Comparison of the Solid and Solution Conformations of Methapyriline, Tripelennamine, Diphenhydramine, Histamine, and Choline. The Infrared–X-Ray Method for Determination of Solution Conformations

Stephen R. Byrn,* Constance Wier Graber, and Sharon L. Midland

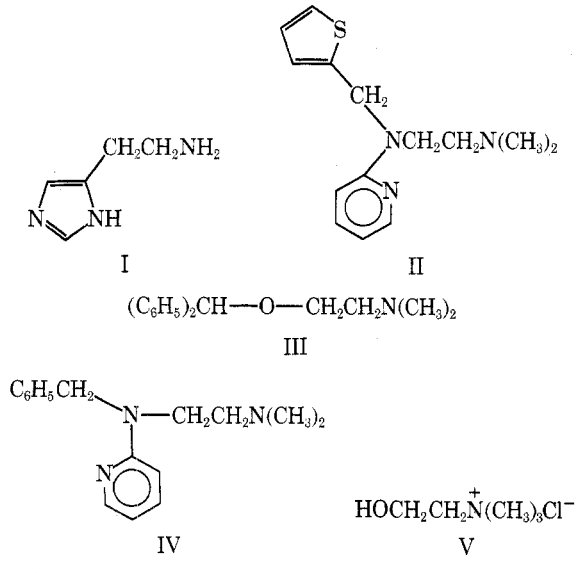
Department of Medicinal Chemistry and Pharmacognosy, School of Pharmacy and Pharmacal Sciences, Purdue University, West Lafayette, Indiana 47907

Received December 11, 1975

The ir–x-ray method for the determination of solution conformation is discussed and illustrated using choline chloride, histamine, and three antihistamines. Infrared spectroscopy was used as an experimental test of the equivalence of conformations in the solid state and solution, provided large groups do not obscure the fingerprint region. If the solid state and solution spectra are identical then a knowledge of the crystal structure gives the conformation in solution. This method indicated that as expected based on reports in the literature the conformation of choline was essentially the same in the solid state and solution. It also showed that, in contrast to reports in the literature, there was a predominance of the trans conformation of methapyriline hydrochloride (2-[(2-dimethylaminoethyl)-2-thenylamino]pyridine hydrochloride) in solution. Tripelennamine hydrochloride (2-[benzyl(2-dimethylaminoethyl)amino]pyridine hydrochloride) was also probably in the trans conformation both in solution and in the solid state. The solid state and solution infrared spectra of histamine were significantly different as expected based on reports in the literature. Similarly, differences in the published infrared spectra of solid acetyl choline chloride and bromide were consistent with x-ray studies which showed that there were different conformations about the ethyl carbon atom–ester oxygen bond in these two salts. The solid state and solution spectra of diphenhydramine hydrochloride are different suggesting that diphenhydramine has a different conformation in crystals and in solutions of this salt.

The conformations of molecules in the solid and solution have been compared using x-ray crystallography and NMR spectroscopy,[1] x-ray crystallography and circular dichroism,[2] x-ray crystallography and dipole moments,[3] infrared and NMR spectroscopy,[4] and infrared spectroscopy.[5]

In this paper we report the use of x-ray crystallography and infrared spectroscopy (the ir–x-ray method) to compare the solid and solution conformations of histamine (I), antihistamines (II–IV), and choline (V). These studies show that x-ray crystallography and infrared spectra can be combined to provide a reliable method for determination of solution conformations.



Experimental Section

X-Ray Powder Diffraction Measurements. The 2-[(2-dimethylaminoethyl)-2-thenylamino]pyridine hydrochloride (methapyrilene hydrochloride) used was a gift from Abbott Laboratories, Chicago, Ill., for which we are grateful. The other compounds used were commercially available. The powder diffraction pattern of ground crystals of the material of interest was measured using a Debye-Scherrer powder camera with Ni-filtered Cu radiation. Intensities were estimated visually. The most intense observed bands of this pattern were then compared to the most intense bands calculated using the atom positions available from the crystal structure determination and the program POWD5.[6]

Infrared Difference Spectra. The ir difference spectra were measured by placing a Nujol mull of the material of interest and $CHCl_3$ solvent (in 0.5-mm NaCl cells) in the sample beam and a reference beam attenuator and a $CHCl_3$ solution of the material in the reference beam. The reference beam attenuator was used to adjust the % T (transmittance) of the mull spectra at 1800 cm^{-1} to approximately that of the solution. Before scanning the spectrum the pen was adjusted to about 50% T. To match the concentration of the $CHCl_3$ solution to that of the mull, the $CHCl_3$ solution was successively diluted, usually 3 parts of solution to 1 part of solvent, and the difference spectrum remeasured. This process was repeated until the difference spectra passed through a point where there was the least deviation from the 50% T line and began to resemble the mull spectra.

Spectral and Powder Diffraction Analysis of Choline Chloride (V) (Ethanaminium, 2-Hydroxy-*N,N,N*-trimethyl Chloride). Choline chloride was dried in a vacuum before use. It was extremely deliquescent and the Nujol mulls were prepared in a glove bag under nitrogen atmosphere. The capillary tubes for powder diffraction were also filled in this glove bag and sealed immediately upon removal.

The infrared spectra of choline chloride in Nujol mull and ca. 40% water solution are shown in Figure 1.

Spectral and Powder X-Ray Diffraction Analysis of Methapyrilene Hydrochloride (2-[(2-Dimethylaminoethyl)-2-thenylamino]pyridine Hydrochloride, II HCl). Methapyriline hydrochloride (II HCl) was recrystallized in plates from 2-propanol, acetone, or ethyl acetate, in fine needles from 1,2-dichloroethane, and in clumps from ethanol or chloroform. The ir spectra (Nujol mull) of these crystals were identical with each other and with the spectrum (Nujol mull) of a cooled melt of II HCl. The ir spectra of a Nujol mull and chloroform solution of II HCl are shown in Figure 2 along with the solution–solid state difference spectra. The ir spectrum of II HCl in these solvents was also virtually identical with the ir spectra of II HCl in H_2O in the regions not obscured by solvent absorption.

