cleavage at the amide bond is a mechanism for iron release in synthetic catechoylamide sequestering agents.

Summary

We have described here a new ligand, Me_3MECAMS, which has a high affinity for ferric ion at physiological pH and can remove iron from transferrin at a significant rate. Moreover, Me_3MECAMS can mobilize iron from ferritin when ascorbate is present. Sulfonation of the ligand affords a high water solubility, as well as lowering the ligand protonation constants. The solution chemistry of Me_3MECAMS is analogous to enterobactin, in that it forms a tris catechol complex at high pH and most likely shifts to a salicylate mode of bonding upon protonation. A second compound, NAcMECAMS, forms a tris(catecholato)iron(III) complex at high pH but undergoes a 2 H^+ step which causes dissociation of an arm to form a bis catecholate coordination geometry. Unlike previously prepared catechoylamides, Me_3MECAMS and NAcMECAMS contain only tertiary amide nitrogens. Although N-substitution presumably does not affect microbial acquisition of iron from FeMe_3MECAMS, this modification may be important for future design of orally efficacious drugs which could encounter nonspecific peptidase activity.

Acknowledgment. We thank Mr. Thomas P. Tufano for experimental aid and discussion of kinetic data, Dr. Geoffrey B. Wong for assistance with the electrochemical apparatus, and Dr. Wesley R. Harris for thermodynamic data on $(i\text{-Pr})_3$MECAMS. This research is supported by the National Institutes of Health through Grant HL 24775 and by the Director, Office of Energy Research, Office of Basic Energy Sciences, Chemical Sciences Division, of the U.S. Department of Energy under Contract Number W-7405-ENG-48. A fellowship from the Guggenheim Foundation to K.N.R. is gratefully acknowledged.

(32) Avdeef, A.; Sofen, S. R.; Bregante, T. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1978**, *100*, 5362-70.

High-Resolution Solid-State ^{13}C NMR. Conformational Studies of NADH and NAD^+ Model Systems

Gary R. Hays,*,† Rob Huis,† Brian Coleman,*,† Derek Clague,† Jan W. Verhoeven,‡ and Frank Rob‡

Contribution from the Koninkliyke/Shell-Laboratorium, Amsterdam (Shell Research B.V.), Badhuisweg 3, 1031 CM Amsterdam, The Netherlands, and the Laboratory for Organic Chemistry, University of Amsterdam, 1018 WS Amsterdam, The Netherlands.
Received November 20, 1980

Abstract: High-resolution solid-state ^{13}C NMR shows that, in the solid form, the Hantzsch ester [3,5-bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine] adopts an asymmetric conformation. A full analysis of the solid-state spectrum was possible by comparison with the available X-ray data. The asymmetric conformation can be rationalized in terms of intramolecular electronic interactions, and similar arguments have allowed us to deduce conformational information from the solid-state spectra of three other symmetrically substituted 1,4-dihydropyridines and their parent pyridinium ions, for which no X-ray data are presently available. All three dihydropyridines display an asymmetric conformation while their pyridinium analogues are symmetrical. In the case of at least one of these [1-benzyl-3,5-bis(methylaminocarbonyl)pyridinium bromide] it is possible to show that both carbonyl groups point away from the ring nitrogen.

In the last couple of years, the combined techniques of high-power proton decoupling, cross-polarization (CP), and magic-angle sample spinning (MAS) have become more and more routinely used in order to obtain high-resolution ^{13}C NMR spectra of solids. Dipolar decoupling eliminates the dipolar interactions; MAS eliminates chemical shift anisotropy (CSA) if the speed of rotation is fast enough;[1] and a cross-polarization pulse sequence[2] enhances the sensitivity of the whole experiment.

Although the isotropic chemical shifts obtained from solids are generally close to those measured in solution, an interesting facet of such experiments on solids can be that dynamic averaging (which occurs readily in solution) is often absent. Hence the "freezing" of free rotation of bulky substituents leads to the formation of fixed conformations. This means that, whereas in solution motion occurs to give average chemical shifts for the various conformers, in the solid state these conformations are "locked" in position. This can create unique chemical environments for nominally equivalent carbon atoms. The chemical shift differences generated by these environments can be seen in the solid-state spectrum as additional line splittings.

Solid-state ^{13}C NMR has already shown this conformational isomerism in solid polymers in the glassy state,[1] examples being poly(phenylene oxide), polysulfone, and a variety of polycarbonates, as well as in crystalline polymers.[3] Less work has been published on small organic molecules. Crystalline 1,4-dimethoxybenzene exists in a single symmetric form rather than as a mixture of the two possible isomers. The preferred anti conformation is locked in position and produces two types of chemical environment for the protonated ring carbons. This difference has been detected by solid-state ^{13}C NMR: a doublet is seen for these carbons in the resultant spectrum.[4,5] 1,3,5-Trimethoxybenzene, on the other hand, produces a spectrum from which it is clear that all three protonated aromatic carbons are chemically nonequivalent in the solid state, giving rise to three lines of nearly equal intensity.[6] This result can only be consistent with the existence of a single asymmetric conformational isomer.

†Shell Research B.V.
‡University of Amsterdam.

(1) Schaefer, J.; Stejskal, E. O.; Buchdahl, R. *Macromolecules* **1977**, *10*, 384.
(2) Pines, A.; Gibby, M. G.; Waugh, J. S. *J. Chem. Phys.* **1973**, *59*, 569.
(3) Fyfe, C. A.; Lyerla, J. R.; Volksen, W.; Yannoni, C. S. *Macromolecules* **1979**, *12*, 757.
(4) (a) Maricq, M. M.; Waugh, J. S. *J. Chem. Phys.* **1979**, *70*, 3300. (b) Maricq, M. M.; Waugh, J. S. *Chem. Phys. Lett.* **1977**, *47*, 327. (c) Waugh, J. S.; Maricq, M. M.; Cantor, R. *J. Magn. Reson.* **1978**, *29*, 183.
(5) Lippmaa, E. T.; Alla, M. A.; Pehk, J. J.; Engelhardt, G. *J. Am. Chem. Soc.* **1978**, *100*, 1929.
(6) Steger, T. R.; Stejskal, E. O.; McKay, R. A.; Stults, B. R.; Schaefer, J. *Tetrahedron Lett.* **1979**, 295.

