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The search for D-glucose derivatives suitable for the study of natural hydrogen isotope fractionation

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ABSTRACT

In order to apply Site-specific Natural Isotope Fractionation (SNIF) to chemical, biochemical, and environmental studies of D-glucose, there is a need for readily prepared compounds which give ^{2}H NMR spectra with all or most signals resolved. Changing substituents at C-1 and/or C-6 of α-D-glucopyranose pentaacetate improved the dispersion of deuterium signals, the best results being achieved with 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-thiocyanato-α-D-glucopyranose, for which only the ^{2}H-2 and ^{2}H-4 signals were not resolved, and with 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl bromide, for which only the ^{2}H-6a and ^{2}H-6b signals were not resolved. Periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside and 4,6-dichloro-4,6-dideoxy-D-galactose was also examined as a possible source of useful compounds. Products obtained from the benzylideneglucoside gave inadequate resolution and broad deuterium signals. The oxidation of 4,6-dichloro-4,6-dideoxy-D-galactose was not straightforward. The α anomer was oxidised more rapidly than the β anomer. The oxidation product, 2,4-dichloro-2,4-dideoxy-3-*O*-formyl-D-threose, was too labile to be isolated pure and its hydrolysis to 2,4-dichloro-2,4-dideoxy-D-threose was accompanied by a slow elimination to form 2,4-dichlorobut-2-enal.

INTRODUCTION

Large variations in the natural abundance levels of deuterium among the different sites of a given molecule have been found[1] and the investigation of Site-specific Natural Isotope Fractionation (SNIF) by deuterium NMR has been shown to provide mechanistic information on the fate of hydrogen in chemical and biochemical syntheses without the need for isotopic enrichment[2]. The pattern of isotopic ratios, $(D/H)_i$, of a molecular species (D and H being the numbers of deuterium and hydrogen atoms at site i) is specially powerful for inferring properties of the precursors and of their transformation pathways[3]. In this respect, access to the isotopic distribution in glucose is highly desirable since this molecule is a key product of photosynthesis. The glucose probe is a rich source of informa-

tion about the influence of metabolic, physiological, and environmental effects which govern plant growth since the amounts of the different monodeuterated isotopomers are conditioned by a number of biosynthetic steps possibly associated with specific kinetic and thermodynamic isotope effects.

To determine the isotope ratios for all the carbon-bound hydrogen nuclei in D-glucose, a derivative is required which gives a deuterium NMR spectrum containing resolved signals for all seven deuterium nuclei. D-Glucose itself gives a poorly resolved ^{2}H NMR spectrum which is also complicated by the presence of α and β anomers. The readily prepared α-D-glucopyranose pentaacetate does not discriminate[4] sites 5,6a,6b or sites 2,3,4. An additional factor which increases the difficulty of resolving signals is the deuterium linewidth. Other derivatives have therefore been studied.

RESULTS AND DISCUSSION

In an attempt to increase the separation between ^{2}H-3 and ^{2}H-2,4, the spectrum of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride (**1**) was measured *, but the signals were too broad to allow the baseline separation that was expected on the basis of the proton chemical shifts (see Fig. 1a). However, at a higher field (at the deuterium frequency of 92.1 MHz), base line separation of ^{2}H-3 from ^{2}H-2,4 was achieved and also the separation of H-6b from H-5,6a (see Fig. 1b).

In order to increase the separation between H-5 and H-6a,6b, replacement of the acetoxy substituent at C-6 by halide or thiocyanate was considered. A bromine substituent was readily introduced into methyl α-D-glucopyranoside by the triphenylphosphine-promoted reaction with tetrabromomethane in pyridine[5]. Acetolysis of the resulting methyl 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranoside gave the α-pyranose tetraacetate (**2**) which, on reaction with hydrogen bromide in acetic acid, gave the known[6] 2,3,4-tri-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranosyl bromide (**3**). The 60-MHz proton NMR spectrum contained resolved signals for all the sites except for the diastereotopic protons at C-6, and a very useful deuterium spectrum was obtained for this compound (see Fig. 1c). The 6-deoxy-6-thiocyanato-α-D-glucopyranose tetraacetate (**4**), which was readily prepared by nucleophilic displacement of the bromide in **2**, gave a ^{2}H spectrum in which the diastereotopic nuclei at C-6 were resolved (see Fig. 1d). Although ^{2}H-2 and ^{2}H-4 were not resolved, a good discriminating potential is exhibited by this compound.

