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HIGH-RESOLUTION ^{1}H- AND ^{13}C-N.M.R. SPECTRA OF D-GLUCOPYRANOSE, 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE, AND RELATED COMPOUNDS IN AQUEOUS MEDIA*

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ABSTRACT

The ^{1}H- and ^{13}C-n.m.r. spectra of D-glucopyranose and 2-acetamido-2-deoxy-D-glucopyranose and its derivatives in D_2O at 25° have been completely interpreted. Iterative analysis allowed accurate determination of the chemical shifts and coupling constants in the 270-MHz ^{1}H-spectra, and these are used to correlate the chemical-shift changes with substitution patterns. The implications of the systematic errors from assuming first-order conditions for the p.m.r. spectra of sugars are discussed in relation to measuring shift changes of sugar–enzyme complexes.

INTRODUCTION

Application of the n.m.r. method in studies of enzymes is well-established, particularly since the advent of instruments with high magnetic fields and Fourier-transform methods. Because n.m.r. spectroscopy permits individual nuclei to be monitored, solution studies, in principle, should give the same kind of structural information as that provided by X-ray crystallography.

In order to compare systematically the solution and crystal structures of the inhibitor complex with hen egg-white lysozyme, it is necessary to assign fully the n.m.r. spectrum of the inhibitor at 25°. We now report on the ^{13}C- and ^{1}H-n.m.r spectra of 2-acetamido-2-deoxy-D-glucopyranose, D-glucopyranose, and some related derivatives. The related sugar, α-D-glucopyranosyl phosphate is included in relation to studies of the enzyme complex with phosphorylase.

Recent reports have dealt with incremental rules for the assignment of n.m.r. signals of sugars in aqueous media[1], acetamido sugars in non-aqueous solvents[2], and lanthanide binding to sugars in aqueous media[3]. However, current literature data are

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insufficient to allow the assignment of the n.m.r. spectra of 2-acetamido-2-deoxy-D-glucose and its derivatives. Additionally, whereas the ^{13}C-spectra for D-glucose[4] and 2-acetamido-2-deoxy-D-glucose[5] have been interpreted, those for the glycosides have not yet been reported.

The best method of obtaining accurate n.m.r. parameters involves the iterative analysis of chemical shifts and coupling constants[6], and whereas this approach is rapidly becoming the standard procedure for carbohydrates, it has not as yet been applied to D-glucopyranose in D_2O at 25°, or to the derivatives reported on here. The addition of 2-acetamido-2-deoxy-D-glucose to hen egg-white lysozyme causes observable changes in the chemical shifts of the sugar protons[7]. These shift changes give information on the enzyme–inhibitor interaction in solution and, as they are generally small, it is necessary to estimate the magnitude of the systematic errors that result on the commonly used assumption that second-order spectra may be treated as first-order.

RESULTS

Assignment of the ^{13}C-spectra

Under the usual conditions of proton decoupling and low enrichment of ^{13}C ($<17\%$ per site), the ^{13}C-spectrum is composed of singlets and at 25.2 MHz they are well resolved. 2-Acetamido-2-deoxy-D-glucose gives 16 signals, eight from each anomer, comprising six from the glucopyranose ring and two from the acetyl group. The methyl α- and β-glycosides each gave 9 signals, the extra signal arising from the methoxyl carbon. Assignments were based on literature data[8], reported assignments[4,5,9], comparative studies with chemically related compounds, and off-resonance proton decoupling.

The ^{13}C-signals of 2-acetamido-2-deoxy-α- and -β-D-glucopyranose were identified by comparison with those of the corresponding methyl glycosides, and by their relative intensities in a spectrum of a freshly prepared solution of 2-acetamido-2-deoxy-α-D-glucopyranose. Of the eight signals, C-1 was assigned from the change of 7 p.p.m. on the replacement of HO-1 with MeO in the α- and β-series. The assignment of the signal for the MeO carbon was by obvious elimination. Assignment of C-2 resulted from comparing the spectra of 2-acetamido-2-deoxy-D-glucose and D-glucopyranose, where the substitution of HO-2 by NHAc caused a shift of 17 p.p.m. (Table I). The signals for the acetyl carbons were assigned by a comparison of the spectra of 2-acetamido-2-deoxy-D-glucose and 2-deoxy-2-fluoroacetamido-D-glucose, using the fluorine–carbon spin coupling. A downfield shift of 58 p.p.m. for the acetyl methyl carbon occurred on replacing one H with F, concomitant with an upfield shift of 4 p.p.m. for the carbonyl carbon. The signals for C-4, C-5, and C-6 were identified by their chemical shifts, which were constant to within ± 0.8 p.p.m. in the range of sugars tested (Table I); C-3 was assigned to the signal that showed a downfield shift of 1.2–2.0 p.p.m. on substituting NHAc for HO-2. There are small differences in the shifts of the signals of C-1, C-2, and C-3 for 2-acetamido-2-deoxy-D-glucose and