0002-7863/81/1503-5140$01.25/0

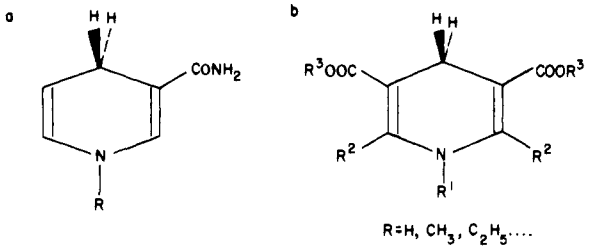


Figure 1. (a) General structure of a 1,4-dihydronicotinamide. (b) General structure of the Hantzsch esters.

X-ray analyses have confirmed the solid-state ^{13}C NMR findings for both compounds.

The ability of high-resolution solid-state ^{13}C NMR experiments to provide conformational information on crystalline organic compounds in a relatively short time has obvious importance in systems where the full X-ray analysis is not available or is difficult to obtain because of an inability to grow large single crystals. In this paper we wish to report the use of high-resolution solid-state ^{13}C NMR for the purpose of obtaining conformational information on a number of substituted 1,4-dihydropyridines and their corresponding pyridinium salts.

In recent years, 1,4-dihydropyridine derivatives have become of increasing interest as model compounds for the NADH coenzyme (dihydronicotinamide adenine dinucleotide). The recognition that a 1,4-dihydronicotinamide (Figure 1a) is the chemically active feature of NADH has led to many reports of reductions by 1,4-dihydropyridines in general.[7] Although derivatives of 1,4-dihydronicotinamide are the most closely related in structure to the coenzyme, the relative instability of such derivatives impedes their application as coenzyme "models".

A partial solution to this problem is found in the symmetrically substituted "Hantzsch esters" (Figure 1b) which can be synthesized with a wide variation in structure. 3,5-Bis(ethoxycarbonyl)-2,6-dimethyl-1,4-dihydropyridine (I) is one of the most stable of these dihydropyridines and is often used for such studies. The X-ray structure of this compound was recently determined[8] and it was found that, disregarding the protons, the complete skeleton is planar and the two double-bonded oxygen atoms of the carboxyl groups point in different directions, thus lowering the symmetry of the molecule. Our first objective, therefore, was to discover whether we could see this asymmetry with solid-state NMR and to compare our findings with the solution-state spectrum. We then extended our investigation to three other substituted 1,4-dihydropyridines and their corresponding pyridinium salts, the X-ray structures of which, to the best of our knowledge, have not been determined, in an attempt to deduce conformations in the solid state.

Experimental Section

Solid-State Spectra. The high-resolution solid-state ^{13}C spectra were obtained with the use of a Bruker CXP-300 NMR spectrometer (7.05 T magnetic field, ^{13}C frequency 75.45 MHz). Our spinning system is at present based upon the Beams-Andrew mushroom rotor.[9] These rotors are hollow and fashioned from Delrin,[10] which gives rise to a large central peak in all our spectra and spinning side bands if one cannot spin fast enough.

For the experiments described here we used a rotor containing about 300 mg of sample. Our driving gas is dry nitrogen and in this series of experiments rotation rates of between 4.2 and 6.5 kHz were used. As this spinning is slow compared to the inherent CSA of both the Delrin rotor and anisotropic carbon atoms in our samples (carbonyls and ring carbons), we sometimes obtained spectra with spinning side bands. Where these obscured sample peaks, the spectrum was reacquired with a different rotation rate.[11]

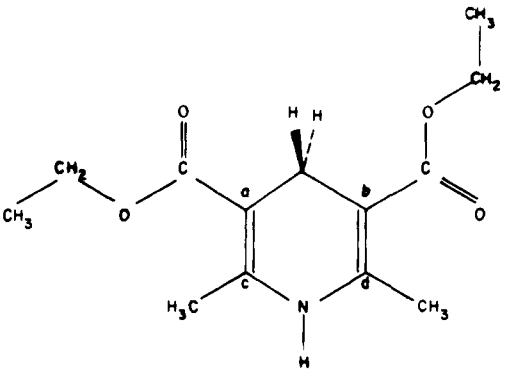
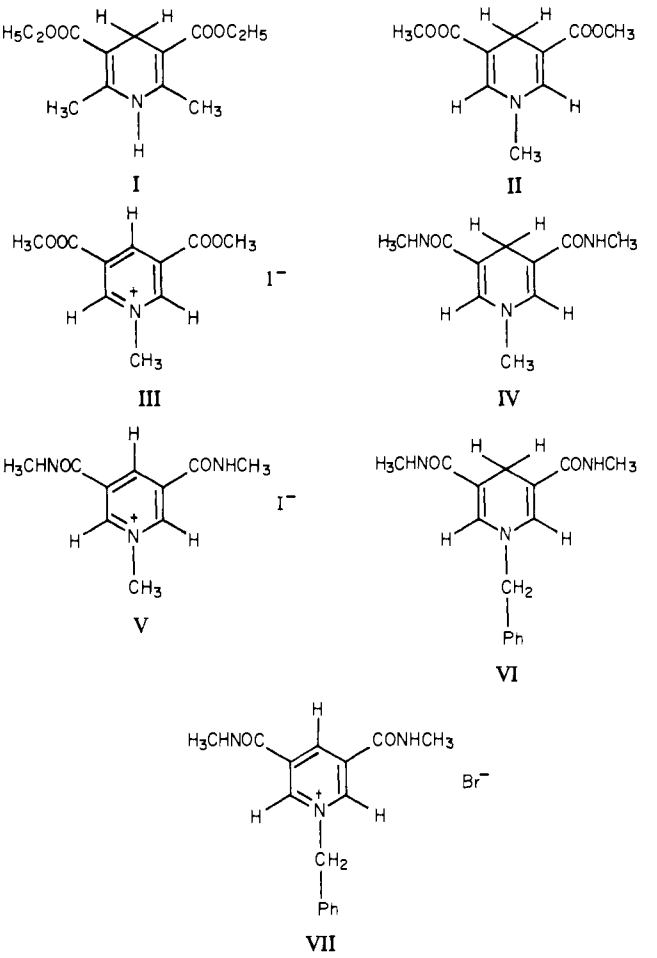


Figure 2. X-ray structure of I (ref 9).

