References and Notes

(1) Previous papers in this series: (a) part 3, J. B. Hendrickson, *J. Am. Chem. Soc.*, **97**, 5763 (1975); (b) part 4, *ibid.*, **97**, 5784 (1975); (c) part 5, *Top. Curr. Chem.*, **62**, 49 (1976).

(2) In his treatment of nucleic acid synthesis design,[3] Powers used time as a unitary criterion, converting material manipulation into units of time.

(3) G. J. Powers, R. L. Jones, M. Carruthers, H. van de Fande, and H. G. Khorana, *J. Am. Chem. Soc.*, **97**, 875 (1975).

(4) A survey of about 90 natural product syntheses from N. Anand, J. S. Bindra, and S. Ranganathan, "Art in Organic Synthesis", Holden-Day, San Francisco, Calif., 1970, was taken as a basis for comparison.

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(7) The Velluz plan[5] has been adapted[6] to create C_{21} steroids by an increase of ten refunctionalization steps at the end, making it less economical. Thus L_K goes to 128 and W increases by almost tenfold to 2622.

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(10) The *order* of operations in a synthetic plan is the reverse of the rank on the plan graph, so that the order of all steps is clearly sequential in a linear plan but arbitrary for steps of equal rank on separate lines in a convergent synthesis. Thus it is unimportant in what order the four initial affixations at $I = 3$ are performed in the convergent plan IV, Figure 3, or whether all the steps on one line are performed before or after those on another before the rank at which the lines join.

(11) A very few highly branched target structures cannot have a fully convergent AP for total synthesis from $n = 1$ synthons, the simplest examples being neopentane and hexamethylethane skeletons. However, even such targets may often have perfect convergent dissections down to synthons of $n = 2$–4 when the branched $n = 4$ (isobutane) skeleton is allowed as a starting material.

(12) The affixation plans (AP) for all these derived fully convergent dissections have identical W but the full W for the CP (including cyclizations) will vary slightly from one dissection to another depending on the detailed size (n) of the intermediate being cyclized in each case.

(13) The plan graph for any bondset indicates its route through the construction grid of all possible construction combinations in ref 1a (Figure 9). The bondset itself defines λ and Δr and so the grid position R_k of the prestruct of k synthons. The order of operations on the plan graph is now a sequence of affixations and cyclizations, i.e., successive lines on the grid leading either down or to the right, respectively. With convergent syntheses several routes through the grid are equivalent,[10] one for the example in Figure 4 being $O_8 \rightarrow O_4 \rightarrow A_4 \rightarrow A_2 \rightarrow B_2 \rightarrow C_2 \rightarrow C_1 \rightarrow D_1$.

(14) The difference Δr is between the number of rings in the target skeleton (r_0) and the total rings of the starting materials,[1a] and is easily counted as the number of target skeleton rings which include any bonds of the bond set.

(15) Since normal synthons are $n = 2$–4 this places a special premium on available aromatic synthons of $n \geq 6$; not only for aromatic targets, but also for nonaromatic synthons in a target. This in turn implies the need for development of more effective methods of dearomatization.

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Detection of the Furanose Anomers of D-Mannose in Aqueous Solution. Application of Carbon-13 Nuclear Magnetic Resonance Spectroscopy at 68 MHz

David J. Wilbur, Carol Williams, and Adam Allerhand*

Contribution No. 3018 from the Department of Chemistry, Indiana University, Bloomington, Indiana 47401.

Received March 28, 1977

Abstract: Natural-abundance ^{13}C NMR spectra (at 67.9 MHz) of aqueous D-mannose (4 M in H_2O, 36 °C) yield identifiable resonances of five carbons of α-D-mannofuranose and three carbons of β-D-mannofuranose. Integrated intensities indicate the presence of 0.6 ± 0.1% α-D-mannofuranose and 0.3 ± 0.1% β-D-mannofuranose.

