cleavage was shown (11) to be a significant reaction in the hydrolysis of II in water.

Samples were analyzed using the three methods described (Table I). The total of I plus II calculated as I determined using Method A agrees with the total content of I determined after complete hydrolysis. This fact, together with the observation that III is the only reaction product formed from II and aniline, strongly indicates that Method A provides an accurate measurement of I and II. With the exception of Sample a, only small deviations can be observed between the content of II determined by Methods A and B. This result may indicate that the value of $\epsilon_{520} = 6200$ M^{-1} cm^{-1} for II (4) is not correct and that using a value of $\epsilon_{520} = 6020 M^{-1}$ cm^{-1} leads to improved agreement between the results of Methods A and B.

The methods described are intended to be applied to II and I in a 0.8 M solution of sodium gentisate (4). The present work shows that the reaction between II and aniline is applicable for the determination of a mixture of I and II in parenteral solutions and that spectrophotometric analysis of the reaction mixture appears to be a simple and reliable method of analysis.

REFERENCES

(1) G. H. Svoboda, G. A. Poore, P. J. Simpson, and G. B. Boder, *J. Pharm. Sci.*, 55, 758 (1966).

(2) G. H. Svoboda, M. J. Sweeney, and W. D. Walkling, *ibid.*, 60, 333

(1971).

(3) D. W. A. Bourne, T. Higuchi, and A. J. Repta, *ibid.*, 66, 628 (1977).

(4) C.-H. Huang, B. Kreilgård, and A. J. Repta, *Bull. Parenteral Drug Assoc.*, 30, 1 (1976).

(5) W. J. Mader, *CRC Crit. Rev. Anal. Chem.*, 1, 193 (1970).

(6) A. Albert and E. P. Sergeant, "Ionization Constants of Acids and Bases," Methuen, London, England, 1962.

(7) A. C. Capomacchia, J. Casper, and S. G. Schulman, *J. Pharm. Sci.*, 63, 1272 (1974).

(8) H. H. Willard, L. L. Merritt, Jr., and J. A. Dean, "Instrumental Methods of Analysis," 5th ed., Van Nostrand, New York, N.Y., 1974, p. 92.

(9) A. B. Hansen and S. S. Larsen, *Dan. Tidsskr. Farm.*, 46, 105 (1972).

(10) K. A. Conners, "Reaction Mechanisms in Organic Analytical Chemistry," Wiley, New York, N.Y., 1973, p. 516 ff.

(11) A. J. Repta, J. R. Dimmock, B. Kreilgård, and J. J. Kaminski, *J. Pharm. Sci.*, 66, 1501 (1977).

Comparison of Solid and Solution Conformations of Hydroxyurea and 3-Ethyl-1-hydroxyurea Utilizing IR-X-Ray Method

GEORGE R. PARKER *× and JAMES D. KORP ‡

Received February 10, 1977, from the *Department of Medicinal Chemistry, College of Pharmacy, and the Department of Chemistry, Crystallography Laboratory, University of Houston, Houston, TX 77004.* Accepted for publication May 12, 1977. *Present address: College of Pharmacy, University of Oklahoma, Oklahoma City, OK 73190. ‡Present address: Department of Chemistry, Crystallography Laboratory, Baylor University, Waco, Tex.

Abstract ☐ The IR–X-ray method for determining solution conformation was used to compare the conformations of the antitumor agents hydroxyurea and its 3-ethyl analog in solutions with conformations in the solid state. X-ray crystal analysis data for hydroxyurea obtained from the literature and the 3-ethyl analog data determined experimentally indicated that similar conformations are present in both compounds, with a carbonyl bond of mostly double bond character and intermolecular hydrogen bonding. The ethyl analog also possessed inter- and intramolecular hydrogen bonding involving the 1- and 3-hydrogens and the carbonyl and hydroxyl oxygens. Comparison of IR spectra (1780–1550 cm^{-1}) of the solid state (mineral oil mulls) with solution spectra (taken in dry octanol, in octanol equilibrated with water and dried, and in wet and dry acetonitrile) indicated that only in dry acetonitrile did the spectrum of hydroxyurea resemble that of the solid. The ethyl analog possessed absorptions similar to the solid only in dry octanol, while spectra of all other solutions for both compounds had strong C=N character and diminished C=O double bond character. The IR solution data were rationalized according to the conformational system of hydroxyureas, which is represented by canonical forms possessing a C=O

