of temperature is shown in Figure 5. These plots were extrapolated to 240°K to obtain a comparison of the rate data for the two reactions. The rate constants and thermodynamic parameters for process II are listed in Table VI.

Table VI. Kinetic and Thermodynamic Data for Process II at 240°*K*

Compd	Solvent	*k*, sec^{-1}	$\Delta G^{\ddagger}$	$\Delta S^{\ddagger}$	$\Delta H^{\ddagger}$
2	Acetone-d_6	1.8×10^3	10.0[a]	−18[a]	5.7[a]
3	$CDCl_3$	0.15×10^3	11.2	−12	8.3
	CS_2	0.19×10^3	11.1	−17	7.1
5	CS_2	4.7×10^3	9.6	−4	8.6
	Acetone-d_6	5.2×10^3	9.5	−7	8.0

[a] Free energies and enthalpies are in kcal/mol. The estimated error is ±1.0 kcal/mol. Entropies are in entropy units. The estimated error is ±4 eu.

Process II is a type of valence tautomerism. The aromatic ring must be twisted with respect to the plane of the quinone ring in order for the ring protons to be magnetically nonequivalent. The interchange of the two halves of the molecule can proceed when the aromatic ring rotates so that the molecule is in a planar conformation. The rate constant which we have measured probably represents the rate of rotation of this ring. The reaction which interconverts the zwitterion and the biradical requires a change in the hybridization of the nitrogen atom. This reaction was slower than the valence tautomerism reaction in each case. The smaller values for k_1 appear to be mainly due to the larger negative entropies for this reaction. The relative values of the entropies probably reflect the geometric requirements of the two reactions.

The values of the k_1's depended on both the steric bulk of the R group and the solvent. Molecules with bulkier R groups had smaller rate constants in each case. The variation in the rate constants with solvent was different for each of the compounds. Both the activation enthalpy and the activation entropy varied when the solvent was changed. Apparently each of the compounds interacts with the solvent in a slightly different manner and the rate data do not show a systematic solvent dependence.

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Sites of Attack of Hydroxyl Radicals on Amides in Aqueous Solution

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Abstract: The reactivity and sites of attack of hydroxyl radicals on simple amides in aqueous solution were studied. The following amides were examined: formamide (F), N-methylformamide (NMF), N,N-dimethylformamide (DMF), acetamide (A), N-methylacetamide (NMA), and N,N-dimethylacetamide (DMA). The rate constants for reaction of OH radical with NMF, DMF, NMA, and DMA fall in the range (1.0–3.5) × 10^9 M^{-1} sec^{-1}, but the reactivity with F and A is significantly lower. The absorption spectra in the wavelength range 225–600 nm and the specific rates of decay of the transient species produced by the reaction of OH radicals with amides were determined and their structures assigned. With F and A, OH radicals attack at both sites on the molecule to give $HCO\dot{N}H$, $\dot{C}ONH_2$, $CH_3CO\dot{N}H$, and $\dot{C}H_2CONH_2$ radicals. With the N-methylated amides, abstraction by OH radicals takes place mainly from the N-methyl group, *e.g.*, $OH + CH_3CON(CH_3)_2 \rightarrow CH_3CON(\dot{C}H_2)(CH_3) + H_2O$. The implications of these results with respect to the radiolysis of peptides are discussed.

A number of investigations have been carried out on the optical absorption and nmr spectral properties of simple amides and peptides in solution (see, *e.g.*, ref 3–5 and references cited therein), containing one or two peptide groups, but very little work appears to have been done on the free radical chemistry of the amide group. Such information is clearly of great interest in the study of the photochemistry and radiation chemistry of peptides in solution.

This paper deals with the reactivity and site of attack of hydroxyl radicals on simple amides in aqueous solution. The technique of pulse radiolysis and kinetic absorption spectrophotometry has been used as a convenient method for generating OH radicals and for observing and identifying the transient species produced. The intermediates produced in the radiolysis of neat liquid amides have been examined and are described[6] separately.

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Related work has been reported by Livingston and Zeldes,[7] who studied by esr the free radicals formed in solution by hydrogen abstraction from simple amides. This was carried out by uv irradiation, in the cavity of the esr spectrometer, of flowing solutions containing acetone or hydrogen peroxide. Smith and Wood,[8] using the titanium–H_2O_2 flow method, have obtained the esr spectra of radicals produced from formamide and acetamide. Fel', Dolin, and Zolotarevskii[9] have briefly reported their results on the pulse radiolysis of aqueous solutions of some amides.

