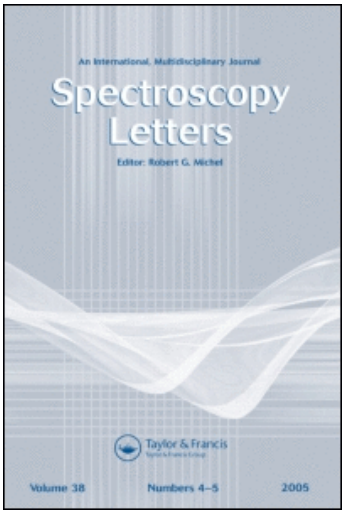

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Low-Temperature Two-Dimensional Heteronuclear Shift Correlation Spectroscopy of A 1, 4-Benzodiazepine

Laurine A. LaPlanche[a]; Robert Rothchild[b]
[a] Department of Chemistry, Northern Illinois University, DeKalb, IL [b] Department of Science Toxicology Research and Training Center, The City University of New York John Jay College of Criminal Justice, New York, NY

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LOW-TEMPERATURE TWO-DIMENSIONAL HETERONUCLEAR SHIFT CORRELATION SPECTROSCOPY OF A 1,4-BENZODIAZEPINE.

KEY WORDS: Ketazolam, ^{13}C NMR Chemical Shifts, Variable Temperature Studies, 2D Long-range ^{13}C-^{1}H shift correlation, Hindered rotation, 1,4-Benzodiazepines

Laurine A. LaPlanche*

Department of Chemistry

Northern Illinois University

DeKalb, IL 60115

Robert Rothchild

The City University of New York

John Jay College of Criminal Justice

Department of Science

Toxicology Research and Training Center

445 West 59th Street

New York, NY 10019-1199

*Author to whom correspondence should be addressed.

ABSTRACT

The unsubstituted phenyl ring in ketazolam, a 1,4-benzodiazepine derivative, has a two-fold rotational barrier about the aryl to tert-alkyl bond connecting this phenyl group to the rest of the molecule. At 50.3 MHz, all twenty of the ^{13}C resonances of ketazolam are resolved below -40°C, including two distinct resonances for the *ortho* carbons and two for the *meta* carbons. Twelve of the twenty ^{13}C nuclei resonate between 138 and 125 ppm, necessitating assignment via a combination of one-bond and long-range heteronuclear shift correlation experiments.

INTRODUCTION

Ketazolam is a 1,4-benzodiazepine derivative, a class of psychoactive drugs which, since the early 1960's has become the most frequently prescribed for their anxiolytic, sedative/hypnotic, anticonvulsant and muscle relaxant properties.

Ketazolam, 1, 11-chloro-8,12b-dihydro-2,8-dimethyl-12b-phenyl-4H-[1,3]oxazino[3,2-d][1,4]benzodiazepine-4,7(6H)-dione, may be prepared (1) by the addition of diketene to diazepam (Valium, Hoffmann-La Roche). ^{13}C NMR chemical shift assignment in these small molecules is more difficult than it at first appears. As recently as 1981, Patra et al. (2) reversed some of the ^{13}C assignments for diazepam and other benzodiazepine drugs made earlier (3). Several workers have published ^{1}H (1,4-5), ^{13}C (2-7) and ^{15}N (8) NMR studies of the 1,4-benzodiazepines. The ^{1}H chemical shift assignments for 1 in solution and the X-

ray crystal structure determination of 1 have been described (1). The ^{13}C spectrum and ^{13}C NMR assignments are not available in the literature. The presence of an unsubstituted phenyl group, bound at an sp^3 chiral carbon atom (C_{12b}), makes ketazolam a most unusual 1,4-benzodiazepine. Even at room temperature, the sharpening of the AB quartet of the methylene protons (H_{6a},H_{6b}) and the increased chemical shift separation of H_{6a} and H_{6b} over that of diazepam indicate that ketazolam has a more rigid structure. The X-ray diffraction results show that the seven-membered ring is in a pseudo-boat conformation and that each of the two amide groups is almost planar (1). This produces an almost flat, rigid structure in which the phenyl group is roughly perpendicular to the rest of the molecule.

Variable temperature ^1H NMR studies at 200.1 MHz of ketazolam (0.031 M in $CDCl_3$) revealed that, indeed, the phenyl group of 1 experiences hindered rotation about the $C_{1'}$-C_{12b} bond (9) even at 20°C, *Fig 1(a)*. Raising the temperature caused the broad *ortho* ^1H resonance centered at approximately 7.15 ppm to sharpen, while cooling the solution resulted in further broadening. Below 1°C, (the approximate *ortho* ^1H coalescence temperature), two *ortho* resonances were apparent; at -59°C, these resonances have a chemical shift separation of 0.27 ppm and doublet structure can clearly be seen for $H_{2'}$, *Fig. 1(b)*. At this temperature, the chemical shifts of the *ortho* protons are $H_{2'}$, 6.98 ppm ($H_{2'}$ is in the shielding region of the chlorophenylene ring) and $H_{6'}$, 7.25 ppm. The *para* and *meta* pro-

