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^{13}C NMR of Crystalline Morphine

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Abstract: The high-resolution ^{13}C NMR spectrum of crystalline morphine obtained with magic angle sample spinning and proton decoupling has resolved resonances from each of the 17 carbon atoms in the molecule. Four of these resonances are split into doublets. The three asymmetric doublets arising from the three carbons bonded to the ^{14}N of the molecule enabled the determination of a value of −1.5 MHz for the nitrogen quadrupole coupling constant. The resonance from C15 is split nearly symmetrically, suggesting that this site exists in two environments.

The characteristics of the receptor bound form of drug molecules are the key to understanding structure–activity relationships. Since large amounts of purified receptors are not generally available for studying the drug–receptor complex directly, it is often necessary to study the drug molecule alone, and infer features of the drug in the complex. Because the structure and electronic properties of these molecules are influenced by their environment, it is valuable to study the molecules in as many situations as possible in order to separate inter- and intramolecular factors.

Direct comparisons between liquid and solid samples are possible by employing high-resolution solution and solid-state NMR methods. In liquids, chemical shifts, spin–spin couplings, and through-space connectivities provide a wealth of information; however, rapid molecular motions remove dipolar and quadrupolar couplings and average anisotropic chemical shift and J couplings to their isotropic values so that much of the information contained in these spin interactions is lost. High-resolution NMR of solids offers complementary chemical shift data, as well as the possibility of measuring unaveraged chemical shift, dipolar, and quadrupolar interactions. In solid-state NMR a variety of techniques are used to selectively suppress one or more of the interactions, so that a single remaining one may be examined in a tractable form.

The quadrupole interaction of ^{14}N reflects the electronic configuration at a nitrogen site through the effect of the electric field gradient on the nuclear energy levels. However, pure quadrupole resonance experiments are technically difficult and have been performed for relatively few molecules. Recently we[1-6] and others[7-14] have described the dipolar coupling between carbon and nitrogen that is apparent in high-resolution solid-state ^{13}C NMR spectra of organic molecules. Under the experimental conditions of proton decoupling and magic angle sample spinning, the resonances from carbon nuclei directly bonded to nitrogen are split into asymmetric doublets. This splitting represents a residual dipolar coupling not removed by magic angle sample spinning because the ^{14}N quadrupole interaction tilts the axis of quantization of nitrogen away from the applied magnetic field. The size of the residual coupling depends on the sign and magnitude of the nitrogen quadrupole coupling constant, the magnitude of the nitrogen quadrupole asymmetry parameter, and the magnitude and relative orientation of the internuclear vector in the principal axis system of the quadrupole interaction. In most cases the residual dipolar coupling can be characterized by the size of the split between the components of the asymmetric double.[6]

Opiate agonism and antagonism are receptor related events which are clearly influenced by the properties of the drug molecules. Apparently the electronic charge and configuration of the piperidine ring fragment of these molecules are important determinants for such binding. Previous efforts to study these factors have included X-ray crystallography and semiempirical quantum mechanical calculations of opiate agonists and antagonists, in-

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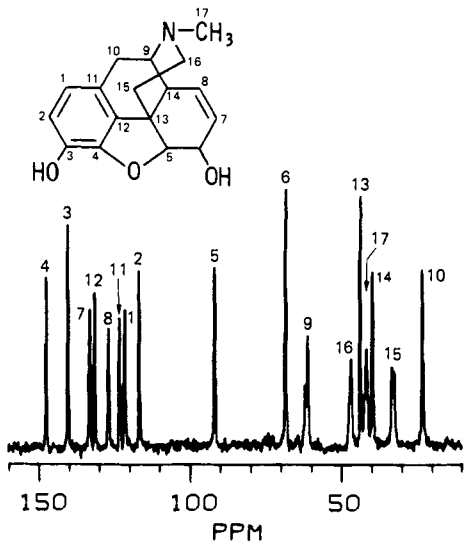

Figure 1. ^{13}C NMR spectrum of polycrystalline morphine sulfate obtained with magic angle sample spinning and proton decoupling.

