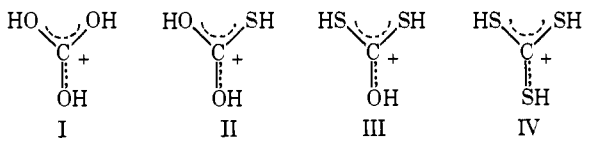
Stable Carbonium Ions. LXIX.[1] Protonation of Ureas, Guanidines, and Biotin in Super Acid Solution

George A. Olah and Anthony M. White[2]

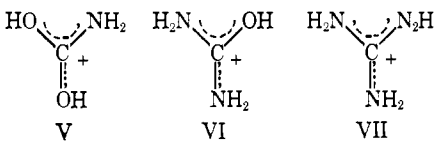
Contribution from the Department of Chemistry, Case Western Reserve University, Cleveland, Ohio 44106. *Received April 6, 1968*

Abstract: Urea, guanidines, and their methylated derivatives are diprotonated in the super acid system FSO_3H–SbF_5 ("magic acid"). ^1H, ^{13}C, and ^{15}N magnetic resonance studies, under conditions where proton exchange with the solvent is slow on the nmr time scale, were used to elucidate the structure of the diprotonated species. D-Biotin is triprotonated under the same conditions on the sulfide sulfur, the carboxyl group, and the urea carbonyl oxygen atom. Study of the structure of protonated D-biotin allows consideration of the preferential bonding of active D-biotin to proteins through the sulfur atom *trans* to the valeric acid side chain.

The direct observation of protonated carbonic acid (I) (trihydroxycarbonium ion) in FSO_3H–SbF_5 solution and the possible importance of this species in biological carboxylation processes[3] have led us to examine some related ions in the same acid system. It was found that successive replacement of hydroxyl by thiol groups can be achieved and a series of thiolhydroxycarbonium ions (protonated thiocarbonic (II), dithiocarbonic (III), and trithiocarbonic acids (IV)) have been observed. The amino analogs of the trihydroxycarbonium ion are also well established. Protonated carbamic acid[5] (aminodihydroxycarbonium ion) (V) has been observed in FSO_3H–SbF_5 solution and the stable crystalline salts of monoprotonated urea (VI) and guanidine (VII) have been extensively investigated.[6–8]



I II III IV



V VI VII

The behavior of urea and guanidine in strong acids has not, however, been clearly established. In sulfuric acid an early investigation, using cryoscopic methods, found tetramethylurea to be diprotonated and guanidine to be tetraprotonated.[9] Subsequent investigation in this acid system, however, has found only monoprotonation for both bases[10,11] although tetraethylurea was found to be diprotonated to the extent of 10% in 100% sulfuric acid.[12] Birchall and Gillespie[13] studied urea and 1,3-dimethylurea in fluorosulfuric acid and in fluorosulfuric acid containing 10% antimony pentafluoride. Using nmr spectroscopy to study these solutions they found no peaks corresponding to NH or OH protons in either acid. The methyl absorption of 1,3-dimethylurea also showed only a singlet. The absence of NH and OH proton absorptions was attributed to diprotonation of the bases. Diprotonation would render these protons highly acidic, leading to rapid exchange with solvent protons and indeed, under these conditions, an exchange-broadened solvent peak was observed. The equimolar mixture of fluorosulfuric acid and antimony pentafluoride used in our previous studies in this series has a much higher acidity than the 20:1 molar acid mixture used by Birchall and Gillespie. It was thus felt of interest to reexamine urea and substituted ureas in the 1:1 molar FSO_3H–SbF_5 acid system ("magic acid") to see if the greater acidity of this acid would result in proton exchange with the solvent being slowed sufficiently to make possible observation of the diprotonated species by nmr. In addition we have examined, by nmr spectroscopy, the behavior of some guanidine bases and the biologically important urea base, biotin, in the same acid system. This study also demonstrates the versatility of the "magic acid" solvent system for structural determinations in relatively complex molecules.