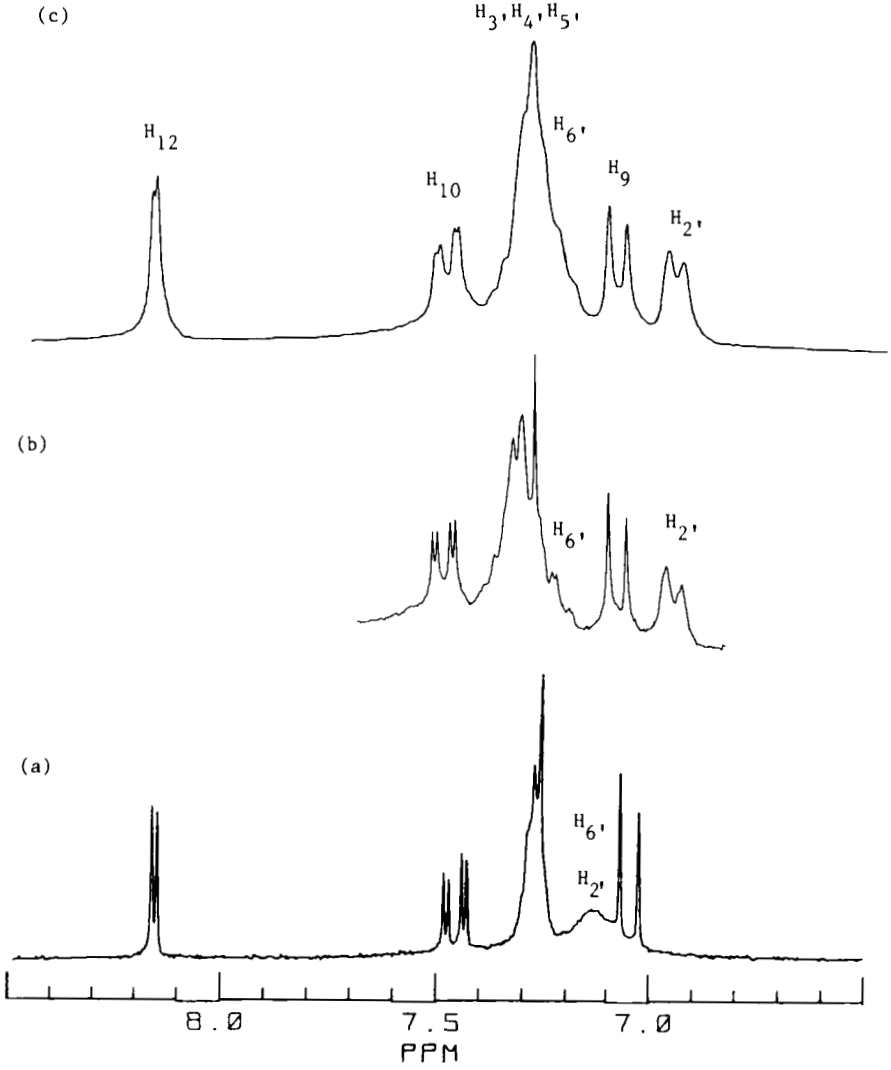


Fig. 1. 200.1 MHz ^1H NMR spectra of ketazolam/$CDCl_3$ solutions: (a) 20°C, 0.031 M; (b) -59°C, 0.031 M; (c) -58°C, 0.600 M. Only aromatic spectral region shown.

tons remain merged within a broad spectral envelope at 7.35 ppm (9). Fig. 1(c) shows the low temperature ^{1}H spectrum of a more concentrated ketazolam solution typical of concentrations used during the 2D experiments.

In this work, the ^{13}C spectra of ketazolam in $CDCl_3$ solution were measured at 50.3 MHz at temperatures between +20°C and -74°C. At temperatures of -43°C and below, the resonance peaks of all twenty of the ketazolam ^{13}C nuclei are resolved, including all six of the ^{13}C nuclei of the unsubstituted phenyl ring. The purpose of this study was to assign all of the ^{13}C nuclei in ketazolam so as to make possible further dynamic studies of the hindered rotation about the aryl to tert-alkyl bond. Direct and long-range ^{13}C-^{1}H shift correlation experiments, carried out at low temperatures, were used to make the assignments. The variable temperature correlation experiments, which were performed at temperatures between -20°C and -74°C, illustrate the effects of an intermediate rate of chemical exchange of the phenyl ring upon cross peak intensities involving phenyl ^{13}C and/or ^{1}H nuclei.

EXPERIMENTAL

Details of solution preparation have been described (9,10). Ketazolam was a gift of the Upjohn Company and was used without further purification. Ketazolam in $CDCl_3$ solution is unstable at room temperature, but solutions kept at -20°C are stable for several weeks.

An IBM Instruments Inc. WP 200/SY FTNMR spectrometer equipped with an Aspect 2000A computer, 10 mm turnable broadband probe and Bruker variable temperature apparatus was used to record ^{1}H spectra at 200.1 MHz and ^{13}C spectra at 50.3 MHz. All solutions were measured in 5-mm tubes. The temperature controller was stable to $\pm$ 1°C for overnight accumulations. The separation of methanol peaks was used to check the calibration of the temperature controller. ^{1}H spectra were referenced to internal TMS; ^{13}C spectra were indirectly referenced to TMS via the $CDCl_3$ triplet (77.0 ppm).

A sweep width of 8333 Hz was used for all ^{13}C measurements, including the 2D ^{13}C-^{1}H shift correlation experiments. The broadband decoupled ^{13}C spectra were collected in 16K data points, with a 5 s delay between transients. The inverse gated decoupled ^{13}C spectrum (broadband decoupling during acquisition) was also collected in 16K points, but with a 15 s delay between transients. The spectral resolution was 1.02 Hz per point.

Two ^{13}C-^{1}H shift correlation experiments were performed using the Bruker microprogram XHCORR.AU (without ^{1}H decoupling, (11)); these were at -20° (A) and -74°C (C). One shift correlation experiment was performed using XHCORRD.AU (with ^{1}H decoupling, (12)); this was at -47°C (B). For each experiment, 2K data points were used for ^{13}C, giving a resolution of 8.14 Hz per point. A sweep width of 1800 Hz was used for ^{1}H, which, with 128 increments of t_1 and one zero filling, gave a resolution of 7.03 Hz per point. A 1 s recycle delay was used

Table 1. Experimental parameters used in heteronuclear shift correlation experiments

Experiment	Temp.(°C)	Conc(M)	Δ_1(ms)[a]	Δ_2(ms)[a]	NS[b]
	One-bond correlation				
A (Fig. 5)	-20°	.474	4.00	2.00	64
B (Fig. 6)	-47°	.600	2.85	2.85	160
C (Fig. 7)	-74°	.592	2.85	2.85	256
	Long-range correlation				
Experiment					
D (Fig. 8)	-43°	.592	45.0	45.0	256

[a] Δ_1 and Δ_2 are the delay times.
[b] NS is the number of transients at each value of t_1, not including two dummy scans.

between transients (with two dummy scans between data blocks) except for (A), in which the recycle delay was 5 s. See Table 1 for number of transients used in each experiment and for lengths of polarization and refocusing delays, Δ_1 and Δ_2.

The Bruker microprogram COLOC.AU (13) was used for the three 2D ^{13}C-^{1}H long-range shift correlation experiments at -23°23°, -43° (D) and -58°C. The 90° ^{1}H transmitter pulse width was 19 μs; the 90° ^{13}C transmitter pulse width was 14 μs. The recycle delay was 1 s.

All 2D datasets were processed using an exponential window function in the ^{13}C dimension (LB=2) and a Gaussian window func-

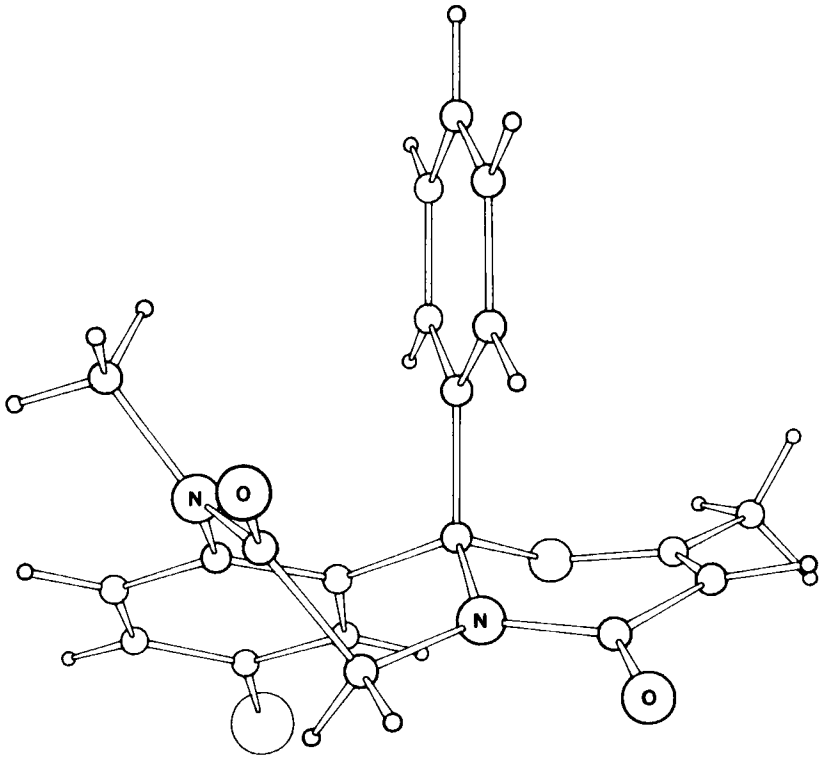


Fig. 2. ORTEP plot of ketazolam.

tion (LB=-3, GB=0.3) in the ^{1}H dimension, except for (C), which was processed using a sine window function in each dimension.

