Table IV

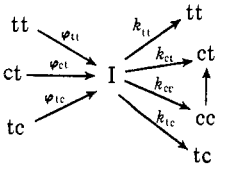
Azulene, M	$\Phi_{tt\rightarrow ct}$	$\Phi_{tt\rightarrow tc}$
all-trans-3,5-Heptadienone (tt) (1.6×10^{-2} M)[a,b]		
0.0	0.28 ± 0.02	0.14 ± 0.01
8.3×10^{-4}	0.28 ± 0.02	0.10 ± 0.01
1.66×10^{-3}	0.29 ± 0.02	0.12 ± 0.02[c]
4.98×10^{-3}	0.31 ± 0.03[d]	0.16 ± 0.01

Azulene, M	t	c
trans-6-Methyl-3,5-heptadienone (6) (1.02×10^{-2} M)[a,b]		
0.0	0.19 ± 0.01	
1.27×10^{-4}	0.19 ± 0.01	
3.80×10^{-4}	0.19 ± 0.01	
1.27×10^{-3}	0.18 ± 0.01	

[a] Uncorrected for back reaction etc. [b] Uncertaintes are those stemming from analytical error. [c] Precision ±13%. [d] Precision ±11%.

corrected for absorption of light by azulene using the following extinction coefficients: at 254 nm $\epsilon_{azulene}$ = 12,080, ϵ_{tt} = 21,800, ϵ_6 = 9830. Results are listed in Table IV.

Derivation of Equation 5. The scheme below was used where φ's are quantum yields for formation of the common excited state or rapidly equilibrating isomeric states (I) and k's are the rates of decay of the reactive excited state(s) to ground-state isomers. The possible formation of cc and its subsequent conversion to ct are included for the sake of completeness.



Observed quantum yields are defined by eq 7–12

$$\Phi_{tt\rightarrow ct} = [\varphi_{tt}(k_{ct} + k_{cc})]/K \quad (7)$$

$$\Phi_{tc\rightarrow ct} = [\varphi_{tc}(k_{ct} + k_{cc})]/K \quad (8)$$

$$\Phi_{tt\rightarrow tc} = \varphi_{tt}k_{tc}/K \quad (9)$$

$$\Phi_{ct\rightarrow tc} = \varphi_{ct}k_{tc}/K \quad (10)$$

$$\Phi_{tc\rightarrow tt} = \varphi_{tc}k_{tt}/K \quad (11)$$

$$\Phi_{ct\rightarrow tt} = \varphi_{ct}k_{tt}/K \quad (12)$$

where

$$K = k_{tt} + k_{ct} + k_{cc} + k_{tc}$$

Now

$$\frac{(7)}{(9)} = \frac{\Phi_{tt\rightarrow ct}}{\Phi_{tt\rightarrow tc}} = \frac{(k_{ct} + k_{cc})}{k_{tc}}$$

$$\frac{(8)}{(10)} = \frac{\Phi_{tc\rightarrow ct}}{\Phi_{ct\rightarrow tc}} = \frac{\varphi_{tc}}{\varphi_{ct}}\frac{(k_{ct} + k_{cc})}{k_{tc}}$$

$$\frac{(12)}{(11)} = \frac{\Phi_{tc\rightarrow tt}}{\Phi_{ct\rightarrow tt}} = \frac{\varphi_{tc}}{\varphi_{ct}}$$

Looking at the right side of the above three equations it can be seen that

$$\frac{(12)}{(11)}\frac{(7)}{(9)} = \frac{(8)}{(10)}$$

or

$$\frac{\Phi_{tc\rightarrow tt}}{\Phi_{ct\rightarrow tt}}\frac{\Phi_{tt\rightarrow ct}}{\Phi_{tt\rightarrow tc}} = \frac{\Phi_{tc\rightarrow ct}}{\Phi_{ct\rightarrow tc}}$$

Acknowledgment. We would like to thank Professors R. A. Caldwell and J. Saltiel for helpful discussions of this work.

Nuclear Magnetic Resonance Spectroscopy. Carbon-13 Spectra of Some Common Oligosaccharides[1]

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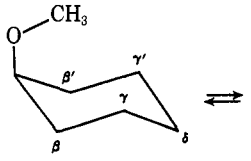
Abstract: The applicability of carbon-13 magnetic resonance spectroscopy to the study of oligosaccharides is demonstrated. The spectra of four disaccharides are discussed and interpreted, and the methods used in peak assignments are explained. The dependences of carbon chemical shifts and carbohydrate conformations upon pH are discussed and, contrary to earlier reports, it is shown that no large changes in the anomeric mixture of glucose are observed in strong base. The spectra of four fully acetylated sugars are presented and partially assigned. Finally, a brief investigation of the carbon-13 spectra of amylose and cellulose acetate is presented, and the potential of this method of studying polysaccharide conformations emphasized.

Several investigations of the ^{13}C nuclear magnetic resonance (cmr) spectra of monosaccharides have shown the feasibility and applicability of this method for structural investigations of simple carbohydrates.[3-6] In general, carbon chemical-shift differences have been

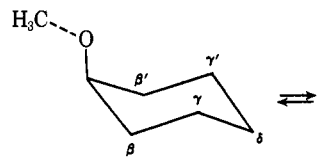
(1) Supported by the Public Health Service, Grant No. 11072, from the Division of General Medical Sciences and by the National Science Foundation.
(2) National Institutes of Health Postdoctoral Fellow, 1967–1970.
(3) D. E. Dorman, S. J. Angyal, and J. D. Roberts, *J. Amer. Chem. Soc.*, 92, 1351 (1970).
(4) D. E. Dorman and J. D. Roberts, *ibid.*, 92, 1355 (1970).
(5) L. D. Hall and L. F. Johnson, *Chem. Commun.*, 509 (1968).
(6) A. S. Perlin and B. Casu, *Tetrahedron Lett.*, 2921 (1969); A. S. Perlin, B. Casu, and H. J. Koch, *Can. J. Chem.*, 48, 2596 (1970); the demonstration by H. J. Koch and A. S. Perlin, *Carbohyd. Res.*, 15, 403 (1970), that the proton-decoupled cmr resonance at 119.5 ppm upfield from CS_2 of α-D-glucose is absent in α-D-glucose-3-*d* proves earlier assignments[4,5] of this resonance to the C-2 are incorrect. We have, therefore, throughout this paper taken the C-2 resonances of α anomers to come at somewhat higher field than the C-3 resonances.

noted to be associated with steric or proximity effects.[3,4] Indeed, the regularity of the chemical-shift differences has allowed the development of a means by which carbon chemical shifts of pyranose and cyclitol carbons can be predicted with a fair degree of accuracy.[3,4]

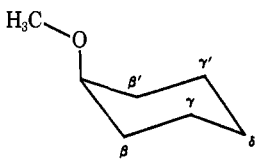
It has also been noted that cmr spectroscopy can provide important information regarding the conformations of the substituents on cyclitol and pyranose rings. Hence, the fact that O-methylations of axial hydroxyl groups in such systems have little effect upon the chemical shift of the γ carbon has been interpreted to indicate that conformations such as Ia do not contribute substantially to the mixture of conformational isomers I.[3]



