for formation of FMF and possibly also compound C) only in such a bent structure and not in a planar conformation. If it is assumed that the N-5 of the triplet is more reactive than N-1 in hydrogen abstraction and that it is also the site of protonation,[38] the pH profile is understandable. Thus, protonation at N-5 could block the most reactive center of the molecule.

The changes in product distribution are harder to explain. In the case of the triplet, although all hydrogens of the side chain can approach the now sp^3-hydridized N-5, the numerous conformational possibilities of the side chain may restrict abstraction to only the nearest hydrogens (1′ or 2′).

If the excited singlet has a planar conformation, N-1 is most likely involved in the abstraction. Products originating from the singlet may reflect preexcitation associations of N-1 and the side chain as, for example, through hydrogen bonding.

A final point concerns the increase in rate below pH 5. Perhaps a second site in the triplet is protonated to give a more reactive species, as has been suggested by Penzer[39] to explain the acceleration of rate of flavin mononucleotide (FMN) catalyzed photooxidation of methionine under acidic conditions in the pH region 5–6.

Acknowledgment. The authors with to express their appreciation to Dr. W. M. Moore of Utah State University, Dr. P. Hemmerich of the University of Konstanz, Germany, and to Dr. O. Chapman of Iowa State University for their critical discussion of some aspects of this work.

(38) P. S. Song (*Ann. N. Y. Acad. Sci.*, **158**, 410 (1969)) has calculated the π-electron density as higher at N-5 than at N-1 in the first excited triplet of 7,8-dimethylisoalloxazine.

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Communications to the Editor

Strategies in the Application of Partially Relaxed Fourier Transform Nuclear Magnetic Resonance Spectroscopy in Assignments of Carbon-13 Resonances of Complex Molecules. Stachyose[1,2]

Sir:

There are at present only two well-known procedures of general applicability for the assignment of resonances in proton-decoupled carbon-13 spectra: comparisons within a series of compounds with similar structures, and the use of splitting patterns arising from incomplete proton decoupling.[3] With the advent of Fourier transform nmr,[4] it has become possible to use partially relaxed[5] Fourier transform (PRFT) spectra[2,6,7] as an additional aid in assignments. We will discuss some strategies and limitations in the use of PRFT spectra for assigning ^{13}C resonances of complex molecules. As an illustration, we will show that the PRFT method is useful in assigning the carbon-13 spectrum of the nonreducing tetrasaccharide stachyose (**1**, Figure 1).

If the ^{13}C nuclei are proton decoupled, then the ^{13}C relaxation is exponential.[8] In this case, intensities in a PRFT spectrum are given by[9]

$$A = A_0[1 - 2\exp(-\tau/T_1)] \qquad (1)$$

where A and A_0 are the observed and equilibrium intensities, respectively, τ is the interval between the 180 and 90° pulses,[5] and T_1 is the ^{13}C spin–lattice relaxation time. A resonance will appear inverted, nulled, or positive (with respect to the normal spectrum) depending on whether τ is smaller, equal to, or larger than T_1 ln 2, respectively. Thus, if two carbons have different T_1 values, the intensity of their resonances will have a different dependence on τ. Successful application of PRFT spectra in assignment of ^{13}C resonances requires an appreciation of how ^{13}C T_1 values vary with molecular site.

Theoretical considerations[8,10–12] and experimental work[2,6,7,13] indicate that the following three principles should be considered when using PRFT spectra for assigning ^{13}C resonances of large and asymmetric molecules. **Principle A**: relaxation of protonated carbons is overwhelmingly dominated by dipolar interactions with the attached protons, with T_1 given by[12]

$$1/T_1 = N\hbar^2\gamma_C^2\gamma_H^2 r_{CH}^{-6}\tau_{eff} \qquad (2)$$

Here γ_C and γ_H are the gyromagnetic ratios of ^{13}C and ^1H, N is the number of directly attached hydrogens, r_{CH} is the CH distance, and τ_{eff} is the effective correlation time for rotational reorientation. Equation 2 is valid under conditions of complete proton decoupling a ıd only when $1/\tau_{eff}$ is much larger than the ^1H resonance frequency. Principle A may not apply to small or very symmetric molecules, which may have an appreciable contribution to $1/T_1$ from the spin–rotation interaction [14] In addition, contributions to $1/T_1$ from chemical shift anisotropy may be important in some