Results and Discussion

Urea in 1:1 *M* fluorosulfuric acid–antimony pentafluoride diluted with an equal volume of sulfur dioxide gave a well-resolved 100-MHz proton nmr spectrum at −80°. Besides two peaks due to the acid solvent, singlets at δ 12.82, 9.38, and 9.01 were observed of relative area 1:2:3, respectively (see Figure 1). This spectrum can only be interpreted as being due to diprotonated urea (VIII).

Two possibilities exist in ion VIII for restricted rotation observed on the nmr time scale. Firstly, restricted rotation about the C–O bond would result in two hydroxyl peaks, unless one of the two possible isomers was present to the exclusion of the other. Secondly, restricted rotation about the C–N bond might result in separate resonances being observed for the

(1) Part LXVIII: G. A. Olah and J. M. Bollinger, *J. Amer. Chem. Soc.*, 90, 6082 (1968).
(2) National Institutes of Health postdoctoral research investigator, 1966–1968.
(3) G. A. Olah and A. M. White, *J. Amer. Chem. Soc.*, 90, 1884 (1968).
(4) G. A. Olah, A. M. White, and A. Ku, to be submitted for publication.
(5) G. A. Olah and M. Calin, *J. Amer. Chem. Soc.*, 90, 401 (1968).
(6) C. R. Redpath and J. A. S. Smith, *Trans. Faraday Soc.*, 462, (1962).
(7) R. Stewart and L. J. Muenster, *Can. J. Chem.*, 39, 401 (1962).
(8) W. Kutzebrugg and R. Mecke, *Spectrochim. Acta*, 17, 530 (1961).
(9) A. Hantzch, *Ber.*, 63B, 1782 (1930).
(10) C. Holstead, A. H. Lamberton, and P. A. H. Wyatte, *J. Chem. Soc.*, 3341 (1953).
(11) G. Williams and M. L. Hardy, *ibid.*, 2560 (1963).
(12) T. Birchall, J. Gauldie, and R. J. Gillespie, unpublished results, quoted by R. J. Gillespie and E. A. Robinson in "Non-aqueous Solvent Systems," T. C. Waddington, Ed., Academic Press, New York, N. Y., 1965, pp 191–192.
(13) T. Birchall and R. J. Gillespie, *Can. J. Chem.*, 41, 2642 (1963).

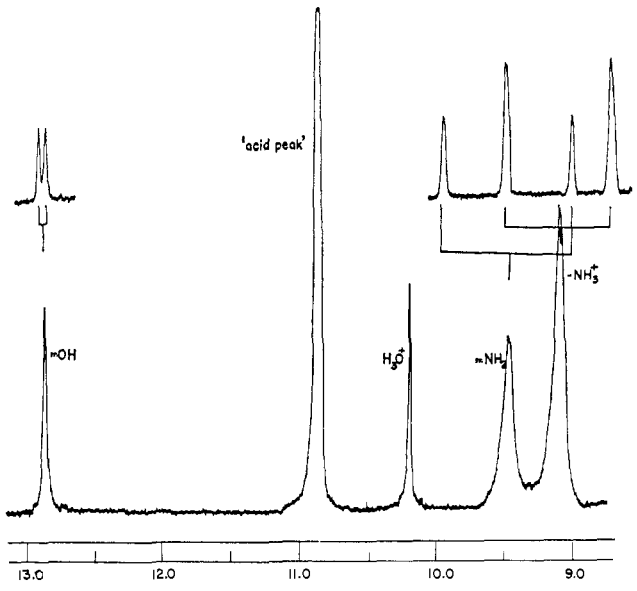


Figure 1. 100-MHz proton nmr spectrum of diprotonated urea (VIII) at −80°. Upper trace shows the corresponding spectrum of the di-^{15}N-enriched compound under the same conditions.