Ia



Ib

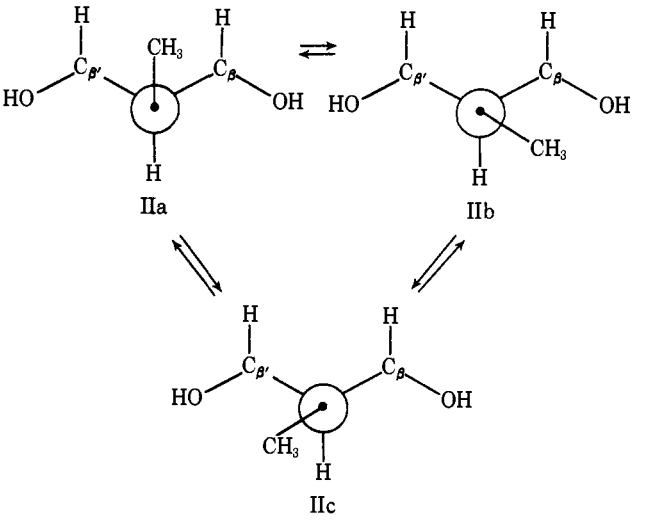


Ic

A reevaluation of the published evidence[3,4] also reveals regularities in the effects of the methylation of equatorial hydroxyls. Thus, if both β and β' bear equatorial hydroxyl groups, II, the chemical-shift change observed at the β carbons is $+0.7 \pm 0.2$ ppm. Since the shielding due to steric perturbations in IIa might be inferred from other work[7] to be much larger than this (perhaps 5 ppm), the population of this conformation must be substantially smaller than that of IIb or IIc which, because of the similarity of the steric environments, would be expected to be equally populated. This is as would be expected,[8] because there are two gauche interactions (C_β and $C_{\beta'}$) experienced by the *O*-methyl group in IIa, and only one each in IIb and IIc. Note also that this explanation requires that shielding at C_β and $C_{\beta'}$ due to steric interactions between the *O*-methyl and the equatorial hydroxyl groups in IIb and IIc must be small.

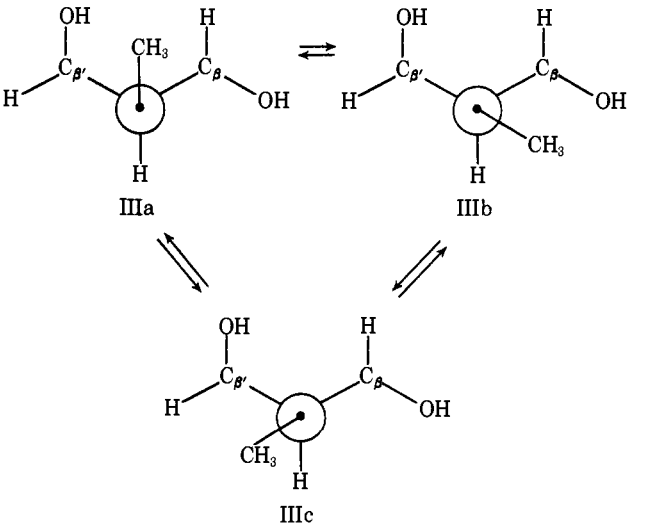
When one of the β hydroxyls is axial, III, there are important differences in the shifts resulting from methylation of the α-equatorial hydroxyl. While the shift at the β carbon bearing the equatorial hydroxyl group remains small and variable (less than ± 1 ppm), the chemical-shift change at β' carbons is $+4.4 \pm 0.1$ ppm. This indicates that IIIc is substantially more populated than is IIIa, again as would be expected.[8a] These conclusions are in agreement with those derived from consideration of the rules for optical rotation.[8b]

Correlations such as this suggest that cmr spectroscopy might give important information regarding the conformations of oligosaccharides. The present work was undertaken to test this notion and reports the cmr spectra of a collection of disaccharides,[8c] as well as of two polysaccharides.



IIa IIb

IIc



IIIa IIIb

IIIc

Experimental Section

The cmr spectra were obtained at 15.1 MHz, using the digital frequency sweep spectrometer described elsewhere.[9,10] The carbohydrates were examined at concentrations of approximately 1–2 *M* in aqueous solutions, and their acetates at about 0.5 *M* in methylene chloride solutions. The chemical shifts so measured were referenced to external carbon disulfide by using the chemical shifts of 10% 1,4-dioxane in water, 10% 1,4-dioxane in 1 *N* sodium hydroxide, and methylene chloride (126.1, 126.0, and 139.1 ppm, respectively) measured from external carbon disulfide. Proton decoupling was effected with noise modulation of a center band at approximately 60 MHz.[10]

The disaccharides used were commercial products and were used without purification. The methyl β-glycosides were prepared using standard procedures,[11,12] as were the acetates.[13] Cellulose acetate and α-cellobiose octaacetate were obtained from cotton by standard procedures.[14]

(7) D. M. Grant and B. V. Cheney, *J. Amer. Chem. Soc.*, 89, 5315, 5319 (1967).

(8) (a) E. L. Eliel, "Stereochemistry of Carbon Compounds," McGraw-Hill, New York, N. Y., 1962, p 126; (b) R. U. Lemieux and J. C. Martin, *Carbohyd. Res.*, 13, 139 (1970).

(8c) NOTE ADDED IN PROOF. N. Yamaoka, T. Usui, K. Matsuda, and K. Tuzimura, *Tetrahedron Lett.*, 2047 (1971), report the C-1′ resonances of some glucobioses.

(9) F. J. Weigert and J. D. Roberts, *J. Amer. Chem. Soc.*, 89, 2967 (1967).

(10) F. J. Weigert, M. Jautelat, and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, 60, 1162 (1968).

(11) F. H. Newth, S. D. Nicholas, F. Smith, and L. F. Wiggins, *J. Chem. Soc.*, 2550 (1949).

(12) G. Zemplen, *Ber.*, 53, 996 (1920).

(13) R. L. Whistler and M. L. Wolfrom, Ed., *Methods Carbohyd. Chem.*, 2, 211 (1962).

(14) R. L. Whistler and M. L. Wolfrom, Ed., *ibid.*, 3, 143, 193 (1963).

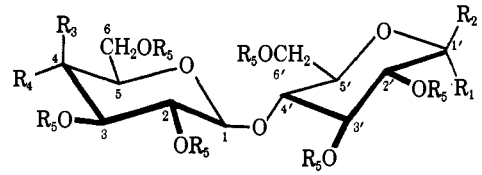
Results and Discussion

A. Disaccharides in Neutral Solution. In cmr spectroscopy the initial and often most difficult task is that of the assignment of the resonances to specific carbon nuclei. In the present case, the availability of the fully assigned spectra of the monosaccharides[4] simplified this problem to some degree. In the first place, the broad generalizations recognized in the earlier study could be expected to be applicable to the disaccharides also. Thus, resonances near 90–100 ppm can be attributed to anomeric carbon nuclei, and in anomeric mixtures the relative heights of the peaks in this region can be used to assign the C-1, C-1′α, and C-1′β resonances specifically. Furthermore, peaks upfield from 130 ppm can be assigned to either hydroxymethyl carbons (6 and 6′) or to *O*-methyl carbons.

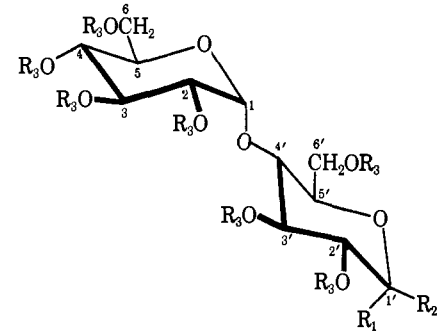
Also, in the study of monosaccharides[4] and cyclitols[3] it was noted that etherification of a hydroxyl group caused a 7–10-ppm downfield shift in the resonance of the carbon bearing this hydroxyl group. A shift of this nature is an example of the β shift, which has been recognized in several widely differing systems.[8,15] In the pyranoses, carbons bearing an etherified oxygen were found to occur in the range of chemical shifts 110–115 ppm,[4] a region downfield from the usual range of shifts for pyranose carbons other than the anomeric nucleus. This result allows the identification of the resonances at 113–115 ppm as arising from the C-4′ nuclei.

