A Non-destructive Reactivity Index of Carbohydrates: The Hydroxy Protonation Rates in Dimethyl Sulphoxide

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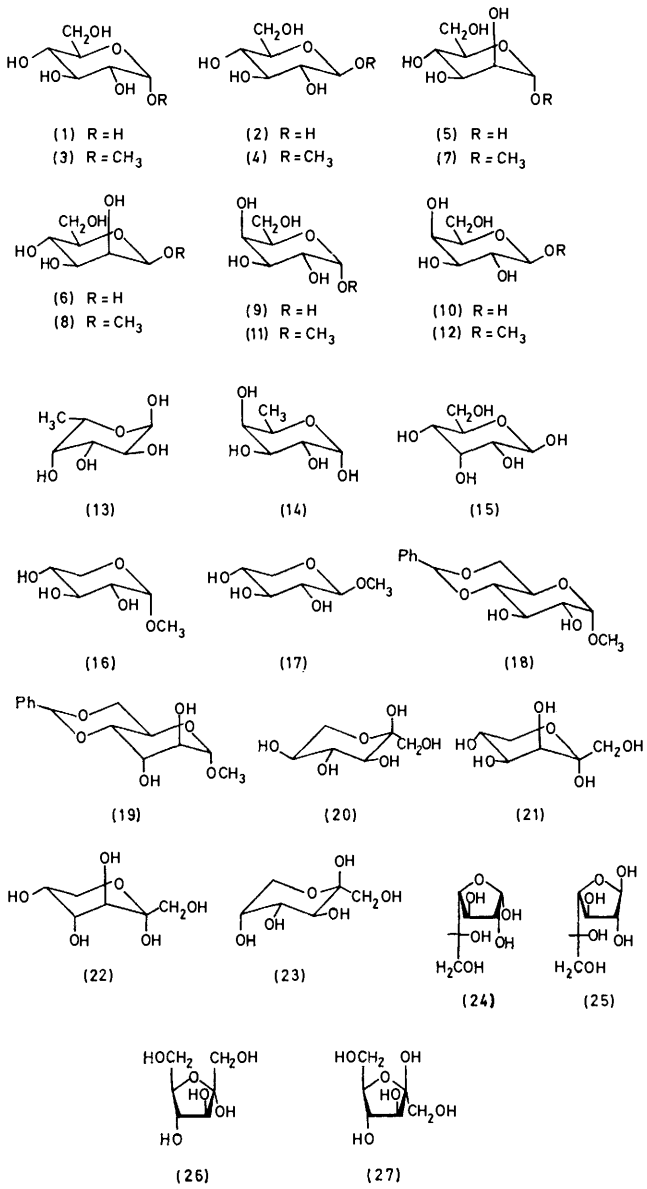
Proton transfers to 26 hydroxylated monosaccharides have been measured in anhydrous dimethyl sulphoxide (DMSO) at 25 °C under acid-catalysed conditions, using dynamic ^{1}H n.m.r. techniques at 250 MHz. The results are summarized by the equation, rate of hydroxylic proton exchange = k[DMSO · · · H$^+$], with k = 1—3 and 4—9 × 10^6 l mol^{-1} s^{-1} for anomeric and non-anomeric hydroxy-groups, respectively. Anomeric hydroxy-groups are protonated slightly faster in ketopyranoses and furanoses than in aldopyranoses, a sequence roughly parallel to that observed for the acid-catalysed hydrolysis of the corresponding glycosides. The protonation rate of an anomeric hydroxy-group is sensitive to the equatorial or axial nature of neighbouring hydroxy-groups. The reverse is true for non-anomeric hydroxy-groups, except if the neighbouring substituent is itself an anomeric hydroxy-group. Steric and electronic effects of 1- and 6-substituents are discussed. The order of kinetic basicities of the hydroxy-groups being protonated is in fairly good agreement with the range of acidities reflected by the chemical shifts of the hydroxy-protons of pyranoses.

