Nuclear Magnetic Resonance and Conformational Studies on Monoacetylated Methyl D-Gluco- and D-Galacto-pyranosides

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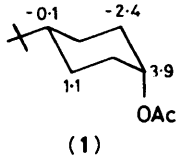
^{1}H and ^{13}C N.m.r. spectra for all mono-*O*-acetylated methyl α- and β-glycopyranosides of D-glucose and D-galactose were obtained and compared with those of the corresponding non-acetylated derivatives. It is shown that the ^{13}C n.m.r. chemical shifts of the carbons in positions α and β to an *O*-acetyl group can be approximated using simple rules, for which the number and location of eventual axial substituents are essential. The chemical shifts of the α- and β-protons are in the same way sensitive to axial substituents. Furthermore the conformation of the *O*-acetyl group was assessed by measurements of $^3J_{C,C}$ and $^3J_{C,H}$ of some selected *O*-acetates, ^{13}C-enriched in the carbonyl carbon, and by theoretical energy calculations.

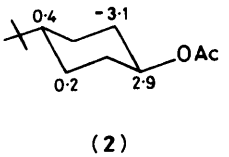
Partially *O*-acetylated sugar residues are frequently found in Nature. Many bacterial polysaccharides contain *O*-acetyl groups[1] which contribute to the immunological activity. Structural studies of bacterial polysaccharides have been undertaken, aimed towards achieving a better understanding of the relationship between immunological reactions and chemical structure. Classically, *O*-acetyl groups have been localised by protection of free hydroxy groups in the polysaccharide with methyl vinyl ether under acidic catalysis followed by methylation using strong base and methyl iodide. This procedure gives, after hydrolysis, methyl ethers of monomeric sugars in which the location of the methyl groups reflects the positions of the *O*-acetyl groups.[2] An alternative route is treatment of the polysaccharide with methyl trifluoromethanesulphonate in trimethyl phosphate. On hydrolysis methyl ethers are obtained in which the methyl groups occupy the positions of the free hydroxy groups in the polysaccharide.[3]

Today n.m.r. spectroscopy is one of the most important methods for structural studies of polysaccharides.[4] N.m.r. spectroscopy gives information on sugar residues, linkages, and substituents but comparison with model substances is important for the interpretation of spectra. ^{13}C N.m.r. spectroscopy has in favourable circumstances been used for determination of the positions of *O*-acetyl groups in polysaccharides.[5] Comparison of spectra from native and de-*O*-acetylated material was essential in these investigations.

The early ^{13}C n.m.r. studies on *cis*- and *trans*-4-t-butylcyclohexanol and their *O*-acetates[6] showed that characteristic chemical-shift changes occurred upon acetylation of the axial and equatorial hydroxy group as depicted in structures (**1**) and (**2**). It is difficult, however, to extrapolate these values to



(**1**)



(**2**)

acetylated sugars, as the chemical shift of each carbon in the latter may be influenced by steric and electronic effects of neighbouring substituents.

Systematic ^{13}C n.m.r. investigations of acylated sugars have been performed for tetradecanoic[7,8] and 3-nitropropanoic[9] acid esters. It is demonstrated that acylation shifts show good additivity for di- and tri-acyl derivatives. For the tetradecanoic acid esters an anomalous chemical shift for C-2 in the 2-*O*-acyl β-anomers was reported and it was suggested to originate from a conformational change of the ester group of the β-anomer. *O*-Acetyl derivatives of L-rhamnose[10] have been investigated by ^{13}C n.m.r. spectroscopy, and ^{1}H and ^{13}C n.m.r. studies on acetylated xylopyranosides[11–13] have been reported. The report on rhamnose acetates is the first on non-glucose derivatives and the authors found a large spread of acylation shifts for the α- and β-carbons. For the xylopyranosides a small proportion of the 1*C*-conformer ($^1C_4$) was found in the β-series[11] and, in addition, acylation-shift rules were found to be different in $CDCl_3$ and in Me_2SO.[13] The predictability of ^{1}H n.m.r. chemical shifts of acetylated xylopyranosides was also found to be poor. We now present ^{1}H and ^{13}C n.m.r. studies on mono-*O*-acetylated methyl D-gluco- and D-galacto-pyranosides. The conformation of the *O*-acetyl group has also been investigated by estimation of torsional angles from the three-bond carbon–carbon and carbon–proton coupling constants using 1-^{13}C-enriched acetyl groups and by theoretical energy calculations.

Results and Discussion

All *O*-acetylated methyl glycosides used in this study were obtained as previously described.[14,15] ^{13}C N.m.r. data of the acetylated glycosides and their parent glycosides are given in Table 1. Assignments were made by comparison with non-acetylated derivatives,[16] single-frequency selective decoupling, and deuterium-induced differential isotope n.m.r. shifts (DIS).[17] The chemical-shift changes induced by the acetylation are also given in Table 1. ^{1}H N.m.r. data and the induced chemical-shift changes for the same derivatives are given in Tables 2 and 3. Assignments were made as described in the Experimental section.

