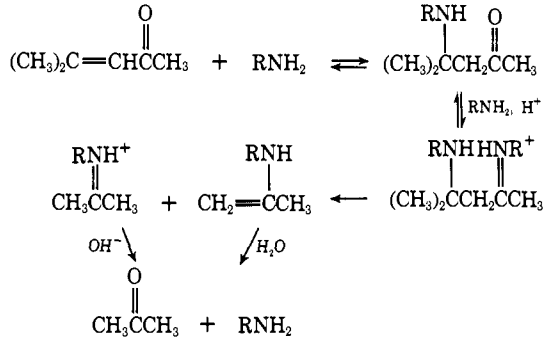
(3 H), and 1.9 (3 H). On addition of mesityl oxide to an aqueous solution of *n*-propylamine, the three signals at highest field are easily observable. The two at δ 2.1 and 2.0 disappear with concurrent appearance of a new signal at δ 1.1. The new signal may be attributed to the *gem*-dimethyl groups of the intermediate Michael adduct. The methyl group α to the carbonyl is expected to absorb about the same place as one of the methyls of mesityl oxide, which accounts for the lack of disappearance of one of the three peaks. Furthermore, if the spectrum is taken in D_2O, all three of the high-field peaks of mesityl oxide disappear since the methyl peak of the intermediate α to the carbonyl is free to exchange with D_2O. The vinyl peak of mesityl oxide also disappears showing that the intermediate is saturated. The multiplet due to the methylene group α to the amine group of *n*-propylamine (δ 2.5) broadens appreciably indicating that there is a change of chemical environment of these protons as well. The time scale of these changes is that expected from the kinetics. At much longer times, the nmr spectrum becomes identical with that of an authentic mixture of acetone and *n*-propylamine (δ 2.1).

Rate constants for the formation of intermediate (k_1) and the breakdown of intermediate (k_2) were obtained by a modification of the technique described by Fersht and Jencks.[4] These rate constants are based on total amine concentration. A plot of log *k* *vs.* pH is given in Figure 1.

The addition reaction depends on a single group of pK_a 10.9, which may be identified as the amine. This result can be accommodated by a slow attack of the nucleophile on unprotonated carbonyl compound. It is also possible that addition proceeds through slow formation of a Schiff base followed by a rapid attack of amine. We are currently investigating this possibility.

The breakdown of the intermediate depends on two groups with pK_a values of 10.8 and 10.9. In the transition state, one of these groups must be protonated and the other free. A reasonable mechanism is given in Scheme I.

Scheme I



The requirement for a single proton in the transition state is in sharp contrast to the analogous cleavage of the Schiff base of diacetone alcohol,[1] which involves breakdown through a neutral transition state. This difference is presumably due to the much greater basicity of the amine than the alcohol. This mechanism for the interconversion of mesityl oxide and acetone bypassing the formation of diacetone alcohol finds support in the report of Yasnikov[5] that amine catalysis of the self-condensation of butyraldehyde occurs in part without the intermediate formation of the aldol product. Additional evidence that diacetone alcohol is not an intermediate is found in the fact that the rate of *n*-propylamine-catalyzed cleavage of diacetone alcohol is too slow to account for the observed cleavage of mesityl oxide in the pH range 9.5–10.5.

(4) A. R. Fersht and W. P. Jencks, *J. Amer. Chem. Soc.*, **92**, 5432 (1970).

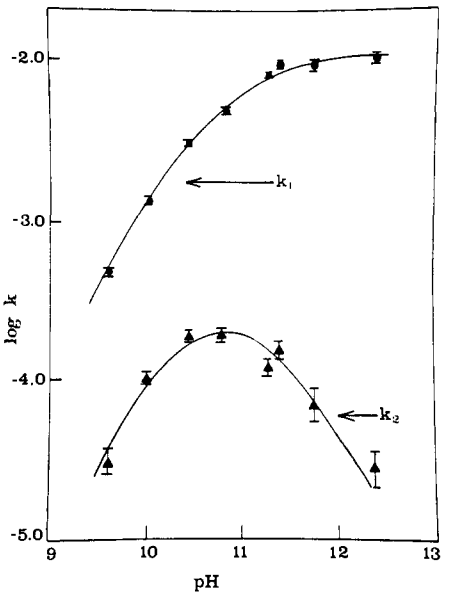


Figure 1. Variation of second-order rate constants (M^{-1} sec^{-1}) with pH for reaction of mesityl oxide with *n*-propylamine (H_2O, μ = 0.2, 25.0°).

