parent peak *m*/3 192; nmr (60 MHz, $CDCl_3$, TMS) 85.2–5.5 (d, 1 H, $J \sim 7.5$), 2.4–3.3 (m, 3 H), 1.96–2.21 (m, 2 H), 2.08 (s, 3 H), 1.38 (s, 3 H), 0.9–2.0 ppm (8 H).

Anal. Calcd for $C_{13}H_{20}O$: C, 81.20; H, 10.48. Found: C, 81.15; H, 10.41.

***syn*-Tricyclo[6.3.0.0^{3,7}]-1,8-dimethyl-2-hydroxyundec-9-ene (Vc):** ir (neat smear) 3500, 3040, 2965, 2900, 1648, 1447, 1373, 1093, 921, 780–90, 706 cm^{-1}; mass spectrum parent peak *m*/*e* 192; nmr (60 MHz, $CDCl_3$, TMS) δ 5.7–6.2 (m, 2 H), 1.80 (s, 3 H), 1.38 (s, 3 H), 1.4–3.6 (12 H).

Anal. Calcd for $C_{13}H_{20}O$: C, 81.20; H, 10.48. Found: C, 81.27; H, 10.50.

Additions of the cyclobutenes to the aromatic compounds were carried out in the same fashion as the addition of benzene to cyclobutene.[5] The spectral properties of the adducts are given in Table III. Combustion analysis are given in Chart II. Details of the equipment and the procedure used in the quantum yield measurements are given elsewhere.[5]

Chart II

Compd	Calcd	Found
VII + VIII	$C_{12}H_{16}$: C, 89.93; H, 10.07	C, 90.01; H, 10.01
IX	$C_{13}H_{18}$: C, 89.59; H, 10.41	C, 89.95; H, 10.30
X	$C_{13}H_{18}$: C, 89.59; H, 10.41	C, 89.20; H, 10.20

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Primary Processes in the Photochemistry of Phenylalanine and Derivatives in Aqueous Solution. Biphotonic Photoionization and Photodissociation Reactions

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Abstract: The photophysical and photochemical processes which phenylalanine and its derivatives phenylalanine amide and *N*-acetylphenylalanine undergo in aqueous solutions at 20° were investigated with the help of the fast reaction technique of flash photolysis. Only the first $^1A_{1g} \rightarrow {}^1B_{2u}$ transition of benzene was optically excited. The transient absorption spectra of the various intermediates observed were characterized and the decay kinetics of these species determined. Both the photodissociation process leading to the formation of the benzyl radical and the photoionization process leading to the hydrated electron e_{aq}^- were found to be strongly dependent on the state of ionization of the free end groups –COOH and $-NH_3^+$, and in particular to the amino group. At pH 5.9, reactions 1–3 are suggested. The dependence of the various processes with pH was obtained as typical "titration" curves which followed the pK_a of the ground state molecule. The addition of low concentrations of characteristic quenchers such as ethyl pyruvate and Ni^{2+} ions indicated that the excited state precursors of these processes must be relatively long lived, probably the triplet excited states. From the dependence upon light intensity, the photodissociation processes leading to the benzyl radical in neutral and acid solutions of phenylalanine, phenylalanine amide, and *N*-acetylphenylalanine were found to be biphotonic in water at 20°. The photoionization process was also found to be biphotonic in nature. In alkaline solutions (above the pK_a of the amino group) of phenylalanine and phenylalanine amide one quantum only was required to bring about the corresponding photodissociation reaction. The quenching experiments showed that a triplet state was also involved as the precursor in alkaline solutions. It is important to note that even though all the excitation is initially absorbed by the aromatic ring, the ejected electron in the photoionization process comes from the $-COO^-$ group, demonstrating the involvement of strong intramolecular interactions.