bond or strong C=N bonds and a CO bond. Hydroxyurea appears to prefer hydrogen bonding to a solvent (water) to bonding with itself, while the ethyl analog possesses internal hydrogen bonding which changes the conformational preferences and conformational equilibrium. In solvents where this inter- or intramolecular hydrogen bonding competes with bonding with the solvent, interconversion of conformers takes place; but this interconversion cannot occur when strong hydrogen bonding (with solvate formation) exists with water. Hydroxyurea tends to exhibit this behavior only when hydrogen bonding to solvent or water is not possible, allowing interconversion through a form possessing a C=O bond. Hydroxyurea and its 3-ethyl analog thus have different solution conformational equilibria depending on the solvent.

Keyphrases ☐ Hydroxyurea and 3-ethyl analog—comparison of solid and solution conformations ☐ Conformations—solid and solution compared, hydroxyurea and 3-ethyl analog ☐ Antineoplastic agents—hydroxyurea and 3-ethyl analog, comparison of solid and solution conformations

The structure or conformation of drugs in solution is a significant property to scientists studying the molecular dynamics involved in absorption, *in vivo* transport, site of action, and enzymatic biotransformations of biologically active compounds (1). Knowledge concerning the actual or potential structure or conformation of drug molecules becomes even more relevant to the molecular pharmacologist or medicinal chemist if a particular structure or conformation is essential for a biological process and that particular molecular species is one of many possible species that may be present *in vivo*.

While the structure of molecules in the solid state can be ascertained with a high degree of accuracy *via* X-ray crystallography, the structure and conformation of molecules in simple solutions are continually being studied and are topics of rigorous debate (2). Various methods have

Figure 1—*X-ray crystal structure of hydroxyurea showing hydrogen bonding between hydroxyl of one molecule and carbonyl oxygen of another.*

been utilized to correlate known structure in the solid state (from X-ray crystal data) to structure and conformation in solution *via* the combination of instrumental methods of analysis such as X-ray crystallography with NMR spectroscopy (3), dipole moments (4), and circular dichroism (5).

This paper reports: (a) the correlation of solid and solution conformations in the hydroxyurea series of antitumor agents (hydroxyurea and 3-ethyl-1-hydroxyurea) utilizing the literature and experimentally determined X-ray crystal structures in combination with the IR spectra of solids and solutions according to the method of Byrn *et al.* (6), and (b) assumptions concerning the structure and conformations of these compounds in solution. The hydroxyurea class was studied since it exhibits

Figure 2—*X-ray crystal structure of 3-ethyl-1-hydroxyurea showing bond distances and inter- and intramolecular hydrogen bonds [estimated standard deviation = ±0.04 Å]. Thermodynamic disorder was observed in the terminal methyl group.*

Table I—IR Absorptions in Carbonyl-Nitrogen Region of Hydroxyurea and 3-Ethyl-1-hydroxyurea in the Solid State (Mineral Oil Mulls) and in Various Solution Conditions

Sample Obtained in	Absorptions in Carbonyl Nitrogen Absorption Region (1780–1550 cm^{-1}), cm^{-1}	
	Hydroxyurea	3-Ethyl-1-hydroxyurea
Mull	1625, 1570	1635, 1580–1560
Octanol (dry)	1655	1680, 1560
Octanol (after water equilibration and drying)	1650	1640
Acetonitrile (wet)	1690	1680
Acetonitrile (dry)	1740, 1640	1680

a solvent conformational dependency which may influence the biological action (7, 8).

EXPERIMENTAL

Compounds—3-Ethyl-1-hydroxyurea was synthesized according to the method of Dresler and Stein (9) with modifications (10, 11). Hydroxyurea[1] was used as received.

X-Ray Crystal Analysis of 3-Ethyl-1-hydroxyurea—A clear, tabular crystal of 3-ethyl-1-hydroxyurea was mounted on an automatic diffractometer[2] equipped with an MoKᾱ X-ray source (Kᾱ = 0.71073 Å) monochromatized by a dense graphite crystal. All measurements were made at ambient room temperature. The crystal symmetry was established as 2/m, space group I2/a. Unit cell parameters were a = 9.981, b = 8.011, c = 14.003 Å, β = 103.32°, and Z = 8 molecules/cell. The calculated density was 1.29 g/cm^3. Using the θ–2θ scan technique in the range 6° < 2θ < 60°, 413 independent reflections with I > 3σ (I) were measured.