Chart I



Single contacts with a CP contact time of 5 ms were used in all cases (except that of compound IV, where 1 ms was used because of the short proton $T_{1\rho}$). Recycle times between acquisitions were between 4 and 8 s; the acquisition time was 80 ms, using a sweep width of 20 kHz. B_1 field strengths were 10 and 40 G for the proton and carbon channels, respectively.

All chemical shifts are given with respect to Delrin at 89.5 ppm,[12] and the numbers of acquisitions were between 1000 and 29000.

Solution-State Spectra. I was measured by using deuterated dimethyl sulfoxide (Me_2SO-d_6) as solvent on a Varian CFT-20 NMR spectrometer (^{13}C frequency 20 MHz). All the other solution spectra were obtained by using a Bruker WM-250 NMR spectrometer (^{13}C frequency 62.89 MHz). The solvent for the 1,4-dihydropyridines was deuterated acetone, and for the pyridinium salts D_2O (Chart I). Hantzsch ester (I) was a generous gift from Professor U. K. Pandit. The pyridinium salts were

(7) See, for example: Van Bergen, T. J.; Hedstrand, D. M.; Kruizinga, W. H.; Kellogg, R. M. *J. Org. Chem.* **1979**, *44*, 4953 and the references therein.

(8) Lenstra, A. T. H.; Petit, G. H.; Dommisse, R. A.; Alderweireldt, F. C. *Bull. Soc. Chim. Belg.* **1979**, *88*, 133.

(9) Andrew, E. R.; Farnell, L. F.; Firth, M.; Gledhill, T. D.; Roberts, I. *J. Magn. Reson.* **1969**, *1*, 27.

(10) Registered trade mark, E. I. DuPont de Nemours Co., for polymethylene oxide: $-(-CH_2O-)_n-$.

(11) The spectra shown in Figures 5a, 6a, 7, and 8 are presented without spinning side bands so that important chemical shift differences are obvious.

(12) Zilm, K. W.; Alderman, D. W.; Grant, D. W. *J. Magn. Reson.* **1978**, *30*, 563.

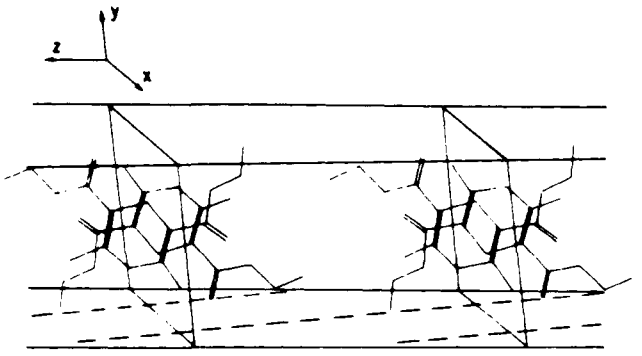


Figure 3. View of the contents of the unit cell of I (ref 9).

Table I. Solid-State and Solution Chemical Shifts (ppm) and Assignments for I

solid state	solution	assignment
16.0	16.0	CH_3 (ethyl)
21.3	19.5	CH_3 (ring)
26.0	26.3	CH_2 (ring)
61.7	60.5	CH_2 (ethyl)
99.5, 102.6	98.7	$=^{*}C<$
146.6, 150.1	148.1	$=^{*}C<$
169.6	168.7	C=O

prepared by quaternization of the appropriate pyridine derivatives with an excess of methyl iodide (III, V) or benzyl bromide (VII). From these the dihydropyridines (II, IV, VI) were obtained by reduction with sodium dithionite in a phosphate buffer (pH 7).

Results and Discussion

As mentioned above, the most interesting features of the molecular geometry of I are that, disregarding hydrogen atoms, the complete skeleton is planar and the two double-bonded oxygen atoms of the carbonyl groups point in different directions. The X-ray determination also showed that the distribution of bond angles in the two halves of the molecule is far from symmetric and that the sum of valency angles about the ring nitrogen atom indicates sp^2 hybridization. This structure is shown in Figure 2. The molecular packing was shown to be basically a layer structure: within one layer an infinite one-dimensional chain of molecules is formed by hydrogen bonding. The unit cell contains two molecules and the view of the contents of the unit cell (depicting the packing of the layers within the crystals) is reproduced in Figure 3. Knowing this crystal structure in such great detail we are able to interpret fully the NMR spectra obtained for I.

The solid-state and solution spectra of I are shown in Figure 4; the chemical shifts and assignments are listed in Table I. It should be noted that, although the chemical shifts obtained from both the solid and the solution spectra reported in this paper are in most cases fairly close to each other, there are differences. In the absence of specific solid-state effects, differences between the isotropic shift values measured in the neat liquid and in a microcrystalline powder have sometimes been shown to be rather small in the case of small organic molecules and do not exceed usual solvent effects.[5] Larger changes in chemical shifts can, however, be observed in molecules with a high chemical functionality such as amino acids, peptides, and proteins.[13]

By a straightforward comparison of the solid-state spectrum with the solution spectrum, one can easily see two doublets in the solid state—assigned to the ring olefinic carbons—compared to two singlets in the solution phase. As we have already noted, dynamic averaging readily occurs in solution and hence the olefinic carbon pairs a and b and c and d are equivalent. In the solid state, however, an asymmetric conformation creates unique environments

