Tritium Nuclear Magnetic Resonance Spectroscopy. Part 9. Specifically Tritiated Glucoses.

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SUMMARY

The strict regiospecificity of the labelling in some mono-tritiated glucoses, available commercially as aqueous solutions, is demonstrated by ^{3}H n.m.r. spectroscopy. After storage there may however sometimes be label also present in the water or as non-glucose impurity. Internally referenced chemical shifts for D-glucopyranose in dilute solution in D_2O at 25°C are provided for the first time.

INTRODUCTION

D-Glucose, labelled specifically, has proved essential for investigating the biochemical processes of glycolysis and glucose synthesis in liver.[2,3] For these purposes, the specificity of labelling is crucial to the detailed biochemical interpretation of experimental results. Schmidt,

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Genovese, and Katz[2] degraded some commercial samples of labelled glucose and apparently found that 5 - 20% of the tritium was not in the specified position.[3] In consequence they stated "It is not known whether in all glucose preparations the nominal localisation of isotope is completely true and whether the observed randomisation could be entirely attributed to triosephosphate isomerisation".[2]

This question of the regiospecificity of the labelling in [^{3}H]glucose was ideal for investigation by ^{3}H n.m.r. spectroscopy. Accordingly we have examined a series of tritiated glucoses by this definitive and non-destructive method. The results are of course not subject to any of the possible uncertainties arising from degradative chemistry. Unequivocally we find that the regiospecificity of labelling is 100% in each of D-[1-, 2-, 3-, 4-, 5- and 6-^{3}H]glucopyranose.

EXPERIMENTAL

Reaction conditions for the specific tritiation of D-glucose are summarised in Table I.

Aqueous solutions of the six mono-tritiated glucoses (20 - 25 mCi ; 2 - 23 Ci mmol^{-1}) (The Radiochemical Centre Ltd) were frozen and lyophilised, and each sample was redissolved in deuterium oxide (105 µl). A trace of sodium 4,4-dimethylsilapentanesulphonate (DSS) was added to each to provide the ^{1}H reference, and the solutions were sealed

in cylindrical microcells (100 µl ; Wilmad). The latter were then inserted into standard n.m.r. tubes (5 mm), which were capped. Triton spectra (with ^{1}H decoupling) were recorded at 96 MHz and 25°C as previously,[4,5] employing a Bruker WH 90 pulse spectrometer.

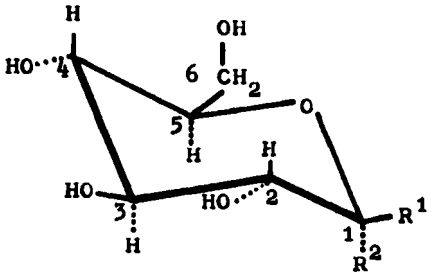
TABLE 1

Preparation of specifically tritiated D-glucopyranose

Position tritiated	Method	Reference
1	Exchange with $T_2/PdO-BaSO_4$.	15
2	Reduction of 2-ketogluconic acid with sodium borotritide.	cf.16
3	Via reduction of 1:2,5:6-di-O-isopropylidene-α-D-ribohexofuran-3-ulose (Ref. 20) with sodium borotritide, then 3-epimerisation of the allo-furano product by benzoate-displacement of the 3-tosylate.	
4	Via reduction of 2,3,6-tri-O-benzyl-α-D-xylohexopyranoside-4-ulose with sodium borotritide.	17
5	Via reduction of 1,2-O-isopropylidene-5-ketoglucuronic acid with sodium borotritide.	cf.18
6	Via reduction of 1,2-O-isopropylidene-glucuronolactone with sodium borotritide.	19

RESULTS AND DISCUSSION

D-Glucopyranose in aqueous solution is an equilibrium mixture of α- and β- anomers [6] (1) and therefore exhibits two chemical shifts for each CH-proton.[7-9] For any mono-CT glucose there will then be separate triton signals arising from the two anomeric compounds present. This expectation is borne out by the ^{3}H n.m.r. results (Table II), each monotritiated glucose showing two ^{3}H resonances with ^{1}H decoupling (e.g. see Figure 1). The relative integrated signal intensities give a measure of the anomer proportions, there being little or no differential nuclear Overhauser effect under the operating conditions.[1,5]



(1) α-D ; R^1 = H, R^2 = OH

β-D ; R^1 = OH ; R^2 = H

The complexity of the ^{1}H n.m.r. spectrum of glucose measured at moderate field strength,[7,8] arising from the narrow range of chemical shifts and the extensive proton-proton spin coupling in the compound, had long precluded

a full analysis, even with the help of INDOR.[10] Only recently was a full analysis achieved.[9] This utilised a 220 MHz spectrum of glucose in deuterium oxide at 60°C. with tetramethylsilane as (presumably) external reference. The chemical shifts so derived[9] (see Table II) are therefore unfortunately not directly comparable with data obtained under normal operating conditions, viz. for dilute solution at ordinary temperature and with respect to an internal reference. Using the set of specifically mono-tritiated glucoses in very dilute solution in deuterium oxide at 25°C, we have obtained the ^3H chemical shifts directly from the

TABLE II

Chemical shifts (p.p.m.) for D-glucopyranose (1) in D_2O

	^3H shift [a]		Anomer proportion (%)		^1H shift [b]	
Position	1-α	1-β	1-α	1-β	1-α	1-β
1	5.15	4.57	37.5	62.5	5.27	4.67
2	3.49	3.20	41	59	3.56	3.29
3	3.66	3.44	42	58	3.75	3.59
4	ca. 3.40	3.36			3.44	3.45
5	3.77	3.41	40	60	3.81	ca. 3.52
6 {	3.71	3.67	21.5	32	4.05	3.91
	3.79	3.84	19	27.5	4.12	4.22

[a] At 96 MHz and 25°C, with respect to DSS

[b] At 220 MHz and 60°C, with respect to TMS (Ref. 9)

sharp singlet lines obtained in the ^{3}H n.m.r. spectra measured with ^{1}H decoupling and with DSS as internal reference. As ^{3}H chemical shifts are virtually the same as ^{1}H shifts,[4] our data (Table II) thus provide the D-glucopyranose ^{1}H chemical shifts, measured under standard conditions, for the first time.

