Practically all of the observations reported in Table I can be accounted for in terms of carbene character associated with C_3H^+. Therefore it would appear that after the long-range ion–molecule interaction has brought the species within "chemical range", it is primarily the carbenoid feature of $:C_3H^+$ rather than the delocalized positive charge that determines the path of reaction. Further insight into such behavior should be forthcoming from studies of the reactivities of other possible carbene cations such as $:C_5H^+$, $:C_2N^+$, $:C_4N^+$, and so on. The gas-phase approach with the SIFT technique adopted here is sufficiently versatile to allow such studies and therefore should provide a new opportunity to improve our understanding of carbene chemistry in general.

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Registry No. C_3H^+, 75104-46-0; H_2, 1333-74-0; CO, 630-08-0; H_2O, 7732-18-5; H_2S, 7783-06-4; CO_2, 124-38-9; CH_4, 74-82-8; CH_3OH, 67-56-1; C_2H_4, 74-85-1; oxygen, 7782-44-7; cyclopropenylium, 26810-74-2; propylene, 115-07-1.

Nuclear Overhauser Effect Measurements Involving the Imino Protons of Yeast tRNA^{Phe} Using Two-Dimensional Proton NMR Spectroscopy

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The potential of nuclear magnetic resonance (NMR) for structural studies of biomacromolecules greatly depends on the possibility to identify individual resonances in the spectrum. Recently, significant progress has been made in this field, particularly by the systematic application of the nuclear Overhauser effect (NOE). Using so-called one-dimensional NOE techniques, two groups[1,2] have now independently obtained a complete assignment of the imino proton spectrum of yeast tRNA^{Phe}, while part of the imino proton spectra of three other tRNAs have been characterized.[3-5]

Notwithstanding these accomplishments, in principle two-dimensional NOE is a more powerful and more efficient method for the detection of NOE's between neighboring protons in biological macromolecules.[6] Moreover, it has been shown[7] that the 2D-NOE experiment provides an immediate qualitative view of the relative rate of exchange of the imino protons in nucleic acid fragments, i.e., a fingerprint of the stability of individual base pairs in a double helical fragment.

However, in the case of a 2D-NOE experiment involving imino protons, e.g., of yeast tRNA^{Phe}, a serious technical problem is posed by the fact that the imino proton resonances are only detectable when the compound is dissolved in (nondeuterated) water. This experimental condition implies a "dynamic range" problem of a factor 10^5 as the solute signal (typically present in 1 mM concentration) is to be recorded in the presence of a huge water signal (proton concentration ca. 110 M). When imino protons of nucleic acids are involved, the "standard" water suppression technique, i.e., (pre-) irradiation of the solvent signal, is prohibited as chemical exchange between water protons and imino protons will give rise to magnetization transfer from the irradiated water to the nucleic acid imino protons, thus leading to a saturation of the latter resonances and impairing their observation.[8]