The structure was solved by application of the multiple-entry tangent formula program MULTAN (12). The ethyl moiety was highly disordered; however, the remainder of the molecule refined well. The three hydrogens not associated with the ethyl group were located. The final R value [R = ($\Sigma||F_0| - |F_c||$)/$\Sigma|F_0|$] of 11% was a disappointingly high value caused by the disorder problem. Since the ethyl portion of the structure was not involved in the hydrogen bonding between C=O and OH, no attempt was made to sort out and clarify the disorder.

This relatively crude crystallographic determination revealed that the title compound has a structure quite similar to that of hydroxyurea itself (Fig. 1), with an analogous intermolecular hydrogen-bonding scheme between the hydroxyl oxygens and the keto oxygens *via* the hydroxyl hydrogens. The approximate distance between oxygens involved in the hydrogen bonding is 2.62 Å (4). There also appears to be hydrogen bonding between the 3-hydrogen and oxygen of the hydroxyl group (intramolecular) and between the 1-hydrogen and carbonyl oxygen of another molecule. No unusual features were noted in the hydroxyurea portion of the molecule. The structure with interatomic bond distances and hydrogen bonds is shown in Fig. 2.

IR Spectra—Spectral data were obtained on double-beam IR spectrophotometers[3], utilizing liquid plates for solutions and mulls[4] for solids taken between sodium chloride plates. Solution spectra were taken of hydroxyurea and 3-ethyl-1-hydroxyurea in octanol[5] (dry), in octanol after water equilibration for 24 hr and drying over sodium sulfate (anhydrous), in acetonitrile[6] (wet), and in acetonitrile dried over sodium sulfate.

Spectra were taken with the solvents as the blank. All spectra were taken with the same instrument readings and at 90% transmission. The spectral data of the compounds in solution and taken as mulls are shown in Table I.