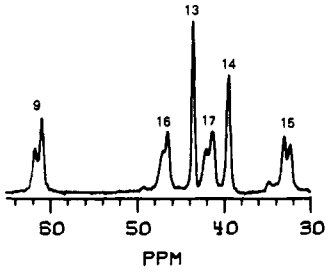

Figure 2. 30–65-ppm region of the ^{13}C NMR spectrum of polycrystalline morphine sulfate.

cluding studies of the prototype agonist, morphine.[15-17] High-resolution NMR spectroscopy is a natural extension of these studies.

Experimental Section

Morphine sulfate was obtained in powdered form without preservatives from Merck Chemical Co. ^{13}C NMR spectra were obtained on a homebuilt double-resonance spectrometer operating at 3.53 T. The ^{13}C magnetization was developed with the spin-lock version of cross polarization with a mix time of 1.0 ms. The proton-decoupling field was 2.5 mT during the data acquisition time of 100 ms. Spin-temperature alteration and phase cycling were used to remove experimental artifacts. A flip-back proton pulse at the end of decoupling allowed the use of recycle times of 3 s. The Andrew-Beams rotor was made of Delrin or Kel-F. The spin rate was ~3.5 or 2.5 kHz and spinning sidebands in Figure 1 were eliminated by using the methods of Dixon.[18] Chemical shifts are relative to external tetramethylsilane. Theoretical calculations of the ^{13}C–^{14}N dipolar splits were performed as described previously[6] on an IBM 4341 computer.

Results

The ^{13}C NMR spectrum of crystalline morphine sulfate in Figure 1 has resolved resonances for each of the 17 carbon atoms in the molecule. The structure and carbon numbering scheme for morphine are also shown in the figure. The resonances of this spectrum are assigned to specific carbons by comparison with previous solution ^{13}C NMR studies.[19] Four out of the 17 carbon resonances show evidence of fine structure in the form of splittings.

Figure 2 contains the ^{13}C resonances of the piperidine ring moiety. The four split ^{13}C resonances are from carbons in the piperidine ring. The resonances of C9, C16, and C17 are split into the asymmetric doublets characteristic of ^{13}C–^{14}N dipolar couplings in high-resolution solid-state spectra. The dipolar split is defined as the center of mass of the more intense component (corresponding to the $n = \pm 1$ state of ^{14}N) minus the center of mass of the less intense component (corresponding to the $n = 0$ state) of the asymmetric doublet.[6] The dipolar splits observed are −31, −21, and −31 Hz for C9, C16, and C17, respectively. C15 is not bonded to nitrogen, therefore the presence of two peaks at the C15 resonance position suggests the presence of two environments for this carbon site. No other resonances, including those from carbons bonded to nitrogen, show evidence of heterogeneity of chemical shift. Therefore, it is probable that the splitting of C15 reflects two conformations at this position rather than the presence of two molecules in the unit cell. The populations of the two states may be assumed to be approximately equal because of the similar intensities of the two peaks.

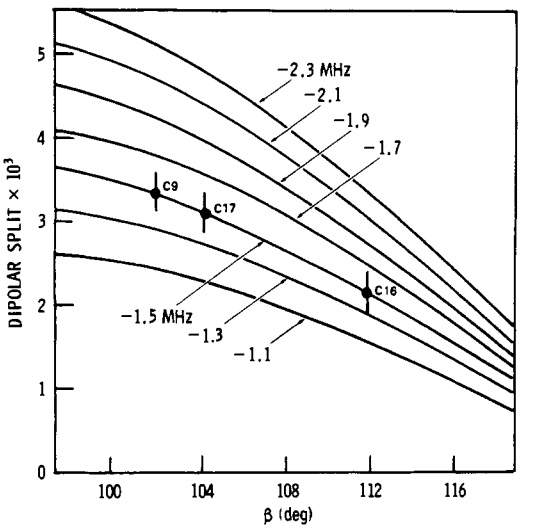

Figure 3. Analyses of the ^{13}C–^{14}N dipolar splittings in morphine sulfate. The lines are theoretical dipolar splittings in fractions of $(3/2)^{1/2}C^D\delta^D$ vs. the angle, β, between the internuclear vector and Z_{efg}, for several values of the ^{14}N quadrupolar coupling constant in the range −1.1 to −2.3 MHz. Circles are mean experimental dipolar splits for labeled carbons of morphine sulfate. Error bars correspond to one standard deviation. The value of the quadrupole asymmetry parameter was taken to be 0.036.