TABLE I

^{13}C CHEMICAL SHIFTS AT 25°[a]

	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	CH_3CO	CH_3CO	*OMe*
α-D-Glucopyranose (1)	92.9	72.3	73.6	70.4	72.3	61.3			
β-D-Glucopyranose (2)	96.7	74.9	76.7	70.4	76.6	61.5			
Methyl α-D-glucopyranoside (3)	100.0	72.5	73.9	70.4	72.1	61.4			55.9
Methyl β-D-glucopyranoside (4)	104.0	73.9	76.5	70.5	76.6	61.6			58.0
2-Acetamido-2-deoxy-α-D-glucopyranose (5)	91.6	54.9	72.4	70.9	71.5	61.5	175.1	22.8	
2-Acetamido-2-deoxy-β-D-glucopyranose (6)	95.7	57.6	74.7	70.7	76.8	61.6	175.3	23.1	
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (7)	99.1	54.5	72.5	70.8	72.0	61.4	175.1	22.8	56.0
Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (8)	102.7	57.9	74.8	70.8	76.7	61.6	175.3	23.1	56.3
2-Deoxy-2-fluoroacetamido-α-D-glucopyranose (10)	91.6	54.5	72.5	70.9	71.4	61.4	171	80	
2-Deoxy-2-fluoroacetamido-β-D-glucopyranose (11)	75.4	57.1	74.5	70.7	76.8	61.6	171	80	

[a] M Solutions, except for methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside, which were 100mM. Chemical shifts are in p.p.m. relative to that of Me_4Si.

2-deoxy-2-fluoroacetamido-D-glucose, which do not occur for C-4, C-5, and C-6. Off-resonance proton decoupling of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside removed ambiguity about the signals for C-2 and the MeO carbon. Quartets were seen for the signals assigned to the acetyl methyl and methoxyl carbons, a triplet for C-6, a singlet for the acetyl carbonyl carbon, and doublets for C-1,2,3,4,5.

The J_{CCF} values for 2-deoxy-2-fluoroacetamido-α- and -β-D-glucose are one of (*a*) 10.3 and 10.9 Hz, (*b*) 17.9 and 18.5 Hz, or (*c*) 28.8 and 7.6 Hz. As J_{CCF} is 25.3 Hz for FCH_2CN and generally[8] not below 20 Hz, (*b*) is the most likely answer. The J_{CF} values are either (*a*) 184.9 and 178.0 Hz, or (*b*) 182.0 and 180.9 Hz, of which (*b*) is the more probable, as the values are more similar (J_{CF} 181 Hz for FCH_2COOH, 172 Hz for FCH_2CN, and 167 Hz for FCH_2CH_2OH)[8]. The shifts are listed in Table I, and their comparisons are recorded in Tables III–V.

Assignments of the ¹H-spectra

The ¹H-spectra of the sugar rings are composed of doublets and double doublets, which arise from the proton spin–spin coupling. They are further complicated through signal overlapping and their second-order nature. Generally, the

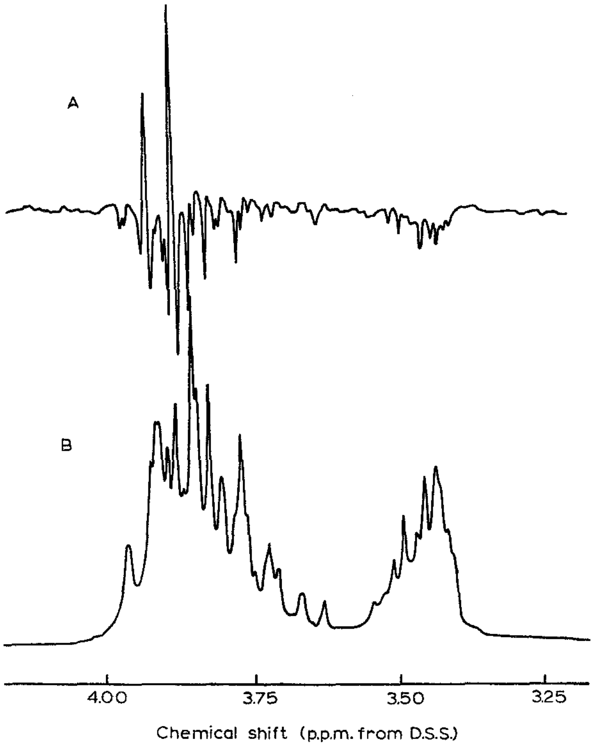

Fig. 1. Spin decoupling of 2-acetamido-2-deoxy-αβ-D-glucopyranose: *A*, decoupled spectrum and the blank; *B*, spectrum on irradiation of H-1α.

assignment proceeds on the basis of an ABCDEF-X system, where X is the anomeric proton. The initial assignments at 270 MHz were made chiefly on the basis of the reported[4] assignments for D-glucopyranose, spin–spin decoupling using difference spectroscopy (Fig. 1), and comparison of the spectra of different sugars.

