Carbohydrate Research, 208 (1990) 23–35
Elsevier Science Publishers B.V., Amsterdam

(1-^{13}C)Alditols: elimination of magnetic equivalence in ^{1}H- and ^{13}C-n.m.r. spectra of symmetric compounds through (^{13}C)-substitution

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(Received March 15th, 1990; accepted with revision June 18th, 1990)

ABSTRACT

(1-^{13}C)Glycerol, D-(1-^{13}C)arabinitol, D-(1-^{13}C)ribitol, D-(1-^{13}C)xylitol, D-(1-^{13}C)glucitol, D-(1-^{13}C)mannitol, and D-(1-^{13}C)talitol have been prepared by $NaBH_4$ reduction of the corresponding (1-^{13}C)aldoses. A comparison of the ^{1}H- (300 and 620 MHz) and ^{13}C (75 MHz) n.m.r. spectra of natural and (1-^{13}C)-substituted dissymmetric alditols has permitted the unequivocal assignments of their hydroxymethyl proton and carbon signals and the measurement of several ^{13}C–^{1}H and ^{13}C–^{13}C spin-coupling constants. Similar spectra of (1-^{13}C)-substituted symmetric alditols, however, are more difficult to interpret since they are composed of overlapping ^{13}C-coupled and ^{13}C-noncoupled subspectra. In some cases, ^{1}H difference spectra and ^{1}H-coupled ^{13}C spectra may be used to extract the ^{13}C–^{1}H and ^{13}C–^{13}C spin couplings from the ^{13}C-coupled component. These couplings have been examined in light of conformational models previously proposed, permitting a preliminary evaluation of standard $^{3}J_{CH}$ and $^{3}J_{CC}$ values for specific coupling pathways in these compounds.

INTRODUCTION

Alditols (polyols) are acyclic carbohydrate derivatives commonly prepared by chemical[1], catalytic[2], or electrolytic[3] reduction of aldoses and ketoses. Polyols have widespread commercial applications. For example, they are used as artificial sweeteners, and as starting materials in the manufacture of emulsifying agents, explosives, pharmaceuticals, and antitumor agents. Alditols also play key roles in biological processes. Glycerol (**1**), D-glucitol (**2**, D-sorbitol), and other alditols mediate the cold tolerance of many animals and are frequently found in high concentrations in the hemolymph and tissues of freeze-sensitive (unable to survive freezing) and freeze-tolerant (able to survive freezing) organisms[4–6a]. In freeze-sensitive species, polyols, sometimes present in 1–5M concentrations, function as antifreezes, whereas in freeze-tolerant animals, they function as cryoprotectants to inhibit freeze damage. Apparently these compounds promote freeze tolerance by lowering the freezing point of intracellular water, increasing intracellular osmotic pressure to reduce cell dehydration during extracellular freezing, protecting and stabilizing protein and membrane structure at low temperatures, and modifying ice crystal growth in the extracellular compartment[6a]. Polyols are used routinely in the cryopreservation of biological materials[6b].

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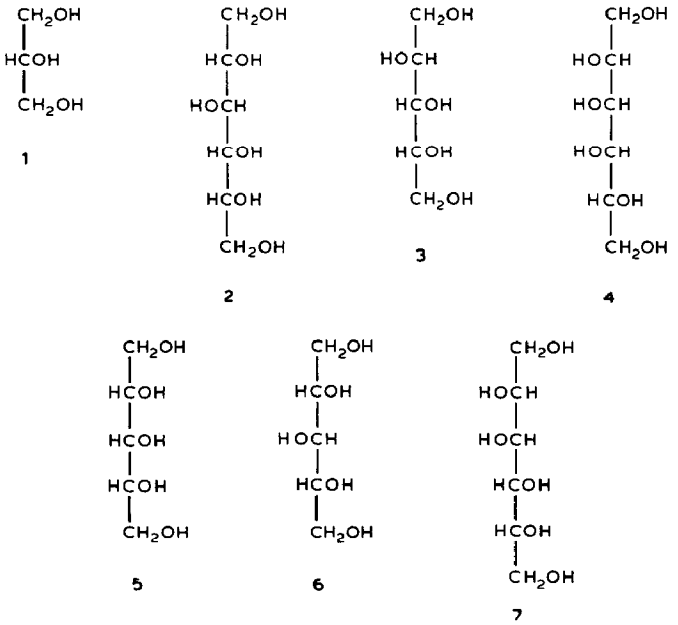
0008-6215/90/$ 03.50

In humans, **2** is produced in the eye lens of diabetic patients through the action of the enzyme aldose reductase, on D-glucose[7,8]. Apparently the unnaturally high intracellular concentration of glucose in this tissue makes its reduction to **2** more favorable. The buildup of D-glucitol (**2**) in the eye lens, and specific covalent modifications of the lens protein, crystallin[9], are believed to be key factors in the formation of cataracts in diabetic patients. Phosphate esters of the alditols play important roles in biological metabolism; for example, -L-glycerol-3-phosphate is a precursor in the biosynthesis of phosphoglycerides. From a chemical standpoint, the alditols are useful model compounds to study the conformational properties of acyclic carbohydrates and the structural factors that stabilize or destabilize potential conformations. Several studies have reported on the solution conformations of these molecules[10–15].