An important problem in the chemistry of carbohydrates is the relative ease of esterification of the various hydroxy-groups contained in monosaccharide molecules, or, conversely, of the hydrolysis of their ethers or esters.[1] Such reactions are acid-catalysed and the rate-determining step in acidic medium often consists in the protonation of the sugar molecule. The comparison of the protonation rates of hydroxy-groups either within a given monosaccharide molecule or between a series of different monosaccharides may therefore constitute a representative index for the reactivity of carbohydrates and provide useful information for a detailed study of carbohydrate reactions. A non-destructive method of measuring these rates is dynamic n.m.r. spectroscopy applied to the hydroxylic protons themselves. The combination of two experimental facilities, namely the improved dispersion and sensitivity brought about by a superconducting spectrometer, and the exceptionally low proton mobility of alcoholic protons in dimethyl sulphoxide (DMSO), allowed us to measure the above mentioned protonation rates in slightly acidic DMSO solutions. The experimental procedure was essentially the same as that already described for thiols,[2] aliphatic alcohols,[3] and cyclohexanols.[4] In acidic medium, proton exchanges are shown to arise exclusively from the protonation of the hydroxy-groups [3,5] (denoted ROH, as in the case of an alcohol) by solvated protons [reaction (1)] rapidly followed by the reverse reaction (2). Chemical exchange (k) is consequently twice as fast as the n.m.r. site exchange $k_{n.m.r.}$ ($k = 2k_{n.m.r.}$). Very small amounts of acid are necessary to bring this exchange on to the n.m.r. time scale, so that the sample investigated may be recovered after these experiments. Applied to polyols, the fundamental advantage of the n.m.r. method, compared with chemical relaxation methods, is the possibility of observing separately the various proton

$$\mathrm{ROH} + \mathrm{DMSO} \cdots \mathrm{H}^+ \xrightarrow{k} \mathrm{ROH_2}^+ + \mathrm{DMSO} \quad (1)$$

$$\mathrm{ROH_2}^+ + \mathrm{DMSO} \xrightarrow{k_D} \mathrm{ROH} + \mathrm{DMSO} \cdots \mathrm{H}^+ \quad (2)$$



exchanges which occur simultaneously on the different hydroxy-groups with closely similar exchange rates. Moreover the chosen reaction involves the same partner (DMSO • • • H^+) for all the investigated hydroxy-groups, thus allowing reliable comparisons. (This is not the case in basic solution, where proton exchange arises from the deprotonation of the alcohol by alkoxide anions.)

Solutions of 26 monosaccharides were examined by ^1H n.m.r. at 250 MHz and 25 °C. All may exist as four isomeric species, the α- and β-furanoid and -pyranoid forms. However, only one isomer was usually observed. The solid carbohydrates used to prepare the DMSO solutions generally exist as one isomeric form only, and the rate of isomerization ($\alpha \rightleftharpoons \beta$) is usually slow in this solvent.[6] An exception is provided by D-mannose where both the α- and β-pyranoid isomers pre-exist in the solid compound. The interconversion between the furanoid and pyranoid forms is much faster [7] and the four possible isomers were observed in significant amounts only in the case of β-D-fructose and α-D-galactose. For this reason, the description and comparison of protonation rates will mainly concern the pyranoid forms of carbohydrates. The investigated compounds were chosen so that the α- or β-pyranoid isomers may assume one conformation only, denoted as *C*1 (or $^4C_1$) and 1*C* (or $^1C_4$) following the nomenclature of Reeves,[8] discarding unsubstituted D-altrose and D-talose. Other criteria for the selected carbohydrates were their availability and stereochemistry. The series of investigated compounds is in Table 1. The hydroxy-chemical shifts

TABLE 1

Nomenclature and conformation of the carbohydrates investigated

α-Anomers	β-Anomers
α-D-Glucose (1) ($^4C_1$)	β-D-glucose (2) ($^4C_1$)
Methyl α-D-glucopyranoside (3) ($^4C_1$)	methyl β-D-glucopyranoside (4) ($^4C_1$)
α-D-Mannose (5) ($^4C_1$)	β-D-mannose (6) ($^4C_1$)
Methyl α-D-mannopyranoside (7)	methyl β-D-mannopyranoside (8)
α-D-Galactopyranose (9) ($^4C_1$)	β-D-galactopyranose (10) ($^4C_1$)
Methyl α-D-galactopyranoside (11)	methyl β-D-galactopyranoside (12)
α-L-Fucose (13) ($^1C_4$)	
α-D-Fucose (14) ($^4C_1$)	
	β-D-allose (15) ($^4C_1$)
Methyl α-D-xylopyranoside (16) ($^4C_1$)	methyl β-D-xylopyranoside (17) ($^4C_1$)
Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (18) ($^4C_1$)	
Methyl 4,6-*O*-benzylidene-α-D-altropyranoside (19) ($^4C_1$)	
α-L-Sorbose (20) ($^1C_4$)	
α-D-Tagatose (21) ($^4C_1$)	
α-D-Fructopyranose (22) ($^4C_1$)	β-D-fructopyranose (23) ($^1C_4$)
α-D-Galactofuranose (24)	β-D-galactofuranose (25)
α-D-Fructofuranose (26)	β-D-fructofuranose (27)

and coupling constants of these compounds have been reported in a previous publication.[9]

EXPERIMENTAL AND RESULTS

Materials.—The investigated monosaccharides were from Sigma, except methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and -α-D-altropyranoside which were prepared according to procedures described previously.[10,11]

The solid compounds were dissolved in [$^2H_6$]dimethyl sulphoxide (99.8%; Commissariat à l'Energie Atomique, France). Chemical shifts and coupling constants were found to be independent of the concentration up to *ca.* 0.7 mol dm^{-3}, thus showing the absence of dimeric carbohydrates in DMSO.[12,13] A standard concentration of 0.5 mol dm^{-3} was used throughout these investigations.

