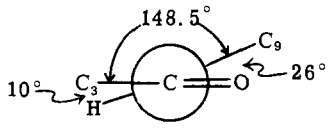

Figure 4. Conformation of the amide portion of **7-trans** in the solid state.[9]

thought to severely underestimate the degree of nonplanarity achievable at a given energy cost.

Pullman, on the other hand, has carried out ab initio self-consistent field molecular orbital calculations for the rotational barrier in formamide[17] and found that the computed total energy as a function of the torsional angle follows the theoretical curve (eq 3) extremely well, deviating by not more than 0.4 kcal/mol up to $\omega = 25°$.

Dunitz found the amide group in the trans isomer of **7** to have the conformation shown in Figure 4. If this is the conformation of the trans isomer in solution, it would appear from our work that this degree of distortion is achieved by the expenditure of approximately 3 kcal/mol in this compound. From eq 3 and a distortion of 26°, we calculate 3.8 kcal/mol. We conclude therefore in contrast to Dunitz and in agreement with Pullman that eq 3 probably does not err significantly in describing the energetics of the amide group. The form of this potential function is of great interest because of the important part it plays in theoretical calculations of polypeptide conformations.

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Proton Localization in Chemical Ionization Fragmentation[1]

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Abstract: The positional integrity of the ionizing proton in fragmentation reactions in chemical ionization mass spectrometry has been examined as a function of sample proton affinity and reaction temperature. In order to distinguish hydrogen atoms originating in the sample from protons transferred from reagent gas, a series of cyclohexyl-d_{11} compounds was used. It is found that hydrogen rearrangements may occur before fragmentation and the amount of scrambling increases with proton affinity of sample and with decreasing temperature. The ease of fragmentation of a series of cyclohexyl derivatives has been related to the proton affinity of the leaving group.

In studies of the chemical ionization mass spectra of deuterium-labeled compounds it has been found that the isotope labels may partially scramble with hydrogen atoms before fragmentation reactions occur.[3-5] Consequently, it has been suggested that the proton transferred in ionization need not be specifically localized in the molecule.[4] To investigate this further we have studied the effects of temperature and proton affinity on the randomization of the ionizing proton.

Cyclohexyl bromide, cyclohexanol, cyclohexyl mercaptan, and cyclohexylamine provide a range of proton affinities and all four compounds form $C_6H_{11}^+$ fragment ions by elimination of appropriate small neutral molecules under methane chemical ionization. In order to distinguish hydrogen atoms originating in the sample from protons transferred from methane reagent gas, the $C_6H_{11}^+$ fragment ions were compared to the corresponding $C_6D_{11}^+$ ions formed by the fully ring deuterated analogues of these compounds at reaction temperatures of 100, 150, and 200 °C.

$$c\text{-}C_6H_{11}X \xrightarrow{(H^+)} \underset{m/e\ 83}{C_6H_{11}^+} + HX$$

$$c\text{-}C_6D_{11}X \xrightarrow{(H^+)} \underset{m/e\ 94}{C_6D_{11}^+} + HX$$

$$X = Br, OH, SH, NH_2$$

Exchange of deuterium atoms out of the ring will be reflected in formation of $C_6D_{10}H^+$ or $C_6D_9H_2^+$ ions in addition to $C_6D_{11}^+$ ions.

Field[6] has predicted that the extent of fragmentation in which a small neutral molecule is eliminated in chemical ionization will be inversely proportional to the proton affinity of

Table I. Methane CI Mass Spectral Data of c-$C_6H_{11}X$ Compounds with the Respective Proton Affinities of HX, Where Known[a]

X		Proton affinity[b] of HX, kcal/mol	$MH^+/C_6H_{11}^+$	Other ions[c]	
Thioacetate	$SCOCH_3$		1.7	$CH_3COSH_2^+$	73%
Amine	NH_2	204–210	1.7	$(M-H)^+$	70%
Isocyanate	NCO		1.3		
Phosphine	PH_2	182–190	1.0	$(M-H)^+$	38%
Isothiocyanate	NCS		0.65		
Benzene	C_6H_5	176–186	0.23	$(M-H)^+$	23%
Propionate	$OCOCC_2H_5$	182–191	0.17	$C_2H_5CO_2H_2^+$	310%
Acetate	$OCOCCH_3$	175–192	0.15	$CH_3CO_2H_2^+$	84%
Thiol	SH	167–173	0.14		
Formate	OCOH	165–182	0.07	$HCO_2H_2^+$	13%
Alcohol	OH	162–171	0.03		
Halide	Cl, Br, I	141–145	0.00	$(M-H)^+$	1–3%
Fluoride	F		0.00	$(M-H)^+$	7%