DISCUSSION

The X-ray crystal structure of hydroxyurea, a clinically effective antitumor agent and the most potent member of the hydroxyurea class, was first determined concurrently by two groups (13, 14). The initial reports were recently confirmed and corrected after reinvestigation at liquid nitrogen temperature (−140°), indicating the crystal structure as shown in Fig. 1 from the atomic coordinate data of Armagan *et al.* (15). Hy-
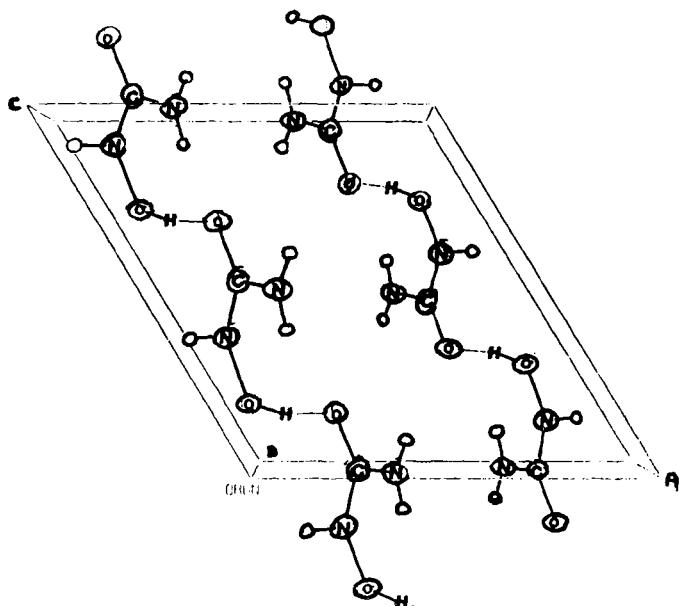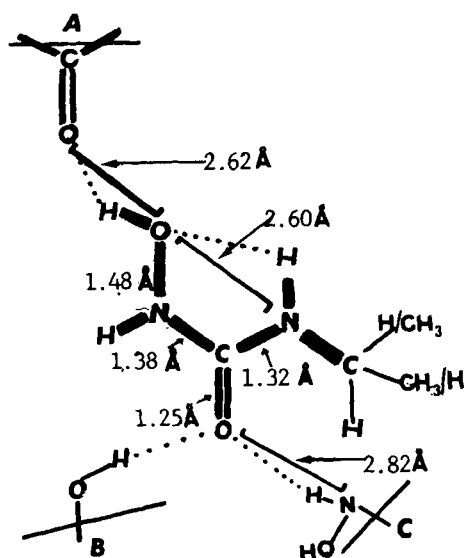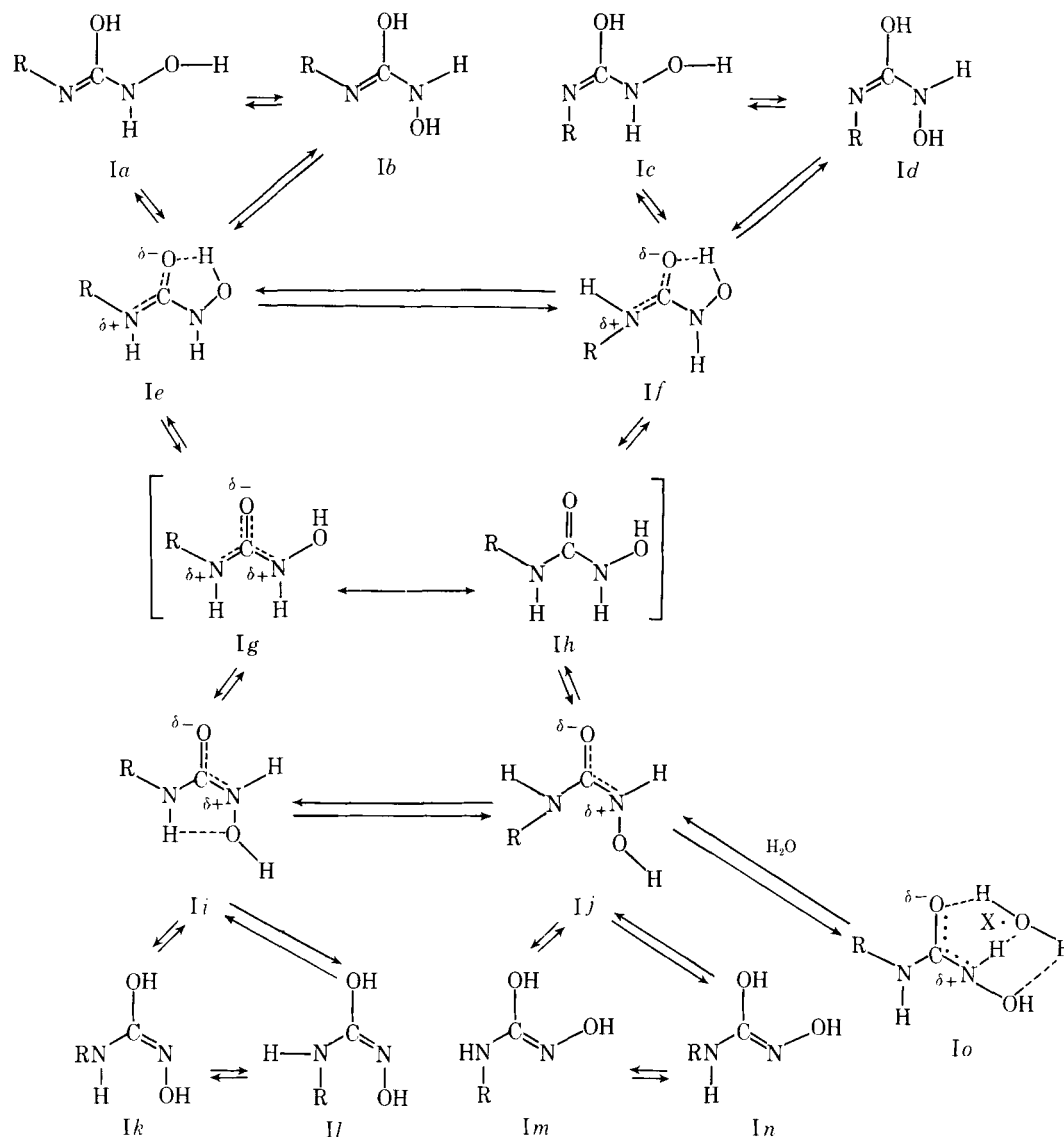
Scheme I—*Conformational and tautomeric system of hydroxyureas. Canonical forms Ig and Ih represent electronic species where the π bond is localized in the C=O bond (Ih) or distributed across the NCN system (Ig). Conformers Ie, If, and Ii are possible because of internal hydrogen bonding, and conformer Ij can hydrogen bond to solvent or water (Io). Tautomers are possible (Ia–Id and Ik–In) if bond breaking and bond making occur.*

droxyurea appears as chains of molecules with hydrogen bonding between the hydroxyl of one molecule and the carbonyl oxygen of another with four molecules in a unit cell. From the bond lengths and bond angles and with Pauling's method of determining bond order from bond length (16), the C=O bond was found to be about 80% double bond in character and both the N^1-C and C-N^2 bonds were found to be about 10% double bond in character.

