Table V. Quantum Yield of Disappearance of Phenyl Azide Data in Air-Saturated Acetonitrile Solutions[a]

concn, M	$\phi(-PhN_3)$	I_0, photons/s	V_0, mL
3.79×10^{-1}	2.71×10^{1}	1.11×10^{15}	4.0
2.18×10^{-1}	2.60×10^{3}	4.02×10^{14}	3.5
2.02×10^{-1}	9.11×10^{3}	7.28×10^{13}	4.0
1.95×10^{-1}	3.78×10^{3}	2.74×10^{14}	3.5
1.84×10^{-1}	3.34×10^{3}	2.42×10^{14}	3.5
1.74×10^{-1}	3.78×10^{3}	3.70×10^{13}	3.5
1.69×10^{-1}	8.82×10^{3}	1.11×10^{14}	4.0
1.69×10^{-1}	1.62×10^{3}	1.87×10^{14}	4.0
1.64×10^{-1}	2.89×10^{3}	2.79×10^{14}	3.5
1.58×10^{-1}	1.42×10^{3}	2.64×10^{14}	3.5
1.37×10^{-1}	4.33×10^{3}	7.64×10^{13}	3.5
1.25×10^{-1}	2.92×10^{3}	2.48×10^{14}	4.0
8.68×10^{-2}	6.03×10^{2}	2.48×10^{14}	4.0
3.25×10^{-2}	9.96	1.07×10^{15}	4.0
3.25×10^{-3}	2.10	9.89×10^{14}	4.0

[a] $A_0 > 3.0$.

shown in Figures 2 and 3 indicate that k_2 and k_3 are essentially equal. Since both reactions 2 and 3 depend upon a phenylnitrene/phenyl azide interaction, that $k_2 \simeq k_3$ is not unexpected. If $k_2 \simeq k_3$, then from the value of the intercept in Figure 2 (24.84) eq 13 yields the relationship $k_2 \simeq (0.1k_4)^{1/2}$; however, in view of the length of the required extrapolation, this value provides only a rough estimate.

Equation 10 also predicts that if $[PhN_3]$ and V_0 are constants, $\phi(-PhN_3)$ will depend upon I_0 such that as I_0 is increased, $\phi(-PhN_3)$ should decrease. While only limited data are available the decrease in $\phi(-PhN_3)$ with increasing I_0 is evident from data at $[PhN_3] = 1.60 \times 10^{-4}$ M (Table IV) and $[PhN_3] = 1.69 \times 10^{-1}$ M (Table V). This relationship is quite notable for the latter case since an increase in I_0 from 1.11×10^{14} to 1.87×10^{14} photons/s results in a decrease in $\phi(-PhN_3)$ from 8.82×10^3 to 1.62×10^3. Also notable is the unusually low $\phi(-PhN_3)$ value of 2.71×10^1 determined at $[PhN_3] = 3.79 \times 10^{-1}$ and $I_0 = 1.11 \times 10^{15}$, the highest value of I_0 utilized in these experiments (Table V).

One measure of the validity of this kinetic treatment of the experimental results is its ability to predict the experimental parameters based upon the simplified four-step reaction mechanism. Indeed, eq 10 correlates (i) all $\phi(-PhN_3)$ values ranging from 0.3 to 15000, (ii) all $[PhN_3]$ data ranging from 1.40×10^{-5} to 7.93×10^{-1} M, (iii) all $\phi(-PhN_3)$ data whether determined in oxygen-purged, oxygen-saturated, or air-saturated solution, and (iv) the variation of $\phi(-PhN_3)$ with I_0.

Finally, eq 10 and Figures 1–3 serve to clarify the photoinitiated autocatalytic (branching) chain decomposition (PACD) reaction of phenyl azide in solution, hence further documentation of the *first molecular explosion in solution.*[8]

Conclusion

Upon irradiation of phenyl azide in dilute acetonitrile solution, molecular nitrogen is lost[4-6] and phenylnitrene is formed.[1-3] $\phi(-PhN_3) = 0.58$ for $[PhN_3] < 10^{-4}$ M. Dimerization of two phenylnitrenes leads to (*E*)-azobenzene formation.[11] At higher $[PhN_3]$ phenylnitrene reacts with phenyl azide to afford two phenylnitrenes,[10,13] which then react further. This autocatalytic branching chain decomposition reaction[9] is manifested as $\phi(-PhN_3)$ values that greatly exceed unit efficiency.[7,8] A kinetic analysis of a simplified four-step reaction mechanism provides a mathematical expression that serves to explain the variation of $\phi(-PhN_3)$ with $[PhN_3]$, I_0, and V_0. $\phi(-PhN_3)$ increases with increasing $[PhN_3]^2$ and decreases with increasing I_0. The mathematical expression fitting the 56 experimental determinations of $\phi(-PhN_3)$ affords further definitive evidence of the *first molecular explosion in solution.*[8]

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Registry No. Phenyl azide, 622-37-7.

Molecular Structure as Reflected in the ^{13}C NMR Spectra of Oligosaccharides with Partially Deuterated Hydroxyls[1]

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Abstract: This paper presents a new approach to the ^{13}C NMR analysis of oligosaccharides. The approach is based on the recently described structural effects on the isotopic multiplets in the spectra of materials with partially deuterated hydroxyls and on some well-known features of the ^{13}C chemical shifts of carbohydrates. It is shown that the kind and number of isotopic multiplets for the nonanomeric methines (carbons 2, 3, 4, and 5 of aldohexoses, carbons 3, 4, and 5 of ketohexoses) form sets characteristic of the position of substitution and can be used in tracking the sequence of glycosidic linkages. In favorable cases, ab initio analysis of di- and trisaccharides is possible by this approach. As examples, the isotopic multiplets in the spectra of α-lactose and a series of fructose-containing oligosaccharides are examined in detail.

Carbon-13 NMR spectroscopy has taken a prominent place beside the traditional chemical and enzymological approaches to the structure elucidation of complex carbohydrates. The use of ^{13}C chemical shifts in structural studies of oligosaccharides in solution requires comparison with the spectra of monosaccharides and knowledge of empirical rules governing substituent effects.[2,3] The application of such approaches is based on known monosaccharide composition and resonance assignments. Several assignment techniques of increasing complexity are available.[2-4] The most elaborate among them is two-dimensional NMR, which relies only on some well-established facts regarding the chemical shifts of the anomeric carbons and hydrogens.[4] Unequivocal spectral assignments can also be obtained from the isotopic multiplets in the spectra of carbohydrates with partially deuterated hydroxyls (in Me_2SO solutions).[5,6] These multiplets are due to small upfield deuterium isotope effects on ^{13}C chemical shifts: 0.09–0.12 ppm for directly bonded hydroxyls (Δ_β) and 0.07 ppm or less for

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Table I. Carbon-13 Chemical Shifts as Elements of Carbohydrate Structure

label	structural element	spectral manifestation
CS1	anomeric carbons	(a) resonate in the 90–100-ppm region (b) quaternary in ketoses
CS2	methylene carbons	(a) resonate in the 60–70-ppm region (b) aldoses have one, ketoses have two such carbons
CS3	C4 of aldofuranoses (C5 of ketofuranoses)[a]	resonate in the 80–88-ppm region
CS4	C5 of β-aldopyranoses	resonate in the 75–78-ppm region
CS5	substitution of hydroxyl hydrogen leads to	(a) downfield shift of up to 11 ppm for α carbon (b) upfield shift of up to 2 ppm for β carbon(s)

[a] Also C2 of aldofuranoses (C3 of ketofuranoses) with a trans–trans configuration of hydroxyls at C1, C2, and C3 (C2, C3, and C4 in ketofuranoses).