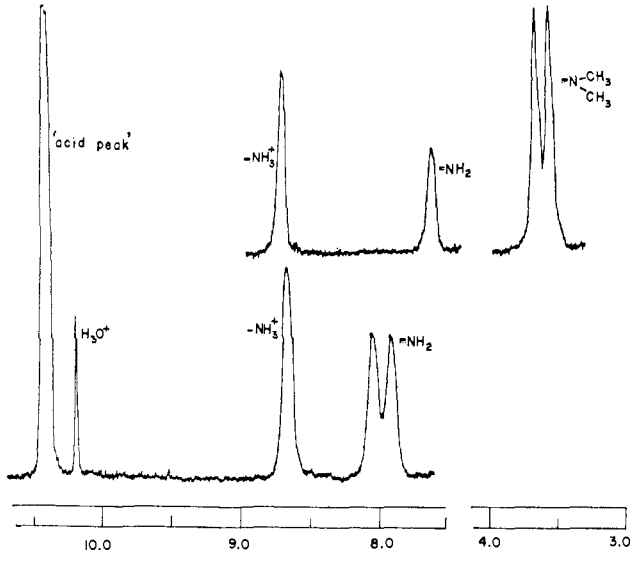
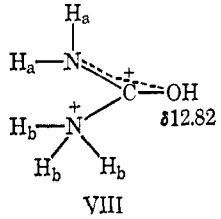


Figure 2. 100-MHz proton nmr spectrum of diprotonated guanidine (XV) at −80°. Upper trace shows the spectrum of diprotonated 1,1-dimethylguanidine (XVII) under identical conditions.

two H_a protons. In practice neither of these possibilities was confirmed experimentally. Evidence will be cited later for their being restricted rotation about the C–N bond but which, however, in the case of urea, does not result in an observable chemical shift difference between the two H_a protons.



VIII

As further confirmation of the assignment of the observed spectrum to the diprotonated species, the proton spectrum of ^{15}N-enriched urea in fluorosulfuric acid–antimony pentafluoride solution was obtained. The proton resonance spectrum (Figure 1) consists of a doublet (J = 4.6 Hz) for the OH protons and two doublets for the NH_3^+ and NH_2 protons ($J_{^{15}NH}$ equal to 76.6 and 96.8 Hz, respectively). ^{15}N–H coupling constants have been related to the state of hybridization of the NH bond[14] and this relationship has recently been confirmed for the sp-hybridized NH bond in protonated nitriles.[15] Using the expression

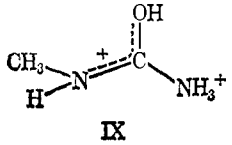
$$s = 0.43J_{^{15}NH} - 6$$

values for the percentage s character of 35.5 and 27.0% for the N–H_a and N–H_b bonds are obtained. These values are in excellent agreement with the structure of the diprotonated species proposed and compare with an intermediate value of 32% (J = 89.0 Hz) found in the free base.[14]

The ^{15}N spectrum of ^{15}N-enriched urea in dimethyl sulfoxide solution was obtained by the indor method and compared to the corresponding spectrum of the diprotonated compound in fluorosulfuric acid–antimony pentafluoride solution. The free base was found to have an ^{15}N chemical shift of 51.2 ppm from $^{15}NH_4^+$ as reference, in good agreement with the ^{14}N shift of 50.5 ppm.[16] Diprotonation leads to nonequivalence of the two nitrogens. The $-NH_3^+$ nitrogen gives a quartet centered at 31.9 ppm and the $=NH_2$ nitrogen a triplet at 104.9 ppm. A heteronuclear decoupling experiment demonstrated that the 4.6-Hz doublet due to the hydroxyl proton arises through coupling to the $-NH_3^+$ nitrogen nucleus.

We are unable at present to account for the magnitude and direction of these ^{15}N chemical shifts. While a linear correlation between nitrogen chemical shifts and calculated π-electron densities has been found for a series of triatomic nitrogen-containing molecules and ions,[17] this correlation has not been extended to other systems. We are currently investigating further examples of the effect of protonation on ^{15}N chemical shifts, comparing the results obtained with charge density calculations, with a view to establishing a more quantitative evaluation of the factors influencing ^{15}N chemical shifts.