The technique of difference spectroscopy involves the subtraction of the n.m.r. spectrum of one compound from that of another obtained under identical instrumental conditions. The subtraction is accomplished with the aid of the spectrometer computer. This method is particularly useful, as it often makes possible the observation of resonance peaks which are otherwise obscured by the envelope of overlapping signals. In the above spin-decoupling experiments, the blank was the spectrum for which the same power of the irradiating frequency F2 was used, but offset upfield of the sugar-proton frequencies. This method is effective, even though the uncoupled proton may be masked by an envelope of coupled and non-coupled signals (Fig. 1). Commercial 2-acetamido-2-deoxy-D-glucopyranose is almost wholly the α anomer. Rapid dissolution of this compound in D_2O and difference spectroscopy against the equilibrium α/β-mixture leads to the spectrum of the β anomer (Fig. 2).

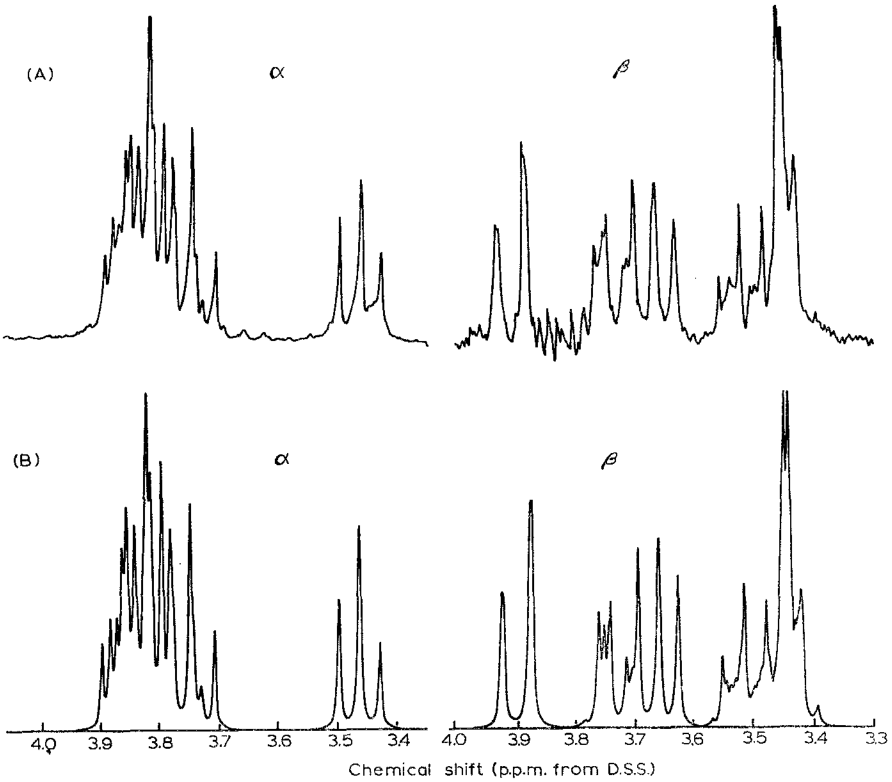

Fig. 2. Spectra of 2-acetamido-2-deoxy-α- and -β-D-glucopyranose at 270 MHz: *A*, observed spectrum; *B*, calculated spectrum.

The reported[4] assignments for α- and β-D-glucopyranose (and hence methyl α- and β-D-glucopyranoside) were confirmed by spin-decoupling experiments. The assignment of the spectra of methyl 2-acetamido-2-deoxy-α- and β-D-glucopyranoside then proceeded on the premise that the replacement of HO-2 by NHAc caused a relatively large downfield shift of the signal for H-2, and this was confirmed by spin-decoupling experiments. The assignment of α-D-glucopyranosyl phosphate was similarly made by a comparison with α-D-glucopyranose.