The free base, methapyriline (II), was prepared by making alkaline a H_2O solution of II HCl and extracting with ether. The ether was dried and then evaporated giving II which had an ir spectrum (in $CHCl_3$) which was virtually identical with that of II HCl except for the 2850-cm^{-1} peak due to the free base and the 990-cm^{-1} peak which was absent from the spectrum of II HCl.

The 60- and 100-MHz spectra of II HCl showed broadened triplets for the ethylene protons and this region of the spectrum is shown in Figure 3. The NMR spectra of II HCl in Me_2SO-d_6, CH_3CN, CD_3OD, and CH_3NO_2 were, in general, quite similar to these two spectra except for slight solvent-induced changes in chemical shift. Broadened triplets were observed for the $-CH_2-$ group when its absorbances were not obscured by absorptions of these solvents. Methapyrilene free base

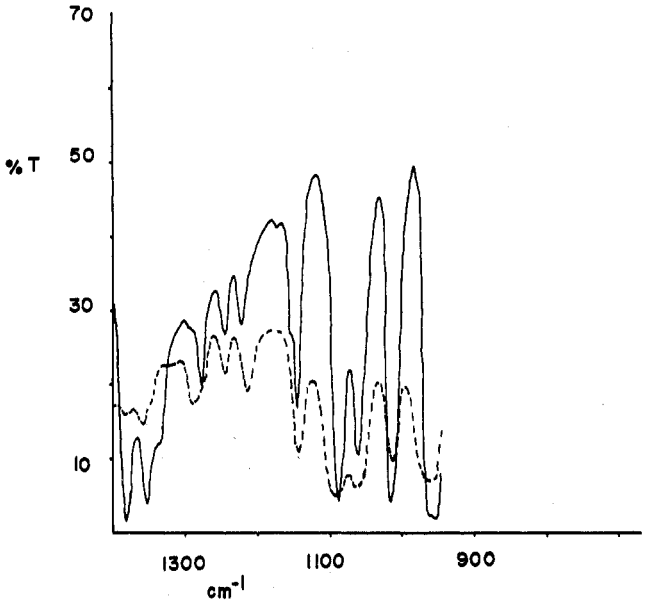


Figure 1. Ir spectra of choline chloride (V) in the solid state and in solution [(—) Nujol mull, (- - -) H_2O solution]. The peak at 1380 (cm^{-1}) in the mull spectrum is due to Nujol.

also exhibited broadened ethylene triplets ($W_{1/2} \approx 4$ Hz, $J \approx 7$ Hz) although their chemical shifts changed substantially as would be expected owing to deprotonation.

Spectral Analysis of 2-[Benzyl(2-dimethylaminoethyl)-amino]pyridine HCl (Tripelennamine HCl). Tripelennamine hydrochloride (IV HCl) was recrystallized from acetone in needles and plates and from 1,2-dichloroethane in plates. This antihistamine (IV HCl) was also recrystallized from ethanol, chloroform, methanol, water, methylene chloride, and from the melt. The ir spectra (Nujol mull) of all of these crystals were identical. The spectra of tripelennamine hydrochloride in $CHCl_3$ and Nujol mull and the solid state and solution difference spectra are shown in Figure 4. The ir spectrum of the free base of IV in $CHCl_3$ prepared in the same way as the free base of II was virtually identical with the spectra of IV HCl in $CHCl_3$ except for differences due to the deprotonation of the $-NMe_2$ group and a peak at 990 cm^{-1} in the free base which was absent in IV HCl.

The ethylene regions of the 60-MHz NMR spectra of IV HCl and IV were similar to the spectra of II HCl (see Figure 3).

Spectral and Powder X-Ray Diffraction Studies of Histamine (I). The free base of histamine was studied in the solid state and in solution. The ir spectra of histamine in Nujol mull and $CHCl_3$ solution along with the solid state and solution difference spectra are shown in Figure 5. The NMR spectrum of histamine in $CDCl_3$ showed the following absorptions (δ): 2.87 (4 H, 8-peak multiplet, $-CH_2CH_2-$), 5.12 (3 H, s, NH), 6.88 (1 H, s, ring CH), 7.12 (1 H, d, ring CH). The NMR spectrum (in $CDCl_3$) of the CH_2CH_2 group of histamine expanded and decoupled was nearly identical with the published decoupled spectrum of this group at pH 7.0 (monocation) in water solution.[7]

Spectral and Single-Crystal X-Ray Studies of Diphenhydramine Hydrochloride (2-Diphenylmethoxy-*N,N*-dimethylethylamine Hydrochloride, III HCl). Diphenhydramine hydrochloride (III HCl) was crystallized from acetone, ethyl acetate, ethanol–ethyl acetate, or benzene–chloroform (5:2) in needles, from ethanol in plates, from chloroform in clumps, and from the melt. The ir spectra (Nujol mull) of all of these crystals were essentially identical but differed from the spectrum of III HCl in $CHCl_3$ (see Figure 6). The ir spectrum of the free base (III) was different from both the solid state and solution spectra of III HCl. The 60-MHz NMR spectra of III HCl and III were also distinctly different. The ethylene protons of III were sharp triplets while those of III HCl were three-peak multiplets. However, these differences may be due to the fact that the chemical shift difference between the two methylene groups was much greater in the free base (III) than the hydrochloride (III HCl).

Diphenhydramine HCl (III HCl) crystallized [from benzene–$CHCl_3$ (5:2)] in hygroscopic needles which belong to the orthorhombic crystal class. Least-squares refinement of the 2θ values of 17 carefully centered reflections gave the following cell parameters (std dev): a = 10.596 (2), b = 14.311 (2), c = 10.773 (2) Å. The ρ calcd for $Z = 4$ molecules of III HCl ($C_{17}H_{21}NO$·HCl, mol wt 291.82) was 1.19 g/cm^3, ρ obsd = 1.18 g/cm^3. The following systematic absences were observed: $0kl$, $k + l = 2n + 1$; $h0l$, $l = 2n + 1$ indicating that III HCl belonged to the space group $Pna2_1$ or to the space group $Pnma$ and the molecule situated on a mirror plane. Several attempts to determine the structure of III HCl in each of these space groups using both the programs of x-ray series[8] and the MULTAN[9] series were unsuccessful.

