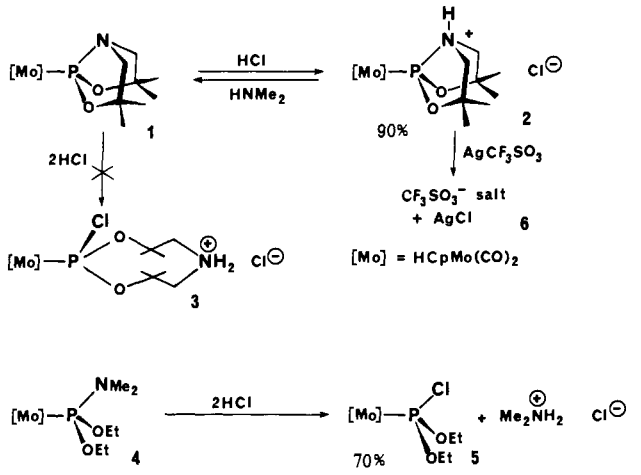
Scheme I



evidence for their formation could be produced.

We therefore wish to report the isolation and unexpected stability of compound **2**, a phosphorammonium salt with a constrained bicyclic structure. The aminophosphine ligand symmetrically tetramethylated at the carbon α to the oxygen atoms was selected for the simplicity expected of its ^1H NMR spectra (Scheme I).

Compound **2** precipitated instantaneously when dry gaseous HCl was bubbled through a solution of the aminophosphane adduct **1**[10] in toluene at −20 °C and was isolated in close to quantitative yields as an unexpectedly stable (dec ~135 °C as compared to ~75 °C for **1**), but highly air-sensitive N-protonated salt, soluble in CH_3CN, $CHCl_3$, and THF, sparingly soluble in ether and toluene. By contrast, when compound **4**—a close, but *non*cyclic analogue of **1**—was treated with HCl in similar experimental conditions, it readily led to the toluene- and ether-soluble chloro phosphite adduct **5** (identified by elemental analysis, IR, NMR, and mass spectroscopy), together with $Me_2NH_2^+Cl^-$, which were isolated in 70% and 90% yields, respectively, thus attesting that the usual P–N bond cleavage reaction took place in this case.

Compound **2** was identified unambiguously. First, its formulation was ascertained by elemental analysis (C, H, Cl, Mo, N, P) and mass spectrometry, which also exclude the formation of the P–N cleavage product **3**. The obtaining of **2** rather than **3** was also established by using a titrated solution of HCl in ether to effect the protonation, which showed that only 1 molar equiv of HCl is needed to convert **1** integrally into **2**. The anion is readily exchanged at −25 °C in MeCN under the action of $AgCF_3SO_3$. The ionic nature of the resulting species **6** is shown by the conductivity of its solutions (Λ = 19.6 Ω cm^2 mol^{-1} in nitrobenzene) compared to that of the starting material **1** (Λ = 0.97 Ω cm^2 mol^{-1}).

Among the most pertinent evidence for the preservation of the P–N bond is the ^1H NMR spectrum (CD_3CN), which at room temperature exhibits an eight-line phosphorus-coupled AB system for the CH_2 groups of the five-membered cycles (δ_A = 3.82, δ_B = 3.44; J_{AB} = 13, $^1/_2(J_{H_AP} + J_{H_bP})$ = 0.4 Hz, a system analogous to, but with chemical shift and coupling parameters slightly different from, those of the free ligand (δ_A = 3.08, δ_B = 2.75; J_{AB} = 12, $^1/_2(J_{H_AP} + J_{H_BP})$ = 0.3 Hz, C_6D_6).

