well known that retinol can change reversibly to its aldehyde form (retinal) and irreversibly to its acid form (retinoic acid) in animal tissues. Retinal is responsible for the vision mechanism, but retinoic acid is not involved in this mechanism. Retinol, retinal, and retinoic acid promote growth, and any other change in structure of these molecules abolishes the growth-promoting activity.

Desmethylretinoic acid does not promote growth since it does not have methyl groups on the side chain. Being an acid, it cannot be involved in the vision mechanism. Desmethylretinoic acid promotes wound healing, and its action on epithelium is still not known. The relationships between healing and supporting a healthy epithelium are being studied.

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ACKNOWLEDGMENTS AND ADDRESSES

Received December 18, 1974, from the *School of Pharmacy, University of California, San Francisco, CA 94143*

Accepted for publication July 17, 1975.

Supported in part by Barnes-Hind Pharmaceuticals, Inc., Sunnyvale, Calif.

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Solvent-Dependent Conformational System in Hydroxyureas Detected by NMR Spectroscopy

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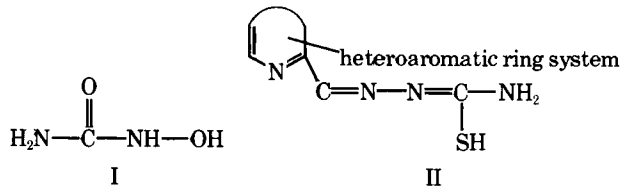
Abstract □ The proton magnetic resonance spectra of the antileukemia agent hydroxyurea and substituted hydroxyureas in several solvents were recorded and correlated with structural features. Solvent-dependent differences in conformational preferences due to effects on internal hydrogen bonding, a temperature-dependent conformational feature, and the exchangeability of protons with deuterium oxide and acetone-d_6 were observed. The conformational features consistent with the spectral data are discussed.

Keyphrases □ Hydroxyurea and substituted hydroxyureas—NMR spectra correlated with structural features, solvent-dependent differences observed □ NMR spectroscopy—hydroxyurea and substituted hydroxyureas, spectra correlated with structural features, solvent-dependent differences observed □ Solvent-dependent conformational system—hydroxyurea and substituted hydroxyureas, detected by NMR spectroscopy □ Antileukemic agents—hydroxyurea, NMR spectra correlated with structural features

NMR spectroscopy has become a powerful tool for the determination of organic molecular structure, configurational stereochemistry, and conformational properties of molecules (1). Knowledge concerning the conformational properties of drugs at their site of action is essential to the understanding of the complex molecular mechanism of action (2).

An NMR analysis was conducted on a series of substituted analogs of the antileukemia agent hydroxyurea (I) to gain some insight into the conformational properties that might explain the relative biological inactivity of the 3-*N*-substituted analogs. Hydroxyurea has been observed to inhibit DNA synthesis in various biological systems (3–5) and exhibits



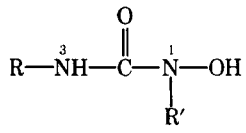
I II

activity against L-1210 lymphoid leukemia (6). The action of this compound has been attributed to the inhibition of the iron-dependent, allosterically controlled enzyme ribonucleoside diphosphate reductase (7–9) *via* a mechanism similar to that observed in a series of α-*N*-formylheteroaromatic thiosemicarbazones (II) (10).

In contrast to the rather large number of active analogs of II (72 of 97 compounds rated active *in vitro* and 51 of 97 active *in vivo*), only the ethyl and *n*-propyl analogs substituted at the 3-*N*-position and the methyl and ethyl 1-*N*-analogs of hydroxyurea were active *in vivo* against L-1210 lymphoid leukemia[1]. These biological data could be rationalized in terms of *in vivo* instability, poor transportability to the site of action, steric effects at the site of action, or conformational factors relevant to drug action, which this paper reports as a possibility in substituted hydroxyureas.

The molecular mechanism of action of I and II has

[1] Personal communication from the Cancer Chemotherapy National Screening Center of the National Cancer Institute.



III

been proposed to involve ligand formation with enzymatic octahedral iron (11, 12), involving conformational planarity in the ligand molecule. NMR spectra of the substituted analogs of hydroxyurea (III) compared to hydroxyurea in various solvents indicated solvent-dependent conformational equilibria sensitive to the π-bond character between the 3-nitrogen and the carbonyl carbon and internal hydrogen bonding between the hydroxyl hydrogen and the carbonyl oxygen. This paper describes the NMR spectral data supporting this assertion.

The properties of internal hydrogen bonding and hindered rotation in hydroxyurea and its substituted derivatives due to this π-bond character may affect the acidity and complexing affinity toward ligand formation as well as *in vivo* stability and transportability. The resultant effect on pKa, lipid solubility, and conformational affinity toward ligand formation may influence the overall biological activity of these molecules. Other investigators may wish to consider these properties in the elucidation of the mechanism of action of hydroxyurea as an antileukemia agent and in the explanation of the reduced activity of substituted analogs as *in vivo* inhibitors of DNA synthesis.

