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IONISATION BEHAVIOUR OF LACTOSE AND LACTULOSE IN WATER AS STUDIED BY ^{13}C-N.M.R. SPECTROSCOPY

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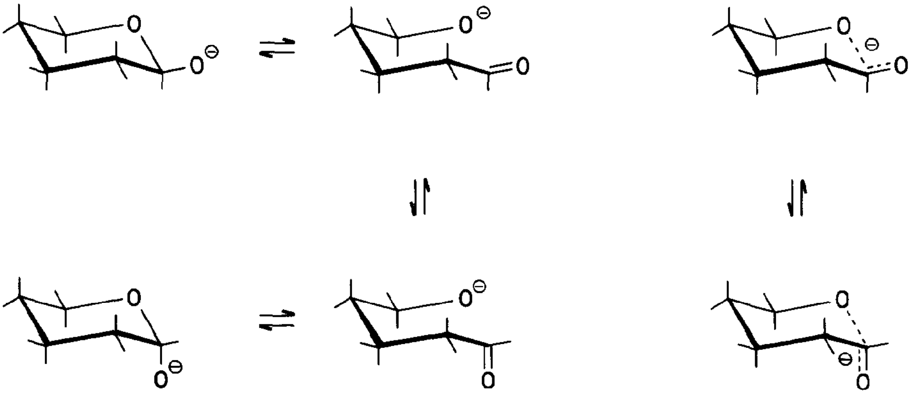
ABSTRACT

From ^{13}C-n.m.r. titration measurements on aqueous solutions of the disaccharides lactose (M) and lactulose (1.1M) at 3–5°, pK_a values for α-lactopyranose (13.5), β-lactopyranose (13.8), and β-lactulopyranose (13.9) have been derived. These values are close to those for D-glucose and D-fructose, respectively, *i.e.*, the attached D-galactosyl group has little influence on the ionisation process. The ionised species are considered to occur either as cyclic and pseudo-cyclic structures in rapid equilibrium or as non-classical anions. This picture is consistent with that for monosaccharides.

INTRODUCTION

^{13}C-N.m.r. titration of aqueous solutions of D-glucose, D-mannose, and D-fructose with potassium hydroxide allowed the ionisation behaviour of the different anomeric forms of these monosaccharides to be described[1,2]. It appeared that the



Scheme 1. Pyranose sugar anions.

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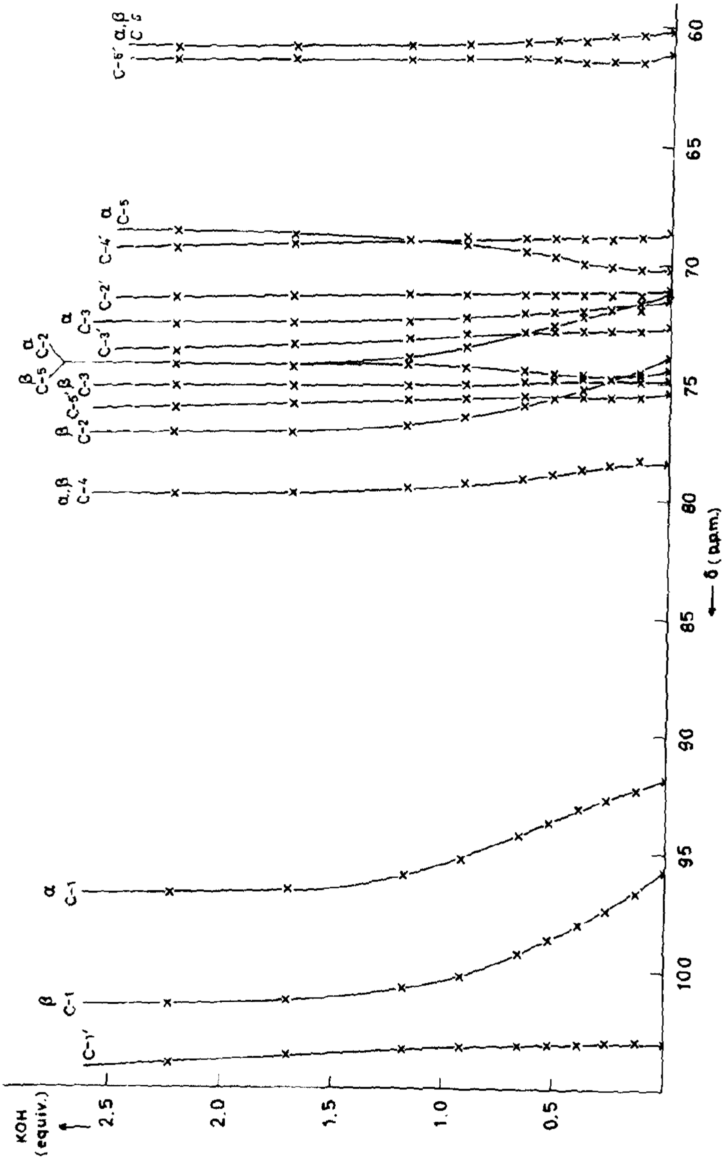