The protein macromolecules contain a large number of chromophoric groupings which are capable of interacting with light both in the free state and as part of the polymer. In most proteins the majority of quanta in the 250–300 nm region are absorbed by the aromatic amino acids tyrosine (Tyr), tryptophan (Trp), and phenylalanine (Phe).[2–5] On the basis of the observed sequence of the singlet excited state energies, it was proposed[6] that electronic energy transfer Phe → Tyr → Trp was feasible as a result of dipolar resonance coupling. Consequently, most of the previous studies have been devoted to tryptophan and to tyrosine since most proteins contain either Trp or Tyr (or both) in addition to Phe. However, destruction of phenylalanine occurs on uv irradiation in the free state[7] or when present in proteins.[8] Furthermore, it has recently been shown that the photodissociation and photoionization processes of tyrosine[9,10] and aromatic alkylcarboxylic

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acids[11] in aqueous solutions occur *via* the triplet excited state. The photophysical and photochemical processes which phenylalanine undergoes may then be of some importance in proteins.

Relatively little amount of work has been carried out on the photochemistry of phenylalanine in aqueous solution.[4,7] Decarboxylation and the formation of aspartic acid and phenyllactic acid have been observed[7] in the absence of oxygen and, in addition, tyrosine, DOPA, and benzoic acid in the presence of oxygen. The ϕ (-Phe) in oxygen increased from 0.027 to 0.055 with increase of pH from 7.3 to 10.8. Photoionization of Phe at pH 11.7 has been observed,[12] as well as the formation of the benzyl radical $PhCH_2\cdot$. The nature of the excited states giving rise to these processes has not been established, though it was suggested[12] that photoionization takes place from the singlet excited state *via* a monophotonic mechanism.

The fluorescence lifetime and quantum yield of Phe in aqueous solutions has been studied.[13-15] In $\sim 10^{-3}$ M concentration and $\sim 20°$, $\phi_F = 0.025$ and $\tau_F = 6.8$ nsec. A marked temperature dependence was found,[14] with ϕ_F and τ_F decreasing with increase in temperature in the range 2-68° (coefficient = $-2.7\%/°C$). This deactivation process was shown[14] to be due mainly to internal conversion (activation energy of 0.25 eV) and to only a small extent to intersystem crossing. The ϕ_F decreases[13] by $\sim 30\%$ on ionization of the -COOH group and by $\sim 15\%$ on ionization of the $-NH_3^+$ group.

The phosphorescence of phenylalanine has been observed[2] only in glasses at 77 °K and has band maximum at 385 nm and $\tau_P \sim 5.5$ sec. The ϕ_P is independent of pH between 2 and 12 at 77 °K. In view of the importance of the triplet excited state and the biphotonic nature of the photoionization and photodissociation processes occurring in tyrosine and phenolic compounds[9,10] and in aromatic alkylcarboxylic acids,[11] the excited states involved in the photochemistry of phenylalanine in aqueous solution were studied using the technique of flash photolysis. Below are presented the results obtained from phenylalanine, phenylalanine amide, and *N*-acetylphenylalanine as a function of pH and of the ionization constants of the compounds. Preliminary results have been published.[16]

Experimental Section

The flash photolysis setup used has been described elsewhere.[17] Most of the work was carried out using flash intensities of ~ 2000 J and flash duration $\tau_{1/2} \sim 10$ μsec. The light output from the four flash lamps was varied by changing the charging voltage across the lamps at constant capacitance. The light output ($^1/_2CV^2$) was found to be directly proportional to the charging $(\text{voltage})^2$ in the wavelength region of excitation.

Quartz optical cells of 20-cm path were used. Solutions were made up in triply distilled water and were degassed by bubbling with prepurified nitrogen gas. All experiments were done at room temperature, 20°. Appropriate solution cut-off filters were placed in the outer jacket of the optical cell. A 15% acetic acid in water mixture gave a 240-nm cut-off filter solution and 3.5:1.0 dimethylformamide and water mixture a 260-nm cut-off filter. Solutions were buffered using perchloric acid, potassium hydroxide, phosphates ($\leqslant 1$ mM), and borate ($\leqslant 1$ mM).