An alternative derivatisation procedure involved periodate oxidation, which when applied to methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**5**) forms the hydrated product **6**, which crystallises from the reaction mixture in high yield[7]. This

* In this initial phase of the work, chloroform was used as a solvent because the compounds examined had the highest solubility in it, and it gives a ^{2}H signal of only moderate intensity. When a promising compound has been identified, variation of temperature and solvent will be studied in attempts to reduce the linewidth.

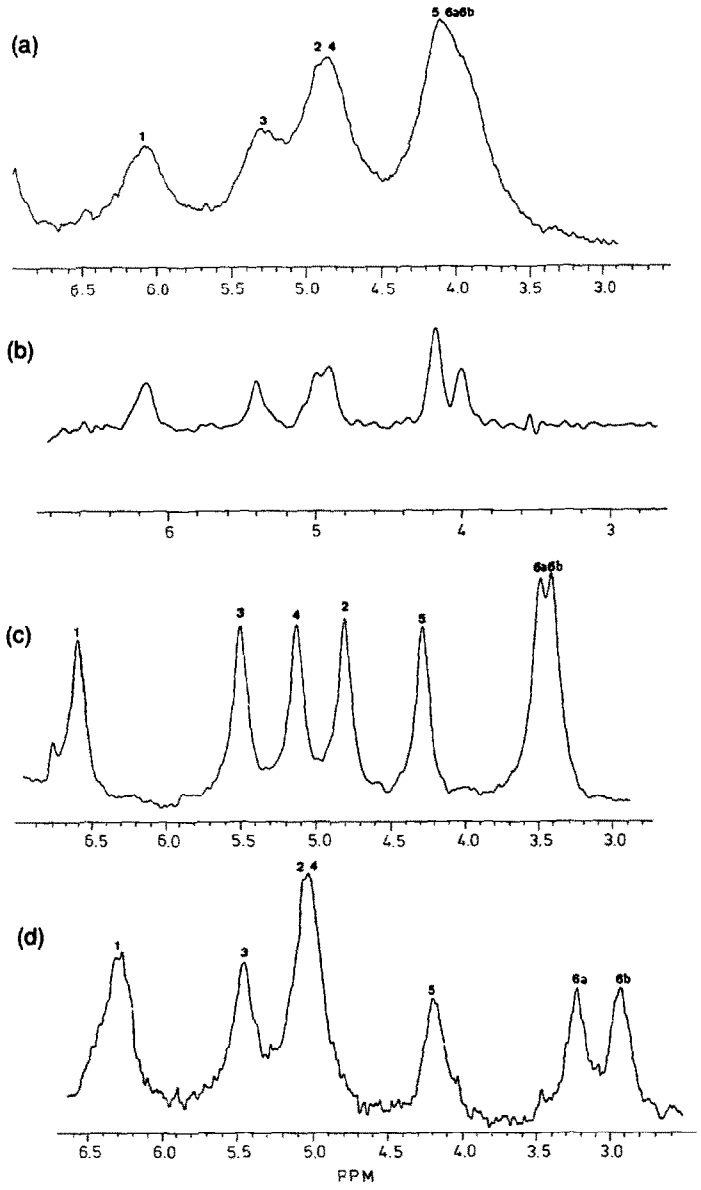
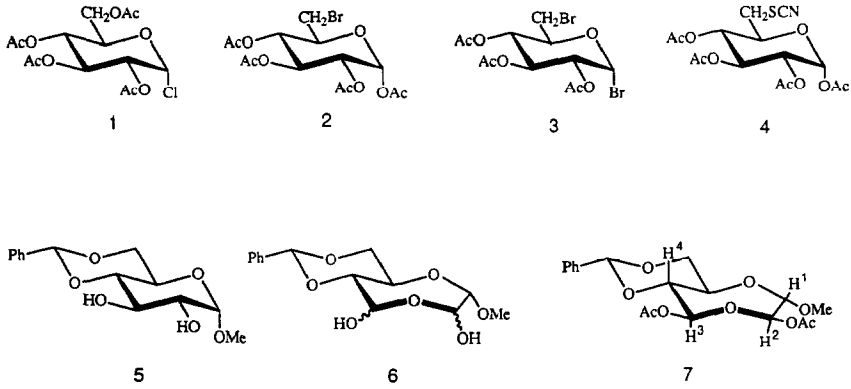