Acknowledgment. Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for support of this research. We are grateful to Dr. V. P. Vitullo for several helpful discussions.

(5) T. S. Boiko, N. V. Volkova, and A. A. Yasnikov, *Ukr. Kim. Zh.*, **29**, 1179 (1963).

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Delayed Fourier Transform Proton Magnetic Resonance Spectroscopy

Sir:

We describe here a simple modification of standard Fourier transform (FT) nmr methods[1] which may be used in analyzing spectra which contain both broad and narrow components. Frequently, line width and intensity measurements on the narrower resonances of such spectra are difficult, because these peaks appear superimposed against the broader resonances. It is possible, however, to filter out the broad resonances from the spectrum by introducing a delay time between the end of the rf pulse and the start of data collection. Each component of the transient signal will then have decayed to $\exp(-\Delta\tau/T_2)$ of its initial value by the time

(1) R. R. Ernst and W. A. Anderson, *Rev. Sci. Instrum.*, **37**, 93 (1966).

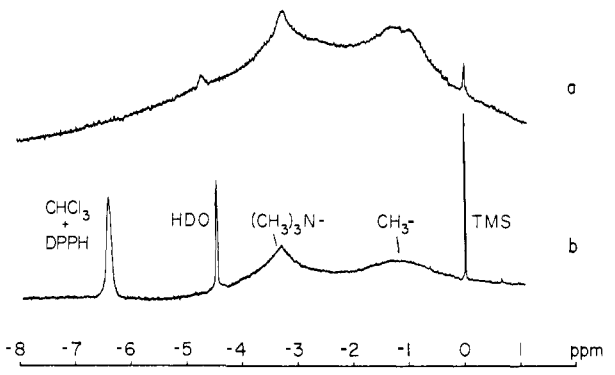

Figure 1. High-resolution pmr spectra of aqueous lecithin bilayer samples: (a) 220-MHz CW spectrum of egg lecithin bilayers at 29°; (b) 100-MHz delayed Fourier transform spectrum of dipalmitoyllecithin bilayers at 60°. The peak centered at −1.14 ppm is assigned to terminal methyl groups on the hydrocarbon side chains of the lecithin molecule. The resonance at −3.32 ppm is from choline methyls of the ionic head groups. The residual protons in the solvent (D_2O) appear at −4.48 ppm. The peak at −6.42 ppm is a calibrated intensity standard of chloroform doped with the free radical, 2,2-diphenyl-1-picrylhydrazyl. Chemical shifts are referred to external TMS (10% in carbon tetrachloride).

the recording of the transient begins. Here $\Delta\tau$ is the length of the pulse plus the chosen delay time, and T_2 is the transverse relaxation time associated with a given component. The resulting transformed spectrum will then exhibit all the components present in the usual continuous wave (CW) nmr spectrum, but with each component weighted by its own factor $\exp(-\Delta\tau/T_2)$. The effect of this weighting process on lines of various widths is shown in Table I.

Table I. Selective Filtering Effect of a Data-Collection-Delay Time $\Delta\tau$ = 500 μsec on Nmr Lines of Various Widths in Fourier Transform Nmr Spectroscopy

$1/\pi T_2$, Hz	Fraction of magnetization remaining	$1/\pi T_2$, Hz	Fraction of magnetization remaining
10	0.98	500	0.46
50	0.92	1000	0.21
100	0.85	2000	0.04
200	0.73		

The advantage of this filtering effect may be seen in our recent nmr work on unsonicated lecithin bilayers. A typical CW pmr spectrum for the protons in a sample of egg lecithin bilayers is shown in Figure 1a. This spectrum reveals several narrow components, but the bulk of the signal is very broad, and obscures the sharper resonances, making it difficult to determine their spectral position and intensity. A delayed Fourier transform (DFT) spectrum of dipalmitoyllecithin at 100 MHz and at 60° with $\Delta\tau$ set at 500 μsec is shown in Figure 1b. In this spectrum the broad component in the usual CW spectrum can be seen to be essentially completely "filtered," and the narrower components can be readily monitored.

The DFT technique has enabled us to monitor the crystalline ⇌ bilayer transition in dipalmitoyllecithin. Above 42°, dipalmitoyllecithin forms a bilayer dispersion in excess D_2O, but below this temperature it exhibits a crystalline phase.[2] For the crystalline phase, the DFT spectrum revealed no sharp components. The DFT spectrum above the phase transition is depicted in Figure 1b. The spectral intensities of the choline methyl and terminal methyl resonances are plotted as a function of temperature in Figure 2. These intensities are presented as a percentage of the expected intensity of each chemical species, calculated from the species' molarity in the sample. It can be seen from Figure 2 that both the choline and terminal methyl groups are simultaneously mobilized *near* the bilayer phase transition temperature.