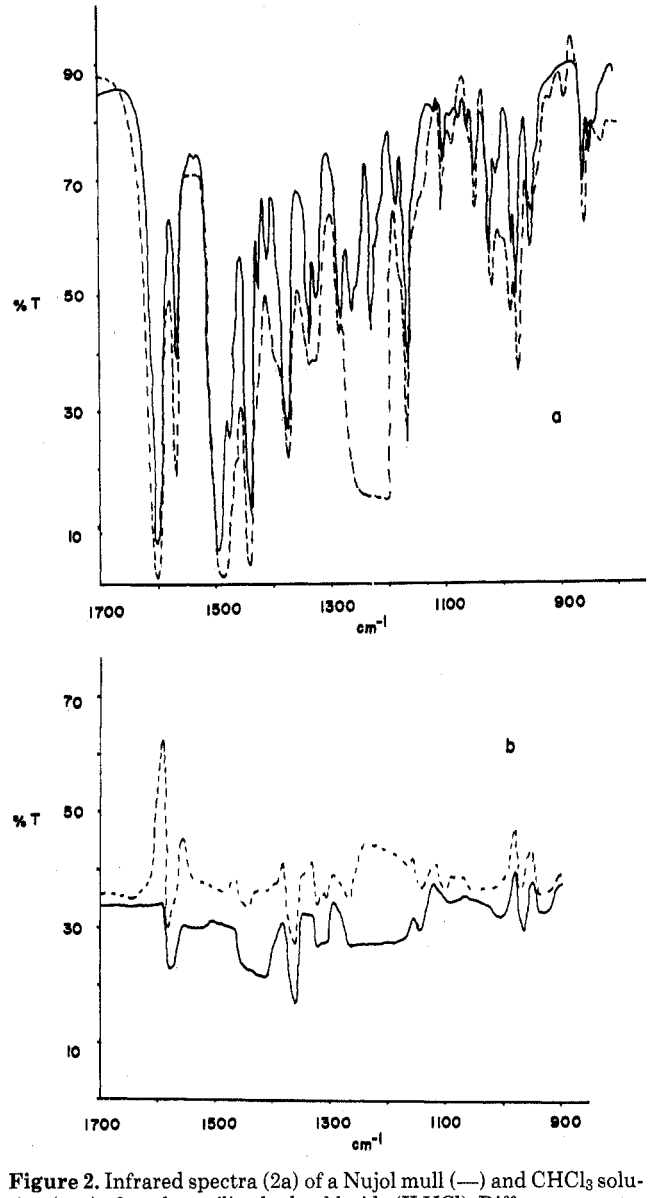


Figure 2. Infrared spectra (2a) of a Nujol mull (—) and $CHCl_3$ solution (- - -) of methapyriline hydrochloride (II HCl). Difference spectra (2b) between a Nujol mull and $CHCl_3$ solution of methapyriline hydrochloride. The dashed line (- - -) corresponds to a solution concentration slightly more than that of the mull and the solid line (—) corresponds to a solution concentration slightly less than that of the mull.

Results and Discussion

General Description of the Method. The method employed to determine the solution conformation of biologically important ethylene derivatives involves three steps: (1) determination of the crystal structure of the compound; (2) establishment that grinding the compound to a powder does not induce a crystalline phase transition; and (3) comparison of the infrared spectra in the solid state and in solution. Step 1 is accomplished using normal single-crystal x-ray techniques.

Since solid-state phase transformations have been reported to occur upon grinding,[10] step 2 was required to establish that the conformation of the compound in the powdered sample was identical with that in single crystals and step 2 was ac-

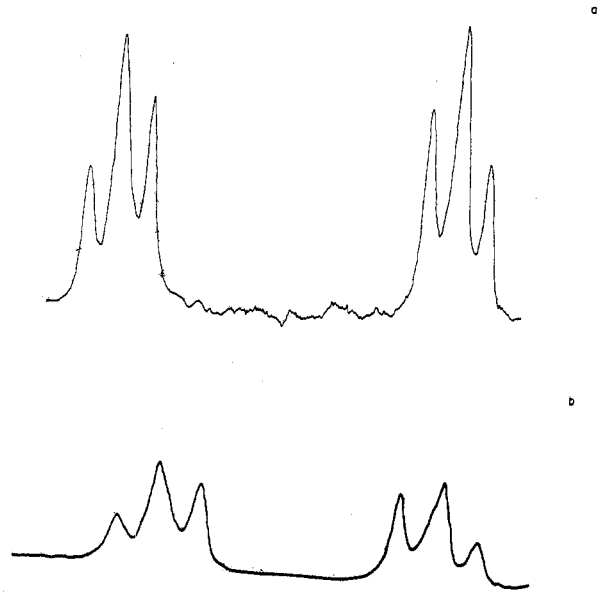


Figure 3. Observed 100-MHz (3a) and 60-MHz (3b) NMR spectra of methapyriline hydrochloride (II HCl) in $CDCl_3$. The scale in hertz is different for the two spectra.

complished by comparing the observed powder diffraction pattern to the theoretical diffraction pattern calculated from the atom coordinates available from the single-crystal x-ray study.

Three methods are available for comparison of solid-state and solution infrared spectra (step 3): (1) visual comparison by superposition of the two spectra; (2) measurement of difference spectra as suggested by Grutzner,[11] and (3) tabular comparison of the absorption maxima. All three methods were used; however, the quickest comparison can often be made using the solid-state and solution difference spectra. Perfect agreement between solid-state and solution spectra was not expected since there are differences in band widths and selection rules between the solid state and solution. We have adopted the working hypothesis that for organic molecules of this size and uneven shape solid state–solution difference spectra will be considered identical if there are three or fewer deviations of greater than 10% T from the center line and no deviation greater than 25% T.