The temperature, solution concentration, length of the polarization transfer (Δ_1) and refocusing delays (Δ_2) and the number of transients at each value of t_1 are summarized in Table 1. For the first XHCORR experiment, (A), the delay times (Δ_1 and Δ_2) were optimized to observe all multiplicities, with $^{1}J_{CH}$=125 Hz for sp^3 type carbons. For (B) and (C), Δ_1 and Δ_2 were optim-

ized for aromatic one-bond couplings, with $^1J_{CH}$=175 Hz. For the COLOC experiment (D), Δ_1 and Δ_2 were set equal to maximize magnetization transfer to quaternary and protonated methine carbons (14) and were optimized for three-bond couplings of 11.1 Hz. For the COLOC experiment carried out at -58°C, Δ_1 was set equal to 70.0 ms to optimize for a smaller $^3J_{CH}$=7.1 Hz, and Δ_1=2Δ_2.

RESULTS AND DISCUSSION

Energy Calculations

Given the unusual structure of ketazolam, with the phenyl ring positioned between the C-methyl and the N-methyl groups (*Fig. 2*), it is not unexpected that the phenyl ring experiences hindered rotation even at 20°C. In order to investigate the nature of the rotational barrier about the $C_{1'}$-C_{12b} bond, the intramolecular energy of the molecule was calculated, starting with crystal structure coordinates (1). The energy was computed from nonbonded energy parameters as described previously (15), employing the crystal-refined nonbonded energy parameters of Momany et al. (16). All hydrogen atoms were included in the calculations. This parameter set does not include chlorine, but because sulfur atoms and chlorine atoms have similar van der Waals radii and since the chlorine atom in ketazolam is not directly involved in the rotation about the $C_{1'}$-C_{12b} bond, the nonbonded parameters of sulfur were used for chlorine in the energy calculations. The energy was calculated at each 10° increment of the torsional angle $C_{2'}$-$C_{1'}$-C_{12b}-O_1. A plot of the

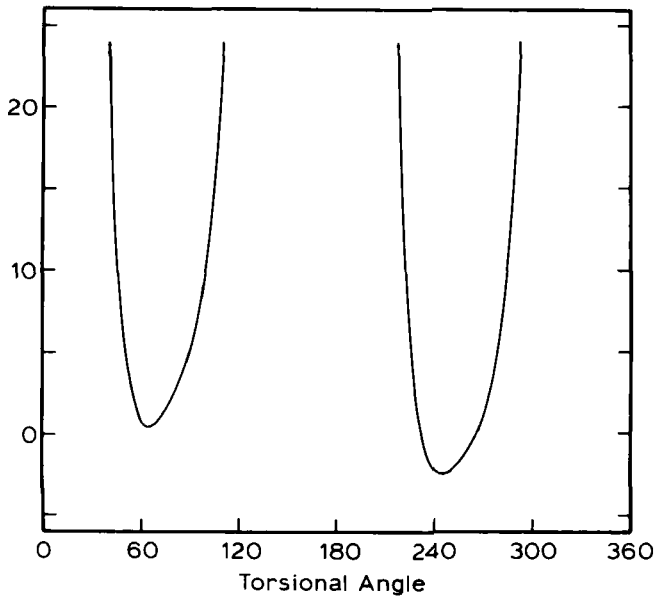


Fig. 3. Calculated nonbonded energy of ketazolam as a function of rotation about the torsional angle $C_{2'}$-$C_{1'}$-C_{12b}-O_1.

energy *vs.* torsional angle is shown in *Fig. 3*. A two-fold barrier is apparent for the phenyl ring with minima in the torsional angle of approximately 60° and 240°. The inequality of the barriers is artifactual, resulting from the fact that the $C_{1'}$-C_{12b} vector is not exactly collinear with the $C_{4'}$-C_{12b} vector in the crystal. When the torsional angle is set to 0°, extremely close contacts (as little as 2Å) are calculated between the $H_{2'}$, $H_{6'}$ protons of the phenyl ring and the 2-methyl and 8-methyl protons. The value of the torsional angle $C_{2'}$-$C_{1'}$-

C_{12b}-O_1, calculated from the crystal structure coordinates, is 238°. Energy minimization about this torsional angle gave a value of 244° with a value of the energy which was 0.34 kcal/mole lower than that calculated for the crystal structure. In this low energy conformation, the plane of the phenyl ring faces the methyl groups on each side of the ketazolam molecule (Fig. 2) with comfortable distances of 3.5-5Å between the $H_{2'}$ or $H_{6'}$ protons of the phenyl ring and the 2-methyl and 8-methyl protons. The coordinates of the minimum energy structure were used to generate the ORTEP plot shown in *Fig. 2*.

^{13}C NMR Spectra

The two-fold energy barrier about the $C_{1'}$-C_{12b} bond has a significant effect upon the chemical shifts of the ^{13}C nuclei in the phenyl ring of ketazolam. *Fig. 4* is a stacked plot of the ^{13}C aromatic resonances from 140-124 ppm at +20°, -20°, -43° and -58°C. An inverse gated decoupling experiment (Fig 4a) performed at 20°C shows that the peaks at 127.7 and 126.2 ppm are twice the intensity of the rest of the ketazolam ^{13}C peaks. One of these peaks may be assigned to the two *ortho* ^{13}C nuclei and the other to the two meta ^{13}C nuclei, although, at this point, it is not possible to distinguish between the *ortho* and $meta_w$ carbons. However, the ^{13}C nuclei resonating at 126.2 ppm are in rapid exchange, while those at 127.7 ppm are broadened by slower exchange on the NMR time scale. As the ketazolam/$CDCl_3$ solution is cooled to -20°C, the resonance at 127.7 ppm separates into two broad peaks (*Fig. 4b*) centered at approximately 128.5 and

