Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Some Inositols and Their O-Methylated Derivatives[1,2]

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Abstract: The ^{13}C nmr spectra of some inositols and their partially O-methylated derivatives are tabulated and discussed. Methods of assignment are detailed. A system of empirical constants which can be used to estimate the spectra of the carbons of these ring systems is derived. The substituent effects are noted to conform generally to the notion that steric or proximity effects are very important sources of chemical-shift differences in these systems.

The substituent effects of hydroxyl groups on ^{13}C chemical shifts have been studied in a series of alkyl-substituted cyclohexanols[6,7] and have been attributed to a combination of steric and electronic effects.[6] There are substantial differences in the substituent effects of axial and equatorial hydroxyl groups at the α, β, and γ carbons.[8] This effect is especially pronounced at the α and γ carbons, the resonances of which are about 5 ppm higher field over what might otherwise be expected[6] when the hydroxyl is axial. This upfield shift may be attributed to a 1,3-diaxial interaction tending to compress the bonds of the α and γ carbons, as has been observed in the case of methylcyclohexanes.[9,10] At the β carbon there is also an upfield shift associated with the change of an equatorial hydroxyl group to the axial disposition. Again, this shift parallels the results of studies of methylcyclohexanes.[9,10] Indeed, the similar behavior of methyl and hydroxyl groups would appear to eliminate any important influence of hyperconjugation of the type considered by Cheney and Grant.[11] The differences in δ shifts produced by axial and equatorial groups are generally small.

In hope of determining the general applicability of the previously determined effects of hydroxyl groups on ^{13}C spectra, we have turned to studies of inositols and some of their O-methyl derivatives. The inositols are of special interest as a unique group of hexasubstituted cyclohexanes in which all of the possible isomers are known.[12] Also, because of the extent of substitution at each carbon remains constant, it should be possible to isolate and define steric or proximity effects apart from inductive effects due to hydroxyl groups. Finally, the ^{13}C spectra of inositols can serve as convenient models for the interpretation of the ^{13}C spectra of the carbohydrates with pyranose ring systems.[13]

Experimental Section

The ^{13}C spectra of the inositols studied in this work were obtained at 15.1 Mcps with the aid of the digital frequency sweep spectrometer previously described.[14] The samples were examined as 2–5 *M* aqueous solutions containing approximately 10% (v/v) of 1,4-dioxane to serve as an internal reference. The chemical shifts so measured were referenced to external carbon disulfide by the relation $\delta_C^{CS_2} = \delta_C^{diox} + 126.1$ ppm. Proton decoupling was achieved with the single-frequency procedure as previously described.[6,14] The proton-decoupling frequencies (pdf) of the inositols and their derivatives were measured relative to the proton-decoupling frequency of the dioxane used as internal reference.

Proton spectra of the inositols were taken with a Varian 220-Mcps spectrometer, again using aqueous solutions (D_2O) with dioxane as internal reference.

Results and Discussion

Assignment of Resonances to Specific Carbons. The chemical shifts of carbons, the specific decoupling frequencies of the directly attached protons, and the spectral assignments for the inositols and their derivatives are given in Table I. It will be seen that all the carbon resonances have been resolved, except those which arise from nuclei which are equivalent by symmetry. This is in marked contrast to the pmr spectra of these compounds, where in general the proton resonances are rather poorly resolved.

The six carbons of *scyllo*-inositol (**1**) are equivalent, and the substance accordingly gives only a single ^{13}C resonance. Because the hydroxyl groups are all equatorial, this resonance provides a convenient reference for discussion of the carbon chemical shifts of the other inositols.

In assignment of the resonances, use was made of the rather general result that equatorial protons of cyclohexane rings come into resonance at lower fields than axial protons.[15] This is also true of chemically similar protons in pyranose rings of the carbohydrates, provided that the chair conformation obtains.[16] As a result, the decoupling frequencies for equatorial protons should come at lower fields than those of axial protons,

(1) Supported in part by the Public Health Service Grant No. 11072-66 from the Division of General Medical Sciences.

(2) Preliminary communication: D. E. Dorman, S. J. Angyal, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, **63**, 612 (1969).

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(6) J. D. Roberts, F. J. Weigert, J. I. Kroschwitz, and H. J. Reich, *J. Am. Chem. Soc.*, **92**, 1338 (1970).

(7) G. W. Buchanan, D. A. Ross, and J. B. Stothers, *ibid.*, **88**, 4301 (1966).

(8) The α carbon is taken to be the carbon to which the hydroxyl group is directly attached.

(9) D. M. Grant and B. V. Cheney, *J. Am. Chem. Soc.*, **89**, 5315 (1967).