This leaves the problem of distinguishing the signals for H-6 and H-6′, the spin–spin coupling relationships of which are identical. This is a controversial problem[10,11]. The solution was based on the following evidence. (*a*) The assignment has been reported[10] for derivatives of 6-deuterio-D-glucose in benzene-d_6. A solvent ring-current effect caused the sequence of chemical shifts of H-6 and H-6′ to be

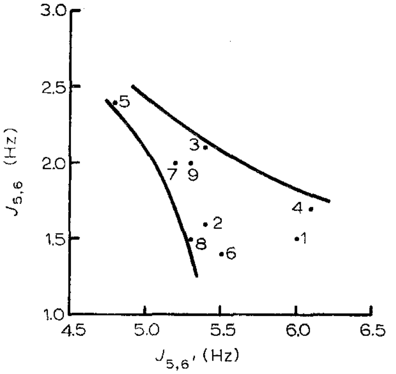

Fig. 3. Correlation of $J_{5,6}$ with $J_{5,6'}$: α-D-glucopyranose (1), β-D-glucopyranose (2), methyl α-D-glucopyranoside (3), methyl β-D-glucopyranoside (4), 2-acetamido-2-deoxy-α-D-glucopyranose (5), 2-acetamido-2-deoxy-β-D-glucopyranose (6), methyl 2-acetamido-2-deoxy-α-D-glucopyranoside (7), methyl 2-acetamido-2-deoxy-β-D-glucopyranoside (8), α-D-glucopyranosyl phosphate (9).

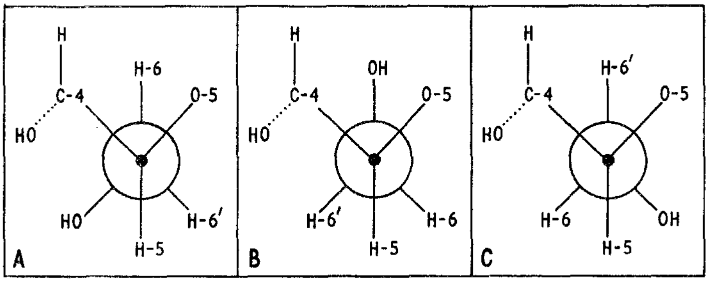

Fig. 4. Rotamers about the C-5–C-6 bond: *A*, $J_{5,6}$ large, $J_{5,6'}$ small; *B*, $J_{5,6}$ small, $J_{5,6'}$ small; *C*, $J_{5,6}$ small, $J_{5,6'}$ large. For D-glucopyranose and 2-amino-2-deoxy-D-glucopyranose, *A* is unfavoured relative to *B* and *C*, because of the proximity of HO-4 and HO-6. Since *B* and *C* preponderate, $J_{5,6}$ is small and $J_{5,6'}$ is large. Hence, the lowfield signal is assigned to H-6 and the highfield signal to H-6′.

reversed relative to those in water[12]. Correcting for this difference, the coupling constants were in agreement with those for the aqueous system, and the stereospecific identification of H-6 and H-6′ was made. (*b*) Assuming that the relative positions of the signals for H-6 and H-6′ are constant in the nine spectra under comment, an inverse correlation between $J_{5,6}$ and $J_{5,6'}$ is observed (Fig. 3). This relationship suggests the viability of the argument in Fig. 4. (*c*) The assignment is consistent with the recently announced rules for the expected relative-shift position of H-6 and H-6′ in the C-5–C-6 fragment[11].

With the exceptions of α-D-glucopyranose and 2-acetamido-2-deoxy-α-D-glucopyranose (see below), a rough assignment was thus completed ready for iterative analysis.

Iterative analysis of the ^{1}H-spectra

The problem of the initial assignment for α-D-glucopyranose and 2-acetamido-2-deoxy-α-D-glucopyranose arose from the similar chemical shifts of the signals of H-5, H-6, and H-6′, and their overlap with those of H-2 and H-3. This situation was resolved by rapidly simulating trial spectra with the spectrometer minicomputer and the Nicolet NMRCAL programme until the observed and calculated spectra agreed approximately. The results showed that there were minor disagreements of up to 0.3 p.p.m. with Table I of the original assignment[4] for H-6 and H-6′ at 60°.