Table II. Isotopic Multiplets as Elements of Carbohydrate Structure

label	structural element	spectral manifestation
IM1	hydroxylated carbon	β effect, Δ_β: 103–118 ppb for $-CH_2OH$, 96–115 ppb for =CHOH, 76–86 ppb for ≡COH
IM2	nonreducing sugar	no β-effect for anomeric carbons
IM3	hydroxyl on vicinal carbon	γ effect, Δ_γ: <70 ppb
IM4	vicinal diol	Δ_γ(trans) > Δ_γ(cis)
IM5	HOC*C*(OH)-C′OH	type $\beta\gamma_2$: (a) octet for $\Delta_\beta > \Delta_\gamma \neq \Delta_{\gamma'}$; (b) septet for $\Delta_\beta = \Delta_\gamma + \Delta_{\gamma'}$, $\Delta_\gamma \neq \Delta_{\gamma'}$; (c) sextet for $\Delta_\beta > \Delta_\gamma + \Delta_{\gamma'}$, $\Delta_\gamma = \Delta_{\gamma'}$; (d) quintet for $\Delta_\beta = \Delta_\gamma + \Delta_{\gamma'}$, $\Delta_\gamma = \Delta_{\gamma'}$
IM6	HOC*C*(OH)-C′OR	type βγ: quartet (Δ_β and Δ_γ)
IM7	HOC*C*(OR)-C′OH	type γ_2: (a) quartet for $\Delta_\gamma \neq \Delta_{\gamma'}$, (b) triplet for $\Delta_\gamma = \Delta_{\gamma'}$
IM8	ROC*C*(OH)-C′OR	type β: doublet (Δ_β)
IM9	HOC*C*(OR)-C′OR	type γ: doublet (Δ_γ)
IM10	ROC*C*(OR)-C′OR	type s: singlet
IM11	2,3,4-cis OH (ribo)	C3 is a $\beta\gamma_2$ sextet with small Δ_γ; C4 is a βγ quartet with small Δ_γ
IM12	2,3-trans/3,4-cis OH (arabino)	C3 is a $\beta\gamma_2$ octet; C4 is a βγ quartet with small Δ_γ
IM13	2,3-cis/3,4-trans OH (lyxo)	C3 is a $\beta\gamma_2$ octet; C4 is a βγ quartet with large Δ_γ
IM14	2,3-trans/3,4-trans OH (xylo)	C3 is a $\beta\gamma_2$ sextet with large Δ_γ; C4 is a βγ quartet with large Δ_γ

hydroxyls on vicinal carbons (Δ_γ). The multiplet type and magnitude of splittings depend on the details of the hydroxylic environment of the carbon atom and can serve as reporters of local structural features such as the number of hydroxyls[5,6] and their steric relationship.[7] Thus, a detailed investigation of the isotopic multiplets in the spectra of a series of monosaccharides has led to the conclusion that the pattern of multiplets is a fingerprint of molecular structure at the pentopyranose level.[1] It could be anticipated that substitution of a hydroxyl, e.g., by the formation of a glycosidic linkage with another monosaccharide, will perturb the multiplet pattern in a way that will reflect the position of substitution.[7]

This paper examines the types and number of isotopic multiplets expected for di- and trisaccharides. An attempt is made to gain detailed information on monosaccharide sequence and composition directly from the ^{13}C NMR spectra of oligosaccharides with

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Table III. List of Isotopic Multiplets and Multiplet Matrix for the Nonanomeric Methines of Monosubstituted Hexoses

substituted carbon	multiplet type C2	C3	C4	C5	number of multiplets $\beta\gamma_2$	$\beta\gamma$	γ_2	β	γ	degen.
				Aldohexopyranose						
1	$\beta\gamma$	$\beta\gamma_2$	$\beta\gamma$	γ_2	1	2	1			
2	γ_2	$\beta\gamma$	$\beta\gamma$	γ_2		2	2			
3	$\beta\gamma$	γ_2	β	γ_2		1	2	1		
4	$\beta\gamma_2$	$\beta\gamma$	γ	γ	1	1			2	
6	$\beta\gamma_2$	$\beta\gamma_2$	$\beta\gamma$	γ	2	1			1	
				Ketohexopyranose						
1		$\beta\gamma_2$	$\beta\gamma_2$	$\beta\gamma$	2	1				
2		$\beta\gamma$	$\beta\gamma_2$	$\beta\gamma$	1	2				
3		γ_2	$\beta\gamma$	$\beta\gamma$		2	1			*a*
4		$\beta\gamma$	γ_2	β		1	1	1		
5		$\beta\gamma_2$	$\beta\gamma$	γ	1	1			1	*b*
				Ketohexofuranose						
1		$\beta\gamma_2$	$\beta\gamma$	γ_2	1	1	1			
2		$\beta\gamma$	$\beta\gamma$	γ_2		2	1			*a*
3		γ_2	β	γ_2			2	1		
4		$\beta\gamma$	γ	γ		1			2	
6		$\beta\gamma_2$	$\beta\gamma$	γ	1	1			1	*b*

[a,b] Identical sets are marked with the same letter.

Table IV. List of Isotopic Multiplets and Multiplet Matrix for the Nonanomeric Methines of Some Disubstituted Hexoses

substituted carbon	multiplet type C2	C3	C4	C5	number of multiplets $\beta\gamma_2$	$\beta\gamma$	γ_2	β	γ	degen.
				Aldohexopyranose						
1,2	γ	$\beta\gamma$	$\beta\gamma$	γ_2		2	1		1	
1,3	β	γ_2	β	γ_2			2	2		
1,4	$\beta\gamma$	$\beta\gamma$	γ	γ		2			2	
1,6	$\beta\gamma$	$\beta\gamma_2$	$\beta\gamma$	γ	1	2			1	
				Ketohexopyranose						
2,1		$\beta\gamma$	$\beta\gamma_2$	$\beta\gamma$	1	2				
2,3		γ	$\beta\gamma$	$\beta\gamma$		2			1	*a*
2,4		β	γ_2	β			1	2		
2,5		$\beta\gamma$	$\beta\gamma$	γ		2			1	*a*
				Ketohexofuranose						
2,1		$\beta\gamma$	$\beta\gamma$	γ_2		2	1			
2,3		γ	β	γ_2			1	1	1	
2,4		β	γ	γ				1	2	
2,6		$\beta\gamma$	$\beta\gamma$	γ		2			1	*a*

[a] Identical sets.

partially deuterated hydroxyls. For this purpose the structural elements reflected in the ^{13}C chemical shift values, the magnitude of the isotope effects, and the pattern and number of isotopic multiplets are used in a concerted fashion. This approach is applied to α-lactose and a series of fructose-containing oligosaccharides. Early ^{13}C NMR work on similar substances has dealt mainly with resonance assignments and anomeric composition.[4,8–14]

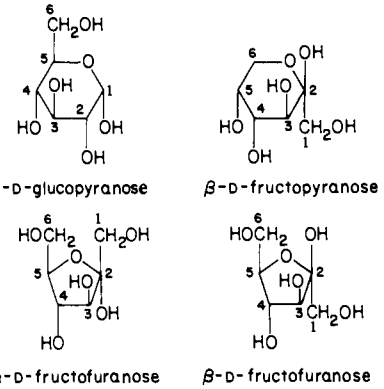

Table V. Multiplet Matrices for Some Hexose Disaccharides

X	$\beta\gamma_2$	$\beta\gamma$	γ_2	β	γ	degen.
		Aldopyranosyl(1→X)aldopyranose				
1	2	4	2			
2	1	4	3			
3	1	3	3	1		
4	2	3	1		2	
6	3	3	1		1	
		Aldopyranosyl(1→X)ketopyranose				
1	3	3	1			
2	2	4	1			
3	1	4	2			*a*
4	1	3	2	1		*b*
5	2	3	1		1	*c*
		Aldopyranosyl(1→X)ketofuranose				
1	2	3	2			
2	1	4	2			*a*
3	1	2	3	1		
4	1	3	1		2	*d*
6	2	3	1		1	*c*
		Ketopyranosyl(2→X)aldopyranose				
2	1	4	2			*a*
3	1	3	2	1		*b*
4	2	3			2	
6	3	3			1	
		Ketofuranosyl(2→X)aldopyranose				
2		4	3			
3		3	3	1		
4	1	3	1		2	*d*
6	2	3	1		1	*c*

[a-d] Identical sets are marked with the same letter.