Experimental Section

Hydroxyl radicals were produced by the radiolysis of aqueous solutions and the intermediates formed observed using the technique of pulse radiolysis. The details of the experimental conditions have been described elsewhere.[10] In brief, a Febetron 705 (Field Emission Corp.) pulsed radiation source was used, which produces an electron beam of 2.3-MeV energy and single pulses of approximately 30-nsec duration. An Osram XBO-450-W xenon lamp was used, and the light output from the lamp increased by a factor of 25–30 by increasing the current to the lamp for short durations of about 1–2 msec. A double monochromator was used[10] to reduce scattered light and improve wavelength resolution, and an EMI 9558-QB photomultiplier and associated circuitry[11] were employed. Dosimetry was carried out as described elsewhere.[10]

The procedure employed in purification of F and NMF is described elsewhere.[12] Formamide is particularly difficult to purify[13] and its hydrolysis to formic acid and ammonia is more rapid than that of other simple amides. It is therefore considered likely that the rate constant $k(\mathrm{OH} + \mathrm{F})$ derived in this work (see Results) is high. N,N-Dimethylformamide and N,N-dimethylacetamide were Eastman "Spectrograde." Acetamide and N-methylacetamide were, respectively, Eastman and Mallinckrodt reagent grade. N-Bromoacetamide, α-chloroacetamide, and α-chlorodimethylacetamide were all recrystallized just previous to use. *t*-Butyl alcohol, potassium thiocyanate, and potassium hydroxide were Mallinckrodt or Baker and Adamson reagent grade products.

Solutions were prepared using water purified by triple distillation, radiolysis, and photolysis. Quartz optical cells of 2-cm optical path were used and filled with a fresh solution for each electron pulse. The optical densities were usually read at ≤ 1 μsec, and the reproducibility of the dose was better than ±5%. Unless stated otherwise, all experiments were carried out in the presence of N_2O (1 atm).

Total doses of approximately 19 krads/pulse were used for formamide, N-methylformamide, N-bromoacetamide, α-chloroacetamide, and α-chloro-N,N-dimethylacetamide; and of approximately 8 krads/pulse for N,N-dimethylformamide, N-methylacetamide, and N,N-dimethylacetamide.

Results

The reactivity of OH radicals in aqueous solution, at pH 5–6, with formamide (F), N-methylformamide (NMF), N,N-dimethylformamide (DMF), acetamide (A), N-methylacetamide (NMA), and N,N-dimethylacetamide (DMA) has been determined in the presence of N_2O (1 atm) using the thiocyanate method.[14] Hydroxyl radicals react with CNS^- ions to produce $(CNS)_2^-$ radical anions

$$\mathrm{OH} + \mathrm{CNS^-} \longrightarrow \mathrm{CNS\cdot} + \mathrm{OH^-} \qquad (1)$$

$$\mathrm{CNS\cdot} + \mathrm{CNS^-} \rightleftharpoons (\mathrm{CNS})_2^- \qquad (2)$$

with $\lambda_{(\mathrm{CNS})_2^-}^{\max}$ 500 nm and $\epsilon_{(\mathrm{CNS})_2^-}^{500}$ 7600 M^{-1} cm^{-1}. The maximum absorption at 500 nm, OD_0, is reduced in the presence of amides in a way which can be represented by

$$\frac{k_1}{k_3} = \frac{\mathrm{OD}}{\mathrm{OD}_0 - \mathrm{OD}} \frac{[\mathrm{amide}]}{[\mathrm{CNS^-}]}$$

where

$$\mathrm{OH} + \mathrm{amide} \longrightarrow \mathrm{product} \qquad (3)$$

Three to five different concentrations of amides were used, the ratio k_1/k_3 was determined, and k_3 was obtained taking[15] $k_1 = 1.1 \times 10^{10}$ M^{-1} sec^{-1} (see Table I).

Table I. Rates of Reaction of OH Radicals with Amides in Aqueous Solution, pH 5.5, as Determined by the Thiocyanate (10^{-2} *M*) Method

System	Concentration range, m*M*	k_1/k_3	$k(\mathrm{S} + \mathrm{OH})$, M^{-1} sec^{-1} [a,b]
Formamide	56–224		[c]
N-Methylformamide	25–100	9.2	1.2×10^9
Dimethylformamide	5–100	6.5	1.7×10^9
Acetamide	20–100	58.0	1.9×10^8
N-Methylacetamide	25–100	6.9	1.6×10^9
Dimethylacetamide	24–72	3.1	3.5×10^9

[a] Derived taking $k(\mathrm{OH} + \mathrm{CNS^-}) = 1.1 \times 10^{10}$ M^{-1} sec^{-1}. [b] Rate constant values ±15%. [c] A high-limit value of 5.0×10^8 M^{-1} sec^{-1} was obtained (see text).