EXPERIMENTAL

Materials—Hydroxyurea was purchased from a commercial source[2], and the 3-*N*-substituted analogs (Table I) were synthesized according to the original procedure (13) with reported modifications (14, 15). The 1-*N*-substituted analogs were made available for this study[3].

NMR Spectra—The NMR spectra were obtained using an NMR spectrometer[4] equipped with a variable temperature accessory. The chemical shift data were measured relative to tetramethylsilane[5] as an internal standard. Location of peaks (δ) is by parts per million downfield from the standard. Solvents for spectral data were acetone-d_6 (99%), pyridine-d_5 (99%), spectral grade pyridine, spectral grade tetrachloroethane, and deuterium oxide; all were obtained from commercial sources[6].

RESULTS

The NMR data for hydroxyurea and substituted analogs in various solvents and after deuterium oxide was added to the solvent appear in Table II. The spectrum of hydroxyurea in acetone-d_6 was somewhat difficult to obtain, since this derivative is the least soluble in this solvent. A low intensity spectrum showed a broad multiplet at 5.75 ppm and multiplets at 7.65 and 7.95 ppm in a ratio of 2:1:1.

Hydroxyurea in pyridine-d_5 appeared as signals in three general chemical shift regions: multiplets at 5.2 and 5.7 ppm, a singlet at 6.7 and a multiplet at 7.7 ppm, and multiplets at 8.4 and 9.1 ppm. The total integration for the signals in the three areas was 2:1:1. In a mixed solvent system of acetone-d_6–pyridine-d_5 (1:1), five distinct signals were observed: a broad multiplet at 6.8 ppm, sharp multiplets at 7.3 and 7.7 ppm, a sharp singlet at 8.7 ppm, and a broad multiplet at 9.1 ppm. A weaker and broad multiplet was also seen at 9.8 ppm, and the ratio of the signals between 6.8 and 7.7 ppm to those at 8.7–9.8 ppm was 3:1.

The addition of deuterium oxide to all of these systems produced an immediate loss of signals due to exchange, except for the deuterated water signal and weak signals at 5.2 and 6.4 ppm with hydroxyurea in pyridine-d_5. All of the spectra of hydroxyureas taken in acetone-d_6 showed a signal at around 3.0 ppm, which was found to be trace water in the acetone-d_6. This signal became larger when hydroxyureas were used that had traces of water in them.

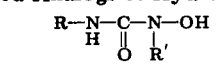
In spectra of the 3-*N*-substituted hydroxyureas (IV–IX), the identification and assignment of three distinct signals downfield (for the two amino and the hydroxyl protons) was difficult for the *n*-propyl and the aromatic analogs of hydroxyurea in acetone-d_6. These signals were all observed for the isopropyl, *n*-butyl, and 1-*N*-substituted analogs of hydroxyurea in acetone-d_6, although the signal assigned to the hydroxyl proton (farthest downfield) rarely integrated as a single proton but rather as a fraction of a proton. The farthest downfield signal (integrated as one or nearly one proton) was observable in the 1-*N*-substituted analogs and in all spectra of compounds taken in pyridine-d_5.

Special attention was given to the isopropyl and *n*-butyl analogs (V and VI) in the 3-*N*-substituted series, since their spectra were different than what was expected. The methyl groups of the isopropyl analog in the solvents acetone-d_6, pyridine-d_5 (anhydrous and after water was added), pyridine (with trace water present), and tetrachloroethane at 36° appear in Fig. 1. The methyl peaks of V in the wet pyridine at 85° also appear in Fig. 1.

The methyl signal in acetone-d_6 appeared as unequal doublets and as three unequal doublets in pyridine rather than the expected doublet for the isopropyl group, and these doublets coalesced into a simple doublet after water was added. The coalescence was dependent on the amount of water or deuterium oxide added. The spectrum taken in spectral grade pyridine (not anhydrous) appeared as three doublets, all of substantial size, which changed relative peak heights at 85° but did not coalesce into two or one doublet.

The methyl groups appeared as a simple doublet in the solvent tetrachloroethane (selected as a suitable solvent for high temperature study) at the normal spectrometer temperature of 36°. The methine proton signal appeared to coalesce to a heptet from the complex signal, which appeared to be two overlapping heptets seen in acetone-d_6 and pyridine-d_5 prior to the addition of water or deuterium oxide.

Table I—Substituted Analogs of Hydroxyurea



Compound	R	R′	Literature (14) Melting Point	Melting Point Found
IV	*n*-Propyl	H	128–129°	127–129°
V	Isopropyl	H	—	108–110°[a]
VI	*n*-Butyl	H	123–125°	122–125°
VII	Phenyl	H	142° dec.	141–142° dec.
VIII	*p*-Tolyl	H	151–153°	151–153°
IX	*p*-Chlorophenyl	H	171° dec.	170–171° dec.
X	H	Methyl	—	—
XI	H	Ethyl	—	—

[a] *Anal.*—Calc.: C, 40.66; H, 8.53; N, 23.72. Found: C, 40.47; H, 8.53; N, 24.04.