If the solution and solid-state infrared spectra are different further studies are required to establish the bonds about which the conformations differ.

Cases Where the Solid State Conformation Predominates in Solution. A. Choline Chloride. Choline chloride has the gauche conformation in the solid state[12] and NMR studies indicated that the gauche conformation also predominated in water solution (ca. 90% gauche, 10% trans).[13] Powder diffraction experiments showed that a phase transition was not induced by grinding. The ten most intense observed peaks corresponded to eight of the ten most intense calculated peaks. Figure 1 shows that the solid and solution ir spectra of choline chloride are identical. Thus it has essentially the same conformation in the solid state and in solution and the ir studies confirm the NMR analysis.

B. Methapyriline Hydrochloride. The crystal structure of methapyriline hydrochloride (II HCl) showed that the NCH_2CH_2N group had the trans conformation in the solid state.[14] There were two molecules per asymmetric unit in crystals of II HCl; these two molecules had different conformations about the C–N–pyridyl(–thenyl) bond. The powder pattern of a sample of plates of II HCl grown from 2-propanol was very similar to the powder pattern calculated from the single-crystal atomic coordinates using the program POWD5.

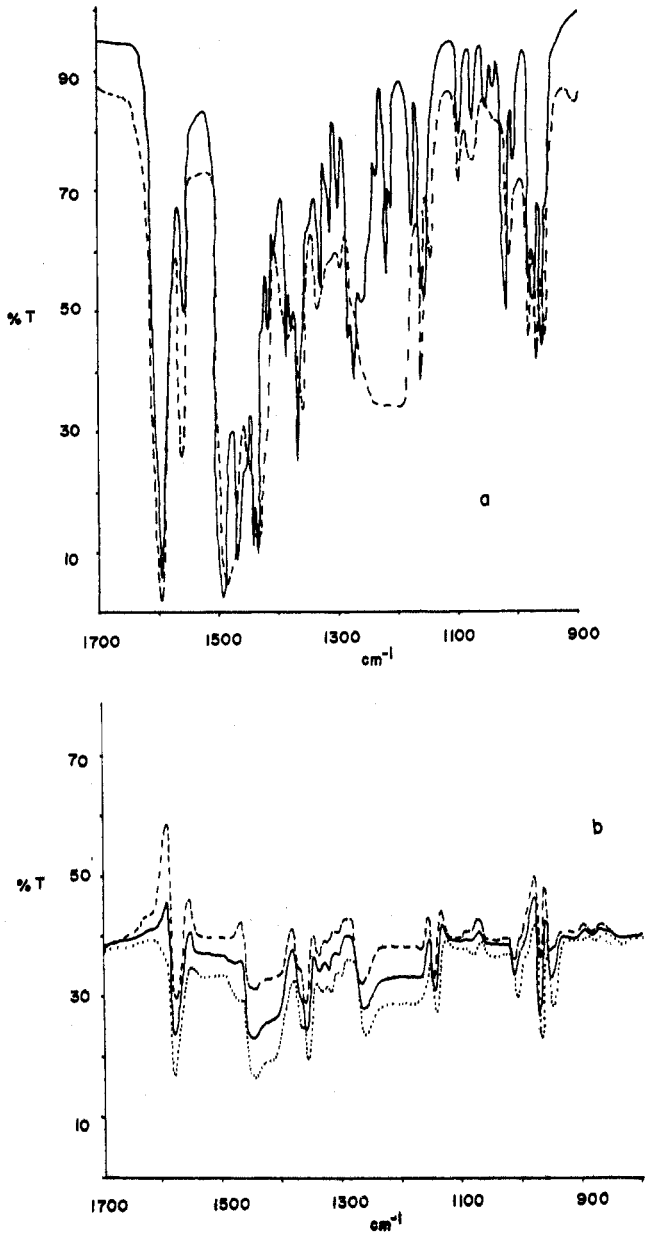


Figure 4. Infrared spectra (4a) of a Nujol mull (—) and $CHCl_3$ solution (- - -) of tripelennamine hydrochloride (IV HCl). Difference spectra (4b) between a Nujol mull and $CHCl_3$ solution of tripelennamine hydrochloride. The dashed line (- - -) corresponds to a solution concentration slightly more than that of the mull, the dotted line (· · ·) corresponds to a solution concentration slightly less than that of the mull, and the solid line (—) corresponds to a solution concentration nearly equal to that of the mull.

The 14 most intense observed lines in the powder pattern of ground II HCl corresponded to 14 of the 15 most intense calculated lines. This experiment showed that a phase transition was not induced upon grinding. The ir spectrum of these plates was virtually identical with the ir spectrum of needles grown from ethyl acetate and also with the ir spectra of crystals obtained from acetone, 1,2-dichloroethane, ethanol, or chloroform. These solid-state ir spectra were also very similar to the solution spectra of II HCl in chloroform, carbon tetrachloride, or water (see Figure 2a). The solid state and solution infrared difference spectra indicated that the spectra were similar although three deviations of greater than 10% T were observed. However, a solution concentration between that used for the difference spectra would almost certainly show no deviations of greater than 10% T. The ir spectrum of the free base, methapyriline (II), was also quite similar to these