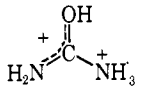
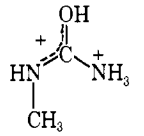
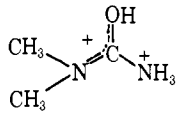
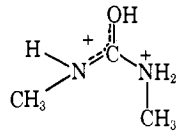
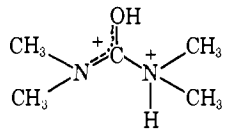
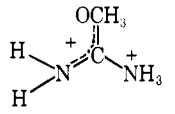
Methyl-, 1,1-dimethyl-, 1,3-dimethyl-, and tetramethylurea were examined under the same conditions as urea. The chemical shifts and coupling constants obtained are summarized in Table I. All the spectra are consistent with diprotonation. Thus the spectrum of methylurea shows a hydroxyl proton (δ 12.55), a methyl doublet (δ 3.74) coupled to one NH proton (δ 9.40), and an NH_3^+ peak (δ 9.01), the structure of the diprotonated base being that of IX.



IX

(14) G. Binsch, J. Lambert, J. D. Roberts, and B. W. Roberts, *J. Amer. Chem. Soc.*, **86**, 5564 (1964).
(15) G. A. Olah and T. Kiovsky, *ibid.*, **90**, 4666 (1968).
(16) M. Witanowski and H. Janouzewski, *J. Chem. Soc., B*, 1062 (1967).
(17) (a) E. L. Wagner, *J. Chem. Phys.* **43**, 2728 (1965); (b) J. E. Kent and E. L. Wagner, *ibid.*, **44**, 3530 (1966).

Table I. Pmr Chemical Shifts[a] and Coupling Constants[b] in Diprotonated Ureas

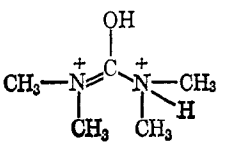
	Temp, °C	>NH+	>NH	-OH	-CH3
	−80	9.01	9.38	12.82	
	−70	9.01	9.40	12.55	3.74 d (5.2)
	−70	9.06		11.94	3.90 3.92
	−70	8.70	9.50	12.81	3.86 t (4.2) 3.82 d (5.1)
	−70	8.60		12.60	3.98 3.92 3.84 d (4.2)
	−70	9.05	9.47		4.73[c]

[a] Chemical shifts are in δ, parts per million, referred to external TMS as standard. [b] Coupling constants, in hertz, are given in parentheses following the multiplicity of the peak. [c] Absorptions due to *p*-toluenesulfonic acid also present in spectrum.

In both the asymmetrically substituted ureas, in which two possible sites for N-protonation exist, protonation was found to occur at the least substituted nitrogen, the result of a combination of steric and electronic factors.

In 1,1-dimethylurea and tetramethylurea, nonequivalence of the methyl groups attached to the sp_2-hybridized nitrogen atom was observed. This result demonstrates the presence of a significant barrier to rotation about the carbon–nitrogen bond.

In the case of diprotonated tetramethylurea, the temperature dependence of the spectrum was observed. At 100°, when slow decomposition began to occur, nonequivalence of the methyl groups was still observed, setting a lower limit for the ΔG for rotation of about 20 kcal. This value indicates that the predominant contribution to the structure is from the immonium form X rather than resonance structures which place

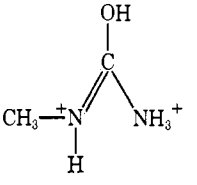


X

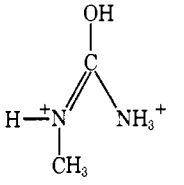
the positive charge on either the central carbon atom or on the oxygen. Some delocalization of charge onto oxygen must occur, however, as evidenced by the methoxyl and hydroxyl proton shifts observed in methylisourea and biotin, to be discussed later.

A similar conclusion was reached in studies of protonated imines[18] in the same acid system and the observed rotational barrier compares with values of the order of 20–25 kcal found in imines which are capable of *cis–trans* isomerism.[19] This comparison between the uncharged imines and immonium-type structures is however of limited significance since it is not clearly established that the mechanism leading to rotation about the C–N bond is the same in both systems.

No evidence was found for *cis–trans* isomerism of diprotonated methylurea or 1,3-dimethylurea, as could be expected in view of the high rotational barrier found for the C–N bond. Apparently one of the isomers is favored to the exclusion of the other, although on the available evidence we cannot distinguish between the two possible species (for example, XI and XII).