N.M.R. Spectra.—Spectra are carried out at 250 MHz and 25 °C on a Cameca 250 spectrometer, operating in the continuous-wave mode, using tetramethylsilane as internal reference and the solvent signal as the heteronuclear deuterium lock signal. The hydroxy-protons give rise to separate lines at 250 MHz, except the non-anomeric hydroxy-groups of β-D-galactose, β-D-glucose, and α-D-tagatose. The concentration of β-D-mannose, which pre-exists in the solid compound, and α-D-fructose, resulting from the fast mutarotation of β-D-fructose, was too small for analysis, with the exception of the anomeric hydroxy-groups which stand apart from the rest of the spectrum. Most hydroxy-protons give rise to doublets because of vicinal coupling with a tertiary proton on the same carbon atom (an exception is the uncoupled anomeric proton of ketohexoses which is represented by a singlet). The observed doublets are easily assigned to the various hydroxy-groups through successive spin-decoupling operations beginning with the anomeric protons 1-OH and 1-H which appear at much lower field. [$^2H_6$]DMSO solutions are used for this purpose so that the non-hydroxylic lines are not obscured by the solvent lines. The four-line spectrum is obtained for 6-OH of aldopyranoses (or 1-OH of ketopyranoses) since the methylene protons of the CH_2OH group are diastereotopic [14] and have slightly different coupling constants with the hydroxy-proton.

Line-shape Measurements.—The n.m.r. rate constant $k_{n.m.r.}$, *i.e.* the reciprocal of the mean lifetime τ_{OH} of one hydroxy-proton, can be expressed by equation (3). As

$$k_{n.m.r.} = \tau_{OH}^{-1} = \tfrac{1}{2}k[\text{DMSO} \cdots \text{H}^+] \qquad (3)$$

$pK_{ROH_2^+}$ is *ca.* −4, [DMSO • • • H^+] ≫ [ROH_2^+], and therefore [DMSO • • • H^+] ≃ C_{H^+}, the analytical concentration of added trifluoromethanesulphonic acid CF_3SO_3H, equation (3) may be recast as (4).

$$k_{n.m.r.} = \tau_{OH}^{-1} = \tfrac{1}{2}kC_{H^+} \qquad (4)$$

Theoretical line-shapes were computed using the density-matrix formalism [15,16] and the EXCH14 program.[17] The rate constants $k_{n.m.r.}$ (or k) were adjusted by trial and error so as to obtain the best fit of the theoretical to the experimental curves. All calculations were performed using a Texas Instruments 980A minicomputer equipped with a Hewlett–Packard 7210A digital plotter.

(a) Doublets for hydroxy-groups coupled to tertiary vicinal protons in the fragment *x*-H–C–*x*-OH are brought to coalescence by small amounts (10^{-6}—10^{-5} mol dm^{-3}) of CF_3SO_3H. The doublets are not perfectly symmetrical and second-order analysis (AB type) was necessary to account for the observed line-shapes. Intermolecular transfer was assumed to take place between identical hydroxy-groups (which represent the A part of the spectrum). This procedure applies to the chemically non-exchanging *x*-H proton (the B part of the spectrum), since the exchange of the *x*-OH proton involves an additional partner, *i.e.* DMSO • • • H^+.

The small fractional population of the latter site, however, ensures the validity of the above approximation. This is made necessary since, in most cases, coalescence of the hydroxy-groups can be easily and accurately measured, only because the tertiary protons *x*-H are coupled together and give rise to complicated multiplets. The validity of this approximation was carefully checked in one case where the A and B parts of the spectrum can be simultaneously observed. This is so for 1-H of aldopyranoses which are clearly separated from those of 2-H, thus allowing 1-H to be spin-decoupled from 2-H. The exchange rates τ_{OH}^{-1} deduced from either the A or the B part of the spectrum were shown to be coincident within 2% up to coalescence. The method is not valid beyond the coalescence range as the coalesced line broadens again through further additions of CF_3SO_3H (see below). Proton transfer rates are not sensitive to the small amount (*ca.* 0.03 mol dm^{-3}) of residual water, an accompanying impurity. This was shown by adding up to 0.2 mol dm^{-3} water to the investigated solutions without observing any alteration of the coalesced spectrum.