Proton NMR spectroscopy has been used to determine the proportions of the predominant (pyranose) anomers of various common aldohexoses in aqueous solution, by taking advantage of the relatively resolved resonances of the anomeric hydrogens.[1,2] Some aldohexoses have also yielded observable resonances for the anomeric hydrogens of the furanose anomers.[2] Notable exceptions are glucose and mannose. Angyal and Pickles[2] estimated that the proportion of the furanose forms in the anomeric equilibrium of each of these sugars in water is considerably less than 1%. Recently, ^{13}C NMR spectra (at 15 MHz) of aqueous D-glucose yielded resolved resonances of carbons 1, 2, and 4 of β-D-glucofuranose.[3] Integrated intensities yielded 0.14 ± 0.02% for the proportion of this anomer at 43 °C.[3] Carbon-13 NMR spectra of D-mannose (**1**), obtained at 15 MHz, exhibited some weak signals attributable to one or both furanose anomers, but interference from sidebands of the strong resonances of α-D-mannopyranose (**1a,** Figure 1) and β-D-mannopyranose (**1b,** Figure 1) prevented an unambiguous interpretation.[4] In this report we show that ^{13}C NMR spectra at 67.9 MHz (63.4 kG) yield identifiable resonances of five carbons of α-D-mannofuranose (**1c,** Figure 1) and three carbons of β-D-mannofuranose (**1d,** Figure 1). The intensities of these resonances yield fairly accurate values for the proportions of the furanose anomers. This study strongly suggests that the resolution and sensitivity available at high magnetic field strengths should greatly facilitate the use of natural-abundance ^{13}C NMR spectroscopy in studies of trace components of complex carbohydrate mixtures.

Experimental Section

Materials. We used three samples of **1:** Sample 1 was obtained by once recrystallizing commercial D-mannose ("Ultrex" grade from J. T. Baker Chemical Co., Phillipsburg, N.J.) into **1a**;[5] sample 2 was obtained by once recrystallizing the "Ultrex" D-mannose into **1b**;[6] sample 3 was D-mannose from Sigma Chemical Co., St. Louis, Mo., used as received.

Methods. Proton-decoupled natural-abundance ^{13}C NMR spectra were obtained at 67.9 MHz (63.4 kG) on a spectrometer consisting of a Bruker high-resolution superconducting magnet, Bruker 10-mm probe, home-built radiofrequency electronics, and a Nicolet 1085 computer. The spectrometer was not equipped with a field-frequency lock. For ^{13}C excitation, 90° radiofrequency pulses of 16 μs duration were used, and the frequency was set 118 ppm downfield from Me_4Si. Time-domain data were accumulated in 8192 addresses, with a spectral width of 73.6 ppm, 16 384 scans, and a recycle time of 1.03 s (5 h accumulation time per spectrum). Fourier transformation was done on 16 384 addresses (by adding 8192 addresses with a zero value at the end of the accumulated time-domain data points), with 0.4 or 0.8 Hz digital broadening. The ^1H irradiation (at 270 MHz) had a peak field strength of about 0.8 G (3.4 kHz). Other proton-decoupling conditions are given in the caption of Figure 2 and in footnote *d* of Table I. Chemical shifts are reported in parts per million downfield

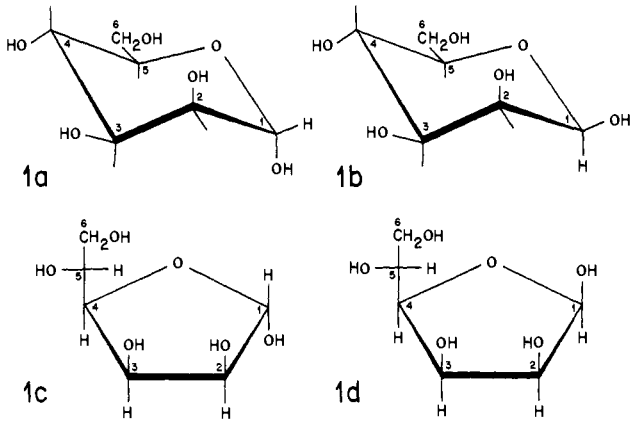

Figure 1. Structures of α-D-mannopyranose (**1a**), β-D-mannopyranose (**1b**), α-D-mannofuranose (**1c**), and β-D-mannofuranose (**1d**).

from the ^{13}C resonance of "external" Me_4Si. They were obtained digitally relative to the chemical shift of C-1 of **1a** (95.4_2 ppm), which was measured relative to internal dioxane (at 67.8_6 ppm) by means of a few scans on a separate sample. No dioxane was present in the samples used for determining the anomeric composition. Estimated accuracy of the chemical shifts is ±0.05 ppm. Integrated intensities were measured digitally.