Interest in studying *in vivo* polyol metabolism in cold-adapted insects with the use of (^{13}C)-substituted compounds and n.m.r. spectroscopy[16,17] prompted a fundamental ^{1}H- and ^{13}C-n.m.r. study of (1-^{13}C)alditols in order to assess the effect of ^{13}C-substitution on their spectral properties. Among the C_4 (tetritols), C_5 (pentitols), and C_6 (hexitols) alditols[18], only three compounds [D-arabinitol (**3**), D-glucitol (**2**), and D-talitol (**4**)] lack molecular symmetry, and ^{13}C-substitution affects their ^{1}H- and ^{13}C-n.m.r. spectra in a predictable fashion. However, ^{13}C-substitution complicates the ^{1}H- and ^{13}C-n.m.r. spectra of symmetric alditols [*e.g.*, the glycerol (**1**), ribitol (**5**), xylitol (**6**), D-mannitol (**7**)] by effectively removing magnetic equivalence to create composite spectra composed of two overlapping subspectra. This paper describes this effect and discusses several n.m.r. methods that may be used to interpret these spectra in order to measure ^{13}C–^{1}H and ^{13}C–^{13}C spin-coupling constants of potential value to conformational studies.



EXPERIMENTAL

Chemicals and reagents. — D-Arabinose, D-ribose, D-xylose, D-glucose, and D-mannose, and Dowex ion-exchange resins were purchased from Sigma Chemical Company. D-Talose and the following (1-^{13}C)aldoses[19] were obtained as a gift from Omicron Biochemicals, Inc.: DL-(1-^{13}C)glyceraldehyde, D-(1-^{13}C)arabinose, D-(1-^{13}C)ribose, D-(1-^{13}C)xylose, D-(1-^{13}C)altrose, D-(1-^{13}C)glucose, D-(1-^{13}C)mannose, and D-(1-^{13}C)talose. Sodium borohydride ($NaBH_4$) was purchased from Aldrich Chemical Company. Deuterium oxide (^2H_2O, 98 atom-% ^2H) was obtained from Cambridge Isotope Laboratories. All other chemicals were reagent grade and were used without futher purification.

Preparation of (^{13}*C)alditols.* — The parent natural or (1-^{13}C)aldose (0.5 g) was dissolved in distilled water to give a final concentration of 0.1M. The solution pH was adjusted to pH 10.5 with dropwise addition of 1M NaOH, and $NaBH_4$ in two-fold molar excess was added batchwise. The solution was left at room temperature for 3 h, after which time glacial acetic acid was added dropwise to decompose unreacted $NaBH_4$. The reaction mixture was treated batchwise with excess Dowex-50 × 8 (200–400 mesh) [H^+] ion-exchange resin, the resin was removed by vacuum filtration, and the acidic filtrate was concentrated to a syrup at 30° *in vacuo*. The resulting syrup was dissolved in methanol (~30 mL), and the solution was evaporated to a syrup *in vacuo* at 30°. Evaporation from methanol was repeated four times to remove the boric acid as methyl-borate. The residue was dissolved in distilled water (~30 mL), and the solution was treated batchwise and separately with Dowex-1 × 8 (200–400 mesh) [OAc^-] and Dowex-50 × 8 (200–400 mesh) [H^+] ion-exchange resins. After removal of the resins, the resulting deionized filtrate was concentrated to a syrup *in vacuo* at 30°.

Instrumentation. — ^{1}H- (300 MHz) and ^{13}C- (75 MHz) n.m.r. spectra were obtained at 25° using a Nicolet NT-300 F.t.-n.m.r. spectrometer equipped with quadrature-phase detection and a 293B pulse programmer. Partially-relaxed ^{1}H-n.m.r. spectra were obtained by the inversion-recovery method[20]. High-resolution rapid-scan cross-correlation ^{1}H-n.m.r. spectra at 620 MHz were obtained at the NMR Facility for Biomedical Studies, Department of Chemistry, Carnegie-Mellon University, Pittsburgh, PA, which is partly supported by NIH grant P41RR00292.

Spectral resolution was enhanced by multiplying f.i.d.s by a double-exponential function prior to Fourier-transformation. The function was selected empirically to optimize spectral resolution and signal-to-noise ratios.

Alditol concentrations were ~50mM and ~0.4M for ^{1}H- and ^{13}C-n.m.r. analyses, respectively, in total solution volumes of 0.5 and 2.0 mL of ^2H_2O, respectively.

RESULTS AND DISCUSSION

Effect of ^{13}*C-substitution on the* ^{1}*H- and* ^{13}*C-n.m.r. spectra of dissymmetric alditols* **2–4**. — The ^{1}H- and ^{13}C-n.m.r spectra of the dissymmetric alditols **2–4** in ^2H_2O contain distinct signals for the non-exchangeable protons and carbons, respectively, in these

molecules. Thus, the ^{1}H-n.m.r. spectrum of D-arabinitol (**3**, Fig. 1A) contains seven multiplets between 3.55–3.95 p.p.m. due to H-1, H-1′, H-2, H-3, H-4, H-5, and H-5′ (throughout this paper the primed hydroxymethyl proton is defined as the more shielded proton of each pair). ^{13}C-Substitution at C-1 causes a large splitting of the H-1 and H-1′ multiplets due to one-bond ^{13}C–^{1}H coupling ($^1J_{C\text{-}1,H\text{-}1} \sim 142$ Hz) (Fig. 1B); thus, a comparison of the ^{1}H-n.m.r. spectra of natural and (1-^{13}C)-substituted dissymmetric alditols allows the terminal diastereotopic hydroxymethyl protons in **2–4** to be distinguished and their chemical shifts assigned (Table I). Depending on the compound, smaller splittings (<5 Hz) may also be observed at H-2 and H-3 due to two-bond ($^2J_{C\text{-}1,H\text{-}2}$) and three-bond ($^3J_{C\text{-}1,H\text{-}3}$) ^{13}C–^{1}H spin coupling (Fig. 1B).