These generalizations, therefore, allowed the identification of the resonances due to C-1, -1′ (α and β), -6, -6′ (α and β), and -4′ (α and β). Extension of this assignment varied with the particular case being considered. In those cases where the two monomeric units were linked through β-glycoside bonds, it was assumed that the resonances of C-1′, -2′, -3, -4, -5, and -6 would be effectively unchanged from their positions in the cmr spectra of the free monomers. The basis of this assumption is the absence of any significant intramolecular steric interactions of the substituents of these carbons, regardless of the dihedral angles around the glycoside ether linkages. Thus, the steric perturbations[8] of these nuclei should not be different from those in the monosaccharides. Tacit in this hypothesis is the assumption that the conformations of the pyranose rings will be the same in both the mono- and disaccharides. The justification of these assumptions lies in the success with which they provide interpretations of cellobiose (**1a, b**) and lactose (**2a, b**).

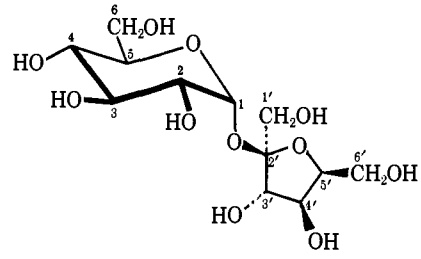
Since the free disaccharides were examined in the form of their anomeric equilibria, one might expect 24 resonances to be observed in their spectra. In practice, however, one finds that the spectrum of the glycosyl portion of the molecule is independent of the configuration of the hydroxyl group at C-1′. Usually, the resonances of C-4′ and -6′ were also the same for both anomers, a result which is in full accord with the monosaccharide study.[4] In the cases studied, no more than 15 resolved resonance lines were detected. In cellobiose (**1a, b**), in which the monomeric units are both glucose, there were observed only 12 resonances; in the spectrum of lactose (**2a, b**), 14 carbon resonances were resolved.



1a, $R_1 = R_4 = OH$; $R_2 = R_3 = R_5 = H$
1b, $R_2 = R_4 = OH$; $R_1 = R_3 = R_5 = H$
1c, $R_2 = OCH_3$; $R_4 = OH$; $R_1 = R_3 = R_5 = H$
1d, $R_1 = R_4 = OCOCH_3$; $R_2 = R_3 = H$; $R_5 = COCH_3$
1e, $R_2 = R_4 = OCOCH_3$; $R_1 = R_3 = H$; $R_5 = CHCH_3$
2a, $R_1 = R_3 = OH$; $R_2 = R_4 = R_5 = H$
2b, $R_2 = R_3 = OH$; $R_1 = R_4 = R_5 = H$
2c, $R_2 = OCH_3$; $R_3 = OH$; $R_1 = R_4 = R_5 = H$



3a, $R_1 = OH$; $R_2 = R_3 = H$
3b, $R_2 = OH$; $R_1 = R_3 = H$
3c, $R_2 = OCH_3$; $R_1 = R_3 = H$
3d, $R_1 = OCOCH_3$; $R_2 = H$; $R_3 = COCH_3$
3e, $R_2 = OCOCH_3$; $R_1 = H$; $R_3 = COCH_3$



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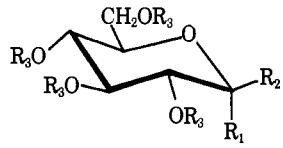
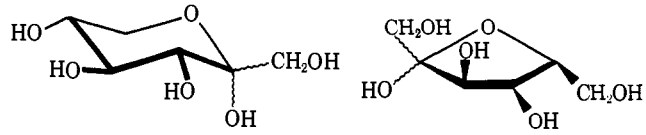


5a, $R_1 = OH$; $R_2 = R_3 = H$
5b, $R_2 = OH$; $R_1 = R_3 = H$
5c, $R_1 = OCH_3$; $R_2 = R_3 = H$
5d, $R_2 = OCH_3$; $R_1 = R_3 = H$
5e, $R_1 = OCOCH_3$; $R_2 = H$; $R_3 = COCH_3$
5f, $R_1 = H$; $R_2 = OCOCH_3$; $R_3 = COCH_3$



7 **8**

Considering first the spectrum of cellobiose (**1a, b**), the assignments are begun with the identification of the

(15) H. J. Reich, M. Jautelat, F. J. Weigert, and J. D. Roberts, *J. Amer. Chem. Soc.*, 91, 7445 (1969); M. Christl, H. J. Reich, and J. D. Roberts, *ibid.*, 93, 3463 (1971).

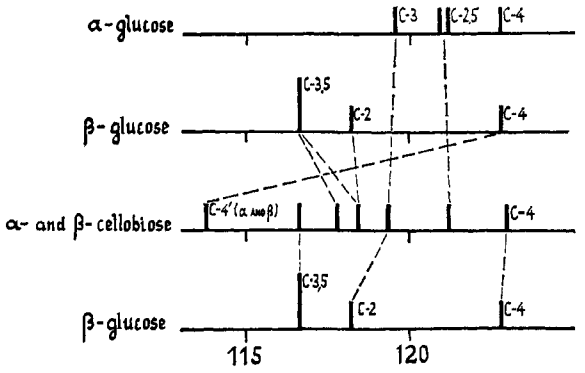


Figure 1. Comparison of the spectra of cellobiose (**1a,b**) and glucose (**5a,b**). The double intensity of the C-3,5 resonance of β-glucose reflects the fact that these peaks are overlapping in this compound.

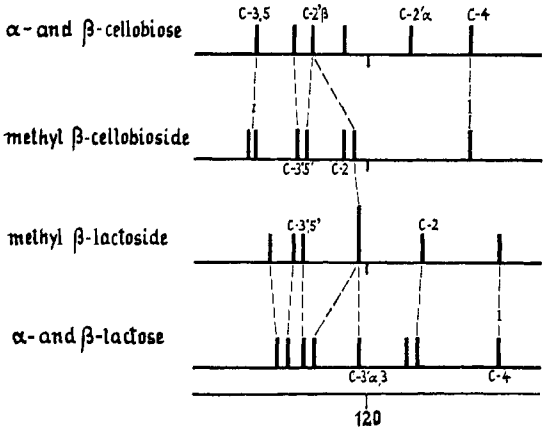


Figure 2. Comparison of the spectra of cellobiose (**1a,b**), methyl β-cellobioside (**1c**), lactose (**2a,b**), and methyl β-lactoside (**2c**).

resonances of C-1, -1′α, and -1′β. The last two were found to come into resonance at chemical shifts very similar to C-1 of the two glucose (**5a, b**) anomers. This lends support to the hypothesis that this portion of the molecule will not suffer intramolecular steric perturbation. Continuing, the peaks near 132 ppm are assigned to the hydroxymethyl groups. One of these resonances is unchanged in chemical shift from that of C-6 of glucose and is hence assigned to the C-6 nucleus. It is noted that this assignment specifies that the intramolecular steric perturbation of C-6′ (α and β) gives rise to an upfield shift, a result fully in accord with earlier research.[4,8] The cmr spectrum of cellobiose shows only one peak within the range of chemical shifts 110–115 ppm. Hence, the two C-4′ resonances are overlapped at 113.8 ppm. The resonances of C-1, -1′ (α and β), -4′ (α and β), -6, and -6′ (α and β) of lactose (**2a, b**) can be similarly assigned. It is noteworthy that the C-1′, -4′, and -6′ resonances of **1** and **2** are quite similar. Because the two disaccharides differ only in configuration at C-4, this result is quite reasonable.

These assignments may be extended by correlation of the cmr spectrum of the disaccharide with those of its monomeric units. The case of cellobiose is demonstrated in Figure 1. It is seen that the resonances of each of the C-2′ (α and β), -3, -4, and -5 can be uniquely identified in this manner. A similar correlation diagram for lactose identified the resonances of C-2′α, -3, and -4 in the spectrum of this sugar. In the case of lactose, however, the C-5 peak can be correlated equally well with either of the peaks at 117.4 or 117.8 ppm, while the resonance of C-2′β is identified as either of the peaks at 118.1 or 118.5 ppm.