The chemicals used were obtained from Cyclochemicals, Eastman, Baker and Adamson, Chemical Samples Company, and Mallinckrodt. These were the best grade available and were used as received.

The pulse radiolysis setup used has been described.[18]

Results and Discussion

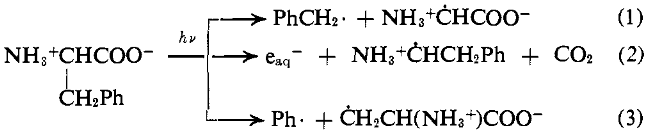
Most of the work to be described below, unless stated otherwise, was carried out using a 240-nm cut-off filter (15% acetic acid in water). Under these conditions, only the $^1A_{1g} \rightarrow {}^1B_{2u}$ benzene transition of the aromatic amino acids was optically excited. The vibronic structure of this absorption band in water has a maximum[19] at 258 nm and ϵ_{258} 190 M^{-1} cm^{-1} (the second transition has λ_{max} 205 nm and ϵ_{max} 9600 M^{-1} cm^{-1}). Small changes with pH in the spectrum of phenylalanine have been observed,[19] indicating that ionization of the amino and carboxyl groups perturbs the absorption spectrum of the aromatic ring.

It is important to remember that not all the photolytic processes occurring can be observed using the technique of flash photolysis and kinetic absorption spectrophotometry.

In the following pages phenylalanine has been abbreviated to Phe and phenylalanine amide to Phe-NH_2.

Phenylalanine. The transient absorption spectra produced on optical excitation of oxygen-free aqueous solutions of phenylalanine ($pK_a^1 = 1.83$ and $pK_a^2 = 9.13$) were found to be strongly dependent upon the pH of the solution. Figures 1 and 2 show the results obtained at pH 0.45, 5.9, and 11.1. These spectra show primarily the transient absorption of the benzyl radical $PhCH_2\cdot$, with maxima at 318 and 307 nm. A closer comparison between these experimental curves and the recently[20] redetermined absorption spectrum of $PhCH_2\cdot$ in water reveals the presence of other absorbing transient species. In addition to the results shown in Figures 1 and 2, the characteristic spectrum of the solvated electron, e_{aq}^-, with λ_{max} at ~ 720 nm was observed on optical excitation of Phe at pH 5.9 and 11.1.

The following primary photolytic processes are considered to occur at pH 5.9.



$$NH_3^+CH(CH_2Ph)COO^- \xrightarrow{h\nu} PhCH_2\cdot + NH_3^+\dot{C}HCOO^- \quad (1)$$

$$\xrightarrow{h\nu} e_{aq}^- + NH_3^+\dot{C}HCH_2Ph + CO_2 \quad (2)$$

$$\xrightarrow{h\nu} Ph\cdot + \dot{C}H_2CH(NH_3^+)COO^- \quad (3)$$

The absorption spectrum of the glycine radical has been determined[21] and has a λ_{max} 250 nm and an extinction coefficient which is strongly dependent upon the pH and the acid-base properties[21,22] of this radical.

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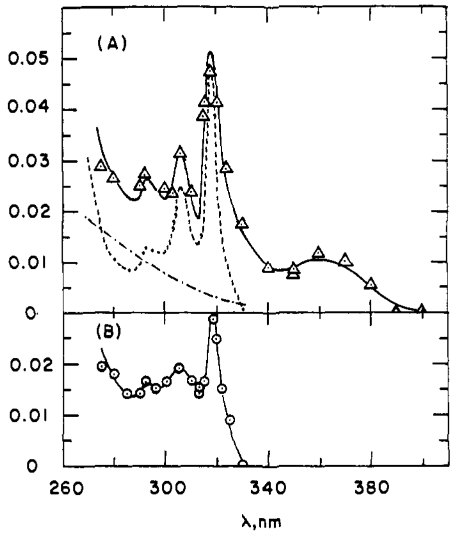


Figure 1. Transient spectra produced in the photolysis of 2 mM phenylalanine: (A) at pH 5.9, N_2 (Δ), spectrum of the $PhCH_2\cdot$ (- - - - -) and the $NH_2\dot{C}HCOO^-$ (-·-·-) radicals used to synthesize the experimental curve, a 240-nm cut-off filter was used; (B) at pH 0.45, N_2 (○), using the same filter.