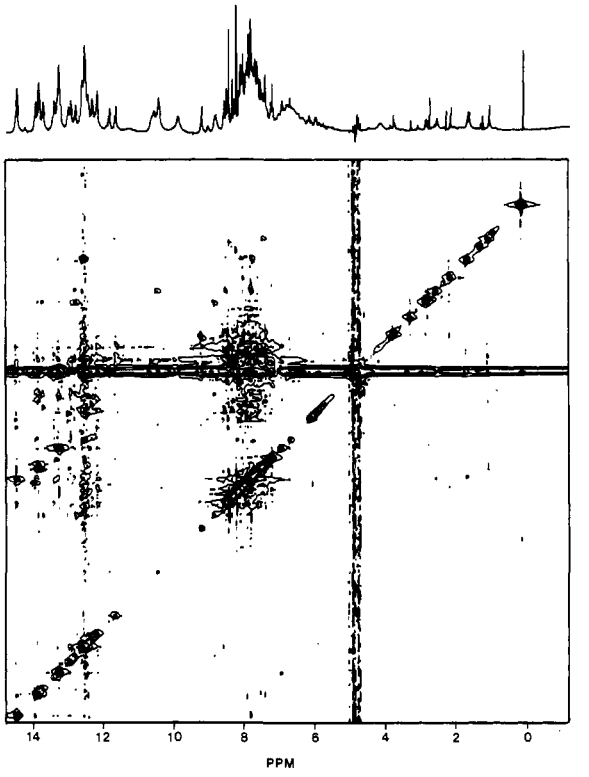

Figure 1. Contour plot of a 500-MHz ^1H NMR 2D-NOE spectrum of yeast tRNA^{Phe} (1.8 mM dialyzed against H_2O/D_2O, 95:5 v/v, containing 5 mM $MgCl_2$, 0.1 mM EDTA, and 12 mM Na_2HPO_4, pH 6.9), T = 28 °C. Spectral width was 20 000 Hz; quadrature detection was used with the carrier at the low-field end of the spectrum. The data set consisted of 4K data points in ω_1-dimension, 512 data points in ω_2-dimension; 256 FID's were accumulated for each value of t_1; total acquisition time was ca. 66 h. Before Fourier transformation, the FID's were multiplied with a sine-bell window[11] and zero filled to 2K data points in the ω_1-dimension. An absolute value plot of the relevant part of the spectrum is shown. The nonsymmetrical appearance of the contour plot is a result of the method of data collection and processing.[7] The vertical and (partly) the horizontal spikes at 4.75 ppm are due to the resonance of residual solvent protons. The top spectrum is a normal 1D spectrum of the same sample recorded by using a time-shared long pulse in combination with DSA.[7] Total accumulation time 6 min, no digital filtering was applied before Fourier transformation.

Recently, an alternative experimental method using a semiselective observation pulse in combination with the digital shift accumulation (DSA) technique was proposed[7] for the present type of measurements. Moreover, it was shown[7] that the method is easily extended to 2D-NOE experiments. Backed by this water suppression technique we explored the limits of 2D-NOE NMR spectroscopy and report here a 2D-NOE experiment involving the imino protons of yeast tRNA^{Phe}. It will be shown that 2D-NOE spectroscopy is indeed feasible for a molecule of the size of a tRNA (molecular weight ~28 000).

Figure 1 shows the absolute value contour plot of the 2D-NOE spectrum of a 1.8 mM solution of tRNA^{Phe}. The spectrum was recorded by using the [90°–t_1–90°–τ_m–90°–Acq(t_2)] pulse sequence introduced by Macura and Ernst[9] except that in our experiment the last pulse (the observation pulse) was replaced by a time-shared long pulse.[7] The mixing time τ_m was 0.3 s. After recording the spectra, the FID's were subjected to a DSA treatment,[10] followed by window multiplication and Fourier transformation in two dimensions. For reference purposes a normal 1D spectrum (recorded using a time-shared long pulse in

(1) Heerschap, a.; Haasnoot, C. A. G.; Hilbers, C. W. *Nucl. Acids Res.* **1982**, *10*, 6981–7000; and in press.
(2) Roy, S.; Redfield, A. G. *Biochemistry* **1983**, *22*, 1386–1390.
(3) Hare, D. R.; Reid, B. R. *Biochemistry* **1982**, *21*, 1835–1842.
(4) Hare, D. R.; Reid, B. R. *Biochemistry* **1982**, *21*, 5129–5135.
(5) Roy, S.; Papastravos, M. Z.; Redfield, A. G. *Biochemistry* **1982**, *21*, 6081–6088.
(6) (a) Wuetrich, K.; Wider, G.; Wagner, G.; Braun, W. *J. Mol. Biol.* **1982**, *155*, 311–319. (b) Billeter, M.; Braun, W.; Wuetrich, K. *Ibid.* **1982**, *155*, 321–346. (c) Wagner, G.; Wuetrich, K. *Ibid.* **1982**, *155*, 347–366. (d) Wider, G.; Lee, K. H.; Wuetrich, K. *Ibid.* **1982**, *155*, 367–388.
(7) Haasnoot, C. A. G.; Hilbers, C. W. *Biopolymers* **1983**, *22*, 1259–1266.
(8) Johnston, P. D.; Redfield, A. G. *Nucl. Acids Res.* **1977**, *4*, 3599–3616.
(9) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95–117.
(10) Roth, K.; Kimber, B. J.; Feeney, J. *J. Magn. Reson.* **1980**, *41*, 302–309.