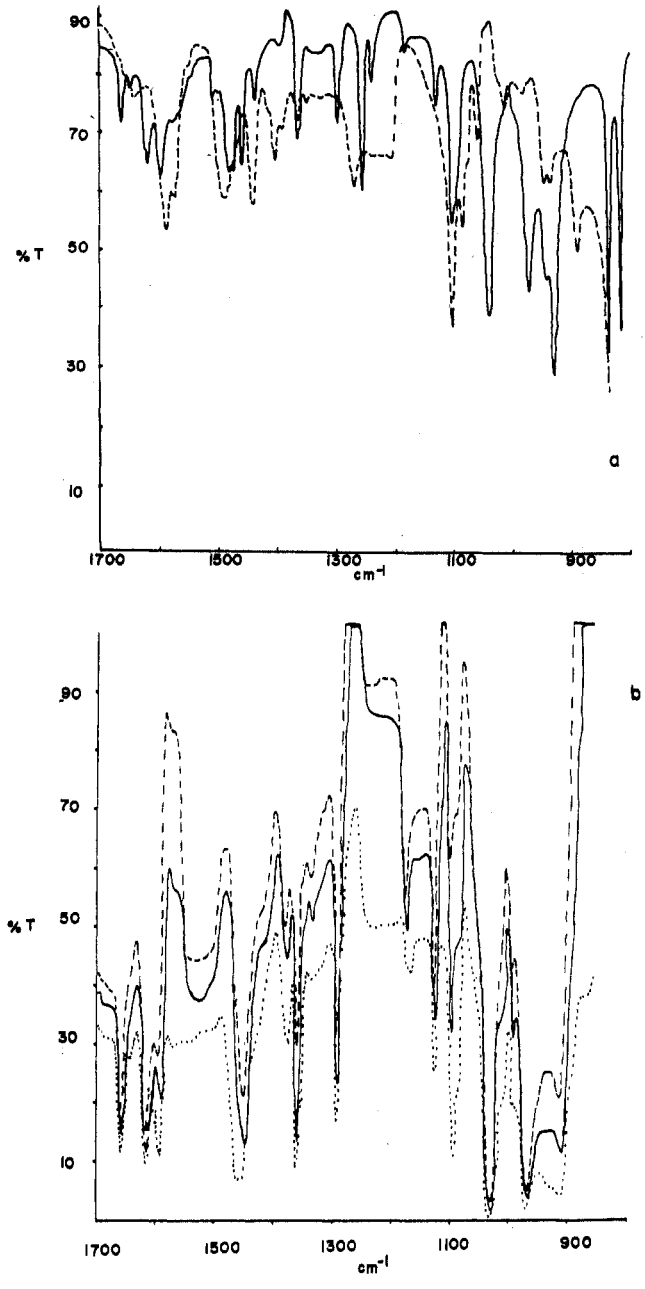


Figure 5. Infrared spectra (5a) of a Nujol mull (—) and $CHCl_3$ solution (- - -) of histamine (I). Difference spectra (5b) between a Nujol mull and a $CHCl_3$ solution of I. The dashed lines (- - -) corresponds to a solution concentration slightly more than that of the mull, the dotted line (· · ·) corresponds to a solution concentration slightly less than that of the mull, and the solid line (—) corresponds to a solution concentration nearly equal to that of the mull.

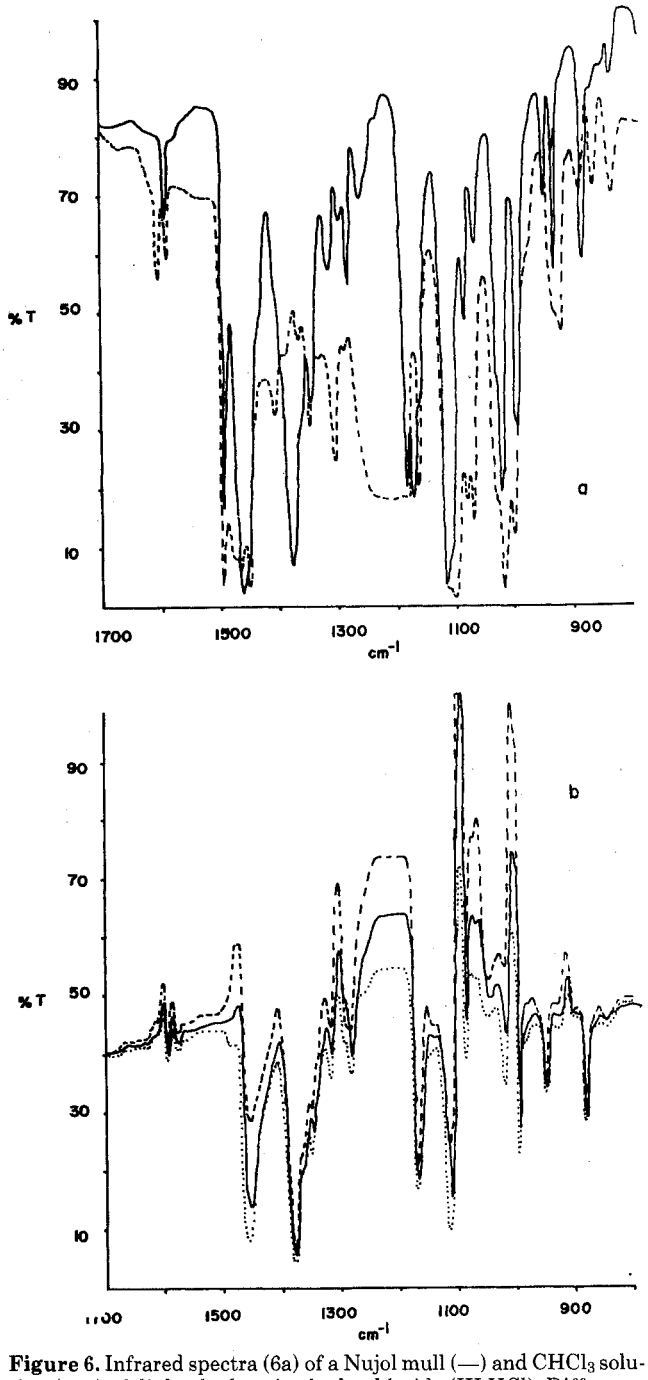


Figure 6. Infrared spectra (6a) of a Nujol mull (—) and $CHCl_3$ solution (- - -) of diphenhydramine hydrochloride (III HCl). Difference spectra (6b) between a Nujol mull and a $CHCl_3$ solution of III HCl. The dashed line (- - -) corresponds to a solution concentration slightly more than that of the mull, the dotted line (· · ·) corresponds to a solution concentration slightly less than that of the mull, and the solid line (—) corresponds to a solution concentration nearly equal to that of the mull.

solution spectra. This data indicates that methapyriline hydrochloride is in the same conformation in the solid state and in solution and the x-ray data show that this is the trans conformation. These data also indicate that there are two conformers about the C–N–pyridyl(–thenyl) bond in solution as in the solid.