The iterative analyses were simplified. For the β anomers, the signals for H-6 and H-6′ were taken separately in an overlapping AB-MNOP-X system. For the α anomers, the procedure employed an ABC-MNO-X system, where A, B, and C were H-5, H-6, and H-6′ (Fig. 5). There was no positive indication of any other than six ^3J

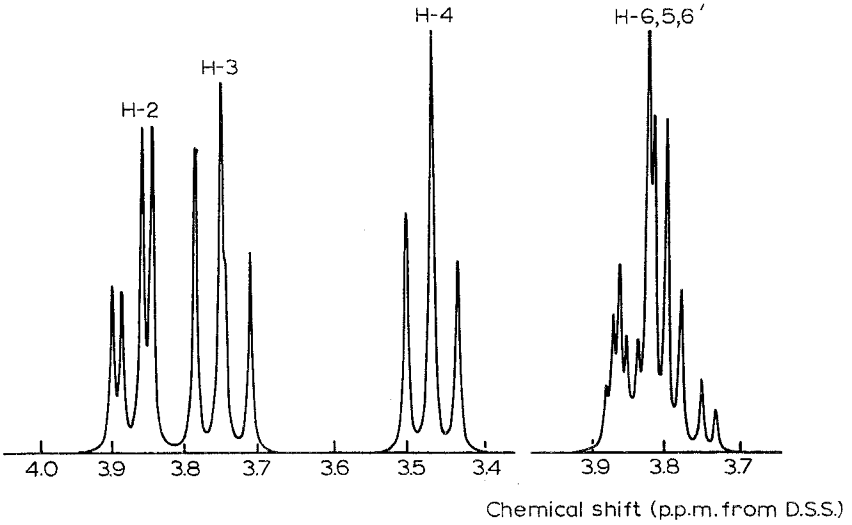

Fig. 5. Simulated spectra for 2-acetamido-2-deoxy-α-D-glucopyranose using the parameters in Table II.

TABLE II

^{1}H CHEMICAL SHIFTS AT 25° (p.p.m. FROM DSS)

	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*	*AcO*	*MeO*
α-D-Glucopyranose	5.216	3.519	3.698	3.395	3.822	3.826	3.749		
β-D-Glucopyranose	4.630	3.230	3.473	3.387	3.450	3.882	3.707		
Methyl α-D-glucopyranoside	4.798	3.551	3.656	3.390	3.637	3.859	3.748		3.410
Methyl β-D-glucopyranoside	4.367	3.247	3.476	3.366	3.451	3.914	3.719		3.561
2-Acetamido-2-deoxy-α-D-glucopyranose	5.190	3.864	3.751	3.476	3.845	3.835	3.776	2.039	
2-Acetamido-2-deoxy-β-D-glucopyranose	4.700	3.664	3.522	3.442	3.457	3.895	3.735	2.037	
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside	4.748	3.902	3.702	3.465	3.658	3.866	3.771	2.024	3.373
Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside	4.435	3.678	3.521	3.428	3.446	3.926	3.740	2.027	3.496
α-D-Glucopyranosyl phosphate	5.435	3.461	3.764	3.376	3.911	3.855	3.726		

TABLE II (*continued*)

$J_{H,H}$ VALUES (Hz) AT 25° AND THE ITERATION PARAMETERS

	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6}$	$J_{5,6'}$	$J_{6,6'}$	*No. of lines assigned*	*No. of frequencies*	*Final RMS error*	*Max. comp. error in any one parameter*
α-D-Glucopyranose	3.8	9.6	9.4	9.9	1.5	6.0	−12.1	392	21	0.22	0.04
β-D-Glucopyranose	8.0	9.1	9.4	8.9	1.6	5.4	−12.3	302	20	0.25	0.05
Methyl α-D-glucopyranoside	4.1	9.8	9.3	9.3	2.2	5.4	−12.3	414	28	0.19	0.03
Methyl β-D-glucopyranoside	8.1	9.2	9.4	9.4	1.7	6.1	−12.4	348	23	0.20	0.04
2-Acetamido-2-deoxy-α-D-glucopyranose	3.6	10.6	9.2	9.6	2.4	4.8	−12.3	361	22	0.17	0.05
2-Acetamido-2-deoxy-β-D-glucopyranose	8.5	9.9	9.1	8.1	1.4	5.5	−12.5	240	18	0.18	0.09
Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside	3.6	10.7	9.8	8.7	2.0	5.2	−12.3	336	20	0.13	0.03
Methyl 2-acetamido-2-deoxy-β-D-glucopyranoside	8.5	10.3	8.9	10.3	1.5	5.3	−12.3	279	21	0.22	0.08
α-D-Glucopyranosyl phosphate[a]	3.4	9.4	9.2	9.8	2.0	5.3	−11.9	367	26	0.27	0.04

[a] $J_{1,P}$ 7.6, $J_{2,P}$ 1.7 Hz.

and one ^2J coupling constants. For methyl 2-acetamido-2-deoxy-α-D-glucopyranoside and α-D-glucopyranosyl phosphate, the signal for H-5 is partly resolved, but again no extra splittings could be observed; examples of long-range, $J_{1,5}$ coupling have been reported[6]. The programmes UEA-NMR and UEA-ITR were used, where a least-squares refinement procedure matched the theoretical frequencies calculated from an approximate spectrum with the observed frequencies[13]. Typically, 300–400 lines were assigned to 20–30 frequencies; 7 chemical shifts and only the 7 coupling constants were allowed to vary.