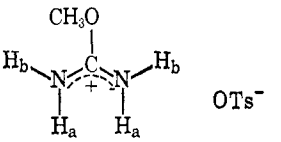


XI



XII

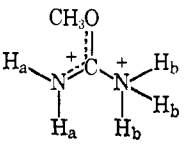
The *p*-toluenesulfonate salt of methylisourea proved to be soluble in sulfur dioxide, enabling a low-temperature nmr spectrum to be obtained. The methoxyl protons appear at δ 3.68, indicative of little or no positive character associated with the oxygen, thus implying that the charge is extensively delocalized onto the two nitrogen atoms. Two NH_2 peaks, at δ 6.19 and 8.02, suggest restricted rotation about the C–N bonds, the inner and outer NH protons (H_a and H_b in XIII) being magnetically nonequivalent.



XIII

No coupling between the NH protons was found. Irradiation of one caused a small (5%) enhancement of the second, but no decrease in the line width could be observed.

In FSO_3H–SbF_5–SO_2 solution, diprotonation occurred, the chemical shifts for the NH protons being very similar to those found in the ureas (Table I). In addition the methoxyl protons were shifted downfield to δ 4.73, showing that in the diprotonated species some charge delocalization onto oxygen is occurring.



XIV

Guanidine was also found to be diprotonated in the "magic acid" system. The nmr spectrum at −80°

(18) G. A. Olah and P. Kreienbuhl, *J. Amer. Chem. Soc.*, **89**, 4756 (1967).
(19) D. Y. Curtin and J. W. Hausser, *ibid.*, **83**, 3474 (1961).

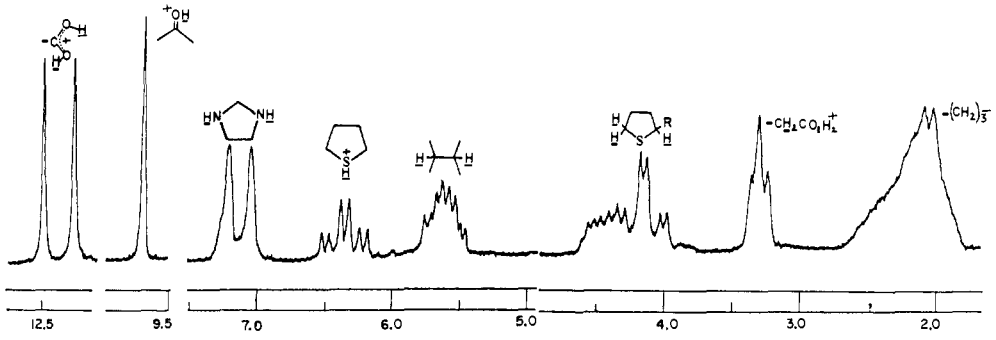
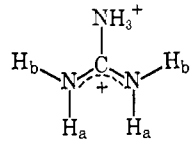


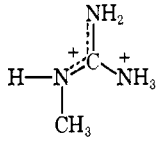
Figure 3. 100-MHz proton nmr spectrum of D-biotin in "magic acid" at −60°.

(Figure 2) consists of an NH_3^+ group at δ 8.68 and two protons at δ 8.07 and 7.85. As in the case of monoprotonated methylisourea, these two absorptions are interpreted as showing restricted rotation about the C–N bond causing nonequivalence of protons H_a and H_b in XV. Again, as in the case of methylisourea, no coupling between these NH protons was detectable. Exchange broadening of the peaks occurred above −60°, prohibiting investigation of the possible temperature dependence of this spectrum.