The hydroxymethyl carbon signals in the ^{13}C-n.m.r. spectra of **2–4** may be assigned unequivocally (Table I) by comparing spectra of the natural compound with that of the natural compound mixed with a small quantity of (1-^{13}C)-substituted derivative and noting which hydroxymethyl carbon signal is enhanced in intensity. In

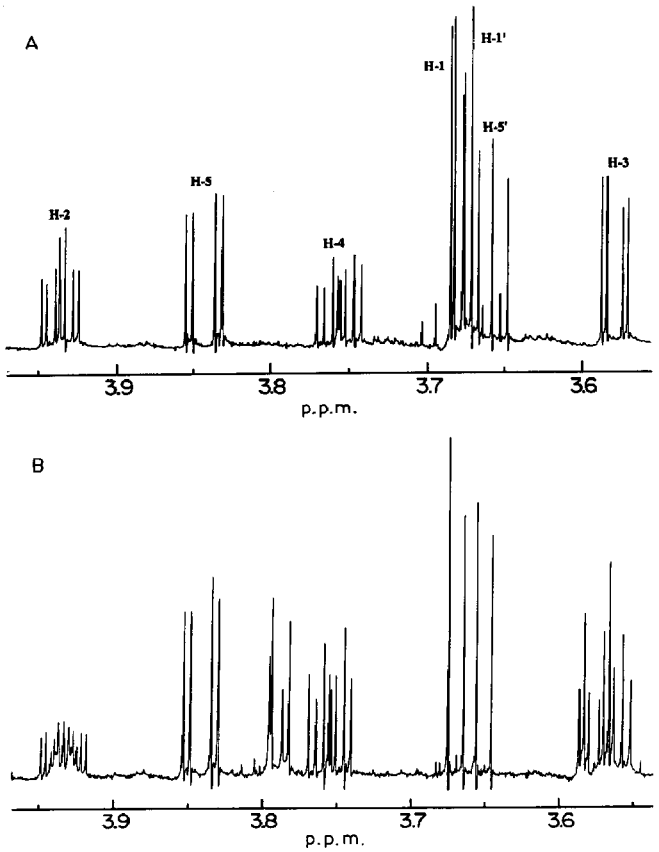


Fig. 1. (A) The 620 MHz ^{1}H-n.m.r. spectrum of the dissymmetric pentitol, D-arabinitol (**3**), showing resonance assignments of the H-1, H-1′, H-2, H-3, H-4, H-5, and H-5′ signals. (B) The 620 MHz ^{1}H-n.m.r. spectrum of D-(1-^{13}C)arabinitol. The H-4, H-5 and H-5′ signals are unaffected by ^{13}C at C-1, while those of H-1, H-1′, H-2, and H-3 show an additional splitting due to $^1J_{C,H}$, $^2J_{C,H}$, and $^3J_{C,H}$.

TABLE I

Proton and carbon signal assignments[a] of the hydroxymethyl protons and carbons of the dissymmetric alditols in ^2H_2O

Nucleus	D-*arabinitol* (3)	D-*glucitol* (2)	D-*talitol* (4)
H-1	~3.676	3.744	3.817
H-1′	~3.676	3.630	3.689
H-5	3.844		
H-5′	3.658		
H-6		3.836	~3.675
H-6′		3.660	~3.675
C-1	64.5	63.9	63.5
C-5	64.4		
C-6		64.3	64.5

[a] ^{1}H and ^{13}C chemical shifts are expressed in p.p.m.: ^{1}H, relative to the internal HOD signal (4.800 p.p.m.) and accurate to ±0.002 p.p.m.; ^{13}C, relative to the C-1 signal of α-D-(1-^{13}C) mannopyranose (95.5 p.p.m.) and accurate to ±0.1 p.p.m.

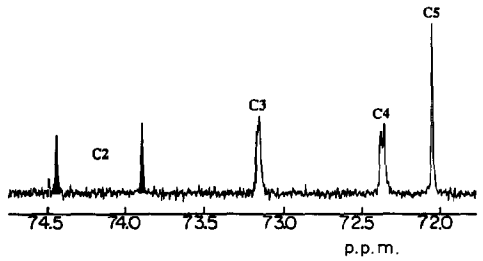


Fig. 2. The partial 75 MHz ^{13}C-n.m.r. spectrum of the dissymmetric hexitol, D-(1-^{13}C)talitol (4), showing the presence of ^{13}C–^{13}C spin-couplings of C-1 to C-2 ($^1J_{C,C}$), C-3 ($^2J_{C,C}$), and C-4 ($^3J_{C,C}$).

addition, the C-2 signals in **2–4** may be assigned by observing the large one-bond ^{13}C-^{13}C coupling constant (~41 Hz) in the ^{13}C-n.m.r. spectra of the (1-^{13}C)alditols (Fig. 2). Longer-range ^{13}C–^{13}C couplings may also be observed, depending on conformation (Figure 2, see below). ^{13}C-Resonance assignments based on spectra of the (1-^{13}C)alditols confirm those reported previously[21].