Fig. 1. ^{13}C-N.m.r. chemical shifts for α- and β-lactopyranose as a function of KOH added to aqueous M lactose at 3–5°.

monosaccharide anions occur either as cyclic and pseudo-cyclic ionised species in rapid equilibrium, or as non-classical anions. This ionisation picture is shown in Scheme 1 for the pyranose ring form.

In extending the above-mentioned approach, we now present ^{13}C-n.m.r. results for lactose and lactulose, in order to verify if such an ionisation picture also holds for disaccharides. Furthermore, ionisation of lactose is of importance as the

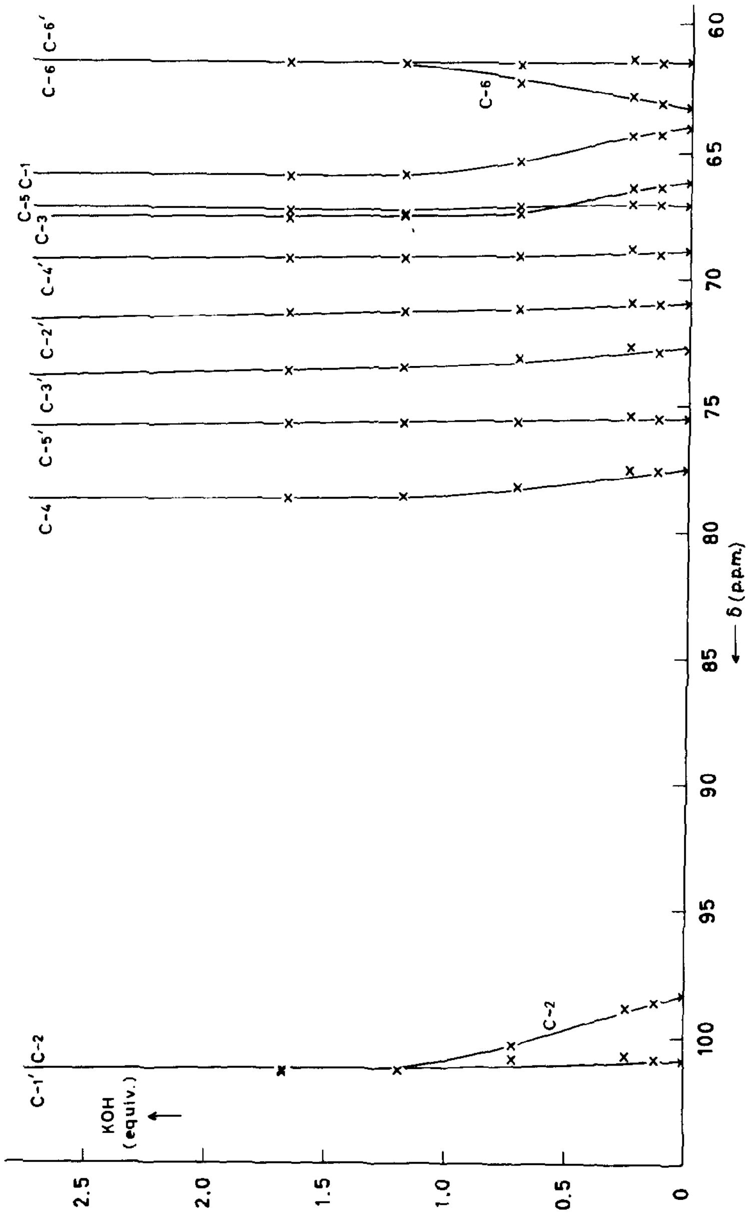


Fig. 2. ^{13}C-N.m.r. chemical shifts for β-lactulopyranose as a function of KOH added to aqueous 1.1M lactulose at 3–5°.

first step in its isomerisation reaction in aqueous alkaline medium, which is the basis of an industrial process for producing lactulose[3]. In this respect, knowledge of the ionisation behaviour of these sugars at practical concentrations (~M), for which ^{13}C-n.m.r. spectroscopy is quite suitable as an analytical tool, would be of value.

EXPERIMENTAL

Samples of lactose and lactulose were provided by Duphar B.V., Weesp, The Netherlands.

^{13}C-N.m.r. spectra (25.2 MHz) were obtained for aqueous solutions at 3–5° with a Varian XL-100-15 spectrometer and, in general, a spectral width of 2500 Hz. The n.m.r. tube was fitted with an inner tube filled with D_2O and 1,4-dioxane as the internal standard.

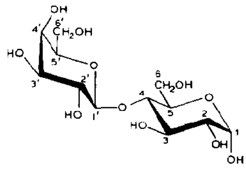
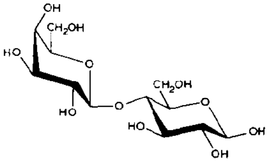
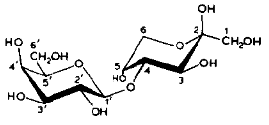