Table VI. Percentage of Pyranose (P) and Furanose (F) Forms during Equilibration in Me_2SO-d_6 Solutions

	α-F	β-F	β-P
fructose	13	30	57[a]
lactulose	24	52[a]	24
palatinose	28	72[a]	
turanose	24	37	39[a]

[a] Isomer in fresh solutions.

Basis of Approach

Structural Elements. Spectral as well as structural assignments are usually based on a set of well-defined structural elements. The elements of molecular structure as reflected in the ^{13}C chemical shifts (resonance positions) of carbohydrates[2,3] are summarized in Table I. It should be pointed out that the resonances of methylene and quaternary carbons can be readily distinguished from the methine carbons by the "attached proton test" (APT).[15] Note that the scope of element CS3 is somewhat limited by the indicated degeneracy and by the possibility that other resonances may become shifted to the 80–88-ppm region by substituent effects. However, the absence of resonances in that spectral region should be taken as a conclusive indication for the absence of furanose structures.

The elements of molecular structure as reflected in the isotopic multiplets in the ^{13}C NMR spectra of carbohydrates with partially deuterated hydroxyls[1,5-7] are summarized in Table II. Elements IM8 and IM9, when applied to C6 of ketoses, provide another means of distinguishing between the pyranose and furanose forms. Note also that substitution at carbons 1 and 6 will leave elements IM11–IM14 intact. On the other hand, substitution at any of carbons 2, 3, or 4 will reduce the multiplicity of the C3 resonance, thereby leading to the loss of important structural information. However, the modification of the multiplet pattern can be used advantageously for the determination of the substituent position, i.e., for the sequencing of oligosaccharides. The logic is analogous to that employed in degradation methods, e.g., methylation analysis.[16]

Multiplet Matrices. The following discussion is concerned with the types of isotopic multiplets for the nonanomeric methines, i.e., carbons 2, 3, 4, and 5 of aldohexoses and carbons 3, 4, and 5 of ketohexoses. It will be shown that the set of numbers for multiplets of each type is characteristic of the position of substitution and can be used in tracing the sequence of glycosidic linkages in di- and trisaccharides. Table III gives a listing of the expected spectral types for monosubstituted hexoses along with the number of multiplets of each type. This latter set of numbers is referred to as "the multiplet matrix".

The remarkable feature in the multiplet matrix for monosubstituted hexoses is that each substitution results in a different set of isotopic multiplets. There are only two degeneracies, which can be easily resolved since they involve pyranose and furanose entries.

The multiplet matrix for disubstituted hexoses given in Table IV was constructed from the point of view of their involvement in oligosaccharides. Therefore, the anomeric carbon is always substituted. There is in Table IV a triple degeneracy involving a ketofuranose and two ketopyranose entries. The latter can be resolved by observing the multiplicity of C6, which should be a γ doublet in the 2,3-disubstituted molecule or a singlet in the 2,5 case.

The multiplet matrix for hexose disaccharides was constructed by summing the corresponding entries from the matrix for monosubstituted monosaccharides (Table III). The results are shown in Table V. There are four (two triple and two double) degeneracies in Table V. However, as will be demonstrated in the following sections, the degeneracies can be readily resolved by invoking other appropriately chosen structural elements.

The multiplet matrix for aldohexopyranosylaldohexopyranoses indicates that *the position of the glycosidic linkage in homodisaccharides can be determined by merely counting the isotopic multiplets of each type.* Partial or complete spectral assignment may be needed in more complex cases. The application of the multiplet matrices for disaccharides will be illustrated when the experimental results are discussed. Multiplet matrices for trisaccharides can be constructed by summing the appropriate entries for two monosubstituted monosaccharides (Table III) and a disubstituted one (Table IV). In the 20-entry matrix (not shown) for trisacchrides composed of aldohexopyranosyl residues, there are only two degeneracies. One of them is easily resolved since one of the entries is a nonreducing sugar. The other degeneracy involves (1→4)(1→6) and (1→6)(1→4) linkages, and its resolution may require a detailed spectral assignment. For homotrisaccharides, as well as for homodisaccharides, the positions of the glycosidic linkages can be determined from the multiplet numbers. While this is also true for heterotrisaccharides, the identification of the individual monosaccharide residues in the sequence will require a more detailed spectral interpretation. It is noteworthy, that the nonreducing end is the easiest to identify since its pattern of isotopic multiplets is the least perturbed.

The matrix (not shown) for trisaccharides composed of two aldohexopyranosyl and one ketohexofuranosyl residues contains 60 entries. There are 16 degeneracies ranging from double (10 of them) to quintuple (only one). Specific examples will be discussed along with the experimental data.

Logical Steps. In structural interpretations, the combined use

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Table VII. Carbon-13 Chemical Shifts[a] and Deuterium Isotope Effects[b] for Some Oligosaccharides