Figure 3 shows the results of theoretical calculations of the magnitude of the splitting of ^{13}C resonances by the ^{13}C–^{14}N dipolar couplings as a function of β, the angle between the carbon–nitrogen internuclear vector and the Z axis of the ^{14}N electric field gradient (Z_{efg}). The dipolar split is given in fractions of $(3/2)^{1/2}C^D\delta^D$ where C^D is a rotationally invariant constant of the dipole interaction equal to $-2\gamma_C\gamma_N\hbar$ and $\delta^D = R_{CN}^{-3}$ where R_{CN} is the internuclear distance. The circles are the mean dipolar splits for C9, C17, and C16 from left to right, respectively, with error bars corresponding to the standard deviation obtained from four separate experiments. Since crystallographic structure data for morphine sulfate have not been obtained, the bond lengths used in this figure are from the X-ray structure determination of morphine hydrochloride trihydrate.[15] The bonding about the nitrogen in morphine is tetrahedral with three approximately equivalent NC bonds, therefore the quadrupole asymmetry parameter, η, is nearly zero, and the bond angles are nearly 109.5°. The calculated curves in Figure 3 used $\eta = 0.036$ taken from the model compound *N*-methylpiperidine[20] and β between 98 and 118°. This immediately limits the values of e^2Qq/h which fit the NMR data to the range of approximately −1.0 to −2.0 MHz. As can be seen in Figure 3, in order to fit the data for all three carbons the angles between Z_{efg} and the respective internuclear vectors must obey the relationship $\beta_{C9} < \beta_{C17} < \beta_{C16}$. Refinement of the data fit is possible by choosing the value of e^2Qq/h which gives an ori-

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entation of Z_{efg} which is most consistent with the established bond angles and distances. When this is done we find that the values of e^2Qq/h smaller than −1.3 MHz fit the data for C9 only at values of B_{C9} which are considerably smaller than the tetrahedral angle. Similarly, values of e^2Qq/h larger than −1.7 MHz fit the data for B_{C16} at angles which are much larger than the tetrahedral angle. The best fit to the data is given by a value of e^2Qq/h of −1.5 MHz. the angles between Z_{efg} and the three internuclear vectors are then given by $\beta_{C9} = 102°$, $\beta_{C17} = 104°$, and $\beta_{C16} = 112°$. The limits are taken to be ±0.2 MHz for e^2Qq/h and ±2° for the angles orienting Z_{efg} in the molecular frame.

Discussion

The value of −1.5 MHz for the quadrupole coupling constant derived from the above analysis is slightly larger than that observed by Brown and co-workers[20] for the protonated form of the tertiary amine 1,2-dipiperdinoethane, and it is much less than the value of 3–5 MHz found for free tertiary amines,[21] therefore it seems to be a resonable value for morphine with the nitrogen protonated. The angles between Z_{efg} and the respective internuclear vectors are all consistent with tetrahedral geometry about nitrogen. Smaller values for β_{C9} and β_{C17} may reflect the fact that the three CNC angles in this compound are all slightly greater than 109.5°.[15] The sign of the quadrupole coupling constant is unambiguously seen to be negative as a positive quadrupole coupling constant for values of β near the tetrahedral angle would give a ^{13}C spectrum with the orientation of the asymmetric doublet reversed.

The most important source of error in the determination of e^2Qq/h from this experiment is the experimental resolution available. The assumption that the asymmetry parameter, η, is close to zero when calculating the theoretical lines in Figure 3 is expected to be quite good for the case where there is no marked deviation from tetrahedral geometry. Substituting a value for η of 0.1 in the calculations would increase the derived coupling constant by 0.1 MHz. The method used to obtain a range of quadrupole coupling constants which will fit the data obviously depends on the choice for reasonable bounds to the values for β. This in turn depends on the accuracy of the bond distances and angles used in making the analysis. The NMR data alone can at least limit the magnitude and sign of the quadrupole coupling constant to relatively narrow ranges.