RESULTS

The ^{13}C-n.m.r. titration curves were measured at 3–5°, in order to prevent undesired, alkali-catalysed isomerisation and degradation. The results for the α- and β-pyranose forms of lactose and for the β-pyranose form of lactulose are given in Figs. 1 and 2, respectively. It should be noted that the α-lactulopyranose form could not be detected, and that the various signals for α- and β-lactulofuranose could not be measured with sufficient accuracy.

The ^{13}C-n.m.r. chemical shift data for neutral lactose and lactulose (δ_0) and

TABLE I

^{13}C CHEMICAL SHIFTS (δ_0) AND THE EFFECT THEREON ($\Delta\delta$) OF IONISATION FOR M LACTOSE AND 1.1 M LACTULOSE IN WATER AT 3–5°[a]

Compound	 *α-Lactopyranose*		 *β-Lactopyranose*		 *β-Lactulopyranose*	
Carbon atom	δ_0	$\Delta\delta$	δ_0	$\Delta\delta$	δ_0	$\Delta\delta$
1	91.9	5.1	95.8	5.7	64.1	2.0
2	71.2	3.3	73.9	3.5	98.3	3.2
3	71.5	1.2	74.9	0.4	66.2	1.5
4	78.4	1.6	78.4	1.6	77.5	1.4
5	70.2	−1.3	74.5	−0.1	67.1	0.2
6	60.2	1.1	60.2	1.1	63.2	−1.5
1′	103.0	1.1	103.0	1.1	100.9	0.5
2′	71.1	0.7	71.1	0.7	71.0	0.6
3′	72.6	1.5	72.6	1.5	72.8	1.2
4′	68.6	1.1	68.6	1.1	68.9	0.5
5′	75.4	1.0	75.4	1.0	75.5	0.4
6′	61.1	0.6	61.1	0.6	61.5	0.2

[a] Positive values denote shifts to low field (p.p.m.).

the shift to low field upon complete ionisation ($\Delta\delta$) of the anomeric hydroxyl group are summarised in Table I. Ionisation is considered to be essentially complete at >2M KOH. Assignments are based on literature data[4,5] and on comparison of the data for lactose, lactulose, D-glucose, and D-fructose[2].