Fig. 1. ^{2}H NMR spectra (excluding OAc groups): (a) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride at 61 MHz; (b) 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride at 92 MHz; (c) 2,3,4-tri-*O*-acetyl-6-deoxy-6-bromo-α-D-glucopyranosyl bromide at 76.6 MHz; (d) 1,2,3,4-tetra-*O*-acetyl-6-deoxy-6-thiocyanato-α-D-glucopyranose at 61 MHz.

can exist in four diastereoisomeric forms, but the ^{1}H NMR spectrum shows that one isomer predominates (See Table I).

An advantage of this derivative is that the benzylidene acetal ring places the diastereotopic nuclei at C-6 in quite different magnetic environments (axial and



equatorial), and their signals are separated by 0.5 ppm in the proton NMR spectrum. However, the signals for H-2 and H-3 overlapped and others (H-4,5,6a and H-1,6b) were not sufficiently resolved. The di-*O*-acetyl derivative[8] was prepared and shown to have the configuration depicted in **7** from vicinal coupling constants and NOE measurements.

The equation of Haasnoot, De Leeuw and Altona[9] was used to determine dihedral angles (ϕ). The expected *trans*-diaxial relationship of H-4 and H-5 was confirmed by the value (9.6 Hz) of $J_{4,5}$ ($\phi_{4,5} = 180°$). The key coupling constants $J_{1,2}$ 5.6 Hz ($\phi_{1,2} = 165°$) and $J_{3,4}$ 7.9 Hz ($\phi_{3,4} = 189°$) implied an anti relationship between H-1 and H-2 and between H-3 and H-4. This was confirmed by NOE difference measurements; irradiation of H-2 increased the intensity of the signal for H-3 by 15% and irradiation of H-3 enhanced the signal of H-2 by 9%. Although the acetylation separated the signals of H-2 and H-3 and increased the separation of H-1 and H-6b, the remaining signals were too close to be resolved in the deuterium NMR spectrum.

The periodate oxidation of the smaller molecule, methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside, which can be readily prepared from methyl α-D-glucopyranoside in a one-pot reaction involving sulphuryl chloride[10], was next examined in the hope that the deuterium signals would be less broad. However, the oxidation product gave a proton spectrum that was much more complicated than that of compound **6**. The oxidation of 4,6-dichloro-4,6-dideoxy-D-galactose[11] (**8**) was also studied since the products, formic ester **9** and formic acid, had the potential for most of the carbon-bound hydrogens to be discriminated. The dichloride was treated with sodium metaperiodate (3 equiv) in deuterium oxide and the reaction was followed by proton NMR measurements. These showed that the α anomer was oxidised more rapidly than the β anomer.

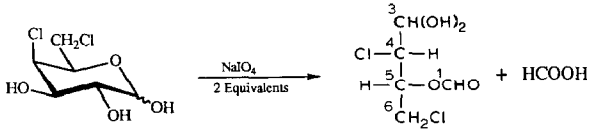
The spectrum after 30 min contained signals for the expected formic ester **9** and for unreacted 4,6-dichloro-4,6-dideoxy-β-D-galactopyranose (see Table I). The first oxidation thus occurred at the *cis*-glycol (C-1–C-2) of the α anomer, but no signals