The NMR spectra of methapyriline hydrochloride are also consistent with the assignment of a predominance of the trans conformation of methapyriline hydrochloride in solution. The spectra of the NCH_2CH_2N group of II HCl in chloroform-d_1, dimethyl sulfoxide-d_6, acetonitrile-d_3, methanol-d_4, and nitromethane were very similar except for slight solvent-induced chemical shift changes. Figure 3 shows expanded scale NMR spectra of the NCH_2CH_2N group of II HCl in $CDCl_3$ at 60 and 100 MHz.

The NMR spectra of methapyriline hydrochloride (II HCl) were calculated using coupling constants based on the solid state H–C–H and H–C–C–H angles and the Karplus[15] and Gutowsky–Karplus–Grant[16] relationships and the program NMRCAL.[17] These spectra were similar but not entirely consistent with the observed 60- and 100-MHz spectra (Figure 3). Nevertheless this exercise showed that these NMR spectra are "deceptively simple spectra"[18] and could be reproduced by a number of coupling constants and/or proportions of conformers. The ir–x-ray method allows clarification of such situations and is recommended in all cases where deceptively simple spectra are suspected.

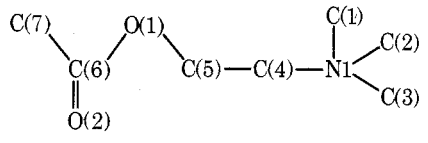
The evidence presented above, which is both experimental and theoretical, strongly suggests that the earlier suggestion[19] of methapyriline hydrochloride existing in an equally populated mixture of gauche and trans isomers in solution should be revised. The ir data in the solid state and in solution suggest that a conservative estimate of the percentage of trans conformer in solution would be 85–90%. The predominance of the trans ethylenediamine linkage is not consistent with experimental and theoretical studies of the parent compound ethylenediamine. These studies showed that gauche conformations were perferred over the trans by 1–2 kcal/mol in part due to the N–H···N hydrogen bond.[20] Electrostatic interactions rather than hydrogen bonding have been suggested to be the factors responsible for the predominance of gauche conformer in crystals and solutions of acetyl choline and derivatives.[13b] Apparently, the relatively weak N–H···N hydrogen bond and/or the H···N electrostatic interaction do not provide sufficient energy to overcome the steric repulsion involved in the gauche conformation of these substituted ethylenediamines. The NMR and ir spectra of the free base of methapyriline suggest that it is also in the trans conformation in solution.

C. Tripelennamine Hydrochloride. The solid-state infrared spectra (Nujol mull) of crystals of tripelennamine hydrochloride (IV HCl) grown from 1,2-dichloroethane, ethanol, chloroform, methanol, water, methylene chloride, or the melt were identical. These solid-state ir spectra were also virtually identical with the spectrum of IV HCl in chloroform (see Figure 4) and the solid-state and solution infrared difference spectra showed only three deviations of just slightly more than 10% T. The NMR spectra of the NCH_2CH_2N group of IV HCl in deuteriochloroform and D_2O were very similar to the spectra of the ethylenediamine portion of methapyriline hydrochloride.

These data suggest that tripelennamine hydrochloride, like methapyrilene hydrochloride, exists in solution as perhaps 85–90% of the trans conformer. This is consistent with the steric arguments used to explain the predominance of trans conformer in solutions of methapyrilene hydrochloride. The confirmation of the presence of trans conformer of tripelennamine hydrochloride in the solid state and in solution must await the determination of its crystal structure, which is in progress. The NMR and ir spectra of the free base of tripelennamine are also consistent with it being the trans conformer in solution.

Cases Where the Solution Conformation Differs from That in the Solid State. A. Histamine. The conformation of histamine (I) has been extensively studied in the solid state and in solution.[7,21,22] The solution NMR spectra of histamine (I) showed complex AA′BB′ multiplets for the ethylene group which led two groups of workers to conclude that I existed in a nearly equal proportion of gauche and trans conformers.[7,21] In the solid state histamine exists in the trans conformation,[22] and powder photographs of a ground sample of histamine matched the pattern calculated using the program POWD5 and crystallographic atom positions. Therefore, no phase transition occurred upon grinding and the mull spectrum was that of the pure trans conformer. If solutions of histamine consist of an equal proportion of gauche and trans rotamers, one would expect significant differences between the solid state and solution ir spectra. Figure 5 compares the spectra of histamine in the solid state (Nujol mull) and in solution ($CHCl_3$)[23] and shows the infrared difference spectra. As expected, there are significant differences between these spectra resulting in 13 deviations of greater than 10% T in the difference spectra. However, attempts to calculate the proportion of gauche and trans conformers from the changes in the relative peak heights of these absorptions have so far failed, perhaps due to deviations from Beer's law or to the fact that some of these absorptions may be due to different imidazole ring –C–C–N conformations between solution and the solid state. The fact that some of the bonds observed in the solid state are absent in solution substantiates this suggestion since both the gauche and trans forms of histamine are present in solution.

The possibility that differences in ir spectra may be due to differences in conformation about bonds other than the $-CH_2CH_2-$ group is consistent with infrared and x-ray crystallographic data on acetyl choline chloride and bromide. Crystallographic studies of these compounds showed that the O(1)–C(5)–C(4)–N(1), C(5)–O(1)–C(6)–O(2), and C(2)–



N(1)–C(4)–C(5) dihedral angles corresponded well, 84.7, 5.2, and 171.4° for the chloride and 78.4, 4.1, and 175.5° for the bromide. However, the C(4)–C(5)–O(1)–C(6) dihedral angle was 168.9° for the chloride and 78.9° for the bromide.[24] The ir spectra of acetyl choline chloride and bromide were different.[25] Absorptions at 916, 1077, 1135, and 1406 cm^{-1} in the Nujol mull spectra of the bromide were either weak or missing in mull spectra of the chloride while the 1456-cm^{-1} absorption in the chloride spectrum was missing in the bromide spectrum. Thus differences in ir spectra of molecules more complicated than the halogenated ethylenes studied by Mitzushima[5f] may arise from differences in conformation about bonds other than the bond of interest.