[a] The spectra were recorded at a source temperature of 150 °C. [b] This is the total range compiled from various sources in ref 6. [c] The intensities of these ions are expressed relative to the MH^+ or $C_6H_{11}^+$ ions, whichever is the greater.

the molecule eliminated. Thus in a series of protonated molecular ions RXH^+, where R is held constant, the rate of elimination of XH should be faster when XH has a lower proton affinity. The validity of this proposed correlation in the cyclohexyl series would have implications for the time dependence of any proton scrambling observed in cyclohexyl bromide, cyclohexanol, cyclohexyl mercaptan, or cyclohexylamine. We have examined this proposed correlation by determining the ratios of $(M+H)^+/C_6H_{11}^+$ ions in the methane chemical ionization spectra of 15 cyclohexyl derivatives.

Experimental Section

The chemical ionization spectra were obtained using a Du Pont 21-491 mass spectrometer with a commercial Du Pont source. All samples were introduced to the source via a gold leak from a heated glass oven. In the temperature studies the stainless steel gas inlet line, which is essentially a reservoir from which reagent gas enters the source via a gold leak, was heated over a 2-m length to the same temperature as the source. The methane reagent gas was dried by passing it through a molecular sieve drying tube to ensure a <5% contribution from H_3O^+ ions in the background methane spectrum.[7]

Cyclohexanol-d_{12} was purchased from Aldrich Chemical Co. and the active deuterium was exchanged out of the molecule by repeated solution in methanol. Cyclohexyl-d_{11} bromide was obtained from Merck Sharpe and Dohme of Canada. The tosylate[8] of cyclohexanol-d_{11} was treated with sodium azide in *N*-methylpyrrolidone to produce cyclohexyl-d_{11} azide which was reduced to cyclohexylamine-d_{11} with lithium aluminum hydride.[9] Cyclohexyl-d_{11} mercaptan was synthesized from cyclohexyl-d_{11} bromide via the alkyl isothiourea.[10]

All compounds were checked for purity by GLC and the isotope incorporation was calculated from their electron impact spectra recorded on a CEC 21-110 mass spectrometer. The compounds purchased had >95% d_{11} and this level of incorporation was maintained in the synthesized compounds since none of the synthetic steps involved the ring deuteriums.

The 15 undeuterated cyclohexyl compounds were purchased from usual commercial sources and were used as received.

Results and Discussion

The correlation between proton affinity and extent or rate of fragmentation has been examined for the 15 cyclohexyl derivatives listed in Table I. Fragmentation has been expressed as the ratio of protonated molecular ions to $C_6H_{11}^+$ ions in the methane chemical ionization spectra recorded at a source temperature of 150 °C. Table I also lists the respective proton affinities of HX where known[11] and other major ions in the spectra expressed as percentage intensity of the MH^+ ions or $C_6H_{11}^+$ ions, whichever is the greater. Although the range of proton affinities reported for these compounds is generally large, the trend is apparent toward relatively larger protonated molecular ions compared to the cyclohexyl fragment ions as the proton affinity of HX increases. However, the presence of other abundant fragment ions in the spectra of some of the molecules, indicating processes with activation energies similar to that required for formation of $C_6H_{11}^+$, cannot be neglected. Thus, thioacetic acid certainly has a lower proton affinity (probably <200 kcal/mol) than ammonia. Cyclohexyl propionate appears low in the order presumably because of the ease of formation of the protonated propionic acid ion, although whether propionic acid can be considered as a small neutral molecule is debatable. Also, the structure of the neutral product may not be readily apparent as with cyclohexyl isocyanate and cyclohexyl isothiocyanate. Field's prediction may only, therefore, be viable in those cases where other complicating fragmentation processes are absent or produce ions of low abundance, where the expelled neutral is, indeed, small, and where the site of protonation is the same in the small neutral (HX) and RX molecules.