Effect of ^{13}C-substitution on the ^{1}H-n.m.r. spectrum of glycerol (**1**). — The ^{1}H-n.m.r. spectrum (300 MHz) of glycerol (**1**, Fig. 3A) contains three multiplets for H-1/H-3, H-2, and H-1′/H-3′, and a first-order analysis of the H-1/H-3 and H-1′/H-3′ multiplets in this spectrum gives the following ^{1}H–^{1}H spin couplings: $^2J_{H\text{-}1,H\text{-}1'} = {}^2J_{H\text{-}3,H\text{-}3'} = 11.7$ Hz, $^3J_{H\text{-}1,H\text{-}2} = {}^3J_{H\text{-}3,H\text{-}2} = 4.3$ Hz, $^3J_{H\text{-}1',H\text{-}2} = {}^3J_{H\text{-}3',H\text{-}2} = 6.5$ Hz.

The introduction of a ^{13}C label at C-1 of **1** significantly complicates the ^{1}H-n.m.r. spectrum, but interpretation is possible at 620 MHz (Fig. 3B). Even at 620 MHz, however, the spectrum is not completely first-order, but the target ^{13}C–^{1}H couplings can be obtained with confidence without resort to computer simulation. In (1-^{13}C)glycerol, H-1 and H-1′ experience large one-bond couplings to C-1 ($^1J_{C\text{-}1,H\text{-}1} = 145.0$ Hz, $^1J_{C\text{-}1,H\text{-}1'}$

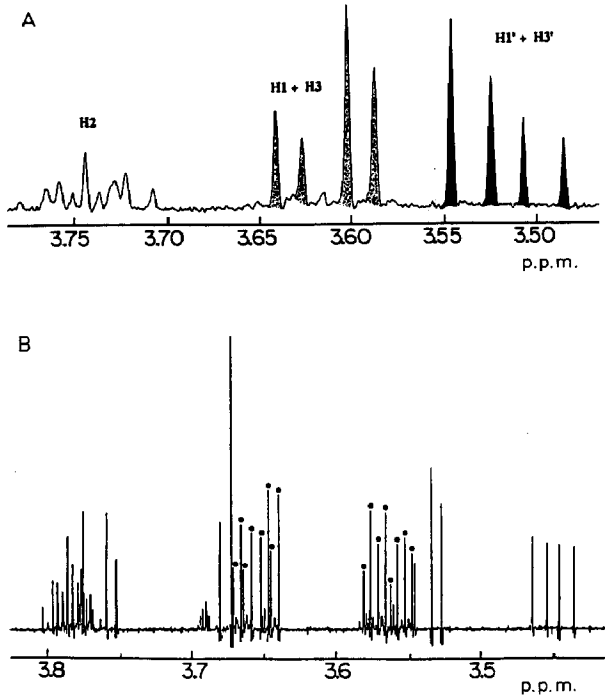


Fig. 3. (A) The 300 MHz ^{1}H-n.m.r. spectrum of glycerol (**1**) showing signal assignments for H-1/H-3, H-2 and H-1′/H-3′. (B) The 620 MHz ^{1}H-n.m.r. spectrum of (1-^{13}C)glycerol. The signals marked by dots are those of H-3 and H-3′ which are split by ^{13}C at C-1 ($^3J_{CH}$). The H-1 and H-1′ signals are displaced from those of H-3 and H-3′, respectively, due to their strong coupling to ^{13}C at C-1 ($^1J_{C,H}$).

= 145.3 Hz), while H-3 and H-3′ experience smaller three-bond couplings to C-1 ($^3J_{C\text{-}1,H\text{-}3}$ = 3.2 Hz, $^3J_{C\text{-}1,H\text{-}3'}$ = 2.9 Hz). Thus, ^{13}C-substitution effectively eliminates the magnetic equivalencies of H-1/H-3 and H-1′/H-3′, and all four hydroxymethyl protons are individually observed.

Effect of ^{13}C-substitution on the ^{1}H-n.m.r. spectra of symmetric pentitols **5** *and* **6**. — Ribitol (**5**) and xylitol (**6**) are symmetric (meso) compounds, and consequently their ^{1}H- and ^{13}C-n.m.r. spectra are simpler than those of D-arabinitol (**3**). ^{1}H spectra of **5** (Fig. 4A) and **6** (Fig. 6A) contain four multiplets due to H-1/H-5, H-2/H-4, H-3, and H-1′/H-5′. In **5**, the H-2/H-4 and H-1/H-5 multiplets, and the H-3 and H-1′/H-5′ multiplets, partially overlap in spectra obtained at 300 MHz, but all four multiplets are resolved in partially-relaxed spectra (Fig. 4B) since in alditols the spin-lattice relaxation times (T_1s) of the hydroxymethyl protons are usually smaller than those of the methine protons.