TABLE I

^{1}H NMR data

Compound	Chemical shifts								Coupling constants						
	H-1	H-2	H-3	H-4	H-5	H-6a	H-6b	Other	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1	6.29	5.02	5.56	5.14	~4.3	~4.3	~4.1	2.11 (6H) 2.05 (3 H) OAc 2.04 (3 H)	4.0	10.1	9.8	9.5	?	?	?
4	6.34	5.09	5.49	5.01	4.22	3.24	2.95	2.22 2.10 2.04 2.03 } OAc	3.7	10.2	9.5	9.7	2.85	8.1	−13.8
6	4.28	4.71 [a]	4.69 [a]	3.46	3.74	3.61	4.11	5.53 (H-8) 6.91 (OH) *J* 7 6.86 (OH) *J* 6.5	5.9		7.6	9.3	10.2	5.0	−10.2
7	4.6	5.82	6.1	3.86	4.09	3.73	4.26	5.49 (H-8)	5.6		7.8	9.6	9.8	5.5	−10.3
8 (α anomer)	5.32	3.92	~4.3	4.63	4.53	~3.8	~3.8		3.9	10.2	3.6	<1	6.6 [b]	6.6 [b]	?
8 (β anomer)	4.72	3.62	3.98	4.57	4.19	~3.8	~3.8		7.9	9.6	3.7	<1	6.6 [b]	6.6 [b]	?
9	8.31		5.22	4.35	5.62	~3.9	~3.9	8.29 HCOOH			5.0	3.6	?	?	?
10			5.34	4.14	~4.3	3.78	3.74	8.29 HCOOH			6.1	3.0	?	?	−11.4

[a] Measured after D_2O exchange. [b] Apparent coupling constant.



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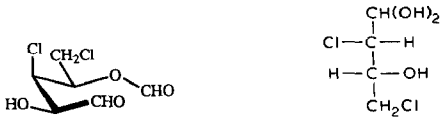

10 11

corresponding to the product (**10**) of this cleavage were detected, and it was concluded that **10** was very rapidly oxidised to **9**.

After reaction for 2 h, there was still some unreacted β-pyranose, but the formic ester had begun to hydrolyse to give **11**. This was indicated by the appearance of a second doublet for H-3 (δ 5.23) slightly different from that for H-3 (δ 5.22) in **9**. After 17 h, the β-pyranose starting material was absent, and a new less deshielded H-5 multiplet for the hydrolysis product (**11**) appeared, overlapping the H-4 multiplet (see Table I). In addition, this spectrum revealed that some elimination had occurred to form 2,4-dichlorobut-2-enal (δ 9.47 s, CHO; 7.35, t, *J* 7.2 Hz, CH; 4.58, d, *J* 7.2 Hz, CH_2). After 41 h, hydrolysis of the formic ester **9** was complete and the products were the aldehyde hydrate **11** and 2,4-dichlorobut-2-enal, in a ratio of 6/1, and formic acid. At the end of the reaction, the pH was ca. 3. An attempt to avoid the elimination side reaction by means of an acetate buffer at pH 5.4 was unsuccessful.

CONCLUSION

A number of glucose derivatives have been prepared in which several mono-deuterated isotopomers associated with the carbon-bound positions are clearly distinguished in the ^2H NMR spectrum. The isotopic discrimination is therefore greatly improved with respect to the case of glucose itself or of its pentaacetate[4]. The dibromide (**3**) and the 6-thiocyanato derivative (**4**) gave the best discrimination of the deuterium nuclei. The synthesis of at least two kinds of glucose derivatives is still necessary for determining the whole set of isotopic ratios. In its present state, the method can therefore be applied to the study of selected glucose samples resulting from typical metabolic pathways. However, in order to investigate envi-

ronmental effects, for instance, it is necessary to have access to the isotope ratios of large numbers of samples. Our search therefore continues for highly soluble derivatives which give less broad signals and hence permit shorter measurement times for the deuterium NMR spectra.

EXPERIMENTAL

General methods.—^{1}H NMR spectra were recorded, for $CDCl_3$ solutions, with a Bruker WM 250 spectrometer, and ^{2}H NMR spectra at 61.4 MHz [pulse width, 24 μs (90°) with broad band proton decoupling; acquisition time = pulse repetition time = 1.7 s] for $CHCl_3$ solutions at 31°C, with a Bruker AM 400 unless otherwise stated. Solutions contained C_6F_6 (8 g L^{-1}) for a fluorine lock, and chemical shifts were measured relative to $CHCl_3$ (δ 7.2 ppm). Spin simulations were performed with the Bruker PANIC programme.

