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Note

Detection of β-D-glucofuranose in aqueous solutions of D-glucose. Application of carbon-13 Fourier-transform n.m.r. spectroscopy

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Proton nuclear magnetic resonance (n.m.r.) has been used to determine the proportions of the four cyclic anomers of various aldoses in aqueous solution, by taking advantage of the relatively resolved resonances of the anomeric hydrogen atoms[1–5]. Carbon-13 n.m.r. has yielded the anomeric composition of aqueous solutions of fructose[6] and other ketoses[6–8]. However, thus far, neither proton nor carbon-13 n.m.r. spectroscopy has yielded detectable signals from a tautomer that constitutes $\lesssim 0.5\%$ of the total concentration[4,6]. For example, although it has been established that aqueous D-glucose contains 38% α-D-glucopyranose and 62% β-D-glucopyranose (at 31° in D_2O), the furanose anomers of D-glucose have not been detected[4]. Angyal and Pickles[4] estimated that the proportion of the furanoses in aqueous D-glucose is considerably less than 1%.

In this study, we take advantage of the high resolving power of ^{13}C-n.m.r. spectroscopy of carbohydrates[9], together with the high sensitivity of the 20-mm probe in our spectrometer[10], to determine the percentage of β-glucofuranose in aqueous solutions of glucose.

Figures 1A and 1B show proton-decoupled, ^{13}C n.m.r. spectra of two samples (described later) of D-glucose having natural isotopic composition, at anomeric equilibrium in H_2O at 42°*. The specific assignments for the resonances of the pyranose forms (given in Fig. 1B) are those of Perlin and co-workers[9]. We shall assign the weak resonances at 103.8, 82.1, and 81.8 p.p.m. downfield from Me_4Si (Figs. 1A and 1B) to carbon atoms of β-glucofuranose (see later), but first we consider the possibility that these very weak signals arise from impurities.

We examined the ^{13}C-n.m.r. spectra of various samples of D-glucose of natural isotopic composition (see later) and compared these spectra with that of D-[1-^{13}C]-

*On the basis of reported data for the rates of interconversion of pyranose anomers of glucose[11], we calculate that, 30 min after dissolving crystalline α- or β-glucopyranose in water at 42°, the proportions of the pyranose anomers differ by less than 0.3% from the equilibrium values. Pyranose–furanose interconversion should be even faster than pyranose anomerization[11,12]. One of the spectral accumulations (Fig. 1B) was started about 30 min after sample preparation. In all other cases, the solutions were incubated for at least 5 h at about 40° before the start of spectral accumulation.

