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Note

Structure of "3-ketolactose" [4-*O*-(β-D-*xylo*-hexopyranosyl-3-ulose)-D-glucopyranose] by ^{1}H- and ^{13}C-n.m.r. spectroscopy

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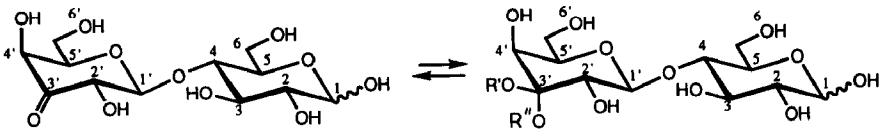
After the oxidation of lactose by bacteria of the genus Agrobacterium[1–4], catalysed by the enzyme hexopyranoside cytochrome c oxidoreductase[5], "3-ketolactose" [4-*O*-(β-D-*xylo*-hexopyranosyl-3-ulose)-D-glucopyranose, **1**] was isolated from the culture medium and crystallised from methanol.

The ^{1}H-n.m.r. spectrum of **1** contained one peak at the methanol frequency that integrated for ~ 6 protons and which could have arisen from a keto form (**1a**) or hydrate (**1b**) with two molecules of crystal methanol, a hemiacetal (**1c** or **1d**) with one molecule of crystal methanol, or a dimethyl acetal (**1e**). For a hemiacetal, the OMe group could be axial (**1c**) or equatorial (**1d**).



1a (keto form)

1b R' = R" = H (hydrate)
1c R' = H, R" = Me (hemiacetal)
1d R' = Me, R" = H (hemiacetal)
1e R' = R" = Me (acetal)

On the basis of a ^{1}H-n.m.r. study[6] of a solution of **1** in Me_2SO-d_6 and the differences expected for the resonance of H-1′ in the keto and the hydrated forms, using the McConnell equation, the occurrence of the hydrated form **1b** was proposed. However, Danneels[7] found that the McConnell equation is not always strictly valid. Therefore, a new study[8] using chemical shift increments, as known at that time, was undertaken and the results indicated the absence of the keto form. This finding was confirmed by polarography of a solution of **1** in 0.1 M lithium chloride which showed that only 1% of **1** was present as the keto form[9].

Larm *et al.*[10] concluded from a study of the ^{13}C-n.m.r. spectra of derivatives of

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methyl glucopyranosides that the 3-oxo derivatives existed in the keto forms and the 2-oxo derivatives existed as hydrates, but no explanation was given. These findings appear to contradict those for **1**. Consequently, **1** has been reinvestigated using more advanced n.m.r. techniques.

The original conclusions on **1** from the ^{1}H-n.m.r. data involved an indirect approach based on differences in chemical shifts, whereas ^{13}C-n.m.r. data provide direct information. Thus, a carbonyl carbon resonates at $\delta \sim 208$, whereas that for a hydrate or hemiacetal resonates at $\delta \sim 92$. The ^{13}C-n.m.r. data for **1** are given in Table I. Although ^{13}C, ^{1}H heteronuclear correlated experiments suffer from second-order effects[11], knowledge of the ^{1}H resonances made the assignment of the ^{13}C resonances straightforward. The presence of a resonance at δ 92.66 and the absence for one at $\delta \sim 208$ confirmed the previous conclusions[7,9] that the keto form was largely absent.

In seeking to determine whether **1** exists as a hydrate (**1b**), hemiacetal (**1c** or **1d**), or a dimethyl acetal (**1e**), the ^{1}H-n.m.r. spectrum at 500 MHz for a solution of **1** in D_2O was obtained and the data are given in Table II. The new assignments were obtained from a COSY experiment and were used to assign the ^{13}C resonances in a ^{13}C, ^{1}H heteronuclear correlated experiment.

The ^{1}H-n.m.r. chemical shift data for the oxoglycosyl moiety of **1** can be explained on the basis of chemical-shift increments caused by disubstitution and the additivity concept[12].