*2,3,4,6-Tetra-*O*-acetyl-α-*D*-glucopyranosyl chloride* (**1**).—This compound, prepared by reaction of β-D-glucopyranose pentaacetate with titanium tetrachloride[12], had mp 73–74°C (lit.[12] mp 73°C); solubility in $CHCl_3$: 1.5 g in 2 mL; for ^{1}H NMR data, see Table I; for ^{2}H NMR, see Figs. 1a and 1b.

*2,3,4-Tri-*O*-acetyl-6-bromo-6-deoxy-α-*D*-glucopyranosyl bromide* (**3**).—This compound, which can be prepared from D-glucose pentaacetate in one step[6], was prepared from methyl α-D-glucopyranoside via methyl 6-bromo-6-deoxy-α-D-glucopyranoside[5], which was subjected to sequential acetolysis (to form **2**) and reaction with HBr in acetic acid[13]; mp decomposed at 170°C (lit.[6] 176°C); ^{1}H NMR (100 MHz): δ 6.64 (d, 1 H, *J* 4 Hz, H-1), 5.58 (t, 1 H, *J* 10 Hz, H-3), 5.16 (t, 1 H, *J* 10 Hz, H-4), 4.84 (dd, 1 H, *J* 4 and 10 Hz, H-2), 4.33 (m, 1 H, H-5), 3.3–3.6 (m, 2 H, H-6a + H-6b), 2.05, 2.02, 1.98 (3 s, each 3 H, Ac); solubility in $CHCl_3$; 0.5 g in 2 mL; for ^{2}H NMR (at 76.7 MHz), see Fig. 1c.

*1,2,3,4-Tetra-*O*-acetyl-6-deoxy-6-thiocyanato-α-*D*-glucopyranose* (**4**).—1,2,3,4-Tetra-*O*-acetyl-6-bromo-6-deoxy-α-D-glucopyranose (5.5 g) and sodium thiocyanate (3.25 g) in Analar DMF (55 mL) were heated at 100°C for 2.5 h. Pouring the cooled mixture into ice-cold water (200 mL) gave a precipitate. Recrystallisation from CH_2Cl_2–light petroleum (bp 60–80°C) gave the thiocyanate **4** (3.5 g, 70%), mp 180–181°C; solubility in $CHCl_3$: 0.6 g in 2 mL; for ^{1}H NMR data, see Table I; for ^{2}H NMR, see Fig. 1d. Anal. Calcd for $C_{15}H_{19}NO_9S$: C, 46.3; H, 4.9; N, 3.6. Found: C, 46.2; H, 4.9; N, 3.9.

*7,9-Diacetoxy-6a-methoxy-2-phenyl-*trans*-m-dioxano[5,4-e][1,4]dioxepane* (**7**).—Periodate oxidation of methyl 4,6-*O*-benzylidene-α-D-glucopyranoside gave the hydrated product[7] **6**, mp 143–146°C, which was shown by ^{1}H NMR data (solvent Me_2SO-d_6) to be predominantly one diastereoisomer; see Table I. The derived diacetate **7** had mp 177–179°C (lit.[8] mp 177–179°C). Spin decoupling and spin simulation were used to confirm the assignments given in Table I.

*4,6-Dichloro-4,6-dideoxy-*D*-galactose* (**8**).—This compound was prepared from methyl 4,6-dichloro-4,6-dideoxy-α-D-galactopyranoside[10] by acetolysis and deacety-

lation; for ^1H NMR data (solvent D_2O), see Table I (assignments were confirmed by spin decoupling).

Periodate oxidation of 4,6-dichloro-4,6-dideoxy-D-galactose (**8**).—4,6-Dichloro-4,6-dideoxy-D-galactose (10 mg) and sodium metaperiodate (29.6 mg) were dissolved in D_2O (1 mL) and the reaction was followed by ^1H NMR (see Table I for data on products).

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