		C1	C2	C3	C4	C5	C6
			α-Lactose				
(1)	β-galactopyranosyl(1→4)-	105.49	72.32	74.90	69.85	77.16	62.11,[g]
		24	103, 38	104, 48, 21	107, 23	27, 27	111
	α-glucopyranose	93.71	73.79	73.05	82.92	71.47	62.26[f,g]
		97, *c*	107, 40, 40	101, 41	*c*	20, 20	110
			Lactulose				
(2)	β-galactopyranosyl(1→4)-	103.00	72.27[h]	74.97[i]	69.85	77.16[j]	62.15[k]
		c	*d*	*d*	*d*	29, 29	115
	β-fructopyranose	65.85	99.79	67.47	79.81	68.16	64.00
		110, 50	80, 11	100, 25	34, 16	105	40
(3)	β-galactopyranosyl(1→4)-	105.36	72.23[h]	74.69[i]	69.85	*e*, *j*	62.48[k]
		18	*d*	~100, 50, 10	*d*		109
	α-fructofuranose	65.36	106.35	83.46	86.89	80.61	62.65[k]
		100, 50	87, *c*	104, 67	41	16	106
(4)	β-galactopyranosyl(1→4)-	104.79	72.29	75.10	69.97	77.00	62.42
		15	102, 34	110, 47, 111	109, 14	30, 30	110
	β-fructofuranose	64.31	104.56	76.22	86.36	82.25	64.93
		136, 33, −21[f]	80, 13	99	32	23	105
			Palatinose				
(5)	α-glucopyranosyl(1→6)-	100.62	73.58	*e*	*e*	*e*	62.58
		23	104, 39				*d*
	α-fructofuranose	65.26	106.05	84.42	78.19	80.64	68.98
		114, 48, 18[f]	88, 12, 12	*c*	*c*	29	
(6)	α-glucopyranosyl(1→6)-	100.67	73.67	75.01	71.89	74.18	62.62
		19	105, 37	99, 43, 43	106, 36	26, 26	117
	β-fructofuranose	64.46	103.98	76.92	77.26	81.26	70.54
		116, 40, 23[f]	79, 13, 13	107. 52, *c*	100, 55	30	10
			Turanose				
(7)	α-glucopyranosyl(1→3)-	103.10	74.39	75.03	70.40	74.79	62.47
		27	102, 33	103, 34, 34	110, 41	22, 22	114
	β-fructopyranose	65.95	99.43	80.04	71.53	71.61	64.78
		120, 50	79, *c*	33, 24, 12[f]	101, 10	100, 15	38
(8)	α-glucopyranosyl(1→3)-	98.10	73.50	*e*	*e*	74.27	62.19
		c	105, 39			*d*	120
	α-fructofuranose	65.33	105.80	87.13	76.50	83.08	62.98
		110, 50	88, 12	64, 46, 13[f]	100	41, 26	115
(9)	α-glucopyranosyl(1→3)-	99.14	73.85	*e*	*e*	74.17	62.22
		33	107, 39			23, 23	120
	β-fructofuranose	64.93	103.70	81.17	76.28	83.49	64.50
		d	83, 14	35, 15	108	35, 35	98
			Sucrose				
(10)	α-glucopyranosyl(1→2)-	93.44	73.33	74.60	71.55	74.52	62.21
		c	102, 34	98, 47, 47	103, 38	27, 27	116
	β-fructofuranose	63.76	105.71	78.71	75.99	84.22	63.84
		99[f]	18	112, 45, −21[f]	100, 49	28, 28	110
			Melezitose				
(11)	α-glucopyranosyl(1→3)-	99.95	73.72	75.31	*e*	74.18	*e*
			105, 36	99, 48, 48		23, 23	
	β-fructofuranosyl(2→1)-	63.67	106.07	83.20	75.02	84.42	64.26
		103[f]	16	22[f]	102	33, 35	105
	α-glucopyranoside	93.59	73.45	74.88	71.77	74.33	62.45
			103, 35	102, 46, 46	110, 39	28, 28	117
			Raffinose				
(12)	α-galactopyranosyl(1→6)-	100.77	70.28	71.20	70.58	72.66	62.33
		c	105, 39	106, 40, 13	103, 15	27, 27	119
	α-glucopyranosyl(1→2)-	93.33	73.19	74.55	71.97	72.98	68.37
		c	100, 34	97, 41, 41	99, 37	29	−8[f]
	β-fructofuranose	63.89	105.73	78.68	75.94	84.05	63.93
		109, −18[f]	17	120, 45, −17[f]	103, 54	28, 28	104
			Stachyose				
(13)	α-galactopyranosyl(1→6)-	100.63[l]	70.24[m]	71.03[n]	70.56[o]	72.81	62.30
		10	107, 39	106, 38, 13	110, 17	23, 23	119
	α-galactopyranosyl(1→6)-	100.46[l]	70.20[m]	71.40[n]	70.76[o]	70.42	68.15
		11	109, 32	114, 50, 13	101, 15	25	
	α-glucopyranosyl(1→2)-	93.43	73.15	74.60	71.87	72.92	68.08
		9	105, 34	102, 41, 41	103, 34	22	−11[f]
	β-fructofuranose	63.87	105.76	78.66	75.96	84.06	63.98
		118, −18[f]	18	109, 46, −20[f]	104, 50	26, 26	110

[a] In ppm downfield from TSP. [b] Upfield shifts (spacings of multiplets) in ppb ± 3. [c] Broad line(s). [d] Obscured. [e] Not resolved. [f] Extra splittings or "noise". [g–o] Assignments are interchangeable for resonances marked with the same letter.

of resonance positions (chemical shifts) and isotopic multiplets is most effective when proceeding along the following logical steps.

(i) Inspection of the anomeric region (90–110 ppm) looking for: (a) number of resonances, which gives the chain length; (b) number of quaternary carbons, which gives the number of ketose residues; (e) β-isotope effects. Only the carbon atom at the reducing end should exhibit a β effect; the presence of more than one such carbon is indicative of a mixture (anomeric or other).

(ii) Counting the isotopic multiplets of each kind for the nonanomeric methine carbons. The numbers, when used with the multiplet matrices, can identify the sequence of glycosidic linkages.

(iii) A detailed structural interpretation should begin with the more prominent and information-rich spectral features such as the $\beta\gamma_2$ multiplets, resonances (multiplets) in the 80–88-ppm region, and methylene resonances (multiplets).

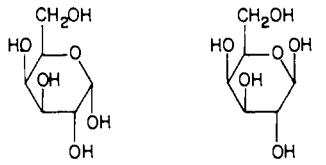
Experimental Section

The oligosaccharides used in this work were crystalline materials obtained from commercial sources. They were dissolved in Me_2SO-d_6 to a concentration of ca. 10% w/v. A calculated amount of D_2O was added to each solution to give a H/D ratio of 1.0 ± 0.2 for the exchangeable hydrogens. The solution was then dried with $CaSO_4$ (noindicating Drierite) and filtered into the NMR tube.

Carbon-13 NMR spectra were recorded at 90.56 MHz and 24 ± 1 °C on a Nicolet 360 WB NMR spectrometer operating in the pulsed Fourier transform mode. Low-power broad-band proton decoupling was achieved using the MLEV-64 pulse sequence.[17] Spectral resolution was enhanced using apodization routines supplied by the instrument manufacturer. The central peak of the solvent resonance was used as an internal reference with a chemical shift of 41.105 ppm downfield from TSP (TSP = trimethylsilylpropinate).

Results and Discussion

The ^{13}C NMR spectra of freshly prepared solutions indicated the presence of only one component. With time the reducing sugars slowly isomerized. The composition of equilibrated solutions was obtained from the integrated intensities in the spectra of week-old samples. The results are given in Table VI. For comparison, also given are the results for the monosaccharide fructose. The chemical shift and isotope effect data are summarized in Table VII. The assignments were made employing the above-outlined approach and using monosaccharide data.[1,3] Given below are details on the application of the approach of structural elements and multiplet matrices to individual cases. These are attempts to obtain ab initio analysis of di- and trisaccharides by extracting the maximum structural information reflected in the ^{13}C NMR spectra with isotopic multiplets.



α-D-galactopyranose β-D-galactopyranose

α-Lactose. The isotopic multiplets in the ^{13}C NMR spectrum of α-lactose with partially deuterated hydroxyls are given in Figure 1. There are two resonances in the anomeric region and two methylenes indicative (elements CS1 and CS2, Table I) of a disaccharide of aldoses. The total number of resonances indicates that these must be hexoses. The multiplicities of the methylene resonances (no γ effects) indicate pyranoses; those of the anomeric carbons (one β effect, elements IM1 and IM2, Table II) indicate that this is a reducing sugar. For the nonanomeric methines one finds two $\beta\gamma_2$ multiplets (at 74.90 and 73.79 ppm), three $\beta\gamma$ quartets (at 73.05, 72.32, and 69.85 ppm), two γ_2 triplets (at 77.16 and 71.47 ppm), and what appears as a broad singlet (at 82.92 ppm). However, since there is no reference to singlets in the multiplet matrices, the singlet must be due to an unresolved γ

(17) Levitt, M. H.; Freeman, R.; Frenkiel, T. *J. Magn. Reson.* **1982**, *47*, 328–330.