With the method of Byrn et al. (6), the IR spectrum of solid hydroxyurea (a mineral oil mull) was compared to spectra taken in solutions. This method compares the important absorptions in IR spectra of solids of known structure with spectra in solution to evaluate the equivalence of conformation in the two systems. Since hydroxyurea can best be evaluated by the absorptions in the carbonyl–nitrogen region (1780–1550 cm^{-1}), only absorptions in this region were analyzed and compared. A powder diffraction X-ray analysis indicated that no phase change occurs on grinding, and thus the IR spectra of the mull represented the structure of hydroxyurea in the solid state.

The IR spectrum of solid hydroxyurea appeared as a broad band from 1650 to 1540 cm^{-1}, with peaks at 1625 and 1570 cm^{-1} representing the amide 1 and II bands of hydroxyurea. The amide I band (1625 cm^{-1}) is primarily C=O stretching vibrations, while the amide II band (1570 cm^{-1}) is due to NH bending and CN stretching (17). Spectra of hydroxyurea in solution were limited by poor solubility in solvents such as chloroform, benzene, ether, tetrahydrofuran, ethyl acetate, and cyclohexane, but spectra could be obtained in alcohols such as 1-octanol. The

IR spectrum of hydroxyurea in dry octanol showed a broad band at 1635–1680 cm^{-1} with the peak at 1655 cm^{-1}. When hydroxyurea in octanol was equilibrated with water and dried with anhydrous sodium sulfate, an identical absorption was observed which peaked at 1645–1655 cm^{-1}.

It is assumed from these data that the structure or apparent conformation(s) (which can be represented by canonical forms Ig and Ih, conformers Ie, If, Ii, and Ij, and/or tautomers Ia–Id and Ik–In in Scheme I) present in solution is different than that present in the solid crystal, but the structure present in dry octanol is the same as that present in octanol equilibrated with water and dried.

Since hydrogen bonding with the solvent can affect the conformations present in solution, IR spectral analysis with a solvent that could not hydrogen bond to hydroxyurea was desirable. Since hydroxyurea was not soluble in most solvents that would not hydrogen bond (benzene, cyclohexane, and alkanes), acetonitrile was selected as a suitable alternative. Because of its miscibility with water, however, the equilibration step (as with octanol) with an aqueous phase was not possible. Therefore, IR spectra were taken in wet and anhydrous acetonitrile (dried over sodium sulfate); absorptions were found at 1690 cm^{-1} in wet acetonitrile and at 1640 and 1740 cm^{-1} in the anhydrous solvent.

Analysis and comparison of the IR spectral data in the solid state *versus* solutions, for hydroxyurea indicate that the conformational or structural species in solution is similar to that of the solid state in solvents that cannot hydrogen bond (dry acetonitrile). In the solid state, the amide I

and II bands are of equal intensity; in dry acetonitrile, the amide I band is more intense and at a lower wavelength, indicating a C=O bond is of greater double bond character and with less hydrogen bonding occurring with the carbonyl oxygen. This finding might be accounted for by the fact that hydroxyurea molecules in the solid state possess intermolecular hydrogen bonding (at a distance of 2.627 Å from C=O to HO) whereas hydrogen bonding of this type in a nonbonding, dry solution is less likely.

In the presence of water or in solvents that can hydrogen bond (such as octanol), the predominant structural or conformational species has minimal double bond CO character because of strong hydrogen bonding with water or solvent and maximal C=N bond character as observed by the intense absorption at 1650 cm⁻¹. This type of structural species could be represented (Scheme I) by tautomers Ia–Id and Ik–In or by a conformer such as Ij, which would hydrogen bond strongly to water or solvent. Tautomeric forms were ruled out through an NMR study[7]. This study showed that the NH protons exchanged slowly in deuterium oxide, which would not occur if tautomeric species were present in solution.