The value of $k(\mathrm{OH} + \mathrm{HCONH_2})$ obtained is considered likely to be high due to the presence of formic acid, $k(\mathrm{OH} + \mathrm{HCOO^-})$[16] $= 2.5 \times 10^9$ M^{-1} sec^{-1}. N-Methylation of the amides increases the rate of reaction 3 in the case of the acetamides by about one order of magnitude. Introduction of a second methyl group, *e.g.*, in DMF and DMA, increases k_3 by a factor of about 2.

The transient optical absorption spectrum produced from the reaction of OH radicals with formamide is shown in Figure 1. Two bands are formed, one with $\lambda_{\max}$ approximately 320 nm and the other with a maximum below 245 nm. The transient absorptions produced from NMF and DMF are shown in Figures 2 and 3. In these cases, the maxima of the far-uv bands were observed and are at 235 and 248 nm for NMF and DMF, respectively.

The reaction of OH radicals with the three formamides was examined in alkaline solutions, up to pH 12–13. No changes in the spectra or extinction coefficients were observed for NMF and DMF, after taking due precautions to minimize the alkaline hydrolysis of these amides. With formamide the alkaline hydrolysis was so fast that it was not possible to study the system in alkaline solutions.

With acetamide, an increased transient absorption was observed in alkaline solution at pH 13.1, as compared to pH 6.0, Figure 4. The OD *vs.* pH curve

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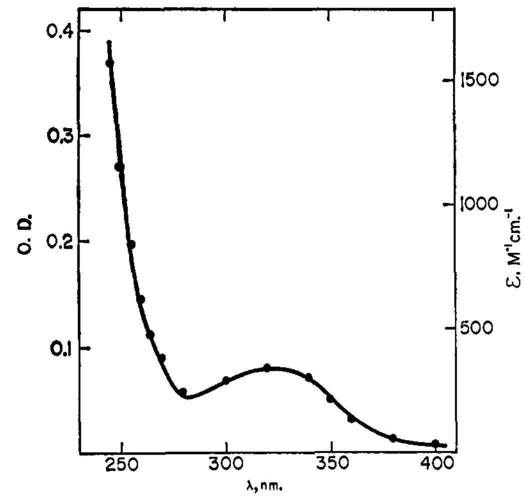

Figure 1. Transient optical absorption produced by the reaction of OH radicals with 1.0 *M* formamide, pH 7.0, N_2O, *D* ~ 19 krads/pulse.

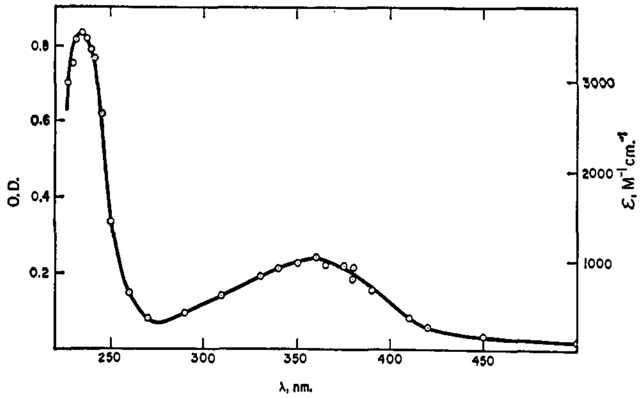

Figure 2. Transient optical absorption produced by the reaction of OH radicals with 0.2 *M* N-methylformamide, pH 5.3, N_2O, *D* ~ 19 krads/pulse.