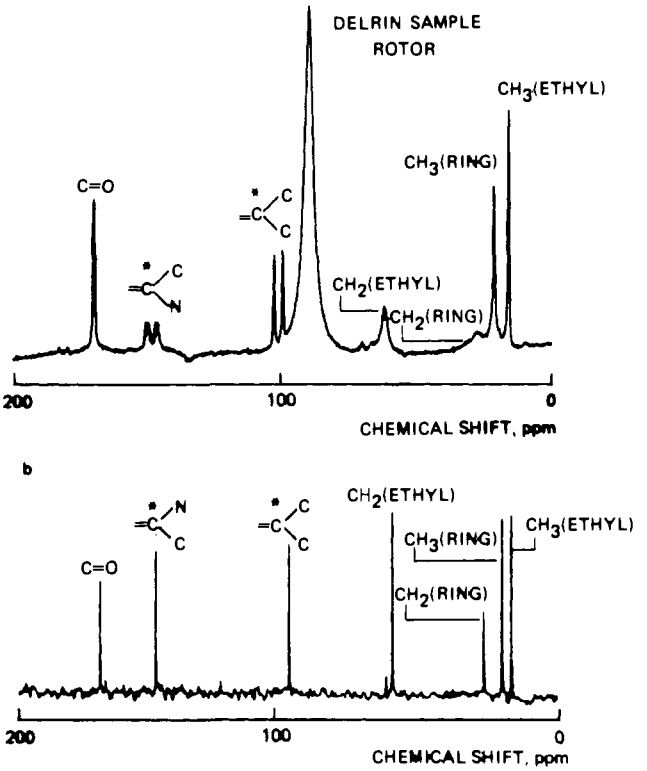


Figure 4. (a) Hantzsch ester (I): solid-state ^{13}C NMR spectrum (sample spun in hollow Delrin rotor). (b) Hantzsch ester (I): solution-state ^{13}C NMR spectrum (solvent: Me_2SO-d_6).

for these atoms, and hence one sees a splitting in the two resonances. This splitting immediately tells us that the olefinic carbons carbons a and b must be different and that c and d are also different, whereas this would not be expected if the molecule possessed a plane of symmetry orthogonal to the ring plane through N-1 and C-4.

We can therefore conclude that, from the solid-state NMR spectrum, one can obtain evidence that the asymmetric conformation is indeed present. Incidentally, conformational data published to date have always involved restricted rotation about a C–O bond,[1,4,5] and we believe this is the first time such a conformational effect about a C–C single bond has been shown clearly in a solid-state ^{13}C spectrum.

The assignments of the two types of olefinic ring carbons can be made on the basis of the line broadening usually observed in carbons bonded directly to nitrogen atoms,[14] which is due to the dipolar interactions from the quadrupolar ^{14}N nucleus in the solid state. This interaction is also known to produce a splitting of lines in some solid spectra,[13,15] but it is not the reason for the splitting of lines we observe. We see not only a splitting of the carbon resonance next to the nitrogen atom but also a similar splitting of the signal for the olefinic carbons β to nitrogen. It has also been shown that such splitting by nitrogen atoms gets smaller the higher the static magnetic field B_0. In addition, glycine and L-alanine, for which a splitting of the signal was observed at 2.1 T,[14] did not give rise to any signal splitting on our instrument (7.05 T), giving only a single broad signal for the carbon bonded to nitrogen.

Other assignments were made as follows. The CH_2 groups may be assigned from the liquid-state spectrum, which is essentially quantitative and hence gives signals in the intensity ratio 2:1 for CH_2(ethyl):CH_2 (ring) as expected from the molecular formula. The ring and ethyl CH_3 groups have been assigned with the aid of the solid-state spectrum of the diethyl ester of terephthalic acid, which yields a chemical shift for the ester CH_3 group of 15.3 ppm.

(13) Frey, M. H.; Opella, S. J. *J. Chem. Soc., Chem. Commun.* **1980**, 474.

(14) Lippmaa, E. Fourth European Experimental NMR conference, Autrans **1979**.

(15) Groombridge, C. J.; Harris, R. K.; Packer, K. J.; Say, B. J.; Tanner, S. F. *J. Chem. Soc., Chem. Commun.* **1980**, 174.

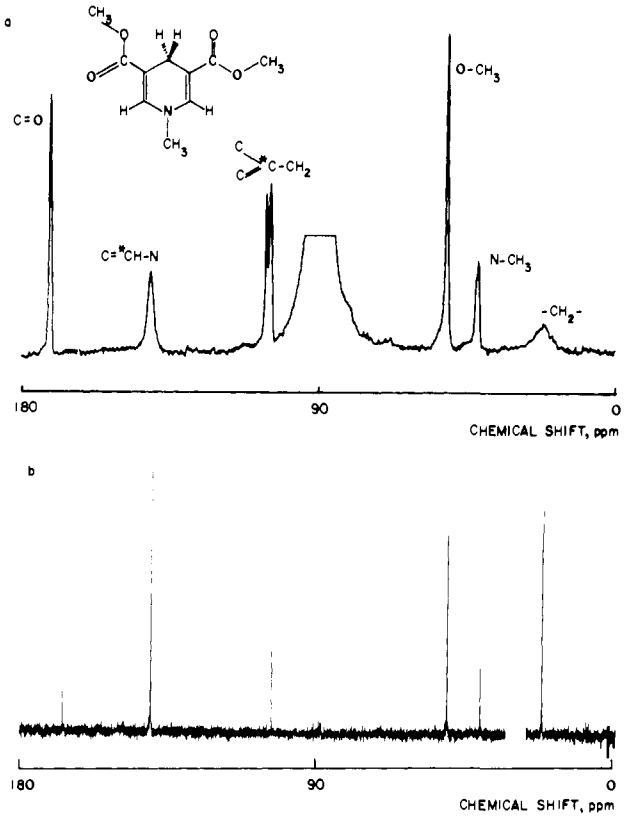


Figure 5. (a) Solid-state ^{13}C NMR spectrum of II. (b) Solution-state ^{13}C NMR spectrum of II (sovent: acetone-d_6).

Since it can be seen from both the X-ray analysis and solid-state ^{13}C NMR that the carbonyl groups are anti to one another, one might naively expect two sets of signals for the alkoxy groups. To understand why only one set is seen, it is necessary to look a little more closely at the ester groups. Rotation about the carbonyl carbon–oxygen single bond gives two limiting conformations:

syn

anti

Oxygen lone-pair/lone-pair interactions will be minimized in the syn conformer, however, and this, therefore, is the expected structure in the solid state.