The substitution of groups on the hydroxyurea molecule, such as alkyl addition at the 1- or 3-nitrogen, produced analogs with appreciably less antitumor activity than hydroxyurea (18). It was of interest to determine if substituted analogs exhibit similar or different structural or conformational characteristics in comparison with hydroxyurea. Since no literature reports of X-ray crystal studies of 1- or 3-alkyl-substituted hydroxyureas could be found, an X-ray crystal analysis of a representative member of the class was initiated.

The physical properties of some substituted hydroxyureas precluded routine crystallographic analysis. For example, the 1-substituted methyl and ethyl analogs have low melting points and require low temperature analysis; and while the methyl analog produces suitable crystals for analysis, the ethyl analog is an amorphous powder unsuitable for crystallographic study. With the higher melting 3-substituted analogs, which could be analyzed at room temperatures, difficulties arose in finding a solvent for optimal growth of suitable crystals. Crystals grown from most solvents (methanol, ether, tetrahydrofuran, and acetone) produced plates or flakes that were too thin for X-ray analysis, as was the case with the biologically inactive 3-n-butyl analog. The 3-ethyl analog could be crystallized from acetone, producing crystals suitable for X-ray analysis; the derived data indicate that the structure is remarkably similar to that of hydroxyurea.

The crystal structure of 3-ethyl-1-hydroxyurea indicated hydrogen bonding between the hydroxyl hydrogen of one molecule and the carbonyl oxygen of another at a distance of about 2.62 Å[8] and internal hydrogen bonding between the 3-hydrogen and the hydroxyl oxygen at a distance of about 2.60 Å[8] (distances calculated between heavy atoms). The carbonyl bond was shorter in length than the bond in hydroxyurea crystals (1.25 Å[8] compared to 1.267 Å), which indicated a double bond character approaching 90%. However, with the relatively high estimated standard deviations[8] accompanying the ethyl disorder, little weight can be given to this figure. The CN¹ bond was slightly longer, indicating that any double bond character in this region was most likely between the CN³ bond.

The IR spectrum of the ethyl analog in the solid state (taken as a mineral oil mull) showed absorptions at 1635 cm⁻¹ (amide I) and in the 1540–1580-cm⁻¹ range (amide II), with peaks at 1560 and 1580 and a shoulder peak at 1540 cm⁻¹. The intensity of the amide I band was slightly stronger than that of the amide II band, and the peak at 1560 cm⁻¹ was the strongest in the amide II range.

Comparison of the crystal structure of the ethyl analog and its IR spectrum with the data for hydroxyurea indicates a good correlation with theoretical principles. The amide I band was shifted to a higher frequency in the ethyl analog, and this compound possessed a greater double bond character in the C=O bond. The amide II band was shifted to a lower frequency, indicative of the lower amount of C=N character in the ethyl analog. Differences in the observed hydrogen bonding of the 3-hydrogen (intramolecular) and 1-hydrogen (intermolecular) in the two compounds might explain the complex absorption in the amide II region (two peaks plus a shoulder peak) in comparison to a broad single absorption for hydroxyurea (with a slight shoulder ridge at 1550 cm⁻¹).

The IR spectra of solutions of the ethyl analog in solvents that could hydrogen bond to the molecules (dry octanol) showed two characteristic peaks observed in all substituted hydroxyurea analogs in dry octanol (8). For the 3-ethyl analog, the two absorptions peaks were at 1680 and 1560 cm⁻¹, with the absorption at the lower frequency being significantly more

intense. These absorptions in dry octanol indicated an identifiable amide I band (for C=O) besides a strong amide II band and thus indicate that the structure(s) or conformation(s) present in dry octanol is different than that of hydroxyurea in the same solvent (a single intense absorption at 1650 cm⁻¹).

It also can be deduced from these data that the structure in dry octanol is similar to that observed in the solid state for the ethyl-substituted analog in that it has a definite degree of C=O character (although less than in the solid state). When the ethyl analog in octanol was equilibrated with water and separated and then the octanol was dried with anhydrous sodium sulfate, the IR spectrum showed only a single absorption (similar to that observed with hydroxyurea in dry octanol) at 1640 cm⁻¹. Spectra taken of the ethyl analog in wet acetonitrile showed a broad band with the peak at 1680 cm⁻¹ and a distinct shoulder at 1630 cm⁻¹. When the spectra was taken in dry acetonitrile, the peak remained at 1680 cm⁻¹ but the shoulder disappeared.