One of the problems inhibiting the extension of these assignments is the fact that peak heights in the region of chemical shifts 115–125 ppm do not fall into any easily recognizable pattern. As will be seen below, this results from heavy overlapping of resonances of the two anomers, the upshot being that it is not possible to use relative peak heights to assign resonances to specific anomers.[4] To overcome this problem, the cmr spectra of the methyl β-glycosides (**1c** and **2c**) were examined. The spectra of these compounds proved to have much narrower and better resolved lines. In the case of methyl β-cellobioside (**1c**), all 13 resonances were clearly resolved. In the spectrum of methyl β-lactoside (**2c**) there were observed 12 peaks, one of which (119.8 ppm) was of clearly twice the intensity of the others. Because the methyl glycosides of the monosaccharides differ only in the chemical shifts of the resonances of C-1 and -2,[4] we should expect the spectra of the β anomers and their methyl pyranosides to be nearly superimposable. In the region of chemical shifts 115–125 ppm, only the position of the C-2′ resonance should change. Other peaks, present in the spectrum of the anomeric mixture but absent in the spectrum of the glycoside, would, therefore, be due to the α anomer.

Figure 2 correlates the spectra of lactose and cellobiose with those of their glycosides. Some of the peaks have been assigned already and these are noted in the figure. The resonances of C-3, -4, and -5 are expected to be unchanged upon glycosidation, and hence the same peaks may be immediately assigned in the spectra of the methyl glycosides. In the spectrum of **1c**, the peak corresponding to that assigned to carbon 2′α of **1a** is missing as would be expected. The peak at 118.4 ppm in the spectrum of cellobiose was assigned to carbon 2′β and it is seen that, as expected, this resonance has been altered in chemical shift in the spectrum of **1c**. From the pyranose study,[4] we would predict an upfield shift of 1–1.5 ppm in the C-2′ resonance due to O-methylation at the anomeric center. Hence, the C-2′ resonance of **1c** is probably at either 119.4 or 119.7 ppm. We may choose between these alternatives by recognizing that the C-2′ resonances of **1c** and **2c** should come at the same chemical shift. Of the two peaks mentioned, only that at 119.7 ppm can be correlated with a resonance in the spectrum of **1c**. We therefore assign this peak to C-2′, and we note that this chemical shift is quite similar to that of methyl β-glucoside (**5c**) (119.4 ppm).[4] Again, this is as might be expected.

In the case of lactose (**2a, b**), the C-2′β resonance could not be specifically assigned (*vide supra*) but was correlated with either of the two peaks, 118.1 or 118.5 ppm. Comparing the spectra of **2a, b** with **2c**, we see that the peak at 118.1 ppm is unchanged and therefore cannot be assigned to C-2′β. This permits the specific assignment of the 118.5-ppm resonance to C-2′ of **2b**.

We may now note that the spectra of the two anomeric mixtures (**1a, b** and **2a, b**) contain only one peak which is not present in the spectra of the two methyl β-glycosides (**1c** and **2c**). These peaks have similar chemical shifts and therefore probably represent the same carbons of **1a** and **2a**. Because all other resonances

Table I. Cmr Spectra of Some Common Disaccharides and Their Methyl β-Pyranosides[a]

Compound	1	2	3	4	5	6	1′	2′	3′	4′	5′	6′	Other
α-Cellobiose	90.2	119.4	116.8	123.0	116.8	131.9	100.8	121.2	119.4	113.8	121.2	132.4	
β-Cellobiose	90.2	119.4	116.8	123.0	116.8	131.9	96.8	118.4	117.9	113.8	118.4	132.4	
Methyl β-cellobioside	90.0	119.4	116.7	123.0	116.8	131.8	89.5	119.7	118.0	113.6	118.2	132.3	CH_3: 135.5
α-Lactose	89.7	121.5	119.8	123.9	117.4	131.6	100.7	121.1	119.7	113.8	121.1	132.3	
β-Lactose	89.7	121.5	119.8	123.9	117.4	131.6	96.7	118.5	117.8	113.8	118.1	132.3	
Methyl β-lactoside	89.6	121.6	119.8	123.9	117.3	131.6	89.6	119.8	117.9	113.9	118.1	132.3	CH_3: 135.5
α-Maltose	92.8	119.9*	119.9*	123.0	120.7*	131.8	100.5	121.1	122.6	114.8	119.4	131.8	
β-Maltose	92.8	119.9*	119.9*	123.0	120.7*	131.8	96.5	118.5	118.0	115.1	116.4	131.8	
Methyl β-maltoside	92.9	119.6*	119.6*	123.1	120.8*	131.8	89.6	119.9*	118.0	115.3	116.4	132.0	CH_3: 135.5
Sucrose[b]	100.6	120.3*	119.8*	123.2	121.5*	132.1	130.3	89.0	111.2	115.7	118.3	130.8	

[a] Chemical shifts in parts per million upfield from external carbon disulfide. Peaks within the same spectrum and designated by asterisks cannot be differentiated in assignment. [b] The resonances of the fructofuranoside portion are not specifically assigned.

persist in all four spectra, the remaining resonances of **1a** and **2a** must be overlapped with resonances of the β anomers. There is little wonder, therefore, that relative peak intensities were of so little aid. Examining previous assignments, we can see that we have accounted for the resonances of all the carbons of the α anomers, except C-3′α and -5′α. Because the intensities of the peaks near 121 ppm are nearly equal to those of the surrounding resonances, it seems likely that the C-2′α, -5′α resonances are overlapping at this position. The C-3′α resonance of cellobiose is assumed to be part of the peak at 119.4 ppm.

In the spectrum of lactose, it was not possible to specifically assign the C-5 resonance. Returning to the assignment of this resonance to either of two peaks and carrying this nonspecific assignment through to **2c** (Figure 2), we conclude that either of the peaks, 117.3 or 117.9, could represent C-5. But the C-5 resonances of **1c** and **2c** should differ in chemical shift, and since the peak at 117.9 ppm can be directly correlated with the 118.0-ppm peak in the cmr spectrum of **1c** (*vide infra*), it cannot represent C-5. Hence, the peak at 117.3 ppm in the spectrum of **2c** and that at 117.4 ppm in the spectra of **2a, b** are assigned to C-5.

There now remain unassigned only three peaks in the spectra of **1c** and **2c**. Reviewing the previous assignments, we see that these peaks must represent C-2,-3′, and -5′. Using our usual assumptions and remembering that these two compounds differ only in configuration at C-4, we can conclude that the resonances of C-3′ and -5′ should occur at the same chemical shifts in the spectra of **1c** and **2c**. Reference to Figure 1 evinces that only the peaks near 118 ppm meet this criterion and these peaks therefore are assigned to C-3′ and -5′, allowing the final assignment of the C-2 resonances by elimination. Leaving the fully elucidated spectra of **1c** and **2c** and going back to the spectra of the anomeric mixtures, a fully assigned spectrum can be given for each of the four spectra as shown in Figure 2.

Carrying this assignment back to Figure 1, we see one curious result which as yet cannot be explained. Thus, the resonances representing C-3′β and -5′β in cellobiose are about 1 ppm upfield from the position of these resonances in β-glucose (**5b**). There is no analogous change, however, in the C-3′α, -5′α resonances. The same phenomenon is observed in the case of lactose. It would seem, therefore, that the steric effects of the glycoside ring and the axial anomeric hydroxyl groups of **1a** and **2a** cancel in some way, a surprising result, because one would expect both effects to be shielding. It is therefore possible that the α and β anomers exist in different conformations about the interannular C–O bonds.