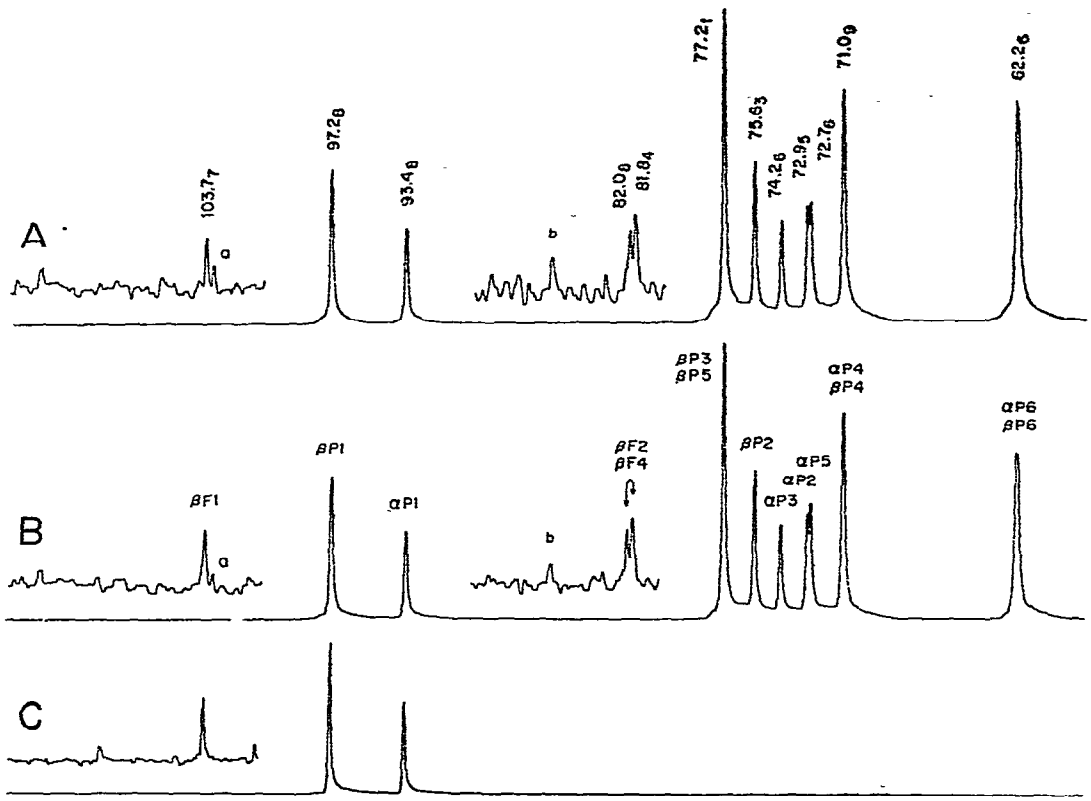

Fig. 1. Proton-decoupled ^{13}C-n.m.r. spectra of D-glucose at anomeric equilibrium in H_2O. The insets have a vertical gain 128 times that of the main spectra. (A) D-Glucose of natural isotopic composition (*sample 2*, see text), 2.0M, pH 4.4, 42°, after 180,224 accumulations with a recycle time of 1.1 sec (55 h total time). Numbers above peaks are chemical shifts in p.p.m. downfield from tetramethylsilane. (B) D-Glucose of natural isotopic composition (*sample 3*, see text), 2.8M, pH 4.7, 41°, after 98,304 accumulations with a recycle time of 1.1 sec (30 h total time). Designations above peaks are assignments. αP = α-D-glucopyranose; βP = β-D-glucopyranose; βF = β-D-glucofuranose; numbers are standard carbon atom designations. Peaks labeled *a* and *b* are either impurities or instrumental artifacts (see text). (C) D-[1-^{13}C]Glucose, 90% ^{13}C-enriched, 0.11M, pH 4.3, 41°, after 4,096 accumulations with a recycle time of 2.1 sec (2.4 h total time).

glucose (Fig. 1C). The following samples of crystalline D-glucose of natural isotopic composition were used for preparing aqueous solutions: *Sample 1*, α-D-glucose, Standard Reference Material 917 (purity $\gtrsim$99.9%) from the National Bureau of Standards (U.S.A.), used as received; *Sample 2*, same as *sample 1*, but once recrystallized into α-D-glucose[13]; *Sample 3*, β-D-glucose from Sigma Chemical Co. (U.S.A.), used as received; *Sample 4*, same as *sample 3*, but three times recrystallized into β-D-glucose[13]; *Sample 5*, α-D-glucose from Sigma, once recrystallized into α-D-glucose[13]. The spectra of Figs. 1A and 1B were obtained on solutions of *sample 2* and *sample 3*, respectively. Within experimental error, the intensities of the resonances at 103.8, 82.1, and 81.8 p.p.m. (relative to the intensities of the glucopyranose

resonances) were independent of the sample. Furthermore, the intensity of the resonance at 103.8 p.p.m. (relative to those of the anomeric carbon atoms of the pyranose forms) did not vary significantly when comparing spectra of glucose of natural isotopic composition to spectra of [1-^{13}C]glucose (Fig. 1 and Table I).

