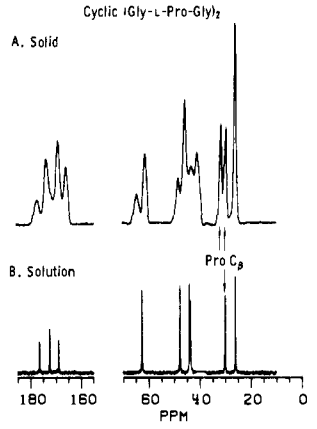


**Figure 3.** $^{13}C$ NMR spectra of *cyclo*-(Gly-L-Pro-Gly) at 38 MHz with chemical shifts relative to external $Me_4Si$. (A) Polycrystalline peptide sample. Same spectrometer and conditions as for Figure 2A using a Kel-F rotor spinning at 2.5 kHz. (B) Peptide in $Me_2SO/H_2O$ solution. Same spectrometer and conditions as for Figure 2B using a recycle time of 5 s. The zeroed points near 40 ppm mark the position of the $Me_2SO$-$d_6$ solvent resonances.

resonances in the 10–40-ppm region, essentially superimposable with those from the solution spectrum. The differentiation of the two proline $C_\beta$'s is clear in the solid-state spectrum, with one resonance at the normal isotropic chemical shift position of 29 ppm and the other shifted upfield about 5 ppm where it overlaps with the two Pro $C_\gamma$'s in the three-carbon resonance at 25 ppm. All carbonyl, $C_\alpha$, and Pro $C_\gamma$ resonances are split into broadened asymmetric doublets because $^{13}C$–$^{14}N$ dipolar couplings of the peptide bonds are not removed by magic-angle sample spinning.[10] The difference in appearance for the 45–65- and 165–175-ppm regions between the solid-state and solution spectra is a consequence of the spin interactions and not of alterations of peptide structure. The chemical shift correlation for the solution and solid-state spectra indicates that the predominant factors leading to the isotropic chemical shifts of the crystalline cyclic pentapeptide are conformational and packing effects play a secondary role; this is in contrast to the situation for amino acids or linear peptides.[3] Inspection of the arrangement of the peptide molecules in the crystal lattice[5] suggests that packing constraints do not contribute strongly to the molecular environment in the solid. There are no solvent molecules in the crystal, and the only intermolecular hydrogen bonding occurs between the Pro-Gly units of two molecules. The highly magnetically anisotropic phenyl rings lie over the molecules to which they belong but do not come into close range of other molecules. The direct correspondence between chemical shifts of the cyclic pentapeptide in the solid and solution seems to derive from the well-defined and highly conserved conformation and the absence of strong packing effects.

The cyclic hexapeptide *cyclo*-(Gly-L-Pro-Gly)$_2$ has an asymmetric structure in the solid state with one intramolecular hydrogen bond.[11] Both Pro-Gly units are in $\beta$ turns, although one has a proline $\psi$ angle of 126° (type II) and the other, without a hydrogen bond, has $\psi$ = 36° (type I). Their $\phi$ angles are similar at −53 and −66°, respectively. The $^1H$ and $^{13}C$ NMR spectra of the cyclic hexapeptide in solution[12] are very simple with the minimum number of lines and are consistent with the $C_2$ symmetric conformation, having the two intramolecular hydrogen bonds drawn in Figure 1B or with rapid averaging of asymmetric conformations.

The $^{13}C$ NMR spectrum of *cyclo*-(Gly-L-Pro-Gly)$_2$ in the solid state in Figure 3A has well-resolved Pro $C_\beta$ resonances, indicative of molecular asymmetry. In contrast, the solution $^{13}C$ NMR spectrum in Figure 3B has a single resonance for the two proline residues. Solution NMR studies on a series of *cyclo*-(X-L-Pro-Y)$_2$ hexapeptides suggest that different Pro $C_\beta$ resonance positions may be associated with the $\psi$ = −30° (type I) and $\psi$ = 120° (type II) $\beta$ turns.[13] However, conclusive interpretation of the observed resonance positions in terms of conformation and change in conformation between the two phases must await the results from additional model compounds, because the relationship between Pro $C_\beta$ chemical shifts and $\beta$-turn conformational states is not rigorously established[14] and because of the competition between inter- and intramolecular factors on chemical shifts in the solid state. Nonetheless, on the basis of the solid-state spectrum of Figure 3B, it can be anticipated that prolines in type I and type II $\beta$ turns will have different NMR parameters, notably $C_\beta$ chemical shifts.

