot, however, accept a *p*-nitrophenol molecule[15].

The stability of all of the complexes is of about the same order. Thus, difference in configuration of the carbohydrates studied does not have much effect on their stability.

The polarity of the solvent has a marked effect on complexes of the proton-transfer type[14]. A threshold dielectric value for maximum complex-formation has been reported[16], but we have not yet explored this matter in detail. However, the formation of the complexes in 50 and in 75% alcohol is decreased, presumably by the depression of the dissociation of the acidic hydroxyl group of the carbohydrates in a medium having a comparatively low dielectric constant.

ACKNOWLEDGEMENTS

Financial support from the USDA [Project No. UR-A7-(10)-144] in the form of a P.L.480 grant is gratefully acknowledged. We thank B. Mukherjee for experimental assistance.

REFERENCES

1 V. A. DEREVITSKAYA, G. S. SMIRNOVA, AND Z. A. ROGOVIN, *Proc. Acad. Sci. USSR, Chem. Sect.* (English Transl.), 136 (1961) 1254.

2 J. A. RENDLEMAN, *Advan. Carbohyd. Chem.*, 21 (1966) 264.

3 S. P. MOULIK, A. K. MITRA, AND K. K. SEN GUPTA, *Carbohyd. Res.*, 19 (1971) 416.

4 E. R. GARRETT AND J. F. YONG, *J. Org. Chem.*, 35 (1970) 3502.

5 C. A. LOBRY DE BRUYN AND W. ALBERDA VAN EKENSTEIN, *Rec. Trav. Chim. Pays-Bas*, 14 (1895) 203.

6 W. PIGMAN, *The Carbohydrates: Chemistry, Biochemistry, Physiology*, Academic Press, New York, 1957.

7 S. NAGAKURA AND H. BABA, *J. Amer. Chem. Soc.*, 74 (1952) 5693.

8 N. D. COGGESHALL AND G. M. LANG, *J. Amer. Chem. Soc.*, 70 (1948) 3283.

9 P. JOB, *Compt. Rend.*, 180 (1925) 928.

10 J. E. PRUE, in R. E. ROBINSON (Ed.), *International Encyclopedia of Physical Chemistry and Chemical Physics*, Vol. 3, Pergamon, London, 1966, p. 14.

11 J. W. SMITH, *J. Chim. Phys.*, 58 (1964) 182.

12 J. J. CHRISTENSEN, J. H. RYTTING, AND R. M. IZATT, *J. Chem. Soc. (B)*, (1970) 1646.

13 B. C. HENDRICKS AND N. H. STEINBACH, *J. Phys. Chem.*, 42 (1938) 335.

14 R. SCOTT AND S. VINOGRADOV, *J. Phys. Chem.*, 73 (1969) 1890.

15 S. P. MOULIK, A. K. CHATTERJEE, AND K. K. SEN GUPTA, unpublished results.

16 R. SCOTT, D. DE PALMA, AND S. VINOGRADOV, *J. Phys. Chem.*, 72 (1968) 3192.