DISCUSSION

The use of NMR spectroscopy in the determination of structural and conformational properties of compounds related to urea and hydroxyurea was reviewed previously (16). The NMR data reported in this paper are similar to those in studies involving the effect of solvent (17) and of internal hydrogen bonding (18) on conformational properties of amides. In the hydroxyurea series in this study, a solvent-dependent conformational system was detected which may be partly due to internal hydrogen bonding.

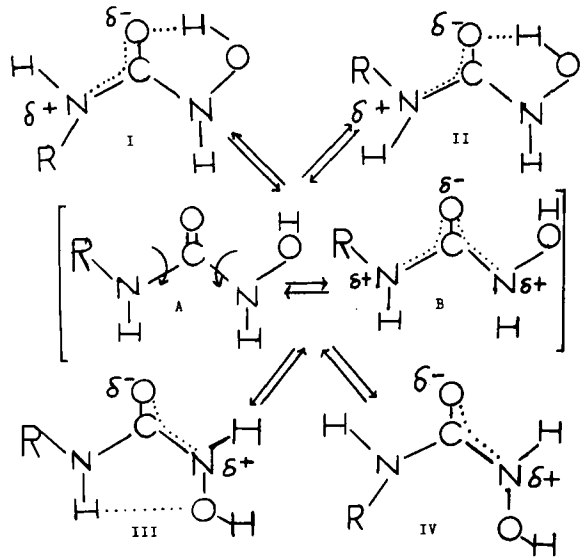
In the hydroxyurea system (Scheme I), if hindrance to rotation

[2] Aldrich Chemical Co.
[3] Squibb Institute for Medical Research.
[4] Varian T60A, 60-MHz spectra.
[5] Stohler Isotope Chemical Co.
[6] Aldrich Chemical Co. and Stohler Isotope Chemical Co.