Hydrogen bromide, water, hydrogen sulfide, and ammonia provide a range of proton affinities and the corresponding derivatives, cyclohexyl bromide, cyclohexanol, cyclohexyl mercaptan, and cyclohexylamine produce substantial $C_6H_{11}^+$ fragment ions by elimination of these small neutral molecules in their methane chemical ionization mass spectra as shown in Table I. We have compared this fragmentation in the undeuterated ($C_6H_{11}X$) and ring-deuterated ($C_6D_{11}X$) molecules using methane reagent gas through a narrow range of reaction temperatures. Table II lists the relative amounts of $C_6D_{11}^+$, $C_6D_{10}H^+$, and $C_6D_9H_2^+$ ions produced in the methane chemical ionization mass spectra of cyclohexyl-d_{11} bromide, cyclohexanol-d_{11}, cyclohexyl-d_{11}, mercaptan, and cyclohexylamine-d_{11} at three source/reagent gas temperatures; 100, 150, and 200 °C. The figures have been calculated for the ion cluster at *m/e* 92–94 by comparing the deuterated and undeuterated methane CI spectra and correcting for ^{13}C contributions and incomplete labeling (<5%). It is apparent that the fragmentation is not simple in all cases.

The fragmentation is specific for loss of Br^- from cyclohexyl bromide at all three temperatures considered here. This may be compared to Field and Munson's observation[3] that in the methane CI mass spectrum of cyclohexane-d_{12}, the only ion in the molecular ion region is $C_6D_{11}^+$, and Hunt and McEwen's observation[5] that no deuterium is incorporated in the

Table II. Percent Hydrogen Incorporation in $C_6D_{11}^+$ Fragment Ions in the CI Mass Spectra of Cyclohexyl-d_{11} Bromide, Cyclohexanol-d_{11}, Cyclohexyl-d_{11} Mercaptan, and Cyclohexylamine-d_{11} at Three Reaction Temperatures

	Br	OH	SH	NH_2
		100 °C		
$C_6D_{11}^+$	100	79	82	65
$C_6D_{10}H^+$		20	14	28
$C_6D_9H_2^+$		1	4	6
		150 °C		
$C_6D_{11}^+$	100	83	84	75
$C_6D_{10}H^+$		16	12	25
$C_6D_9H_2^+$		1	4	
		200 °C		
$C_6D_{11}^+$	100	86	84	84
$C_6D_{10}H^+$		14	10	16
$C_6D_9H_2^+$			6	

$C_{10}H_{21}^+$ ion in the methane-d_4 CI mass spectrum of *n*-decane. The removal of the deuterium and hydrogen respectively in these cases is considered to be an abstraction reaction which generally occurs in molecules of low basicity.[12] This abstraction or dissociative proton transfer is certainly occurring with cyclohexyl bromide and is too fast to allow exchange processes to occur.

With cyclohexanol-d_{11} and cyclohexyl-d_{11} mercaptan, however, some scrambling of ring deuteriums with reagent or heteroatom hydrogen is occurring. Deuterium–hydrogen exchange does not take place between $C_6D_{11}^+$ ions and the methane plasma as shown by Field and Munson's cyclohexane-d_{12} result[2] and our cyclohexyl-d_{11} bromide result. Therefore, the comparable amounts of scrambling for both of these substrates reflects the similar proton affinities of H_2O and H_2S and the consequent similar lifetimes of the protonated molecular ions, RXH^+, or other intermediate complexes such as $(M + C_2H_5)^+$. There is also a trend toward slightly more scrambling on going from high to low reaction temperature. This trend is definite for cyclohexanol-d_{11} but is not so convincing for cyclohexyl-d_{11} mercaptan for this temperature range. This temperature trend reflects the decomposing ion lifetimes which are longer at lower temperature and hence more time is available for rearrangements to occur before decomposition.

These points are supported by the results of cyclohexylamine-d_{11} whose protonated molecular ion or electrophilic addition complex is more stable than for the other three deuterated compounds considered here since the proton affinity of NH_3 is higher than HBr, H_2O, or H_2S. That is, there is extensive hydrogen–deuterium rearrangement before decomposition and the scrambling increases significantly at lower temperatures. Of course, the un-ionized amine has two hydrogens attached to the heteroatom compared to one for the alcohol and thiol and, therefore, more scrambling may be considered as being a statistical origin. Also the number of pathways through which an intermediate ion might rearrange may vary with the functional group.