Comparison of the $\Delta\delta$ values for the resonances of H-1 and H-4 for 2-deoxy-β-D-*lyxo*-hexopyranose[13] and β-D-galactopyranose[14] on the one hand, and 2-deoxy-β-D-*arabino*-hexopyranose, β-D-glucopyranose[14], and β-D-mannopyranose[14] on the other, indicates the following increments for the substitution of a proton by a hydroxyl group on C-2: β-effects (p.p.m.) for H-1, syn ~ −0.25, anti ~ −0.05; γ effects (p.p.m.) for H-4, γOH (aa)H ~ +0.15, γOH (ag)H ~ +0.07, γOH (g^+g^-)H ~ +0.30 (ref. 13).

The δ effects are known[15] to be ~ −0.04 p.p.m.

When the chemical shifts of the ^{1}H resonances of lactose[8] are taken as references and accepting that the conformations about the glycosidic bonds in lactose and **1** are similar, then, on the addition of an axial OH group at C-3 in **1**, the chemical shift for the

TABLE I

^{13}C-N.m.r. chemical shift data for a solution of "3-ketolactose" (**1**) in D_2O

Sugar moiety	*C-1'*	*C-2'*	*C-3'*	*C-4'*	*C-5'*	*C-6'*	*OCH_3*
β-D-*xylo*-Hexopyranosyl-3-ulose	103.79	72.56	92.66	72.42	75.63	61.92	49.74
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	
α-D-Glucopyranose[a]	95.28	72.00	72.29	79.62	70.00	61.45	
β-D-Glucopyranose[a]	96.60	74.66	74.98	79.49	75.63	61.61	

[a] α,β-Ratio ~ 1:2 based on integrated intensities.

TABLE II

^{1}H-N.m.r. data for a solution of "3-ketolactose" (**1**) in D_2O

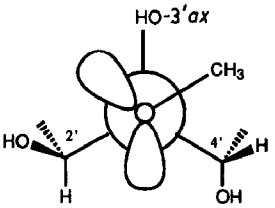
Chemical shifts (p.p.m.)							
β-D-*xylo*-Hexopyranosyl-3-ulose moiety							
H-1′	H-2′	H-3′	H-4′	H-5′	H-6′A	H-6′B	OMe (acetal)
4.57	3.61	–	3.66	3.99	3.78	3.78	3.37
α-D-Glucopyranose moiety							
H-1	H-2	H-3	H-4	H-5	H-6A	H-6B	
5.25	3.60	3.85	3.67	3.96	3.90	3.90	
β-D-Glucopyranose moiety							
H-1	H-2	H-3	H-4	H-5	H-6A	H-6B	
4.68	3.31	3.67	3.67	3.64	3.97	3.84	

H-5′ resonance is expected at δ 4.00 (δ 3.74 + 0.30 − 0.04; found, δ 3.99) and for that of H-1′ at δ 4.68 (δ 4.42 + 0.30 − 0.04; found, δ 4.57). The methyl group has only a minor δ effect on the resonances of H-1′ and H-5′.

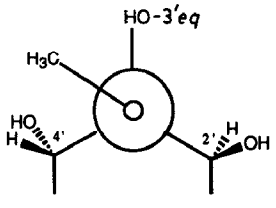
In contradiction to the previous findings (at 300 MHz using homo-INDOR), the chemical shift of the H-4′ resonance was found to be δ 3.66 not δ 3.98.

The chemical shift for the H-4′ resonance at δ 3.66 is not surprising, although for D-galactopyranose (or D-galactopyranosyl units) it is usually found[8] at δ 3.95. The introduction of an axial hydroxyl group at C-3 of β-D-galactopyranose would cause a β effect of −0.25 p.p.m. on the resonance of the equatorial H-4 and ~ −0.05 p.p.m. on that of axial H-2.

The γ effects[15] allow a distinction to be made between the hydrate (**1b**), the hemiacetal (**1c** and **1d**), and the dimethyl acetal (**1d**). If the MeO group is equatorial, only one rotamer (**2a**) need be considered, which causes a downfield shift [γCH$_3$ (g$^+$g$^-$)H] of +0.20 for the resonance of H-2′ and almost no effect [γCH$_3$(ag)H] for H-4′. These data agree qualitatively with the experimental data (δ 3.56 for H-2′ and δ 3.94 for H-4′ in lactose; δ 3.61 for H-2′ and δ 3.66 for H-4′ in **1**). If the methyl group is axial, only conformer **2b** need be considered. The methyl group would cause a downfield shift [γCH$_3$(g$^-$g$^+$)H] of +0.20 p.p.m. for H-4′ and have no effect on H-2′ [γCH$_3$(aa)H]. The latter possibility does not agree with the experimental data, which also do not accord with the hydrate **1b** and the dimethyl acetal **1e**. Thus, it is concluded that **1** exists as a hemiacetal (**1d**) with the MeO group equatorial.