Table II—Data of Hydroxyureas in Various Solvents

Compound (Solvent)	Chemical Shift of Signals[a], δ
Hydroxyurea (I) (acetone-d_6)	5.75 (broad m, 1–2H, NH_2), 7.7 (m, 0.5H, NH), 8.0 (m, 1.5H, NH and OH)
I (acetone-d_6 plus deuterium oxide)	4.2 (DOH)
I (pyridine-d_5)	5.2 and 5.7 (m, 2H, NH_2), 6.7 (s) and 7.7 (m, 1H, NH), 8.4 (m) and 9.1 (broad s, 1H, OH)
I (pyridine-d_5 plus deuterium oxide)	3.2 (DOH), 5.2 and 6.4 (weak signals, ratio of 1:1)
I [pyridine-d_5–acetone-d_6 (1:1)]	6.8 (m), 7.3 (m) and 7.7 (m, 3H, NH_2 and NH), 8.7 (s), 9.1 (m), and 9.8 (broad m, 1H, OH)
3-*N*-(*n*-Propyl)hydroxyurea (IV) (acetone-d_6)	1.0 (t, 3H, CH_3), 1.55 (sextet, 2H, CH_2), 3.0 (m, 2H, CH_2), 6.2 and 7.0 (m, 1–2H, NH and/or OH)
IV (acetone-d_6 plus deuterium oxide)	1.0 (t, 3H, CH_3), 1.6 (sextet, 2H, CH_2), 3.15 (t, 2H, CH_2), 3.9 (DOH)
IV (pyridine-d_5)	0.9 (t, 3H, CH_3), 1.6 (sextet, 2H, CH_2), 3.5 (q, 2H, CH_2), 7.3 (m, 1H, NH), 9.6 (s, 1H, NH), 11.0 (m, 1H, OH)
IV (pyridine-d_5 plus deuterium oxide)	0.8 (t, 3H, CH_3), 1.5 (sextet, 2H, CH_2), 3.3 (complex t, 2H, CH_2), 5.4 (DOH), 7.3 (m, 0.3H, NH), 8.6 (s, 0.1H, NH), 9.4 (m, 0.2H, OH)
3-*N*-(Isopropyl)hydroxyurea (V) (acetone-d_6)	1.2 (2 pairs d, 6H, CH_3), 3.2 (H_2O), 3.9 (2 pairs heptet, 1H, CH), 6.3 (m, 1H, NH), 8.0 (m, 0.5H, NH), 8.4 (s, 0.5H, OH)
V (acetone-d_6 plus deuterium oxide)	1.1 (2 pairs d, 6H, CH_3), 3.8 (DOH), 3.9 (heptet, 1H, CH)
V (pyridine-d_5 anhydrous)	1.3 (2 pairs d, 6H, CH_3), 4.3 (octet, 1H, CH), 6.9 (d, 1H, NH), 8.8 (m, 1H, NH), 10.9 (m, 1H, OH)
V (pyridine-d_5 plus deuterium oxide)	1.2 (d, 6H, CH_3), 4.2 (m, 1H, CH), 5.3 (DOH), 6.9 (d, 0.3H, NH), 9.3 (m, 0.3H, NH or OH)
V (pyridine, trace water)	1.1 (3 pairs d, 6H, CH_3), 4.1 (complex nonet, 1H, CH)
V (tetrachloroethane)	1.1 (d, 6H, CH_3), 3.8 (heptet, 1H, CH)
3-*N*-(*n*-Butyl)hydroxyurea (VI) (acetone-d_6)	0.9 (m, 3H, CH_3) 1.5 (m, 4H, CH_2CH_2), 3.1 (m, 2H, CH_2 and H_2O), 6.5 (m, 1H, NH), 7.8 (m, 1H, NH), 8.3 (m, 1H, OH)
VI (acetone-d_6 plus deuterium oxide)	0.9 (t, 3H, CH_3), 1.4 (m, 4H, CH_2CH_2), 3.2 (m, 2H, CH_2N), 4.0 (DOH)
VI (pyridine-d_5)	0.9 (m, 3H, CH_3), 1.7 (m, 4H, CH_2CH_2), 3.5 (complex q, 2H, CH_2), 7.3 (m, 1H, NH), 9.3 (m, 1H, NH), 10.8 (broad m, 1H, OH)
VI (pyridine-d_5 plus deuterium oxide)	0.8 (m, 3H, CH_3), 1.4 (m, 4H, CH_2CH_2), 5.8 (DOH), 7.3 (m, 0.3H, NH)
3-*N*-(Phenyl)hydroxyurea (VII) (acetone-d_6)	3.2 (H_2O), 7.3 (m, 5H, C_6H_5), 8.3 (m, 1H, NH), 9.2 (m, 0.6H, NH), 10.0 (m, 0.3H, OH)
VII (acetone-d_6 plus deuterium oxide)	3.65 (DOH), 7.3 (m, 5H, C_6H_5)
3-*N*-(*p*-Tolyl)hydroxyurea (VIII) (acetone-d_6)	2.3 (s, 3H, CH_3), 3.0 (H_2O), 7.3 (m, 4H, C_6H_4), 8.7 (m, 0.5H, NH and/or OH)
VIII (acetone-d_6 plus deuterium oxide)	2.3 (s, 3H, CH_3), 3.8 (DOH), 7.3 (m, 4H, C_6H_4)
3-*N*-(*p*-Chlorophenyl)hydroxyurea (IX) (acetone-d_6)	3.0 (H_2O), 7.5 (m, 4H, C_6H_4), 8.4 (m, 0.5H, NH or OH)
IX (acetone-d_6 plus deuterium oxide)	3.9 (DOH), 8.5 (m, 4H, C_6H_4)
1-*N*-(Methyl)hydroxyurea (X) (acetone-d_6)	3.1 (s, 3H, CH_3), 6.2 (m, 2H, NH_2), 9.1 (m, 1H, OH)
X (acetone-d_6 plus deuterium oxide)	3.1 (s, 3H, CH_3), 3.8 (DOH), 6.2 (m, 0.25H, NH_2), 9.6 (m, 0.12H, OH)
X (pyridine-d_5)	2.7 (s, 3H, CH_3), 6.5 (m, 2H, NH_2), 8.7 (m, 1H, OH)
X (pyridine-d_5 plus deuterium oxide)	3.4 (s, 3H, CH_3), 5.8 (DOH), 6.2 (broad m, 1H, NH), 7.0 (m, 1H, NH)
1-*N*-(Ethyl)hydroxyurea (XI) (acetone-d_6)	1.1 (t, 3H, CH_3), 3.5 (q, 2H, CH_2), 6.2 (m, 2H, NH_2), 9.1 (m, 1H, OH)
XI (acetone-d_6 plus deuterium oxide)	1.1 (t, 3H, CH_3), 3.6 (complex q, 2H, CH_2), 3.9 (DOH)
XI (pyridine-d_5)	0.6 (t, 3H, CH_3), 3.2 (q, 2H, CH_2), 6.5 (m, 2H, NH_2), 10.7 (m, 1H, OH)
XI (pyridine-d_5 plus deuterium oxide)	1.2 (t, 3H, CH_3), 3.7 (q, 2H, CH_2), 5.8 (DOH), 6.8 (m, 0.3H, NH)

[a] Chemical shifts reported as δ in parts per million downfield from tetramethylsilane.