(b) The two doublets of the methylene protons, 6-CH_2OH of aldopyranoses are not perfectly symmetrical and second-order analysis (AB_2 type) was again necessary. Using the same assumptions as above, computed spectra simulated the exchange of proton A between identical AB_2 molecules. The observation of the spectrum was again restricted to the A part.

(c) The singlets representing the anomeric 2-OH of ketopyranoses cannot be treated in this way. We used a method already described in the case of proton transfers to phenols.[3] Such transfers may, however, be studied because the 2-OH singlet is much broadened even for very low concentrations (*ca.* 10^{-5} mol dm^{-3} of added acid). This can be explained by the presence of a small amount of residual water (*ca.* 0.03 mol dm^{-3}) whose protons are averaged on the n.m.r. time scale with those of trifluoromethanesulphonic acid. The fractional population p of the exchanging site is therefore increased to a value $p = (2[H_2O] + C_{H^+})/[$carbohydrate$]$. Proton exchange is then followed by observing the line-broadening $\Delta\nu$ (Hz) of the unique, dominant 2-OH singlet. Rates of proton exchange were derived using relationship (5) due to Meiboom [18] (in which τ is replaced by $p\tau_{OH}$), where δ is the chemical shift (in rad s^{-1}) between exchanging sites.

$$\pi\Delta\nu = \delta^2p^2\tau_{OH}/(1 + \delta^2p^2\tau_{OH}^2) \quad (5)$$

Line-broadenings were found to be proportional to the concentration of acid C_{H^+}. This point is consistent with a slow proton transfer ($\delta^2p^2\tau_{OH}^2 \gg 1$) in which case equation (5) is reduced to (6). The n.m.r. rate constant τ_{OH}^{-1} was

$$\pi\Delta\nu = \tau_{OH}^{-1} = \tfrac{1}{2}kC_{H^+} \quad (6)$$

thus obtained from the linewidth of the 2-OH singlet

TABLE 2

Protonation rate constants (10^6 dm^3 mol^{-1} s^{-1}) of the hydroxy-groups of aldoses and ketoses in 0.5M-DMSO solution at 25 °C

	α-Anomers					β-Anomers				
Aldopyranoses	1-OH [a]	2-OH	3-OH	4-OH	6-OH	1-OH [a]	2-OH	3-OH	4-OH	6-OH
(1), (2)	1.81	7.74	6.87	5.13	7.17	2.88	*b*	*b*	*b*	7.51
(3), (4)		7.04	6.28	5.31	7.31		5.03	6.14	5.40	7.58
(5), (6)	1.04	4.07	7.50	3.78	5.95	2.47	*c*	*c*	*c*	*c*
(7), (8)		*d*	6.74	*d*	6.36	*c*	*c*	*c*	*c*	*c*
(9), (10)	1.93	*d*	*d*	*d*	6.95	2.92	*d*	*d*	*d*	*d*
(11), (12)		8.05	6.35	4.52	7.16		5.06	6.15	4.12	7.06
(13) + (14)	2.21	9.44	8.89	5.55		*c*	*c*	*c*	*c*	*c*
(15)	*c*	*c*	*c*	*c*	*c*	3.72	*d*	*d*	*d*	*d*
(16), (17)		7.42	8.28	9.28			5.25	8.20	9.29	
(18)		3.56	2.86				*c*	*c*		
(19)		5.00	1.30				*c*	*c*		
Ketopyranoses	1-OH	2-OH	3-OH	4-OH	5-OH	1-OH	2-OH	3-OH	4-OH	5-OH
(20)	10.4	2.44	11.3	6.74	12.2	*c*	*c*	*c*	*c*	*c*
(21)	*d*	0.92	*d,e*	*d,e*	*d,e*	*c*	*c*	*c*	*c*	*c*
(22), (23)	*c*	1.64	*c*	*c*	*c*	12.6	2.56	13.3	6.87	10.2
Furanoses	1-OH [a]					1-OH [a]				
(24), (25)	2.55	*d,e*	*d,e*	*d,e*	*d,e*	2.70	*d,e*	*d,e*	*d,e*	*d,e*
(26), (27)	2.26	*d,e*	*d,e*	*d,e*	*d,e*	2.90	*d,e*	*d,e*	*d,e*	*d,e*

[a] Anomeric hydroxy-group. [b] One doublet only is observed for 2-, 3-, and 4-OH. [c] In too small quantity at equilibrium, or not available. [d] Overlapping lines. [e] Unidentified hydroxy-groups.

measured before and after the addition of known quantities of acid.