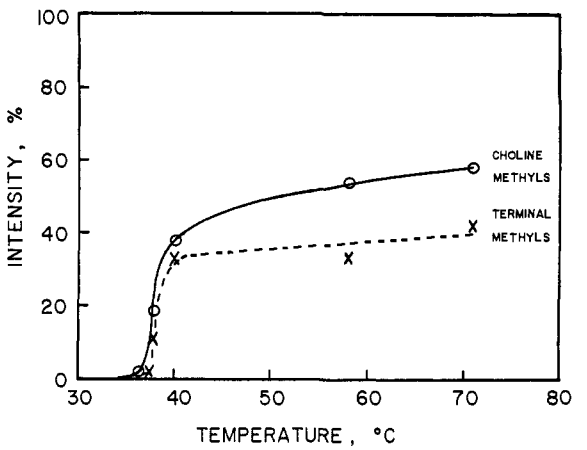

Figure 2. Variation of the choline and terminal methyl signal intensities with temperature for a dipalmitoyllecithin bilayer sample, using delayed Fourier transform pmr at 100 MHz.

Changes in the lipid bilayer pmr spectrum upon addition to the bilayer of ion-specific transport carriers such as the cyclic oligopeptide antibiotic, valinomycin, can also be observed by this technique. In Table II is displayed the effect of valinomycin, in the amount one molecule of valinomycin per 50 lecithin molecules, in an egg lecithin bilayer dispersion. We observed that the addition of valinomycin reduces the line width of the choline methyl resonance by 50% but has little effect on the resonance of the terminal methyl groups. The intensity of the choline methyl resonance is also reduced by 50%. These results suggest that valinomycin interacts with the bilayer predominantly at the polar-head region.[3]

Table II. Effect of Valinomycin on the DFT-Pmr Spectrum[a] of an Egg Lecithin Water Dispersion

	Lecithin		Lecithin + 2% valinomycin	
	Choline methyl	Terminal methyl	Choline methyl	Terminal methyl
Line width, Hz	120	160	68	160
% of protons observed	30	50	17	50

[a] 100 MHz.

These examples illustrate the value of delayed FT nmr in distinguishing peaks which are difficult to analyze in ordinary CW nmr spectra because they appear against a background of stronger and broader reso-

(2) D. Chapman, R. M. Williams, and B. D. Ladbrooke, *Chem. Phys. Lipids*, 1, 445 (1967).
(3) S. M. Johnson and A. D. Bangham, *Biochim. Biophys. Acta*, 193, 82 (1969).

nances. This method also allows the measurement of T_1 as well as T_2 of these otherwise obscured lines by the method of Freeman and Hill.[4] We have, for example, recently determined the T_1's and the line widths of both the choline and terminal methyls of egg lecithin bilayers.[5] Hopefully, further applications can be made to nmr studies of liquid-crystalline and gel-like samples in general.

Acknowledgment. This work was supported in part by Grant GM 14523-05 from the National Institute of General Medical Sciences, U. S. Public Health Service, and by Grant GP-8540 from the National Science Foundation.

(4) R. Freeman and H. D. W. Hill, *J. Chem. Phys.*, **54**, 3367 (1971).
(5) G. W. Feigenson, C. H. A. Seiter, and S. I. Chan, *J. Amer. Chem. Soc.*, submitted for publication.

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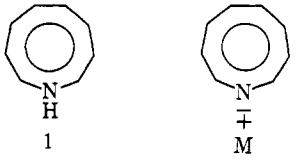
Alkali Metal Azonides. Acidity of 1*H*-Azonine

Sir:

Recently we described the preparation of 1*H*-azonine[1] and a variety of N-substituted derivatives[2] by the low-temperature alcoholysis of *N*-carbethoxyazonine followed by appropriate quenching, and indicated that the overall conversion is best reasoned by the initial generation of the azonide system, *i.e.*, **2**.[2] Presently, we describe the isolation and spectral characterization of various alkali metal azonides and also record information relating to the acidity of 1*H*-azonine (**1**).