NMR spectroscopy can bridge the gap between solid-state and solution studies of peptide conformation. When intramolecular effects determine chemical shifts, then the multiplicities and positions of resonances can be correlated with structural features present in solution and in the solid state. In particular, in the two cyclic peptides studied, the carbon chemical shifts of proline resonances were found to reflect the participation of this residue in local hydrogen-bonded conformations with consequent differences in ring geometries and backbone dihedral angles.

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## 1-Azetine: Thermal Ring Opening to 2-Azabutadiene

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The thermal decomposition of cyclopropyl azides leads usually to ring-expanded 1-azetine derivatives with minor amounts of nitriles and olefins. However, no azetine was observed when the carbon substituent $\alpha$ to the azido group is a hydrogen atom.[1] Similarly, 2-phenyl-1-azetine was obtained from the base-promoted 1,2-elimination of HCl from 2-phenyl-*N*-chloroazetidine,[2] whereas the unknown parent compound was too unstable to be isolated under the same conditions. We now wish to report the preparation, spectroscopic data, and some chemical properties of 1-azetine (**2**).

We have recently shown[3] that highly strained olefins could be easily obtained by vacuum gas-phase elimination of the halogenated precursor over silica supported *KO-t-Bu*. By use of the same apparatus,[4] dehydrohalogenation of *N*-chloroazetidine (**1**)[2]

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Scheme I

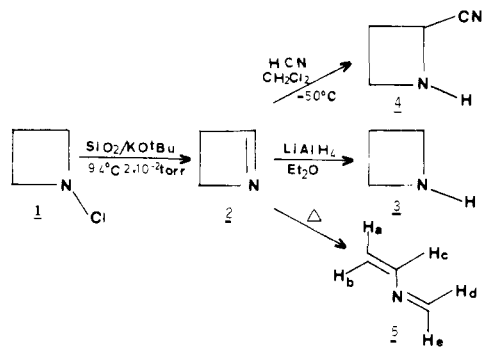


Table I. $^{13}C$ Chemical Shifts and Coupling Constants of 2, 6, and 7

| compound | chemical shift, $\delta_C$[a] | | | $J_{^{13}C-H}$, Hz | | |
|---|---|---|---|---|---|---|
| | C-2 | C-3 | C-4 | $^{13}C$-2-H | $^{13}C$-3-H | $^{13}C$-4-H |
| 1-azetine (2)[b] | 187.0 | 36.1 | 58.8 | 182.0 | 142.0 | 150.5 |
| cyclobutene (6) | 162.3[c] | 56.5[c] | 56.5[c] | 170[d] | 140[d] | 140[d] |
| 3-phenyl-1-azirine (7)[e] | 160.6 | 28.6 | | 242.5 | | |

[a] Relative to internal $Me_4Si$. [b] Sample concentration in $CDCl_3$, 15% at -55 °C, recorded on a WP 60 Bruker NMR spectrometer. [c] Reference 10a. [d] Reference 10b. [e] Reference 10c.

led to 1-azetine (2), partially at 15 °C (5%), completely at 94 °C (yield >98%, purity >95%).[5] The stability of the pure compound is somewhat greater than initially expected; it can be trapped at liquid nitrogen temperature and revaporized. It is a colorless liquid at -70 °C and polymerizes in a few seconds even in sealed degassed tubes at 20 °C. The half-life of 2 in solution in sealed degassed tubes ($CFCl_3$, 36 °C) is about 90 min or 3 days in the presence of hydroquinone. However, polymerization occurs rapidly with traces of oxygen or acid. Conclusive structural proof for 2 was afforded by (1) its reduction with $LiAlH_4$ in ether at 0 °C to azetidine (3) and (2) the addition of HCN at -50 °C in $CH_2Cl_2$ solution to give the nitrile derivative 4 (Scheme I).[6] Further evidence is provided by the $^1H$ NMR spectrum ($CD_2Cl_2$, -60 °C) which exhibits two sharp triplets at $\delta$ 3.09 ($J$ = 2.8 Hz) and 4.00 and a singlet at $\delta$ 8.22.[7] The $^{13}C$ NMR parameters are compared with those observed for cyclobutene (6) and 3-phenyl-1-azirine (7)[8] (Table I). The $^{13}C$-2-H and $^{13}C$-4-H coupling constant values are larger than those observed in cyclobutene (6). This phenomenon could be due to the presence of the adjacent electronegative atom which is known to increase the $^{13}C$-H coupling constant.[9]