(10) D. K. Dalling and D. M. Grant, *ibid.*, **89**, 6612 (1967).

(11) B. V. Cheney and D. M. Grant, *ibid.*, **89**, 5319 (1967).

(12) See S. J. Angyal, *Quart. Rev.* (London), **11**, 212 (1957).

(13) D. E. Dorman and J. D. Roberts, *J. Am. Chem. Soc.*, **92**, 1355 (1970).

(14) F. J. Weigert and J. D. Roberts, *ibid.*, **89**, 2967 (1967); **90**, 3543 (1968).

(15) See J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Pergamon Press, Ltd., London, 1966, p 696.

(16) R. U. Lemieux and J. D. Stevens, *Can. J. Chem.*, **44**, 249 (1966).

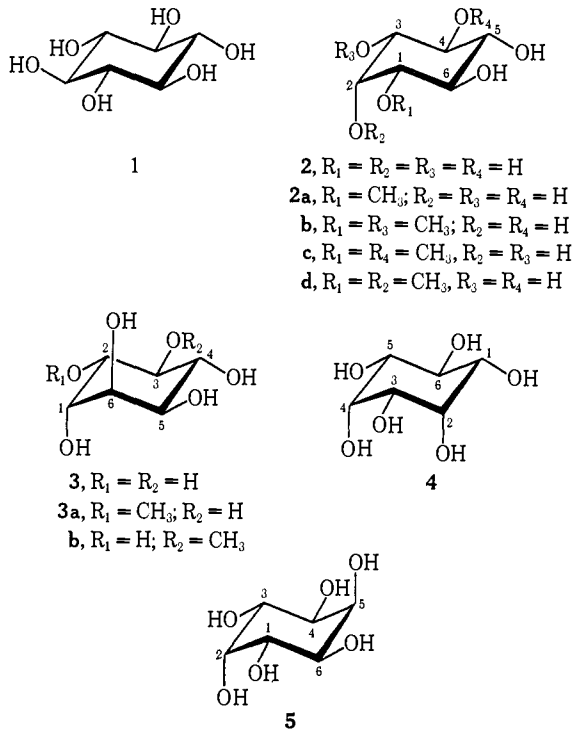

Figure 1. Inositol isomers.

and this was useful in the identification of the resonances of carbons bearing hydroxyl groups.

For *myo*- (**2**), *epi*- (**4**), and 1,3-di-O-methyl-*myo*-inositols (**2b**), the plane of symmetry gives pairs of equivalent carbons and hence some of the resonances of these spectra can be distinguished by their intensities. Thus, one of the single carbon resonances of **2** can be assigned to C-2 by its pdf, while the other must arise from C-5. Similar arguments lead to the identification of the resonances of carbons 2 and 5 of **2b**, and carbons 1 and 5 of **4**.

The assignments may be further extended by use of the results of the cyclohexanol study,[6] which suggested that the resonance of a carbon which is γ to an axial hydroxyl group should be shifted to higher field than the resonance of a chemically similar β carbon. On this basis, the 121.4 ppm resonance of **2** is assigned to carbons 4 and 6, leaving the lower field, doubly intense resonance at 120.1 ppm to be assigned to carbons 1 and 3. For **4**, the line at 125.7 ppm, which is the highest field resonance observed for any of the unsubstituted inositols, is assigned to carbon 6, which is γ to two axial hydroxyl groups. This leaves the singly intense resonance at 122.4 ppm to carbon 3, which is β to both axial hydroxyls.

In **2**, carbon 5 which is δ to the axial hydroxyl group has, as expected from the cyclohexanol study, its resonance at 118.2 ppm, only slightly different from the chemical shift of the resonance of the carbons of **1**. These data can be used to assign the remaining resonances of *chiro*-inositol (3). Carbons 2 and 5 of **3** are each β to one axial hydroxyl group and γ to another. Carbons 3 and 4, on the other hand, are each γ to one and δ to the other of the two axial hydroxyl groups. Because the δ effect is expected to be smaller than the β effect, C-2 and C-5 should come into resonance at higher fields than carbons 3 and 4. We therefore have taken C-2 and C-5 to come at 122.0 ppm and C-3 and C-4 at 119.7 ppm.

