Comparisons of Rotamer Populations of Nialamide, Azaperone, and Chloroquine in Solid State and in Solution

GERALD P. MIGLIACCIO * and STEPHEN R. BYRN ˣ

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Abstract □ IR and NMR spectroscopy were combined with previously published X-ray crystallographic data to determine the solution conformations of the $-(CH_2)_n-$ fragments of nialamide, azaperone, and chloroquine. The solution conformation of these compounds then was compared to the solid-state conformation. In addition, the limits of the IR–X-ray method are discussed. This paper shows that a combination of IR, NMR, and X-ray crystallographic data can lead to a complete picture of the conformations available to drugs. In addition, the danger of using solid-state conformational data alone to make pharmacological suggestions is illustrated.

Keyphrases □ Nialamide—comparison of rotamer populations in solid state and in solution, conformation determined by IR and NMR spectroscopy and X-ray crystallography □ Azaperone—comparison of rotamer populations in solid state and in solution, IR and NMR spectroscopic and X-ray crystallographic determinations of conformation □ Chloroquine—comparison of rotamer populations in solid state and in solution, IR and NMR spectroscopic and X-ray crystallographic determination of conformation □ Conformation—in solid state and in solution, azaperone, nialamide, chloroquine

An understanding of the relationship between the conformation of a flexible drug at the receptor and its conformation in the liquid (solution) and solid state is a major goal of molecular pharmacology.

BACKGROUND

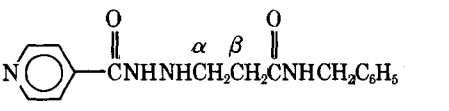
A relationship (1) between the proportion of rapidly equilibrating conformers in solution and biological activity has been proposed for histamine, antihistamines, acetylcholine and its analogs, and several hormones (1–10). For example, the ability of histamine to stimulate two distinct biological responses initially was suggested to be due to the presence of *gauche*- and *trans*-conformers (1). The observation that ethylenediamine antihistamines blocked only one of these responses was attributed to their preference for the *trans*-conformation (2). However, more recent studies led to other suggestions, including the conclusion that there is no clearcut conformation–activity relationship for histamine and its derivatives (3, 4).

A relationship between the solid-state conformation about the phenyl–piperidine bond and analgesic potency was suggested (8), and the solid-state conformation of anticonvulsant drugs was suggested to be related to their activity (9).

Finally, the most stable conformer of several drugs in solution was suggested to be the one bound to the receptor (11). This suggestion led to numerous attempts to correlate differences in the proportion of the most stable conformers with activity.

In contrast to these suggestions, it was postulated that rapid substrate isomerism should not affect enzymatic reaction rates (12). Furthermore, rapid substrate isomerism had no effect on the rate of chymotrypsin-catalyzed hydrolysis of DL-thiazoline (13), and the enzyme thermolysin bound the lowest energy solution conformer of one dipeptide inhibitor and the highest energy solution conformer of a second dipeptide inhibitor[1].

In addition to these examples of the trapping of a rotamer by a receptor, there are numerous examples of the separation of a rotamer from a mixture of rotamers by crystallization (14–22). For example, crystallization results in the trapping of a single rotamer of chlorocyclohexane (14), tetramethylthiatane (15), cyclo-gly-gly-*d*-ala-*d*-ala-gly-gly (16, 17), isobutyramide (18), 3′-isopropyo-3,5-diiodo-*l*-thyronine (19), 1,4-diphenyl-1,4-dithiabutane-1,4-dioxide (20), and histamine (21). In addition, the phenomenon of conformational polymorphism was reviewed (22). Conformational polymorphism occurs when a compound crystallizes in different conformations in different polymorphs.



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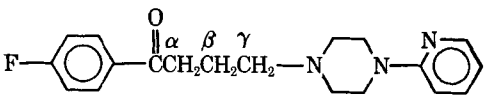

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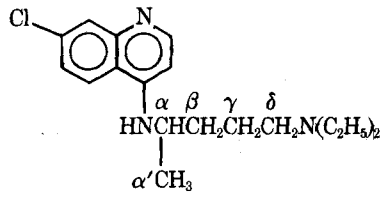

III

These reports show that crystallization can result in the selection of one rotamer from a mixture; thus, the development of methods for investigating whether crystallization results in the trapping of one of a mixture of rotamers is an important step toward understanding the relationship (if any) between the solid-state, solution, and receptor-bound conformations of a drug. This paper reports studies aimed at developing such an understanding. Three flexible drugs were chosen for investigation using an extension of the IR–X-ray method (21): nialamide (I), a monoamine oxidase inhibitor (23); azaperone (II), a sedative neuroleptic (24); and chloroquine (III), an antimalarial (25).