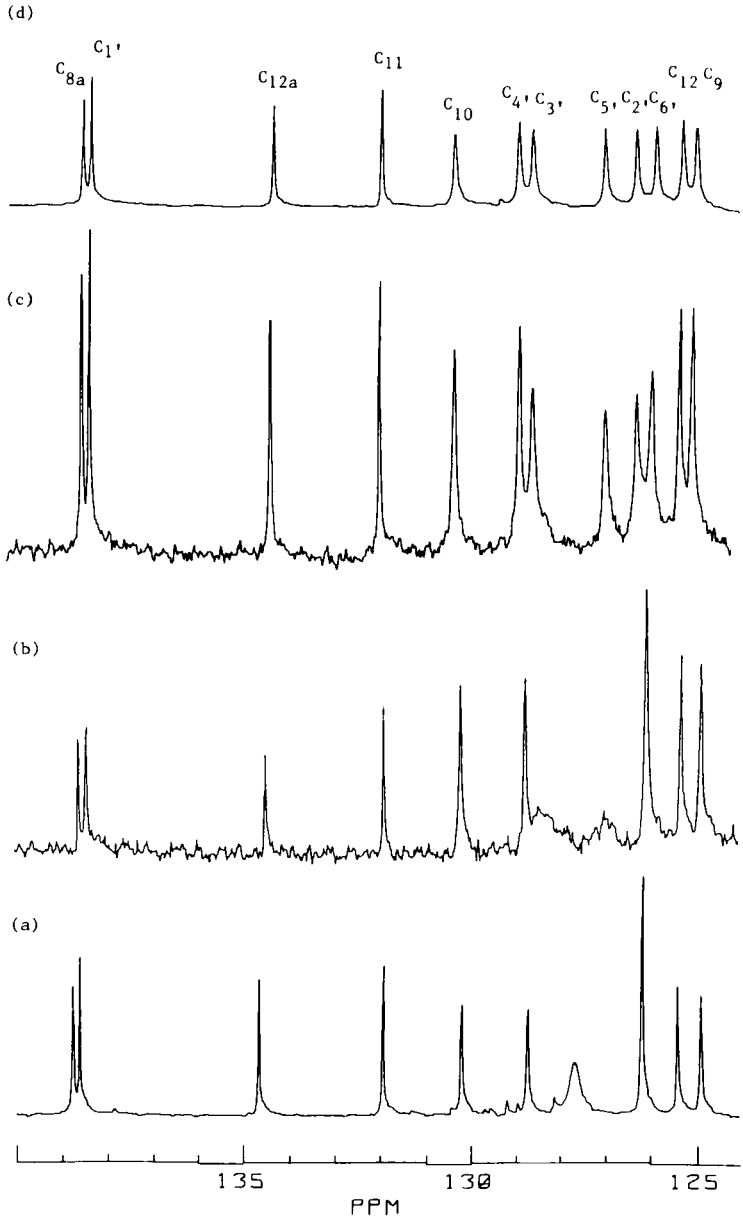


Fig. 4. 50.3 MHz ^{13}C NMR spectra of ketazolam/$CDCl_3$ solutions: (a) 20°C (b) -20°C (c) -43°C (d) -58°C. Only aromatic spectral region shown. See Table 2 for chemical shifts and concentrations.

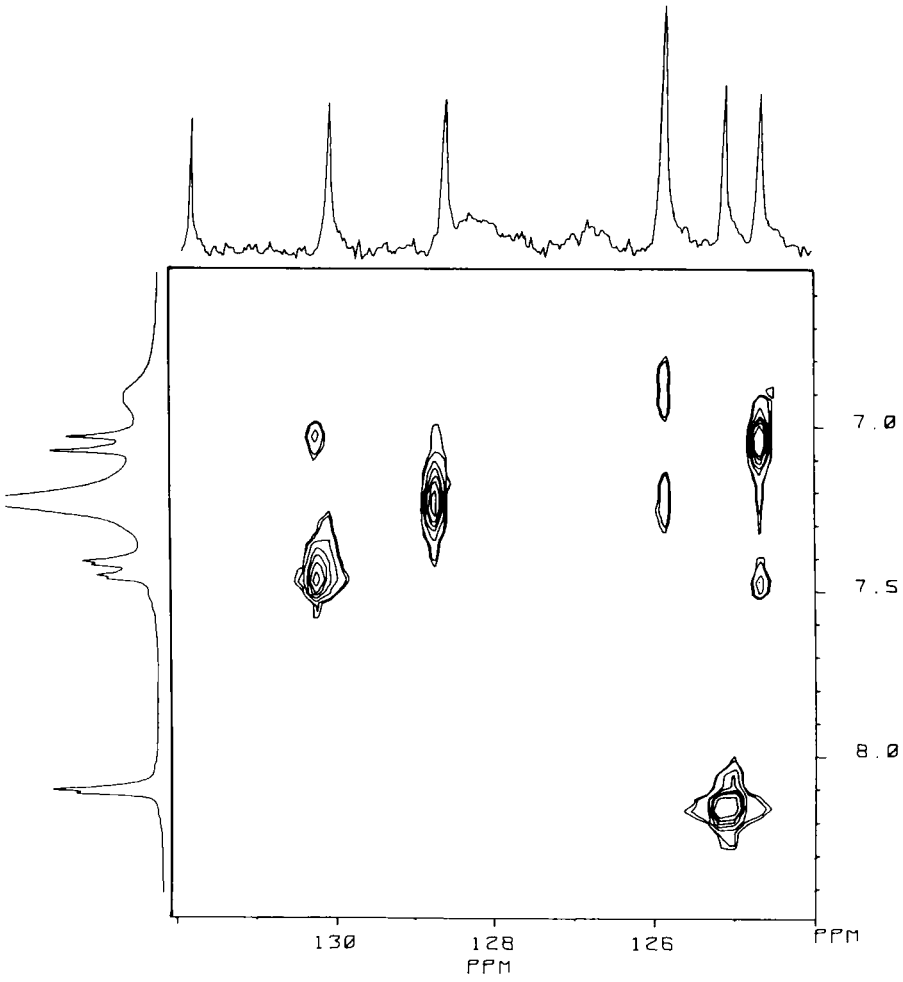


Fig. 5. Contour plot of the two-dimensional ^{13}C-^{1}H correlated NMR spectrum of 0.474 M ketazolam/ $CDCl_3$ at -20°C (A). Optimized for one-bond couplings; see Experimental section and Table 1.

127.0 ppm. The peak at 126.2 ppm remains a singlet at this temperature. Upon further cooling to -43°C, however, this singlet becomes two peaks, at 126.1 and 125.8 ppm (*Fig. 4c*). The two resonances which were extremely broad at -20°C, now sharpen to peaks at 128.4 and 126.8 ppm (Fig. 4c). At -58°C, (Fig. 4d), all aromatic resonances are well resolved, with a chemical shift separation of 0.5 ppm for the upfield set of exchanging ^{13}C nuclei and 1.6 ppm for the downfield set. All other resonances shift somewhat with concentration and temperature changes, but none so dramatically as the *ortho* and *meta* ^{13}C nuclei of the phenyl ring.