Considering now the cmr spectrum of the anomeric mixture of maltose (**3a, b**), it is necessary to recognize the possible steric interaction between the glycoside moiety and the substituents of C-3 and -5. Such a circumstance dictates that it is no longer possible to assume that the C-3,5 resonances will be unchanged from their positions in the monosaccharide analogs. Similarly, it is not possible to extend uncritically the results from the cases of lactose and cellobiose to the case of maltose. The assignment of the resonances of the maltose (**3a, b**) must, therefore, be approached with a minimum of prejudice.

We can, however, assign the resonances of C-1, -1′α, -1′β, -4′α, -4′β, -6, and -6′ (α and β) using our prior experience. In the spectrum of maltose, 15 of the expected 18 peaks are resolved (Table I). Above 130 ppm, there is observed only one resonance, a result which dictates that the resonances of C-6, -6′α, and 6′β must overlap. Therefore, 17 resonances are accounted for at the outset. The resonances of C-1, -1′α, and -1′β are routinely assigned and it is noted that the last two are unchanged in their positions in cellobiose and lactose. In the case of maltose, however, the resonances of C-4′α and -4′β are apparently different in chemical shift. They are assigned on the basis of their lower chemical shifts and their relative peak heights.

By the comparison of the spectra of maltose and methyl β-maltoside (**3c**) it can be established that the peaks at 100.5, 114.8, 121.1, and 122.6 ppm are due to the α anomer, a result in accord with the relative peak heights observed in the spectrum. By further comparisons of the cmr spectra of the similar methyl β-glucoside portions of **1c**, **2c**, and **3c** it is apparent that the methyl and anomeric carbon resonances are not different in these three compounds. By making the reasonable assumption that the C-2′ resonances will also be similar in chemical shift, the peak at 119.9 ppm in the spectrum of **3c** is assigned to this carbon.

To facilitate further assignments, we will focus attention on the spectrum of the methyl β-maltoside, **3c**. In Figure 3, the cmr spectrum of this compound is compared to those of the two methyl glucosides, **5c** and **5d**. There are definite similarities in these three spectra.

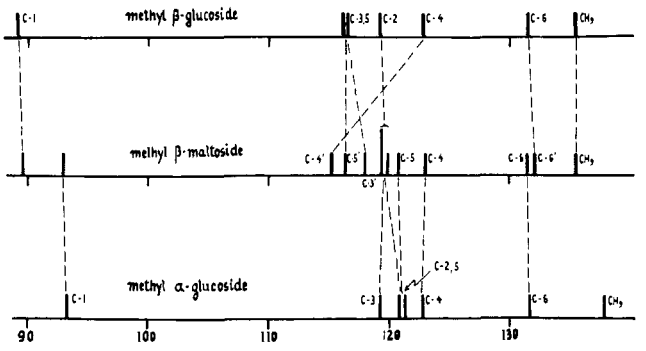


Figure 3. Comparison of the spectra of methyl β-maltoside (**3c**), methyl α-glucoside (**5c**), and methyl β-glucoside (**5d**).

The methyl resonance of **3c** corresponds quite closely to that of methyl β-glucoside, and the C-1′ resonance of **3c** is also similar in chemical shift to C-1 of **5c**. It seems safe to correlate the C-4 resonance of **3c** to the peak at 123.1 ppm, in agreement with the position of the analogous carbon of methyl α-glucoside (**5d**). The striking result obvious in Figure 3 is the small difference in the chemical shifts of C-6 and -6′. Intramolecular steric interactions of the latter might have been expected to shield this carbon resonance, but this effect must be small. It seems reasonable to hypothesize that the conformation of **3c** does not bring this hydroxymethyl group into proximity with the glucosyl ring.

There remain five resonances unassigned in the spectrum of **3c**. Unless we assume a downfield shift of almost 3 ppm, and such shifts seem rare in the absence of α or β shifts,[2-4,8] we must assign the peak at 116.4 ppm to either C-3′ or -5′ (Figure 3). We tentatively assign it to C-5′ on the assumption that this carbon nucleus will suffer steric perturbations similar to C-6′ which we have already noted to show only small interactions. Note also from Figure 3 that either C-2 or -5 (or both) must be shifted downfield by at least 1 ppm.

Extension of specific assignments at this point is very difficult. We feel that the peak at 118.0 ppm is most reasonably assigned to C-3′, but even this assignment cannot be defended. The peaks at 119.6, 119.9, and 120.8 ppm remain to be correlated with C-2′, -2, -3, and -5, and though we favor the assignments shown in Table I, those peaks within a single spectrum which are designated with asterisks cannot be specifically assigned.

The conformation of methyl β-maltoside (**3c**) in the crystal has been elucidated[16] and closely approximates that shown in **3c**. We suggest that in aqueous solutions the compound equilibrates between **3c** and a conformation in which rotation of the ether linkages has brought C-3′ into closer proximity with C-2 and -3. The steric environments of C-5, -5′, and -6′ would not be much different from those of the analogous carbons in the monosaccharides. Though this interpretation is tentative, it derives some support from studies of the specific solvation effects with somewhat simpler models for α-glucopyranosides.[16b]

Although the cmr spectrum of sucrose **4** has already been reported,[10] there has been no attempt at its interpretation. Comparisons of the sucrose spectrum with that of maltose (Table I), however, make a partial assignment possible. It is seen, for example, that the peaks tentatively assigned to the glucoside portion of **3** can be correlated with peaks of similar chemical shifts in the spectrum of **4**. Furthermore, the nuclei of the furanoside carbons seem to come into resonance generally at lower field than do those of the pyranose examples. This result is in broad agreement with studies concerned with the ribose portions of nucleotides.[17a]

In an attempt to extend this assignment, the cmr spectrum of fructose (**7**, **8**) was examined in aqueous solution. The spectrum indicated that the mixture consisted of two major constituents in the ratio of approximately 2:1 (Table II). The major isomer possessed a cmr spectrum quite similar to those of pentapyranose systems,[4] while that of the minor isomer conformed broadly to a furanose spectrum.[17] These results are in agreement with polarimetric studies[18] which have shown that at equilibrium the concentration of fructopyranose (**7**) is about twice that of fructofuranose (**8**). Comparisons show that the cmr spectrum of **8** is quite similar to that of the furanoside portion of sucrose (**4**). The differences in these spectra can be attributed to the differing steric environments of the carbons, as well as to the possibly different conformations of the furanoside and the free fructofuranose.

Table II. The Cmr Spectrum of Fructose (**7**, **8**) in Aqueous Solution[a]

Peak no.	7	8
1	94.6	91.0
2	122.7	111.8
3	123.3	116.7
4	124.7	117.8
5	128.4	129.4
6	129.3	130.1

[a] Chemical shifts in parts per million upfield from external carbon disulfide.

A remarkable feature of the cmr spectrum of **4** is the position of the C-1 resonance which is far upfield from its position in maltose (**3a**, **b**), methyl β-maltoside (**3c**), or methyl α-glucoside (**5c**). This peak is also rather broad and of diminished intensity. The resonance of C-2′ is, by contrast, very sharp and intense. We attribute the position of the C-1 signal to strong steric perturbation of H-1 by the furanoside ring. The large peak width of this resonance, and indeed of several resonances throughout the spectrum, may be due to rotations around the glycosidic linkages at rates approximating the nmr time scale.

While the interpretations of the above spectra are in some degree tentative, they permit some general conclusions. First, the conformations of cellobiose (**1**) and lactose (**2**) do not seem to involve forms which permit strong intramolecular steric interactions between the two rings. Indeed, the similarities between the spectra of these disaccharides and their monomers are quite striking. This is true even of C-2, -3′, -5′, and -6′, and although steric interactions be-

(16) (a) S. C. C. Chu and G. A. Jeffrey, *Acta Crystallogr.*, **23**, 1038 (1967); (b) R. U. Lemieux, A. A. Pavia, J. C. Martin, and K. A. Watanabe, *Can. J. Chem.*, 47, 4427 (1969).

