Relationship between the Structure of the Simple Sugars and their Behaviour on the Paper Chromatogram

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(*Received* 28 *July* 1950)

The object of the present study was to relate the chemical structure of the sugars to their R_F values on the paper chromatogram.

In the case of a homologous series the relationship between the R_F value and the structure is particularly simple. Consden, Gordon & Martin (1944), in their work on the separation of the amino-acids, have shown that the R_F values of members of a homologous series change in a regular manner with each additional group in the molecule. Martin (1949), treating the paper chromatogram as a liquid/liquid extraction column, has predicted on theoretical grounds that a linear relationship should exist between the logarithm of the partition coefficient of a substance between the two phases on the paper and the number of active groups of any one kind in the molecule, provided that the various active groups do not interfere with each other. The partition coefficient is calculated from the relation (Consden *et al.* 1944)

$$\alpha = \frac{A_L}{A_S}\left(\frac{1}{R_F} - 1\right), \qquad (1)$$

in which

$$R_F = \frac{\text{movement of band of substance under investigation}}{\text{movement of the advancing front of liquid}},$$

A_L = cross-sectional area of solvent phase,

A_S = cross-sectional area of water phase,

$$\alpha = \text{partition coefficient}: \frac{\text{concentration of solute in aqueous phase}}{\text{concentration of solute in the organic phase}}.$$

Since A_L/A_S is assumed to be constant for a given solvent and paper at a given temperature, α is directly proportional to $([1/R_F]-1)$, and the relationship deduced by Martin (1949) mentioned above can be rewritten with $\log([1/R_F]-1)$ in place of $\log \alpha$. Bate-Smith & Westall (1950) have tested this relationship for a number of homologous series of substituted aromatic compounds and have found good agreement. It is probable, however, that the picture of the paper chromatogram as a liquid/liquid extraction column is over-simplified, because the water imbibed into the cellulose fibres is almost certainly bound to the cellulose and is not free in the sense required by Martin's theory. In addition, it is probable that, in certain cases at least, the free hydroxyl groups of the cellulose are active. A more comprehensive picture, especially for hydrophilic solutes, has been suggested by Hanes & Isherwood (1949). These authors regard the operation of the chromatogram as depending upon the relative ease with which the solutes can enter into the structure of the water imbibed on the cellulose and into the structure of the water in the mobile phase. Such a picture gives a more concrete expression to the influence of the molecular size, shape and disposition of active groups in the solute on the R_F value. The partition coefficient α in equation (1) is then regarded as a measure of the distribution of a solute between the mobile phase and the water-cellulose complex.

The purpose of the present study is to relate the chemical structure of the sugars to their movement on the chromatogram. An important part of the study is the elucidation of the influence of the configuration of the hydroxyls of the sugars, for a preliminary survey of the experimental data shows that the separation of the simple sugars must depend to a large extent upon the spatial arrangements of the hydroxyl groups and the interaction between them, and only to a small extent upon the number of hydroxyl groups present. This follows from the fact that whole groups of sugars, which are separable on the chromatogram, have the same number of hydroxyls and probably even the same ring structure. Study of the sugars offers, therefore, the possibility of exploring the effect on the R_F value of different arrangements of the same groups in the molecule. However, before a more detailed analysis of the experimental data can be made, it is essential to obtain some idea of the probable structure of the sugars in solution. In the case of some of the common pentoses and hexoses which exist in the crystalline state as pyranoses, Cox, Goodwin & Wagstaff (1935)

suggest that there is little change in ring form during the process of solution. They point out that there is a close correspondence between the molecular volume in the solid state and the aqueous solution volume, which indicates that the form of the molecule is constant. This suggestion receives some support from work on the oxidation of the aldoses to the corresponding δ-lactones (Pigman & Goepp, 1948) which indicates that the common hexoses have pyranose ring structures in solution. Additional evidence that a number of sugars are almost entirely in the pyranose form in solution has been provided by a study of the mutarotation of sugars in aqueous solution. Isbell & Pigman (1937) have shown that for a number of aldohexoses the reaction follows a first-order equation, and that this fact makes it probable that the main constituents of the equilibrium solution are the α- and β-pyranose modifications. However, a number of sugars exhibit mutarotations which do not follow the first-order equation, and it is suggested by Isbell & Pigman (1937, 1938) that this must represent the establishment of equilibria in which three or more components are present in appreciable amounts. In fact the anomalous mutarotation in such cases is often explained in terms of a pyranose-furanose interconversion. Apart from the furanose ring form, one of the components present in solution is probably the open chain aldehydo form which is usually postulated as the intermediary between the various pyranose and furanose forms. There is no direct proof of its existence, but Cantor & Peniston (1940) have shown that sugar solutions contain an isomer which is reducible at the dropping mercury electrode of the polarograph. The proportion of this reducible form is small (< 0·2 %) in the case of sugars which, according to mutarotation data, exist almost entirely in the pyranose form, but may be large with sugars such as D-altrose (1·1 %) and D-ribose (8·5 %) which exhibit complex mutarotations. In the arrangement of the results in Table 2 we have assumed that the sugars exist in solution almost entirely as the pyranose forms, though it is clear that in a number of cases the solutions must contain appreciable amounts of furanose and open chain aldehydo forms which will affect the observed R_F value.