1

M

2a, M = Li
b, M = Na
c, M = K
d, M = Rb
e, M = Cs

The azonides shown in **2b–2e** were prepared in good yield (>80%) on treatment of *N*-carbethoxyazonine with a slight deficiency of the appropriate metal *tert*-butoxide in tetrahydrofuran at *ca.* −20°, while lithium azonide (**2a**) was generated in the same medium on the reaction of 1*H*-azonine with *n*-butyllithium at *ca.* −30°; 61% yield. In each case, **2** was isolated pure as a white hygroscopic solid, the relative sensitivity to water increasing in the order **2e** → **2a** and invariably resulting in the formation of the common conjugate acid, *i.e.*, **1**, on exposure to air. In their thermal behavior the azonides are strongly reminiscent of 1*H*-azonine in that they are inert to prolonged heating. For example,

(1) A. G. Anastassiou and J. H. Gebrian, *Tetrahedron Lett.*, 825 (1970).
(2) A. G. Anastassiou, S. W. Eachus, R. P. Cellura, and J. H. Gebrian, *J. Chem. Soc. D*, 1133 (1970).

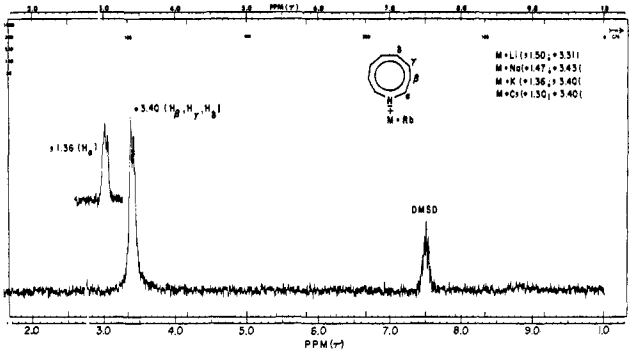


Figure 1. Nmr spectral characteristics of azonides **2a–2e** in DSMO-d_6.

each member shown in **2** was recovered quantitatively (nmr) after being heated neat for 2 days at *ca.* 100° *in vacuo* (*ca.* 0.005 mm). The nmr spectra of the azonides are well exemplified by that of **2d** shown in Figure 1, with the low-field resonance invariably possessing one-third the area of the high-field signal. We note that these spectra differ substantially from those of all other heteronins[2] in two respects, (1) the appearance of the α pair of hydrogens at exceedingly low fields (τ 1.30–1.50) and (2) the essential coincidence of the β, γ, and δ resonances (τ 3.40–3.51). The first phenomenon obviously arises from the deshielding influence that the positive gegenion exerts on the most closely located protons, while the second, *i.e.*, the coincidence of resonances, is no doubt due to the more extensively delocalized frame of the azonide compared to 1*H*-azonine, owing to the greater availability of the nitrogen lone pair for overall contribution into the "π" system. Further, the assignment in each case of the low-field resonance to the α hydrogen pair receives strong support from the nmr characteristics of **2a–2d** in a less ionizing solvent such as acetone.[3] In this medium, unlike DMSO, the position of the low-field signals was found to be a sensitive function of gegenion size, an increase in the alkali metal ionic radius invariably leading to a shift of this resonance to lower fields. Specifically, the nmr characteristics of **2** in acetone are as follows: for M = Li, τ 2.40 (2 H), 3.30 (6 H); for M = Na, τ 1.74 (2 H), 3.20 (6 H); for M = K, τ 1.36 (2 H), 3.37 (6 H); for M = Rb, τ 1.30 (2 H), 3.30 (6 H). Obviously the nmr spectra of the azonides are largely those of solvent-separated species in DMSO and of tight ion pairs in acetone.

The azonide system closely resembles its conjugate acid **1** in its uv spectral characteristics, *e.g.*, for **2c**, λ_{max}^{THF} 339 nm (sh) (ϵ 3900), 330 (4500), and <280 (>10,000).[4] Significantly, the position of the low-energy absorptions is, for reasons detailed earlier,[2] indicative of a planar geometry.

Acidity measurements relating to the N–H function of π-excessive heterocycles provide yet another means

(3) We were unable to record the nmr spectrum of **2e** in acetone-d_6 because of insufficient solubility.
(4) While this band represents the strongest absorption in the spectrum of **2c** its position and intensity could not be accurately assessed owing to the fact that the high dilution necessary here invariably leads to the generation of some 1*H*-azonine (λ_{max}^{THF} 342 nm (sh) (ϵ 1400), 327 (sh) (2200), 298 (3450), and 234 (24,800)).