Jelus et al. have recently shown[13] that the $(M - 17)^+$ ion in the isobutane CI mass spectrum of cyclohexanol can be accounted for largely by decomposition of an $(M + 57)^+$ association complex that is relatively long lived. Using cyclohexanol-*1*-*d* and cyclohexanol-*2,2,6,6*-d_4 they showed that partial scrambling occurs before decomposition of $(M + 57)^+$ to $(M - 17)^+$ ions. Table III lists the relative amounts of C_6D_{11}, $C_6D_{10}H$, and $C_6D_9H_2$ ions found by us in the isobutane CI mass spectra of cyclohexanol-d_{11} at three reaction temperatures. These data support the aforementioned result[13] and further, more rearrangement is apparent at lower temperature. However, less scrambling occurs than for the methane CI mass spectra of cyclohexanol-d_{11} because of a combination of factors such as the relative lifetimes and energies[13,14] of the decomposing intermediates. That is, the abstraction of hydroxide from cyclohexanol by *tert*-$C_4H_9^+$ is slightly endothermic,[13] whereas dissociative proton transfer from CH_5^+ and $C_2H_5^+$ to cyclohexanol and abstraction of hydroxide from cyclohexanol by $C_2H_5^+$ are exothermic. The pathways through which rearrangements may occur will also play a role and the question is then whether the scrambling observed in these systems represents random selection from the ring or specific exchange mechanisms. Studies with specifically deuterated species and other reagent gases are underway to answer this point.

Table III. Percent Hydrogen Incorporation in $C_6D_{11}^+$ Fragment Ions in the Isobutane CI Mass Spectrum of Cyclohexanol-d_{11} at Three Reaction Temperatures

	100 °C	150 °C	200 °C
$C_6D_{11}^+$	90	96	99
$C_6D_{10}H^+$	6	4	1
$C_6D_9H_2^+$	3		

The isobutane CI mass spectrum of cyclohexyl mercaptan produces an $(M - SH)^+$ ion of only 20% abundance relative to the $(M + H)^+$ ion at 200 °C reaction temperature, so measurements of $C_6D_{10}H^+$ ions produced by rearrangements in cyclohexyl-d_{11} mercaptan are uncertain and, if present, are certainly small.[14] Cyclohexylamine produces no appreciable $(M - NH_2)^+$ ions with isobutane reagent gas at 200 °C reaction temperature and the isobutane reagent gas does not react significantly with cyclohexyl bromide.

Conclusion

In chemical ionization mass spectrometry using methane or isobutane reagent gas, the positional integrity of the ionizing proton may not be maintained in fragmentation reactions. That is, hydrogen rearrangements may occur in protonated molecular ions, MH^+, or intermediate electrophilic addition complexes such as $(M + C_2H_5)^+$ with methane or $(M + C_4H_9)^+$ with isobutane before decomposition occurs. Care should, therefore, be taken in the analysis of deuterium-labeled compounds by chemical ionization mass spectrometry when the protonated molecular ion[15] or fragment ions are being examined. It would be of theoretical interest to examine which reagent gas species are responsible for the rearrangements and the specificity of the rearrangement processes. That is, which hydrogens are involved, especially with relevance to analogous processes occurring in electron impact and field ionization mass spectrometry.[16]

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An ESR Study of the Acid Dissociation of NH Protons.[1] 1. Linear Peptide Radicals and Related Radicals