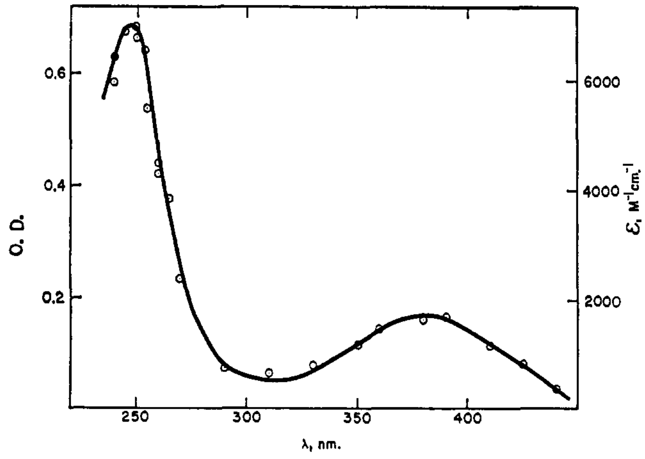

Figure 3. Transient optical absorption produced by the reaction of OH radicals with 0.2 *M* dimethylformamide, pH 5, N_2O, *D* ~ 8 krads/pulse.

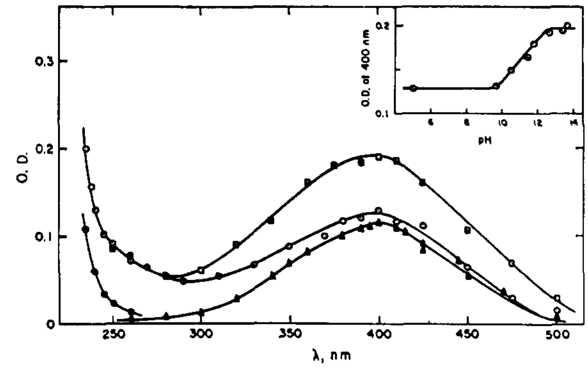

Figure 4. Transient optical absorption produced by the reaction of OH radicals with 1.0 *M* acetamide, pH 6.0 (○) and 13.1 (□), N_2O; of e_{aq}^- with N-bromoacetamide, pH 5, Ar, in presence of 0.5 *M* *t*-butyl alcohol (●), *D* ~ 19 krads/pulse. The symbol △ represents data given in Figure 7 for the reaction of e_{aq}^- with α-chloroacetamide at pH 5.5 and 12.4. The insert shows absorption at 400 nm *vs.* pH of species produced from acetamide.

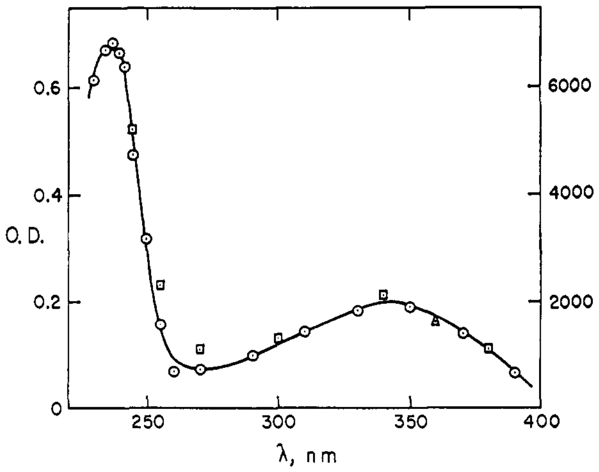

Figure 5. Transient optical absorption produced by the reaction of OH radicals with 0.2 *M* N-methylacetamide, pH 5 (○) and 13 (□), N_2O, *D* ~ 8 krads/pulse.

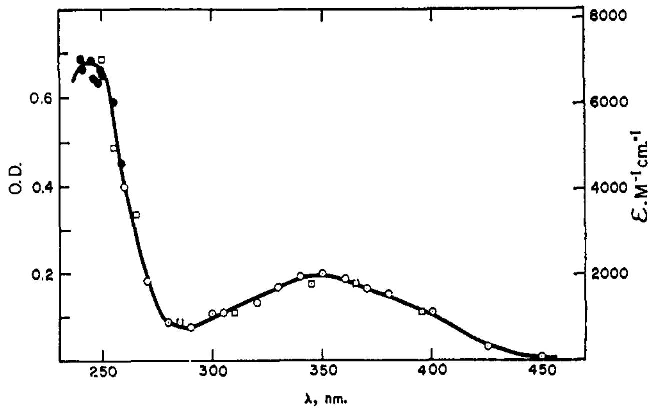

Figure 6. Transient optical absorption produced by the reaction of OH radicals with 0.2 *M* dimethylacetamide, pH 5 (○) and 13.2 (□), N_2O, *D* ~ 8 krads/pulse.

for the transient at λ_{max} 400 nm is shown as the insert in Figure 4.