(17) (a) D. E. Dorman and J. D. Roberts, *Proc. Nat. Acad. Sci. U. S.*, 65, 19 (1970); (b) A. J. Jones, M. W. Winkley, D. M. Grant, and R. K. Robins, *ibid.*, 65, 27 (1970).

(18) F. Grønlund and B. Anderson, *Acta Chem. Scand.*, 20, 2663 (1966).

tween these sites might be expected to be rather severe, the chemical-shift differences between the disaccharides and their monomers are, in fact, not large. Thus, adding a pyranose moiety equatorially to the anomeric center of glucose has no more effect on the chemical shift of C-2 than does the addition of an *O*-methyl group at the same position.[4]

By contrast, cmr spectra of disaccharides joined through α-glycosidic linkages show the effects of either large changes in conformation or important intramolecular steric interactions. Thus, comparisons of the cmr spectra of maltose (**3**) and sucrose (**4**) with those of their monomers evince the presence of both up- and downfield shifts. Since the latter shifts are rare in systems wherein steric perturbation is important,[4,8] their elucidation may provide important information regarding the conformations of these molecules.

Finally, the simplicity of proton-decoupled cmr spectra of carbohydrates is again emphasized. Although the spectra of anomeric mixtures show several overlapping resonances, in those of pure compounds nearly all the resonances are clearly resolved. It is evident that cmr spectroscopy can make important contributions in carbohydrate chemistry.

B. Carbohydrates in Alkaline Solutions. It has been known for some time that certain of the properties of carbohydrates in aqueous solution are dependent upon the pH. Thus, Reeves and Blouin[19] noted a pH dependence of the optical rotation of several sugars and attributed this effect to changes in the conformation. A more recent study involving proton magnetic resonance (pmr) measurements, however, has shown that the conformations of sugars possessing no free anomeric hydroxyl group are not different in aqueous and alkaline solutions.[20] However, for pyranoses with a free anomeric hydroxyl function, it was reported[20] that the anomeric mixture is shifted strongly toward the β anomer. The rationale[19,20] of this behavior was the notion that axial hydroxyl groups become more bulky upon ionization, due to increased solvation. This would disfavor the α anomer with respect to the β, thereby shifting the equilibrium toward the latter.

Such a phenomenon has obvious application to the present study of disaccharides. Thus, if the β anomer could be strongly favored in solution, the accompanying simplification of the cmr spectrum would be of great aid in its interpretation. It is first necessary, however, to determine the effect of high pH on the carbon chemical shifts of carbohydrates. Accordingly, the spectra of methyl α-glucoside (**5c**), methyl β-glucoside (**5d**), and sucrose (**4**) were studied in 1 *N* sodium hydroxide solutions. It was found that the effect of the basic solution was quite small, there being at most a 0.2-ppm downfield shift in most of the carbon resonances.

This method of simplifying the spectra of carbohydrates therefore seemed of great promise. Unfortunately, it was found that the spectrum of glucose (**5a, b**) in 1 *N* sodium hydroxide was almost unchanged from that in neutral solution. In 1.5 *N* potassium hydroxide, the base used by Rao and Foster,[20] there was very little, if any, enhancement of the proportion of β anomer. Again, there were general and relatively small downfield shifts in most of the carbon resonances. Reexamination of the pmr spectrum of glucose in 1.5 *N* potassium hydroxide published by Rao and Foster[20] suggests that their failure to detect the H-1 resonance of the α anomer might have been due to poor adjustment of the detector phase.

There were, however, quite remarkable changes in the spectra of maltose (**3a, b**) and methyl β-maltoside (**3c**) in 1 *N* sodium hydroxide. While the spectrum of the former was very complex, there was no indication that the relative proportions of the two anomers had been significantly altered. The changes in the chemical shifts were, however, generally larger and more variable than observed in the other carbohydrates. Changes in the spectrum of maltose were also evident in 0.5 *N* sodium hydroxide (Table III). In both solutions, as in the case of glucose, the development of yellow-brown colorations and new peaks near 128 ppm gave indication of the operation of the Lobry de Bruyn–van Eckenstein rearrangement.[21] In the 0.5 *N* sodium hydroxide solution, however, this difficulty was less pronounced and the cmr spectrum was less complex.

Table III. The pH Dependence of the Cmr Spectra of Maltose (**3a, b**) and Methyl β-Maltoside (**3c**)

Peak no.	Maltose (**3a, b**) Neutral	Maltose (**3a, b**) 0.5 *N* NaOH	Maltose (**3a, b**) 1 *N* NaOH	Methyl β-maltoside (**3c**) Neutral	Methyl β-maltoside (**3c**) 1 *N* NaOH
1	92.8	92.5	92.7	89.6	89.0
2	96.6	95.1	95.8	92.9	90.7
3	100.5	99.9	100.2	115.3	113.0
4	114.8	115.0	114.8	116.4	116.3
5	115.1	116.1	115.0	118.0	117.9
6	116.4	117.5	116.7	119.6[b]	118.2
7	118.0	118.2	118.1	119.9	119.2
8	119.4	119.2	119.6	120.8	119.5
9	119.9	119.4	119.9	123.1	119.9
10	120.7	119.7	120.3	131.8	122.9
11	121.1	120.6	120.9	132.0	131.7[a]
12	122.6	122.9	122.9	135.5	135.5
13	123.0	131.7	131.8		
14	131.8				

[a] Chemical shifts in parts per million upfield from external carbon disulfide. [b] Doubly intense.

Even clearer, however, was the pH dependence of the cmr spectrum of methyl β-maltoside (**3c**), and it is possible to identify tentatively some of the resonances of this spectrum. It is notable, for example, that peaks assigned above to the methyl carbon and C-1′, -2′, -3′, and -5′ can be closely correlated with peaks in the spectrum taken in alkaline solution. The resonances of C-1, -3, and -4′, however, are obviously shifted by significant downfield increments. This possibly signals large changes in the conformation of the glucosyl ring. This is not entirely clear, however, because similar changes were detected in the cmr spectrum of amylose in which the shifts may be attributed to disruption of the helix conformation (*vide infra*).

C. The Acetates. Studies involving the *O*-methyl ethers of cyclitols[3] and pyranoses[4] were instrumental in the eventual interpretation of those spectra. The cmr spectra of acetylated carbohydrates have not been studied, however. In an effort to find a means of interpreting the cmr spectrum of cellulose acetate (*vide infra*),

(19) R. E. Reeves and F. A. Blouin, *J. Amer. Chem. Soc.*, 79, 2261 (1956).

(20) V. S. R. Rao and J. F. Foster, *J. Phys. Chem.*, 69, 636 (1965).

(21) W. Pigman, Ed., "The Carbohydrates," Academic Press, New York, N. Y., 1957, p 60.

a brief examination of the cmr spectra of the two peracetylated isomers of glucose and cellobiose was undertaken.

There are few extant data to aid in the interpretation of the acetylated carbohydrates. Christl, Reich, and Roberts[15] have studied the cmr spectra of four simple acetates (Table IV). Their results indicate that acetylation of a primary or secondary group is accompanied by small downfield shifts in the resonance of the carbinyl carbon; only in the case of *tert*-butyl alcohol and its acetate, however, was there a shift of the magnitude usually observed for O-methylation of primary and secondary alcohols.[3,4] At the carbon adjacent to the carbinyl carbon, there were observed reasonably constant but not very large (3.5–5) upfield shifts. In all cases, the acetate carbonyl carbon came at 22–23 ppm, while the methyl resonance observed occurred near 171–173 ppm.

Table IV. Comparison of the Carbon Chemical Shifts of Simple Alcohols and Their Acetates[a,b]

Alcohol	Carbon	δ(OH)	δ(OAc)	Δδ
Methanol		143.5	141.8	−1.7
Ethanol	α	135.5	132.7	−2.8
	β	174.9	178.7	+3.8
Isopropyl	α	129.1	125.7	−3.4
alcohol	β	167.4	171.1	+3.7
tert-Butyl	α	124.1	113.1	−11.0
alcohol	β	161.2	164.7	+3.5

[a] Data from Christl, Reich, and Roberts.[15] [b] Chemical shifts in parts per million upfield from external carbon disulfide.