Each freshly prepared solution of **1** was incubated at 38 °C for at least 8 h before the start of spectral accumulation (see caption of Figure 2 and footnotes *e–g* of Table I).

Results and Discussion

Figure 2 shows the proton-decoupled natural-abundance ^{13}C NMR spectrum of aqueous **1** at anomeric equilibrium in H_2O at 36 °C. Indicated assignments of the pyranose resonances (Figure 2A) are taken from the literature.[7] Before assigning any of the weak resonances (Figure 2B) to the furanose anomers, we must consider the possibility that these signals arise from impurities. We examined spectra of solutions of **1**

Table I. Equilibrium Composition (in percent[a]) of 4 M D-Mannose in H_2O at 36 °C

Sample[b]	1a	1b	1c	1d
1[c]	63.24	35.96	0.55	0.25
1[d,e]	62.67	36.36	0.70	0.27
2[d,f]	63.67	35.49	0.60	0.24
3[d,g]	62.54	36.46	0.64	0.36

[a] Percentages obtained from four separate spectra are given. The values for **1a** and **1b** were obtained from the arithmetic averages of the integrated intensities of the resonances of carbons 1–5 of each of these anomers (see Figure 2A). The percentage of **1c** was obtained from the arithmetic average of the integrated intensities of the resonances of carbons 1, 2, and 4 of this anomer (see Figure 2B). The percentage of **1d** was obtained from the arithmetic average of the integrated intensities of the resonances of carbons 1 and 4 of this anomer (see Figure 2B). [b] Sample designations refer to the types of crystalline **1** used in the preparation of each solution. Samples 1, 2, and 3 are recrystallized **1a**, recrystallized **1b**, and commercial **1**, respectively (see Experimental Section). [c] Percentages were obtained from the spectrum of Figure 2. [d] The ^1H irradiation was centered 5 ppm downfield of Me_4Si and had a noise-modulation bandwidth of 1000 Hz. [e] The solution was the same as used for Figure 2, except that spectral accumulation was started 16 h after dissolving sample 1. [f] After sample 2 was dissolved, the solution was incubated for 3 days at 38 °C before the start of spectral accumulation. [g] After sample 3 was dissolved, the solution was incubated for 8 h at 38 °C before the start of spectral accumulation.