As well as reducing this electronic interaction, the syn arrangement also has the important effect of placing the alkoxy group at the maximum possible distance from the ring. Thus the alkoxy group chemical shifts are dominated by the carbon–oxygen double bond. Since each alkoxy group is essentially the same with regard to the proximate carbonyl group, we expect the signals for the two alkoxy groups to be accidentally isochronous.

Finally, a surprising feature of the solid-state NMR spectrum of I is that the signal from the ring CH_2 group is extremely broad. As this broadening is not present in the solution spectrum it must be a result of either specific solid-state phenomena or the characteristics of the solid-state NMR experiment.[16]

The solid-state spectrum of II is shown in Figure 5a. The chemical shifts of both the solid and solution samples and our assignments are listed in Table II. In this compound, the solid-state spectrum shows a splitting in the nonprotonated ring olefinic carbons, whereas in the solution-state spectrum (Figure 5b) a singlet is present. This indicates that these carbon atoms are again nonequivalent in the solid state, which in turn must mean that the carbonyl groups are again asymmetric as drawn, in exactly the same way as in I.

Table II. Solid-State and Solution Chemical Shifts and Assignments for II

solid state	solution	assignment
24.1	21.3	ring CH_2
43.4	40.6	N-CH_3
52.6	50.6	O-CH_3
104.1 105.4	103.6	nonprotonated ring C
139.3	140.4	ring C-N
168.5	167.3	C=O

Table III. Solid-State and Solution Chemical Shifts and Assignments for III

solid state	solution	assignment
54.8	49.5	N-CH_3
58.5	54.5	O-CH_3
130.1	131.2	nonprotonated ring C
145.2	145.7	ring C-H
153.5	149.9	ring C-N
164.5	163.0	C=O

Many of the assignments can be made by comparison with I, in particular the ring CH_2, the nonprotonated ring carbons, the olefinic ring carbons next to the nitrogen, and the carbonyl carbon. The O-CH_3 and the N-CH_3 methyl groups can be assigned with the aid of the reported liquid chemical shifts for the methyl groups in dimethyl oxalate and trimethylamine, and also from the quantitative solution spectra, giving the intensity ratio O-CH_3:N-CH_3 as 2:1. One could even make a tentative assignment from solely the solid-state spectrum. As we have previously mentioned, the band shape for carbons directly bonded to nitrogen atoms is likely to be broad.

It is apparent in this case, however, that the other olefinic ring carbon signal is not split in the solid-state spectrum. When one considers the different gross molecular structures of I and II it is possible to see why this might be so. Whereas the α-olefinic ring carbon is nonprotonated in I, the methyl group has been replaced with a proton in II and it is well-known that although nonprotonated carbons tend to give sharp and narrow solid-state resonances, proton-bearing carbons give broader lines. Second, the carbons in question are directly bonded to the nitrogen atom and this also gives a broadening effect. If it is then realized that the splitting of the nonprotonated carbon resonance seen in II is smaller than that in I, it is possible that any conformational splitting that may have been present has been lost.

Only one methoxy-carbon signal is seen in the solid-state spectrum and, as before, this is indicactive of a syn conformation for the methyl group with respect to the carbonyl bond, the conformation expected if oxygen lone-pair/lone-pair interactions are minimized. Finally the ring CH_2 signal is again broad, suggestive of broadening by a nearby nitrogen nucleus. This points to molecular packing similar to that found in I.

It is interesting to compare II with the corresponding pyridinium salt III. The solid-state spectrum shown in Figure 6a shows only one sharp line for the corresponding nonprotonated ring carbons, indicating this time a symmetric conformation with respect to the carbonyl groups. This can be compared to the very similar solution-state spectrum shown in Figure 6b. The chemical shifts for both the solid- and the liquid-state spectra, together with the assignments, are listed in Table III. The methyl groups and carbonyl carbons may be easily assigned by comparison with the results for II, and the ring carbons by comparison with the reported liquid chemical shifts for pyridine. The nonprotonated ring carbon

(16) A referee has suggested that insufficient decoupling power could be the reason for the unexpectedly large line width of the methylene ring carbon. It has been shown that although B_1 fields of 10 G may be adequate for experiments at 15 MHz, off-resonance broadening effects could be an important source of line braodening at 75 MHz, thereby requiring fields greater than 10 G for adequate decoupling. (VanderHart, D. L.; Earl, W. L.; Garroway, A. N. *J. Magn. Reson.*, submitted).

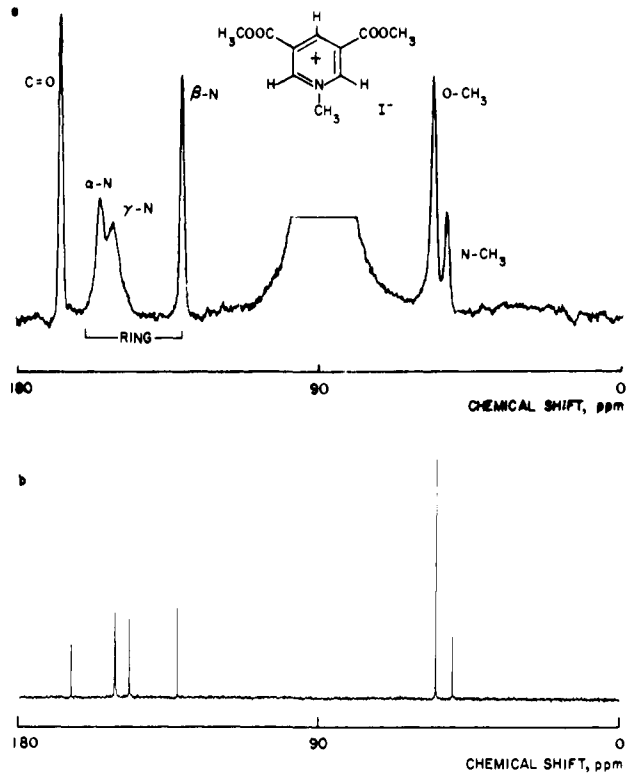


Figure 6. (a) Solid-state ^{13}C NMR spectrum of III. (b) Solution-state ^{13}C NMR spectrum of III (solvent: D_2O).