One-bond ^{13}C-^{1}H shift correlation

Heteronuclear shift correlation experiments at -20°(A), -47°(B) and -74°C(C) were used to assign all protonated carbons and to identify the ^{13}C nuclei of the unsubstituted phenyl ring as their chemical shifts changed due to the decrease in rate of rotation of the ring as the temperature was lowered. ^{13}C nuclei which are easily identified by their one-bond correlations with attached protons are 2-CH_3, 8-CH_3, C_3, C_6, C_9, C_{10} and C_{12}. Only the aromatic spectral region from 132 to 124 ppm is shown in the contour plots of *Fig. 5-7* since this is the region most affected by chemical exchange. The peak at 128.8 ppm, which has a cross peak to an aromatic proton from the unsubstituted phenyl ring at 7.3 ppm, and which experiences a very small chemical shift (0.1 ppm) as the temperature is reduced from 20°C to -74°C, is the *para* carbon, $C_{4'}$. This ^{13}C does not undergo

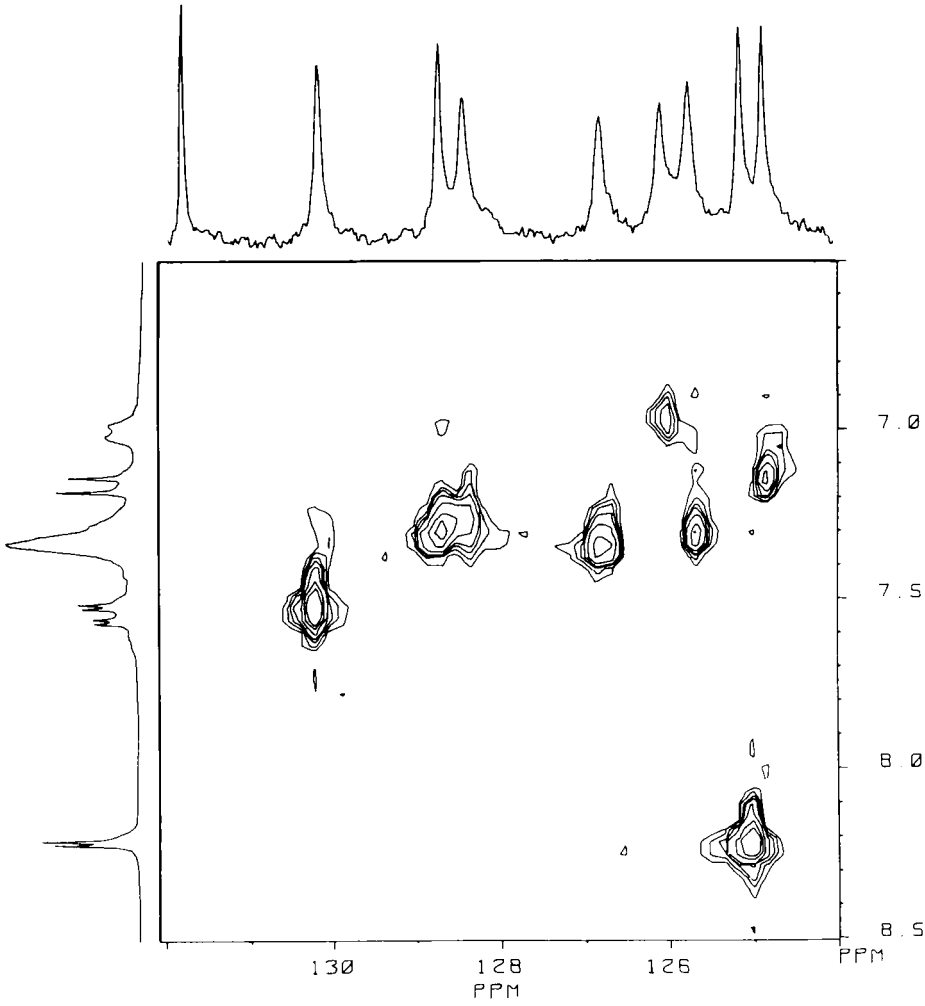


Fig. 6. Contour plot of the two-dimensional ^{13}C-^{1}H correlated NMR spectrum of 0.600 M ketazolam/$CDCl_3$ at -47°C (B). Optimized for one-bond couplings; see Experimental section and Table 1.

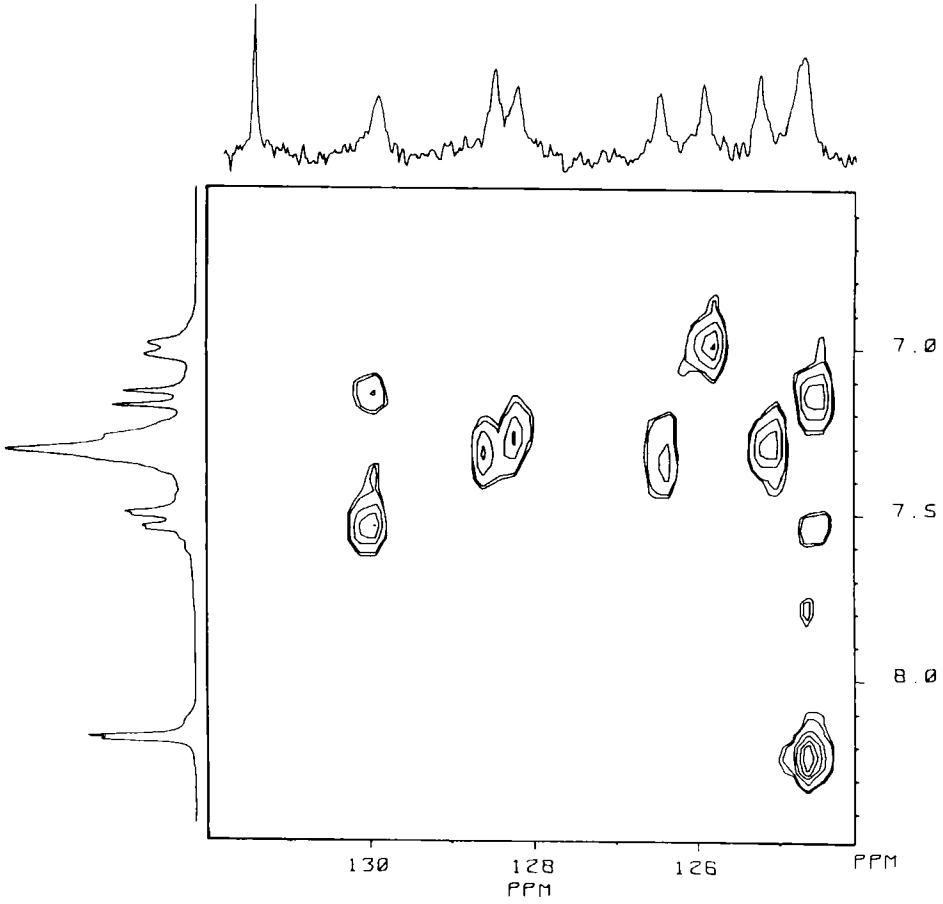


Fig. 7. Contour plot of the two-dimensional ^{13}C-^{1}H correlated NMR spectrum of 0.592 M ketazolam/$CDCl_3$ at -74°C (C). Optimized for one-bond couplings; see Experimental section and Table 1.