Scheme I—Conformers (I–IV) produced by rotation around 1-N-carbonyl and 3-N-carbonyl bonds by passing through some intermediate. Hypothetical intermediates A and B possess all sp^3 *bonds (A) or distribution of π-bond character all along the N—C—N system.*

around the nitrogen–carbonyl bonds is minimal, all four conformers are present in proportion to their thermodynamic stability in solution, but individual conformers are not discernible by NMR due to rapid interconversion between conformers. Hindered rotation about either the 1-*N*- or 3-*N*-carbonyl bond produces conformational isomers due to slow rotation with respect to the NMR time scale, and these isomers are observable in the NMR spectra.

The most conclusive proofs of conformational isomerization in this hydroxyurea series are the NMR spectra of the isopropyl analog (V) in various solvents. The addition of water and deuterium oxide to the systems produced changes in the spectra, which appear to indicate that internal hydrogen bonding is reduced by hydrogen bonding to solvent water (or deuterium oxide). This finding indicates that the conformational isomerization in the isopropyl analog, which is observed on the NMR time scale as complex doublets, is due to internal hydrogen bonding (I, II, and III in Scheme I); this bonding increases the amount of π-bond character between the 3-nitrogen and the carbonyl carbon.

The spectrum of the 3-*N*-(*n*-butyl) analog was also more complex than expected for a simple *n*-butyl group, and decoupling of the methylene group from the nitrogen (—CH_2CH_2NH) simplified the spectrum to some extent. Nevertheless, the uncoupled spectrum also can be interpreted as one derived from a hindered system where conformational isomers are present.

The isopropyl methyl groups of V appeared as a set of doublets in acetone-d_6, as three sets of doublets of about equal size in spectral grade pyridine (trace water present), and as three sets of doublets (one pair large and two pairs of much smaller but equal size)