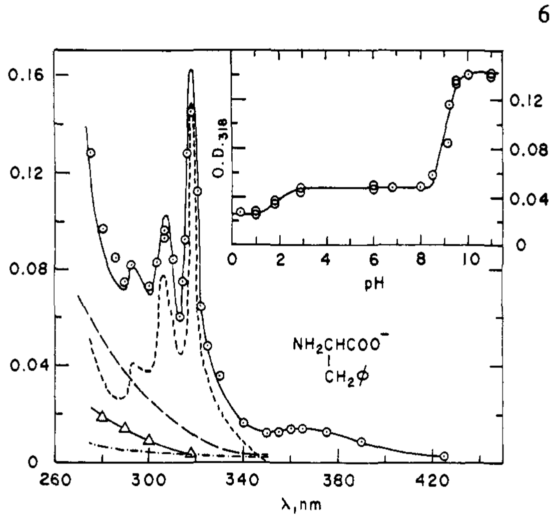


Figure 2. Transient spectrum produced in the photolysis of 2 mM phenylalanine at pH 11.1, N_2 (⊙). Spectrum of the $PhCH_2\cdot$ (- - - -), $NH_2\dot{C}HCOO^-$ (– – –), and $NH_2\dot{C}HCH_2Ph$ (-·-·-) radicals used to synthesize the experimental curve. A 240-nm cut-off filter was used. Insert: dependence upon pH of the absorbance at 318 nm. The spectrum produced on photolysis of 5 mM phenylalanine in air (1 atm) at pH 11.1 (Δ) using a 260-nm cut-off filter.

$$NH_3^+\dot{C}HCOOH \xrightleftharpoons[pK_a \leqslant 1.0]{-H^+} NH_2\dot{C}HCOOH \xrightleftharpoons[pK_a = 6.6]{-H^+}$$

$$NH_2\dot{C}HCOO^- \xrightleftharpoons[pK_a > 11.5]{-H^+} NH^-\dot{C}HCOO^- \qquad (4)$$

Based on an equimolar formation (reaction 1) of the benzyl and glycine radicals and using the known[20,21] spectra and extinction coefficients of these radicals, the experimental curve obtained at pH 5.9 was synthesized (in part); see Figure 1A.

It has been shown[11] (see also below) that the photoionization of aromatic carboxylic acids leads to the ejection of the electron from the $-COO^-$ group, *e.g.*

$$PhCH_2COO^- \xrightarrow{h\nu} PhCH_2\cdot + e_{aq}^- + CO_2 \qquad (5)$$

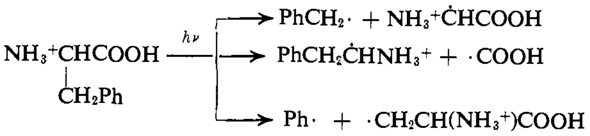
and $[PhCH_2\cdot] = [e_{aq}^-]$. The radical $NH_3^+\dot{C}HCH_2Ph$ (or $NH_2\dot{C}HCH_2Ph$, since the pK_a is not known) produced on photoionization of Phe, process 2, is expected to have a relatively very weak extinction coefficient at $\lambda > 280$ nm and an absorption maximum at < 220 nm. The spectrum of the $NH_3^+\dot{C}HCH_3$ radical has been observed[23] and that of the substituted radical NH_3^+-$\dot{C}HCH_2Ph$ is expected to be much the same. Due to the reactivity of $k(e_{aq}^- + \text{Phe}) = 1.6 \times 10^8\ M^{-1}\ cm^{-1}$ at pH 6.9 (ref 24), the amount of e_{aq}^- produced in process 2 was determined at a lower ($2 \times 10^{-4}\ M$) concentration of Phe. Taking[25] ϵ_{700} $1.8 \times 10^4\ M^{-1}\ cm^{-1}$ for e_{aq}^-, the $[e_{aq}^-] = 0.22\ \mu M$, using a water cut-off filter, at pH 7.0. Under the identical experimental conditions, the $[PhCH_2\cdot] = 0.21\ \mu M$. From this experiment it is concluded for processes 1 and 2 that $\phi_{(1)} \simeq \phi_{(2)}$.