EXPERIMENTAL

Apparatus and Spectra—All IR spectra were analyzed as solids in potassium bromide pellets or as solutions in 0.5-mm sodium chloride cells[2]. Preliminary NMR spectra were measured on 60-, 80-, or 90-MHz spectrometers[3]. All samples then were analyzed on a 360-MHz superconducting Fourier transform spectrometer[4]. All spectra were analyzed by computer[5].

Chemicals—Chloroquine was obtained as the diphosphate salt[6]. It was used without further purification in aqueous solution and was converted to the free base for analysis in organic solvents according to a literature method (26). The diphosphate salt (25 g) was dissolved in distilled water (500 ml). The free base was separated by addition of 0.1 *N* NaOH (200 ml). A white viscous solution formed and was extracted with methylene chloride (4 × 100 ml). The extract was dried over anhydrous sodium sulfate for 1 hr with stirring. This solution then was filtered, and

[1] T. L. Shieh and S. R. Byrn, unpublished data.

[2] Beckman IR4230 spectrometer.

[3] Varian EM-360 or FT-80 and Perkin-Elmer R-32, 90 MHz.

[4] Nicolet Technology Corp. (at the Purdue University Regional Biochemical Magnetic Resonance Laboratory).

[5] Purdue University CDC computer system.

[6] Sigma Chemical Co.

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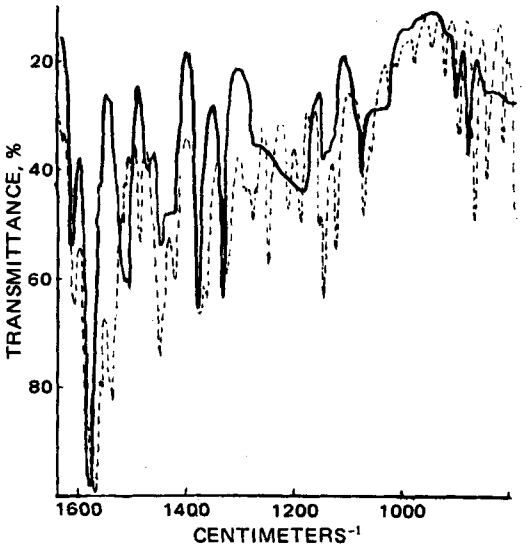


Figure 1—*Solid (---) and solution ($CHCl_3$) (—) IR spectra of chloroquine.*

the methylene chloride was removed by vacuum distillation. A yellow oil remained, which crystallized after 12 hr, mp 86–88°.

Solutions—All IR solutions were prepared with spectral grade chloroform. Concentrations were adjusted to maximize the signal in the fingerprint region.

The following solvents[7] were used for NMR studies: chloroform-d_1 (99.8 atom % D), acetone-d_6 (99.5 atom % D), dimethyl sulfoxide-d_6 (99.5 atom % D), deuterium oxide (99.7 atom % D), and pyridine-d_5 (99 atom % D). Tetramethylsilane was used as the internal standard in organic solvents. In deuterium oxide, the chemical shifts were usually reported relative to the HDO resonance position.

The following solution concentrations were used: 10–30 mg/0.5 ml for the 60- and 90-MHz spectra, 5–10 mg for the 80-MHz spectra, and 2–3 mg for the 360-MHz spectra. All solutions were filtered with a fritted-glass filter and were placed immediately in 5-mm tubes.

Computer Analysis—Chemical shifts were extracted from the spectra, and representative coupling constants were submitted to the LAOCN3 computer program (27). These calculated frequencies then were assigned to experimentally observed frequencies. The same input data along with those assignments then were submitted to LAOCN3. Calculated chemical shifts and coupling constants were produced that best fit the experimental spectrum. After most assignments were made, the calculated spectral data were submitted as input data to LAOCN3 and the process was repeated until a suitable simulation was obtained.