EXPERIMENTAL

Many sugars used in this investigation were not available commercially and we are indebted to Prof. T. Reichstein, Pharmazeutisches Anstalt der Universität Basel, and Prof. C. S. Hudson, National Institute of Health, Bethesda, Maryland, for the gift of a number of the rarer sugars. These are marked in Table 2 with an asterisk and a dagger respectively. Of the remainder, most were synthesized by the epimerization in dry pyridine of the appropriate available sugar (Fischer, Taube & Baer, 1927; Danilow, Venus-Danilowa & Schantarowitsch, 1930). Normally two aldoses and a ketose are present in the mixture after epimerization which makes the chemical isolation of any particular sugar difficult. In the present study, however, this was not necessary as the mixture of sugars was separated on the paper chromatogram and the R_F value of the desired sugar measured at the same time. The various sugar spots were identified by spraying comparable papers (three was the maximum necessary) with reagents which reacted either with aldose or ketose sugars, and by comparison with such genuine specimens of the appropriate ketose and aldose sugars as were available. L-Gulose and L-idose produced by the epimerization of L-sorbose were identified after separation on the chromatogram by comparison with a genuine specimen of idose.

Aldoses. These gave a definite colour (red, green, brown, blue and yellow) if a paper was sprayed with 0·1 M-aniline hydrogen phthalate in moist *n*-butanol and then heated at 105° for 5 min. (Partridge, 1949). This reagent also reacts with ketoses if during the removal of the solvent used on the chromatogram they are heated with basic substances such as pyridine. Apparently some of the ketose is epimerized to aldose. As ethyl acetate-pyridine-water was used as the solvent for the majority of the observations in this study, the papers were dried at room temperature in a stream of air for at least 2 hr. to remove the pyridine before spraying with the aniline hydrogen phthalate. Under these conditions the reagent gave no reaction with ketoses.

Ketoses. These gave a coloration (red-brown or purple) if the paper was sprayed with a mixture of equal volumes of 0·2 % (w/w) naphthoresorcinol in ethanol and 2 % (w/w) trichloroacetic acid in water, and then heated at 105° for 5 min.

Aldoses and ketoses. The total number of sugar spots present was revealed by spraying a paper with 0·1 M-$AgNO_3$ in 5 M-NH_4OH and then heating at 105° for 5–20 min.

Methyl glycosides. These were prepared by dissolving the sugar (10 mg.) in 0·5 % (w/w) HCl in methanol (1 ml.). Furanosides were produced by leaving the solution at room temperature for a period depending upon the sugar (2 hr. for pentoses and 15 hr. for hexoses) (Levene, Raymond & Dillon, 1932) and pyranosides by heating the solution at 70° for 3–4 hr. The solutions were then neutralized with Ag_2CO_3. The AgCl and excess Ag_2CO_3 were filtered off and the clear filtrates concentrated to about 0·3 ml. The mixture of glycosides was separated on the paper chromatogram and the R_F values of each individual glycoside measured at the same time. The position of the glycosides was revealed by spraying the paper with 0·1 M-$AgNO_3$ in 5 M-NH_4OH and then heating at 105° for 30 min. The paper darkened considerably, but the positions of the glycoside spots were easily visible. The silver oxide was reduced by the glycosides under these conditions. The free sugars present reduced the reagent within the first few minutes and could be distinguished readily from the glycosides which only reduced the reagent very slowly. The identification of furanoside and pyranoside was made on the assumption that the treatment at room temperature produced both furanoside and pyranoside, the furanoside usually in much greater amount, whereas the treatment at 70° produced only the pyranoside. Comparison of the glycoside spots produced by the two treatments indicated which was furanoside and which was pyranoside. In one case genuine methyl pyranosides were run on the paper at the same time. These were α- and β-methyl glucopyranoside. The result with these glycosides confirmed the previous identification of certain glucoside

spots as pyranose. There was a small difference between the R_F values of the α- and β-isomers.

A completely satisfactory determination of the R_F values of the methyl glycosides must await the provision of authentic specimens of each of the glycosides because, with the method described above, there is always some doubt about the identification of the various glycoside spots on the chromatogram. The doubt arises because it is known that a number of sugars can form derivatives with an anhydro ring in addition to the normal furanose or pyranose ring, and the presence of such derivatives will tend to confuse the identification of the various glycoside spots on the chromatogram. In certain cases the anhydro ring derivative is the main product of the action of acids on the free sugar. D-Altrose readily gives D-altrosan which is a 1:6-anhydro-D-altropyranose (Richtmeyer & Hudson, 1940). This carries only three hydroxyl groups and may be expected to have a higher R_F value than normal pyranosides.

The apparatus and the general procedure used for the accurate measurement of the R_F values was the same as that described earlier by Jermyn & Isherwood (1949). The measurements were carried out at a temperature of $20 \pm 0{\cdot}25°$. The solvent, which was the water-poor phase from a two-phase mixture of ethyl acetate (2 vol.), pyridine (1 vol.) and water (2 vol.), was freshly prepared for each run because it was found that the ethyl acetate present slowly hydrolysed. However, the change in composition in the first 1–2 days, the time necessary for the development of the chromatogram, had no appreciable effect on the R_F values. The mixed phases at the bottom of the chromatogram were also renewed each time because it was essential that the solvent on the paper and the liquid at the bottom of the jar were in equilibrium; any difference would tend to cause the composition of the solvent to change as it flowed down the paper.