The transient species produced from the reaction of OH radicals with NMA and DMA are shown in Figures 5 and 6. In each case two maxima are produced, one in the near-uv and the other in the far-uv region of the spectrum. No observable change in the transient absorption was found on studying the reaction at pH 13.2; see Figures 5 and 6.

Table II. Absorption Maxima, Extinction Coefficients, and Decay Kinetics of Transient Species Produced from the Reaction of OH Radicals with Amides

Amide	Suggested radical	λ_{max}, nm	ϵ, M^{-1} cm^{-1}	$2k$, M^{-1} sec^{-1} [b]
$HCONH_2$	$HCO\dot{N}H$	<245	>1500	$\sim 1 \times 10^{10}$
	$\dot{C}ONH_2$	320	350	$1.3 \pm 0.1 \times 10^9$
$HCONH(CH_3)$	$HCONH(\dot{C}H_2)$	235	3500	$1.4 \pm 0.2 \times 10^9$
	$HCONH(\dot{C}H_2)$	360	1000	$1.4 \pm 0.2 \times 10^9$
$HCON(CH_3)_2$	$HCON(\dot{C}H_2)(CH_3)$	248	6900	$2.3 \pm 0.2 \times 10^9$
	$HCON(\dot{C}H_2)(CH_3)$	380	1600	$2.3 \pm 0.2 \times 10^9$
CH_3CONH_2	$CH_3CO\dot{N}H$	<235	>2000	$>2.6 \pm 0.1 \times 10^9$
	$\dot{C}H_2CONH_2$	400		$2.2 \pm 0.2 \times 10^9$
$CH_3CONH(CH_3)$	$CH_3CONH(\dot{C}H_2)$	238	7000	$1.3 \pm 0.4 \times 10^9$
	$CH_3CONH(\dot{C}H_2)$	340	2000	$1.3 \pm 0.4 \times 10^9$
$CH_3CON(CH_3)_2$	$CH_3CON(\dot{C}H_2)(CH_3)$	245	7000	$1.6 \pm 0.3 \times 10^9$
	$CH_3CON(\dot{C}H_2)(CH_3)$	350	2000	$1.6 \pm 0.3 \times 10^9$
$ClCH_2CONH_2$[a]	$\dot{C}H_2CONH_2$	400	1050	$3.0 \pm 0.4 \times 10^9$
$CH_3CONHBr$[a]	$CH_3CO\dot{N}H$	<235	1000	
$ClCH_2CON(CH_3)_2$[a]	$\dot{C}H_2CON(CH_3)_2$	490	1000	$4.0 \pm 0.3 \pm 10^9$

[a] Transient species produced from the reaction with e_{aq}^-, in the presence of 1.0 *M t*-BuOH to scavenge OH radicals. [b] Uncertainties are based on probable errors in experimental values of $2k/\epsilon$ and do not take into account uncertainties in ϵ.

Discussion

The radiolysis of water and aqueous systems generates OH radicals, hydrated electrons, and H atoms.

$$H_2O \rightsquigarrow OH, e_{aq}^-, H$$

In the presence of N_2O (1 atm, approximately 2.3×10^{-2} *M*) the hydrated electrons are all (>98%) converted to OH radicals

$$e_{aq}^- + N_2O \longrightarrow OH + N_2 + OH^- \quad (4)$$

with $k_4 = 5.6 \times 10^9$ M^{-1} sec^{-1}.[16] Under these conditions [*e.g.*, $k(e_{aq}^- + CH_3CONH_2) = 1.7 \times 10^7$ M^{-1} sec^{-1}][16] the OH radicals are the main species reacting with the amides present in solution. Hydrogen atoms, present with a yield of 10% that of OH radicals, are expected to react with amides in the same manner as the OH radicals.

Acetamides. Acetamide. The results obtained with the three acetamides are discussed first, since these provide a clear interpretation of the sites of attack of OH radicals. Abstraction of hydrogen atoms from A by OH radicals can take place at two sites.

$$CH_3CONH_2 + OH \longrightarrow \cdot CH_2CONH_2 + H_2O \quad (5)$$

$$CH_3CONH_2 + OH \longrightarrow CH_3CO\dot{N}H + H_2O \quad (6)$$

In order to identify the transient absorptions due to $\cdot CH_2CONH_2$ and $CH_3CO\dot{N}H$ radicals, the corresponding halogenated compounds were studied. These were made to react with e_{aq}^- when dehalogenation occurs predominantly.