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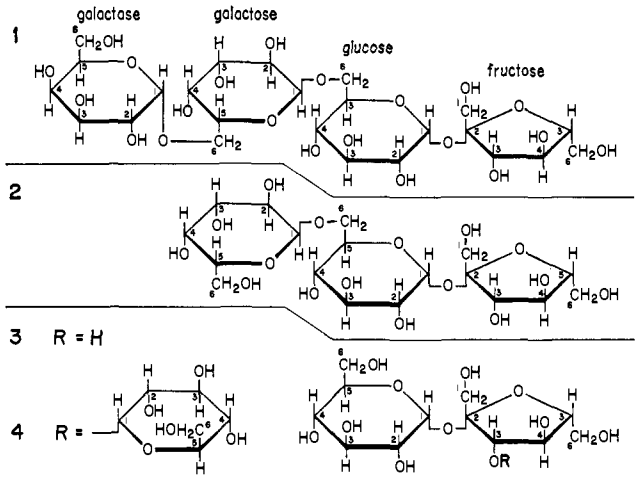

Figure 1. Structures of stachyose (1), raffinose (2), sucrose (3), and melezitose (4).

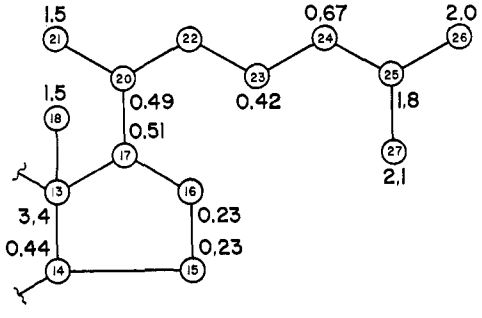

Figure 2. Some carbon-13 spin–lattice relaxation times (sec) (large numbers) of 1 *M* cholesteryl chloride in CCl_4 at 42°, determined at 15.08 MHz from proton-decoupled PRFT spectra. Small numbers are standard carbon designations.

cases at very high magnetic field strengths. We wish to emphasize that because of the inverse sixth power dependence of $1/T_1$ on r_{CH}, contributions from long-range $^{13}C-^{1}H$ dipolar interactions to $1/T_1$ of a protonated carbon will be negligible. Lack of appreciation of this fact can easily lead to incorrect assignments.[15,16] **Principle B**: a nonprotonated carbon with a τ_{eff} comparable to that of a protonated carbon will have a much longer spin–lattice relaxation time than the latter. **Principle C**: different carbons within the same molecule do not necessarily have the same τ_{eff}. Differences in τ_{eff} may arise from anisotropic reorientation of the molecule as a whole[11] and/or from the effect of internal reorientation.[7,13,17] As a consequence, $1/NT_1$ is not necessarily equal for all protonated carbons in a molecule.

We illustrate the experimental consequences of the above principles in Figure 2, which shows T_1 values of representative carbons of 1 *M* cholesteryl chloride in CCl_4. Experimental details will be given elsewhere.[13] Protonated carbons on the ring backbone, including those not shown in Figure 2, all have the same $1/NT_1$ value and hence the same τ_{eff}. The overall reorientation of this molecule can thus be considered isotropic. When this is the case, one can use T_1 values to distinguish CH from CH_2 carbons. The nonprotonated carbon on the ring backbone (C-13) has a much longer T_1 value than protonated carbons on the backbone, but not much longer than the methyl carbons, which show the effect of internal reorientation. Mathematical details will be given elsewhere.[13] A comparison of all the T_1 values on the group attached at C-17 indicates that the effect of internal motion increases toward the free end of the side chain.