0002-7863/83/1505-5483$01.50/0

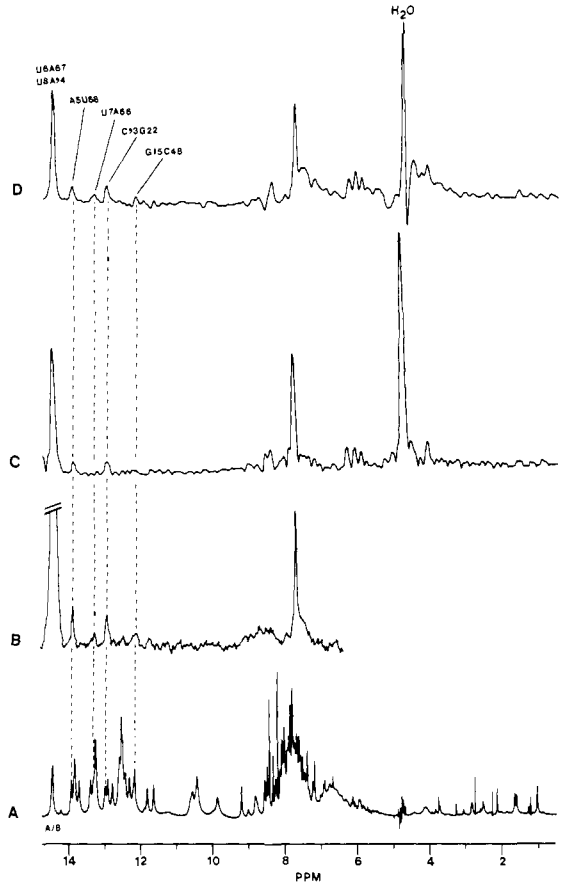

Figure 2. Comparison of NOE results obtained for the A/B imino proton resonance of yeast $tRNA^{Phe}$ using different techniques. The assignments of the imino protons of adjacent Watson–Crick base pairs are indicated. The large NOE observed at 7.7 ppm is from the A14H8 and the A67H2 proton resonances, which at this temperature coincide. (A) Reference spectrum (same as in Figure 1). (B) 1D-NOE spectrum recorded with a "Redfield 2-1-4" pulse in combination with ADA.[1,7] (C) Cross section (parallel to ω_1) through the contour plot given in Figure 1 taken for a value of ω_2 corresponding to the position of resonance A/B. (D) Same cross section as in (C), however, after "phase sensitive" Fourier transformation in both dimensions.

combination with DSA[7]) is presented above the contour plot.

The peaks along the diagonal of the contour plot in Figure 1 represent the normal 1D-NMR spectrum, while the cross peaks depict the transfer of magnetization that occurs between several resonances during the mixing period τ_m. As explained elsewhere[7] the 2D spectrum is not symmetrical with respect to the diagonal due to the use of a semiselective observation pulse in combination with the DSA technique.

A detailed analysis of all the cross peaks observed in the 2D-NOE spectrum is clearly beyond the scope of the present communication; however, one general feature merits further discussion. In Figure 2C a cross section along the f_1 axis of the 2D spectrum is shown, which was taken at the f_2 position coinciding with the resonance at 14.4 ppm marked A/B in the 1D spectrum (Figure 2A). The latter resonance was shown[1] to originate from two imino protons of the $tRNA^{Phe}$: base pair U6A67 and the (tertiary) base pair U8A14. Using 1D-NOE techniques (Figure 2B), the resonances arising from the base pairs adjacent to these U6A67 and U8A14 pairs were identified[1,2] as indicated in Figure 2.