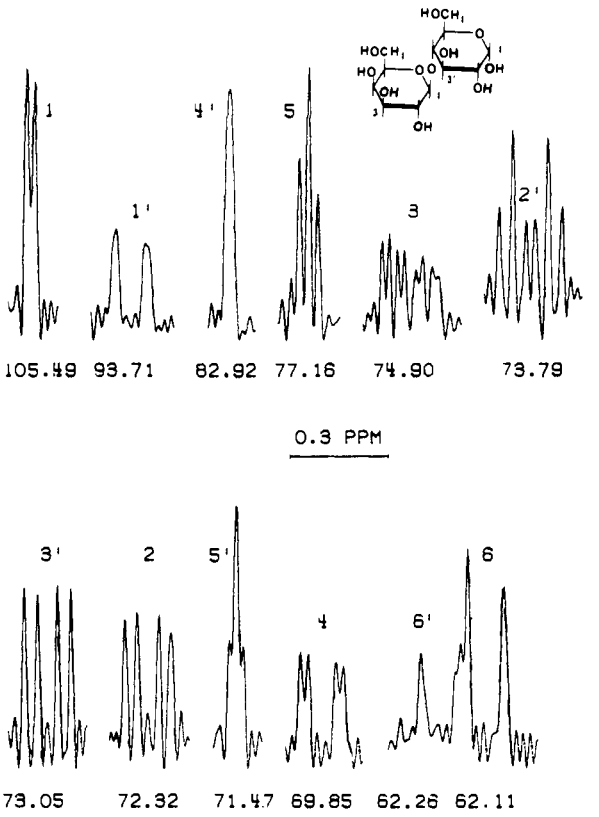
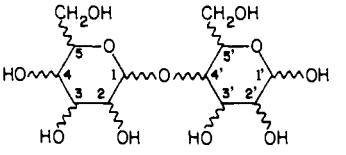


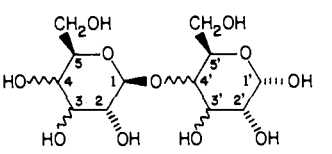
Figure 1. Isotopic multiplets in the ^{13}C NMR spectrum (resolution enhanced) of α-lactose with partially deuterated hydroxyls. The chemical shift of the protio form is given under each multiplet.

doublet or γ_2 triplet. Referring to the multiplet matrix for a disaccharide composed of aldohexopyranoses (see Table V), one finds that the closest entry contains two $\beta\gamma_2$, three $\beta\gamma$, one γ_2, and two γ multiplets and is characteristic of an aldopyranosyl (1→4) aldopyranose. (It is instructive and helpful to refer at this point to the flat structural formula A.) Since[1] Δ_γ (C1,2)$_{cis}$ <



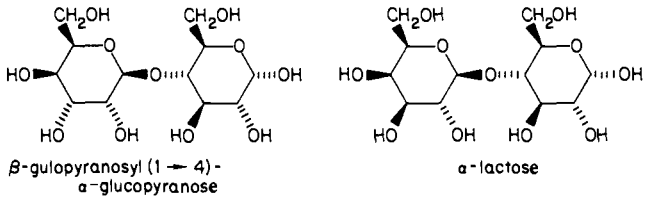
A

14 ppb, the absence of further splitting in the resonance (at 93.71 ppm) of the anomeric carbon at the reducing end indicates a cis arrangement of the hydroxyls at Cl′ and C2′. The γ isotope effect for the resonance at 105.49 ppm suggests a trans configuration at C1 and C2. The γ_2 triplet at 77.16 ppm is due to C5 at the nonreducing end. The chemical shift value suggests (element CS4) a β anomer, thus establishing the stereochemistry of the glycosidic linkage. The collapsed γ doublet at 82.92 ppm is the resonance of C4′. This leaves the triplet at 71.47 ppm for the remaining nonhydroxylated carbon, C5′. Its unusual multiplet appearance (doublet expected) will be discussed later on. The above assignments are based on structure A and on the expectations from substituent effects on chemical shifts (element CS5). The absence of other resonances in the 75–78-ppm region suggests that the reducing end is an α anomer. At this point in the assignment and interpretation process one is in a position to draw structure B. In



B

doing so, the D enantiomers were arbitrarily chosen. Of the remaining multiplets, the ones of type $\beta\gamma_2$ are due to C3 and C2′ while the $\beta\gamma$ quartets are due to C2, C4, and C3′. *If* the $\beta\gamma_2$ sextet at 73.79 ppm is assigned to C3, its form would indicate (element IM14, Table II) a xylo structure; i.e., the nonreducing end is β-glucopyranosyl. In this case, according to monosaccharide data,[1,3] the resonances at 73.05 and 72.32 ppm must be due to C2 and C4, respectively. These should be compared with the chemical shift values for methyl β-glucopyranoside: 75.02, 78.28, and 71.71 ppm for C2, C3, and C4, respectively.[1] The discrepancies of 1.97, 4.49, and −0.61 ppm are too large for a correct assignment. This argument leads to the assignment of the $\beta\gamma_2$ sextet at 73.79 ppm to C2′ and the $\beta\gamma_2$ octet at 74.90 ppm to C3. The octet for C3 indicates (elements IM12 and IM13) either an arabino or a lyxo structure; i.e., the nonreducing end is either β-galactopyranosyl or β-gulopyranosyl. Unfortunately, the differences in chemical shift values between these two monosaccharide residues, as judged from the differences between the corresponding methyl glycosides,[3] are insufficient for the resolution of this degeneracy. Turning to the reducing end, the magnitude of the γ- isotope effect for C2′ indicates that the hydroxyl arrangement at C2′/C3′ must be trans. Depending upon the configuration at C3′/C4′, the reducing end can be either α-galactopyranose (for a cis arrangement) or α-glucopyranose (for a trans arrangement). Referring to the chemical shifts for these monosaccharides,[1,3] one notes that C5 in α-galactopyranose is downfield from the other nonanomeric methines, whereas in α-glucopyranose C5 is upfield from both C2 and C3. Since here C5′ resonates at the relatively high-field position of 71.47 ppm and is upfield from both C2′ and C3′, the reducing end is assigned as α-glucopyranose. Thus, without recourse to information regarding monomer composition, the interpretation of the spectrum in Figure 1 has led to the conclusion that the spectrum is due to either β-galactopyranosyl(1→4)-α-glucopyranose or β-gulopyranosyl(1→4)-α-glucopyranose. The final assignments of the resonances at 72.32



and 73.05 ppm were based on those for α-glucose and methyl β-galactopyranoside.[1,3]

The methylene resonances are markedly different from one another in their appearance. The reduced intensity of the one at 62.26 ppm is due to extra splittings. Such effects could be a reflection of isotopic perturbation of chemical equilibria involving intramolecular hydrogen bonding.[1] The unusual appearance of the resonance of C5′ is probably due to the same phenomenon.

Lactulose. The structural analysis of the isotopic multiplets in the ^{13}C NMR spectrum (not shown) of freshly dissolved lactulose with partially deuterated hydroxyls could proceed in a way similar to that described for α-lactose. From the number and kind of carbon atoms (resonances) and from the splitting patterns in the methylene and anomeric regions, one could readily determine that the spectrum is that of a reducing aldohexopyranosylketohexofuranose. One of the multiplets (at 76.22 ppm; see Table VII, entry 4) appeared as a somewhat broadened β doublet. However, an entry for the set of multiplets thus obtained could not be found in the multiplet matrix given in Table V. The set obtained with the assumption that the multiplet at 76.22 ppm is a $\beta\gamma$ quartet with unresolved Δ_γ splittings corresponds to an aldopyranosyl(1→4)ketofuranose. With this, the $\beta\gamma_2$ octet at 75.10 ppm could be readily assigned to C3 of the nonreducing end. As with α-lactose, it was impossible to distinguish between the epimeric-at-C3 galactose and gulose structures. Tracing the relative stereochemistry of the substituents on the furanose ring reached a point where, after determining a trans configuration at C3′/C4′ from the relatively large Δ_γ on C4′ (at 86.36 ppm),