Interpretation of ^{13}C *N.m.r. Spectra.*—The introduction of an *O*-acetyl group in any position of a glycopyranoside causes deshielding of the substituted carbon (the α-carbon) by 0.7—3.5 p.p.m. and shielding of the carbons next to this (the β-carbons) by 1.2—2.8 p.p.m. Smaller effects on the γ-carbons (−0.8 to 0.3 p.p.m.) and δ-carbons (−0.3 to 0.3 p.p.m.) are also observed. Overall the results broadly support the characteristic changes in chemical shift shown in structures (**1**) and (**2**) although variations depending upon position of *O*-acetylation and stereochemistry of the sugar residue occur. All α-carbons in the ring are deshielded more (~ 1 p.p.m.) in the D-galactopyranosyl derivatives than in the analogous D-glucopyranosyl derivatives. A comparable effect of an axial substituent (especially for C-2 and C-3) is seen for the α-glycopyranosides as compared with

Table 1. Observed ^{13}C n.m.r. chemical shifts[a] of mono-*O*-acetylated methyl α- and β-D-gluco- and -D-galactopyranosides. The values in parentheses are the chemical-shift differences[b] relative to the parent glycosides

Compound	C-1	C-2	C-3	C-4	C-5	C-6	OMe	O–C	Me
Methyl α-D-Glc*p*	100.1	72.1	74.0	70.4	72.4	61.4	55.9		
2-*O*-Acetyl-	97.4	73.8	71.6	70.3	72.4	61.3	55.6	174.0	21.1
	(−2.7)	(1.7)	(−2.4)	(−0.1)	(0.0)	(−0.1)	(−0.3)		
3-*O*-Acetyl-	100.0	70.4	76.4	68.6	72.2	61.2	56.0	174.6	21.5
	(−0.1)	(−1.7)	(2.4)	(−1.8)	(−0.2)	(−0.2)	(0.1)		
4-*O*-Acetyl-	100.0	71.9	71.9	71.9	70.3	61.1	56.0	174.1	21.3
	(−0.1)	(−0.2)	(−2.1)	(1.5)	(−2.1)	(−0.3)	(0.1)		
6-*O*-Acetyl-	100.2	72.0	73.8	70.4	70.1	64.3	56.0	174.9	21.1
	(0.1)	(−0.1)	(−0.2)	(0.0)	(−2.3)	(2.9)	(0.1)		
Methyl β-D-Glc*p*	104.1	74.0	76.5	70.5	76.5	61.6	58.1		
2-*O*-Acetyl-	102.0	74.6	74.6	70.4	76.8	61.4	57.8	174.0	21.2
	(−2.1)	(0.6)	(−1.9)	(−0.1)	(0.3)	(−0.2)	(−0.3)		
3-*O*-Acetyl-	103.9	72.3	78.3	68.8	76.5	61.4	58.2	174.6	21.4
	(−0.2)	(−1.7)	(1.8)	(−1.7)	(0.0)	(−0.2)	(0.1)		
4-*O*-Acetyl-	104.1	73.9	74.6	71.9	74.6	61.2	58.2	174.1	21.3
	(0.0)	(−0.1)	(−1.9)	(1.4)	(−1.9)	(−0.4)	(0.1)		
6-*O*-Acetyl-	104.3	73.9	76.4	70.3	74.2	64.1	58.1	174.9	21.1
	(0.2)	(−0.1)	(−0.1)	(−0.2)	(−2.3)	(2.5)	(0.0)		
Methyl α-D-Gal*p*	100.3	69.1	70.4	70.1	71.6	62.1	55.9		
2-*O*-Acetyl-	97.5	71.8	68.1	70.1	71.6	62.0	55.6	174.2	21.2
	(−2.8)	(2.7)	(−2.3)	(0.0)	(0.0)	(−0.1)	(−0.3)		
3-*O*-Acetyl-	100.1	66.8	73.9	68.0	71.2	61.9	56.0	174.3	21.4
	(−0.2)	(−2.3)	(3.5)	(−2.1)	(−0.4)	(−0.2)	(0.1)		
4-*O*-Acetyl-	100.3	69.2	68.9	72.5	70.4	61.3	56.1	174.4	21.1
	(0.0)	(0.1)	(−1.5)	(2.4)	(−1.2)	(−0.8)	(0.2)		
6-*O*-Acetyl-	100.3	68.9	70.1	70.0	69.1	65.0	55.9	174.9	21.1
	(0.0)	(−0.2)	(−0.3)	(−0.1)	(−2.5)	(2.9)	(0.0)		
Methyl β-D-Gal*p*	104.6	71.6	73.6	69.5	76.0	61.8	58.0		
2-*O*-Acetyl-	102.5	73.3	71.9	69.6	76.2	61.8	57.8	174.3	21.4
	(−2.1)	(1.7)	(−1.7)	(0.1)	(0.2)	(0.0)	(−0.2)		
3-*O*-Acetyl-	104.4	69.4	76.4	67.4	75.7	61.6	58.1	174.1	21.3
	(−0.2)	(−2.2)	(2.8)	(−2.1)	(−0.3)	(−0.2)	(0.1)		
4-*O*-Acetyl-	104.6	71.9	72.2	71.8	74.6	61.1	58.1	174.4	21.1
	(0.0)	(0.3)	(−1.4)	(2.3)	(−1.4)	(−0.7)	(0.1)		
6-*O*-Acetyl-	104.7	71.4	73.5	69.6	73.3	64.7	58.1	174.8	21.1
	(0.1)	(−0.2)	(−0.1)	(0.1)	(−2.7)	(2.9)	(0.1)		