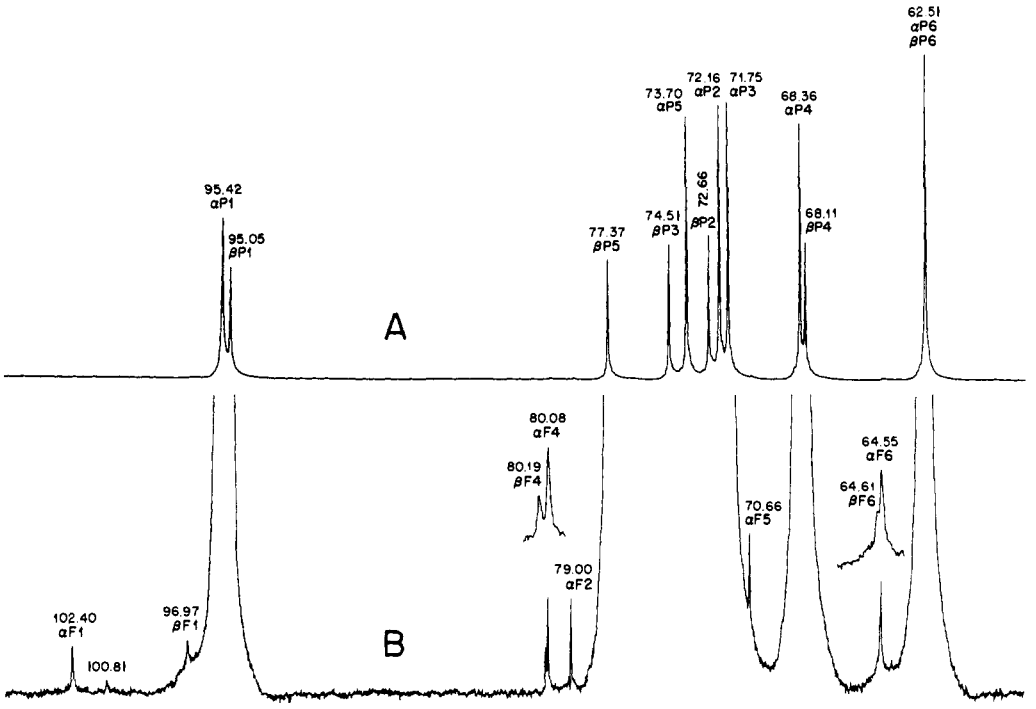

Figure 2. Proton-decoupled natural-abundance ^{13}C NMR spectrum (at 67.9 MHz) of 4 M D-mannose in H_2O at 36 °C, after 5 h of accumulation (see Experimental Section). Spectrum B is the same as A, but with a 32-fold vertical expansion. The insets in spectrum B present a fourfold horizontal expansion of the peaks directly below these insets. Spectral accumulation was initiated after the freshly prepared solution had been incubated at 38 °C for 30 h (Table I indicates that samples incubated for 8, 16, 30, and 72 h all have the same anomeric composition). The ^1H irradiation was centered 3.6 ppm downfield from the ^1H resonance of Me_4Si and had a noise-modulation bandwidth of 300 Hz. Symbols directly above the peaks indicate assignments (αF1 = C-1 of α-D-mannofuranose, etc). Numbers above indicated assignments are chemical shifts in parts per million downfield from Me_4Si. Note that the anomeric carbon resonances are somewhat broader than other peaks, as a consequence of differences in the effectiveness of proton decoupling. When the ^1H irradiation was centered 5 ppm downfield from Me_4Si (see footnote *d* of Table I), the resonances of the anomeric carbons became narrower than those of other carbons. These differences in line widths are consistent with the reported proton chemical shifts (of the pyranose anomers).[1] As expected, in each spectrum the resonance of C-1 of each anomer has essentially the same integrated intensity as other resolved resonances of that anomer.

(at anomeric equilibrium) prepared with the use of recrystallized **1a,** recrystallized **1b,** and unpurified commercial **1** (see Experimental Section). The intensity of the resonance at 100.8 ppm (Figure 2B) was about three times greater in the spectrum of the unpurified **1** than in spectra of the other two samples, while the intensities of all other resonances were independent of sample (Table I). We conclude that the peak at 100.8 ppm arises from an impurity, and that the other weak resonances of Figure 2B may arise from minor anomers of **1.** The chemical shifts of these resonances are fully consistent with their assignment to the furanose anomers (see below).

Perlin and co-workers have reported the ^{13}C chemical shifts of methyl α-D-mannofuranoside and methyl β-D-mannofuranoside.[8] Consider first carbons 2–6. The chemical shifts of these carbons should not change appreciably when going from a methyl furanoside to the free furanose. On this basis, the resonance at 79.0_0 ppm is assigned to C-2 of **1c.** The resonances at 80.0_8 and 80.1_9 ppm (Figure 2B) are assigned to C-4 of **1c** and **1d,** but not on a one-to-one basis. However, the resonance at 80.0_8 ppm has the same intensity as that at 79.0_0 ppm and is therefore assigned to C-4 of **1c.** The resonance at 70.6_6 ppm is assigned to C-5 of **1c.**[8] The two resonances at about 64.6 ppm have chemical shifts charateristic of C-6 of furanose forms of aldohexoses.[8] On the basis of their intensities (relative to those of the resonances assigned above to C-2 and C-4 of **1c** and to C-4 of **1d**), we assign the peak at 64.5_5 ppm to C-6 of **1c** and the shoulder at 64.6_1 ppm to C-6 of **1d.** The resonance of C-3 of **1c** and those of carbons 2, 3, and 5 of **1d** are not identified in our spectra. This result is consistent with the expected close proximity of these resonances to the intense signals of pyranose anomers.[8]