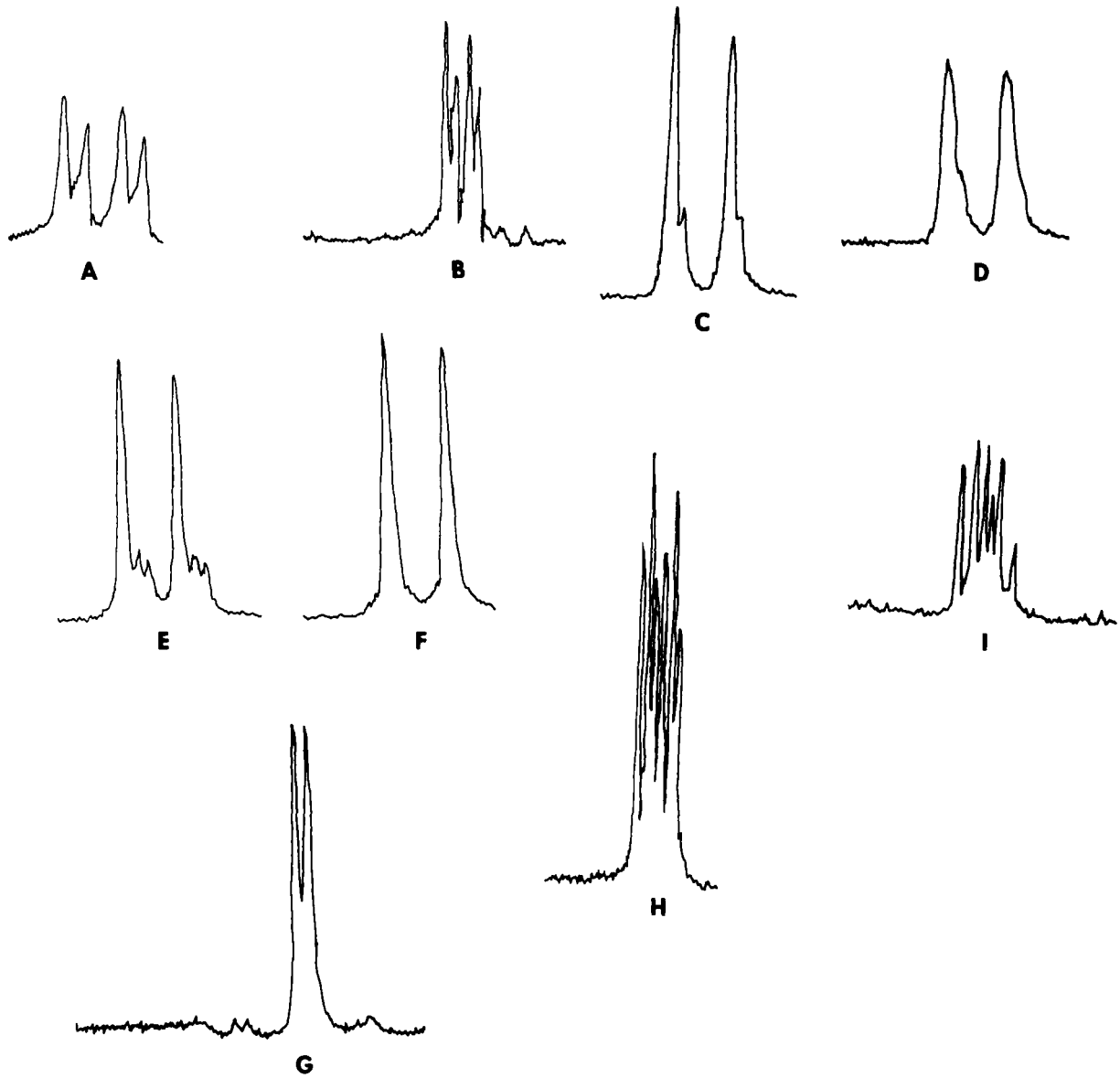


Figure 1—*Spectra of methyl peaks of 3-*N*-(isopropyl)hydroxyurea in various solvents. Key: A and B, in acetone-*d_6* with sweep width of 100 and 250 Hz, respectively; C and D, in the same solvent after water was added; E and F, in pyridine-*d_5* in solvent with trace water (E) and after water was added; H, in pyridine (trace water) at 85°; and I, in same solvent at 36°, as are all other spectra. G is spectrum in tetrachloroethane at 36°. Spectra C, D, E, and F had a sweep width of 100 Hz and G, H, and I had a sweep width of 500 Hz.*

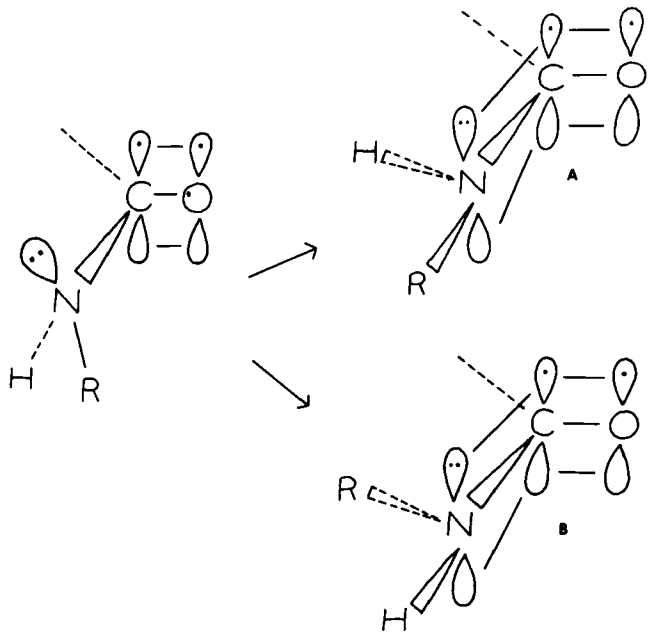
in pyridine-d_5 at 36° (Fig. 1). All of these spectra coalesced into a simple doublet after sufficient water or deuterium oxide was added.

The NMR spectrum of the isopropyl analog in acetone-d_6 (Fig. 1; A and B were taken almost immediately after the acetone-d_6 was opened) can be rationalized as due to conformers where rotation was slowed on the NMR time scale and the isopropyl group was thus in the proximity of the carbonyl carbon or the carbonyl oxygen (Scheme II) for longer periods than would be expected if rotation around bonds was unhindered. In this system, hydrogen bonding internally between the hydroxyl and the carbonyl oxygen would promote an increase in π-bond character (3-nitrogen to carbonyl), separation of charge, and a change of hybridization state of the 3-nitrogen from sp^3 to sp^2 (Schemes I and II). When more water or deuterium oxide was added to this system, a simple doublet was observed, which might be rationalized as due to hydrogen bonding between the hydroxyl group and water and the carbonyl oxygen and water (or deuterium oxide).

If the π-character between the 1-nitrogen and carbonyl carbon is increased with a corresponding decrease in π-character between the 3-nitrogen and carbonyl *via* this hydrogen bonding with water, then faster rotation around the 3-*N*-carbonyl bond by the isopropyl group would be possible, and this result would be observed on the NMR time scale as a simple doublet. The observation of three sets of doublets in pyridine (I in Fig. 1) can be rationalized as due to a rotating conformational system (Scheme I), where four conformers are possible (I–IV). These conformers are due to rotations around the 1-*N*—carbonyl—3-*N* bonds and pass through some transition state existing somewhere between A and B.

The canonical form shown as A depicts the hydroxyurea system as entirely made up of sp^3 bonds between nitrogens and carbonyl carbon, and free and unhindered rotation would be possible. This system would be characterized as fast rotation on the NMR time scale, and the spectrum would be observed as a simple doublet in the case of the isopropyl group. The canonical form shown as B depicts a system where the π-bond character is equally distributed across the nitrogen–carbon–nitrogen system, with a resultant separation of charge. In this case, rotation is restricted due to the π-character of the bonds; if no rotation occurs, discrete conformers exist.