B. Diphenhydramine Hydrochloride. Infrared spectra of mulls of crystals of diphenhydramine hydrochloride (III HCl) grown from acetone, ethyl acetate, benzene–ethanol, chloroform, and the melt were identical with each other. However, these spectra were not identical with the ir spectrum of diphenhydramine hydrochloride in chloroform solution (see Figure 6). There were at least seven deviations of greater than 10% T in the difference spectra. Crystallographic studies indicate that there was either one or one-half molecule per asymetric unit ruling out the possibility that both the trans and gauche conformers were present in the solid state. Thus, in contrast to the ethylenediamine antihistamines there are different conformers present in the solid state and in solution. It is not clear whether these differences are due to rotation about the ethylene bond or some other bond in the molecule. There were also significant differences between the NMR and ir spectra of diphenhydramine hydrochloride and the free base; however, the reason for these differences in unclear.

Limits of the Method. The most serious limitation of this method is that it can probably only be successfully applied to relatively small molecules. Compounds with many functional groups which have conformations in the solid state and solution which differ by only a rotation about one bond would probably have identical infrared spectra. For example, our preliminary studies of diphenhydramine picrate indicate that the picrate anion obscures almost the entire fingerprint region of the spectrum. However, it is interesting to note that infrared spectra have been used to show that the solid state and solution conformations of some polypeptides are different.[26]

A second limitation is that infrared spectra cannot easily detect the presence of a few percent of a minor conformer. The large differences between the spectra of histamine in the solid and solution (Figure 5) showed that this method can easily detect 50% of another conformer; however, the similarities of the solid and solution spectra of choline chloride indicate that this method cannot detect the presence of a few percent of the minor conformer. Further studies are required to precisely

establish lower detection limits for the infrared–x-ray method; however, a conservative estimate is 15% of a minor conformer.

Acknowledgments. The support of the donors of the Petroleum Research Fund, administered by the American Chemical Society, and the National Institutes of Health is gratefully acknowledged.

Registry No.—I, 51-45-6; II HCl, 135-23-9; III HCl, 147-24-0; IV HCl, 22306-05-4; V, 67-48-1.

Supplementary Material Available. (1) Tables comparing the absorption maxima of the solid and solution ir spectra of choline chloride, methapyriline hydrochloride (II HCl), tripelennamine hydrochloride (IV HCl), histamine (I), and diphenhydramine hydrochloride (III HCl); and (2) detailed chemical shift data for the NMR spectra of methapyriline hydrochloride (II HCl), tripelennamine hydrochloride (IV HCl), and diphenhydramine hydrochloride (III HCl) (7 pages). Ordering information is given on any current masthead page.