$$ClCH_2CONH_2 + e_{eq}^- \longrightarrow \cdot CH_2CONH_2 + Cl^- \quad (7)$$

$$CH_3CONHBr + e_{aq}^- \longrightarrow CH_3CO\dot{N}H + Br^- \quad (8)$$

Using 0.5–1.0 *M t*-butyl alcohol to scavenge all the OH radicals produced, pulse radiolysis of oxygen-free aqueous solutions of halogenated compounds has been shown[10,17] to be a convenient method for producing specific radicals. The transient species produced from reaction 7 was found to have an absorption maximum at 400 nm and ϵ_{400} 1050 M^{-1} cm^{-1}, Figure 7. The transient species $CH_3CO\dot{N}H$, reaction 8, starts absorbing below approximately 300 nm, its maximum lies below 235 nm, and it has ϵ_{235} ~1000 M^{-1} cm^{-1}; see Figure 4 and Table II. Thus it would seem that about 50% of the OH radicals reacts with A to produce the $\cdot CH_2CONH_2$ radical, with λ_{max} approximately 400 nm. This value was arrived at on the basis of the extinction coefficient of $\cdot CH_2CONH_2$ derived from the pulse radiolysis of $e_{aq}^- + ClCH_2CONH_2$ (see Table I and Figure 7). The remaining OH radicals attack the NH_2 group to produce the $CH_3CO\dot{N}H$ radical. The increase at 400 nm observed in alkaline solution (insert, Figure 4) appears to follow the dissociation constant of the OH radical, $OH \rightleftharpoons O\cdot^- + H^+$, p$K$ = 11.9,[18] and suggests that $O\cdot^-$ radicals attack the α-CH_3 of acetamide more preferentially than do OH radicals. An alternative possibility is that increased absorption at 400 nm is due to $\dot{C}H_2CONH^-$, formed by acid dissociation of $\dot{C}H_2CONH_2$. Since pK_A for the corresponding dissociation of acetamide is 15.1,[19] this explanation implies that removal of an α-hydrogen

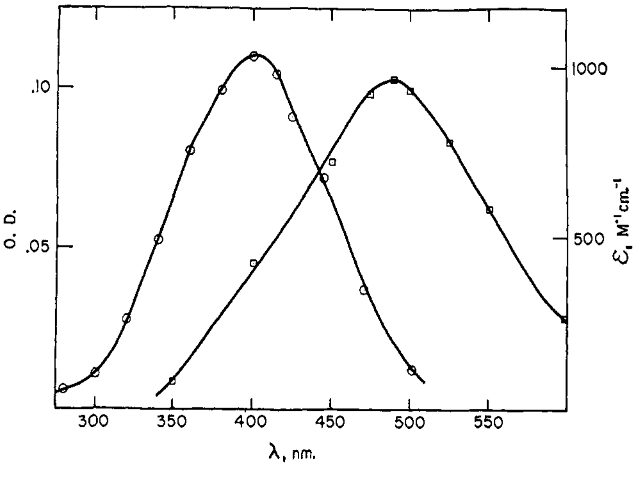

Figure 7. Transient optical absorption produced by the reaction of e_{aq}^- with 2×10^{-2} *M* α-chloroacetamide at pH 5.5 (○), and 2×10^{-2} *M* α-chloro-N,N-dimethylacetamide at pH 5.5 (□); both systems in the presence of 1.0 *M t*-butyl alcohol, Ar, *D* ~ 19 krads/pulse.

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atom reduces the pK_A by about three orders of magnitude, an effect which is consistent with the known[10, 17] acidities of a number of other radicals. However, this possibility is contradicted by the fact that the absorption of $\dot{C}H_2CONH_2$ produced by means of reaction 7 is the same at pH 5.5 and 12.4 (see Figure 4). The formation of $\cdot CH_2CONH_2$ radicals is confirmed by the observation[20] of succinamide, $G = 2.6$, in the γ-radiolysis of 0.25 *M* acetamide in the presence of N_2O at pH 7.