The value of e^2Qq/h allows use of the modified Townes and Daily[22] analysis described by Brown and co-workers[20] to compute electron occupation numbers for the orbitals about nitrogen.[20,23] In this analysis the quadrupole coupling constant is given by $e^2Qq/h = e^2Qq_0/h\cdot(3/4)(\sigma - \delta)$, where σ is the occupancy number of the nitrogen lone pair orbital and δ is the occupancy number of the N–C orbitals. δ is assumed to increase by an inductive effect when the nitrogen lone pair orbital goes from the free to a protonated state and is given by $\delta = \delta_0 + 0.15(2 - \sigma)$ where δ_0 is the N–C orbital occupancy of the free base which is calculated to be 1.259, with the factor 0.15 an estimate of the proportionally constant of the inductive effect.[20] Using our value for both the sign and magnitude of the observed quadrupole constant, and a value of −9.0 MHz for e^2Qq_0/h,[21] we calculate a value of σ of 1.5 e. This finding unambiguously shows that σ is larger than δ, a result that would have been difficult to predict due to the similarity of the electronegativities of the hydrogen and alkyl groups.[21] For example, this contrasts with the result of a semiempirical quantum mechanical calculation where the bond polarity of the NH orbital in protonated morphine was approximately equal to those of the N–C bonds.[17]

The NMR experiment provides an unambiguous determination of the sign of the quadrupole coupling constant, as well as its magnitude and orientation in the molecular frame, the accuracy of which depends on the experimental resolution available and the accuracy of the bond distances and geometry used to calculate the curves for fitting the experimental data.

Glasel[24] in a high field solution ^{13}C and ^1H NMR study of morphine described the prsence of major and minor components for the C15, C16, and C17 carbons and protons in an acidified sample. He attributed these components to nitrogen invertomer equilibrium "frozen out" at low pH. The ^1H coupling constants were nearly identical for both components. Since the conformation difference at C15 in our spectrum is seen only at that site, with approximately equal populations of two conformations present, it is unlikely that this represents the same phenomenon. However, in combination with the solution study, the solid-state spectrum draws attention to the possibility of the presence of two conformations of morphine at the receptor.

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Registry No. Morphine sulfate, 64-31-3.

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Structure and Reactivity of Gas-Phase Ions: $C_4H_4^{+\cdot}$

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Abstract: The structure and reactivity of $C_4H_4^{+\cdot}$ ions in the gas phase have been investigated with use of collision-induced dissociation (CID) and tandem ion cyclotron resonance spectroscopy. Two stable isomers were identified, one linear $C_4H_4^{+\cdot}$ and one cyclic $C_4H_4^{+\cdot}$, in agreement with observations of Ausloos. It is shown that the m/z 26/27 ratio in the CID pattern of $C_4H_4^{+\cdot}$ is strongly dependent on the isomeric content of the $C_4H_4^{+\cdot}$ beam. This ratio was calibrated against isomeric composition, and the percent cyclic and linear $C_4H_4^{+\cdot}$ arising from a variety of sources was explored. The ion chemistry of $C_4H_4^{+\cdot}$ with C_2H_2 was examined in the tandem ICR. The linear form reacted with a rate constant of $\sim(3 \pm 1.5) \times 10^{-10}$ cm^3/s, while the cyclic form reacted extremely slowly if at all. It was shown that acetylene "catalyzes" isomerization of linear $C_4H_4^{+\cdot}$ to cyclic $C_4H_4^{+\cdot}$ with a rate constant of $\sim 1 \times 10^{-10}$ cm^3/s, presumably via a long-lived $C_6H_6^{+\cdot}$ intermediate.

It has been pointed out repeatedly in the past that structure elucidation of small and medium sized hydrocarbon ions is not an easy task.[1] Isomerization barriers are often small, and thus the decomposition of the ions may be slow compared to the isomerization rates. Experimental methods which sample excited ions, therefore, often show either one or a mixture of interconverting structures. For example, according to isotopic labeling experiments, metastable ion spectra, field ionization kinetics, and photoion–photoelectron coincidence (PIPECO) measurements, $C_4H_8^{+\cdot}$ and $C_4H_6^{+\cdot}$ ions with sufficient energy to decompose have

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