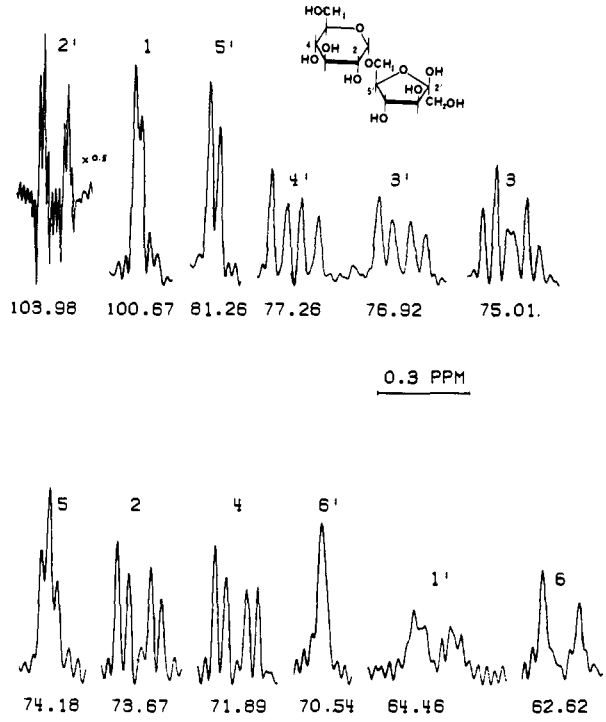


Figure 2. Isotopic multiplets in the ^{13}C NMR spectrum (resolution enhanced) of a fresh solution of palatinose, α-glucopyranosyl(1→6)β-fructofuranose, with partially deuterated hydroxyls. The chemical shift of the protio form is given under each multiplet.

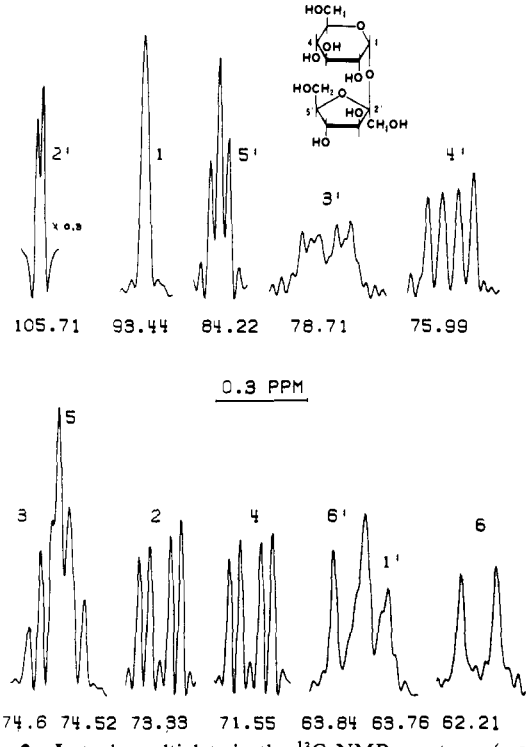


Figure 3. Isotopic multiplets in the ^{13}C NMR spectrum (resolution enhanced) of sucrose with partially deuterated hydroxyls. The chemical shift of the protio form is given under each multiplet.

one could not distinguish between β-D-fructofuranose and the epimeric-at-C5′ α-L-tagatofuranose. This ambiguity, however, could be resolved using the information in the isotopic multiplets of the aldopyranosyl–ketopyranosyl isomer, which appeared in the spectrum of an aged solution of lactulose. The resonance of C4′ (at 79.81 ppm) in that spectrum exhibited a large and a small γ effect indicating a trans–cis configuration at C4′, thus estab-

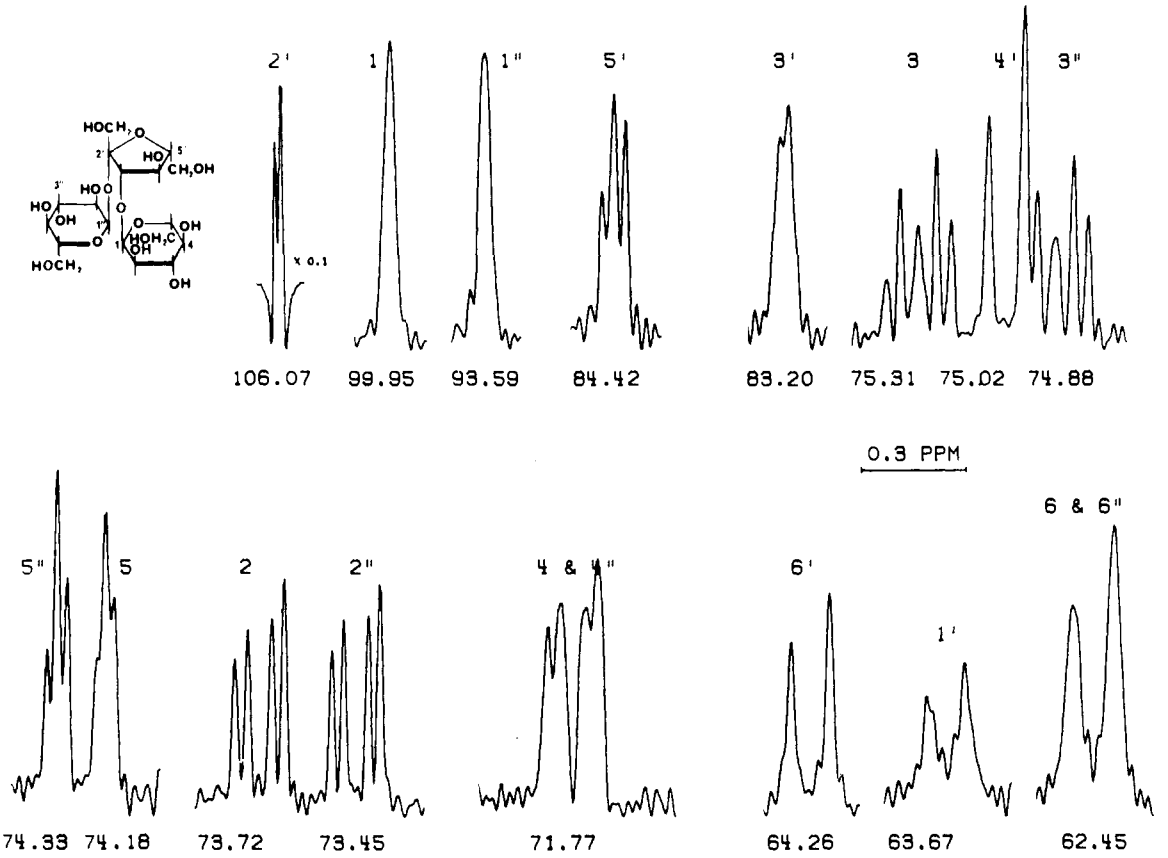
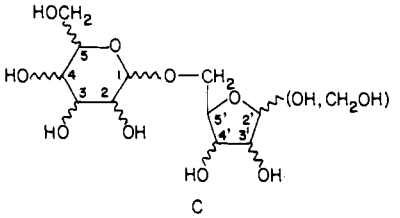


Figure 4. Isotopic multiplets in the ^{13}C NMR spectrum (resolution enhanced) of melezitose with partially deuterated hydroxyls. The chemical shift of the protio form is given under each multiplet.

lishing the reducing end as fructopyranose. It is noteworthy that the resonance of C1 of the β-fructofuranose moiety exhibited extra splittings due to a long-range *downfield* isotope effect (vide infra).