N,N-Dimethylacetamide. In this case two radicals, one of which has two conformational isomers, could be formed on reaction with OH radicals.

$$CH_3CON(CH_3)_2 + OH \to \cdot CH_2CON(CH_3)_2 + H_2O \tag{9}$$

$$CH_3CON(CH_3)_2 + OH \to CH_3CON(CH_2)(CH_3) + H_2O \tag{10}$$

The $\cdot CH_2CON(CH_3)_2$ radical was produced independently by the reaction of e_{aq}^- with α-chloro-N,N-dimethylacetamide.

$$e_{aq}^- + ClCH_2CON(CH_3)_2 \longrightarrow \dot{C}H_2CON(CH_3)_2 + Cl^- \tag{11}$$

This radical was found, Figure 7, to have a λ_{max} at approximately 490 nm and an ϵ of approximately 1000 M^{-1} cm^{-1}. From the transient absorption obtained from DMA, Figure 6, it would appear that more than 95% of the OH radicals reacts according to reaction 10. The fact that the decay kinetics of the two absorption bands at 245 nm and 350 nm, Table II, are identical is consistent with the presence of only one species.

N-Methylacetamide. With NMA, three constitutionally isomeric radicals can be produced by abstraction of hydrogen.

$$CH_3CONHCH_3 + OH \to \cdot CH_2CONHCH_3 + H_2O \tag{12}$$

$$CH_3CONHCH_3 + OH \to CH_3CO\dot{N}CH_3 + H_2O \tag{13}$$

$$CH_3CONHCH_3 + OH \to CH_3CONH\dot{C}H_2 + H_2O \tag{14}$$

By comparison with the spectra of $\cdot CH_2CONH_2$ and $\cdot CH_2CON(CH_3)_2$ the radical produced in reaction 12 is expected to have a λ_{max} between 400 and 490 nm and ϵ_{max} about 1000 M^{-1} cm^{-1}. From the data of Figure 5 it can be concluded that no more than 10% of the reaction proceeds *via* reaction 12. Some occurrence of reaction 13 cannot be ruled out by the available data. A possible interpretation of the fact that NMA reacts about ten times as fast as A is that N-methylation activates N–H to abstraction. The great similarity of transient spectra produced from NMA and DMA and their twofold difference in reactivity toward OH radicals support the view that (14) is the dominant reaction with NMA. The fact that the kinetics of decay of the maxima at 238 and 340 nm are identical is also consistent with the conclusion that the spectrum is that of $CH_3CONH\dot{C}H_2$.

It is interesting to note that the independent conclusions reached above for A, NMA, and DMA are in agreement with the esr results of Livingston and Zeldes[7] who report the presence of a pair of conformational isomers (due to the hindered rotation of the N-carbonyl bond) for the radical $HCON(\dot{C}H_2)(CH_3)$ produced from DMF.

Formamides. Formamide. Interpretation of the results for the formamides is somewhat speculative, as specific radicals could not be produced independently due to the instability of the corresponding halogenated derivatives. However, by analogy with the acetamides, the interpretation given below would seem acceptable.

Hydroxyl radicals can abstract hydrogen from F at two positions.

$$HCONH_2 + OH \to HCO\dot{N}H + H_2O \tag{15}$$

$$HCONH_2 + OH \to \dot{C}ONH_2 + H_2O \tag{16}$$

The radical with the free electron on the nitrogen is expected to absorb strongly, with a maximum below 240 nm (by analogy with $CH_3CO\dot{N}H$), as can be seen in Figure 1. The absorption at about 370 nm is presumably due to the $\dot{C}ONH_2$ radical. The $\dot{C}ONH_2$ radical has been observed[7] by esr in the acetone-photosensitized decomposition of formamide, but the $HCO\dot{N}H$ radical was not reported.[7]

N-Methylformamide. The main species produced from the reaction of OH radicals with NMF is considered to be the $HCONH(\dot{C}H_2)$ radical, Figure 2. This radical should have two absorption bands (by analogy with $CH_3CONH(\dot{C}H_2)$) and the decay rates of the two bands indicate one species (Table II). This is also supported by the relative reactivity to OH radicals of F, NMF, and DMF (Table I).

Dimethylformamide. Hydroxyl radicals dehydrogenate mainly the N-substituted methyl group to produce one radical present in two conformationally isomeric forms.

$$HCON(CH_3)_2 + OH \longrightarrow HCON(\dot{C}H_2)(CH_3) + H_2O \tag{17}$$

The transient absorption spectrum shown in Figure 3 has maxima at 248 and 380 nm, and the identical decay kinetics (Table II) of the two bands are consistent with the presence of only one species.