Rate Measurements.—The validity of equation (1) was checked using two concentrations of β-D-glucose (0.5 and 0.25 mol dm^{-3}) and four concentrations of trifluoromethanesulphonic acid at 25 °C and observing the anomeric 1-OH doublet. A plot of τ_{OH}^{-1} against C_{H^+} is linear with a slope ($k/2$) of 1.44×10^6 dm^3 mol^{-1} s^{-1}. This slope does not depend on the concentration of β-D-glucose, in accord with equations (1) and (4), and also on the addition of a second monosaccharide to the β-D-glucose solution, confirming that proton transfers to the different hydroxy-groups do not influence each other in the acidity range used in these investigations. The latter property allowed us to improve the accuracy of rate comparisons by using a mixture of each monosaccharide (0.25 mol dm^{-3}) with an equivalent amount of β-D-glucose which played the role of a H^+ indicator. Four concentrations of trifluoromethanesulphonic acid were used for each compound. All the hydroxy-groups of one compound were examined in each experiment (Table 2), except in the case of overlapping lines (spin decoupling was used to disentangle some complicated spectra).

DISCUSSION

The Anomeric Hydroxy-groups.—The protonation of the anomeric hydroxy-group of aldopyranoses and ketopyranoses is clearly slower, by a factor of 3—4, than that of the other hydroxy-groups. This is in line with the higher acidity of the anomeric protons, or, conversely, the lower basicity of the anomeric hydroxy-groups, because of the inductive effect of the adjacent ring oxygen atom. The acidity to which we refer is related to the ionization equilibrium (7). This equilibrium repre-

$$ROH_2^+ + DMSO \overset{K_{ROH_2^+}}{\rightleftharpoons} ROH + DMSO \cdots H^+ \quad (7)$$

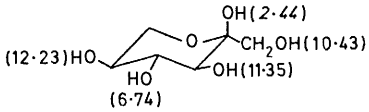
sents the balanced sequence of reactions (1) and (2), and therefore [3] $K_{ROH_2^+} = k_D[DMSO]/k = 7.03 \times 10^{10}/k$ if we assume that the deprotonation step is diffusion controlled. As $k \simeq 10^6$ dm^3 mol^{-1} s^{-1}, the $pK_{ROH_2^+}$ values are large and negative (pK_{ROH_2} *ca.* −4) and cannot be measured by potentiometry. In fact potentiometric measurements yield the ionization constant of the neutral hydroxy-group, $K_{ROH} = [RO^-][H^+]/[ROH]$. The pK_{ROH} variations along a series of alcohols or carbohydrates [9,19-22] do not necessarily reflect the variations of $pK_{ROH_2^+}$, although some parallelism may be observed between the two sets of values. In fact, some anomalies were noted in the parallelism between the pK_{ROH} values of the investigated monosaccharides and the chemical shift δ_{OH} of the anomeric hydroxy-groups,[9] because of the possibility of intramolecular hydrogen bonding in sugar anions.[20,23] Taking the chemical shift scale as a reliable acidity index, the anomeric hydroxy-groups can be compared to trichloroethanol [3] which has δ_{OH} 6.78, k 1.55×10^6 dm^3 mol^{-1} s^{-1}, and $pK_{ROH_2^+}$ −4.65. At the other end of the acidity range, the 6-hydroxymethyl groups are very similar to ethanol [3] which has δ_{OH} 4.36, k 6.26×10^6 dm^3 mol^{-1} s^{-1}, and $pK_{ROH_2^+}$ −4.05.

The order of increasing protonation rates k follows the order reported for the hydrolysis reaction (k_H) of the corresponding glycosides, *e.g.* for the methyl glycosides in 0.01 mol dm^{-3} hydrochloric acid at 95 °C,[24] as shown by the following sequences: $10^{-6}k$, 1.04 (α-D-mannose) < 1.81 (α-D-glucose) < 1.93 (α-D-galactose); $10^5 k_H$, 0.38 (α-D-mannoside) < 0.88 (α-D-glucoside) < 0.96 (α-D-galactoside).

The anomeric 2-OH of ketopyranoses are shifted by *ca.* 1 p.p.m. with respect to those of aldopyranoses.[9] We should therefore expect a higher basicity of ketopyranoses and consequently a faster protonation rate. This is indeed the case for the following pairs of compounds which differ only by the position of the hydroxymethyl substituent.

(i) α-L-*Sorbose* (20) (δ_{OH} 5.24) *compared with* α-D-*glucose* (1) (δ 6.18). The protonation rate of the anomeric hydroxy-groups of α-L-sorbose is 1.35 times faster than that of α-D-glucose.