The composition of the mixed phases at the bottom of the jar was also affected by the differential evaporation of the components to saturate the papers and the atmosphere of the jar in the preliminary equilibration. To minimize this effect at least 200 ml. of the mixed phases were added to a jar of the usual size (30–40 l.). The paper strip after the sugars had been applied was equilibrated for 24 hr. in contact with the mixed phases at the bottom of the jar before the solvent was allowed to run down the paper. For most solvents whose boiling points are about 100° this ensures not merely that the atmosphere in the chromatogram jar is saturated, but that the water-cellulose complex is in equilibrium with the solvent mixture. A simple experiment in which a paper (Whatman no. 1) was hung over a mixture of equal volumes of glacial acetic acid (b.p. 118°) and water in a chromatogram jar and the acetic acid present in equal areas of paper titrated after 24, 48 and 72 hr. showed that 4·3, 4·92 and 4·94 ml. of alkali were required respectively. Equilibration was practically complete after 48 hr. Using ethyl acetate-pyridine-water as solvent 24 hr. was sufficient. Recently Hanes & Isherwood (1949) have described a magnetic fanning device in which the papers are made to wave gently to and fro in the jar and thus stir the air. This greatly accelerates the process of equilibration so that a period of a few hours is sufficient.

Preliminary trials with D-glucose under the conditions described above indicated that the R_F values on the majority of papers varied by $\pm 0{\cdot}015$ about a mean value of 0·195 though some of the papers gave figures outside this range. Even this variation represents a difference between extremes of about 15%. It was essential to have a standard on each paper in order to determine whether the R_F values of the sugars on different papers could be compared and in practice D-glucose was used for this purpose. If the R_F value fell outside the range 0·20–0·19, the paper was rejected. No attempt was made to correct the results for variations in the R_F value of the D-glucose standard.

RESULTS

General relationship between the R_F value and the water content of the organic phase. In a previous paper Jermyn & Isherwood (1949) suggested that the R_F value of a sugar was related to the water content of the solvent, though no attempt was made

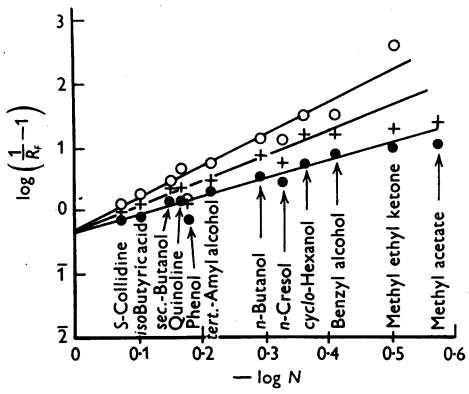


Fig. 1. Relationship between R_F value and molar fraction of water in non-aqueous phase. ○, Rhamnose; +, xylose; ●, glucose.

to formulate any definite relationship. In the present paper we have attempted to find a more precise relationship. By a re-examination of the data of Jermyn & Isherwood (1949) it has been found empirically that the graph of $\log([1/R_F]-1)$ against $-\log N$ gives a characteristic linear relationship for each sugar. N is the molar fraction of water in the solvent at 20°. In Fig. 1 the data for the three sugars rhamnose, xylose and glucose have been plotted. R_F values below 0·1 have not been included because a very small error in the measurement of the R_F value has a very large absolute effect on the value of $([1/R_F]-1)$. Plotting the data on a logarithmic scale has the advantage that the experimental errors in the measurement of the R_F value and the water content of the solvent do not assume too great a prominence at one end of the graph as compared with the other.

The results for phenol and *m*-cresol are particularly interesting because they do not agree with those obtained with other solvents. They give R_F values which are higher than would be expected from the amount of water dissolved in the organic solvent. It is possible that they can form loose compounds with the sugars which increase their solubility in the phenolic solvent.

Comparison between the configuration of the sugars and methyl glycosides and their R_F values in ethyl acetate-pyridine-water. A preliminary examination of the results indicated that if the sugars were arranged in groups on the basis of the ring structure assumed to exist in solution, the R_F values of corresponding members of each groups were similar. The mean figures for groups of sugars are given in Table 1. The individual results for most of the common sugars are given in Table 2.

tion of a DL mixture of sugars has been successful (Flood, Hirst & Jones, 1948).

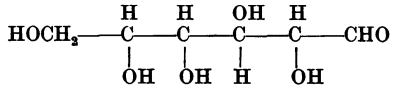
A detailed comparison of the various sugars has been made in Table 3.

In Table 3(*a*) the R_F values of sugars which differ only in the disposition of the hydroxyl group on one carbon atom of the ring are compared so as to show the effect of changing one hydroxyl group on a particular carbon atom from above the ring to below the ring.

Table 1. *Mean R_F values of various groups of sugars in ethyl acetate-pyridine-water at* 20°

Class of sugars	Ring form	Range of R_F values	Mean R_F value
Aldotetrose	Furanose	0·41 –0·43	0·42
Aldopentose	Pyranose	0·23 –0·33	0·285
Ketopentose	Furanose	0·35 –0·37	0·36
Aldohexose	Pyranose	0·175–0·31	0·24
Ketohexose	Pyranose	0·24 –0·30	0·26
Heptose	Pyranose	0·14 –0·23	0·18
Hexitol			
Sorbitol	.	0·18, 0·18,	0·19
Dulcitol	.	0·20	
Mannitol	.		
Aldohexomethylose	Pyranose	0·265–0·48	0·40
Ketohexomethylose	Furanose	0·43 –0·47	0·44
Disaccharide			
Sucrose, Trehalose	.	0·16, 0·15	0·13
Lactose, Cellobiose	.	0·07, 0·125,	
Maltose	.	0·13	
Trisaccharide			
Raffinose	.	.	0·044
Methyl glycosides of			
Aldopentoses	Pyranose	0·40 –0·48	0·44
	Furanose	0·52 –0·55	0·54
Methyl glycosides of			
Aldohexoses	Pyranose	0·23 –0·37	0·31
	Furanose	0·43 –0·48	0·46

The configuration of the sugars is described according to the Fischer convention, thus D-glucose



is written + + − +. The configuration of the ring is shown in the bracketed column, e.g. $\overbrace{+\,+\,-\,+}$.