Fel', *et al.*,[9] have reported an absorption maximum at 380 nm from pulse radiolysis of air-free aqueous solutions of DMF, but no absorption below 300 nm is shown. The absorption at 380 nm is ascribed[9] to a transient produced by the reaction of e_{aq}^- with DMF. The results presented above were obtained with N_2O-saturated solution ($\sim 2.3 \times 10^{-2}$ *M*) under conditions such that all the e_{aq}^-'s reacted according to reaction 4. The present results and interpretations disagree with the assignment given by Fel', *et al.*[9] No comparison with the data of Fel', *et al.*, for NMF and F can be made since they did not characterize transients derived from these amides.

Selectivity of OH Radical Reactions

The present work establishes that abstraction from the N-methyl group of amides is at least one order of magnitude faster than from the α-methyl group (see Table I). Comparison of the results for the acetamide series with the reactivity of OH radicals[16] with acetic acid and its methyl ester suggests that amide nitrogen is much more effective in activating the methyl group

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than is ester oxygen. Values of $k(OH + S)$ in units of $10^7\ M^{-1}\ sec^{-1}$ are

CH_3CONH_2	$CH_3CON(CH_3)_2$	CH_3CO_2H	$CH_3CO_2CH_3$
19	350	1.4	8

Implications for Radiolysis of Peptides

The determination of the sites of attack by OH radicals on simple amides and the observed activation of N-methyl groups by the amide nitrogen have been found to be of considerable importance in the study of more complex peptides.[21] From a comparison with the transient absorption spectra and the extinction coefficients of radicals derived from α-CH_3 and N-CH_3 certain generalities are apparent. For instance, the radicals $\dot{C}H_2CONHR$ (where R = H, $CH_2CO_2^-$, or $CH_2CONHCH_2CO_2^-$) and $\dot{C}H_2CON(CH_3)_2$ have absorption maxima above 400 nm with $\epsilon \sim 1000\ M^{-1}\ cm^{-1}$. On the other hand, the radicals $CH_3CONH\dot{C}HR$ (where R = H or CO_2^-) and $CH_3CON(CH_3)\dot{C}H_2$ have main absorption maxima in the region 235–270 nm, and much higher extinction coefficients, $\epsilon \sim 6000$–$14{,}000\ M^{-1}\ cm^{-1}$. These differences are being exploited further to establish sites of free radical attack on peptides.

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Interaction of Anhydrous Formic Acid with Model Amides[1,2]

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Abstract: The interaction of anhydrous formic acid with model amides has been studied using infrared, nmr, viscosity, conductance, and density measurements. Several equilibria are needed to describe this interaction over the entire mixing range of acid and amide: hydrogen bonding is prevalent over the entire mixing range, protonation of the amides occurs at high acid concentrations, and ion pair formation is present at intermediate acid concentrations. The data are consistent with protonation and hydrogen bonds involving the acidic proton of formic acid and the amide carbonyl oxygen. The degree of protonation, as measured directly by the specific conductance, reflects the basicities of the different amide carbonyls.

The use of nonaqueous solvent systems containing organic acids in the study of polypeptide conformational stability is well documented.[4] Solvent systems containing halogenated acetic acids, such as trifluoroacetic and dichloroacetic acids, have been widely used in the study of the helix $\rightleftharpoons$ random chain interconversion.[5,6] The interaction of these acids with model amides[5,7] and polypeptides[8,9] has been investigated by a variety of physicochemical techniques. Although conflicting results have been presented for polypeptide systems[7,9,10] there is general agreement that trifluoroacetic and dichloroacetic acids act as strong acids toward amides and protonate extensively.

In contrast, less attention has been given to the weaker, unsubstituted fatty acids, such as formic and acetic. Conformational transitions have been reported in formic[11] and acetic[12] acids but no detailed study of the action of these acids on amides or polypeptides has been carried out. From previous investigations in this laboratory involving gelatin[13] and derivatives of poly-L-proline[11] in formic acid, the usefulness of anhydrous formic acid in the study of polypeptide conformational stability became apparent. Formic acid appeared to interact in some specific manner with polypeptides, the degree of interaction being easily regulated by the addition of basic solvent components, such as amides and dimethylsulfoxide. In this respect, the formic acid–polypeptide systems seemed ideal models for the study of solvent–backbone interactions. In addition, Chao, Veis, and Jacobs[14] have shown that formic acid is a good solvent for differentiating the basicity of various model amides. The high dielectric constant of formic acid, *viz.* ϵ 58,[15] and the resistance to solvolytic degradation shown by polypeptides in it further recommended its use as a general

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(2) Presented in part at the 158th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969.

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