[a] Chemical shifts (δ) are reported in p.p.m. using dioxane (δ 67.4) as internal reference. [b] Positive differences indicate downfield shifts.

the β-glycopyranosides. Thus axial substituents influence the magnitude of deshielding of the α-carbon except when they have a 1,4-relationship to the *O*-acetyl group. The 2-*O*-acetates experience a further shielding of ~ 1 p.p.m. due to the proximity of the anomeric centre. An acetoxy group in a primary position causes a shift of 2.9 p.p.m. except for the β-glucoside, for which the shift is 2.5 p.p.m.

The shielding of the β-carbons depends, *inter alia*, upon whether the acetylated hydroxy group is equatorial or axial. Substitution of an axial hydroxy group causes less shielding of the β-carbons than does substitution of an equatorial hydroxy group. If both adjacent hydroxy groups are equatorial almost the same magnitude of shielding is obtained for both β-carbons. For the 2-*O*-acetyl derivatives though, the anomeric carbon is shielded more than is C-3. The proximity of the ring oxygen and the orientation of the methoxy group at C-1 are probably responsible for this effect. The magnitude of deshielding is also influenced by 1,2- and 1,3-disposed axial substituents.

Only small changes in the chemical shift of the γ-carbons are generally observed on acetylation. In the 4-*O*-acetyl derivatives, however, C-6 is significantly shielded for the D-galactopyranosides due to the axial hydroxy group at C-4. A similar but smaller effect is observed for the corresponding D-glucopyranosides. The chemical shift of the carbonyl carbon of the *O*-acetyl group varies within 0.9 p.p.m. The most deshielded carbon is found in the 6-*O*-acetyl derivatives. The chemical shift of the C–Me groups varies within 0.4 p.p.m. only. The highest chemical-shift values are found for the 3-*O*-acetates and the lowest for the 6-*O*-acetates.

The effect on the α-carbon upon acetylation can be summarised and the chemical shift approximated as follows, starting from the chemical shift of the non-acetylated parent glycoside.

(1) If the carbon under consideration is a secondary carbon add 1.8 p.p.m. (This is the acylation shift of 3-*O*-acetyl-β-D-glucopyranoside, in which there are only equatorial substituents.)

(2) For each axial substituent except those in a 1,4-relationship add 1.0 p.p.m.

(3) For 2-*O*-acetyl derivatives subtract 1.1 p.p.m.

(4) For the α-anomers of 3-*O*-acetates add 0.3 p.p.m.

(5) For all 4-*O*-acetates subtract 0.4 p.p.m.

For the primary acetates add 2.9 p.p.m. except for the β-glucoside for which 2.5 p.p.m. is added.

It was mentioned earlier that for tetradecanoic acid esters anomalous chemical shifts were obtained for the α-carbon of 2-*O*-acyl β-anomers.[7] Using the above approximations the induced shift from the anomeric centre is of the same magnitude for α- and β-glycosides, *viz.* −1.1 p.p.m.

In a similar manner the chemical shift of a β-carbon can be approximated as follows.

(1) If the carbon under consideration is secondary substract 1.7 p.p.m. (This is the induced deshielding for 3-*O*-acetyl-β-D-glucopyranoside.)

Table 2. ^{1}H N.m.r. chemical shifts and $^3J_{H,H}$ coupling constants (in parentheses) of acetylated compounds and parent glycosides