DISCUSSION

The observed ^{13}C shifts upon ionisation are consistent with the postulated ionisation picture for monosaccharides[1,2]. Firstly, the signals for most of the carbon atoms, including those of the D-galactopyranosyl group, are shifted, which is due, at least in part, to a change in hydration on going from the neutral sugar to the sugar anion. Secondly, the (extra) downfield shifts of the signals for the anomeric carbon atom and the adjacent carbon atom(s) can be understood in terms of enhanced C=O bond character in the anionic species, whereas the small upfield shifts for C-5 (C-6 for **3**) are due to the partial negative charge on the ring oxygen as well as the diamagnetic anisotropy of the (partial) carbonyl group.

The titration curves obtained may be described by the following equation:

$$\frac{[L^-]}{[L]} = \frac{(\delta - \delta_o)}{(\delta_i - \delta)} = K[HO^-], \qquad (1)$$

in which L^- and L denote the ionised and neutral species of lactopyranose or lactulopyranose, respectively, δ is the actual chemical shift at the hydroxyl concentration $[HO^-]$, δ_o and δ_i are the shifts of L and L^-, respectively, and K is the dissociation constant of the equilibrium

$$L + HO^- \rightleftharpoons L^- + H_2O.$$

With the use of $K_w = 1.5 \times 10^{-15}$ at 3°, we obtain

$$K_a = \frac{[L^-]\,1.5 \times 10^{-15}}{[L]\,[HO^-]} \qquad (2)$$

as the apparent concentration ionisation constant. Here, $[HO^-]$ represents the true concentration derived from

$$[HO^-] = [K^+] - [L^-] \qquad (3)$$

The apparent pK_a values thus derived are important for the description of the ionisation behaviour under practical alkaline conditions at high concentrations (~M). In this respect, it may be noted that the thermodynamic pK_a values at infinite dilution will be ~1.3 log units lower than the apparent pK_a values, as indicated earlier for D-glucose[2].

During the ^{13}C-n.m.r. titration, it was found that the anomeric composition varied (see below). Direct calculation of the ionisation constants for each anomer from the titration curves by equations *2* and *3* is therefore not possible. However, the use of $\Sigma\Delta\delta$ for all detectable anomeric carbons allows determination of the overall pK_a value for the sugar. The overall pK_a of lactose (M in water at 3–5°) thus obtained (13.6) was slightly less than that of D-glucose (13.9; 1.1M in water at 3–5°). For lactulose, it was assumed that the different anomeric forms have comparable acid strengths. In this way, the overall pK_a value, as derived from the titration curve of β-lactulopyranose (1.1M in water at 3–5°), was found to be 13.9, which is slightly less than that of D-fructose (14.2; 1.1M in water at 3–5°). Apparently, the D-galactopyranosyl group has a minor effect (0.3pK unit) on the ionisation of the D-glucose and the D-fructose residues.

The shift of the anomeric equilibrium on ionisation will be dealt with in more detail for lactose. Lactose is solely present as its α- and β-pyranose forms, so that the ionisation may be completely described by the following set of equilibria:

$$\begin{tikzcd}
\beta L + HO^- \arrow[r, leftrightharpoons, "K_\beta"] \arrow[d, leftrightarrows, "K^{o}"] & \beta L^- + H_2O \arrow[d, leftrightarrows, "K^-"] \\
\alpha L + HO^- \arrow[r, leftrightharpoons, "K_\alpha"] & \alpha L^- + H_2O
\end{tikzcd}$$

Application of equation *1* to α- and β-lactopyranose yields equation *4*.

$$\frac{[(\delta - \delta_o)/(\delta_i - \delta)]_\alpha}{[(\delta - \delta_o)/(\delta_i - \delta)]_\beta} = \frac{[\alpha L^-]\,[\beta L]}{[\beta L^-]\,[\alpha L]} = \frac{K^{o}}{K^-} = \frac{K_\alpha}{K_\beta} \qquad (4)$$