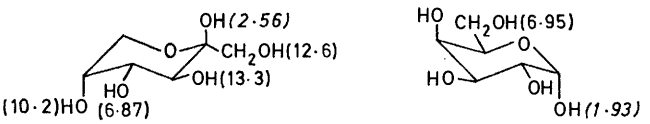
(ii) β-D-*Fructose* (23) (δ_{OH} 5.14) *compared with* α-D-*galactose* (10) (δ 6.10). Again the rate constant ratio is 1.33, in line with the high rate of hydrolysis of fructopyranosides,[1] although another factor of much greater importance, *i.e.* the structure of the anomeric carbon, should intervene in this comparison.



(20) (1)

Values in parentheses are 10^{-6} k

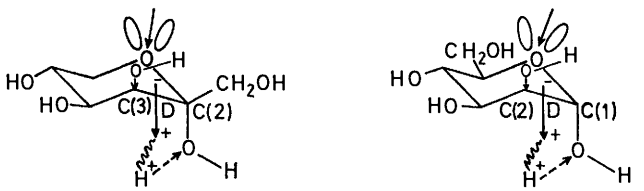
The upfield shift of the anomeric hydroxy-group of ketoses has been tentatively assigned to the steric strain of solvation brought about by the geminal hydroxymethyl group.[9] This partial desolvation makes the approach of the solvated proton easier. Another favourable factor is anchimeric assistance to the leaving proton through



(23) (10)

Values in parentheses are 10^{-6} k

hydrogen-bonding to (or protonation of) the geminal hydroxymethyl substituent. However other factors may play a major role in compounds such as α-D-tagatose (21) and α-D-mannose (5), a pair in which the protonation rate is slightly faster in the aldose than in the ketose. The main point is the relatively slow proton transfer rate for both compounds, each one being considered within its own class, *i.e.* among the set of aldoses or ketoses, respectively (Table 2). This anomaly should then proceed from a structural feature common to both compounds, *i.e.* the presence of an axial anomeric hydroxy-group vicinal to an axial hydroxy-group. Moreover the ano-



(21) (5)

meric O–H bond is parallel to the adjacent ring C–C bond (as shown by the presence of a long-range coupling constant) and is therefore directed outside the pyanose ring.[9] The dipoles of the oxygen ring atom and of the adjacent axial C–OH bond are then roughly parallel and yield a reinforced resultant dipole D pointing to the anomeric oxygen atom, thus repelling the incoming proton. The presence of a large dipole around the C(2)–OH bond has also been invoked to account for the higher acidity of the α-anomer compared with that of the β-anomer of methyl D-mannopyranoside [25] (see later). The anomaly

disappears in the β-isomer of D-mannose as the anomeric OH bond has moved away from the dipole area.

The protonation of the anomeric hydroxy-groups of furanoses is slightly faster than that of the corresponding pyranoses (except for β-D-galactose), in line with the greater ease of hydrolysis of furanosides,[1] although many other factors, such as the steric strain of the five-membered ring, are of greater importance to account for the rates of hydrolysis.

The Effect of Substitution.—Four types of substitution are present in the carbohydrates investigated.

(a) The *O*-methylation of the anomeric hydroxy-groups of aldopyranoses is accompanied by a small decrease of the protonation rate of the adjacent 2-OH (by a factor of 1.1 in α-D-glucose compared to methyl α-D-glucoside) and of the hydroxy-group one bond further away (3-OH) (again by a factor of 1.1). Inductive effects account only for a part of the 2-OH rate decrease, by a factor of *ca.* 1.04, if we use the Taft–Ingold σ* values [26] of 0.64 and 0.56 for CH_3OCH_2 and CH_2OH (taken to simulate approximately the situation in methyl glucoside and glucose, respectively) and the slope of the Taft–Ingold relationship ρ* of −0.227 (as deduced from a previous study of aliphatic alcohols [3]). Other factors should be important to account for the 3-OH rate decrease. The steric hindrance of 1-OH is slightly increased by methylation, and this factor could add to inductive effects to decrease proton transfer to the 2-OH. Neither of these two effects can, however, account for the rate decrease of 3-OH where another factor, as yet unknown, is probably important.

(b) Substituting a hydroxymethyl group by hydrogen has a large positive influence upon neighbouring hydroxy-groups lying up to three bonds away. This is shown by the methyl α- and β-D-xylopyranosides compared to the methyl α- and β-D-glucosides, respectively. The 4-, 3-, and 2-OH protonation rates are multiplied by *ca.* 1.7, 1.25, and 1.05, respectively. The rate enhancements should be assigned to a large steric effect, since the σ* values of the two substituents are close together (0.49 and 0.56). The size difference between the two substituents is still enhanced by the solvation of the hydroxymethyl group.