When the 1D-NOE difference spectrum (Figure 2B) is compared with the aforementioned cross section in Figure 2C, it is seen that not all interimino proton NOE's detected in the 1D-experiment have counterparts in the analogous 2D experiment. The reason for this fact must be sought in the method of 2D-data processing. In general, 2D spectra are presented in absolute value mode (as is the case in Figures 1 and 2C) in order to avoid phase correction in two dimensions. However, as absolute value mode signals normally display line shapes with long "tails" on both sides, 2D spectra must be heavily resolution enhanced (e.g., by multiplying the FID's with a sine-bell window[11]) in order to remove these "tails" from the spectrum.[12] The loss in sensitivity introduced by this type of resolution procedures is considerable and in the case at hand it even leads to an intolerable loss of information (cf. Figure 2, B and C). The solution for this problem is found in a "phase sensitive" 2D-FT procedure. Recently, States et al.[13] have shown that pure absorption phase 2D spectra have a much better intrinsic resolution, thus abolishing the need for resolution enhancement digital filtering. In fact, it is possible to enhance the signal-to-noise ratio by applying line-broadening digital filters without significant loss in resolution. This is shown in Figure 2D, which depicts the same cross section as given in Figure 2C, but now after the (same) 2D data file was Fourier transformed after applying a Gaussian line-broadening filter in both dimensions. It is seen that all four expected interimino proton NOEs are present in the spectrum (Figure 2D). The magnitude of these NOE's depends somewhat on the position where the cross section is chosen, because the A/B imino proton resonances are not coinciding exactly. The spectrum represents a compromise in the sense that for the given cross section all four NOE's are visible.

In summary, we have demonstrated that 2D-NOE spectroscopy is indeed possible for tRNA molecules having a molecular weight of ~28 000 even when large dynamic range problems are involved. Moreover, it is shown that, using this experimental technique, NOEs between imino protons of adjacent Watson–Crick base pairs are detectable, but full information can only be extracted after a "phase sensitive" 2D Fourier transformation of the data.

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(11) de Marco, A.; Wuethrich, K. *J. Magn. Reson.* **1976**, *24*, 201–206.
(12) Bax, A.; Freeman, R.; Morris, G. A. *J. Magn. Reson.* **1981**, *43*, 333–338.
(13) States, D. J.; Haberkorn, R. A.; Ruben, D. J. *J. Magn. Reson.* **1982**, *48*, 286–292.

Methyleneoxonium and Hydroxymethylene Dications: Dicationic Analogues of Ethylene and Acetylene

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There has been considerable recent theoretical and experimental interest in the methyleneoxonium ($CH_2OH_2^{+\cdot}$)[1-4] and hydroxymethylene ($HCOH^{+\cdot}$)[4-8] radical cations. The methyleneoxonium

(1) Bouma, W. J.; Nobes, R. H.; Radom, L. *J. Am. Chem. Soc.* **1982**, *104*, 2929.
(2) Bouma, W. J.; MacLeod, J. K.; Radom, L. *J. Am. Chem. Soc.* **1982**, *104*, 2930.
(3) Holmes, J. L.; Lossing, F. P.; Terlouw, J. K.; Burgers, P. C. *J. Am. Chem. Soc.* **1982**, *104*, 2931.
(4) Frisch, M. J.; Raghavachari, K.; Pople, J. A.; Bouma, W. J.; Radom, L. *Chem. Phys.* **1983**, *75*, 323.
(5) Bouma, W. J.; MacLeod, J. K.; Radom, L. *Int. J. Mass Spectrom. Ion Phys.* **1980**, *33*, 87.
(6) Osamura, Y.; Goddard, J. D.; Schaefer, H. F.; Kim, K. S. *J. Chem. Phys.* **1981**, *74*, 617.
(7) Vas Pires, M.; Galloy, C.; Lorquet, J. C. *J. Chem. Phys.* **1978**, *69*, 3242.

0002-7863/83/1505-5484$01.50/0