The ^1H spectrum also shows a singlet at 5.53 ppm for the cyclopentadienyl protons, a doublet at −6.73 ppm (J_{HP} = 61.7 Hz) for the MoH hydride proton, and two signals at 1.55 and 1.31 ppm for the nonequivalent methyl groups. Contrary to what is observed with the unprotonated complex **1** no splitting of the signals, attributable to cis and trans isomers at molybdenum, was observed upon cooling at −80 °C in CD_2Cl_2. This means either that only one isomer is present for **2** in these conditions or that the cis–trans interchange is still rapid on the NMR time scale. The significantly smaller apparent coupling observed for **2** at −80 °C in CD_2Cl_2 (54 Hz) compared to that measured at room temperature in CD_3CN (61.7 Hz) points rather to a fast temperature-dependent equilibrium between the two isomers, with predominance of the cis isomer in view of the larger J_{HP} coupling expected,[11] comparable to that found for *cis*-**1** (61.0 compared to 27.1 Hz for *trans*-**1** in toluene-d_8 at −40 °C).

The retention of the bicyclic structure is further ascertained by the close resemblance of the mass and infrared spectra of **2** to those of **1** and their difference with respect to those of **5**. The modification undergone by the ligand is nevertheless evidenced by the presence of the broad signal (~100 Hz) at 11.55 ppm in the ^1H NMR spectrum, as expected for an ammonium proton, and by a slight displacement, by ca. 5 ppm, of the ^{31}P signal (δ 209.5, J_{PH} = 62.5 Hz in CD_2Cl_2) toward higher fields, smaller than would be expected for **3**. Comparable NMR patterns are observed for the trifluoromethanesulfonate salt **6**. Also noteworthy is the absence in the IR spectrum of **2** of the characteristic ν(PCl) stretching vibration found around 535 cm^{-1} for **5**.[12]

Protonation causes a decrease in electron density on phosphorus, both because of the presence of the positively charged quaternarized nitrogen and because N(pπ)–P(dπ) donation is no longer possible. The decrease (by 10 and 16 cm^{-1}) in frequency of the two ν(CO) vibrations upon protonation is interpreted to mean that the diminution in electron density in the bonding orbitals of the CO groups outweighs that which occurs in their π^* orbitals.

Compound **2** can be deprotonated under the action of diethylamine (1 equiv is required to complete the process) to restore adduct **1** (80% isolated) and the ammonium salt H_2NEt_2Cl (85% isolated).

Attempts to protonate the uncomplexed bicyclic aminophosphane ligand led to the immediate formation, even at −80 °C, of a white precipitate, whose ^{31}P spectrum exhibited numerous signals.

These results illustrate the drastic changes in behavior of the P–N entity when subjected to constraints that force the nitrogen atom to keep a pyramidal configuration.

(9) See, for example: Höfler, M.; Schnitzler, M. *Chem. Ber.* **1972**, *105*, 1133.
(10) Febvay, J. Thesis, Université de Nice, France, 1981.
(11) Faller, J. W.; Anderson, A. S.; Jakubowski, J. *J. Organomet. Chem.* **1971**, *27*, C45.
(12) Thomas, L. C. "Interpretation of the Infrared Spectra of Organophosphorus Compounds"; Heyden: London, 1974; p 74.

Determination of Molecular Structure in Solution via Two-Dimensional Nuclear Overhauser Effect Experiments: Proflavine as a Rigid Molecule Test Case

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The two-dimensional NMR nuclear Overhauser effect (2D NOE) experiment is potentially the most powerful method for determining molecular structures in solution.[1,2] Wüthrich's lab has elegantly exploited the 2D NOE technique semiquantitatively in studies of small protein structure.[3,4] We have recently examined some aspects of molecular structure determination via 2D NOE theoretically.[5] Proflavine, a rigid molecule with X-ray crystal structure determined, was studied here as a test case. The in-

(1) Macura, S.; Ernst, R. R. *Mol. Phys.* **1980**, *41*, 95.
(2) Jeener, J.; Meier, B. H.; Bachmann, P.; Ernst, R. R. *J. Chem. Phys.* **1979**, *71*, 4546.
(3) Wider, G.; Macura, S.; Kumar, A.; Ernst, R. R.; Wüthrich, K. *J. Magn. Reson.* **1984**, *56*, 207.
(4) Billeter, M.; Braun, W.; Wüthrich, K. *J. Mol. Biol.* **1984**, *155*, 321.
(5) Keepers, J. W.; James, T. L. *J. Magn. Reson.* **1984**, *57*, 404.