From these IR spectral data, it is assumed that, for the 3-ethyl analog, the structural or conformational species present (with a definite C=O character) is different in dry octanol from that present in dry acetonitrile (which lacks C=O character) but that the species present does not change appreciably in wet acetonitrile as was observed with hydroxyurea. The species present in the octanol solution after water equilibration, observed via the IR spectra, also appears to resemble the form(s) present in acetonitrile whether water is present or not.

It is apparent from these IR spectral data that, while the structural forms of hydroxyurea and its 3-ethyl analog are quite similar in the solid state, the species present in solutions differ appreciably. The spectral data can be visualized as due to changes in the equilibrium proportions of Ie, If, Ii, and Ij and canonical forms Ig and Ih. These canonical forms depict the extreme structural forms where C=O character is 100% and the CN bonds are single bond in character, allowing free rotation and thus fast interconversion through conformers Ie, If, Ii, and Ij (form Ih), or where the CO bond is substantially single bond in character and the remaining electron density is distributed across the CN bonds, producing restriction of rotation and separation of charge (form Ig).

For hydroxyurea (where R = H), a structural form similar to Ig is present in octanol regardless of whether the solvent is dry or has been equilibrated with water and dried. In dry acetonitrile, a form similar to Ih appears to be present; but when water is added, the structural form reverts back to a species similar to Ig. With the ethyl analog (where R = C₂H₅), the equilibrium is shifted in the direction of Ih in dry octanol; but after contact with water (water equilibration), the equilibrium is shifted to a form resembling Ig. In acetonitrile, a form similar to Ig is present whether water is present or not.

This behavior could be attributed to the ability of the ethyl analog to have strong enough inter- or intramolecular hydrogen bonding when hydrogen bonding is not possible with the solvent (dry acetonitrile) so that the species present is similar to Ig and, therefore, the addition of water would not change the species present. With respect to the IR results, hydroxyurea does not seem to possess the internal hydrogen bonding seen in the ethyl analog, and interactions with octanol and/or water predominate (form Ig). In solvents that do not hydrogen bond (dry acetonitrile), interconversions through the conformers via a form such as Ih would occur; this process would not continue when strong hydrogen bonding with water occurred (wet acetonitrile). Another possible explanation is that differences in solvation of hydroxyurea and the 3-ethyl derivative exist and change the relative stability of the conformers.

In conclusion, it is apparent that the addition of an ethyl group to the hydroxyurea molecule adds a property that influences the conformational preferences in solutions besides the added lipophilicity and bulk expected. The effect could influence the species present in vivo at various biological sites (at membranes, at site of action, etc.) and thus influence the process involved, depending on which species is stabilized due to the ability of hydrogen bonding with the hydroxyurea molecule by macromolecules or water.

REFERENCES

(1) E. J. Ariens, Farmaco, 24, 6 (1969).

(2) I. W. Mathison, W. E. Solomons, P. H. Morgan, and R. R. Tidwell, in "Principles of Medicinal Chemistry," W. O. Foye, Ed., Lea & Febiger, Philadelphia, Pa., 1974, pp. 89–92.

(3) B. Pullman, P. Courriere, and H. Berthod, J. Med. Chem., 17, 439 (1974).

(4) I. J. Borowitz, S. Liverles, K. Megerle, and R. D. Rapp, Tetrahedron, 30, 4209 (1974).

[7] Unpublished data.
[8] Estimated standard deviation = ±0.04 Å.

(5) V. Cody, W. L. Duax, K. Yasuda, Y. Osawa, and D. A. Noeton, *ibid.*, **28**, 5683 (1972).

(6) S. R. Byrn, C. W. Graber, and S. L. Midland, *J. Org. Chem.*, **41**, 2283 (1976).

(7) G. R. Parker, N. K. Hilgendorf, and J. G. Lindberg, *J. Pharm. Sci.*, **65**, 585 (1976).

(8) G. R. Parker and E. C. Moore, *ibid.*, **66**, 1040 (1977).

(9) W. Dresler and R. Stein, *Justus Liebigs Ann. Chem.*, **150**, 242 (1869).

(10) R. E. Harmon, J. C. Dabrowiak, D. J. Brown, S. K. Gupta, M. Herbert, and D. Chitharanjan, *J. Med. Chem.*, **13**, 577 (1970).

(11) G. Clifton, S. R. Bryant, and C. G. Skinner, *ibid.*, **13**, 377 (1970).

(12) G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr.*, **A27**, 368 (1971).

(13) I. K. Larsen and B. Jerslev, *Acta Chem. Scand.*, **20**, 983 (1966).