Table I. ^{13}C Chemical Shifts of Inositols and O-Methylinositols[a]

Compound	Carbon	$\delta_C^{CS_2}$	Pdf[b]	δ_H
scyllo- (**1**)	1–6	118.8		
myo- (**2**)	5	118.2	0.43	0.49
	1,3	120.1[c]	0.17	0.14
	2	120.3	−0.27	−0.29
	4,6	121.4[c]	0.27	0.24
L-*chiro*- (**3**)	3,4	119.7	0.13	0.19
	1,6	120.9	−0.27	−0.26
	2,5	122.0	0.0	0.02
epi- (**4**)	2,4	118.0[c]	−0.27	−0.30
	1,5	120.8[c]	0.23	0.28
	3	122.4	0.03	0.02
	6	125.7	0.0	−0.08
D-1-O-Methyl-*myo*- (**2a**)	1	112.0	0.50	
	5	118.1	0.27	
	3	120.2	0.10	
	6	120.9	0.10	
	4	121.4	0.17	
	2	124.5	−0.43	
	CH_3	135.6	0.33	
1,3-Di-O-methyl-*myo*- (**2b**)	1,3	112.1[c]	0.33	
	5	118.1	0.40	
	4,6	121.1	0.07	
	2	129.2	−0.77	
	CH_3	135.1	0.30	
1,4-Di-O-methyl-*myo*- (**2c**)	4	110.3	0.33	
	1	112.2	0.50	
	5	118.8	0.23	
	3	120.8	0.07	
	6	122.0	0.30	
	2	124.7	−0.63	
	CH_3 (4)	132.8	0.13	
	CH_3 (1)	135.8	0.30	
1,2-Di-O-methyl-*myo*- (**2d**)	1	111.5	*e*	
	2	114.4		
	5	118.1		
	3	119.9		
	6	120.7		
	4	121.1		
	CH_3 (2)	131.0		
	CH_3 (1)	135.1		
L-2-O-Methyl-*chiro*- (**3a**)	2	112.4	0.23	
	4	119.7	0.20	
	3	120.6	−0.10	
	6	121.2	−0.33	
	5	122.1	−0.07	
	1	125.3	−0.47	
	CH_3	135.7	0.27	
D-3-O-Methyl-*chiro*- (**3b**)	3	110.0	0.47	
	4	120.4	0.10	
	1	120.8	−0.23	[d]
	6	121.1	−0.23	[d]
	5	121.9	0.17	
	2	122.7	0.07	
	CH_3	133.1	0.17	

[a] The ^{13}C chemical shifts are upfield in parts per million relative to CS_2. [b] In parts per million relative to the proton decoupling frequency of dioxane. [c] Peak intensity twice that of the resonances from carbons expected to be unique from the molecular symmetry. [d] Assignments uncertain within this pair. [e] Noise modulation of proton-decoupling frequency used in this case.

The completed assignments of the spectra of the unsubstituted inositols are supported by the comparison of the proton-decoupling frequencies with the pmr spectra (*cf.* Table I). In all cases, the pdf values correlate with the proton chemical shifts to within 0.1 ppm, a margin which is probably within the experimental error involved in obtaining the former values.

With regard to the O-methylated inositol derivatives, the undecoupled spectra clearly distinguish the ring

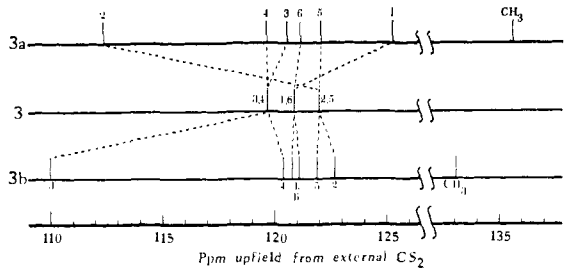

Figure 2. Correlation of the spectra of *chiro*-inositol (**3**), 2-O-methyl-*chiro*-inositol (**3a**), and 3-O-methyl-*chiro*-inositol (**3b**).

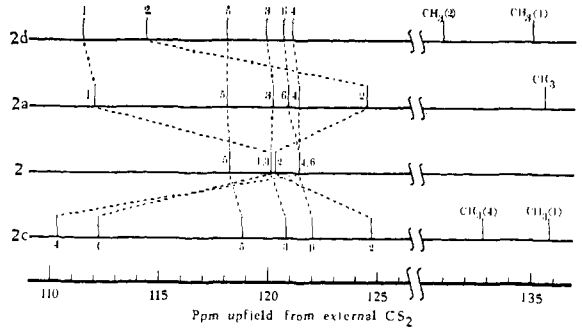

Figure 3. Correlation of the spectra of *myo*-inositol (**2**), 1-O-methyl-*myo*-inositol (**2a**), 1,4-di-O-methyl-*myo*-inositol (**2c**), and 1,2-di-O-methyl-*myo*-inositol (**2d**).

carbons from the methyl carbons, the resonances of the latter being split into quartets by the three attached protons. The available evidence[17-19] indicated that methylation of a hydroxyl group results in a 7–10-ppm downfield shift in the resonance of the α carbon. This permits the assignment of the unusually low-field resonances in the spectra of the O-methylinositols to carbons carrying methoxyl groups. For **2b**, the other doubly intense resonance must arise from carbons 4 and 6.