There were two errors in the iteration. (*a*) The double doublets for some protons fused into triplets, so that the precision of measurement for the frequencies of the two middle lines of the four deteriorated. (*b*) Another error arose from the occurrence of virtual coupling, which is reflected in the extra splittings and broadenings that may occur in the signal of a nucleus A if it is coupled to a nucleus B that is strongly coupled [large ratio of $J_{BC}/(\nu_B - \nu_C)$] to a third nucleus C, even if $J_{AC} = 0$. This situation occurred in β-D-glucopyranose and methyl 2-acetamido-2-deoxy-β-D-glucopyranoside for the strongly coupled H-4 and H-5 signals, and the accurate assignment of frequencies was less certain.

From the analyses, the change in the chemical shift of a proton could vary as much as 3 Hz or 0.01 p.p.m. from start to end of analysis at 270 MHz. Thus, the value of the method is the ability to add at least one order of magnitude of accuracy to the measured chemical shifts. Alternatively expressed, there is a clear visual discrepancy between the spectra if the observed spectrum is compared to a trial spectrum calculated on the basis of first-order analysis. The results are presented in Table II.

On the addition of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside to hen egg-white lysozyme, the shifts of the signals for H-3 and H-5 were almost unaffected, whereas that for H-4 was strongly shifted upfield. This system was used as the model in a series of spectral calculations to estimate the systematic error in following these shifts. Fig. 6 shows that this error rises to a maximum of 2.6 Hz or 0.01 p.p.m., which should be added to the experimentally observed shift change for the H-4 signal. For the protons directly or virtually coupled to H-4, the shift changes are <0.6 Hz.

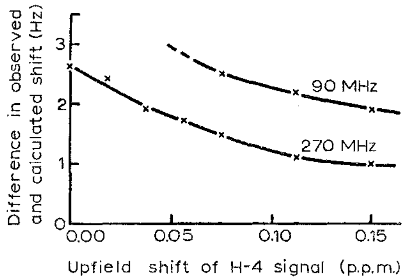

Fig. 6. Illustration, for binding of methyl 2-acetamido-2-deoxy-β-D-glucopyranoside to lysozyme, of the systematic error at 270 and 90 MHz inherent in a shift titration in which the second-order spectrum of H-4 is treated as first-order.

DISCUSSION

Chemical shifts

The change in orientation at the anomeric centre of D-glucopyranose influences the n.m.r. signals substantially. Koch and Perlin[4] have observed that there is a steric relationship between the chemical shifts for the proton and carbon nuclei. An increase in the shielding of carbon associated with a steric interaction is generally accompanied by a decrease in shielding of the attached proton[14]. These results are reflected generally by the other three pairs of sugars given in Table IV.

There are large shift differences for the protons and carbons at sites 1, 2, 3, and 5, which are opposite in direction between the proton and carbon nuclei. However, in the work reported herein, the higher precision of the proton data shows that systematic changes of chemical shift of an order of magnitude less than those for the 1, 2, 3, and 5 sites occur at positions 4 and 6. In particular, the signal for H-6 changes in the same direction as that for C-6, whereas that for H-6′ changes in the opposite direction.

Further analogous conclusions follow from the data in Table III. On substitution of the *N*-acetyl group, the changes in shift for the protons are generally in the opposite direction to those for the carbon nuclei. The quantitative changes for particular carbons are similar in the four pairs of compounds listed; the precision of the proton data shows a sub-division of the four pairs into groups of two α and two β pairs. As to be expected (and used as one guide in the assignment procedure), the magnitude of the changes is much more pronounced at position 2 than elsewhere.

Finally, the effect of changing HO-1 to MeO-1 (Table V) is clearly dependent on whether an axial or an equatorial orientation is involved. The changes are greater for the axial than the equatorial position; it is especially marked for H-5 in the α anomer. Moreover, the effect of substitution on the direction of shift change is not consistently opposite between the carbon and proton nuclei.

Biochemical application

The assignment of the spectra of 2-acetamido-2-deoxy-D-glucopyranose and its methyl glycosides permits solution studies of the interaction of the sugars with hen egg-white lysozyme. Such studies usually involve measurement of the chemical shifts of the sugar protons in the presence of lysozyme, and the results are usually presented in the form of a shift titration[7]. We have shown that the systematic error inherent in the shift titrations, by assuming that the second-order spectra are first-order, is of the order of 0.01 p.p.m., but can be greater if the total shift change in the titration is only 0.05 p.p.m. (Fig. 6).