chemical exchange upon rotation about the $C_{1'}$-C_{12b} bond. The only other one-bond correlations which appear in *Fig. 5* (-20°C) are C_9/H_9 at 124.9/7.06 ppm; C_{10}/H_{10} at 130.2/7.50 ppm; C_{12}/H_{12} at 125.4/8.18 ppm; and the ^{13}C resonance at 126.1 ppm

which has cross peaks to two ^{1}H resonances, one at about 7.0 ppm ($H_{2'}$) and one at about 7.3 ppm ($H_{6'}$). This ^{13}C resonance at 126.1 ppm, which becomes two peaks at temperatures below -23°C (Fig. 4), must be $C_{2'}$ *and* $C_{6'}$, the *ortho* carbons of the unsubstituted phenyl ring. This leaves only two unassigned resonances in this aromatic spectral region, the two very broad peaks at 128.5 ppm and 127.0 ppm, which are $C_{3'}$ and $C_{5'}$, the *meta* carbons. The rate of exchange of these carbons at -20°C makes their spin-spin relaxation times (T_2) so short that transfer of polarization from the *meta* protons in the 2D experiment is not observed. The *meta* protons also undergo exchange, of course, which reduces their T_2 values, thereby reducing the amount of transverse polarization which is available for transfer to the ^{13}C nuclei; thus the absence of $C_{3'}$ or $C_{5'}$ cross peaks in *Fig. 5*. Small two-bond correlations are visible for C_9/H_{10} and C_{10}/H_9 in the chlorophenylene rings.

To be certain that the assignments of the *ortho and meta* carbons of the phenyl ring do not change due to peak crossing as the temperature is lowered, the one-bond correlation experiment was repeated at -47°C and -74°C. The ^{13}C peak which correlates with $H_{2'}$ resonates at 126.1 ppm at -20°C and -43°C; at 126.3 ppm at -58°C and at 126.2 ppm at -74°C. A plot of all four *ortho* and *meta* ^{13}C chemical shifts as a function of temperature (not shown) reveals that crossover of resonances is very unlikely.

One major change which occurs at -47°C (*Fig. 6*) is the separation of the *ortho* ^{13}C resonances (126.1 ppm at -20°C) into two cross peaks: $C_{2'}/H_{2'}$ at 126.1/7.0 ppm and $C_{6'}/H_{6'}$ at 125.8/7.3 ppm. The other striking change is that the *meta* ^{13}C resonances $C_{3'}$ and $C_{5'}$ are now visible at 128.4 and 126.8 ppm, each correlating with the merged proton resonances ($H_{3'}$,$H_{5'}$) at 7.3 ppm. The *meta* ^{13}C cross peak at 128.4 ppm is not completely resolved from that of $C_{4'}$ at 128.7 ppm in *Fig. 6*, (see below for results at -74°C). The contour diagram shown in Fig. 6 is the only one of the three one-bond correlation experiments which does not show C_9/H_{10}, C_{10}/H_9 cross peaks. The pulse sequence used for this experiment included proton-proton decoupling (see Experimental Section).

At -74°C, the ^{13}C peaks of ketazolam begin to broaden due to the increased viscosity of the solution; it is indeed surprising that, at this temperature and at a concentration of 0.592 M ketazolam in $CDCl_3$, the ketazolam does not come out of solution. Nevertheless, the resolution in the contour plot (Fig. 7) is quite good. All eight one-bond cross peaks are resolved, including those of C_9 and C_{12}, (although at -74°C, the C_9 and C_{12} peaks merged at 125.0 ppm in the normal broadband decoupled ^{13}C NMR spectrum). The separation of resonances reached at -74°C is 0.7 ppm for the *ortho* ^{13}C nuclei and 1.8 ppm for the *meta* ^{13}C nuclei. It was not expected that the *meta* ^{13}C resonances should have a larger chemical shift difference than the *ortho* ^{13}C resonances, for two reasons: (a) the *meta*

carbons are farther from magnetically anisotropic groups, and (b) the *ortho* ^1H resonances separate at low temperatures (Fig. 1b), while the *meta* ^1H resonances remain merged with the *para* ^1H resonances even at -74°C. Nevertheless, the one-bond correlation experiments make it clear that this is indeed the case. The delay times used in these experiments are shown in Table 1 and discussed in the Experimental Section.

Long-range ^{13}C-^1H shift correlation

After the protonated carbons of ketazolam have been assigned via one-bond correlation experiments, eight quaternary carbons remain to be assigned. It was necessary to carry out the long-range correlation (COLOC) experiments at low temperatures so that shift correlations to the unsubstituted phenyl ring could be observed. (Also, for the 12-hour duration of these COLOC experiments, the ketazolam in $CDCl_3$ solution would decompose if not kept at -20°C or below). The COLOC experiments were carried out at -23°, -43° and -58°C, with somewhat different values of the delay times. Only the data obtained at -43°C will be discussed; -43°C is a sufficiently low temperature to slow the rotation of the phenyl ring so that $C_{2'}$, $C_{3'}$, $C_{5'}$ and $C_{6'}$ are resolved. The spectral region from 140 to 124 ppm is shown in *Fig. 8*; the rest of the correlation diagram does not change significantly with temperature.

The delay times for the COLOC experiment at -43°C were set equal to optimize for methine and quaternary $^3J_{CH}$ couplings of 11.1 Hz. (A large three-bond coupling was expected for C_{11}, the