The introduction of ^{13}C at C-1 of **5** significantly complicates ^{1}H-n.m.r. spectral data (Fig. 5A). In symmetric pentitols, C-1 is coupled to H-1 and H-1′, and potentially to H-2 and H-3, whereas H-4, H-5 and H-5′ will be unperturbed. Since the latter three protons are magnetically equivalent to H-2, H-1, and H-1′, respectively, the ^{1}H spectrum of the (1-^{13}C)pentitol will be composed of two overlapping subspectra, one

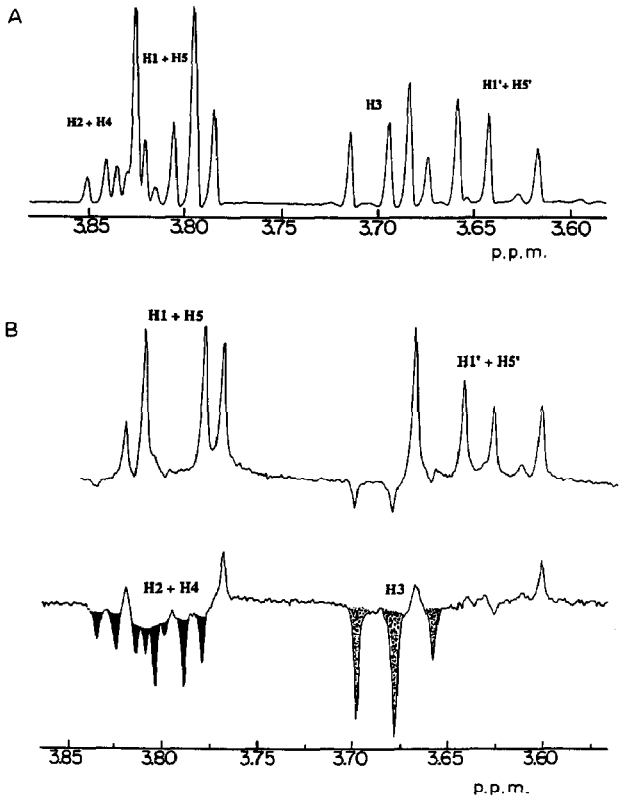


Fig. 4. (A) The 300 MHz ^{1}H-n.m.r. spectrum of ribitol (**5**), showing the overlapping signals of H-2/H-4 and H-1/H-5, and H-3 and H-1′/H-5′. (B) Partially-relaxed ^{1}H-n.m.r. spectra of **5** obtained by inversion recovery with τ values of 0.7 s (bottom) and 1.2 s (top). Careful selection of τ allows the selective detection of the methine protons, H-2, H-3, and H-4 (bottom spectrum) and the hydroxymethyl protons, H1/H5 and H1′/H5′ (top spectrum).

^{13}C-coupled and the other non-^{13}C-coupled. As shown in Fig. 5A, H-1′, being directly bonded to C-1, is coupled strongly to the ^{13}C ($^1J_{C\text{-}1,H\text{-}1}$ = 143.8 Hz), and H-1′ quartets are observed at 3.35 and 3.85 p.p.m. Since H-5′ is far removed (*i.e.*, five bonds) from C-1, it will not couple to C-1, and its quartet remains at 3.60 p.p.m. Similar behavior is observed for H-1 and H-5, with only H-1 coupled to C-1 ($^1J_{C\text{-}1,H\text{-}1}$ = 142.5 Hz). The H-3 signal appears as a sextet (Fig. 5A) since H-3 is coupled to H-2/H-4 (6.0 Hz) and C-1 (3.7 Hz). The H-2/H-4 multiplets [one potentially coupled to C-1 ($^2J_{C\text{-}1,H\text{-}2}$), the other not] are superimposed at ~3.76 p.p.m. This superimposition makes it difficult to determine $^2J_{C\text{-}1,H\text{-}2}$ by a direct inspection of the 1D spectrum.

$^2J_{C\text{-}1,H\text{-}2}$ in **5** may be obtained, however, by analyzing the ^{1}H-coupled ^{13}C-n.m.r. spectrum of D-(1-^{13}C)ribitol (Fig. 5B). In this spectrum, only the enriched carbon (C-1) is observed, and signal multiplicity is determined by the ^{13}C–^{1}H couplings between it and nearby protons (H-1, H-1′, H-2, H-3). Since $^1J_{C\text{-}1,H\text{-}1}$, $^1J_{C\text{-}1,H\text{-}1'}$, and $^3J_{C\text{-}1,H\text{-}3}$ were obtained from an analysis of the ^{1}H spectrum, these values can be used to calculate the ^{1}H-coupled