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Abstract: Free radicals formed by the reaction of OH or O^- radicals with aliphatic compounds containing a peptide group (–CONH–) and structurally related compounds have been studied by the in situ radiolysis–steady state ESR method. Linear peptide radicals (–CONHĊ<) from *N*-acetylglycine, *N*-methylacetamide, *N*-ethylacetamide, *N*-methylpropionamide, ethyl methylcarbamate, *N*-methylformamide, *N*-acetylalanine, glycylglycine, and hippuric acid have been found to result from hydrogen abstraction from the C–H bond adjacent to the NH group. In strongly basic solutions significant changes occur in ESR parameters of six peptide radicals. These changes occur in α-, β-, δ-, or γ-proton coupling constants and can be interpreted in terms of the dissociation of the NH proton. The pK_a values for the NH proton dissociation have been determined to be 14.0 for $CH_3CONH\dot{C}HCOO^-$, 14.5 for $CH_3CONH\dot{C}H_2$, $CH_3CH_2CONH\dot{C}H_2$, and $CH_3CH_2OCONH\dot{C}H_2$, 14.6 for $CH_3CONH\dot{C}HCH_3$, and 13.3 for $HCONH\dot{C}H_2$. The pK_a values for the peptide radicals derived from *N*-acetylglycine, *N*-methylacetamide, and *N*-methylformamide are respectively estimated to be 5.4 to 5.6, 4.9, and 5.2 units lower than those for the parent compounds. Since the pK_a value of $CH_3NH\dot{C}HCOO^-$ from sarcosine appears to be larger than 17, the acidity of peptide radicals results from the electron-withdrawing effect of the carbonyl group adjacent to the NH group. Upon dissociation of the NH proton, α- and β-proton coupling constants decrease while δ- or γ-proton coupling constants increase. Analysis of the changes for four radicals suggests that dissociation is accompanied by a decrease in spin density on the α carbon by 0.09 to 0.12 while that on the γ carbon increases by 0.06 to 0.10. An accompanying slight increase in g factor is also observed upon the dissociation, indicating an increase in the spin density on the oxygen and/or nitrogen atom in the dissociated form. The changes in the ESR spectrum of the radical from *N*-acetylglycine have been studied in considerable detail and the dynamics of the equilibrium $CH_3CONH\dot{C}HCOO^- + OH^- \rightleftarrows CH_3CON^-\dot{C}HCOO^- + H_2O$ have been investigated by means of the pH dependence of line width. The forward and reverse rate constants are found to be 8×10^8 and 1.6×10^7 M^{-1} s^{-1}, respectively. The deviation of the rate of the forward reaction from the value expected under diffusion control is discussed. The coupling constants of the NH protons of peptide radicals are found to be accurately predicted by the following equation which includes not only the contribution from the spin density on the nitrogen atom but also the canceling effects of spin densities on the α- and γ-carbon atoms: $a^H(NH) = |25.0\rho_N - 0.6(\rho_{\alpha\text{-}C} + \rho_{\gamma\text{-}C})|$. Sigma radicals (ĊONH–) were also detected from three compounds with formyl groups. They were not, however, observable in the strongly alkaline region, probably because of hydrolysis of the parent molecule. With *N*-acetylethanolamine, the α-hydroxy radical was found rather than the peptide radical. Dissociation of the OH proton was observed in weakly basic solution.

The reactions of OH radicals with compounds which have the peptide linkage have been investigated by various workers using optical pulse radiolysis[3-6] and ESR spectroscopy.[7-12] One of the most important radicals produced in these reactions is that resulting from hydrogen abstraction from the carbon atom adjacent to the peptide nitrogen, i.e., –CONHĊH–. This radical is sometimes called the peptide radical.[6] Since the acid–base properties of the peptide radical[13] may be of importance in understanding radiation damage to polypeptides and proteins in the α-helical configuration where hydrogen bonding, CO···HN, plays an important role, it is desirable to establish the acidity of NH protons of peptide radicals. Using optical pulse radiolysis, a number of pK values have been found for free radical intermediates formed by the reaction of OH radicals with linear and cyclic peptides.[4,6] Several pK values are concluded to be due to the dissociation of the peptide protons. They are 9.6 for two cyclic peptide radicals[6] and 10.9 to 12.1 for four linear peptide radicals.[4] Little appears to be known regarding the acidity of linear peptide radicals with pK > 13. In an optical pulse radiolysis study[4] it was speculated that a few linear peptide radicals would dissociate peptide protons with pK values larger than 13 while ESR experiments[11] suggested that the radical from *N*-acetylglycine ($CH_3CONH\dot{C}HCOO^-$) might dissociate an acetyl proton instead of the NH proton. The present paper describes a detailed in situ radiolysis–ESR study[14] of the acidity of some linear peptide and related radicals formed by hydrogen abstraction with OH or O^- radicals. Advantage is taken of the ability of ESR spectroscopy to follow the acid–base properties of a radical in a more direct way[15] and over a wider acidity range[16-18] than the optical pulse radiolysis method.

Experimental Section

Chemicals were of the highest grade available from Eastman, Aldrich, J. T. Baker, Sigma Chemical, Cyclo Chemical, Linde, and Matheson and were used without further purification. Water was distilled and freed from organic impurities by passing the vapor with oxygen through a silica oven at ~600 °C. Experiments were carried out by the in situ radiolysis–ESR method.[14] Radicals were produced by irradiating an aqueous solution of the desired compound (5–10