If π-bond character between 1-nitrogen and carbonyl carbon increases with a corresponding decrease in π-bond character at the 3-*N*-carbonyl bond in B, rotation around the 3-*N*-carbonyl bond would be allowed as long as internal hydrogen bonding (seen in Conformers I and II) did not prevent it. Changes in the hybridization state of the nitrogen–carbon–nitrogen system would allow



Scheme II—Change of hybridization state of 3-nitrogen from sp^3 *to* sp^2*, with resultant* π*-character of 3-N-carbonyl carbon bond producing conformational isomers A and B, which have the R group held rigid either in the environment of the carbonyl oxygen (A) or the carbonyl carbon (B).*

Conformers I–IV to form *via* rotation due to the sp^3 character of the bonds (similar to that in A). The speed of rotation between these conformers would be observable on the NMR time scale, and they would be seen as a single conformer if rotation and interconversion are fast or as individual conformers if rotation and interconversion are slow.

Conformer IV would probably be in the lowest population, since it is not stabilized by internal hydrogen bonding. Conformer III might not be observed if hydrogen bonding to solvent (such as acetone) is stronger than internal hydrogen bonding. The three sets of signals in pyridine then might be interpreted as due to Conformers I–III, and the two sets of peaks in acetone-d_6 might be interpreted as due to I and II. When a polar solvent (such as water or deuterium oxide) is added to the system, the amount of internal hydrogen bonding is decreased due to hydrogen bonding with solvent; thus, Conformers I–III are not as stable, and rotation and interconversion are more rapid. This result might explain why the addition of water or deuterium oxide to these systems produces NMR spectra where a simple doublet is observed rather than the complex sets of two or three doublets.

Conformer III, which depicts a restriction of rotation due to an increase in π-bond character between the 1-nitrogen and carbonyl and internal hydrogen bonding between the 3-*N*-proton and hydroxyl oxygen, might only be observable when the carbonyl carbon is stabilized by solvent such as the case of pyridine. The best representations of the system are not A and B but rather systems where there is some degree of sp^3 bond character, some π-bond character between 1-*N*-carbonyl and 3-*N*-carbonyl at all times, and the increase and/or decrease of the π-bond character allows the rotation in the systems through Conformers I–IV.

When a spectrum of the isopropyl analog was taken in tetrachloroethane (for a high temperature study), the isopropyl group appeared as a simple doublet at 36°. The coalescence of the peaks in a relatively nonpolar solvent (a dielectric constant of 8.2 compared to 78.5, 20.7, and 12.3 for water, acetone, and pyridine, respectively) was unexpected. The fact that the isopropyl group of V in tetrachloroethane produced a doublet could mean that: (*a*) the conformational mobility is fast on the NMR time scale, possibly due to fast interconversion (faster than that observed in acetone and pyridine); or (*b*) there is a greater preponderance of one conformation in this solvent.

Since the least polar solvent in this series produces a spectrum similar to that observed when the most polar agent (*i.e.*, water, *etc.*) is added to the solvents, both of these possibilities may be the explanation. Possibly, tetrachloroethane is the least able to stabilize the separation of charge that occurs in conformers where π-character is increased between the nitrogens and carbonyl carbon of the system (Conformers I–IV in Scheme I); thus, rotation about bonds and interconversion are very fast, and a simple doublet appears in the NMR spectrum. Tetrachloroethane could possibly be acting as a proton donator similar to chloroform, which would then stabilize Conformer IV (Scheme I), or possibly act by some mechanism to stabilize one particular conformer of the system in preference to all of the others. But these explanations seem less likely than the increase in speed of interconversion where charge is not separated and the conformers are not stabilized.

Although it is not possible to determine from the data presented whether conformational isomers due to restricted rotation are present in all of the substituted analogs, the NMR spectra of hydroxyurea (I) and other compounds of the series in various solvents indicate that the likelihood of conformational isomerization in the hydroxyurea series exists. The molecule hydroxyurea is structurally or conformationally different in the two solvents, acetone-d_6 and pyridine-d_5. Hydroxyurea may exchange with one solvent (acetone-d_6) and not with pyridine, but pyridine is the better base.

In acetone-d_6, it was difficult to determine whether the signal assigned to the hydroxyl proton represented one proton. However, it appeared that less than one proton was present, possibly due to immediate exchange with solvent. In pyridine, hydroxyurea appeared as three distinct signals in the ratio of 2:1:1, as would be expected for hydroxyurea. The spectrum of hydroxyurea in a mixed solvent system [acetone-d_6–pyridine-d_5 (1:1)] possessed three downfield signals equal to a single proton. This proton could be the hydroxyl proton in three different environments, as would be expected in Conformers I–III (Scheme I) where internal hydrogen bonding would stabilize these conformers.