Palatinose. A quaternary carbon and three methylenes in the 12-line ^{13}C NMR spectrum of freshly dissolved palatinose are evidence (elements CS1 and CS2, Table I) for the presence of hexoaldose and hexoketose structures. The isotopic multiplets shown in Figure 2 reveal a $\beta\gamma_2$ sextet for the quaternary carbon and no β effect for the other anomeric carbon. This indicates (elements IM1 and IM2, Table II) a reducing aldosylketose in which carbons 1 and 3 at the reducing end are also unsubstituted. The multiplet set for the nonanomeric methines consists of one $\beta\gamma_2$, four $\beta\gamma$, one γ_2, and one γ multiplets. However, in the multiplet matrices in Table V there is no entry for such a set. Probably, the broadened quartet at 76.92 ppm is a degenerate $\beta\gamma_2$ octet. Thus, the multiplet set becomes two $\beta\gamma_2$, three $\beta\gamma$, one γ_2, and one γ. For such a set there are three entries in Table V, two of which have the ketose at the reducing end. The downfield position of the methylene (at 70.54 ppm) with no β effect (element CS5) and the γ doublet at 81.26 ppm (element CS3) suggest that these are the resonances of carbons 6 and 5, respectively, of a 6-substituted ketohexofuranose, i.e., that the molecule is an aldohexopyranosyl(1→6)ketohexofuranose. Reference to the flat structural formula C should be helpful in



C

the following discussion. Clearly the only candidate for the γ_2 -triplet at 74.18 ppm is C5. The chemical shift value suggests (element CS4) an α anomer. The magnitudes of the β and γ

Table VIII. Multiplet Matrices for Some Nonreducing Hexose Trisaccharides Containing a Ketofuranose Residue

X	$\beta\gamma_2$	$\beta\gamma$	γ_2	β	γ	degen.
		Ketofuranosyl(2→X)aldopyranosyl(1→1)-aldopyranoside				
2	1	6	3		1	*a*
3	1	4	4	2		*b*
4	1	6	2		2	*c*
6	2	6	2		1	*d*
		Aldopyranosyl(1→X)ketofuranosyl(2→1)-aldopyranoside				
1	2	6	3			
3	2	4	3	1	1	
4	2	4	2	1	2	
6	2	6	2		1	*d*
		Aldopyranosyl(1→X)aldopyranosyl(1→2)-ketofuranoside				
2	1	6	3		1	*a*
3	1	4	4	2		*b*
4	1	6	2		2	*c*
6	2	6	2		1	*d*

[a-d] Identical sets are marked with the same letter.

isotope effects on the chemical shift of C2′ suggest a cis configuration at C2′/C3′.[1] Thus, the degenerate $\beta\gamma_2$ octet at 76.92 ppm must be due to C3′, with the larger Δ_γ splitting arising from a trans C3′/C4′ configuration (element IM4). This leaves the sextet at 75.01 ppm for C3. This multiplet pattern is characteristic (element IM14) of a xylo structure: α-glucopyranose or α-idopyranose. The chemical shift values are clearly those of the former, with the $\beta\gamma$ quartets at 73.67 and 71.89 ppm corresponding to C2 and C4, respectively.[3] These assignments leave the $\beta\gamma$ quartet at 77.26 ppm for C4′. The identity of the reducing end, either β-fructofuranose or the epimeric-at-C5′ α-L-tagatofuranose, can be established on the basis of the chemical shifts: in D_2O, carbon 4 of α-tagatofuranose resonates at 71.90 ppm, whereas the fructofuranoses have no methines at such high-field positions.[3]

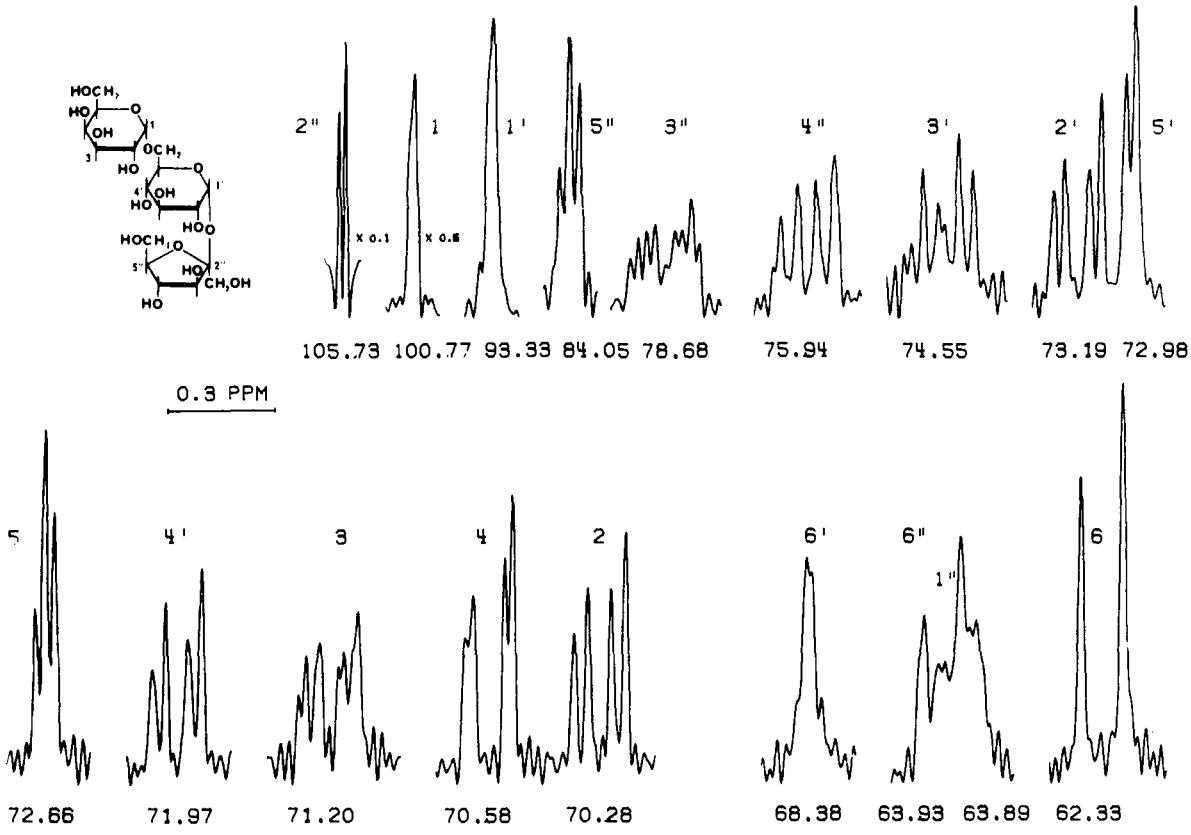


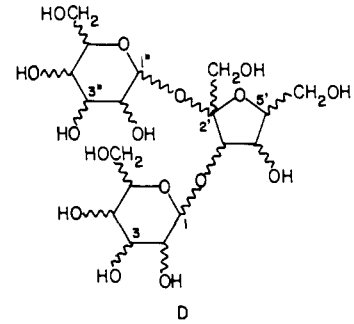
Figure 5. Isotopic multiplets in ^{13}C NMR spectrum (resolution enhanced) of raffinose with partially deuterated hydroxyls. The chemical shift of the protio form is given under each multiplet.

Thus, the complete primary structure (including monosaccharide composition) of palatinose as α-glucopyranosyl(1→6)-β-fructofuranose was determined ab initio from the ^{13}C NMR spectrum with isotopic multiplets. The extra splittings or "noise" in the methylene resonance at 64.46 ppm will be discussed later on.

Turanose. Overlap of some of the isotopic multiplets, even for freshly dissolved turanose, considerably hampered the structural analysis of the spectrum. However, spectral assignments based on the known monosaccharide composition and sequence were readily made (entries 7, 8, and 9 in Table VII).