(c) Substituting a 6-hydroxymethyl group by methyl (in α-D-fucose) has the expected effect on the adjacent 4-OH from the σ* values (0.59 and 0, respectively). Again, significant rate increases are observed for 3- and 2-OH, which can be traced to the smaller steric hindrance of the poorly solvated methyl group.

(d) Substituting oxygens in both 4- and 6-positions of methyl α-D-glucoside by a benzylidene substituent results in an important decrease of the protonation rate of 3- and 2-OH, by a factor of 2.20 and 1.98, respectively, as a result of the steric hindrance brought about by the presence of a second ring fused to the glucoside.

Comparison of α- and β-Anomers.—(a) The protonation rates $k^{\beta}_{1\text{-OH}}$ of β-aldopyranoses are systematically larger than those ($k^{\alpha}_{1\text{-OH}}$) of the corresponding α-anomers. The ratio $r_{\alpha\beta} = k^{\beta}_{1\text{-OH}}/k^{\alpha}_{1\text{-OH}}$ is 1.59 and 1.51 in D-glucose and D-galactose, respectively. These values are close to that (1.43) found in an analogous comparison of isomeric cyclohexanols [4] which differ only by the position, equatorial or axial, of the hydroxy-group ($k_{\text{equatorial}}/k_{\text{axial}}$ 1.43). This clearly shows that the difference of reactivity between the α- and β-anomers arises from the steric hindrance between the axial hydroxy-group of the α-anomer and the tertiary protons 3- and 5-H. The total interaction may be estimated as *ca.* 0.9 kcal mol^{-1}, assuming an incremental free energy difference of *ca.* 0.45 kcal mol^{-1} for each 1,3 or 1,5 diaxial H–OH interaction.[7] The ratio $r_{\alpha\beta}$ is higher still for D-mannose ($r_{\alpha\beta}$ 2.38) because of the anomalous value of $k^{\alpha}_{1\text{-OH}}$, as discussed above. These results are clearly parallel to those obtained for the hydrolysis of methyl D-glucopyranoside [27] ($r_{\alpha\beta}$ 1.77) and methyl D-mannopyranoside [28] ($r_{\alpha\beta}$ 2.40). They are also in agreement with the greater acidity of the neutral molecule (K_{ROH} values) which is observed for the β- with respect to the α-anomer in aqueous solution.[25,29,30] The position of the anomeric hydroxy-group still influences the protonation rate of the adjacent 2-OH bond of aldopyranoses: $r_{\alpha\beta}$ is 1.40 and 1.41 for the pair of anomeric methyl D-glucopyranosides and methyl D-xylopyranosides, respectively. This may result from steric hindrance to the approach of the solvated proton which is presumably more important when the two vicinal 1-OCH_3 and 2-OH substituents are *gauche,trans* than when they are *gauche,cis*. A piece of evidence supporting this assumption is the magnitude of the mutual interaction between the vicinal hydroxy and methyl substituents in pairs of isomeric cyclohexanols, expressed as free energy increments: [31] $-\Delta G^{\ominus}$ 0.38 and 0.66 kcal mol^{-1} in the following configurations of 2-methylcyclohexanol, CH_3(eq)OH(eq) and CH_3(eq)OH-(ax), respectively.

(b) A comparison of α- and β-ketopyranoses can be carried out in one case only, *i.e.* for D-fructose. The β-anomer has the $^4C_1$ configuration and an *axial* anomeric 2-OH (Table 1). This results unambiguously from the existence of long-range couplings through a zig-zag arrangement of four bonds H–O–C–O–H between 2-OH and 3-H on the one hand, and 5-OH and 6-H on the other.[9] The configuration of the α-anomer is rather uncertain, as the total steric strains of conformations $^4C_1$ and $^1C_4$, estimated by using the appropriate free energy increments, are not clearly different from each other. Our kinetic data may help to solve this problem. The local steric interactions around the anomeric hydroxy-group amount to 2.35 and 0.35 kcal mol^{-1} in conformations $^1C_4$ and $^4C_1$, respectively (using $-\Delta G^{\ominus}$ 1.9, 0.35, 0.0, and 0.45 for two diaxial 1- and 3-OH, two equatorial or axial vicinal hydroxy-groups, and the axial 1-OH and 5-H, respectively [7]). These values are clearly larger or smaller, respectively, than that computed for the β-anomer (conformation $^1C_4$). The smaller protonation rate obtained for the α-anomer (with respect to the β-anomer) is therefore consistent with the conformation $^4C_1$ of the α-anomer and the axial anomeric 2-OH.