Compound	1-H	2-H	3-H	4-H	5-H	6-H	6-H′	OMe	OAc
Methyl α-D-Glc*p*[a]	4.815 (4.0)	3.566 (10.0)	3.672 (10.0)	3.406 (10.0)	3.655 (2.2) (5.8)	3.878 (12.8)	3.763	3.425	
2-*O*-Acetyl-	4.973 (3.7)	4.676 (10.3)	3.862 (9.8)	3.525 (9.8)	3.680 (2.0) (5.4)	3.896 (12.5)	3.786	3.412	2.168
3-*O*-Acetyl-	4.860 (3.8)	3.753 (10.0)	5.138 (9.3)	3.596 (9.9)	3.725 (2.0) (5.1)	3.883 (12.1)	3.787	3.450	2.178
4-*O*-Acetyl-	4.857 (4.0)	3.668 (10.0)	3.864 (9.8)	4.829 (10.3)	3.833 (2.4) (5.1)	3.710 (12.5)	3.604	3.436	2.163
6-*O*-Acetyl-	4.807 (3.7)	3.579 (9.8)	3.680 (9.0)	3.462 (10.0)	3.861 (2.4) (5.1)	4.406 (12.2)	4.304	3.421	2.139
Methyl β-D-Glc*p*[a]	4.385 (8.2)	3.263 (9.6)	3.493 (9.6)	3.380 (9.6)	3.470 (2.4) (6.4)	3.934 (12.8)	3.727	3.577	
2-*O*-Acetyl-	4.570 (8.0)	4.676 (9.5)	3.685 (9.0)	3.465[b] (9.7)	3.482[b] (1.7) (5.6)	3.931 (12.5)	3.737	3.510	2.156
3-*O*-Acetyl-	4.488 (8.1)	3.447 (9.5)	4.985 (9.5)	3.574[b] *c*	3.566[b] (1.7) (5.4)	3.935 (12.5)	3.748	3.587	2.183
4-*O*-Acetyl-	4.429 (8.1)	3.358 (9.5)	3.718 (9.5)	4.805 (9.8)	3.663 (2.0) (5.4)	3.740 (12.0)	3.593	3.585	2.162
6-*O*-Acetyl-	4.404 (8.1)	3.273 (8.8)	3.488 (9.0)[b]	3.488 (8.0)[b]	3.667 (2.5) (5.1)	4.423 (12.2)	4.305	3.576	2.139
Methyl α-D-Gal*p*[a]	4.845 (3.0)	3.840 (9.8)	3.800 (2.3)	3.974 (1.0)	3.908 (8.2) (4.6)	3.790 (12.0)	3.730	3.422	
2-*O*-Acetyl-	~5.00 *c*	~5.00	~4.04	~4.04	3.939 (7.1) (5.1)	~3.78	~3.78	3.399	2.158
3-*O*-Acetyl-	4.912 (3.9)	4.068 (10.5)	5.030 (2.4)	4.147 (n.r.)[d]	3.983 (7.1) (5.3)	~3.75	~3.75	3.450	2.174
4-*O*-Acetyl-	4.892 (3.9)	3.858 (10.3)	4.009 (2.9)	5.330 (n.r.)	4.061 (7.1) (5.1)	~3.60	~3.60	3.425	2.164
6-*O*-Acetyl-	4.845 (4.0)	~3.83	~3.83 (2.0)	4.018 (n.r.)	4.129 (8.3) (3.7)	4.250[b] (11.5)	4.320[b]	3.417	2.134
Methyl β-D-Gal*p*[a]	4.322 (8.0)	3.515 (10.0)	3.651 (3.8)	3.926 (0.8)	3.701 (7.6) (4.4)	3.796 (11.2)	3.757	3.579	
2-*O*-Acetyl-	4.520 (8.1)	4.918 (10.0)	3.867 (3.2)	3.987 (n.r.)	~3.74[b] (~8) (~4)	~3.82[b] (11.7)	~3.78[b]	3.526	2.168
3-*O*-Acetyl-	4.448 (7.8)	3.733 (10.0)	4.876 (3.4)	4.117 (n.r.)	~3.74[b] (6.1) (6.1)	~3.80[b] (11.5)	~3.77[b]	3.600	2.184
4-*O*-Acetyl-	4.412 (7.8)	3.528 (10.0)	3.862 (3.4)	5.309 (n.r.)	3.889 (6.4) (6.4)	3.636	3.636	3.591	2.182
6-*O*-Acetyl-	4.338 (7.8)	3.518 (10.0)	3.659 (3.7)	3.965 (n.r.)	3.924 (6.1) (6.1)	~4.29	~4.29	3.572	2.132

[a] Assignments are taken from K. Bock and H. Thøgersen, *Annu. Rep. NMR Spectrosc.*, 1982, **13**, 1. [b] Obtained through ABX calculations. [c] Not obtained owing to the complexity of the spectrum. [d] Not resolved.

(2) If the β-carbon is axially substituted subtract 0.5 p.p.m.

(3) If the β-carbon has a 1,3-disposed axial substituent and the α-carbon is equatorially substituted subtract 0.5 p.p.m.

(4) If the β-carbon is anomeric subtract 0.5 p.p.m.

(5) If the α-carbon is axially substituted add 0.3 p.p.m.

(6) If the carbon under consideration is adjacent to a primary acetate subtract 2.5 p.p.m.

With these approximations calculated values do not deviate from observed values by more than 0.1 p.p.m. for α-carbons or by more than 0.3 p.p.m. for β-carbons.