The strong IR band of 2 at 1570 $cm^{-1}$ ($CCl_3F$, 25 °C or -196 °C with an optical cryostat) is consistent with the presence of a C=N group in a four-membered ring;[10c] the UV spectrum (*n*-pentane, -60 °C) exhibits a single band at 237 nm ($\epsilon$ 80[11]). In spite of numerous attempts, no mass spectrum of 2 could be obtained, presumably due to rapid polymerization. Flash vacuum pyrolysis (FVP) of 2 (450 °C, $10^{-4}$ torr) in a furnace equipped with an optical cryostat[12] formed the unknown 2-azabutadiene (5), as witnessed by its IR spectrum ($\nu_{C=N}$ 1610, $\nu_{C=C}$ 1628 $cm^{-1}$; the product was trapped on a liquid nitrogen cooled KBr target window). Polymerization of the solid film was observed on warming. Larger samples of 5 were obtained by fitting the FVP apparatus on the vacuum line after the dry ice trap.[4] The $^1H$ NMR spectrum of 5 [$(CD_3)_2CO$, -60 °C][13] confirms its structure [$\delta_{H_a}$ 5.24 (dd, $J_{H_aH_c}$ = 7.1, $J_{H_aH_b}$ = 0.8 Hz); $\delta_{H_b}$ 5.56 (dd, $J_{H_bH_c}$ = 14.8 Hz), $\delta_{H_c}$ 6.97 (dd); $\delta_{H_d}$ 7.31 (d, $J_{H_dH_e}$ = 16.7 Hz; $\delta_{H_e}$ 7.59 (d)]. Its mass spectrum exhibits the molecular ion at $m/e$ 55. Polymerization of a dilute solution of 5 takes only a few minutes at 25 °C, but no dimer or trimer products were detected. The thermal transformation of 1-azetine to 2-azabutadiene was predicted by molecular orbital theory to proceed along a pathway similar to that for hydrocarbon analogues.[14] Preceding experimental data[1b,15] support these observations. For the parent compound 2 no retro [2 + 2] process with formation of HCN could be observed even by FVP at higher temperatures (500-700 °C).[16]

The use of $SiO_2$/*KO-t-Bu* as a solid base on a vacuum line proves to be a powerful technique for the direct synthesis of high-purity samples of very reactive intermediates. Further work is in progress to elaborate the chemical reactivities of 1-azetine and 2-azabutadiene and synthesize other reactive intermediates by similar methods.

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(4) *tert*-Butyl alcohol formed in the reaction was efficiently eliminated by a dry-ice trap fitted on to the vacuum line after the elimination apparatus.

(5) Several grams could be obtained with the same solid base if the addition of chloro amine 1 was regular and slow (1 g/h).

(6) $^1H$ NMR ($CDCl_3$) $\delta$ 4.2 (m, 1 H), 3.6 (m, 2 H), 2.6 (m, 2 H); IR ($CD_2Cl_2$) $\nu_{N-H}$ 3680, 3595, $\nu_{C\equiv N}$ 2208 $cm^{-1}$. Due to the instability of this product, combustion analysis could not be carried out; by high-resolution MS the molecular weight corresponds to $C_4H_6N_2$. (Anal. Calcd: $m/e$ 82.0531. Found: 82.0530.)

(7) The $^1H$ NMR spectrum of 2 was deceptively simple. The coupling constant between the vinylic proton and the proton of the adjacent methylene group was too small (<0.2 Hz) to be detected by using a Nicolet NTC 200 NMR spectrometer.

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## Use of Singly Modified Cytochrome *c* Derivatives To Determine the Site for Electron Transfer in Reactions with Inorganic Complexes

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Cytochrome *c* (MW = 12400) is a component of the mitochondrial respiratory chain and transfers electrons from cytochrome $c_1$ to cytochrome *c* oxidase. The heme prosthetic group of horse cyctochrome *c* is almost completely enfolded by a polypeptide chain of 104 amino acids, leaving the edge containg pyrrole rings II and III partly exposed at the "front" surface of the protein. It has been suggested that electron transfer in and out of the protein is via this exposed heme edge.[1] However, the evidence for such a mechanism is only indirect.[2] At pH 7 many amino

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