0002-7863/84/1506-7986$01.50/0

Table I. Proflavine Proton–Proton Distances

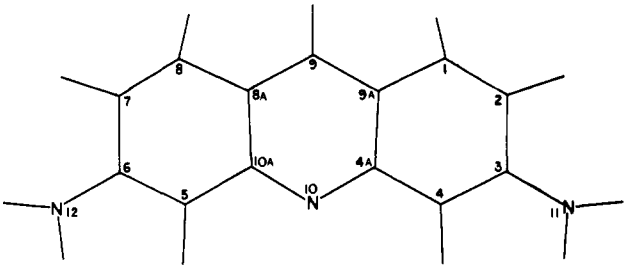
	2D NOE calcd fit, Å				cryst struct,[a] Å			
	H1	H2	H4	H9	H1	H2	H4	H9
H1	0.00	2.39	4.68	2.34	0.00	2.64	4.68	2.49
H2	2.39	0.00	3.78	4.16	2.64	0.00	4.18	4.96
H4	4.68	3.78	0.00	5.08	4.68	4.18	0.00	5.48
H9	2.34	4.16	5.08	0.00	2.49	4.96	5.48	0.00

[a] From ref 9.

terproton distances obtained for proflavine in solution by 2D NOE agree well with those from the X-ray crystal structure.

The theory for 2D NOE was largely developed by Ernst's lab. Following this development,[1,2,6] we[5] recently described a matrix method relating all interproton distances in a molecule to peak intensities in a theoretical 2D NOE spectrum which can be iteratively compared with the experimental pure absorption 2D NOE spectrum. The method of calculation is similar to that previously used to examine effects of spin diffusion in one-dimensional NMR.[7,8] Unlike previous 2D NOE studies, this method is not limited by assumptions regarding the number or geometry of proton spins, spin diffusion, the details of molecular motions, or the range of mixing times employed in the experiment. The purpose of the present communication is to demonstrate the validity of the technique by comparing interproton distances determined with the 2D NOE method for a rigid molecule in solution with those distances in the X-ray crystal structure.

Proflavine was chosen as a test case because it is a simple, rigid molecule of known crystal structure.[9] The number of interacting



protons is limited by exchanging the amino protons with deuterons. Furthermore, the molecule has no substantial internal motions other than ammonium group bond rotation; this facet plus the molecular dimensions allows use of a simple isotropic motional model as a decent approximation. (We note that theoretical calculations indicate an "effective" isotropic motion may reasonably be used for present purposes even with substantial internal motions.[5])

Peak intensities in a pure absorption proton 2D NOE spectrum depend on the experimental mixing time, the molecular tumbling rate manifest in the spectral density, and interproton distances (between all protons, not just any two). As an experimental parameter, the mixing time is known. There are several ways of dealing with the spectral density, including leaving it as an adjustable parameter. Here we have calculated the spectral density from the spin–lattice relaxation time (T_1) value of 1.51 s determined for the H1 proton of proflavine,[10] assuming simple isotropic