The proton work described herein has been carried out at 270 MHz, and it is interesting to note that the analogous experiment at 90 MHz would have had a systematic error of up to 0.04 p.p.m. if the ring protons had been studied. Thus, caution is necessary in interpreting small shift changes of <0.1 p.p.m. at low frequencies as the spectra become more second-order with consequential, larger shift errors. Such

TABLE III

CORRELATION OF THE DIFFERENCES IN p.p.m. IN CHEMICAL SHIFTS TO SHOW THE INFLUENCE OF CHANGING HO-2 TO AcNH-2

	Difference							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*		*H-6′*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*		*C-6*	
(α-D-Glucopyranose) −	+0.026	−0.345	−0.053	−0.081	−0.023	−0.009		−0.027
(2-acetamido-2-deoxy-α-D-glucopyranose)	+1.3	+17.4	+1.2	−0.5	+0.8		−0.2	
(Methyl α-D-glucopyranoside) −	−0.050	−0.351	−0.046	−0.075	−0.021	−0.007		−0.023
(methyl 2-acetamido-2-deoxy-α-D-glucopyranoside)	+0.9	+18.0	+1.4	−0.4	+0.1		0.0	
(β-D-Glucopyranose) −	−0.070	−0.434	−0.049	−0.055	−0.007	−0.013		−0.028
(2-acetamido-2-deoxy-β-D-glucopyranose)	+1.0	+17.3	+2.0	−0.2	−0.3		−0.1	
(Methyl β-D-glucopyranoside) −	−0.068	−0.431	−0.045	−0.062	+0.005	−0.012		−0.021
(methyl 2-acetamido-2-deoxy-β-D-glucopyranoside)	+1.3	+16.0	+1.7	−0.3	−0.1		0.0	

TABLE IV

CORRELATION OF THE DIFFERENCES IN p.p.m. IN CHEMICAL SHIFTS ON $\alpha \rightarrow \beta$ CONVERSION

	Difference						
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*	*H-6′*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*	*C-6*	
(α-D-Glucopyranose) –	+0.586	+0.289	+0.225	+0.008	+0.372	−0.056	+0.042
(β-D-glucopyranose)	−3.8	−2.6	−3.1	0.0	−4.3	−0.2	
(2-Acetamido-2-deoxy-α-D-glucopyranose) –	+0.490	+0.200	+0.229	+0.034	+0.388	−0.060	+0.041
(2-acetamido-2-deoxy-β-D-glucopyranose)	−4.1	−2.7	−2.3	+0.2	−5.3	−0.1	
(Methyl α-D-glucopyranoside) –	+0.431	+0.304	+0.180	+0.024	+0.186	−0.055	+0.029
(methyl β-D-glucopyranoside)	−4.0	−1.4	−2.6	−0.1	−4.5	−0.2	
(Methyl 2-acetamido-2-deoxy-α-D-glucopyranoside) –	+0.313	+0.224	+0.181	+0.037	+0.212	−0.060	+0.031
(methyl 2-acetamido-2-deoxy-β-D-glucopyranoside)	−3.6	−3.4	−2.3	0.0	−4.7	−0.2	

TABLE V

EFFECT OF CHANGING HO-1 TO MeO-1

	Difference							
	H-1	*H-2*	*H-3*	*H-4*	*H-5*	*H-6*		*H-6′*
	C-1	*C-2*	*C-3*	*C-4*	*C-5*		*C-6*	
(α-D-Glucopyranose) –	+0.418	−0.032	+0.042	+0.005	+0.185	−0.033		+0.001
(methyl α-D-glucopyranoside)	−7.1	−0.2	−0.3	0.0	+0.2		−0.1	
(2-Acetamido-2-deoxy-α-D-glucopyranose) –	+0.442	−0.038	+0.049	+0.011	+0.187	−0.031		+0.005
(methyl 2-acetamido-2-deoxy-α-D-glucopyranoside)	−7.5	+0.4	−0.1	+0.1	−0.5		+0.1	
(β-D-Glucopyranose) –	+0.263	−0.017	−0.003	+0.021	−0.001	−0.032		−0.012
(methyl β-D-glucopyranoside)	−7.3	+1.0	+0.2	−0.1	0.0		−0.1	
(2-Acetamido-2-deoxy-β-D-glucopyranose) –	+0.265	−0.014	+0.001	+0.014	+0.011	−0.031		−0.005
(methyl 2-acetamido-2-deoxy-β-D-glucopyranoside)	−7.0	−0.3	−0.1	−0.1	+0.1		0.0	

errors may be avoided if the spectra are computed at the experimental frequency to set up a calibration curve to allow the appropriate correction to be made. However, the shift correction at 90 MHz is greater than the experimental error in measuring these shifts; thus, measurements made at 90 MHz or lower should be undertaken with extreme caution if the induced shifts in second-order spectra are <1 p.p.m. Any such shift changes observed in a titration would be underestimated, sometimes seriously.