Sucrose. The isotopic multiplets in the ^{13}C NMR spectrum of sucrose with partially deuterated hydroxyls are shown in Figure 3. The absence of β isotope effects for the anomeric carbons indicates (element IM2, Table II) that this is a nonreducing disaccharide. The three methylenes and the fact that all three exhibit β effects are indicative (element CS2, Table I) of a ketofuranose. The intensity distribution in the multiplet at 78.71 ppm suggests that this is a nominal $\beta\gamma$ quartet with extra negative splittings. While it is clear that the band at 74.60 ppm is a $\beta\gamma_2$ multiplet, the one superimposed on it at 74.52 ppm could be either a γ_2 triplet or a γ doublet. Thus, the multiplet set for the nonanomeric methines is one $\beta\gamma_2$, four $\beta\gamma$, and either two γ_2 or one γ_2, and one γ multiplets. The multiplet matrices in Table V contain no entries for the latter set. On the other hand, there are three entries for the former set; only the one for aldopyranosyl-(1→2)ketofuranose is for a nonreducing sugar. From this point on the structural analysis proceeds in a fashion similar to the one employed for palatinose. Using this approach the spectrum is unequivocally identified as that of α-glucopyranosyl(1→2)-β-fructofuranose. The assignments given in Table VII (entry 10) are in excellent agreement with those of Jarrell et al.[13]

Melezitose. In the ^{13}C NMR spectrum of melezitose there are 16 resolved resonances corresponding to 18 carbon atoms, of which three are anomeric. One of the latter is quaternary, indicating (element CS1, Table I) that the trisaccharide contains a ketose residue. One of the methylene (62.45 ppm) and one of the methine (71.77 ppm) resonances correspond to two carbon atoms each. The isotopic multiplets in the spectrum of melezitose with partially deuterated hydroxyls are shown in Figure 4. None of the anomeric carbons exhibits a β-isotope effect, indicating (element IM2, Table II) that the sugar is nonreducing. All of the methylenes are β doublets, indicating that the ketose must be a furanose. The multiplet set of the nonanomeric methines contains two $\beta\gamma_2$ sextets, four $\beta\gamma$ quartets (the one at 71.77 ppm counts as two), three γ_2 triplets, one β doublet, and one γ doublet. In the multiplet matrix for ketofuranose-containing nonreducing trisaccharides given in Table VIII there is one entry corresponding to this set: aldopyranosyl(1→3)ketofuranosyl(2→1)aldopyranoside. One can now draw the flat structural formula D,



D

which should be useful in the continuation of the analysis. A number of assignments can be readily made. The γ_2 triplets at 74.33 and 74.18 ppm are due to C5″ and C5. The one at 84.42 ppm is due to C5′ (element CS3). The γ doublet at 83.20 ppm can be assigned to C3′ (element CS5) and the β doublet at 75.02 ppm to C4′. The $\beta\gamma_2$ sextets at 75.31 and 74.88 ppm are due to C3 and C3″. Their pattern is characteristic (element IM14) of the xylo structure. Reference to the chemical shift tables[3] brings one to the conclusion that both residues are α-glucopyranosyls. With this in mind, the resonances at 73.72 and 73.45 ppm can be assigned to C2 and C2″, those overlapping at 71.77 ppm to

C4 and C4″, and the ones overlapping at 62.45 ppm to C6 and C6″. This leaves the β doublets at 64.26 and 63.67 ppm for C1′ and C6′. The identification of the ketose residue relies heavily on the chemical shift values since there are no vicinal diol interactions to serve as structural clues. However, the unequivocal spectral assignment of the furanose-ring carbons allows one to use the chemical shift tables[3] with confidence. Based on the low-field position of C4′ the presence of psicose or tagatose can be ruled out. The position of C2′ suggests further that the ketose is either α-sorbofuranose or β-fructofuranose. The discrimination between these two in favor of the latter is based on the low-field position of C5′.

This example shows that, in favorable cases, one can determine ab initio the monosaccharide composition, sequence, and stereochemistry of glycosidic linkages of a trisaccharide from the ^{13}C NMR spectrum with isotopic multiplets. It is noteworthy in this regard that the individual assignments of the glucosyl resonances to individual residues remained unresolved up to this point. The final assignments given in Table VII (entry 11) were based on the analogy of the glucosyl residue attached at C2′ with that in sucrose. Extra "noise" in the spectrum in Figure 4 is observed for the C1′ and C3′ resonances.

Raffinose. The isotopic multiplets in the ^{13}C NMR spectrum of raffinose with partially deuterated hydroxyls are shown in Figure 5. They permit the ready determination that this is a ketofuranose-containing nonreducing trisaccharide. However, the multiplet set of nonanomeric methines (two $\beta\gamma_2$, six $\beta\gamma$, two γ_2, one γ) corresponds to three possible sequences (see Table VIII). One of them, aldopyranosyl(1→6)ketofuranosyl(2→1)aldopyranside, can be ruled out since C5 of the ketofuranose moiety at 84.05 ppm exhibits two γ effects, indicating that C6 must be unsubstituted. Unfortunately, the other two possibilities, aldopyranosyl(1→6)aldopyranosyl(1→2)ketofuranoside and ketofuranosyl(2→6)aldopyranosyl(1→1)aldopyranoside, cannot be resolved on the basis of multiplet patterns and monosaccharide chemical shifts. A comparison with the spectrum of sucrose indicates that this is a derivative of sucrose obtained by substitution at C6 of the glucosyl residue. Now the multiplet pattern and chemical shift values of the resonances of the third residue lead to its positive identification as α-galactopyranosyl. The possibility of it being α-gulopyranosyl can be ruled out on the basis of the high-field positions of the C2 and C5 resonances. Here again one observes extra splittings in the C1 and C3 resonances of the fructose residue.

Stachyose. The stachyose spectrum was assigned by comparison with that of raffinose. It should be pointed out that in the analysis of tetrasaccharides (and higher oligosaccharides) by the approach described in this paper one faces an additional problem: the sequence ABCD cannot be distinguished from ACBD.

Long-Range Isotope Effects. Spectral effects in the form of extra splittings, excessive "noise", or line broadening were observed for some of the resonances in a number of sugars, in particular, for carbons 1 and 3 of the β-fructofuranose residues. In some of the cases, these long-range isotope effects are of opposite sign to the normal β and γ effects. Similar phenomena in the case of monosaccharides have been rationalized in terms of isotopic perturbation of equilibria involving structures with intramolecular hydrogen bonds.[1] There, as well as here, the hydroxyls responsible for these effects form a β-diol array in which intramolecular hydrogen bonding leads to the closure of a stable six-membered ring. However, further investigation is needed in order to clarify this phenomenon.

Conclusions

The isotopic multiplets in the ^{13}C NMR spectra of oligosaccharides with partially deuterated hydroxyls are a rather detailed reflection of molecular structure. Taken together with the chemical shift values and using comparisons with data on monosaccharides, it is possible to obtain chemical and structural identifications from the spectrum alone. According to the examples discussed above, this appears to be a viable new approach to the analysis of oligosaccharides.[18,19]

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Registry No. 2, 58166-23-7; **3**, 58166-25-9; **4**, 58166-24-8; **5**, 58166-27-1; **6**, 15132-06-6; **7**, 58166-22-6; **8**, 58096-73-4; **9**, 20749-95-5; α-lactose, 14641-93-1; lactulose, 4618-18-2; palatinose, 13718-94-0; turanose, 547-25-1; sucrose, 57-50-1; melezitose, 597-12-6; raffinose, 512-69-6; stachyose, 470-55-3.

(18) After the completion of this manuscript a paper by Christofides and Davies[19] appeared, which describes the use of isotopic multiplets for the spectral assignment of stachyose and its subunits. Their results are in excellent agreement with the ones presented here, except for the transposed assignments of carbons 1 and 6 of the β-fructofuranose moiety. The present assignments are based on the results for lactulose and palatinose, for which the particular positions of the glycosidic linkages dictate the multiplet patterns and force the assignments.

(19) Christofides, J. C.; Davies, D. B. *J. Chem. Soc., Perkin Trans. 2* **1984**, 481–488.