Table 3. ^{1}H N.m.r. chemical-shift differences induced by acetylation. Positive differences indicate downfield shifts. The values were obtained by comparison with the corresponding parent glycosides.

Compound	1-H	2-H	3-H	4-H	5-H	6-H	6-H′	OMe
Methyl α-D-Glc*p*								
2-*O*-Acetyl-	0.158	1.110	0.190	0.119	0.025	0.018	0.023	−0.013
3-*O*-Acetyl-	0.045	0.187	1.466	0.190	0.070	0.005	0.024	0.025
4-*O*-Acetyl-	0.042	0.102	0.192	1.423	0.178	−0.168	−0.159	0.011
6-*O*-Acetyl-	−0.008	0.013	0.008	0.056	0.206	0.528	0.541	−0.004
Methyl β-D-Glc*p*								
2-*O*-Acetyl-	0.185	1.413	0.192	0.085	0.012	−0.003	0.010	−0.067
3-*O*-Acetyl-	0.103	0.184	1.492	0.194	0.096	0.001	0.021	0.010
4-*O*-Acetyl-	0.044	0.095	0.225	1.425	0.193	−0.194	−0.134	0.008
6-*O*-Acetyl-	0.019	0.010	−0.005	0.108	0.197	0.489	0.578	−0.001
Methyl α-D-Gal*p*								
2-*O*-Acetyl-	~0.15	~1.16	~0.24	~0.07	0.0	~−0.01	~0.05	−0.022
3-*O*-Acetyl-	0.067	0.228	1.230	0.173	0.082	~−0.04	~0.02	0.028
4-*O*-Acetyl-	0.047	0.018	0.209	1.356	0.153	~−0.19	~−0.13	0.003
6-*O*-Acetyl-	0.0	~−0.01	~0.03	0.044	0.221	0.460	0.590	−0.005
Methyl β-D-Gal*p*								
2-*O*-Acetyl-	0.198	1.403	0.216	0.061	~−0.17	~0.03	~0.05	−0.053
3-*O*-Acetyl-	0.126	0.218	1.225	0.191	~−0.17	~0.01	~0.04	0.021
4-*O*-Acetyl-	0.090	0.013	0.211	1.383	0.188	−0.154	−0.094	0.012
6-*O*-Acetyl-	0.016	0.003	0.008	0.039	0.016	~0.50	~0.56	−0.007

Interpretation of ^{1}H *N.m.r. Spectra.*—Most of the ^{1}H n.m.r. spectra (Tables 2 and 3) could be interpreted on a first-order basis, but for some of the derivatives **ABX** calculations or simulations had to be performed. From the coupling constants (Table 2) it can be concluded that a $^4C_1$ chair conformation prevails, as expected.

A general downfield shift is obtained upon acetylation (Table 3) for both the ring proton on the acetoxylated carbon (α-proton) and on vicinal protons (β-protons). Downfield shifts are also observed for most other protons except for 6-H and 6-H′ of the 4-*O*-acetyl derivatives, for which significant upfield shifts are observed.

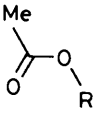
The chemical shifts of the α-protons are dependent on the number and location of axial substituents and the proximity to the anomeric centre. The largest downfield shift, 1.49 p.p.m., is obtained for methyl 3-*O*-acetyl-β-D-glucopyranoside which is devoid of axial substituents. The introduction of an axial substituent in a β-position gives a downfield shift which is approximatedly 0.25 p.p.m. smaller.

The 6-H and 6-H′ protons are less deshielded by acetylation of O-6 (*ca.* 0.5 p.p.m.), and the sum of the two downfield shifts is smaller than the shift for any secondary *O*-acetyl derivative.

The deshieldings obtained for the β-protons are all of the same order, ~0.20 ± 0.05 p.p.m. The influence on β-protons from axial substituents is small. The smallest deshielding is observed for the anomeric protons of 2-*O*-acetyl-α-glycosides. Other significant chemical-shift changes (>0.1 p.p.m.) are found for some γ-protons. Downfield shifts are observed for all γ-protons, except for 6-H and 6-H′ of the 4-*O*-acetyl derivatives in which they are shifted upfield by from 0.09 to 0.19 p.p.m. It is reasonable to assume that these shifts derive from through-space effects which depend upon the proximity of the acetyl group.

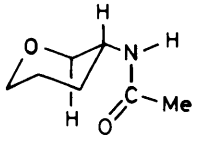
The preferred conformation of the hydroxymethyl group can be deduced from the coupling constants $J_{5,6}$ and $J_{5,6'}$ which represent the time-averaged values from the contributions of the various conformations of the hydroxymethyl group. Several investigators have studied the distribution of conformers of simple hexopyranoses and their methyl glycopyranosides,[18] and it is generally observed, for D-glucose, D-galactose, and their derivatives, that the two rotamers, in which no 1,3 parallel interactions occur, are about equally populated. The observed values of $J_{5,6}$ and $J_{5,6'}$ are similar to those of the parent glycosides for most of the acetates (Table 2). This indicates that only smaller changes in the rotamer distribution have occurred. A comparison between the 6-*O*-acetyl derivatives and the parent glycosides is difficult, however, as the electron density along the coupling path is changed by acetylation.