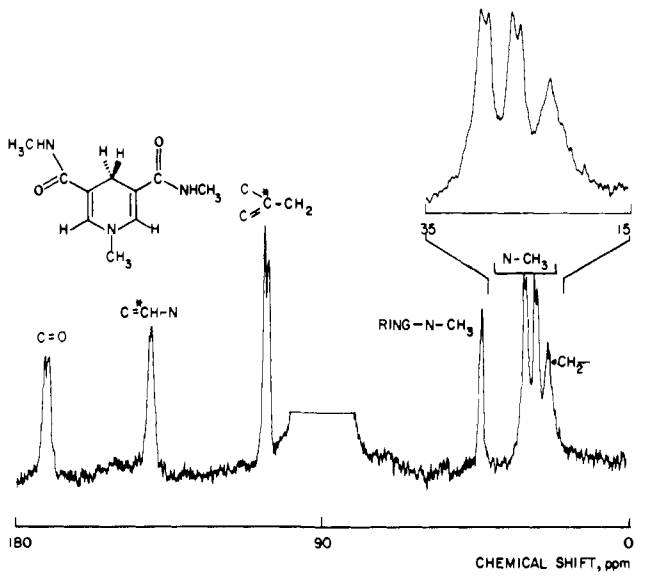


Figure 7. Solid-state ^{13}C NMR spectrum of IV.

can also be assigned directly from the solid-state spectrum as it would be expected to be the narrowest ring carbon resonance.

The solid-state spectrum of IV (where the methoxy group in II has been replaced by a methylamino group) is shown in Figure 7. It is immediately obvious that we are now dealing with a far more complicated spectrum than might have been expected by comparison with compounds I and II. Not only do we see the splitting of the nonprotonated ring carbons that we now expect if the carbonyl groups are asymmetric, but we also see a splitting of the carbonyl peak and what appears to be two doublets attributable to the methylamino carbon atoms. The ring carbons attached to nitrogen, the ring methyl carbon, and the ring CH_2 continue to give only single resonances. The solution-state spectrum displays a single resonance line for each of these carbons. The chemical shifts of both the solid and solution samples and their assignments are listed in Table IV. Many of the peak assignments can be made by comparison with the spectra for I and II, in particular the ring CH_2, ring N–CH_3, olefinic carbons, and carbonyl carbons. This leaves only the methyl groups in the amino function as the remaining unassigned carbon atoms.

Table IV. Solid-State and Solution Chemical Shifts and Assignments for IV

solid state	solution	assignment
23.0	24.2	ring CH_2
26.3, 27.2, 29.1, 30.2	26.7	NH–CH_3
42.2	42.4	ring N–CH_3
105.2, 106.4	107.7	non protonated ring C
140.3	138.2	ring C–N
170.4, 171.4	169.5	C=O

It would appear, therefore, that to give these line splittings more than one factor must now be influencing the solid-state spectrum. If we first consider the methylamino groups it is possible to see how one could obtain two main resonances. If it is assumed that the splitting in the nonprotonated ring carbon signals is again due to the carbonyl groups being asymmetric, then if these methyl groups were anti to the carbonyl two main signals would be expected. If the methyl groups were syn, as is the case with the alkoxy compounds I and II, then, as we have seen, the chemical shifts of the methylamino carbons would be dominated by the carbonyl bond. If they were anti, they would be mainly affected by their immediate environment and hence show separate resonances.

If we were to see two main methylamino signals in our solid-state spectrum, this would also confirm that the carbonyl groups were indeed asymmetric. It would not matter if the methylamino function was syn or anti to the carbonyl bond in a symmetric molecule as both cases would give rise to only one single methyl resonance. (Two resonances would, however, arise if one methyl was anti and one syn in a "symmetric" molecule, but this conformation would be extremely unlikely.)

Although we see to first order a pair of distinct resonances for these methylamino carbons, on closer inspection it is obvious that each of the pair is itself a doublet. It is also obvious that the carbonyl signal is split in a similar way. We therefore believe that we are seeing not only the effects of asymmetric carbonyl groups and restricted rotation about the amide bond, yielding an anti conformation for the methylamino group with respect to the carbonyl bond, but also the existence of noncongruent molecules in the unit cell.

An example of this phenomenon has been found recently[17] in the analysis of 2,4-dinitrotoluene. Here there is a splitting of the methyl signal, indicating two noncongruent molecules in the unit cell. The aromatic region of this spectrum, although not straightforward to interpret, does not appear to give any simple doubling of the peaks and so variations in local environment would appear largest for the methyl groups. This noncongruency in 2,4-dinitrotoluene has since been confirmed by X-ray analysis.

We suggest, therefore, that the small splittings of both methyl peaks and the splitting of the carbonyl resonance could also be due to this effect: noncongruent molecules in the unit cell, with the carbon atoms on the periphery of the molecule, in this case the methyl and carbonyl carbons, showing the effect more than ring at the center of the molecule.

In conclusion, therefore, we believe that the greater complexity of the solid-state spectrum of IV is due to the influence of three factors. First, the splitting of the nonprotonated ring carbons, indicates that the carbonyl groups are again asymmetric. This would not be as conclusive evidence as with I or II were it not, second, for the confirmation from the pattern of the methylamino

(17) Balimann, G. E.; Groombridge, C. J.; Harris, R. K.; Packer, K. J.; Say, B. J.; Tanner, S. F. "Nuclear Magnetic Resonance Spectroscopy in Solids", Proceedings, June 1980; The Royal Society: London, in press.

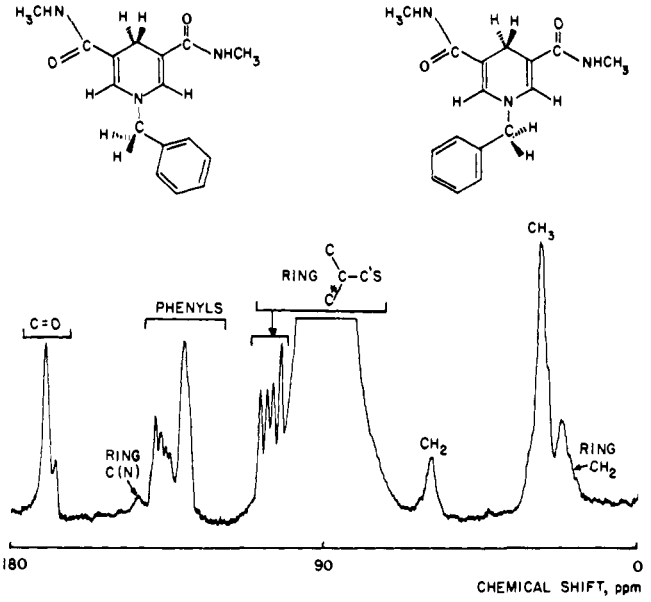


Figure 8. Solid-state ^{13}C NMR spectrum of VI.