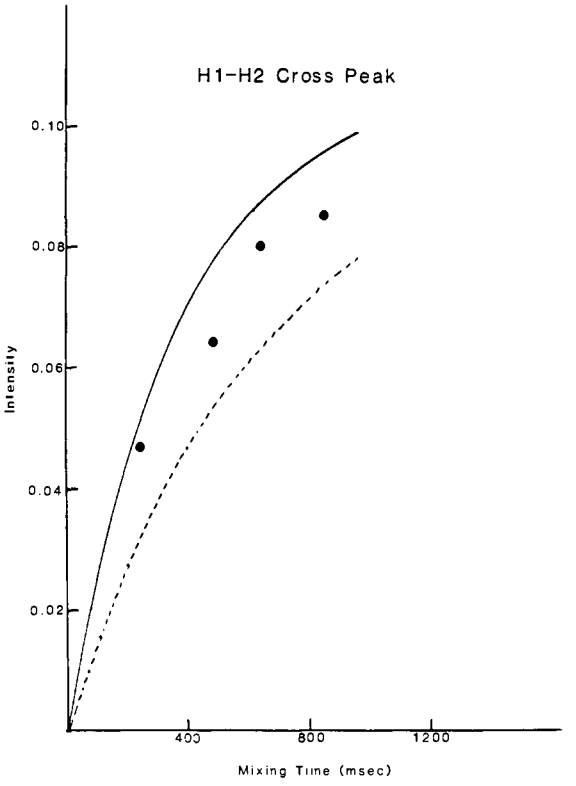


Figure 1. H1–H2 cross-peak intensity. Experimental values (●) with calculated curves using the crystal-structure distances (broken line) and distances calculated by simultaneously fitting all cross-peak and diagonal-peak intensities (solid line). Intensities are expressed as a fraction of the sum of diagonal- and cross-peak intensities.

motion and intramolecular dipole–dipole relaxation. The latter assumption is justified by the two protons close to H1 and a series of concentration dilutions showing that intermolecular effects were negligible. The isotropic correlation time was determined to be 2.0 ns under present experimental conditions.

Six cross-peak intensities and four diagonal-peak intensities measured from each 2D NOE spectrum at four different mixing times yielded 40 pieces of experimental data from which the six interproton distances in proflavine can be determined. The 2D NOE spectra calculated for each mixing time by the matrix method[5] were compared with the experimental spectral intensities. After several iterations, the residuals were minimized yielding interproton distances, listed in Table I, which permitted the simultaneous best fit of the calculated to the experimental spectral intensities for all four mixing times. Interproton distances for crystal-structure protons, located by a Fourier difference synthesis,[9] can be compared with those from 2D NOE on proflavine in solution (Table I). The difference is <10% in most cases. Cross-peaks disappear into the noise for distances greater than 4.5–5 Å, thus establishing the upper limit on distance determinations at the moment.

Figure 1 shows, for H1–H2 cross-peaks, observed intensities and intensities calculated both for the crystal structure and for the internuclear distances obtained in a best fit of all 40 intensity data. The two curves in Figure 1 represent a change in H1–H2 distance of only 0.25 Å, i.e., about 10%. Although changes in other distances also are influential, Figure 1 illustrates the sensitivity of the method to changes in internuclear distance. With only H1–H2 cross-peak intensities, the experimental points could be fit very well with an H1–H2 distance increase of only 0.10 Å.

Although X-ray crystal-structure distances and 2D NOE solution structure distances generally agree within 10%, there are discrepancies. First, X-ray crystallography, even with Fourier difference synthesis, may not have placed the hydrogens accurately.

(6) Bodenhausen, G.; Ernst, R. R. *J. Am. Chem. Soc.* **1982**, *104*, 1304.
(7) Hull, W. E.; Sykes, B. D. *J. Chem. Phys.* **1975**, *63*, 867.
(8) Bothner-By, A. A. Noggle, J. H. *J. Am. Chem. Soc.* **1979**, *100*, 5152.
(9) Achari, A.; Neidle, S. *Acta Crystallogr., Sect. B* **1976**, *B32*, 2537.
(10) Proflavine hemisulfate was lyophilized twice from 99.8 atom % D_2O and dissolved in 1:1 (v/v) 99.96 atom % D_2O/ethylene-d_6 glycol (98 atom % D). The solvent mixture and −20 °C temperature were used so proflavine would have motions similar to larger biomolecules of interest to us. Slower motion enhances cross-peak intensities, but slower motion is not required for use of the technique. 2D NOE spectra were recorded at 240 MHz with mixing times of 240, 480, 640, and 850 ms and phase cycling to produce pure absorption phase spectra (modification of States et al. technique[11]). The data at each mixing time consisted of 256 points in t_2 and 64 points in t_1. Thirtytwo free induction decays were accumulated at each t_1. During processing the t_1 dimension was zero filled to 256 points. The sweep width was ±630 Hz. Diagonal and cross-peak intensities were estimated by summing integrals from the four highest intensity cross sections along f_1 for each diagonal peak.
(11) States, D. J.; Haberkorn, R. A.; Ruben, D. J.; *J. Magn. Reson.* **1982**, *48*, 286.