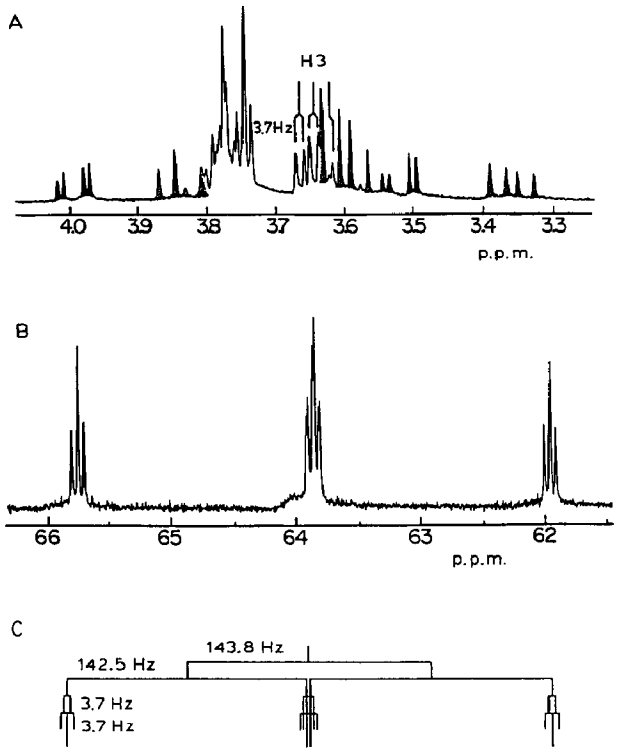


Fig. 5. (A) The 300 MHz ^{1}H-n.m.r. spectrum of D-(1-^{13}C)ribitol (**5**). Hatched signals are those of H-1′/H-5′, and filled signals are those of H-1. The sextet at ~3.65 p.p.m. is H-3, which is coupled to C-1 ($^3J_{C\text{-}1,H\text{-}3}$ = 3.7 Hz). The H-2 signal is buried at ~3.76 p.p.m. (B) The ^{1}H-coupled ^{13}C-n.m.r. spectrum of D-(1-^{13}C)ribitol (**5**). (C) The manual simulation of (B) using $^1J_{C,H}$ and $^3J_{C,H}$ values obtained from (A). This analysis permits $^2J_{C\text{-}1,H\text{-}2}$ to be determined from the outer triplets (see text).

^{13}C spectrum (Fig. 5C). This analysis shows that the observed triplet pattern for the outer multiplets of this spectrum can only be generated when $^2J_{C\text{-}1,H\text{-}2} = {}^3J_{C\text{-}1,H\text{-}3}$. Thus, $^2J_{C\text{-}1,H\text{-}2}$ = 3.7 Hz. It should be appreciated that the outer multiplets of the spectrum in Fig. 5B contain information about $^2J_{C\text{-}1,H\text{-}2}$ and $^3J_{C\text{-}1,H\text{-}3}$ only. The center multiplet contains information about these couplings and the *difference* between $^1J_{C\text{-}1,H\text{-}1}$ and $^1J_{C\text{-}1,H\text{-}1'}$, and thus it is less useful in determining $^2J_{C\text{-}1,H\text{-}2}$.

The ^{1}H-n.m.r. spectra of xylitol (**6**) and D-(1-^{13}C)xylitol are shown in Fig. 6. As observed for **5**, the incorporation of ^{13}C at C-1 causes H-1 and H-1′ to be split ($^1J_{CH}$), whereas H-5 and H-5′ are unperturbed. The H-3 signals overlap those of H-5′, while the H-2 signals overlap those of H-4. Since H-5′ and H-4 are not coupled to C-1, the H-2 and H-3 multiplets could be resolved by subtracting the ^{1}H spectrum of **6** from that of D-(1-^{13}C)xylitol. The resulting difference spectrum for H-2 (Fig. 7A) reveals a twelve-line multiplet containing spin couplings to H-1, H-1′, H-3, and C-1. Comparison with the H-2 sextet in the unenriched spectrum gives $^2J_{C\text{-}1,H\text{-}2}$ = 4.2 Hz. The difference spectrum of H-3 (Fig. 7B) gives a sextet with $^3J_{C\text{-}1,H\text{-}3}$ = 2.2 Hz. The ^{1}H-coupled ^{13}C-n.m.r. spectrum of D-(1-^{13}C)xylitol confirmed these $J_{C,H}$ values.

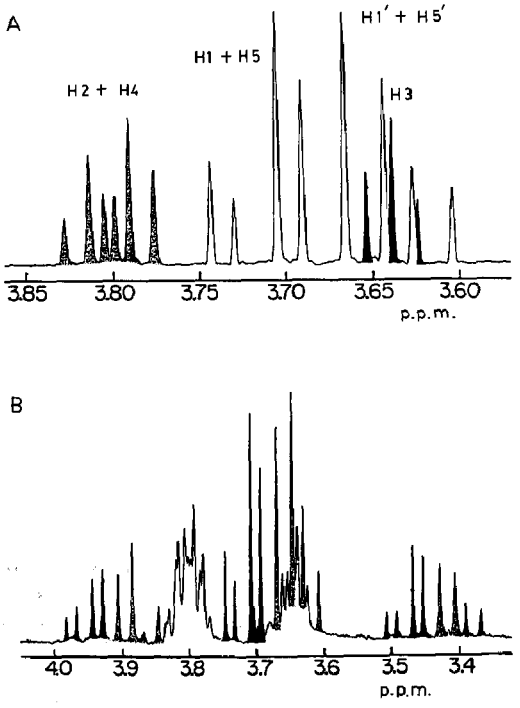


Fig. 6. (A) The 300 MHz ^{1}H-n.m.r. spectrum of xylitol (**6**) showing signal assignments for H-1/H-5, H-1′/H-5′, H-2/H-4, and H-3. (B) The 300 MHz ^{1}H-n.m.r. spectrum of D-(1-^{13}C)xylitol. Hatched signals are those of H-1′/H-5′, and filled signals are those of H-1/H-5. The H-2/H-4, and H-3 multiplets are obscured at ~3.80 and ~3.65 p.p.m., respectively.

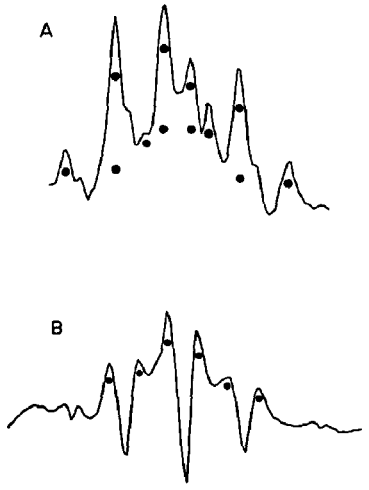


Fig. 7. (A) The ^{1}H-n.m.r. difference spectrum of the H-2/H-4 multiplet in Figure 6B. The 12-line pattern gives $^2J_{C\text{-}1,H\text{-}2}$ = 4.2 Hz. (B) The ^{1}H-n.m.r. difference spectrum of the H-3 multiplet in Figure 6B. The sextet gives $^3J_{C\text{-}1,H\text{-}3}$ = 2.2 Hz.