The comparison of some compounds of this series in acetone-d_6 and pyridine-d_5 indicates that the hydroxyl proton is recognizable in the spectra of all compounds taken in pyridine, while only the 1-*N*-substituted analogs and the 3-*N*-isopropyl and *n*-butyl compounds show a recognizable hydroxyl signal. This observation might be attributed to a property of these analogs, such as a greater degree of internal hydrogen bonding or a stronger hydrogen bond, which protects these analogs from exchanging with the solvent acetone-d_6. While this process might be taking place, it is difficult to verify in this system since the exchange of the hydroxyl proton with deuterium of acetone-d_6 is dependent on the enolization of the acetone and the rate of this process can be increased if trace amounts of acid are added to these systems and observed as relatively fast exchange of the hydroxyl proton in the NMR spectra. Since the hydroxyureas in this series are not stable for long periods at room temperature and in solvents, trace amounts of acidic (or basic) residues might be catalyzing the enolization of the acetone-d_6, producing the exchange of the hydroxyl proton in certain analogs.

It is concluded from these data that conformational isomers are possible in hydroxyurea systems due to possible internal hydrogen bonding between the carbonyl oxygen and hydroxyl proton. This hydrogen bonding appears to be further stabilized in nonhydrogen bonding solvents, which are polar enough to stabilize the resultant separation of charge, and by substitution at the 3-nitrogen by bulky groups such as isopropyl.

The internal hydrogen bonding can be reduced by solvents such as acetone, which can hydrogen bond to hydroxyl, but this bonding continues to stabilize the conformers that are still hydrogen bonded. The internal hydrogen bonding is appreciably reduced by the addition of a polar hydrogen bonding solvent, such as water, which appears to promote separation of charge between the 1-nitrogen and the carbonyl carbon and thus allows free rotation around the 3-*N*-carbonyl axis. The use of a relatively nonpolar solvent such as tetrachloroethane appears to promote the conformational form that is most nonpolar (similar to canonical form A in Scheme I), which allows fast interconversion between Conformers I–IV due to free rotation around both the 1-*N*-carbonyl and the 3-*N*-carbonyl bonds.

Therefore, conformational isomers are likely in hydroxyurea systems. This property is more or less prevalent in various analogs under various conditions. Although the effect of this form of isomerization, if any, in biological systems cannot be ascertained at this time, the property is being considered as a possible factor in ex-

plaining the low *in vivo* antileukemic activity of substituted hydroxyureas. The potential interrelationship between internal hydrogen bonding and conformational isomerization as well as the resultant effect on the pKa of hydroxyurea molecules and biological transport to the site of action is currently being studied. Initial *in vitro* tests on ribonucleotide reductase (mammalian) by analogs IV–VI indicate enzyme inhibition activity[7] at about 10 times the concentration required with hydroxyurea (I). Therefore, some property involving transport of these molecules to their site of action might be involved in their pharmacodynamics.

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ACKNOWLEDGMENTS AND ADDRESSES

Received October 30, 1974, from the *College of Pharmacy, Drake University, Des Moines, IA 50311*

Accepted for publication July 18, 1975.

Presented in part at the Medicinal Chemistry Section, APhA Academy of Pharmaceutical Sciences, Chicago meeting, August 1974.

Supported by the Drake University Research Council.

The authors thank Dr. David Abraham of the National Cancer Institute for supplying *in vivo* antileukemia data on hydroxyurea and 3-*N*-substituted analogs, Miss Barbara Stearns of the Squibb Institute for supplying the 1-*N*-substituted compounds and *in vivo* data on these compounds, and Dr. Lawrence Brown, Department of Chemistry, Drake University, for helpful suggestions.

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NOTES

Rapid Determination of Dimethyl Polysiloxane by Proton Magnetic Resonance Spectroscopy

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Abstract □ A new procedure is described for the quantitative extraction and determination of dimethyl polysiloxane by ^{1}H-NMR spectroscopy, using tetrachloroethylene as the solvent and dioxane as the internal reference.

Keyphrases □ Dimethyl polysiloxane—coatings, NMR analysis □ NMR spectroscopy—analysis, dimethyl polysiloxane coatings □ Silicones—NMR analysis of dimethyl polysiloxane coatings

The increasing use of silicones in drug, cosmetic, and food industries has given rise to various chemical and physical methods for the identification of this material. Several methods allow quantitative determinations of siloxane. Gravimetric (1) or volumetric (2) assays, visible or UV determination after mineralization (3–7), potentiometric titration (8), and IR (3, 6, 9–12) or atomic absorption spectroscopy (5, 12–14) determination after extraction have been reported.

A simple assay by NMR spectroscopy of the dimethyl polysiloxane (dimethicone) coating on needles of syringes is presented. This new technique compares favorably with the usual IR method.

EXPERIMENTAL

Method—Commercially available dimethyl polysiloxanes (I) [range of viscosity 0.65–10^6 centistokes (cS)] are silicone polymers in which each silicon atom of the polysiloxane chain is bonded to two methyl groups.