TABLE I

EQUILIBRIUM COMPOSITIONS (IN PERCENT[a]) OF VARIOUS SAMPLES OF D-GLUCOSE IN H_2O AT 43°

Sample	*Conc.* (M)	*α-Pyranose*	*β-Pyranose*	*β-Furanose*
[1-^{13}C][b]	0.11	37.5	62.4	0.13
[1-^{13}C][c]	0.11	36.9	62.9	0.15
[1-^{13}C][d]	0.24	37.5	62.4	0.14
[1-^{13}C][e]	0.24	37.2	62.6	0.13
Average[f]		37.3	62.6	0.14
2[g]	2.0	37.6 (37.9)	62.3 (61.9)	0.08 (0.13)
3[h]	2.8	38.0 (38.8)	61.9 (61.1)	0.13 (0.15)
1,2,4[i]	2.8	37.8 (38.4)	62.1 (61.5)	0.10 (0.13)
4,5[j]	4.0	40.3 (40.2)	59.5 (59.7)	0.13 (0.16)

[a]Estimated experimental error is ±1 for the percentages of pyranose anomers, ±0.02 for the percentages of β-glucofuranose in D-[1-^{13}C]glucose solutions, and ±0.04 for the percentages of β-D-glucofuranose in D-glucose solutions of natural isotopic composition. Values outside parentheses were obtained from the intensities of the anomeric carbon resonances only. Values inside parentheses are averages obtained from the resonances of carbons 1, 2, 3, and 5 of the pyranose anomers and carbons 1, 2, and 4 of β-glucofuranose (see Fig. 1B). [b]D-[1-^{13}C]Glucose at 41°. Intensities were taken from the spectrum in Fig. 1C (4,096 accumulations with a recycle time of 2.1 sec). [c]D-[1-^{13}C]Glucose at 42°, with 49,152 accumulations and a recycle time of 2.1 sec. [d]D-[1-^{13}C]Glucose at 45°, with 16,384 accumulations and a recycle time 2.2 sec. [e]D-[1-^{13}C]Glucose at 43°, with 16,384 accumulations and a recycle time of 2.2 sec. [f]Arithmetic averages of the values obtained from the four spectra of D-[1-^{13}C]glucose described in footnotes *b–e*. [g]*Sample 2* (see text) at 42°. Intensities were taken from the spectrum of Fig. 1A (180,224 accumulations with a recycle time of 1.1 sec). [h]*Sample 3* (see text) at 41°. Intensities were taken from the spectrum of Fig. 1B (98,304 accumulations with a recycle time of 1.1 sec). [i]Intensities were measured on the digital sum of spectra of three samples (each 2.8M, at 42°). Recycle time was 1.1 sec. The number of scans was 32,768 for *sample 1*, 65,536 for *sample 2*, and 32,768 for *sample 4* (sample descriptions are given in text). [j]Solution contained 5.24 g of D-glucose (*sample 4*) and 3.41 g of D-glucose (*sample 5*) (sample descriptions are given in text). Spectrum was recorded at 43°, with 81,920 accumulations and a recycle time of 1.1 sec.

On the basis of the foregoing observations and chemical-shift considerations[9], we assign the resonance at 103.8 p.p.m. (Figs. 1A, 1B, and 1C) to C-1 of a minor anomer of glucose. The chemical shifts of the resonances at 82.1 and 81.8 p.p.m. (Figs. 1A and 1B) are in the range expected for C-2 and C-4 of β-glucofuranose, on the basis of the reported chemical shifts of C-2 and C-4 of methyl β-D-glucofuranoside (80.6 and 82.3 p.p.m., respectively[14]). Furthermore, the chemical shifts of carbon atoms 2–4 of methyl α-D-glucofuranoside are all upfield of 79 p.p.m.[14]. We assign the peaks at 82.1 and 81.8 p.p.m. in Figs. 1A and 1B to C-2 and C-4 of β-D-glucofuranose, but not on a one-to-one basis. If conversion of β-D-glucofuranose into methyl β-D-glucofuranoside changes the chemical shift of C-2 more than that of C-4, then it would follow that the peak at 82.1 p.p.m. in Figs. 1A and 1B arises from C-4.