For the purpose of description the carbon atoms of the ring are numbered from the reducing end and the numbers do not necessarily correspond with the usual numbering for the free sugar, thus D-fructopyranose would be described as having a —CH_2OH group attached to carbon atom 1 of the pyranose ring, this carbon atom normally being regarded as number 2 of the sugar molecule.

In certain cases the D-sugars were not available and the results given are for the corresponding L-sugars. No difference between the R_F values for enantiomorphs in optically inactive solvents is likely. Even in optically active solvents no resolu-

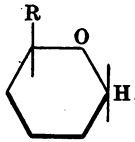
In Table 3(*b*) the R_F values of sugars which differ only in the disposition of the hydroxyl groups on carbon atoms 2 and 3 are compared. If the hydroxyl groups are on the same side of the ring, the arrangement is regarded as being *cis* and if on opposite sides as *trans*.

In Table 3(*c*) the effect of adding the group —CH_2OH to either carbon atom 5 or 1 of the pyranose ring is studied by comparing aldohexoses and ketohexoses with aldopentoses which possess the same disposition of hydroxyl groups on carbon atoms 2, 3, 4 of the pyranose ring, irrespective of whether the sugar belongs to the L or the D series. In the case of the aldohexoses the sugars have been subdivided into two groups depending whether the group —CH_2OH attached to carbon atom 5 is above the ring or below.

DISCUSSION

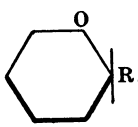
In a previous paper Jermyn & Isherwood (1949) suggest that the R_F values of the sugars run roughly parallel with the water content of the solvent and

Table 2. *Relationship between the configuration and the R_F value of sugars and glycosides in ethyl acetate-pyridine-water at 20°*

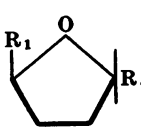
(*a*) Sugars with the general formula  , where R = —CH_3, —CH_2OH, —COOH, —$CHOH.CH_2OH$

Configuration (Ring)				Aldomethylose sugars			Aldohexose sugars			Aldohexuronic acids‡			Aldoheptose sugars		
				R = —CH_3	M.p. (°)	R_F	R = —CH_2OH	M.p. (°)	R_F	R = —COOH	M.p. (°)	R_F	R = —$CHOH.CH_2OH$	M.p. (°)	R_F
+	+	+	+	Allomethylose*	146	0·36	Allose*	128	0·22	.	.	.	.	.	.
+	+	+	−	Altromethylose	.	0·45	Altrose†	108	0·27	.	.	.	.	.	.
+	+	−	+	Glucomethylose	139–145	.	Glucose	146–150	0·195	Glucuronic	165	0·15	D-Gala-L-glucose†	190–194	0·16
+	−	+	+	Gulomethylose*	.	0·36	Gulose	.	0·23	.	.	.	D-Gluco-D-guloset	210	0·19
+	+	−	−	Mannomethylose	123	0·38	Mannose	132	0·24	Mannuronic	165	0·18	D-Gala-L-mannose†	.	0·21
+	−	+	−	Idomethylose*	.	0·51	Idose	.	0·31	.	.	.	.	.	.
+	−	−	+	Galactomethylose	145	0·265	Galactose	165	0·175	Galacturonic	160	0·13	D-Gulo-L-galactose†	.	0·14
													D-Manno-D-galactose†	134	0·15
+	−	−	−	Talomethylose*	.	0·48	Talose*	128	0·285	.	.	.	D-Gulo-L-talose†	.	0·23

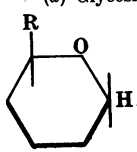
Sugars marked * were a gift from Prof. T. Reichstein and those marked † were from Prof. C. S. Hudson.
‡ These were run in ethyl acetate-acetic acid-water as solvent (Jermyn & Isherwood, 1949).

(*b*) Sugars with the general formula  , where R = —H, CH_2OH

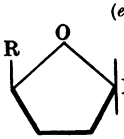
Configuration (ring)			Ketohexose sugars			Aldo-pentose sugars		
			R = —CH_2OH	M.p. (°)	R_F	R = —H	M.p. (°)	R_F
+	+	+	Allulose	Syrup	0·30	Ribose	95	0·33
+	−	−	Tagatose	124	0·27	Lyxose	106–117	0·30
+	−	+	Sorbose	164	0·24	Xylose	145	0·28
+	+	−	Fructose	102	0·24	Arabinose	159	0·23

(*c*) Sugars with the general formula  , where R = —H, —CH_2OH and R_1 = —H, —CH_3