The reported structure has the hydrogens well out of the plane of the ring.[9] If we place the hydrogens in the ring plane of the crystal structure, many internuclear distances are in better agreement with those found here. Another possibility is that librational motions in the dissolved molecule may yield slightly smaller effective distances. Of course, there can be some real difference between solution and crystal structures; the three rings in the crystal structure are not quite coplanar. A final source of discrepancies lies in the 2D NOE technique. Dilution experiments indicate that a very small amount of intermolecular dipolar interactions exist (estimated <10%) at the concentration used. Also, analysis of 2D NOE peak intensities needs refining.

The test case explored here justifies cautious use of 2D NOE for quantitative determination of several interproton distances. The combination of these distances and other geometric constraints (bond distances, bond angles, steric limitations) can be utilized with the distance geometry algorithm[12] to calculate a family of acceptable molecular structures (hopefully, closely related).

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Registry No. Proflavine, 92-62-6.

(12) Havel, T. F.; Crippen, G. M.; Kuntz, I. D. *Biopolymers* **1979**, *18*, 73.

Photocyclizations of *o*-(Benzyloxy)acetophenone and -benzophenone: Effects of Variable Rotational Freedom on Biradical Behavior

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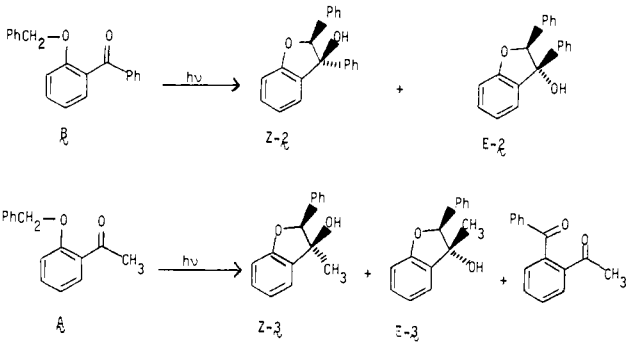
In all bimolecular photoreactions, acetophenone and benzophenone behave similarly.[1] Their n,π* triplets react with comparable rate constants, and they form the same kinds of products with comparable quantum efficiencies. We wish to report this similarity does not extend to intramolecular photoreactions, in particular the cyclization of *o*-(benzyloxy) ketones induced by triplet-state δ-hydrogen abstraction.[2] The benzophenone derivative reacts in a 50-fold greater quantum efficiency than the corresponding acetophenone, apparently because of much different rotational barriers and rates of intersystem crossing in the two 1,5-biradical intermediates.

o-(Benzyloxy)benzophenone (B), like some 4-alkoxy derivatives,[2c] cyclizes to the benzofuranols **2** upon UV irradiation. In benzene, the *Z*/*E* ratio is 8/1 and the combined quantum yield is 0.95. In the presence of Lewis base solvents, the product quantum yield falls to 0.60–0.65 and the *Z*/*E* ratio changes to 1.2:1. The reaction is readily quenched by typical triplet quenchers. Laser flash spectroscopy[3] revealed a transient triplet (λ_{max} = 540 nm) with a lifetime of 52 ns. No transient identifiable as a 1,5-biradical was apparent in benzene with enough diene to lower the triplet lifetime to <5 ns.[4] However, with added pyridine



typical biradical decay (at 550 nm) could be observed with τ = 13 ns.

o-(Benzyloxy)acetophenone (A) also photocyclizes to benzofuranols, but in much lower quantum efficiency. In benzene, only (*Z*)-3 is detectable with Φ = 0.02. With 2 M pyridine present, the *Z*/*E* ratio is 1.5/1 while Φ = 0.20. In both solvent systems, 2-benzoylacetophenone is formed with Φ = 0.05–0.07. The rearrangements are readily quenchable in benzene. Flash spectroscopy reveals an identically quenchable triplet with a lifetime of 455 ns. This lifetime is too long to allow reliable detection of any substantially shorter lived biradical.