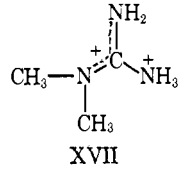


XV

Methyl and 1,1-dimethylguanidine were diprotonated in FSO_3H–SbF_5–SO_2 solution (XVI and XVII). In both bases, second protonation is at one of the primary amine groups, as in the case of the asymmetrically substituted ureas studied. In 1,1-dimethylguanidine, the two methyl groups are magnetically nonequivalent. A coalescence temperature of +16° was found corresponding to a $\Delta G^{\ddagger}$ value for the rotational barrier



XVI

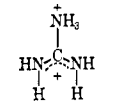
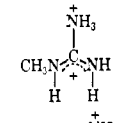
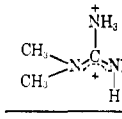


XVII

about the C–N bond of 15 kcal. This is significantly lower than the corresponding barrier in diprotonated ureas and demonstrates the greater extent of charge delocalization and hence lower C–N bond order in the diprotonated guanidines.[20] Chemical shifts of the protons in the guanidine bases studied are summarized in Table II.

D-Biotin contains three functional groups capable of being protonated under strong acid conditions. In FSO_3H–SbF_5–SO_2 solution D-biotin was found to be soluble, without decomposition, and the 100-MHz spectrum of this solution at −60° was well resolved (Figure 3). Double and triple irradiation were used to obtain a complete assignment of the spectrum. Chemical shifts and coupling constants at −60° are summarized in Table III and are compared to those found in trifluoroacetic acid solution.[21]

Table II. Pmr Chemical Shifts and Coupling Constants in Diprotonated Guanidines

	Temp, °C	>NH⁺	>NH	−CH₃
	−80	8.68	8.07 7.85	
	−60	8.71	7.88 8.05 q (40)	3.54 d (40)
	−60	8.63	7.54	3.62 3.69

Protonation at all three sites was found (XVIII), at the sulfide group, the carboxyl group, and the urea carbonyl oxygen. Only monoprotonation of the urea portion of the molecule is found, and the OH proton absorbs at much higher field than in the diprotonated ureas described previously. This is to be compared with the large downfield shift of the methoxyl protons on second protonation of methylisourea tosylate.

The SH proton consists of a triplet (J = 14.5 Hz) of doublets (J = 5.5 Hz). The vicinal S–H coupling constants in protonated thiane have been discussed and 14.1-Hz axial–axial and 2.3-Hz axial–equatorial couplings found.[22] Based on these results for the six-membered ring system, the magnitude of the vicinal S–H coupling constants in protonated D-biotin indicate that the conformational preference of the SH proton is *trans* to the valeric acid side chain. At the present time, no suitable model systems are available for establishing a Karplus relation for H–C–S–H coupling constants, and thus the coupling constants observed cannot be directly related to the dihedral angles involved. The coupling constants will clearly be dependent on the extent to which the ion is puckered and also the extent to which the sulfur deviates from tetrahedral hybridization.

(20) Proton exchange between the NH_3^+ and NH_2 groups could lead to magnetic equivalence of the methyl groups in diprotonated 1,1-dimethylguanidine. That this was not the case is demonstrated by the fact that at the coalescence temperature separate signals for these protons can still be observed.

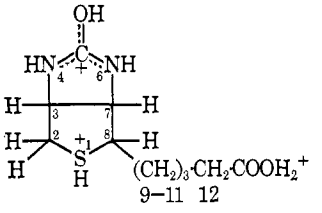
(21) J. A. Glasel, *Biochemistry*, **5**, 1851 (1966).

(22) J. B. Lambert, R. G. Kreske, and D. K. Weary, *J. Amer. Chem. Soc.*, **89**, 5921 (1967).

Table III. Pmr Chemical Shifts for D-Biotin[a] and Its Triprotonated Form

Solvent	C_2	C_8	C_3C_7	$C_9C_{10}C_{11}$	C_{12}	$CO_2H_2^+$	OH	NH
FSO_3H–SbF_5–SO_2	4.0–4.5		5.60	1.7–2.6	3.29	12.28 12.54	9.64	7.04 7.21
CF_3COOH[11]	3.10	3.47	4.81	1.73	2.62	...		...

[a] Chemical shifts in FSO_3H–SbF_5–SO_2 solution are given in parts per million from external TMS. Shifts in CF_3COOH are referred to internal TMS as standard.