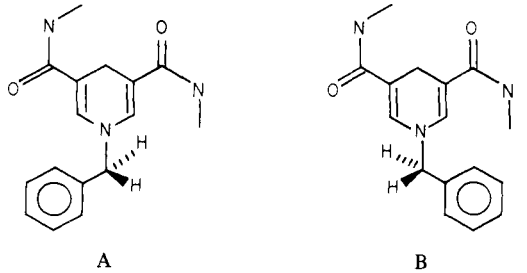
Table V. Solid-State and Solution Chemical Shifts and Assignments for V

solid state	solution	assignment
26.4	26.7	$NH-CH_3$
48.7	49.0	ring $N-CH_3$
134.1	134.4	nonprotonated ring C
141.0	141.3	ring C-H
146.3	146.6	ring C-N
163.2	163.5	C=O

resonances that the carbonyls are indeed asymmetric. The two main peaks can only arise if these methyl groups are anti to asymmetric carbonyl groups. Third, the further splitting of the two main methylamino doublets and the splitting of the carbonyl group resonances can arise from the existence of noncongruent molecules in the unit cell.

The interpretation of the spectra of the corresponding pyridinium salt V is, however, straightforward. The solid-state and liquid spectra both show single lines for the chemically nonequivalent carbon atoms. The chemical shifts and assignments for both phases are given in Table V. Again we can directly deduce that, in the salt, the conformation of the carbonyl bonds is symmetric.

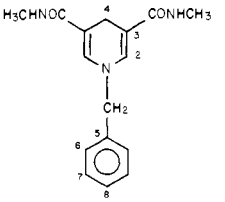
If the ring methyl group is replaced by a benzyl group one obtains VI, the solid-state spectrum of which is shown in Figure 8. It can be clearly seen for the olefinic carbons β to nitrogen that, in the solid state, the asymmetric arrangement of the carbonyls is again present, but that the prochiral group which has now been introduced leads to four signals. It is clear that two distinctly different conformers A and B must be present. We cannot, however, say whether the two rings are coplanar.



A B

The existence of both conformers is substantiated by the solid-state carbonyl resonances. Only one of the four different carbonyl oxygens is close to the benzene ring, and this proximity causes the chemical shift of the carbonyl group concerned to be different from that of the other three, and so two carbonyl signals are seen, with intensities of about 3:1.

Table VI. Solid-State and Solution Chemical Shifts and Assignments for VI



solid state	solution	assignment[a]
22.1	22.6	ring CH_2 4
asym line shape centered on 28.5	25.7	$NH-CH_3$
59.4	57.2	$\rangle\!\rangle$-CH_2-N
102.7, 104.9, 106.6, 108.5	106.5	3
129.4, 130.5, 134.5, 135.7, 137.0, 137.4, 138.8, 141.8	127.7	6
	128.0	8
	129.1	7
	135.6	2
	138.5	5
167.3, 170.1	167.3	C=O

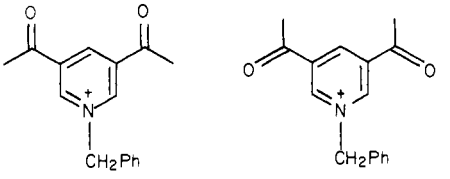
[a] For numbering sequence see structure.

We also expect more signals for the aminomethyl groups because of the presence of the benzyl group. In IV, two main amino-group resonances were observed due to the anti conformation with respect to the carbonyl bond, but in VI one methyl group might be expected to have a slightly different chemical shift because of the close proximity of the aromatic ring. Three signals with intensities 1:1:2 could therefore be predicted. Unfortunately the broad methyl signal is not resolved into separate peaks.

The two orientations of the benzyl group with respect to the dihydropyridine ring, which itself has asymmetric carbonyl groups, can also be expected to result in differences within the phenyl ring for nominally equivalent ring carbon atoms. This is again manifested by a splitting of some of the phenyl resonances and this is apparent in the solid-state spectrum, although it is not possible to assign the peaks with any degree of certainty. In any event there are too many signals for the two rings in the molecule to be orthogonal.

The solid-state and solution chemical shifts for VI are given in Table VI. As expected, the solution-state spectrum gives only one line for each chemically nonequivalent carbon atom in the molecule. Many of the assignments are obvious from comparison with compounds I, II, and IV. The phenyl ring assignments have been made with the aid of the quantitative solution spectrum and by comparison with the chemical shifts for similar compounds.

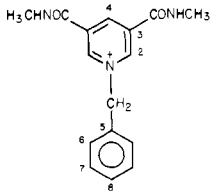
The solid-state spectrum of VII, on the other hand, is qualitatively the same as the solution spectrum. The chemical shifts and assignments are shown in Table VII. The carbonyl groups are thus symmetric and the conformation must be C or D.



C D

Although with III and V it has not been possible to determine

Table VII. Solid-State and Solution Chemical Shifts and Assignments for VII



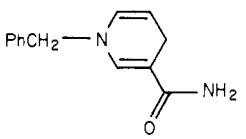
solid state	solution	assignment[a]
26.9, 30.3	26.4	N-CH_3
66.8	65.4	$\rangle\!\rangle$-CH_2-
129.5	129.1	6
	129.5	7
133.3	130.1	8
147.0	131.5	5
149.2	134.6	3
	141.8	4
	145.3	2
161.7	163.0	C=O

[a] For numbering sequence see structure.