EXPERIMENTAL

Solutions (M or 0.1M) of the sugars were prepared in 99.8% D_2O. ^{13}C-N.m.r. spectra were recorded at 25° at neutral pH with a Varian XL-100 spectrometer operating at 25.2 MHz and tubes of outside diameter 12 mm. Spectra were accumulated under conditions of complete proton decoupling, storing the decay over 8 K core, with an acquisition time of 0.8 sec and pulse delay of 1.0 sec, for at least 1 K transients. The free-induction decay was exponentiated using a time constant of 0.4 sec before Fourier transformation. Shifts were measured[8] relative to that of *p*-dioxane at 0 p.p.m., as a secondary chemical reference at 67.4 p.p.m. downfield of that of Me_4Si. The digitisation is 1.2 Hz/point.

^{1}H-N.m.r. spectra were recorded at 25° at 270 MHz, using a Bruker spectrometer assembly belonging to the Oxford Enzyme Group, incorporating a Nicolet 1085 computer, a 294 disc storage system, and a 293 pulse controller. For a typical 25mM solution of sugar, 64 or 128 transients were accumulated over 8 K core, with dwell time (interpoint time) of 500 or 666 μsec, and a pulse repetition rate of 10–12 sec (*i.e.*, greater than 5 T_1's). The pulse angle was set low at $\sim 25°$. The Fourier transform was calculated over 16 K, using the zero-addition technique to improve the resolution; similarly, the free-induction decay was not exponentiated. The spectral width was thus 1000 Hz (0.122 Hz per point) or 750 Hz (0.092 Hz per point), respectively; the digitisation is the practical error of the iterative analyses. Chemical shifts were measured relative to acetone, at 2.215 p.p.m. downfield of DSS. The iterations were performed with the Oxford University ICL 1906A Computer, using the programmes UEA-NMR and UEA-ITR (SRC Library of NMR computer programmes, c/o Dr. P. Anstey, School of Chemical Sciences, University of East Anglia, Norwich, NOR 88C, U.K.). Most of the sugars were commercial samples. Methyl 2-acetamido-2-deoxy-α- and -β-D-glucopyranoside were gifts from Dr. K. Hamaguchi, Osaka University, Japan (Nakarai Chemical Corporation). 2-Deoxy-2-fluoroacetamido-D-glucose was prepared in our laboratory[15].

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REFERENCES

1 A. De Bruyn, M. Anteunis, R. De Gussem, and G. G. S. Dutton, *Carbohydr. Res.*, 47 (1976) 158–163, and references therein.

2 T. J. Schamper, *Carbohydr. Res.*, 36 (1974) 233–237.

3 S. J. Angyal, G. L. Bodkin, and F. W. Parrish, *Aust. J. Chem.*, 28 (1975) 1541–1549, and references therein.

4 H. J. Koch and A. S. Perlin, *Carbohydr. Res.*, 15 (1970) 403–410.

5 D. R. Bundle, H. J. Jennings, and I. C. P. Smith, *Can. J. Chem.*, 51 (1973) 3812–3819.

6 B. Coxon, *Adv. Carbohydr. Chem. Biochem.*, 27 (1972) 7–83.

7 R. A. Dwek, *N.M.R. In Biochemistry*, Oxford University Press, 1973.

8 J. B. Stothers, *Carbon-13 N.M.R. Spectroscopy*, Wiley, New York, 1972.

9 D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, 92 (1970) 1355–1361.

10 D. Gagnaire, D. Horton, and F. R. Taravel, *Carbohydr. Res.*, 27 (1973) 363–372.

11 A. De Bruyn and M. Anteunis, *Carbohydr. Res.*, 47 (1976) 311–314.

12 L. M. Jackman and S. Sternhell, *Applications of NMR Spectroscopy in Organic Chemistry*, Pergamon Press, 1969, 112–113.

13 R. B. Johannesen, J. A. Ferretti, and R. K. Harris, *J. Magn. Reson.*, 3 (1970) 84–93.

14 R. G. S. Ritchie, N. Cyr, and A. S. Perlin, *Can. J. Chem.*, 54 (1976) 2301–2309, and references therein.

15 C. G. Butchard, R. A. Dwek, P. W. Kent, R. J. P. Williams, and A. V. Xavier, *Eur. J. Biochem.*, 27 (1972) 548–553.