RESULTS

Chloroquine—The IR spectra of chloroquine in solution and in the solid state are shown in Fig. 1. The 360-MHz NMR spectrum of chloroquine in acetone gave the following data: 0.98 (t, 6H), 1.32 (d, 3H), 1.60 (m, 3H), 1.80 (m, 1H), 2.07 [m, $(CH_2H)_2CO$], 2.42 (t, 2H), 2.47 (m, 4H), 3.30 (s, H_2O), 3.93 (m, 1H), 6.49 (d, 1H), 6.60 (d, 1H), 7.37 (dd, 1H), 7.88 (d, 1H), 8.25 (d, 1H), and 8.47 (d, 1H) ppm.

The region of interest is the aliphatic portion of the spectrum, and the following assignments were made for that area. The triplet at 0.98 ppm was assigned to the methyl protons of the ethyl group, and the multiplet at 2.47 ppm was assigned to the methylene protons of this group. The methylene protons adjacent to the nitrogen on C_δ were assigned to the triplet at 2.42 ppm. The multiplet at 1.60 ppm was from the two C_γ protons and one C_β proton, while the other C_β proton was assigned to the multiplet at 1.80 ppm. The multiplet at 3.93 ppm was assigned to the C_α proton, and the doublet at 1.32 ppm was assigned to the $C_{\alpha'}$ methyl protons.

LAOCN3 analysis gave the following set of vicinal coupling constants: $J_{\alpha,\beta} = 8.28$ Hz, $J_{\alpha,\beta'} = 8.28$ Hz, $J_{\beta,\gamma} = 8.77$ Hz, $J_{\beta',\gamma} = 7.35$ Hz, and $J_{\gamma,\delta} = 5.92$ Hz. The simulated spectrum obtained from these coupling constants and the observed spectrum are shown in Fig. 2.

These coupling constants can be used to estimate the population of

[7] Aldrich Chemical Co.

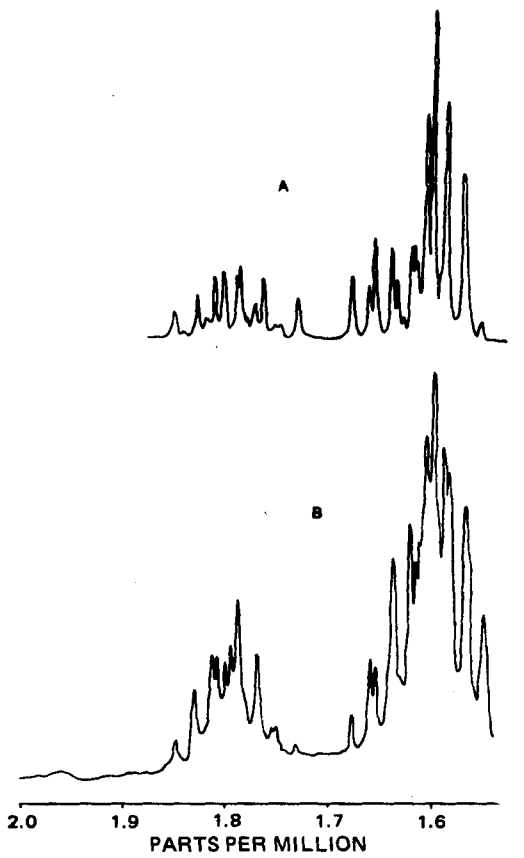


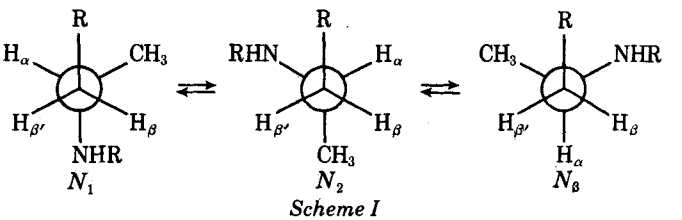
Figure 2—*Observed (B) and simulated (A) 360-MHz spectra of chloroquine in the range of 550–675 Hz. The simulated spectrum was obtained from the LAOCN3 stick plot. A 1.5-Hz linewidth was used.*

rotamers according to published procedures (28–31). The conformation about the α–β bond can be approximated using Eqs. 1–3 and Scheme I as derived by Abraham and Pachler (28):