Figure 1 shows a typical ^{3}H spectrum - that from the mixed anomers of [5-^{3}H]glucose. Only with [4-^{3}H] glucose were the lines from the two anomers so close as to be unresolved at 96 MHz. The position of the weaker,

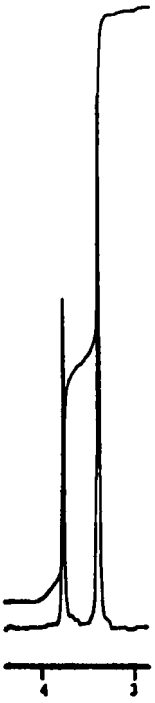


Figure 1

Figure 2

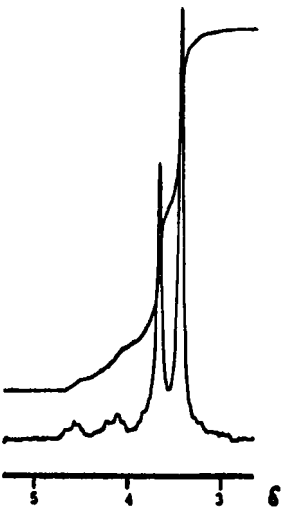


Figure 3

lower-field line from the 4-triton in the 1-α anomer (Table II) was therefore estimated from the line shape of the composite signal. In the spectrum of the [6-^{3}H] glucose there were four sharp lines (Figure 2), in contrast to the broadened signal observed without ^{1}H spin decoupling.[11] These give the chemical shifts of the two 6-methylene positions in each of the 1-β and 1-α anomers. The highest and lowest field lines of the group were assigned to the non-equivalent 6-positions in the major 1-β anomer on the basis of the line intensities (Table II). The non-equivalence of the methylene hydrogens has its origin, of course, in the asymmetry at the adjacent 5-position.

The radiochemical purity of each labelled glucose solution was confirmed by the absence or presence of other ^{3}H n.m.r. signals, as recorded in Table III. Thus the solution of [1-^{3}H]glucose showed two extra, minor signals: a sharp one at δ 4.9 was attributed to tritiated water, whilst a weak broadened signal centred at δ 5.3 was from unknown purity. Although [1-^{3}H]glucose appeared stable in solution,[12] it showed loss of the label by exchange with solvent water in the presence of base[13] - for which process there is a straightforward mechanism[14] - so the presence of tritiated water in the present sample was perhaps not surprising. Comparison with the data in Table II indicates that the unknown impurity cannot be a C-tritiated glucose. Whereas the [1-^{3}H]glucose itself was 100% regiospecifically labelled, the solution was not

TABLE III

^3H N.m.r. analysis of solutions of tritiated D-glucopyranose in D_2O (with ^1H decoupling)

Position tritiated	^3H N.m.r. signals (p.p.m.)	Relative intensity (%)	Assignment (and anomer)	Regiospecificity of label in glucose (%)	Total radiochemical purity of sample (%)
1	4.57 s [a]	50	1β	100	80
	4.9 s	10	DTO		
	5.15 s	30	1α		
	5.3 br	10	?		
2	3.20 s	59	2 (1-β)	100	100
	3.49 s	41	2 (1-α)		
3	3.44 s	48	3 (1-β)	100	86
	3.66 s	38	3 (1-α)		
	4.1 br	9	?		
	4.5 br	5	?		
4	3.36 s	} 64	4 (1-β)	100	64
	ca. 3.40		4 (1-α)		
	3.7 br	30	?		
	4.8 s	6	DTO		
5	3.41 s	60	5 (1-β)	100	100
	3.77 s	40	5 (1-α)		
6	3.67 s, 3.84 s	59.5	6 (1-β)	100	100
	3.71 s, 3.79 s	40.5	6 (1-α)		

[a] s = singlet, br = broad.

radiochemically pure (Table III) and so the significance of the non-glucose radioactivity formed on storage would need consideration before biochemical tracer experiments were undertaken. The [2-^{3}H]glucose solution and also the solutions of the [5-^{3}H] and [6-^{3}H] samples were radiochemically pure and each glucose was 100% regiospecifically labelled. The solution sample of [3-^{3}H]glucose contained 14% of the total tritium as unknown impurities (see Figure 3 and Table III) : these could hardly be other C-tritiated glucose (see Table II) because the extraneous signals were broad in spite of the ^{1}H decoupling. The solution of [4-^{3}H]glucose at the time of examination had some 36% of the tritium content other than as the labelled glucose, with a small proportion of this as tritiated water. The breadth of the main impurity signal (ca. 0.8 p.p.m.) makes it most unlikely to be from other C-tritiated glucose. Indeed the conclusion to be drawn from these ^{3}H n.m.r. analyses is that each of the commercial samples of C-tritiated D-glucopyranose examined was 100% regiospecifically labelled.

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