In the spectra of **2a**, **3a**, and **3b** it is possible to identify the resonances of carbons bearing axial hydroxyl groups through the comparison of pdf values. In the cases of carbons which are γ or δ to the methoxyl groups, the methylation shifts are generally smaller than ± 0.3 ppm. It seems safe to presume that γ and δ carbons which carry equatorial hydroxyl groups will be similarly shifted by methylation of an equatorial hydroxyl group at the α carbon, and this conclusion is useful in the assignment of the resonances of these relatively remote carbons. For β carbons which bear axial hydroxyl groups, there is a methylation shift of approximately 4.5 ppm per methoxyl group. The remaining β carbons, many of which can be assigned by the process of elimination, show smaller and more variable methylation shifts (*cf.* Figures 2 and 3).

The case of **2d**, with its axial methoxyl group, is unique in this study. It might be expected that steric perturbation of the axial protons at the γ carbons 4 and 6 would lead to significant shifts in the resonances of these carbons.[9,10] Consequently, the generalities which emerge from the spectra of the other O-methylinositols cannot be uncritically applied to the problem of assigning the resonances of the spectrum of **2d**. Because of the paucity of this substance, it was not practical to obtain specific pdf values, and the spectrum was taken using noise decoupling.[20]

A tentative assignment of the resonances in this spectrum results from comparison of the spectra of **2a** and **2d** (Figure 3). The two lowest field peaks must be associated with the methoxyl-bearing carbons. The lower of these resonances is unlikely to be due to carbon 2, because that would require an α methylation shift of unprecedented magnitude (13 ppm). This peak is accordingly assigned to C-1, and it is notable that the position of the C-1 resonance does not change much throughout the series **2a**–**2d**. This leaves the peak at 114.4 ppm to be assigned to carbon 2. Carbon 5 of **2d** is δ to the axial methoxyl group and γ to the other, and hence might be expected to be unchanged from its position in **2a**. The resonance at 118.1 ppm is accordingly assigned to C-5.

Comparison of the three remaining ring carbon resonances of **2d** with the spectrum of **2a** shows that methylation of the C-2 hydroxyl group cannot have had a shielding effect on the C-4 resonance. It seems, therefore, that 1,3-diaxial interaction due to an axial methoxyl group does not lead to upfield shifts at the γ carbon. This result is in accord with conclusions derived from the study of carbohydrates,[13,19] which indicated that methoxylation of axial hydroxyl groups led to significant shifts only in the chemical shifts of the α and β carbons. On this basis, the remaining resonances at 119.9, 120.7, and 121.1 ppm are assigned to carbons 3, 6, and 4, respectively.

Quantitative Correlations. It has become common in ^{13}C nmr spectroscopy to discuss chemical shifts in terms of substituent shifts.[6,9,17-19] Such substituent effects can be used to estimate spectral parameters for other related substances, and have been helpful in theoretical rationalization of ^{13}C chemical shifts.[11] We have been able to derive a series of substituent shift constants which have utility in the interpretation of the spectra of both the inositols and the pyranose carbohydrates.[13]

The treatment assumes that ^{13}C chemical shift differences in the inositols are associated with the variation in steric perturbation which must accompany epimerization of any center in the molecule. A reference point for the chemical shift of the α carbon is adopted, and the changes in its chemical shift are correlated with epimerization of the hydroxyl groups on the remaining carbons from the equatorial to the axial configuration. It is assumed that such steric changes at the β, γ, and δ carbons will be associated with an independent and additive constant representing the change in chemical shift in each case. The symbols β, γ, and δ are used to represent the shifts associated with such epimerization of the β, γ, and δ carbons, respectively. The subscripts a or e are added to these symbols to specify whether the hydroxyl group at the α position is axial or equatorial. It is necessary to adopt reference points for both of these latter situations. For the case in which an α carbon bears an equatorial hydroxyl group, the chemical shift of the single carbon resonance of *scyllo*-inositol (**1**) is used, while the C-2 resonance of *myo*-inositol (**2**) is a convenient reference for cases in

(17) F. J. Weigert, Ph.D. Thesis, California Institute of Technology, Pasadena, Calif., 1968, p 211.
(18) H. J. Reich, M. Jautelat, F. J. Weigert, and J. D. Roberts, *J. Am. Chem. Soc.*, **91**, 7445 (1969).
(19) L. D. Hall and L. F. Johnson, *Chem. Commun.*, **1968**, 509.
(20) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, 60, 1162 (1968).

which the hydroxyl group at the α carbon is axial. On this basis, the difference in chemical shift between the C-1 resonance of *epi*-inositol (**4**) and the resonance of **1** will be $\beta_e + \delta_e$, while the chemical-shift difference between the C-2 resonances of **2** and **4** can be represented by γ_a.