$$N_1 = \frac{J_{\alpha\beta} - J_g}{J_t - J_g} \qquad \text{(Eq. 1)}$$

$$N_2 = \frac{J_{\alpha\beta'} - J_g}{J_t - J_g} \qquad \text{(Eq. 2)}$$

$$N_3 = 1 - N_1 - N_2 \qquad \text{(Eq. 3)}$$



Scheme I

In these equations, N_1, N_2, and N_3 are the populations of the rotamers shown and $J_t = 13.11$ Hz and $J_g = 3.63$ Hz. Solving these equations gives $N_1 = N_2 = 0.49$ and $N_3 = 0.02$. While these numbers should be considered as estimates due to the assumptions involved, the sterically hindered conformer N_3 is obviously energetically unfavorable.

The conformation about the β–γ bond can be determined using the following procedure (28–30). Let $N = J_{\beta\gamma} + J_{\beta'\gamma}$ and $L = J_{\beta\gamma} - J_{\beta'\gamma}$. Since it is impossible to differentiate $J_{\beta\gamma}$ and $J_{\beta'\gamma}$, L can have either a positive or negative value. The equation $\frac{1}{2}N + \frac{1}{6}L = 17.97 - 0.80\Sigma E$ can be used to determine whether L is positive or negative. The value of ΣE is calculated with the assumption that L is both positive and negative and ΣE is calculated from tables provided in the literature (32). Usually, a clear indication that L is positive or negative is obtained. If L is positive, the *gauche*-rotamers predominate; if L is negative, the *trans*-rotamers predominate. If L is zero, there is a nearly equal proportion of all rotamers. For the β–γ bond, $N = 16.12$ and $L = \pm 1.42$. This small value of

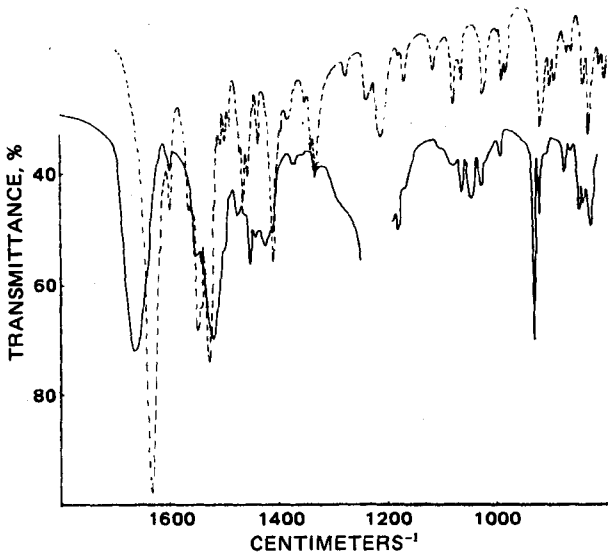


Figure 3—*IR spectra of nialamide in the solid state (- - -) and in solution ($CHCl_3$) (——).*

L indicates that the *gauche*- and *trans*-rotamers are of nearly equal energy. If L is positive, ΣE is calculated to be 12.09; if L is negative, ΣE is calculated to be 12.69. The theoretical ΣE is 14.00. Since L may be negative, this exercise indicates that the *trans*-rotamer may be of slightly lower energy than the *gauche*-rotamers.

The conformation about the γ–δ bond can be analyzed in the same way. In this case, $L = 0$, indicating that the *gauche*- and *trans*-isomers are of equal energy.

Nialamide—The IR spectra of nialamide (I) in the solid state and in solution can be compared in Fig. 3. The 80-MHz NMR spectrum of nialamide in pyridine gave the following data: 2.85 (t, 2H), 3.66 (t, 2H), 4.68 (d, 2H), and 5.17 (s) ppm. The triplets at 2.85 and 3.66 ppm were assigned to the methylene protons on C_β and C_α, respectively. The doublet at 4.68 ppm was assigned to the benzyl protons. The broad singlet at 5.17 ppm was thought to be due to all of the nitrogen protons in the molecule. The coupling constant for $J_{\alpha,\beta}$ was 6.5 Hz. This coupling constant gave $L = 0$ and indicates that all three rotamers are equally populated in the absence of the deceptively simple catastrophe.