XVIII

Relatively strong interactions between the sulfur atom in D-biotin and the protein to which it is attached have been suggested.[21,23] The fact that protonation of the sulfur occurs *trans* to the valeric acid side chain suggests that binding of the sulfur with the protein should occur from the same direction.[24] Attack from this side of the thiolane ring is particularly favorable in D-biotin in which the valeric acid side chain is *cis* to the ring junction. In L-biotin, any interaction with the sulfur would have to occur with approach from either the same side as the side chain or the same side as the ureido ring. Both of these modes of attack would be less favorable, on steric grounds, than in D-biotin, and indeed L-biotin shows no physiological activity.[28]

(23) A. C. Mildvan and M. C. Scrutton, *Biochemistry*, **6**, 2978 (1967).

(24) The steric requirements of the proton in such cases is a question that has not been completely resolved. In the protonation of ketones[25] and cyclic ethers[26] protonation from the least hindered side is always found, and our results accord with the view that in a conformational rivalry between the proton and nonbonding electrons, the final equilibrium position is primarily determined by the interaction properties of the proton.[27]

(25) G. A. Olah, M. Calin, and D. H. O'Brien, *J. Amer. Chem. Soc.*, **89**, 3586 (1967).

(26) G. A. Olah, P. J. Szilagyi, and J. Sommer, to be published.

(27) Footnote 17 in ref 17a.

Experimental Section

1,1-Dimethylurea was prepared from nitrourea and dimethylamine. Methylisourea *p*-toluenesulfonate was prepared from urea and methyl *p*-toluenesulfonate. Other urea and guanidine bases used were commercially available and, where necessary, were recrystallized before use.

Solutions of the bases in fluorosulfuric acid–antimony pentafluoride–sulfur dioxide were prepared as described earlier.[3] Proton spectra were obtained using a Varian Associates Model HA-100 spectrometer with a capillary tube of TMS as the internal lock signal. Chemical shifts, referred to this standard, were obtained using a frequency counter. Temperature measurements were made by means of a thermometer constructed to fit into a 5-mm nmr tube and are considered accurate to within $\pm 1°$.

^{15}N spectra were obtained by the indor method using the apparatus described previously.[3] The reference used for determination of ^{15}N shifts was a saturated aqueous solution of $^{15}NH_4NO_3$ for which ν_H/ν_N was found to be 9.8686442.

Rotational rates were computed at coalescence using the relationship

$$k = 1/\tau = \pi\left(\frac{\Delta\nu^2}{2}\right)^{1/2}$$

where $\Delta\nu$ is the chemical shift separation in hertz in the absence o exchange.

Acknowledgment. This work was possible through a grant of the National Institutes of Health. Hoffman LaRoche, Inc., is thanked for a gift of D-biotin.

(28) P. Gyorgy, *Vitamins*, **1**, 527 (1954).

The Stereochemistry of Protonation. XI

Howard E. Zimmerman and Patrick S. Mariano

Contribution from the Chemistry Department of the University of Wisconsin, Madison, Wisconsin 53706. *Received March 15, 1968*

Abstract: The stereochemistry of ketonization of exocyclic cyclohexane enols and tautomerism of *aci*-nitro compounds was reinvestigated. The enol of 1-acetyl-4-phenylcyclohexane was found to afford predominantly the less stable *cis* isomer on ketonization. Similarly, 1-*aci*-nitro-4-phenylcyclohexane tautomerized preferentially to *cis*-1-nitro-4-phenylcyclohexane when large proton donors were used. With smaller donors, the *trans* isomer was preferred. It was concluded that substituents at carbon 2 are not necessary for kinetic control leading to the less stable stereoisomer. The relationship of ground-state conformations to the transition states utilized is presently discussed. It is concluded, in keeping with our earlier postulate concerning kinetic control of ketonization, that approach of the proton donor in the least hindered way is usually the most important factor.

Some years ago, when the difference between kinetic and thermodynamic control of reactions was just becoming clear, we noted that many organic reactions involved unstable enol intermediates and that stereochemical preferences observed in these reactions are controlled by the kinetics of ketonization. Our hypothesis, which seems to be generally accepted, was that in many cases the less stable stereoisomeric product