The remarkable observation that we must explain is the far greater efficiency with which the substituted benzophenone undergoes cyclization. Since the π,π* lowest triplet of A is less reactive than the n,π* lowest triplet of B,[5] A's low quantum yield could be partially due to radiationless decay competing with δ-hydrogen abstraction. However, the triplet lifetime ratio for A/B is the same as the ratio of rate constants for γ-hydrogen abstraction for valerophenone/*o*-methoxyvalerophenone, models for n,π* and π,π* triplets undergoing the same internal reaction.[6] Therefore δ-hydrogen abstraction is concluded to be the major mode of triplet decay for both A and B, and the lower quantum yields for A must involve competing reactions of the 1,5-biradical formed from the triplet.

Added Lewis bases destroy the stereoselectivity of cyclization for both ketones. This effect is well established for both 1,4-biradicals[7] and 1,5-biradicals,[2d,8] since the solvated OH is much larger than a free OH. Most hydroxy biradicals previously studied undergo a large amount of disproportionation back to starting ketone, which is suppressed by solvation of the OH.[7,8] The 10-fold enhancement of the cyclization quantum yield for A by added pyridine is thus typical behavior. What is unusual about A is the low overall quantum efficiency and the significant amount of diketone product.

The behavior of B is also unusual in that its biradical intermediate undergoes none of the usual disproportionation back to ground-state ketone. Solvation apparently impedes overall cyclization sufficiently that another process becomes competitive.[9] In fact, flash spectroscopy reveals a byproduct which has an absorption profile in the near-UV very similar to those of the LATs formed by para coupling of radicals during photoreduction of ketones.[10] Moreover, this byproduct is destroyed by addition of acid to the solution. We conclude that the biradicals also cyclize at the ortho position to form spiro enols. This competitive cyclization is minor with B but major with A. The benzoylaceto-

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‡ National Research Council; NRCC Contribution No. 23898.

(1) Wagner, P. J. *Top. Curr. Chem.* **1976**, *66*, 1. Guttenplan, J. B.; Cohen, S. G. *J. Am. Chem. Soc.* **1972**, *94*, 4040.

(2) (a) Pappas, S. P.; Blackwell, J. E. *Tetrahedron Lett.* **1966**, 1175. (b) Pappas, S. P.; Pappas, B. B.; Blackwell, J. E. *J. Org. Chem.* **1967**, *32*, 3066. (c) Lappin, G. R.; Zannucci, J. S. *J. Org. Chem.* **1971**, *36*, 1808. (d) Pappas, S. P.; Zehr, R. D. *J. Am. Chem. Soc.* **1974**, *96*, 7112.

(3) For a description of the apparatus and methodology, see: Scaiano, J. C. *J. Am. Chem. Soc.* **1980**, *102*, 7747.

(4) Small, R. D.; Scaiano, J. C. *J. Am. Chem. Soc.* **1978**, *100*, 4512.

(5) Yang, N. C.; McClure, D. S.; Murov, S. L.; Houser, J. J.; Dusenbery, R. *J. Am. Chem. Soc.* **1967**, *89*, 5466.

(6) Wagner, P. J.; Kemppainen, A. E.; Schott, H. N. *J. Am. Chem. Soc.* **1973**, *95*, 5604.

(7) (a) Wagner, P. J. *J. Am. Chem. Soc.* **1967**, *89*, 5898. (b) Wagner, P. J.; Kelso, P. A.; Kemppainen, A. E.; McGrath, J. M.; Schott, H. N.; Zepp, R. G. *J. Am. Chem. Soc.* **1972**, *94*, 7506.

(8) Wagner, P. J.; Chiu, C. *J. Am. Chem. Soc.* **1979**, *101*, 7134.

(9) Previous investigators[2c,d] have correctly concluded that biradical solvation would slow down cyclization; we have shown that quantum yields are lowered only if another biradical process unaffected by solvation is competitive.[8]

(10) (a) Schenk, G. O.; Cziesla, M.; Eppinger, K.; Matthias, G.; Pape, M. *Tetrahedron Lett.* **1967**, 193. (b) Filipescu, N.; Minn, F. L. *J. Am. Chem. Soc.* **1968**, *90*, 1544. (c) Scaiano, J. C.; Abuin, E. B.; Stewart, L. C. *J. Am. Chem. Soc.* **1982**, *104*, 5673.

0002-7863/84/1506-7988$01.50/0