Configuration (ring)		Aldotetrose sugars		Ketopentose sugars		Keto-hexomethylose sugars	
		$R = R_1$ = —H	R_F	$R = R_1$ = —CH_2OH	R_F	R_1 = —CH_3, R = —CH_2OH	R_F
+	+	Erythrose	0·41	Araboketose	0·35	Allomethylo-ketose + + +	0·44
						Galactomethylo-ketose + − −	0·43
+	−	Threose	0·43	Xyloketose	0·37	Mannomethylo-ketose + + −	0·43
						Idomethylo-ketose + − +	0·47

(*d*) Glycosides with the general formula  , where R = —H, —CH_2OH

Configuration (Ring)			Aldohexose glycosides		Aldopentose glycosides	
			R = —CH_2OH	R_F	R = —H	R_F
+	+	+	Allose	0·23	Ribose	0·40
+	+	−	Altrose	0·36	Arabinose	.
+	−	+	Glucose	0·29	Xylose	0·46
+	+	+	Gulose	.	.	.
+	−	−	Mannose	0·37	Lyxose	0·48
+	−	+	Idose	.	.	.
+	+	−	Galactose	0·34	.	.
+	−	−	Talose	0·29	.	.

(*e*) Glycosides with the general formula  , where R = —CH_2OH, —$CHOH.CH_2OH$

Configuration Ring			Aldohexose glycosides		Aldopentose glycosides	
			R = —$CHOH.CH_2OH$	R_F	R = —CH_2OH	R_F
+	+	+	Allose	0·45	Ribose	0·54
+	+	−	Altrose	0·48	Arabinose	0·55
+	−	+	Glucose	0·43	Xylose	0·52
−	+	+	Gulose	.	.	.
+	−	−	Mannose	0·48	Lyxose	0·55
−	+	−	Idose	.	.	.
−	−	+	Galactose	0·47	.	.
−	−	−	Talose	0·44	.	.

Table 3. *Influence of the configuration of the hydroxyls on each carbon atom of the pyranose ring on the R_F values of the sugars*

(*a*) Comparison of sugars in which the hydroxyl on one carbon atom has been changed from above to below the ring

Hydroxyl inverted on carbon atom no.	Sugars compared	Difference in R_F value
4	Gulose, allose	+0·01
	Idose, altrose	+0·04
	Galactose, glucose	−0·02
	Talose, mannose	+0·055
3	Glucose, allose	−0·025
	Mannose, altrose	−0·03
	Galactose, gulose	−0·055
	Talose, idose	−0·025
2	Altrose, allose	+0·05
	Mannose, glucose	+0·045
	Idose, gulose	+0·08
	Talose, galactose	+0·11
3	Xylose, ribose	−0·05
	Lyxose, arabinose	+0·07
2	Arabinose, ribose	−0·10
	Lyxose, xylose	+0·02

(*b*) Interaction of hydroxyls on adjacent carbon atoms

Sugar	Configuration of hydroxyls on carbon atoms 3	2		R_F	Sugar	Configuration of hydroxyls on carbon atoms 4	3	2		R_F
Ribose	+	+	*cis*	0·33	Allose	+	+	+	*cis*	0·22
					Altrose	+	+	−	*trans*	0·27
Arabinose	+	−	*trans*	0·23	Glucose	+	−	+	*trans*	0·195
					Mannose	+	−	−	*cis*	0·24
Xylose	+	−	*trans*	0·28	Idose	−	+	−	*trans*	0·31
					Gulose	−	+	+	*cis*	0·23
Lyxose	−	−	*cis*	0·30	Galactose	−	−	+	*trans*	0·175
					Talose	−	−	−	*cis*	0·285

(*c*) The effect of adding the group —CH_2OH to either carbon atom 1 or 5 of the pyranose ring

Configuration of ring	Aldohexoses —CH_2OH on carbon atom 5	R_F		R_F	Ketohexoses —CH_2OH on carbon atom 1	R_F	Aldopentoses No substituent	R_F
+ + +	Allose	0·22	Talose	0·285	Allulose	0·30	Ribose	0·33
+ − −	Mannose	0·24	Gulose	0·23	Tagatose	0·27	Lyxose	0·30
+ − +	Glucose	0·195	Idose	0·31	Sorbose	0·24	Xylose	0·28
+ + −	Altrose	0·27	Galactose	0·175	Fructose	0·24	Arabinose	0·23

that the order in which the sugars separate is not affected by the composition of the solvent. In the present paper this suggestion has been investigated more thoroughly and the results given in the earlier paper have been interpreted so as to show in a more striking manner the exact relation which exists between the movement of the sugars on the chromatogram and the water content of the solvent. The water content of the solvent (N) is defined as the molar fraction of water in the non-aqueous phase at 20°. It has been found empirically that if $\log([1/R_F]-1)$ is plotted against $-\log N$ for each sugar then the graph is a straight line. Examples of this are shown in Fig. 1, for the three sugars L-rhamnose, D-xylose and D-glucose. The graphs show clearly that the value of $([1/R_F]-1)$, which can be regarded as equivalent to the distribution of the sugar between the water-cellulose complex and the solvent, cf. equation (1), is governed almost entirely by the water content of the solvent. The graphs also show that the relative values of $([1/R_F]-1)$ for the various sugars remain in the same order independent of the water content of the solvent. The value of this general relationship be-

tween the movement of the sugars on the chromatogram and the water content of the solvent is that the results of a detailed study in one particular solvent can now be applied to a variety of solvents. The order of R_F values will be similar in each solvent except phenols which will be referred to later. However, it should be pointed out that this generalization, so far as this study is concerned, applies to a temperature of about 20° and solvents which are the water-poor phase of a two-phase mixture.