It is difficult, of course, to extrapolate these data to the acetylated carbohydrates. Each carbon of the latter can be subject to the influences, steric and electronic, of two or three proximal acetyl groups. It seems reasonable, however, to assume that the steric effects will outweigh the smaller deshielding mechanism to give a net upfield shift. This is indeed as observed, there being a general shift in the cmr spectrum of the pyranose carbons toward higher field (*cf.* Table V).

The interpretation of the spectra of α- and β-glucose pentaacetate (**5c** and **5f**) may be initiated by the assignment of the resonances near 100 and 131 ppm to C-1 and -6, respectively. It is noted that the C-6 resonances, unlike all others in the spectrum, are deshielded with respect to their positions in the free pyranoses. This behavior is apparently related to the primary nature of the C-6 hydroxyl group. The resonances of the anomeric carbons are noted to undergo large upfield shifts with respect to the free sugars.

In comparing the spectra of α- and β-glucose pentaacetates, it is noted that only one resonance other than that already assigned to C-6 remains unchanged in the two spectra. This peak is assigned to C-4 on the basis that this carbon is nearly as remote from the anomeric center as is C-6.[4] As in the cmr spectra of the free sugars, this resonance is the highest field of those assignable to pyranose ring carbons.

There remain only two unassigned resonances in the spectra of the glucose pentaacetates, and one of these is twice as intense as the other in each spectrum. By elimination, these peaks must represent C-2, -3, and -5, and in comparing the spectra of the two isomers it can be noted that all three of these nuclei must be shielded in the α relative to the β anomer.

A similar partial assignment of the spectra of α- and β-cellobiose octaacetates (**1d** and **1e**) is possible. Comparing the spectra of these compounds (Table V), it is noted that the resonance near 92 ppm is essentially unchanged in the two spectra. The resonances between 100 and 105 ppm, however, correspond very closely to those of the C-1 resonances of the glucose pentaacetates. Hence, the lower field peak is assigned to C-1, while those at higher field must be due to the C-1′ resonances of the octaacetates. At higher fields, it is somewhat surprising to learn that the C-6, -6′ resonances are clearly resolved in the α-cellobiose octaacetate but overlapping in the β isomer. The remaining spectra of these two octaacetates are superimposable with the exception of only three peaks. Because only the resonances of C-1′, -2′, -3′, and -5′ should be different in these compounds, it is safe to conclude that these three resonances so identified in this region of these spectra represent C-2′, -3′, and -5′.

It is also possible to assign the resonances of C-4 and -4′ of the octaacetates. The peak near 125 ppm is assigned to C-4 in each spectrum on the basis that the chemical shifts of the resonances of these carbons would

Table V. The Cmr Spectra of the α and β Anomers of Glucose Pentaacetate and Cellobiose Octaacetate[a]

Compound	Carbonyl carbons	Carbon 1	2	3	4	5	6	1′	2′	3′	4′	5′	6′	Methyl carbons
α-Glucose pentaacetate	22.7, 23.1, 23.3, 23.7, 24.2	103.9	123.6	123.0	124.8	123.0	131.4							172.2, 172.7,[b] 173.2
β-Glucose pentaacetate	22.7, 23.2, 23.6, 23.8, 24.1	101.2	122.5	120.2	124.9	120.2	131.2							172.6, 172.7[b]
α-Cellobiose octaacetate	22.6, 22.8, 23.0, 23.1, 23.2, 23.6, 23.8, 23.9	92.1	120.1[d]	120.9[d]	124.9	121.4[d]	131.3	104.1	123.6 or 123.7	123.6[e] or 123.7	117.0	122.1[e]	131.7	172.6
β-Cellobiose octaacetate	22.7, 22.8, 23.0, 23.3, 23.6,[c] 23.9, 24.2	92.4	120.1[d]	120.8[d]	124.8	121.4[d]	131.3	101.2	122.5	119.3 or 120.8	117.1	119.3 or 120.8	131.2	172.6, 172.9

[a] Chemical shifts in parts per million upfield from external carbon disulfide. [b] Most intense band in methyl region. [c] Of double intensity. [d,e] Peaks so symbolized and within the same spectrum cannot be differentiated in assignment.

Table VI. Sequence of Peaks in Cmr Spectra of Some Common Polysaccharides[a]

	Solvent	1	2	3	4	5	6
Soluble starch	H_2O	93.0	115.5	119.4	121.2	121.5	132.1
	0.01 *N* NaOH	93.0	115.5	119.2	120.9	121.3	131.9
	0.1 *N* NaOH	92.6	115.1	119.2	120.9	121.3	132.1
	1 *N* NaOH	91.1	113.0	118.4	120.1	121.3	131.8
	6.5 *M* LiBr	93.3	115.4	119.3	121.2	121.7	131.7
Cellulose acetate	CH_2Cl_2	92.2	116.7	120.3[b]	130.9		
		CH_3, 172.0; C=O, 22.4, 22.8, 23.2					

[a] Chemical shifts in parts per million upfield from external carbon disulfide. Peak 1 arises from C-1, peak 2 from C-4, and peak 6 from C-6. The other peaks are *not* assigned to specific carbons. [b] Peak more intense and broader than any other below 150 ppm.

not be expected to differ largely from the chemical shifts of the C-4 resonances of the glucose pentaacetates. The unusually low-field peaks near 117 ppm are assigned to the C-4′ resonances, and it is instructive that the usual large downfield β shift persists in the peracetates.

The spectra of the glucose pentaacetates and the cellobiose octaacetates have all progressed to the point where only the resonances of 2, 3, 5, 2′, 3′, and 5′ remain unassigned. Assuming that the C-2′ resonance of α-cellobiose octaacetate (**1d**) will not differ in chemical shift from the C-2 resonance of α-glucose pentaacetate (**5e**), comparison of these two spectra indicates that these two resonances occur at approximately 123.6 ppm. A similar argument establishes the peaks at 122.5 ppm to represent the analogous carbon resonances of the β isomers. The bases of these arguments are, of course, the similarities in the chemical shifts of the anomeric carbons which bear acetate groups. Note that this assignment specifies that the doubly intense peaks in the spectra of α- and β-glucose pentaacetates represent the resonances of C-3 and -5 of each isomer, and the cmr spectra of these compounds are therefore fully assigned.

It is not possible to specify any further assignments of the cellobiose octaacetates with certainty. Certain features regarding these spectra, however, are obvious even from this partial assignment. It is seen from Figure 4, for example, that the resonances of C-3′ and -5′ of β-cellobiose are shifted in opposite directions, away from the C-3, -5 resonance of β-glucose pentaacetate. Further, the resonances of C-2, -3, and -5 occur in a relatively narrow range centered at approximately 120.7 ppm. It is rather surprising that the C-2 resonance of the octaacetate is at lower field than is the analogous resonance of β-glucose pentaacetate; this seems to imply greater steric interactions at this center in the pentaacetate than in the octaacetate. It is also striking that at least one of the resonances assigned to C-3 and -5 of β-cellobiose octaacetate is significantly shielded relative to its position in β-glucose pentaacetate. Clearly, the interpretation of the cmr spectra of the acetates of carbohydrates would require a more extensive study of model compounds.

D. The Polysaccharides. To test the extent to which cmr spectroscopy is applicable to the higher molecular weight carbohydrates, a preliminary study of the cmr spectra of some common polysaccharides was undertaken. The pmr spectra of these compounds have already been shown to be useful in the identification and correlation of polysaccharides from various sources. The pmr spectra are somewhat complex, however, and often only the H-1 proton signal is useful in structure elucidation and correlations. The results above suggest that cmr spectra of these compounds might be somewhat less complex.