Azaperone—Figure 4 shows the comparison of the solid and solution IR spectra of azaperone. The 90-MHz NMR spectrum in chloroform gave the following data: 2.00 (m, 2H), 2.45 (t, 2H), 2.55 (t, 4H), 3.00 (t, 2H), and 3.49 (t, 4H) ppm. The multiplet at 2.00 ppm was assigned to the C_β methylene protons. The signals due to the C_γ and C_α methylene protons were determined to be the triplets at 2.45 and 3.00 ppm, respectively. The triplets at 2.55 and 3.49 ppm were assigned to the piperazinyl protons. The coupling constants were $J_{\beta,\gamma} = 7.0$ Hz and $J_{\alpha,\beta} = 7.1$ Hz. These data indicate that $L = 0$ for both the β,γ and α,β bonds, thus suggesting that the *gauche*- and *trans*-rotamers are of equal energy.

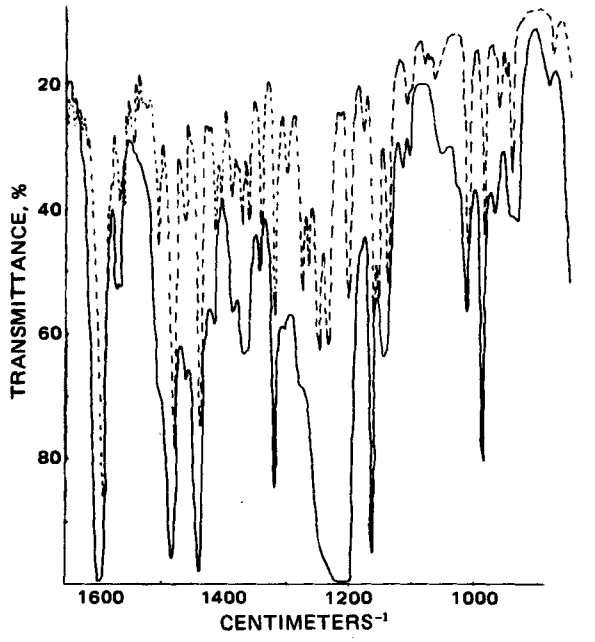


Figure 4—*IR spectra of azaperone in the solid state (- - -) and in solution ($CHCl_3$). (——)*

DISCUSSION

Comparison of the IR spectra of azaperone (II) in the solid state and in solution (Fig. 4) indicates that the solid and solution conformations are the same. An extremely good correlation can be seen between peak positions as well as between relative intensities. Since the *trans*-rotamer about both the α–β and β–γ bond is frozen in the solid, this rotamer probably predominates in solution. However, the NMR spectrum of azaperone is characteristic of a rapidly rotating A_2B_2 system about both the C_α–C_β bond axis and the C_β–C_γ bond axis. The coupling constants produce rotamer percentages that indicate (within experimental error) that the molecule exists one-third of the time as each rotamer about each bond. In other words, the molecule is rapidly rotating and there is no preference for any one of the three rotamers. In addition, theoretical calculations of the populations of the rotamers of butyrophenones related to azaperone showed that the *gauche*- and *trans*-conformers about the β–γ and γ–δ bonds were nearly equally populated (33). If it is rotating rapidly, it would be impossible for the solid-state conformation to be the same as the solution conformation; thus, the general utility of the IR–X-ray method must be questioned.

The earlier analysis of methapyrilene intensifies this contradiction (21). Comparison of the solid-state and solution IR spectra indicates the same results as with azaperone; the conformations in both states should be the same. Once again, the NMR spectrum is in disagreement. The methylene protons of methapyrilene produce two triplets in chloroform solution, indicative of rapid rotation. The NMR results for diphenhydramine in chloroform also indicate that the *gauche*- and *trans*-rotamers are of nearly equal energy; however, in this case, the solid-state and solution spectra are different, substantiating these results (21) and indicating that the solid-state and solution conformations are different.

To investigate these contradictions, the peaks in the fingerprint region of the IR spectrum were characterized. The theory behind the IR–X-ray method (21) states that conformational changes are reflected by changes in the absorption frequency of a given vibration. Only vibrations that are intimately involved with rotations about the ethane fragment or that come in close contact with it upon rotational change reflect any conformational change. Therefore, it is necessary to decide whether the fingerprint region contains enough absorbances that can reliably reflect conformational change or whether the region is dominated by absorbances of rigid structural components whose vibrations are not significantly affected by rotation.