On the basis of a comparison of the ^{13}C chemical shifts of β-D-glucofuranose,[3] α-D-galactofuranose,[4] and β-D-galactofuranose[4] with those of the corresponding methyl furanosides,[8] we expect that the resonance of C-1 of each methyl mannofuranoside will be shifted about 6 to 8 ppm upfield upon conversion to the free mannofuranose. The peak at 102.4_0 ppm (Figure 2B) is 7.3 ppm upfield from the reported chemical shift of C-1 of methyl α-D-mannofuranoside.[8] The peak at 96.9_7 ppm (Figure 2B) is 6.6 ppm upfield from the reported chemical shift of C-1 of methyl β-D-mannofuranoside.[8] Therefore, we assign the resonances at 102.4_0 and 96.9_7 ppm to the anomeric carbons of **1c** and **1d,** respectively. The intensities of these resonances are consistent with those of other peaks assigned above to each furanose anomer (see caption of Figure 2).

We used integrated intensities to determine the proportion of each anomer (Table I). We took into account possible systematic errors caused by inadequate digital resolution, differences in spin–lattice relaxation times (T_1), and differences in nuclear Overhauser enhancements (NOE).[9] Digital resolution was 0.6 Hz, and line widths were typically 3 Hz (but 4 to 6 Hz for the resonances of the anomeric carbons under the proton-decoupling conditions of Figure 2). As for the effect of T_1, our recycle time (interval between successive 90° radiofrequency excitation pulses) of 1.0 s was at least three times the T_1 value of each pyranose carbon: The PRFT method[10] yielded a T_1 of about 0.2 s for C-6 and about 0.3 s for all methine carbons of the pyranose anomers (4 M mannose in H_2O, 37 °C). It is reasonable to assume that the T_1 values of **1c** and **1d** are not very much longer than those of **1a** and **1b.** Therefore, differences in T_1 values should not be a significant source of systematic errors in our determination of the anomeric composition of **1.**[3] In considering the effect of the NOE, it is safe to assume that at low magnetic field strengths (such as 14 kG) all protonated carbons of an aqueous monosaccharide (or small oligosaccharide) have the full NOE of 3.0.[10,11] The extrapolation of this statement to ^{13}C NMR studies at 63 kG is subject to the assumptions that chemical shift anisotropy is not a significant relaxation mechanism for ^{13}C resonances of carbohydrates at 63 kG,[12] and that the "extreme narrowing condition" is still applicable.[9] The first assumption can be readily justified for all carbons of pyranose and furanose anomers (but not for the anomeric carbon of the *linear* form of a ketose), on the basis of published data.[12] Our measured T_1 values (see above) indicate that the NOE of all ^{13}C resonances of **1** should be about 2.9.[13] This expectation is supported by the observation that each of the 11 protonated carbons of sucrose (1 M in H_2O at 38 °C) has an NOE of 2.9 ± 0.1 at 63.4 kG.[13]

Our values of 63 ± 1 and 36 ± 1% for the proportions of **1a** and **1b,** respectively, in 4 M D-mannose in H_2O at 36 °C (arithmetic averages of the values in Table I) are in excellent agreement with the values of 65.5 ± 2 and 34.5 ± 2%, respectively, obtained by Angyal and Pickles[2] from proton NMR measurements in D_2O at 44 °C. Our results indicate the presence of 0.6 ± 0.1% α-D-mannofuranose and 0.3 ± 0.1% β-D-mannofuranose in aqueous D-mannose at 36 °C (Table I).

Acknowledgment. This work was supported by the National Science Foundation (Grant CHE 76-17572) and the U.S. Public Health Service (Grant GM 22620-01).

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