Proceeding in this way, one can write a series of expressions to represent the chemical shifts, measured in parts per million from the two defined reference points, of the resonances of the carbons of unsubstituted inositols. The expressions are shown in Table II under the heading $\Delta\delta$. By treating these expressions as twelve equations in six unknowns, a least-squares solution can be fit to the experimental data. Such a solution leads to the following best values of the constants: β_e = 1.7 ± 0.3 ppm; γ_e = 2.8 ± 0.3 ppm; δ_e = −0.7 ± 0.5 ppm; β_a = 0.6;[21a] and γ_a = −2.3.[21] The chemical shifts calculated using these constants are tabulated in Table II, and the standard deviation of these calculated values is about 0.8 ppm.

Table II. Calculated and Empirical ^{13}C Chemical Shifts in Unsubstituted Inositols

Compound	Carbon	Ref point	$\Delta\delta^a$	$\delta_{calcd}{}^b$	$\delta_{exp}{}^b$	Error
scyllo- (**1**)					118.8	
myo- (**2**)	5	118.8	δ_e	118.1	118.2	−0.1
	1,3	118.8	β_e	120.5	120.1	+0.4
	2				120.3	
	4,6	118.8	γ_e	121.6	121.4	+0.2
chiro- (**3**)	3,4	118.8	$\gamma_e + \delta_e$	120.9	119.7	+1.2
	1,6	120.3	β_a	120.9	120.9	0
	2,5	118.8	$\beta_e + \gamma_e$	123.3	122.0	+1.3
epi- (**4**)	2,4	120.3	γ_a	118.0	118.0	0
	1,5	118.8	$\beta_e + \delta_e$	120.9	120.8	+0.1
	3	118.8	$2\beta_e$	122.2	122.4	−0.2
	6	118.8	$2\gamma_e$	124.4	125.7	−1.3

[a] Chemical shift measured in parts per million from the relevant reference point. [b] Chemical shift measured in parts per million from external carbon disulfide.

While the correlation of calculated and empirical chemical shifts is not ideal, it is interesting to note that the chemical-shift differences predicted by these constants within the spectrum of a single inositol are somewhat better. The expressions of Table II, for example, predict that the C-3,4 and C-2,5 resonances of **3** will differ by $(\beta_e + \gamma_e) - (\gamma_e + \delta_e) = 2.4$ ppm; the empirical chemical-shift difference is 2.3 ppm. Similarly, the C-1,5 resonance of **4** is predicted to differ from that of C-6 by 4.6 ppm, while the empirical difference is 4.9 ppm. The explanation of this phenomenon may be related to the failure to adopt a standard concentration throughout this study, and experiments designed to test this hypothesis are under way in these laboratories.

The shifts due to O-methylation seem too variable to yield to an analysis of the type above. Thus, while there is a quite constant upfield β methylation shift of about 4.5 ppm when the hydroxyl at the β carbon is axial, the analogous shifts when the hydroxyl group is equatorial fall into no presently recognizable pattern. The α methylation shifts are also variable. A reliable system of substituent shifts for these derivatives must accordingly await further study.

Conclusions

The substituent shifts derived above for the unsubstituted inositols are in accord with the general recognition that steric hindrance or a proximity effect is a very important factor in determining ^{13}C chemical-shift differences in reasonably related compounds. The fact that the magnitudes of γ_e and γ_a are the largest of the series of substituent constants probably reflects the importance of 1,3-diaxial interactions, while the small and variable δ_e shifts show the expected attenuation of steric effects that would accompany increasing distance between interacting groups.

The small methylation shifts observed at the γ and δ carbons are also in accord with the hypothesis that steric effects are an important source of chemical-shift differences in these systems. In the case of 1,2-di-O-methyl-*myo*-inositol (**2d**), the absence of a significant γ shift suggests very different populations of the three staggered rotamers of the O-methyl group. It is possible that the variable β methylation shifts are also associated with rotation around the C–O ether bond. Continuing research in these laboratories will attempt to clarify these questions.

(21) (a) Too few examples were available to obtain estimates of error in these cases. (b) The low solubility of *neo*-inositol (**5**) has thus far precluded its study, and hence no value for δ_a is available.