Evaluation of the Conformation of the O-*Acetyl Group.*—For steric and other reasons esters are generally found in a planar conformation with the two alkyl groups *trans* to each other as in structure (**3**).[19] Rotation around the R–O bond can occur more freely, and for different acetylated sugars, studied by *X*-ray crystallography,[20] the torsional angle defined by H, C, O, and C(=O) usually has a value between 0° and ±40°.

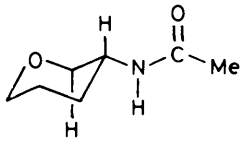


(**3**)

In a ^{1}H n.m.r. study[21] of the trimethylsilyl derivatives of 2-acetamido-2-deoxyhexopyranoses the conformation of the *N*-acetyl group was also investigated. It was found that for 2-acetamido-2-deoxy-β-D-gluco- and -D-galacto-pyranosides both the *cis* and *trans* conformations [NH, CH φ 0° and 180°, resp., for (**4**) and (**5**)] are present in a dynamic equilibrium. It was proposed that this situation should also be present in the



(**4**)



(**5**)

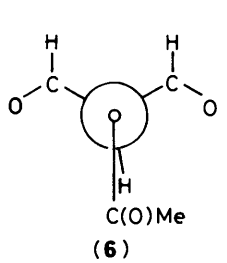
Table 4. $^2J_{C_*,C}$, $^3J_{C_*,H}$, and $^3J_{C_*,H}$ of selected mono-*O*-acetyl derivatives ^{13}C-labelled in the carbonyl carbon

Compound	C-1	C-2	C-3	C-4	C-5	C-6	$^3J_{C_*,H}$
Methyl a-D-Glc*p*							
2-*O*-Acetyl-	0.7	2.6	2.0				3.7
6-*O*-Acetyl-					2.4	2.4	2.9, 2.2
Methyl a-D-Glc*p*							3.9
2-*O*-Acetyl-	1.1	2.6	1.3				
3-*O*-Acetyl-		1.1	2.8	0.9			3.7
6-*O*-Acetyl-					2.4	2.4	2.7, 2.4
Methyl a-D-Gal*p*							
2-*O*-Acetyl-	0.7	2.6	2.0				n.r.[a]
6-*O*-Acetyl-					2.4	2.4	2.9, 3.2
Methyl a-D-Gal*p*							
2-*O*-Acetyl-	1.3	2.6	1.1				3.9
3-*O*-Acetyl-		2.2	2.6	n.r.			3.4
6-*O*-Acetyl-					2.2	2.6	2.7

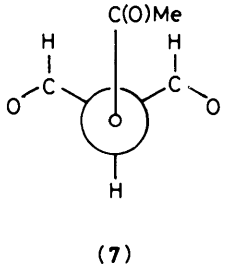
[a] n.r. = not resolved.

α-pyranosidic derivatives. It is reasonable to assume that a similar equilibrium is present for most *O*-acetyl derivatives.

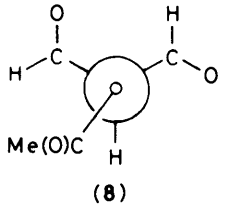
In order to obtain information on the conformation of the *O*-acetyl group some selected 1-^{13}C-labelled acetates were synthesised. The coupling constants, between the ^{13}C-labelled carbonyl carbon and the α-carbon ($^2J_{C,C}$), the β-carbons ($^3J_{C,C}$), and the α-proton ($^3J_{C,H}$) respectively, given in Table 4, were obtained from the ^{1}H-decoupled ^{13}C n.m.r. spectrum and the ^{1}H n.m.r. spectrum, respectively. $^3J_{C,H}$ and $^3J_{C,C}$ Depend on the dihedral angle φ in a manner similar to $^3J_{H,H}$.[22] Variations caused by heteroatoms in the coupling path, different hybridisation on coupling carbons, and type and geometry of substituents are large. Therefore the obtained information should be interpreted with caution. As the coupling path and hybridisation are the same for all derivatives in this study they can be compared with each other but not necessarily with those for other derivatives. As seen from Table 4, $^3J_{C,C}$ for the 2-*O*- and 3-*O*-acetyl derivatives vary between less than 0.5 Hz and 2.2 Hz. For derivatives in which both vicinal substituents are equatorial, the two coupling constants are almost equal (~1.1 Hz). This may indicate the presence of a large population of molecules in which both dihedral angles, defined by C, C, O, C(=O), are similar as in structures (**6**) (120°) or (**7**) (60°). For derivatives having one axial vicinal substituent, the coupling to the carbon bearing the axial substituent is 0.7 Hz or less and to the other carbon ~2 Hz. The dihedral angle between the carbonyl carbon and the carbon with the axial substituent is thus approaching 90° and that between the carbonyl carbon and the carbon with the equatorial substituent is approaching 150° (**8**) or 30° (**9**). The expected $^3J_{C,H}$ value for structure (**6**) is *ca.* 4 Hz, whereas for structure (**7**) a value of 7—8 Hz is expected. The observed value, ~3.8 Hz, indicates a high proportion of rotamer (**6**), the conformation found in crystal structures. For the conformers (**8**) and (**9**) values of *ca.* 3.5 and 6 Hz, respectively, are expected. The observed values (3.4 and 3.7 Hz) indicate that rotamer (**8**) predominates. Similar results were obtained by ^{1}H n.m.r. analysis for D-glucopyranose pentaacetates, which were ^{13}C-labelled in the carbonyl carbon.[23]