TABLE II

^{13}C–^{13}C Spin-coupling constants[a] in some alditols in ^2H_2O

Compound	*Coupled carbons*			*Preferred conformation*[b]
	1,2	1,3	1,4	
Glycerol (**1**)	41.2			
D-Arabinitol (**3**)	42.3	1.6	2.3	e
Ribitol (**5**)	41.4	1.1	1.7[c]	e,b
Xylitol (**6**)	41.6	1.8	2.0	e,b
D-Glucitol (**2**)	41.3	2.3	1.2	b
D-Talitol (**4**)	41.4	1.0	1.4	b
D-Mannitol (**7**)	41.5	1.7[d]	1.7[d]	e

[a] In Hz, accurate to ±0.1 Hz. [b] e = extended; b = bent. [c] Tentative coupling. [d] Overlapping doublets.

Effect of ^{13}C-substitution on the ^{13}C-n.m.r. spectra of symmetric alditols. — The ^{13}C-n.m.r. spectra of symmetric pentitols contain three signals from C-1/C-5, C-2/C-4, and C-3. Upon substitution of ^{13}C at C-1, the magnetic equivalence of C-2 and C-4 is removed (Fig. 8A) due to the large one-bond ^{13}C–^{13}C coupling at C-2, allowing the measurement of $^3J_{C\text{-}1,C\text{-}4}$. Thus, all available ^{13}C–^{13}C spin couplings involving C-1 can be determined in symmetric pentitols, and these couplings in D-(1-^{13}C)ribitol and D-(1-^{13}C) xylitol are listed in Table II.

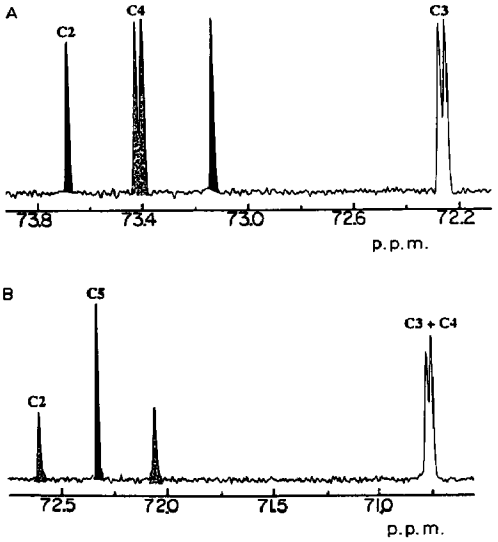


Fig. 8. (A) The partial 75 MHz ^{13}C-n.m.r. spectrum of D-(1-^{13}C)xylitol (**6**), showing splitting of the C-2, C-3, and C-4 signals by ^{13}C at C-1. (B) The partial 75 MHz ^{13}C-n.m.r. spectrum of D-(1-^{13}C)mannitol (**7**), showing splitting of the C-2 signal ($^1J_{C,C}$). The overlapping C-3 and C-4 signals prevent an evaluation of $^2J_{C\text{-}1,C\text{-}3}$ and $^3J_{C\text{-}1,C\text{-}4}$ (see text).

In contrast to the symmetric pentitols, the complete interpretation of the ^{13}C-n.m.r. spectra of symmetric hexitols is not straightforward. For example, in D-(1-^{13}C) mannitol (**7**), C-1 is coupled to C-2 ($^{1}J_{C,C}$), and potentially coupled to C-3 ($^{2}J_{C,C}$) and C-4 ($^{3}J_{C,C}$) (Fig. 8B). The C-2 and C-5 signals can be readily distinguished, but those of C-3 and C-4 overlap. For D-(1-^{13}C)mannitol, the C-3/C-4 signal appears as a doublet (Fig. 8B). This doublet could be produced if (*a*) C-3 and C-4 are *not* coupled to C-1, but their chemical shifts are different due to differential ^{13}C isotope shift effects at these two carbons, or (*b*) both C-3 and C-4 have similar couplings to C-1. A third possibility, namely, that only one of the two carbons is coupled to C-1, can be excluded since in this case the signal would appear as a triplet with the outer signals arising from the coupled carbon and the center signal arising from the non-coupled carbon. A 1D INADEQUATE[19,22] spectrum obtained on D-(1-^{13}C)mannitol (data not shown) revealed an out-of-phase doublet for the C-3/C-4 signal, thus eliminating option (*a*). Thus, it is likely both C-3 and C-4 are coupled to C-1 in **7**, but $^{2}J_{C\text{-}1,C\text{-}3}$ and $^{3}J_{C\text{-}1,C\text{-}4}$ cannot be evaluated without additional labeling (*e.g.*, deuteriation at H-3 or H-4, or (^{12}C)-enrichment at C-3 or C-4) to suppress one of the signals.

^{13}C–^{1}H and ^{13}C–^{13}C spin-coupling constants in the alditols: conformational implications. — Like the cyclic furanoses, the acyclic alditols may assume numerous potential conformations in solution, and it is likely that several of these conformations have relatively similar energies. Thus, alditols are conformationally flexible molecules. Hence, experimental structural parameters such as chemical shifts and spin-spin coupling constants will reflect this conformational heterogeneity, and in the absence of a detailed knowledge of potential conformations and their energies, it is difficult to translate these parameters into a reliable conformational model. It is possible, however, to examine the limited number of available ^{13}C–^{1}H and ^{13}C–^{13}C couplings obtained in this study in light of previously-proposed conformational models to assess their level of consistency.