Indeed, a plot of $[(\delta - \delta_o)/(\delta_i - \delta)]_\alpha$ against $[(\delta - \delta_o)/(\delta_i - \delta)]_\beta$ shows a linear relationship with a slope $K^{o}/K^- = 0.54$ (Fig. 3). This ratio implies that the overall β/α ratio should increase on ionisation of lactose. This could be confirmed from the β/α ratio determined from the integrals of the signals for the α- and β-anomeric carbon atoms (Fig. 4). Using $K^{o} = 2.0$, as determined separately for neutral lactose by both ^{13}C- and ^{1}H-n.m.r. spectroscopy, the values $K^- = 3.7$ and $\Delta pK_a = pK_a^\alpha - pK_a^\beta = -\log K^{o}/K^- = 0.27$ are obtained. The latter value is in good agreement with ΔpK_a obtained from Fig. 4, *i.e.*, $\Delta pK_a = \Delta \log (\beta/\alpha) = 0.28$, and appears to be close to that for D-glucose[2] (0.30; 1.1M in water at 3–5°). Consequently, the D-galactopyranosyl group has a negligible influence on the degree of shift of the anomeric equilibrium on ionisation. The $\alpha \rightarrow \beta$ shift on ionisation shows that either (*i*) an equatorial–equatorial arrangement of the anomeric hydroxyl group and the vicinal hydroxyl group is more favourable for ionisation than the axial–equatorial arrangement, or (*ii*) the anomeric effect is smaller in the ionised species because the electronegativity of C-O$^-$ is lower than that of C-OH. The $\alpha \rightarrow \beta$ shift is consistent with the results obtained for monosaccharides[2].

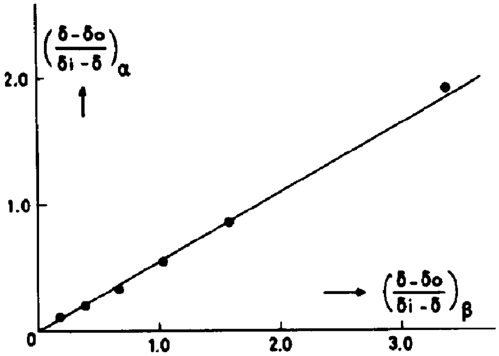


Fig. 3. Ionisation of α-lactopyranose *versus* the ionisation of β-lactopyranose, according to equation *2*; M lactose in water at 3–5°.

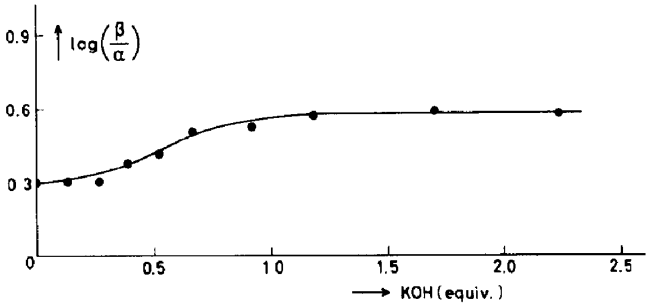


Fig. 4. Pyranose log (β/α) ratio for M lactose in water at 3–5° as a function of KOH added.

From the equivalence points for both α- and β-lactopyranose, the total amount of KOH added at these points, and the anomeric equilibrium constants $K^0 = 2.0$ and $K^- = 3.7$, the dissociation constants for the individual anomeric forms were calculated to be $K_\alpha \approx 10$ and $K_\beta \approx 20$. These data correspond with pK_a values of 13.8 ±0.1 and 13.5 ±0.1 for α- and β-lactopyranose, respectively. It should be mentioned, however, that the difference in pK_a values for these anomeric forms as calculated from Figs. 3 and 4 is much more accurate than that obtained from the individual titration curves.

In conclusion, the results show that ^{13}C-n.m.r. spectroscopy is a valuable analytical tool for the study of the ionisation behaviour of sugars at concentrations of practical significance. Information on both the degree of ionisation and the structure of the ionised species can thus be obtained.

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