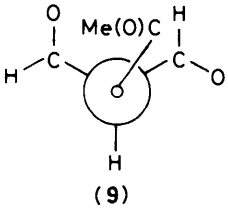
(**6**)



(**7**)



(**8**)



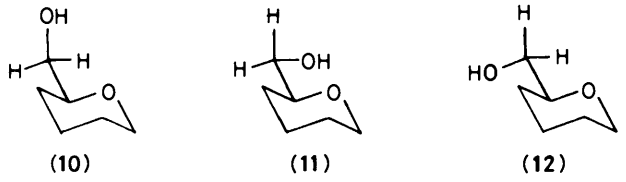
(**9**)

Theoretical Calculations.—Molecular modelling was performed for all β-D-glycopyranosides and for the 2-*O*-acetyl-α-D-glycopyranosides. Two different methods were used. To start with, hard-sphere molecular modelling using the HSEA program[24,25] was used. This is based on the Kitaigorodsky expression and *ab initio* molecular orbital calculations. The latter, which are used for the calculation of the exoanomeric effect, are omitted when bonds other than glycosidic bonds are investigated.

Molecular mechanics calculations (MM2),[26] although more time-consuming, were also used in order to obtain more reliable energy values.

Co-ordinate sets for starting points of the calculations were obtained as described in the Experimental section. In both methods rotations around the bond C(H)–O were investigated and the torsional angle, φ, was defined by the atoms H, C, O, C(=O).

In the HSEA calculations a minimum-energy molecule is obtained at $\varphi \sim -30°$ for the secondary acetates. For many derivatives the energy well is not symmetrical and for these a major part of the population may have a numerically smaller φ-value. By estimation of the position of the centre of the well, two categories of compounds can be identified. The first has two equatorial vicinal substituents and a centre of the well at $\varphi \sim 0°$. The second category has one equatorial and one axial vicinal substituent and has a centre of the well at $\varphi \simeq -30°$. Thus the 2-*O*-acetyl-α-D-glycosides and the 3-*O*-acetyl-D-galactopyranosides belong to this category. For both categories the axial



(10) (11) (12)

group is similarly oriented to give a negative φ-value. In addition a minimum at φ *ca.* 180° was obtained for most derivatives. This conformation had a much higher energy and the barrier for rotation to this minimum was high. The conformation of the 6-*O*-acetyl derivatives was also studied. As two conformers for the hydroxymethyl group prevail for *gluco*- [conformers (**10**) and (**11**)] and *galacto*- [conformers (**11**) and (**12**)] derivatives, the *O*-acetyl derivative of each conformer was investigated. For the *gauche-trans* conformer (**11**) of the D-*gluco*-derivative, which is the one found in the crystal structure,[27] three minima with approximately the same energy were found, at *ca.* 60°, 180°, and 270° (C-5, C-6, O-6, C=O). In the crystal structure a value of 72.2° is found. For the other conformer, the *gauche-gauche* (**10**), two minima at *ca.* 90° and 180° were found. The energies of these were also approximately the same. The *galacto*-derivative gave for the *gauche-trans* conformer (**11**) the same minima as the *gluco*-derivative, and for the other conformer (**12**) two minima at *ca.* 180° and 270° were obtained. For all four compounds conformations with φ ~0° were of high energy, but the barrier to rotation between the other minima was less than 10 kJ.

Molecular mechanics calculations, performed as described in the Experimental section, gave further indication of the presence of two main conformers of the secondary acetates. The energy of the minimum at ~180° was significantly lower than obtained by the HSEA calculations, with a barrier for rotation of less than 40kJ. Either the energy of the conformer in the bottom of this well was 0—8 kJ higher than the first minimum or the well was narrower. Thus the major part of the population should be present in the first energy well. Comparison of different conformers between the two extremes showed that an increase of the bond angle C–O–C had occurred for the 180° conformation. The 4-*O*-acetyl-D-galactopyranoside derivative differed somewhat from the others, lacking the second minimum at 180°. This is easily understandable from examination of a stick-and-ball model in which severe interactions between several atoms are indicated for this conformer. For the 6-*O*-acetyl derivatives the molecular mechanics calculations showed the same pattern as the HSEA calculations. The barrier to rotation over the maximum at ~0° was up to 40 kJ and those between the minima were less than 8 kJ. The *O*-acetyl group in these derivatives thus seems to have a large degree of freedom.