There are obvious problems involved in the cmr spectra of polysaccharides, however. The first is the high solubility generally required to overcome the low inherent sensitivity of carbon-13. In the studies discussed below, the solutions were often quite viscous because of the high concentrations used. In the case of soluble

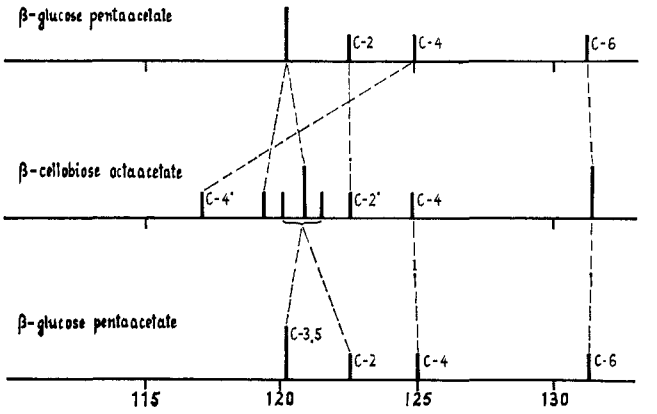


Figure 4. Comparison of the spectra of β-cellobiose octaacetate (**1e**) and β-glucose pentaacetate (**5f**). The line positions in the upper curve are correlated with the glycoside portion of the β-cellobiose octaacetate while those of the lower curve are correlated with the glycosyl part.

starch in neutral solutions, the samples studied were gels of varying rigidity. Another problem is that of the excessive line widths encountered. Because of the latter problem, the reliability of the chemical shifts presented is somewhat lower, the uncertainty in some cases approaching 0.3 ppm.

The first example studied was amylose. The sample used was soluble starch, a commercial product containing 10–15% amylopectin. In the present preliminary study, the presence of a 15% impurity, especially one which is so similar in structure, would probably have a rather minor effect on the results. The spectra obtained are presented in Table VI. It is evident that all six resonances are resolved, although the separation of the peaks near 121 ppm is of the same order as their uncertainties.

A series of studies has indicated that amylose in solution exists in the form of a helix established through hydrogen bonding between the C-2 hydroxyl of one monomeric unit with the C-3 hydroxyl of the next.[23]

(22) P. A. J. Gorin, J. F. T. Spencer, and R. J. Magus, *Can. J. Chem.*, 47, 3569 (1969), and references therein.

(23) (a) S. R. Erlander and R. Tobin, *Makromol. Chem.*, 111, 212 (1968); (b) S. R. Erlander, R. M. Purvinas, and H. L. Griffin, *Cereal Chem.*, 45, 140 (1968).

At high pH or at high concentrations of certain ionic species, the internal hydrogen bonding is thought to be disrupted, with the amylose adapting a random-coil conformation.[23] To investigate the effect of this phenomenon on the cmr spectrum of amylose, we have measured the carbon chemical shifts of amylose as a function of basicity (Table VI). It is observed that the changes are rather minor except when 1 *N* sodium hydroxide was used. In 6.5 *M* lithium bromide, a system known[23] to have significant effects upon the optical rotation of amylose, there were only small changes in the cmr spectrum compared to neutral solutions.

The interpretation of the spectrum of soluble starch is difficult in that we have no really adequate model for comparison. Once the resonances of C-1, -4, and -6 have been assigned in the usual way, there can be no more specific assignments. It is interesting, however, to observe that the positions of the three specifically assigned resonances of amylose are very similar in chemical shift to the analogous nuclei of maltose (**3a, b**) and methyl β-maltoside (**3c**). Furthermore, the changes observed in these chemical shifts in 1 *N* sodium hydroxide approximate closely those changes observed in the spectrum of **3c** in the same milieu. It therefore seems probable that similar changes in conformation are being observed in both cases.

The constancy in the chemical shift of the C-6 resonance throughout the systems studied is also interesting. While in a helical conformation such as the one described above, the hydroxymethyl group would be expected to experience no intramolecular steric interactions. The disruption of the helix, however, could lead to increased interactions of this carbon and, hence, to changes in its chemical shift. In fact, no significant changes are observed.

An attempt was also made to measure the chemical shifts of the carbons of glycogen. After some 2400 spectral accumulations, peaks were evident at approximately 119.7, 120.9, and 121.7 ppm. However, no resonances corresponding to C-1, -4, and -6 could be detected.

Finally, the cmr spectrum of cellulose acetate was measured (Table VI). In this case, the peaks other than the easily assigned resonances of C-1, -4, and -6 overlap in one large broad peak at about 120.3 ppm. As often observed in the glucose and cellobiose acetates, the acetate methyl resonances could not be resolved. The resonances due to the acetate carbonyl carbons, however, were clearly resolved and easily observable. Again, it is recognized that the spectrum of this polysaccharide closely approximates that of its closest disaccharide relative, β-cellobiose octaacetate.

While these results present no really new data about the conformations of these molecules, the fact that the spectra were measurable heralds a new and important method in carbohydrate chemistry. The development of pulse spectroscopic methods in conjunction with variable-temperature capabilities should make the cmr spectra of disaccharides routinely available to chemists concerned with their study.

Nuclear Magnetic Resonance Spectroscopy. Barriers to Internal Rotation in Some Halogenated Methylbutanes[1]

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Abstract: Barriers to internal rotation about the C(2)–C(3) bonds have been determined for ten closely related halogenated methylbutanes by detailed line-shape analysis of their nuclear magnetic resonance spectra. The experimental barriers range from 9 to 16 kcal/mol. In addition, for some of the compounds investigated, free-energy differences were obtained for rotational isomers under conditions of slow conformational exchange. The results are discussed in terms of possible contributing nonbonded interactions.

Molecular structure and reactivity are governed to a large extent by both attractive and repulsive nonbonded interactions between atoms or groups of atoms within the molecule. Extensive studies of conformational equilibria have been especially informative in revealing the nature and magnitudes of intramolecular interactions.[2] Nuclear magnetic resonance (nmr) spectroscopy is particularly well suited for studying conformational equilibria and equilibration because, in favorable cases, it provides not only barriers to conformational equilibration, but also direct measurements of ground-state conformational energy differences.[3]

Rotation about C–C bonds in substituted ethanes is of considerable current interest,[4,5] and has been

(1) Supported by the National Science Foundation; presented at the 161st National Meeting of the American Chemical Society, Los Angeles, Calif. March 31, 1971.

(2) E. L. Eliel, N. L. Allinger, S. J. Angyal, and G. A. Morrison, "Conformational Analysis," Interscience, New York, N. Y., 1965; M. Hanack, "Conformation Theory," Academic Press, New York, N. Y., 1965.

(3) (a) G. Binsch, *Top. Stereochem.*, 3, 97 (1968); (b) J. W. Emsley, J. Feeney, and L. H. Sutcliffe, "High Resolution Nuclear Magnetic Resonance Spectroscopy," Vol. 1, Pergamon Press, Elmsford, N. Y., 1965, Chapter 9.

(4) (a) F. J. Weigert, M. B. Winstead, J. I. Garrels, and J. D. Roberts, *J. Amer. Chem. Soc.*, **92**, 7359 (1970); (b) W. J. Orville-Thomas, *J. Mol. Struct.*, 6, 1 (1970); (c) N. Sheppard, *ibid.*, 6, 5 (1970); (d) A. V. Cunliffe, *ibid.*, 6, 9 (1970); (e) P. N. Brier, *ibid.*, 6, 23 (1970); (f) N. L. Owen, *ibid.*, 6, 37 (1970); (g) R. J. Abraham, *ibid.*, 6, 49 (1970); (h) J. Bailey and S. Walker, *ibid.*, 6, 53 (1970); (i) E. Wyn-Jones, *ibid.*, 6,