No evidence is available for process 3. The absorption spectrum of the $Ph\cdot$ radical in water is relatively weak,[11,26] ϵ_{260} ~630 $M^{-1}\ cm^{-1}$. Similarly the absorption of the $CH_2CH(NH_3^+)\dot{C}OO^-$ radical is expected[21,27] to be very weak at $\lambda > 270$ nm. However, the rupture Ph-$(CH_2)_nCOO^-$ has been shown[11] to occur in the photolysis of aromatic carboxylic acids. Furthermore, aspartic acid ($^-OOCCH_2CH(NH_3^+)COO^-$) has been observed[7] as a photoproduct of phenylalanine. Its formation is suggested to occur *via* reactions 6 and 7

$$CO_2 + e_{aq}^- \longrightarrow CO_2\cdot^- \qquad (6)$$

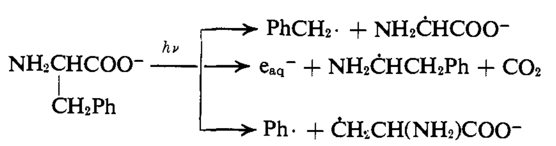
$$CO_2\cdot^- + \dot{C}H_2CH(NH_3^+)COO^- \longrightarrow {}^-OOCCH_2CH(NH_3^+)COO^- \qquad (7)$$

where $k_6 = 8 \times 10^9\ M^{-1}\ sec^{-1}$ (ref 25) and $k_7 \sim 2 \times 10^9\ M^{-1}\ sec^{-1}$ (estimated from ref 27). No evidence is also available for the rupture $PhCH_2CH(NH_3^+)$-COO^-. The corresponding process in acid solutions $PhCH_2$-$CH(NH_3^+)$-$COOH$ might be occurring, based on the results found[11] from the photolysis of aromatic carboxylic acids. In acid solutions these processes are suggested.



$$NH_3^+\underset{\substack{|\\CH_2Ph}}{C}HCOOH \xrightarrow{h\nu} \begin{cases} PhCH_2\cdot + NH_3^+\dot{C}HCOOH & (8)\\ PhCH_2\dot{C}HNH_3^+ + \cdot COOH & (9)\\ Ph\cdot + \cdot CH_2CH(NH_3^+)COOH & (10)\end{cases}$$

On optical excitation of phenylalanine at pH 11.1, the quantum yield of the intermediates produced is appreciably higher (Figure 2). The following processes are suggested.



$$NH_2\underset{\substack{|\\CH_2Ph}}{C}HCOO^- \xrightarrow{h\nu} \begin{cases} PhCH_2\cdot + NH_2\dot{C}HCOO^- & (11)\\ e_{aq}^- + NH_2\dot{C}HCH_2Ph + CO_2 & (12)\\ Ph\cdot + \dot{C}H_2CH(NH_2)COO^- & (13)\end{cases}$$

Most of the experimental curve can be synthesized based on the formation of the radicals in (11–13). Following the same procedure as described above, at pH 11.2, $[e_{aq}^-] = 0.45\ \mu M$ and $[PhCH_2\cdot] = 0.47\ \mu M$ in $2 \times 10^{-4}\ M$ Phe, using a water cut-off filter. This result shows that $\phi_{(11)} \simeq \phi_{(12)}$.