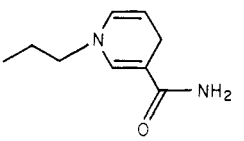
which of the two symmetrical conformations might be present, in this case it is possible to distinguish between them from the solid-state spectrum. If D were the preferred structure we would expect two carbonyl signals since one carbonyl bond is now in close proximity to the phenyl ring. Since only one signal is observed, C must be the conformation adopted in the crystal. This conclusion is supported by the signals of the *N*-methyl groups. Two signals are expected if C is the structure because only in C can the phenyl ring have a major effect on the chemical shift. Two signals are indeed seen in the solid-state spectrum.

Thus far it has been shown, on the basis of ^{13}C NMR data, that, in the solid state, the 1,4-dihydropyridines adopt the asymmetric carbonyl configuration, whereas the pyridinium analogues adopt a symmetric conformation. Since there are no X-ray data for relevant 3,5-disubstituted pyridinium ions and only limited X-ray data for disubstituted 1,4-dihydropyridines it is necessary to consider known structures of monosubstituted compounds.

X-ray data have been published for two 1,4-dihydronicotinamides, E[18] and F.[19] In both cases the carbonyl oxygen points



E



F

toward the ring nitrogen but, unlike the Hantzsch ester I, the carbonyl groups do not lie in the plane of the ring. This nonplanarity is smaller for E (less than 4°), and it has been postulated[18] that this is a result of the anti amide proton being able to fit between the ring methylene protons, making possible a nearly planar molecule with almost maximum conjugation. If this is correct, there must be other factors operating in the case of the *n*-propyl compound F, in which the carbonyl group lies 22° out of the plane of the ring.[19] The fact, however, that the carbonyl oxygen points toward the ring nitrogen can be rationalized in terms of the intramolecular electrostatic attraction.

In the case of the disubstituted 1,4-dihydropyridines in this study, the situation is naturally more complicated. Regardless of substituents, the lone pair on N-1 will again be delocalized into the π system. This will lead to an increase in electron density on the oxygen atoms of the carbonyl substituents in the 3 and 5 positions. The conformation with both carbonyl groups pointing toward the ring nitrogen would be expected to minimize the dipole moment of the molecule but would lead to large repulsive forces (both steric and electronic) between the two alkoxy or amido groups and the ring methylene group. If, however, both the carbonyls remained symmetrical but pointed away from the ring, there would again be large electron–electron repulsion between the carbonyl oxygens. It would appear, therefore, that an asymmetric conformation is a compromise between competing effects, avoiding most of the aforementioned problems.

There are more X-ray data available for monosubstituted nicotinamidium ions.[20] In most cases the carbonyl oxygen points toward the ring nitrogen, with a deviation from planarity with the ring, which can be as large as 38°. This conformation again maximizes intramolecular electrostatic attraction between the positive ring nitrogen and the carbonyl oxygen. In solution, such electrostatic attraction will be minimized by solvation and it has recently been shown[21] for NAD^+ that the two planar conformations are roughly equally populated and interconvert rapidly on the NMR time scale.

Electrostatic forces would also be expected to be important for the 3,5-disubstituted pyridinium ions studied in the solid state. *Intra*molecular attraction would be maximized if both carbonyl oxygens pointed toward the ring nitrogen while *inter*molecular attraction is perhaps greater if the oxygens point away. Since we have shown that the latter is indeed the preferred conformation in at least one sample (VII) it appear possible that intermolecular forces might dominate the solid-state structure of the pyridinium ions. It is quite likely that the intramolecular forces in the case of the pyridinium ions are weaker than in the case of their dihydropyridine counterparts: the electron density on the carbonyl oxygens will be less because the ring π system is electron deficient.

Conclusions

High-resolution solid-state ^{13}C NMR has proved a valid means of obtaining basic conformational information on the Hantzsch ester for which a full X-ray analysis exists. We have shown that details of the gross structure can be derived from the NMR data.

This has enabled us to deduce the conformations of three 1,4-dihydropyridines for which no X-ray analysis exists.

We have also obtained information regarding the conformations of the corresponding pyridinium ions. For one of these (VII) we have been able to show that the molecule adopts the symmetric conformation with both carbonyl oxygens pointing away from the ring nitrogen.

Work on the X-ray structure analysis of VI and VII is currently in progress at the University of Amsterdam which, it is hoped, will confirm our conclusions.

Acknowledgment. We should like to express our gratitude and appreciation to Dr. D. G. Gillies (Royal Holloway College, University of London) for his help during a 6-month sabbatical period at our laboratory. L. P. Blaauw is thanked for obtaining some of the solution state spectra shown in this publication. The able assistance of J. J. Scheele (University of Amsterdam) in the preparation of the compounds is gratefully acknowledged. One of the authors (F.R.) thanks the Netherlands Foundation for Chemical Research (SON) for financial support.

(18) Karle, I. L. *Acta Crystallogr.* **1961**, *14*, 497.
(19) Koyama, H. *Z. Kristallogr.* **1963**, *118*, 51.
(20) (a) Saenger, W.; Reddy, B. S.; Mühlegger, K.; Weimann, G. *Nature (London)* **1977**, *267*, 225. (b) Herriott, J. R.; Camerman, A.; Deranleau, D. A. *J. Am. Chem. Soc.* **1974**, *96*, 1585. (c)Ash, R. P.; Herriott, J. R.; Deranleau, D. A. *J. Am. Chem. Soc.* **1977**, *99*, 4471. (d) Johnson, P. L.; Maier, C. A.; Paul, I. C. *J. Am. Chem. Soc.* **1973**, *95*, 5370. (e) Johnson, P. L.; Frank, J. K.; Paul, I. C. *J. Am. Chem. Soc.* **1973**, *95*, 5377. (f) Frank, J. K.; Thayer, N. N.; Paul, I. C. *J. Am. Chem. Soc.* **1973**, *95*, 5386. (g) Voet, D. *J. Am. Chem. Soc.* **1973**, *95*, 3763. (h) Sakaki, T.; Inove, M.; Senda, S.; Tomita, K. *Biochem. Biophys. Res. Commun.* **1978**, *83*, 21.
(21) Redfield, A. G.; Waelder, S. *J. Am. Chem. Soc.* **1979**, *101*, 6151.