A plausible theoretical basis for the empirical relationship described above can be deduced from the fact that sugars in solution have a strong affinity for water molecules which are presumably bound to the hydroxyl groups of the sugars. The sugars in aqueous solution are in fact very heavily hydrated and may be expected to approximate in behaviour to water itself. The affinity of sugars for neutral organic molecules is small, and it seems that it is the heavily hydrated sugar molecules which are soluble in the aqueous organic solvents. It follows, therefore, that the distribution of a sugar between an aqueous solvent and the water-cellulose complex will depend to a large extent on the readily available water in the two phases. It is probable, however, that the water bound to the cellulose which is available to the sugar on the chromatogram, is only slightly affected by changes in the composition of the solvent so that the distribution of a sugar between the two phases is largely controlled by the water content of the solvent. If one regards both the solubility of the water and the heavily hydrated sugar as manifestations of the affinity of molecules of the solvent for water hydroxyls, then the distribution of the sugar between the two phases may be expected to run parallel with the solubility of water in the organic phase.

In the present paper the R_F values of the sugars were measured using the water-poor phase of a two-phase mixture of ethyl acetate-pyridine-water. Accurate R_F values for a number of the sugars using this solvent had already been obtained before this study was started and as the solvent was readily available and convenient to use, it was adopted as standard.

In Fig. 1 it will be noticed that the results for phenol and *m*-cresol are not consistent with those obtained with other solvents, the R_F values being greater than would be expected from the water content. In addition it has been found that the order in which the sugars separate on the chromatogram is different, thus the order of R_F values in phenol and in ethyl acetate-pyridine-water is glucose, galactose, mannose and galactose, glucose, mannose respectively. The explanation probably lies in the fact that phenols can form compounds with sugars analogous to those formed by water so that the distribution of a sugar between the aqueous phenolic solvent and the water-cellulose complex will depend not only on the water present in each phase but also on the phenol. However, apart from phenols, the order in which the sugars separate on the paper chromatogram is independent of the solvent.

In Table 1 the mean R_F values for various groups of sugars are listed. The comparable figures for the methyl glycosides and for the di- and tri-saccharides are also given. A brief examination of the figures indicates that while the number of hydroxyl groups in a monosaccharide has some relation to the R_F value, other factors contribute largely to the observed results. The mean R_F values for the hexo-methyloses (0·40 and 0·44) and pentoses (0·29 and 0·36) and for the methyl pyranosides (0·44 and 0·31) and furanosides (0·54 and 0·46), show that even if the number of hydroxyl groups is the same, the R_F values may be very different. Grouping the sugars on the basis of the number of hydroxyls alone, would mean in the case of the pentoses, hexoses and heptoses, that the range of R_F values in each group considerably overlapped those in the other groups. Comparison of the mean R_F values for the mono-, di-, and tri-saccharides illustrates the effect of varying the number of sugar residues in the molecule.

In Table 2 the sugars have been arranged in groups on the basis of the configuration of the atoms which compose their ring forms. Thus the pyranose forms of D-xylose, D-glucose, D-glucomethylose, and L-gala-D-glucoheptose all belong to the D-glucose type. These sugars differ only in the group attached to carbon atom 5 of the pyranose ring, the configuration of the atoms of the ring being the same in each case. Such a grouping of sugars has been termed homomorphous (Hann, Merrill & Hudson, 1935), and as would be expected from the identity of the configurations of the rings, members of each homomorphous series often show marked chemical and physical similarities with each other. In such a series it is often possible to predict the properties of an unknown member from those of the basic type. In the present study the ring structures were assumed to be pyranose, except where the chemical structure of the sugar prevented this being formed. Thus, the ketopentoses and aldotetroses were formulated as furanose.

Comparison between the various groups in Table 2 shows that the R_F values of corresponding members of each group are markedly dependent on the configuration of the ring. In addition the residue attached to either carbon atom 5 of the pyranose ring or 4 of the furanose ring exercises the same general influence on the R_F value independent of the configuration of the ring. This means that the same sequence of R_F values is preserved in each of the homomorphous groups. Comparison of the sugars in Table 2(*a*) shows that the replacement of

—CH_2OH of any of the aldohexose sugars by —CH_3 or —$CHOH.CH_2OH$ changes the R_F value by about +50 and −15% respectively. A similar effect is observed with the residue attached to the reducing end of the ring. Comparison of the ketohexose and aldopentose sugars in Table 2(*b*) shows that the replacement of —H on carbon atom 1 of any of the aldopentose sugars by —CH_2OH changes the R_F value by about −10%. The sugars which can only form furanose rings have much higher R_F values than those which can form pyranose, and the differences between individual sugars are less. The influence of the ring form is very clearly demonstrated in the case of the aldohexose methyl pyranosides and furanosides. Reference to Table 2(*d*) and (*e*) shows that the range of R_F values for the pyranosides 0·23–0·37 is wider, and the mean value 0·31 smaller, than for the corresponding furanosides (0·43–0·48 and 0·46 respectively).

Comparison of the various homomorphous groups is probably helped by the fact that the influence of the configuration of the ring on other properties such as the proportion of the various ring isomers in solution is automatically brought into line with the R_F values. Sugars which have the same configuration for the first four carbon atoms will give very similar mixtures of the various ring forms. Evidence on this point is rather scanty, but it is known that D-altrose (++++) and D-ribose (+++) are characterized in solution by the presence of large amounts of the aldehydo forms, approximately twenty times that of other sugars in their class. This fact suggests that the pyranose ring with the configuration (+++) is rather unstable.