We used integrated intensities of the ^{13}C resonances of glucose solutions to determine the proportions of α-pyranose, β-pyranose, and β-furanose anomers (Table I). We took into account three common sources of errors that arise when the intensities of ^{13}C resonances are used for quantitative analysis, namely, inadequate digital resolution, differences in nuclear Overhauser enhancements, and differences in spin–lattice relaxation-times (T_1)[15]. A digital resolution of 0.46 Hz was used; the linewidths of the glucopyranose resonances were typically 2.5 Hz. It is safe to assume that all protonated carbon atoms of an aqueous carbohydrate have the full nuclear Overhauser enhancement[16] of 3.0. When the recycle time (interval between 90° radiofrequency pulses) is not infinitely long relative to T_1, the observed intensity[17] is a function of T_1:

$$I_t/I_\infty = 1 - \exp(-t/T_1), \qquad (1)$$

where I_t is the observed intensity when the recycle time is t, and I_∞ is the unattenuated intensity (which would be observed in the limit of an infinitely long recycle-time). The T_1 values of the ^{13}C resonances of the pyranose anomers of glucose solutions were measured at various concentrations, by the partially-relaxed, Fourier-transform n.m.r. method[16]. These T_1 values (Table II) were then introduced into Eq. *1* to convert the observed intensities (I_t) to unattenuated intensities (I_∞)*. The proportions of the anomers shown in Table I were computed with the use of I_∞. These proportions are not measurably different from those computed directly from the observed intensities, because the T_1 values of α-glucopyranose do not differ significantly from those of β-glucopyranose (Table II), and because we assumed that the β-glucofuranose anomer has similar T_1 values to those of the pyranose anomers. It should be noted that, in the case of 4M glucose, the recycle time was sufficiently long (relative to the T_1 values of the pyranose carbon atoms) to yield a negligible difference between I_t and I_∞. In this instance, there would be no measurable effect on the intensities of the resonances of the β-furanose anomer, even if its T_1 values differed by as much as a factor of two from those of the pyranose anomers. Although the measured T_1 values (of the pyranose anomers) exhibit a strong concentration-dependence, at any given concentration all of the T_1 values (of *methine* carbons) are practically the same (Table II). We conclude that the rotational reorientation of each pyranose anomer is fairly isotropic[16], and that the rotational correlation-time of α-glucopyranose is about the same as that of β-glucopyranose[16]. It is thus reasonable to assume that the change in molecular shape when going from a pyranose to a furanose anomer will not significantly alter the rate of rotational reorientation. Thus, we expect very similar ^{13}C T_1 values for pyranose and furanose anomers.

The values of 37.3 ±1 and 62.6 ±1% for the proportions of α-glucopyranose and β-glucopyranose, respectively, in dilute aqueous glucose at 43° are in agreement with the values of 37 ±2 and 63 ±2%, respectively, obtained by Angyal and

*We assumed that the ^{13}C T_1 values of β-glucofuranose are the same as those of β-glucopyranose (see text).

TABLE II

SPIN–LATTICE RELAXATION-TIMES (IN SECONDS[a]) OF INDIVIDUAL CARBON ATOMS OF THE PYRANOSE ANOMERS OF D-GLUCOSE IN H_2O AT 45°

Conc. (M)	*α-Pyranose*[b]				*β-Pyranose*[b]		
	1	2	3	5	1	2	3,5[c]
0.07[d]	1.98				2.07		
2.0	0.94	0.98	0.95	0.95	0.99	0.98	0.99
2.8	0.64	0.65	0.65	0.64	0.68	0.65	0.66
4.0	0.35	0.34	0.35	0.34	0.36	0.34	0.34

[a]Estimated accuracy is ±5%. [b]Column headings are standard carbon designations. T_1 values of carbons 4 and 6 are not indicated, because the resonance of each of these carbons atoms of α-D-glucopyranose is not resolved from the corresponding resonance of β-D-glucopyranose (see Fig. 1B). [c]Carbons atoms 3 and 5 of β-D-glucopyranose yield a single resonance (see Fig. 1B). [d]D-[1-^{13}C]Glucose.