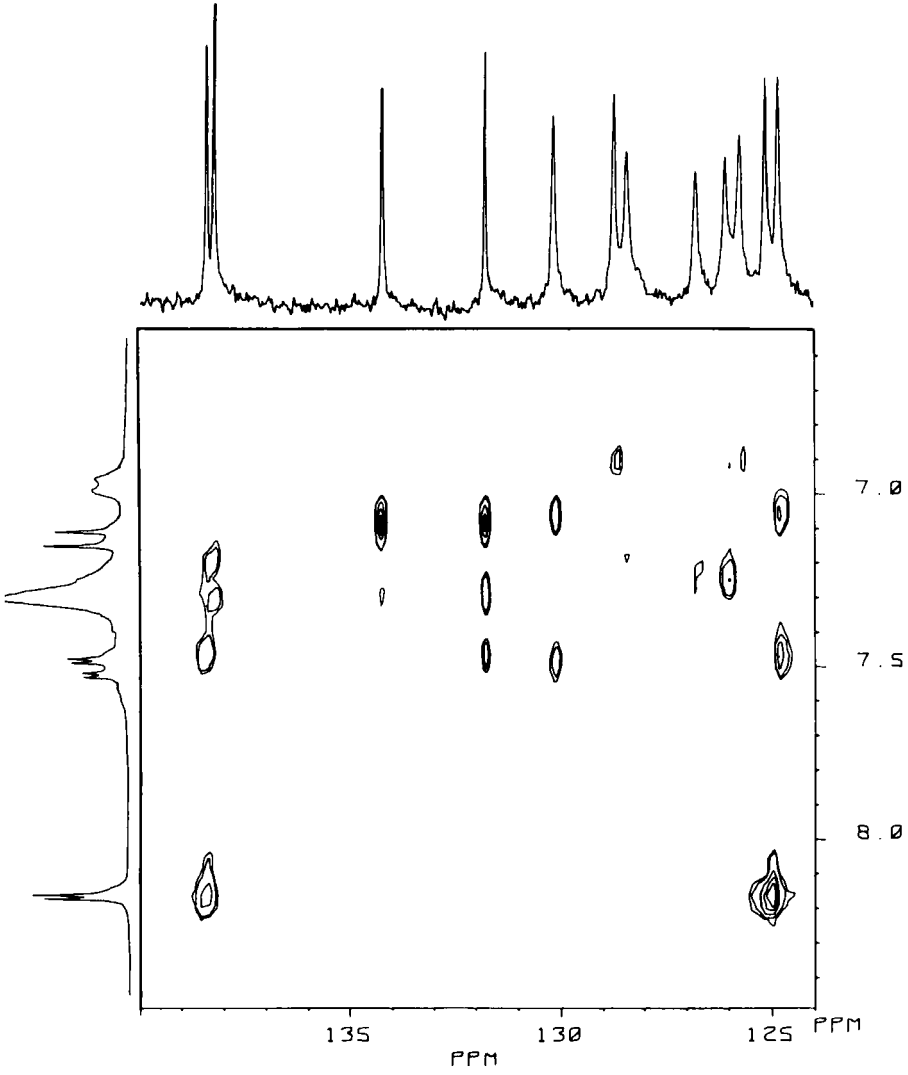


Fig. 8. Contour plot of the two-dimensional ^{13}C-^{1}H correlated NMR spectrum of 0.592 M ketazolam/$CDCl_3$ at -43°C (D). Optimized for long-range couplings; see Experimental section and Table 1.

chlorinated carbon. (17)). The quaternary carbons whose cross peaks may be seen in Fig. 8 are C_{8a}, $C_{1'}$, C_{12a} and C_{11}. The remaining carbons in this Figure are protonated and have been assigned (Table 2). The ^{13}C peak to lower magnetic field (138.3 ppm) is C_{8a}, with cross peaks reflecting three-bond coupling to H_{10} (7.50 ppm) and H_{12} (8.18 ppm). The next ^{13}C peak, at 138.1 ppm, is $C_{1'}$, with cross peaks to aromatic protons $H_{3'}$, $H_{5'}$ and possibly $H_{6'}$ at 7.2 and 7.3 ppm. Continuing to higher magnetic field, the next ^{13}C peak is C_{12a} at 134.2 ppm with a strong cross peak to H_9 at 7.12 ppm. The last quaternary ^{13}C in Fig. 8 is C_{11} at 131.8 ppm, with a strong cross peak to H_9 ($^3J_{CH}$) at 7.12 ppm, and a weaker cross peak to H_{10} ($^2J_{CH}$) at 7.50 ppm. The results of the COLOC experiments are not completely unambiguous regarding the assignments of these four quaternary carbons. However, considered together with the ^{13}C spectrum of ketazolam obtained without broadband decoupling, the assignments are certain: C_{11}, for example, is split into a multiplet of at least six lines due to coupling to H_9, H_{10} and H_{12}, while C_{12a} is approximately a doublet of doublets due to coupling to H_9 and H_{12}. C_{8a} and $C_{1'}$ are merged and broadened in the undecoupled spectrum due to the proximity of C_{8a} to a quadrupolar nitrogen nucleus. The COLOC experiment at -43°C, however, makes clear that C_{8a} is the downfield resonance while $C_{1'}$ is 0.2 ppm upfield. The separation of C_{8a} and $C_{1'}$ is obvious when an expanded region of the contour plot of Fig. 8 is viewed between 137 and 140 ppm.

Table 2
^{1}H and ^{13}C Chemical shifts of ketazolam at different temperatures[a]

Assignment	^{1}H	^{13}C				
	+20°C	-74°C	-58°C	-43°C	-20°C	+20°C
2	----	162.7	162.6	162.4	162.3	162.1
2-CH_3	1.89	20.0	20.1	19.9	20.0	19.6
3	5.36	100.4	100.7	100.6	100.7	100.8
4	----	161.9	161.9	161.6	161.6	161.4
6a	3.34	43.3	43.4	43.3	43.5	43.5
6b	5.36					
7	----	165.2	165.3	165.1	165.2	165.1
8-CH_3	2.51	34.0	34.1	33.8	33.8	33.6
8a	----	138.0	138.3	138.3	138.6	138.8
9	7.12	125.0	125.0	124.9	124.9	124.9
10	7.50	130.1	130.3	130.2	130.2	130.2
11	----	131.6	131.9	131.8	131.9	131.9
12	8.18	125.0	125.3	125.2	125.4	125.4
12a	----	133.8	134.2	134.2	134.5	134.7
12b	----	91.0	91.3	91.3	91.6	91.7
1'	----	137.8	138.2	138.1	138.4	138.6
2'	7.15[c]	126.2	126.3	126.1	126.1	126.2
3'[b]	7.28[c]	128.5	128.6	128.4	128.5[d]	127.7
4'	7.28[c]	128.7	128.9	128.7	128.8	128.7
5'[b]	7.28[c]	126.7	127.0	126.8	127.0[d]	127.7
6'	7.15[c]	125.5	125.8	125.8	126.1	126.2

[a] The concentration of the solution for the ^{1}H at +20°C and ^{13}C assignments at -43°C was 0.600 M ketazolam in $CDCl_3$. The concentrations used at the other temperatures were 0.592 M (-74°C and -58°C), 0.474 M (-20°C) and 0.664 M (+20°C). The reference for ^{1}H was internal TMS. ^{13}C resonances were referenced to internal $CDCl_3$ at 77.0 ppm.

[b] ^{13}C Assignments may be reversed. *Meta* ^{13}C peaks cannot be distinguished because *meta* ^{1}H peaks overlap at all temperatures.

[c] Very broad peaks. At low temperatures (-20°C and below, $H_{2'}$ resonates at about 7 ppm, while $H_{3'}$-$H_{6'}$ are merged at 7.3 ppm).

[d] Very broad *meta* ^{13}C peaks.