In Table 2 we have included the melting points of a number of the sugars for comparison with their R_F values. In the case of the aldopentoses and aldohexoses the melting point is roughly inversely proportional to the R_F value. Talose is exceptional in that its melting point is somewhat higher than the other sugars with a similar R_F value. In the case of other classes of sugars the melting point does not correspond in any simple manner to the R_F value; of the ketohexose sugars, allulose, tagatose and sorbose behave like the aldohexoses, but fructose is very different. From its position in the table it ought to have a melting point higher than 164° and an R_F value of less than 0·24, whereas it has a melting point of 102° and an R_F value of 0·24. The important fact about the comparison between the melting point and the R_F value is that a close relationship does exist within a restricted group of sugars. This is sufficiently remarkable to stimulate speculation as to what fundamental property of the sugar molecule forms the link between the melting point and the R_F value. The melting point of an organic crystal is a measure of the strength of the internal bonds between the molecules in the lattice and in the case of the sugars which are mainly linked together by their hydroxyl groups, must represent at least approximately the extent and strength of the association of these hydroxyl groups. The R_F value, as described previously, is a measure of the affinity of a sugar for water, the affinity being inversely proportional to the R_F value. In solution the sugar molecules are largely hydrated. Now Cox *et al.* (1935) have pointed out that for certain common pentoses and hexoses (figures were not available for any others) the molecular volume in the crystalline state is very similar to the aqueous solution volume which they suggest means that the association of a sugar with water molecules in solution is of the same nature and extent as the association of neighbouring sugar molecules in the solid. We may expect, therefore, the affinity of a sugar for water molecules to be closely related to the strength of the internal bonds between the sugar molecules in the crystal and correspondingly the R_F value to be inversely proportional to the melting point. However, any discrepancy between the form of the molecule in the solid state and in solution will cause the relationship to break down. It is perhaps significant that the sugars for which figures were available of the molecular volume in the crystal state and in solution (Cox *et al.* 1935) were aldopentoses and aldohexoses. These give the simple relationship between the melting point and the R_F value. The examination of the other classes of sugars which do not give this relationship might show considerable discrepancies between the form of the molecule in the solid state and in solution.

A more detailed comparison of the various sugars has shown that in many cases the R_F value can be related even more closely to the configuration of the ring. In Table 3(*a*) the R_F values of sugars which differ only in the disposition of the hydroxyl group on one carbon atom of the ring are compared so as to show the effect of changing one hydroxyl group on a particular carbon atom from above the ring to below the ring. In the case of the aldohexoses, the figures for the various pairs of sugars, with the exception of galactose-glucose, show that the change alters the R_F value in the same sense for any one particular carbon atom, but that the direction in which the R_F value is altered is different for each carbon atom. In the case of the aldopentoses, no clear relationship is discernible between the R_F value and the disposition of the hydroxyl groups above or below the ring. In Table 3(*b*) the R_F values of sugars which differ only in the disposition of the hydroxyl groups on carbon atoms 2 and 3 are compared. If the hydroxyl groups are on the same side of the ring, the arrangement is regarded as being *cis* and if on opposite sides *trans*. In the case of the aldopentoses the figures indicate that the *cis* arrangement has a higher R_F value than the *trans*, but that in the case of the aldohexoses no such simple relationship exists.

Confirmatory evidence of the significance of the relationship in the case of the aldopentoses which are admittedly a small sugar group, is provided by a study of the ketohexoses, Table 3(*c*). The order of R_F values corresponds to that expected from analogy with the aldopentoses, so that in this case also the *cis* arrangement has a higher R_F value than the *trans*. The effect of the configuration of the hydroxyl groups on carbon atoms 3 and 4 has also been examined, but the most careful scrutiny has not shown any sign of regularity. In Table 3(*c*) the effect of adding the group —CH_2OH to either carbon atom 5 or 1 of the pyranose ring is studied by comparing aldohexoses and ketohexoses with aldopentoses which possess the same disposition of hydroxyl groups on carbon atoms 2, 3, 4 of the pyranose ring. It is noticeable that the order of R_F values for the first group of aldohexoses (—CH_2OH above the ring) is the exact opposite of that for the second (—CH_2OH below ring). This suggests that the disposition of the —CH_2OH group on carbon atom 5 must have a very strong influence on the R_F value. The comparison between the aldohexoses, ketohexoses and aldopentoses indicates that adding the group —CH_2OH to carbon atom 5 completely changes the influence of the ring hydroxyl groups on the R_F value whereas adding it to carbon atom 1 has very little effect.