Pickles[4] from proton n.m.r. measurements in D_2O at 44°. The ratio of α- to β-pyranose seems to increase slightly at high concentration of glucose (Table I).

Our results indicate that dilute aqueous glucose at 43° contains 0.14 ±0.02% of β-glucofuranose (Table I). We do not detect any resonances clearly assignable to α-glucofuranose. The very small peak labeled *a* in Figs. 1A and 1B is absent from spectra of [1-^{13}C]glucose (0.11–2.9M solutions), and is therefore not assignable to C-1 of a glucose anomer (the chemical shift of peak *a* is inconsistent with an assignment to any carbon atom other than the anomeric one[9,14]). The very small peak labeled *b* in Figs. 1A and 1B has a chemical shift inconsistent with an assignment to any carbon atom of α-glucofuranose[14]. Peaks *a* and *b* may be ^{13}C resonances of impurities, or they may be instrumental artifacts. The lack of detectable resonances of α-glucofuranose does not necessarily imply that the proportion of this anomer is significantly less than that of β-glucofuranose. The ^{13}C chemical shifts of methyl α-D-glucofuranoside[14] suggest that all of the resonances of α-glucofuranose may overlap with the relatively intense, spinning sidebands of the resonances of the pyranose anomers. However, it is possible that α-glucofuranose contributes to the peak at 81.8 p.p.m., because this peak is consistently somewhat more intense than those at 82.1 and 103.8 p.p.m. (Figs. 1A and 1B). If this is the case, then the intensities in Figs. 1A and 1B imply that the proportion of α-glucofuranose is $\lesssim 0.05\%$.

EXPERIMENTAL

Materials. — α-D-Glucose of natural isotopic composition was purchased from the National Bureau of Standards, Washington, D.C. 20234, U.S.A., and from Sigma Chemical Co., St. Louis, Missouri 63178, U.S.A. These materials were used without further purification and also after recrystallization (into α-D-glucose) by the method of Hudson and Dale[13]. β-D-Glucose was purchased from Sigma and used as received or after recrystallization (into β-D-glucose) by the method of Hudson and Dale[13].

D-[1-^{13}C]Glucose (90% enriched) was purchased from Merck Co., St. Louis, Missouri 63116, U.S.A., and used as received.

Methods. — Proton-decoupled, ^{13}C Fourier-transform, n.m.r. spectra were obtained at 15.18 MHz with the use of 20-mm, spinning sample-tubes, as described previously[10]. A spectral width of 3,788 Hz was used. Time-domain data were accumulated in 8,192 or 16,384 addresses of a Nicolet 1085 computer. Fourier transformation was carried out on 16,384 time-domain points in all cases (when only 8,192 time-domain addresses were used for data accumulation, 8,192 addresses with a zero value were placed at the end of each block of accumulated data-points). Digital broadening of 0.88 Hz was used. Chemical shifts were obtained digitally, and are reported in parts per million downfield from the ^{13}C resonance of tetramethylsilane (Me_4Si). Estimated accuracy is ± 0.05 p.p.m. For each concentration of D-glucose, dilute aqueous 1,4-dioxane (at 67.8_6 p.p.m. downfield from external Me_4Si) was used as the internal reference for the chemical shifts of the pyranose anomers (64–2048 scans per spectrum were used). No 1,4-dioxane was present in the samples used for determination of the proportion of β-D-glucofuranose; the chemical shifts of this anomer were referenced to the values of the pyranose anomers. Integrated intensities were measured digitally from spectra recorded with the use of 90° radio-frequency pulse-excitation. Spin–lattice relaxation-times of pyranose carbon resonances were measured from partially-relaxed, Fourier-transform, n.m.r. spectra[16]. At least five partially-relaxed spectra were used for each T_1 measurement. The number of accumulations per spectrum was 16 for 0.07M D-[1-^{13}C]glucose, 64 for 2M and 2.8M D-glucose, and 32 for 4M D-glucose.

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