(14) H. Berman and S. H. Kim, *Acta Crystallogr.*, **23**, 180 (1967).

(15) N. Armagan, J. P. G. Richards, and A. A. Uraz, *ibid.*, **B32**, 1042 (1976).

(16) L. Pauling, "The Nature of the Chemical Bond," 3rd ed., Cornell University Press, Ithaca, N.Y., 1960, p. 281.

(17) N. L. Alpert, W. E. Keiser, and H. A. Szymanski, "The Theory and Practice of Infrared Spectroscopy," Plenum, New York, N.Y., 1970, p. 281.

(18) R. H. Adamson, *Proc. Soc. Exp. Biol. Med.*, **119**, 456 (1965).

ACKNOWLEDGMENTS

GLC Determination of Phenylbutazone in Human Plasma

ANTOINE SIOUFI [x], FRANÇOISE CAUDAL, and FRANÇOISE MARFIL

Received March 1, 1977, from *Ciba-Geigy Biopharmaceutical Research Center, 92506 Rueil-Malmaison Cedex, France.*   Accepted for publication May 11, 1977.

Abstract □ A GLC method for phenylbutazone at concentrations down to 10 ng/ml in human plasma is described. After addition of an internal standard, phenylbutazone is extracted at pH 5 into benzene. The dry extract is dissolved in benzene, and phenylbutazone is determined by GLC using a [63]Ni-electron-capture detector.

Keyphrases □ Phenylbutazone—GLC analysis in human plasma □ GLC—analysis, phenylbutazone in human plasma □ Antirheumatic agents—phenylbutazone, GLC analysis in human plasma

Many methods have been proposed for the quantitative assay of phenylbutazone[1] (I) in biological fluids. The first UV method (1, 2) (extraction of phenylbutazone with heptane in acidic medium) was modified (3, 4) and automated (5), for the serial determination of phenylbutazone in human plasma. The UV determination of phenylbutazone after oxidation to azobenzene by alkaline permanganate was described (6) and applied to small-volume samples (7). The method of Burns (1, 2) was also applied to 1 drop of capillary blood (8). Stevens (9) determined phenylbutazone by UV absorption after fast extraction in hexane from an acidic medium.

Several investigators studied the determination of phenylbutazone by GLC. McGilveray *et al.* (10) described a procedure using 1 N HCl and heptane for extraction and diphenyl phthalate as the external standard. Midha *et al.* (11) published an elaborated method for determining phenylbutazone and oxyphenbutazone. These two compounds were derivatized by flash-heater methylation with trimethylanilinium hydroxide, but each yielded two peaks.

A GLC technique was also used to determine phenylbutazone and oxyphenbutazone in the plasma and urine of horses and dogs (12). The extraction was performed with 2 N H_2SO_4 and 3 × 20 ml of benzene. Phenylbutazone was assayed by GLC without derivatization, but oxyphenbutazone was separated as its heptafluorobutyryl derivative.

Tanimura *et al.* (13) determined phenylbutazone, oxyphenbutazone, and γ-hydroxyphenylbutazone in human or rabbit plasma, using fluoranthene as the internal standard and N,O-bis(trimethylsilyl)trifluoroacetamide as the silylating reagent. A GLC method in rat serum or urine, using promethazine as the external standard, was also described (14). The retention times were between 15 and 18 min. This method was checked for its specificity. Finally, a high-pressure liquid chromatographic (HPLC) technique was recently reported (15).

The GLC method described here is simple and fast, and it specifically determines phenylbutazone in human plasma with a high sensitivity. A phenylbutazone analog, 4-butyl-1,2-bis(*p*-tolyl)-3,5-pyrazolidine (II), is used as the internal standard.

EXPERIMENTAL

Reagents—A pH 5 buffer[2] was prepared by diluting the contents of seven vials with 1000 ml of water. Benzene was analytical grade[3]. The

I

II

---
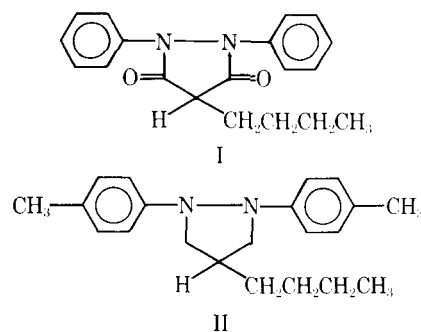
[1] Active ingredient of Butazolidin (Geigy).