The overall picture obtained from the results in Table 3 is that the presence of a substituent on carbon atom 5 of the pyranose ring appears to have a profound influence on the interaction between the various hydroxyl groups attached to the ring and on the affinity of the sugar for water molecules. In the absence of a substituent, the association of a sugar with water molecules is limited only by the interaction between the hydroxyls on neighbouring carbon atoms. In the presence of a substituent, the association of a sugar with water molecules is governed by whether the hydroxyl groups are on the same side of the ring as the substituent or not. The fact that the order of R_F values for the methylose sugars is the same as for the corresponding aldohexoses indicates that the group —CH_3 influences the R_F value in the same general manner as —CH_2OH and suggests that the substituent does not act through an association of hydroxyl groups between carbon atom 6 and the ring, but rather through some form of steric hindrance. This interference with the interaction of the hydroxyl groups of the pyranose ring with each other and with water molecules disappears if the substituent is attached to carbon atom 1 instead of carbon atom 5. Comparison of the ketohexoses in which the group —CH_2OH is attached to carbon atom 1 of the pyranose ring, and the aldopentoses, shows that the order of R_F values in each group of sugars corresponds very closely. This implies that the group —CH_2OH has very little effect on the interaction of the hydroxyl groups of the pyranose ring which are the controlling influence in the case of the aldopentoses. It is possible, however, that in solution the ring form of the ketohexoses adjusts itself so as to eliminate largely any steric hindrance, an adjustment which is probably impossible when the group —CH_2OH is attached to carbon atom 5 of the pyranose ring. It is noteworthy that in solutions of the ketohexoses considerable amounts of the reducing open chain forms are present and that in the case of fructose (Gottschalk, 1943) about 20 % of the sugar exists as the furanose form. These observations suggest that the pyranose ring is unstable with the substituent on carbon atom 1 of the ring and it may be that a steric factor is responsible.

The furanose sugars have not been examined in the same detail as the pyranose sugars because it is apparent from a brief inspection of the results in Table 2(*c*) that the configuration of the hydroxyl groups on the ring has less influence on the R_F value. In Table 2(*c*) the sugars have been grouped so as to bring out similarities in R_F value for similar types of ring structure. Thus D-galacto-methylo-ketose + − − has been compared with D-ethythrose + +. Strictly L-galacto-methylo-ketose − + + should be compared.

The R_F values for the glycosides given in Table 2(*d*) and (*e*) probably represent the mean values for the α- and β-isomers. In many cases the amount of sugar at our disposal was too small to enable us to make a detailed investigation and to that extent the results must be regarded as provisional. The R_F values for genuine specimens of α- and β-methyl-glucopyranoside (0·29 and 0·305) were sufficiently close to suggest that no separation of the αβ-isomers would occur on the paper chromatogram using ethyl acetate-pyridine-water as solvent. The results for the methyl glycosides have been arranged on the basis of the configuration of the ring in a similar manner to the sugars. It is not our intention to analyse these results in detail owing to possible uncertainties in some of the results. However, comparison between the various groups shows that the R_F values of corresponding members of each group are markedly dependent on the configuration of the ring. In addition, it is significant that the order of R_F values for the methyl pyranosides is not the same as that for the parent sugars. D-Galactose which has the lowest R_F value of the aldohexoses gives a methyl pyranoside which has a R_F value well above the lowest of its class. Furanosides have much higher R_F values than pyranosides.

SUMMARY

1. An empirical relationship has been discovered between the movement of the sugars (R_F value) on the paper chromatogram and the molar fraction of water (N) in the solvent. The graph of log $([1/R_F] - 1)$

against $-\log N$ is a straight line for each sugar. The relationship holds over a wide range of solvent mixtures, the only exceptions being those containing phenols as the organic component. The relationship is given a theoretical basis in terms of the strong association of the hydroxyl groups of the sugars with the water molecules in mixed solvents containing water. The sugars separate in the same order in all solvents, except phenols.

2. If the sugars are arranged in the form of a homomorphous series on the basis of an assumed preferential formation of a pyranose ring in solution (furanose when pyranose is impossible) then it is found that members of each homomorphous series show close similarity in behaviour on the paper chromatogram. The sequence of R_F values for each group of sugars (aldohexose, aldomethylose, etc.) depends only on the configuration of the hydroxyls of the ring. The same appears to be true of the methyl glycosides.

3. A detailed analysis has been made of the contribution of each hydroxyl group to the observed R_F value in the case of the aldohexoses, aldopentoses and ketohexoses. The analysis shows that if a substituent (—CH_2OH) is attached to carbon atom 5 of the pyranose ring then the influence of a hydroxyl group on any particular carbon atom largely depends upon whether the hydroxyl is on the same side of the ring as the substituent or not. The effect on the R_F value is different for each carbon atom. In the absence of a substituent (aldopentoses) or if the substituent is attached to carbon atom 1 of the pyranose ring (ketohexoses), it is the interaction between the hydroxyl groups on neighbouring carbon atoms which governs the R_F value, a *cis* disposition of hydroxyls giving a higher R_F value than a *trans*.

4. Sugars with a furanose ring have a much higher R_F value than those with a pyranose ring.

5. The R_F values of the simple pentoses and hexoses are roughly inversely proportional to their melting points.

The work described in this paper was carried out as part of the programme of the Food Investigation Organization of the Department of Scientific and Industrial Research.

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The Mechanism of the Formation of Organic Acids by Mould Fungi

5. THE INFLUENCE OF ARSENITE ON *ASPERGILLUS NIGER*

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(*Received* 19 *June* 1950)

Chughtai, Pearce & Walker (1950) showed recently that acetic acid is produced in the mycelial felts of cultures of *Aspergillus niger* growing on a glucose medium and that, following the addition of sodium arsenite to such cultures, pyruvic acid may be detected in the medium. A quantitative study of the effects of arsenite on the utilization of sugar and on the formation of acids in this mould has now been carried out.

EXPERIMENTAL METHODS AND RESULTS

The strain of *A. niger* (designated N 1) used generally in previous studies in this series was again employed. The medium in the quantitative experiments consisted of