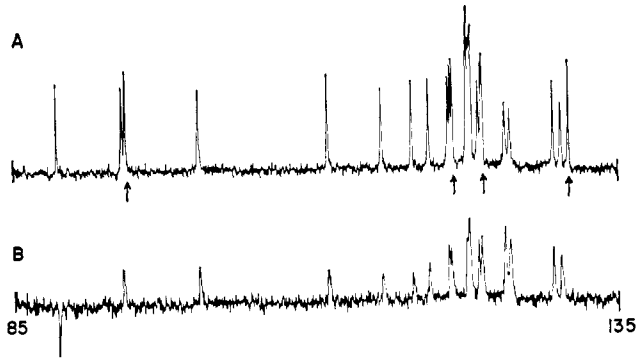

Figure 3. Proton-decoupled natural-abundance carbon-13 nmr spectra of 0.5 *M* aqueous stachyose at 65°, obtained at 15.08 MHz by the Fourier transform method, with 4096 points in the time domain and 62.5-ppm sweep widths. Horizontal scale is in parts per million upfield from carbon disulfide: (A) normal spectrum after 256 scans, using a recycle time of 2.72 sec (total time 11.6 min); (B) PRFT spectrum with τ 0.34 sec, after 512 scans with a recycle time of 2.72 sec (total time 23.2 min). Arrows in top spectrum indicate terminal galactose resonances that were identified using the PRFT spectrum.

The normal proton-decoupled natural-abundance carbon-13 Fourier transform spectrum of 0.5 *M* aqueous stachyose is shown in Figure 3A. Assignments (Table I) were made as follows. The resonances were divided into those originating from fructofuranose, glucopyranose, and galactopyranose rings by comparisons with the spectra of raffinose (2) and sucrose (3). Differentiation of resonances from the two galactose rings was not possible by conventional procedures. However, one galactopyranose ring is terminal *and* is attached to the rest of the molecule by means of a $-O-CH_2-$ linkage. Thus, it *may* have faster internal reorientation and measurably longer carbon-13 T_1 values than the other rings. Examination of PRFT spectra of **1** reveals that the already identified galactose resonances can indeed be divided into two sets on the basis of appreciably different T_1 values. Figure 3B shows a PRFT spectrum with τ 0.34 sec. One set of galactose resonances is nulled ($T_1 \approx 0.5$ sec), while the other set is positive ($T_1 < 0.5$ sec). The resonance of the nonprotonated carbon of the fructofuranose ring is still negative ($T_1 > 0.5$ sec). The nulled resonances were assigned to the terminal galactose (Table I). It should be noted that it was not necessary to determine T_1 values from sets of PRFT spectra to make these assignments. Comparisons of intensities within a single PRFT spectrum were sufficient. As a check, PRFT spectra with other τ values were recorded. After the resonances were allocated to individual monosaccharide moieties, specific assignments were made using the ^{13}C chemical shifts of monosaccharides[18] and disaccharides[19] given by Dorman and Rob-

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Table I. Assignments in the Proton-Decoupled Natural-Abundance Carbon-13 Spectra of Some Oligosaccharides[a]

Assignment[b]	Chemical shift[c] Stachyose[d]	Raffinose[e]	Sucrose[f]
F-2	88.9	88.9	89.1
IG-1	94.3		
TG-1	94.6[g]	94.2	
G-1	100.6	100.7	100.7
F-4	111.4	111.4	111.4
F-3	115.8	115.8	116.1
F-5	118.4	118.4	118.6
G-2,3,5	119.8, 121.4, 121.6	119.8, 121.3, 121.6	120.0, 120.4, 121.6
TG-5	121.7[g]	121.8[h]	
j	123.0[i], 123.1, 123.3[i]	123.1[i], 123.4	123.4
IG-3,4	123.9, 124.2		
TG-3	124.3[g]	124.1	
IG-6	126.2		
G-6	126.6	126.5	132.4
F-1,6	130.2, 130.8	130.2, 130.8	130.3, 131.1
TG-6	131.5[g]	131.5	