The results from the theoretical calculations were thus in accord with the coupling constants obtained for the ^{13}C-labelled compounds. Both methods show two categories of compounds, one with two equatorial vicinal substituents and one with one axial vicinal substituent. Both methods also show that the two conformations (**6**) and (**8**) dominate.

Conclusions.—The empirical rules for estimating the chemical-shift differences in the ^{13}C n.m.r. spectra on *O*-acetylation of different positions of glucopyranosides and galactopyranosides may be useful for the location of *O*-acetyl groups in polysaccharides. The proton linked to an acetoxylated, secondary carbon appears in the region for anomeric protons and is easily recognised. This is not true for the protons of an acetoxylated primary carbon. This carbon, however, gives a typical signal in the ^{13}C n.m.r. spectrum at ~65 p.p.m., a region in which few other signals appear.

Experimental

The various acetylated methyl glycosides were synthesized as described previously.[14,15] For the ^{13}C-enriched compounds C-1-labelled acetyl chloride (92.4% isotopic purity; Prochem; B.O.C. Ltd) in toluene (0.6 mM) was used. To a solution of a sugar derivative containing one free hydroxy group (150 mg, 0.35 mmol) in pyridine (1 ml) was added a solution of labelled acetyl chloride in toluene (0.6 ml, 0.38 mmol). The reaction was followed by t.l.c. and was complete after 4 h. Subsequent work-up was performed as for the unlabelled compounds.

^{13}C N.m.r. spectra with complete proton decoupling were recorded at ambient temperature on a JEOL FX-100 spectrometer using dioxane as internal reference (67.4 p.p.m.). The use of a spectral width of 5 kHz with a data memory of 16K gave a digital accuracy of ±0.032 p.p.m. The pulse width used was 7 μs (45°). ^{13}C N.m.r. spectra of labelled compounds were recorded using a digital resolution of 0.18 Hz.

Deuterium-induced differential isotope shift (DIS) spectra were obtained according to Pfeffer *et al.*[17] in a 10 mm coaxial dual cell.

^{1}H N.m.r. spectra were obtained at ambient temperature on a JEOL GX-400 spectrometer with sodium 3-trimethylsilyl-[$^{2}H_4$]propanoate (TSP) as internal reference (0.000 p.p.m.). Each spectrum was recorded with a spectral width of 4 kHz, a 45° pulse angle, and 32K data points, giving a digital accuracy of 0.0024 p.p.m. To assign ^{1}H n.m.r. signals homonuclear decoupling was used. For spectra with signals having higher-order couplings a spin-simulation program provided by JEOL for the FX-series spectrometers was used to obtain the chemical shifts. Vicinal coupling constants were taken as positive and geminal as negative. Chemical shifts given are accurate within ±0.01 p.p.m. as the simulation program was devoid of an iteration procedure. ^{1}H N.m.r. spectra of ^{13}C-labelled acetates were recorded as described above but with a digital resolution of 0.18 Hz. ABX spectrum calculations were performed according to standard procedures.

The energy of the possible rotamers of the *O*-acetyl group was evaluated with the HSEA[24,25] and the MM2 program.[26] Rotations around the linkage between the ester oxygen and the carbonyl carbon was considered insignificant and the torsional angle formed by the ring carbon, ester oxygen, and the carbonyl carbon and oxygen was thus set to 0°.

The torsional angle φ was defined by the ring proton, the ring carbon, the ester oxygen, and the carbonyl carbon. The starting co-ordinates for the minimisation were obtained by taking the crystal co-ordinates from the 6-*O*-acetyl derivative of methyl β-D-glucopyranoside[27] and methyl β-D-galactopyranoside[28] changing the *O*-acetyl group co-ordinates to the appropriate hydroxy group with a φ angle of 0° or for the 6-*O*-acetyl derivatives at 180° (C-5, C-6, O-6, C). The co-ordinates for 2-*O*-acetyl-α-D-glucopyranosides were obtained by combination of the acetyl co-ordinates and the crystal co-ordinates of methyl α-D-glucopyranoside. The co-ordinates for methyl α-D-galactopyranoside were obtained from the combination of α-D-galactopyranose and a methyl group. The resulting co-ordinates were then used as for the *gluco*-derivatives. For the molecular mechanics calculations lone pairs and when possible a hydrogen were added to every oxygen, without considering their orientation, with the exception of the carbonyl oxygen which in the MM2 program is treated like an oxygen with an enlarged sphere.

The energy was minimised for all possible rotamers by using the MM2 angle-driver with 10° increments from 0° to 180° and −180°, and minimising to the closest local minimum.

Acknowledgements

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