(c) The protonation rates of the α- and β-anomers of

D-galactofuranose and D-fructofuranose are not very different from each other ($r_{\alpha\beta}$ 1.06 and 1.28, respectively). This is also the case for the hydroxy-group chemical shifts (δ 6.22 and 6.14 and 5.60 and 5.33, respectively). Presumably the steric strain of the five-membered ring becomes the major structural factor and plays the same role in the α- and β-anomers.

Comparison of Equatorial and Axial Non-anomeric Hydroxy-groups.—An interesting point is to examine again the rate variation $r_{ax,eq}$ induced by changing the observed hydroxy-group from an equatorial to an axial position. Comparisons of appropriate pairs of aldohexoses or ketohexoses, in which all the substituents are left unchanged except the observed hydroxy-group, lead to conclusions in good agreement with those already drawn with anomeric hydroxy-groups. This is shown by consideration of the following pairs: (i) 4-OH of methyl α-D-glucoside and methyl α-D-galactoside, $r_{ax,eq}$ 1.17; (ii) 5-OH of α-L-sorbose and β-D-fructose, $r_{ax,eq}$ 1.20; (iii) 2-OH of α-D-mannopyranose and α-D-glucose, $r_{ax,eq}$ 1.90.

These ratios are smaller than those obtained with pairs of anomeric axial and equatorial hydroxy-groups. This decrease may be assigned to the presence in each pair of one diaxial interaction only of the axial hydroxy-group with a tertiary axial proton (instead of two, as previously, because of the presence of the ring oxygen in the appropriate position). An opposite situation would be the presence of a large axial substituent, *e.g.* a hydroxy- or a methoxy-group. In fact, this could be observed in one case, namely for 3-OH of methyl 4,6-*O*-benzylidene-α-D-glucoside compared with methyl 4,6-*O*-benzylidene-α-D-altroside (ignoring the influence of the 2-OH bond which is simultaneously changed from an axial to an equatorial position), $r_{ax,eq} = 2.20$.

Influence of Neighbouring Axial or Equatorial Hydroxy-groups.—The influence of the axial or equatorial position of an anomeric hydroxy-group upon the protonation of its neighbours has been examined above. This influence is limited to the vicinal 2-OH of aldopyranoses. Conversely, the protonation rate of an anomeric hydroxy is sensitive to the equatorial or axial nature of a substituent S attached to an α- or β-carbon. This is demonstrated by considering the two pairs (i) β-D-glucose and β-D-mannose, S = 2-OH, $r_{ax,eq} = K(\text{glucose})/k\text{-mannose}) = 1.16$ and (ii) β-D-glucose and β-D-allose, S = 3-OH, $r_{ax,eq} = 0.77$. This influence becomes negligible for γ-substituted carbons, as in α-L-sorbose compared with β-D-fructose (S = 5-OH, $r_{ax,eq} = 0.95$) or in β-D-glucose compared to β-D-galactose (S = 4-OH, $r_{ax,eq} = 0.98$).

The protonation rate of non-anomeric hydroxy-groups is not clearly sensitive to the equatorial or axial position of a substituent S, except if S is an anomeric hydroxy-group (see the above example of the 2-OH bond of methyl α- and β-D-glucopyranosides, $r_{ax,eq} = 0.71$, and of methyl α- and β-D-xylopyranosides, $r_{ax,eq} = 0.70$). This is shown by considering the following pairs: (i) the 3-OH protonation of α-D-glucoside and α-D-galactoside (S = 4-OH, $r_{ax,eq} = 0.99$), of α-D-glucose and α-D-mannose (S = 2-OH, $r_{ax,eq} = 0.92$) or their *O*-methyl derivatives ($r_{ax,eq} = 0.93$) and (ii) the 4-OH protonation of α-L-sorbose and β-D-fructose (S = 5-OH, $r_{ax,eq} = 0.98$).

The exceptional role of neighbouring anomeric hydroxy-groups should be traced to the so called anomeric effect,[32] *i.e.* the overlap of the heterocyclic orbitals with those of the axial 1-OH oxygen in the α-anomer. The 2-OH proton is more acidic in the β-anomer,[23,25] in agreement with a downfield shift in their n.m.r. spectrum [9] and a smaller protonation rate.

In conclusion, proton transfer rates are a good basis to estimate the relative bascities of the various hydroxy-groups of carbohydrates. The n.m.r. method allows simple and reliable comparisons, even between closely similar groups. These rates offer, as well as the chemical shifts and coupling constants of the static hydroxy spectra, interesting parallelisms with the inductive and steric effects of neighbouring substituents, and can constitute a good index of reactivity to characterize the first step of acid-catalysed reactions of carbohydrates.

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