Table 3. $^{13}C/^{1}H$ Correlations of Ketazolam.[a]

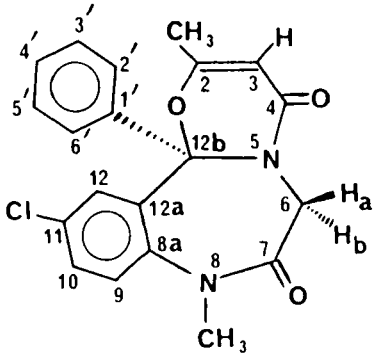
Carbon	Bonded H	Long-range connected H
2	----	2-CH_3, H_3
2-CH_3	CH_3	----
3	H_3	2-CH_3
4	----	H_{6a}
6	H_a,H_b	----
7	----	8-CH_3, H_{6a}, H_{6b}
8-CH_3	CH_3	----
8a	----	8-CH_3, H_{10},H_{12}
9	H_9	H_{10}
10	H_{10}	H_9, H_{12}
11	----	H_9, H_{10}
12	H_{12}	----
12a	----	H_9, $H_{6'}$
12b	----	H_{6b}, H_{12}, $H_{2'}$, $H_{6'}$
1'	----	$H_{3'}$, $H_{5'}$, $H_{6'}$
2'	$H_{2'}$	b
3'	b	b
4'	b	$H_{2'}$
5'	b	b
6'	b	b

[a] Not all correlations may be seen in each experiment (see text).

[b] Aromatic ^{13}C resonances correlate with aromatic ^{1}H resonances; however, in the concentrated solutions used for these low-temperature experiments, only $H_{2'}$ resonates at a different frequency; $H_{3'}$-$H_{6'}$ are merged.

The quaternary carbon C_{12b}, being sp^3 hybridized, resonates upfield (91.3 ppm at -43°C) from the other sp^2 quaternary carbons and may be identified via cross peaks from three-bond couplings to H_{6b}, H_{12}, $H_{2'}$ and $H_{6'}$.

In the COLOC experiment carried out at -58°C (not shown), the three ^{13}C resonances between 162 and 165 ppm are easily assigned to the remaining two carbonyl carbons and the quater-



nary C_2. The peak to lowest field, at 165.3 ppm, has cross peaks to the 8-methyl protons at 2.51 ppm and to H_{6a} and H_{6b} and is therefore the carbonyl carbon, C_7. The other carbonyl carbon, at 161.9 ppm, has cross peaks to H_3 at 5.36 ppm and H_{6a} at 3.34 ppm and is C_4. This leaves C_2 at 162.6 ppm, with cross peaks to H_3 and to the 2-methyl protons at 1.89 ppm.

The ^1H and ^{13}C chemical shift assignments at various temperatures are shown in Table 2. The observed one-bond and long-range correlation cross peaks are collected in Table 3.

CONCLUSION

All twenty ^{13}C resonances of ketazolam are resolved (at 50.3 MHz) at temperatures of -40°C and below, including those of the unsubstituted phenyl ring. Low-temperature one-bond ^{13}C-^1H correlation experiments make possible the assignment of all protonated carbons. The phenyl carbons may be identified starting from the one-bond cross peak in the 2D spectrum which

correlates $C_{2'}$ and $H_{2'}$. $H_{2'}$ is the only proton of the phenyl ring possessing a unique chemical shift (at the concentrations and temperatures used). Variable-temperature ^{13}C studies then allow the coalescing *ortho* ^{13}C resonances to be distinguished from the coalescing *meta* ^{13}C resonances.

Low-temperature long-range correlation experiments were used to assign the eight quaternary ^{13}C resonances. With the ^{13}C assignments completed, detailed studies of the activation energy for internal rotation about the aryl to tert-alkyl bond in ketazolam are now possible.

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REFERENCES

1. Szmuszkovicz J., Chidester C.G., Duchamp D.J., MacKellar F.A., Slomp G. Synthesis and proof of structure of a novel benzodiazepine. Tetrahedron Lett. 1971; (39):3665-3668.

2. Patra A., Mukhopadhyay A.K., Mitra A.K., Acharyya A.K. Carbon-13 NMR signals of some benzodiazepine drugs. Org. Magn. Reson. 1981; 15(1):99-101.

3. Singh S.P., Parmar S.S., Farnum S.A., Stenberg V.I. Fourier transform carbon-13 NMR analysis of benzodiazepines. J. Heterocyclic Chem. 1978; 15(7):1083-1087.

4. Haran R., Tuchagues J.P. Carbon-13 and proton NMR studies of 1,4-benzodiazepines. J. Heterocyclic Chem. 1980; 17(7):1483-1488.

5. Scahill T.A., Smith S.L. Carbon-13 and hydrogen NMR data for a series of 1,4-benzodiazepines. Magn. Reson. Chem. 1985; 23(4):280-285.

6. Cazaux L., Vidal C., Pasdeloup M. NMR of some benzodiazepine drugs: structure elucidation of lanthanide-induced shifts of N-1 substituted benzodiazepinones. Org. Magn. Reson. 1983; 21(3):190-195.

7. Paul H.-H., Sapper H., Lohmann W., Kalinowski H.-O. Analysis and applications of carbon-13 NMR lanthanide induced shifts of 1,4-benzodiazepines. Org. Magn. Reson. 1982; 19(1):49-53.

8. Scahill T.A., Smith S.L. Nitrogen-15 NMR studies of 1,4-benzodiazepines:1. Org. Magn. Reson. 1983; 21(10):621-623.

9. LaPlanche L.A., Rothchild R. Unusual hindered rotation of an unsubstituted phenyl group. Variable temperature ^{1}H NMR studies and preliminary ^{13}C assignments in ketazolam. Spectrosc. Lett. 1990; 23(8):in press.

10. LaPlanche L.A., Rothchild R. Improved method for optical purity determination of ketazolam with chiral shift reagent. Spectrosc. Lett. 1990; 23(1): 45-64.

11. Bax A., Morris G. An improved method for heteronuclear chemical shift correlation by two-dimensional NMR. J. Magn. Reson. 1981; 42(3): 501-505.

12. Bax A. Broadband homonuclear decoupling in heteronuclear shift correlation NMR spectroscopy. J. Magn. Reson. 1983; 53(3):517-520.

13. Kessler H., Griesinger C., Zarbock J., Loosli H.R. Assignment of carbonyl carbons and sequence analysis in peptides by heteronuclear shift correlation via small coupling constants with broadband decouping in t_1 (COLOC). J. Magn. Reson. 1984; 57(2):331-336.

14. Martin G.E., Zektzer A.S. Long-range two-dimensional heteronuclear chemical shift correlation. Magn. Reson. Chem. 1988; 26(8):631-652.

15. LaPlanche L.A., Vanderkooi G., Jasmani H., Mat-Suki M. The solution structure of lidocaine: conformational analysis and NMR lanthanide-induced shifts. Magn. Reson. Chem. 1985; 23(11):945-951.

16. Momany F.A., Carruthers L.M., McGuire R.F., Scheraga H.A. Intermolecular potentials from crystal data. III. Determination of empirical potentials and application to the packing configurations and lattice energies in crystals of hydrocarbons, carboxylic acids, amines, and amides. J. Phys. Chem. 1974; 78(16):1595-1620.

17. Wehrli F.W., Marchand A.P., Wehrli S. Interpretation of Carbon-13 NMR Spectra, New York: John Wiley & Sons, 1983, p. 75.

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