Jeffrey and Kim previously concluded[12] that the carbon chain of alditols adopts an extended, planar, zigzag conformation when configurations at alternate carbon atoms differ, whereas bent, non-planar conformations are preferred when these configurations are the same. Based on this rule, D-arabinitol (**3**) should prefer an extended, planar zigzag conformation (Fig. 9A), and ^{1}H–^{1}H coupling data appear to confirm this expectation[10]. This being the case, $^{3}J_{C\text{-}1,C\text{-}4}$ (2.3 Hz) (Table II) in **3** may be considered a crude value (since **3** is not conformationally pure[10]) for this coupling in alditols having C-1 and C-4 antiperiplanar.

D-Glucitol (**2**) and D-talitol (**4**) each possess a destabilizing 1,3-O-2–O-4 interaction in their extended, planar, zigzag conformations. This interaction may be eliminated by rotating the C-2–C-3 bond by 120°, producing a "bent" or "sickle" conformation. In "bent" **2**, C-1 and C-4 are gauche, and the observed $^{3}J_{C\text{-}1,C\text{-}4}$ (1.2 Hz) (Table II) is consistent with this preferred conformation. Likewise, C-1 and C-4 are gauche in the bent conformation of **4** (Fig. 9B), and a comparable $^{3}J_{C\text{-}1,C\text{-}4}$ (1.4 Hz) is observed. Furthermore, $^{3}J_{C\text{-}1,H\text{-}3}$ (4.0 Hz) is consistent with a bent conformation in which C-1 and H-3 are trans (Fig. 9B), while C-6 and H-4 are gauche in "bent" **4**, giving a value of $^{3}J_{C\text{-}6,H\text{-}4}$ of 1.9 Hz. [D-(6-^{13}C)talitol was prepared from D-(1-^{13}C)altrose.]

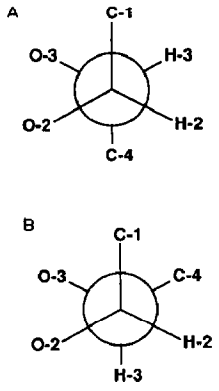


Fig. 9. Newman projections for the C-2–C-3 bond in the preferred conformations of **3** (A) and **4** (B).

In ribitol (**5**) and xylitol (**6**), $^3J_{C\text{-}1,C\text{-}4}$ is 1.7 and 2.0 Hz, respectively (Table II). These values are intermediate between $^3J_{C\text{-}1,C\text{-}4}$ (gauche) (~1.3 Hz) and $^3J_{C\text{-}1,C\text{-}4}$ (trans) (~2.3 Hz), and suggest that **5** and **6** exist in solution as a mix of extended and bent conformers[10].

In glycerol (**1**), $^3J_{C\text{-}1,H\text{-}3} = 3.2$ Hz and $^3J_{C\text{-}1,H\text{-}3'} = 2.9$ Hz. These values are intermediate between the related gauche and trans couplings in **4** (1.9 Hz and 4.0 Hz, respectively), suggesting the presence of conformational heterogeneity. Values of $^3J_{H\text{-}1/H\text{-}3,H\text{-}2}$ (4.3 Hz) and $^3J_{H\text{-}1'/H\text{-}3',H\text{-}2}$ (6.5 Hz) also appear to support this conclusion.

SUMMARY

In contrast to the ^{1}H- and ^{13}C-n.m.r. spectra of dissymmetric (1-^{13}C)alditols [*e.g.*, D-(1-^{13}C)arabinitol], those of symmetric alditols [*e.g.*, (1-^{13}C)glycerol] contain uncommon characteristics that complicate their interpretation (in fact, these characteristics will be encountered in (^{13}C)-substituted symmetric compounds in general). By introducing a ^{13}C isotope at C-1, the hydroxymethyl proton and carbon magnetic equivalencies are effectively eliminated, and the resulting ^{1}H- and ^{13}C-n.m.r. spectra are composed of two overlapping subspectra, one ^{13}C-coupled and the other non-coupled. Future n.m.r. studies of isolated, labeled alditol intermediates and end-products generated from the *in vivo* metabolism of ^{13}C-labeled precursors [*e.g.*, D-(1-^{13}C)glucose] will need to contend with these complicating features, especially if the aim is to identify and quantify the isotopomers present.

In some cases, ^{1}H-n.m.r. difference spectra and ^{1}H-coupled ^{13}C-n.m.r. spectra may be used to extract ^{13}C–^{1}H and ^{13}C–^{13}C spin-couplings from ^{1}H- and ^{13}C-n.m.r. spectra of symmetric (1-^{13}C)alditols. These couplings are potentially useful in assessing the conformational properties of the acyclic alditols, but further studies of conformationally-constrained polyols will be required to define the Karplus relationships appropriate for particular coupling pathways before they may be quantitatively treated.

ACKNOWLEDGMENTS

This work was supported by the National Institutes of Health (GM 33791) and the Research Corporation (10028). The authors thank the NMR Facility for Biomedical Studies, Department of Chemistry, Carnegie-Mellon University, Pittsburgh, PA, for use of their 620 MHz n.m.r. spectrometer.

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