Characterization of the azaperone IR spectrum leads to the finding that most major absorbances are due to rigid structural components. They dominate the entire region and mask any absorbances due to the ethane fragment. For example, three positions of absorbance of the fluorobenzene component are at 1600, 1480, and 1160 cm^{-1}. These peaks are all dominating in the fingerprint region. Many more absorbances can be assigned to the fluorobenzene portion of the molecule along with contributions from the pyridine and piparazine ring systems. Actually, most peaks in the IR spectrum are due to these three components. Likewise, it is evident that the absorbances in the methapyrilene IR spectrum cannot reflect changes in conformation. These absorbances can be attributed to the pyridine and thiophene portions of the molecule. Thus, in these cases, the IR–X-ray method probably is unreliable.

Similar characterizations of the IR spectra of nialamide (I), diphenhydramine, and chloroquine (III) provide different results. The IR spectra of nialamide indicate that the solid and solution conformations differ, and the NMR data are in agreement with these results. Once again, equal proportion of rotamers about the ethane fragment is found in solution while the crystal structure shows that the *gauche*-rotamer is trapped in the solid state (24). Some major spectral lines are due to pyridine. The phenyl ring contributes only a small number of sharp absorbances to the spectrum. Therefore, there is a sufficient number of spectral lines to reflect conformational change, and the method is valid. Similar results for diphenhydramine explain the agreement between the IR and NMR data. The two phenyl rings create little interference in the IR spectrum, and once again the analysis is valid.

The quinoline ring system of chloroquine is similar to the phenyl ring in contributing a few sharp peaks to the IR spectrum; thus, the IR–X-ray

method is valid for analysis of this compound. Indeed, the IR spectra of chloroquine in the solid state and in solution are quite different. This result is confirmed by comparison of the NMR data and the crystal structure. The NMR data indicate that rotamers N_1 and N_2 predominate for the α–β bond while nearly an equal proportion of rotamers exists for the β–γ and γ–δ bonds. In the solid state, the crystal structure shows that rotamer N_2 is frozen for the α–β bond, the *trans*-rotamer is frozen for the β–γ bond, and the *gauche*-rotamer is frozen for the γ–β bond.

In addition, the solution conformation of chloroquine was determined without shift reagents and the results of this analysis were similar to those obtained with shift reagents (26), indicating that the shift reagent did not greatly alter the conformation of chloroquine.

In the NMR experiment, many of the compounds produced sets of triplets with coupling constants that indicate equal proportions of rotamers. These spectra may be deceptively simple (28), which would make the NMR results unreliable. Deceptively simple means that there is more than one set of chemical shifts and coupling constants that could produce the same NMR spectrum; therefore, there could be different proportions of rotamers. To ensure the reliability of the NMR data, this problem is attacked using nialamide as the example. If the two triplets in the nialamide spectrum arose from a conformation that was strongly preferred due to intramolecular or intermolecular interaction, this conformation probably would be similar to the conformation that is frozen upon crystallization. By taking the hydrogen atom coordinates from the crystal structure, the solid-state dihedral angles can be calculated using the BONDLA computer program (34). Solid-state coupling constants then are determined from the Karplus equation and are: $J_{\alpha,\beta} = 2.97$ Hz, $J_{\alpha,\beta'} = 7.23$ Hz, $J_{\alpha',\beta} = 12.8$ Hz, and $J_{\alpha',\beta'} = 3.31$ Hz. These coupling constants produce a complex *AA'BB'*-simulated NMR spectrum, which has little resemblance to the experimentally observed spectrum. Therefore, the NMR technique apparently is reliable.

In conclusion, these results indicate that if a compound contains two or more heteroatomic rings, any IR spectral changes due to conformational changes probably are masked by the absorption of these ring systems. The fingerprint region of the IR spectrum for all compounds containing rigid structures must be characterized prior to the use of the IR–X-ray method to ensure its validity.

This paper also shows how a combination of IR and NMR spectroscopy and X-ray crystallography can be used to provide a complete picture of the conformational features of drugs. In addition, the study illustrates the importance of comparing solid-state and solution conformations before making pharmacological suggestions based on the conformation in the solid state. In nialamide, azaperone, and chloroquine, the solid-state conformation represents only one of several nearly equally energetic solution conformers, which clearly shows that the conformer frozen in the solid state cannot be assumed to be the pharmacologically active or lowest energy solution conformer.

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