2a



2b

The experimental value of the resonance for H-2′ is ~ 0.10 p.p.m. smaller than that calculated, which accords with the suggestion of Danneels[12] that such a deviation will be found when the proton is syn-axial to an electron lobe of the oxygen (see **2a**).

The structure **1d** proposed for **1** was confirmed by a 2D NOESY experiment. When the MeO group is equatorial, n.O.e. effects are to be expected on the resonances of H-2′ and H-4′; when it is axial, n.O.e. effects are to be expected on the resonances of H-1′, H-4′, and H-5′. The first situation was found in practice and confirmed the earlier conclusions based on chemical-shift increments; the assignment[8] based on the chemical shifts of the methyl resonances was rejected.

The occurrence of a hemiacetal should also be reflected in the ^{13}C-n.m.r. chemical shift data. Although increments for dialkyl substitution have been proposed[16], to the best of our knowledge, this is not true for dihydrates, hemiacetals, or acetals.

Considering **1** and the model compounds studied by Thiem and his co-workers[10], it can now be postulated that a hexopyranosidulose in the $^1C_4$ conformation mainly (or exclusively) occurs in the keto form when both the neighbouring substituents are equatorial. For hexopyranosid-4-uloses, an equilibrium of 65% keto/35% hydrate was found[10]. When one of the neighbouring substituents is axial, the hydrate is formed. For mixtures of $^4C_1$ and $^1C_4$ conformers, this postulate does not hold[20].

EXPERIMENTAL

"3-Ketolactose" (**1**) was a gift of Dr. M. J. Bernaerts. Its preparation, purification, and physical properties are fully described[1–5].

The ^{1}H-n.m.r. spectrum at 20° was obtained with a Bruker AM500 spectrometer operating at 500.12 MHz, using a pulse angle of 19° and a resolution of 0.33 Hz/point. The ^{13}C-n.m.r. spectrum was run on a Bruker WH360 spectrometer operating at 90.556 Hz, with a pulse width of 18°, a sweep width of 21739 Hz, and 32 K data points. No relaxation delay was used. The resolution was 1.327 Hz/point.

The DQF phase-sensitive COSY experiment involved the sequence 90°(^{1}H)–t_1–90°(^{1}H)90°(^{1}H)–Acq[17]. The 90°(^{1}H)pulse was 7.90 μs and the relaxation delay was 1.5 ms. A $\pi/2$-shifted sine-bell function was used in each dimension. A 4 × 1K matrix was obtained using 16 scans. The resolution was 1 Hz/point in each dimension.

The ^{13}C,^{1}H heteronuclear correlated experiment involved the Bax–Morris sequence 90°(^{1}H)–$t_{1/2}$–180°(^{13}C)–$t_{1/2}$–Δ_1–90°(^{1}H)90°(^{13}C)–Δ_2–Acq[18]. The 90°(^{1}H) pulse was 8.20 μs, the 90°(^{13}C) pulse was 10.20 μs, Δ_1 (to obtain optimum polarisation of the ^{13}C,^{1}H nucleus doublet) was 3.2 ms, Δ_2 (to rephase the antiphase ^{13}C nucleus multiplets) was 2.1 ms, and there was no relaxation delay. The spectral width for ^{13}C was 15151 Hz and 1200.19 Hz for H-1. A matrix of 4 × 1K data points was obtained using 88 scans.

For the 2D-NOESY experiment, the sequence proposed[19], 90°(^{1}H)–t_1–90°(^{1}H)–τ_m–χt_1–90°(^{1}H)–Acq, was used with a relaxation delay of 1.5 s, a 90°(^{1}H) pulse of 7.9 μs, and a mixing time τ_m of 120 ms randomly varied by 15% in order to suppress the zero quantum J cross-peaks. A matrix of 2 × 1K data points was obtained using 16 scans, zero-filled in the F1 direction. A 45°-shifted sine-bell function was used in each direction.

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