[a] Spectra were obtained at 15.08 MHz using the Fourier transform method. [b] F = fructose, IG = inner galactose, TG = terminal galactose, G = glucose. Numbering system is indicated in the structural formulas. Carbons written on the same line could not be assigned on a one-to-one basis. [c] In parts per million upfield from carbon disulfide. Measured with respect to internal dioxane, which was taken as 126.2 ppm. Estimated accuracy ± 0.1 ppm. [d] 0.5 *M* aqueous solution at 65°. [e] 0.6 *M* aqueous solution at 65°. [f] 1 *M* aqueous solution at 36°. [g] Specific assignment based on PRFT spectra. [h] Not a specific assignment. Interchange with a glucose carbon (2, 3, 5) assignment is possible. [i] Two-carbon resonance. [j] This region contains TG-2, TG-4, IG-2, IG-5, and G-4 in stachyose; TG-2, TG-4, and G-4 in raffinose; G-4 in sucrose.

erts. However, their assignments for C-3 and C-4 of the fructofuranose ring in sucrose were reversed, on the basis of the ^{13}C spectrum of melezitose (**4**).[20]

We believe that PRFT spectra will be an extremely useful addition to the existing methods used for assigning ^{13}C resonances, especially when internal motion contributes measurably to $1/T_1$ of some carbons. It should be emphasized that internal reorientation will have a measurable effect on $1/T_1$ only if the correlation times for internal motion are comparable to or shorter than the correlation times for overall reorientation of the molecule.[13]

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(20) The methine carbons of the fructofuranose ring in melezitose resonated at 109.6, 111.4, and 119.2 ppm upfield from CS_2, indicating a large downfield shift of the resonance at 116.1 ppm when going from sucrose (Table I) to melezitose. Since alkylation has a deshielding effect on a hydroxylated carbon,[18,19] the resonance at 116.1 ppm in sucrose must be carbon 3 of the fructofuranose ring.

Adam Allerhand,* David Doddrell

Contribution No. 1929, Department of Chemistry Indiana University, Bloomington, Indiana 47401

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Study of Anomeric Equilibria of Ketoses in Water by Natural-Abundance Carbon-13 Fourier Transform Nuclear Magnetic Resonance. D-Fructose and D-Turanose[1,2]

Sir:

Proton nuclear magnetic resonance has been used successfully to study anomeric equilibria of aldoses in aqueous solution[3-5] by observing the relatively isolated downfield resonance of the proton attached to carbon 1 of each anomer.[3,4] Proton nmr has not been used to study anomeric equilibria in reducing ketoses such as fructose and turanose (Figure 1), presumably because

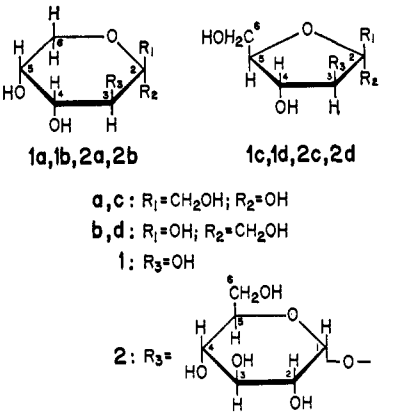

Figure 1. Structures of D-fructose (**1**) and D-turanose (**2**) anomers.

the anomeric carbon is nonprotonated, and thus there are no easily assignable resonances (Figure 2). Proton-decoupled natural-abundance ^{13}C spectra of saccharides are well resolved and much simpler to analyze than the corresponding proton spectra.[2,6-9] Recently, two anomeric forms of fructose have been observed by Dorman and Roberts[9] using continuous-wave carbon-13 nmr. We wish to demonstrate that the enhanced sensitivity of carbon-13 Fourier transform nmr[10] permits the observation of even minor components in anomeric mixtures of saccharides, with sufficient signal-to-noise ratio for quantitative studies. We present some results for D-fructose (**1a-d**) and 3-*O*-(α-D-glucopyranosyl)-D-fructose (turanose, **2a-d**). A spectrum obtained a few minutes after dissolving commercial β-D-fructose[11] in water (Figure 3A) indicated the presence of two components. The equilibrium spectrum (Figure 3B) contained resonances from four components (A, B, C, and D, given in order of decreasing concentration). On the basis of chemical

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(11) Obtained from Sigma Chemical Company, St. Louis, Mo.