References and Notes

(1) (a) E. Culbertson, P. D. MacNicol, and P. R. Mallison, *Tetrahedron Lett.*, 1345 (1973); (b) A. Welter, M. Marlier, and G. Dardene, *Bull. Soc. Chim. Belg.*, **84**, 243 (1975); (c) B. Pullman, P. Courriere, and H. Berthod, *J. Med. Chem.*, **17**, 439 (1974); (d) W. Guschlbauer, D. Tran, M. Baldin, and J. C. Catlin, *Nucleic Acids Res.*, **1**, 855 (1974); (e) A. E. Tonelli and A. I. Brewster, *J. Am. Chem. Soc.*, **94**, 285 (1972); (f) J. F. McConnell, S. J. Angyal, and J. D. Stephens, *J. Chem. Soc., Perkin Trans. 2*, 2039 (1972); (g) S. Kukolja, P. V. Demarco, M. D. Jones, and J. W. Puschal, *J. Am. Chem. Soc.*, **94**, 7592; (1972); (h) R. A. Moriarty, E. L. Yeh, V. A. Curtis, C. L. Yeh, J. L. Flippen, J. Karle, and K. C. Ramey, *ibid.*, **94**, 6872 (1972).
(2) (a) G. Jung, M. Ottnad, P. Hartter, and H. Lachmann, *Angew. Chem.*, **87**, 448 (1975); (b) V. Cody, W. L. Duax, K. Yasuda, Y. Osawa, and D. A. Norton, *Tetrahedron*, **28**, 5683 (1972).
(3) (a) O. A. Ravenskii, A. N. Vereshchagin, F. G. Khalitov, and E. N. Tsvetkov, *Izv. Akad. Nauk SSSR, Ser. Khim.*, 995 (1973); (b) I. J. Borowitz, S. Liverles, K. Megerle, and R. D. Rapp, *Tetrahedron*, **30**, 4209 (1974).
(4) (a) H. S. Gutowsky, G. G. Belford, and P. E. McMahon, *J. Chem. Phys.*, **36**, 3353 (1962); (b) Z. Malarski, *Mol. Cryst. Liq. Cryst.*, **25**, 259 (1974).
(5) (a) J. Elguero, C. Marzin, and J. Merthou, *Bull. Soc Chim. Fr.*, **12**, 3303 (1973); (b) T. Fujiyama, *Bull. Chem. Soc. Jpn.*, **44**, 3317 (1971); (c) J. W. Brasch, *J. Chem. Phys.*, **43**, 3473 (1965); (d) P. Klaeboe, *Acta Chem. Scand.*, **23**, 2641 (1969); (e) D. Y. Curtin and S. R. Byrn, *J. Am. Chem. Soc.*, **91**, 6102 (1969); (f) S.-I. Mizushima, "Structure of Molecules and Internal Rotation", Academic Press, New York, N.Y., 1954; (g) H. F. Van Woerden and E. Havinga, *Recl. Trav. Chim. Pays-Bas*, **86**, 341 (1967); (h) N. L. Allinger, H. M. Blatter, L. A. Freiberg, and F. M. Karkowski, *J. Am. Chem. Soc.*, **88**, 2999 (1966).
(6) C. M. Clark, D. K. Smith, and G. G. Johnson, Department of Geosciences, The Pennsylvania State University, University Park, Pa.
(7) N. S. Ham, A. F. Casy, and K. R. Ison, *J. Med. Chem.*, **16**, 470 (1973).
(8) The X-Ray System-Version of June 1972, Update April 1974, Technical Report TR-192 of the Computer. Science Center, University of Maryland, June 1972.
(9) M. M. Wolfson and P. Main, University of York, England.
(10) See, for example, F. Dachille and R. Roy, *Nature (London)*, **186**, 34, 71 (1960); M. A. Moustafa, A. R. Ebian, S. A. Khalil, and M. M. Motawi, *J. Pharm. Pharmacol.*, **23**, 868 (1971).
(11) The idea of measuring ir difference spectra was suggested to us by Dr. J. M. Grutzner, Department of Chemistry, Purdue University. We are grateful for his interest in this work.
(12) J. Hjortas and H. Sorum, *Acta Crystallogr., Sect. B*, **27**, 1320 (1971).
(13) (a) D. Lichtenberg, P. A. Kroon, and S. I. Chan, *J. Am. Chem. Soc.*, **96**, 5934 (1974); (b) Y. Terui, M. Ueyama, S. Satoh, and K. Tori, *Tetrahedron*, **30**, 1465 (1971), and references cited therein.
(14) G. R. Clark and G. J. Palenik, *J. Am. Chem. Soc.*, **94**, 4005 (1972).
(15) A. A. Bothner-By, *Adv. Magn. Reson.*, **1**, 195 (1965); J. A. Pople and A. A. Bothner-By, *J. Chem. Phys.*, **42**, 1339 (1965).
(16) H. S. Gutowsky, M. Karplus, and D. M. Grant, *J. Chem. Phys.*, **31**, 1278 (1959).
(17) We are grateful to the Chemistry Department[11] and Mr. John Koslowsky, Purdue University, for making these facilities available to us.
(18) R. J. Abraham and K. G. R. Pachler, *Mol. Phys.*, **7**, 165 (1964); R. J. Abraham, "The Analysis of High Resolution NMR Spectra", American Elsevier, New York, N.Y., 1971.
(19) N. S. Ham, *J. Pharm. Sci.*, **60**, 1764 (1971).
(20) L. Radom, W. H. Lathan, W. J. Wehre, and J. A. Pople, *J. Am. Chem. Soc.*, **95**, 693 (1973); M. S. Jhon, U-L Cho, L. Biker, and H. Eyring, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 121 (1972); P. Krueger and H. D. Mettee, *Can. J. Chem.*, **43**, 2970 (1965).
(21) C. R. Ganellin, E. S. Pepper, G. N. J. Port, and W. G. Richards, *J. Med. Chem.*, **16**, 610 (1973).
(22) J. J. Bonnet and J. A. Ibers, *J. Am. Chem. Soc.*, **95**, 4829 (1973).
(23) The ir spectrum of histamine in water showed broadened peaks and was rather nondescript forcing us to use $CHCl_3$ as solvent. The decoupled NMR spectrum of histamine in $CDCl_3$ was nearly identical with the decoupled NMR spectrum of histamine in D_2O,[7] suggesting that there is no appreciable solvent or protonation effect under these conditions.
(24) T. Svinning and H. Sorum, *Acta Crystallogr., Sect. B*, **31**, 1581 (1975), and references cited therein.
(25) D. Aslanian, A. Lautie, and M. Balkanski, *J. Chim. Phys.*, **71**, 1028 (1974).
(26) H. Brumberger and B. Cheng, *Biopolymers*, **13**, 2653 (1974).

20-Methylcholesterol[1]

Yves Letourneux, Güniz Büjüktür, Maria T. Ryzlak, Ajit K. Banerjee, and Marcel Gut*

The Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts 01545

Received December 17, 1975

Three syntheses of 20-methylcholesterol (**8a**), resting on the alkylation of a C-20 anion with iodomethane, are described. The NMR signals of the 21- and the 28-methyl group are discussed in relation to the stereochemistry at C-20 of cholesterol and of 20-isocholesterol.

Within the framework of our studies[2] on the mechanism of the biodegradation of cholesterol to pregnenolone, we have investigated modifications[3] of the side chain of cholesterol. This paper describes syntheses of 20-methylcholesterol, a compound which cannot readily be metabolized by adrenals to pregnenolone and therefore was tested as a possible inhibitor both for the biosynthesis of cholesterol and for the biodegradation of cholesterol to pregnenolone.

The essence of the three syntheses described below consists in the generation of a carbanion at C-20, followed by its alkylation. Although there could be, a priori, a choice between the alkylation of a C-22 carbaldehyde with iodomethane, followed by the extension (of the aldehyde) to the complete cholesterol side chain, and the alkylation of a C-22 aldehyde with isohexyl bromide, followed by the conversion of the aldehyde to methyl, the second variant did not work: either the yield was poor, or the aldehyde suffered O-alkylation to give the enol ether **5**. This was revealed by its NMR spectrum, which lacked an aldehyde proton signal (δ 9–11) but indicated an olefinic methyl at δ 1.63, and also by its ir spectrum, giving a peak at 1660 cm^{-1}, commensurate with structures of similar enol ethers.[4]

The starting material for the first synthesis was the *i*-steroid aldehyde[5] **1**, which was alkylated with excess iodomethane (potassium *tert*-butoxide in *tert*-butyl alcohol) to give the α,α-dimethyl aldehyde **2** in 55% yield. The NMR spectrum of **2** shows a singlet at 9.66 ppm, in distinction to the doublet of the starting material **1**, where coupling (–CH–CHO) was observed. The stereoselectivity of this alkylation was studied by using iodomethane-d_3. The 250-MHz NMR indicates two