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Table I. Decay Kinetics of Intermediate Species Produced in the Flash Photolysis of O_2-Free Aqueous Solutions of Aromatic Amino Acids (2 m*M*)

Compd	pH	Suggested radical	$\lambda_{\text{monitored}}$	$2k/\epsilon$	$2k$, M^{-1} sec$^{-1\,a}$
Phenylalanine	11.1	$PhCH_2\cdot$	318	7.2×10^5	6.5×10^9
Phenylalanine amide	9.5	$PhCH_2\cdot$ + some $NH_2\dot{C}HOONH_2$	318	6.0×10^5	5.4×10^9
Phenylalanine amide	9.5	$PhCH_2\cdot + NH_2\dot{C}HCONH_2$	280	4.3×10^5	
N-Acetylphenylalanine	6.15	$PhCH_2\cdot + CH_3CONH\dot{C}HCOO^-$	318	2.5×10^5	2.3×10^9
N-Acetylphenylalanine	1.25	$PhCH_2\cdot + CH_3CONH\dot{C}HCOOH$	318	3.1×10^5	2.8×10^9

[a] Derived based on ϵ_{318} 9.0 × 10^3 M^{-1} cm^{-1} (ref 20) for $PhCH_2$ radical; decay rates may be due to $R_1\cdot + R_1\cdot$ and $R_1\cdot + R_2\cdot$ reactions.

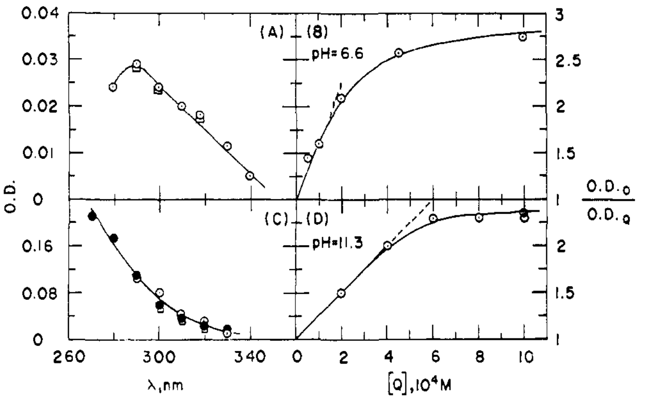

Figure 3. Quenching of the excited state of phenylalanine by $Ni(ClO_4)_2 \cdot 6H_2O$ and ethyl pyruvate: (A) a transient spectrum produced on flash photolysis of 2 m*M* phenylalanine, 1 m*M* $Ni(ClO_4)_2 \cdot 6H_2O$, pH 6.6, in the presence of N_2 (⊙) and N_2O plus 1.0 *M* *t*-BuOH (□), 15% acetic acid filter; (B) O.D.$_0$/O.D.$_Q$ as a function of [Ni^{2+}] monitored at 318 nm; (C) a transient spectrum produced on flash photolysis of 5 m*M* phenylalanine, 1 m*M* ethyl pyruvate, pH 11.3, 260-nm cut-off filter, in the presence of N_2 (⊙) and N_2O plus 1.0 *M* *t*-BuOH (□). Symbols ● are normalized O.D. produced from the *direct* photolysis of 2 m*M* ethyl pyruvate, pH 3.9, 218-nm cut-off filter; (D) O.D.$_0$/O.D.$_Q$ as a function of [ethyl pyruvate] monitored at 318 nm.

The increase in photodecomposition was monitored at 318 nm as a function of pH, and the results obtained are shown in the insert, Figure 2. Two titration-type curves can be seen, from which the midpoint values are 1.9 ± 0.1 and 9.1 ± 0.1. These values are similar to the $pK_a^1 = 1.83$ and $pK_a^2 = 9.13$ ionization constants of phenylalanine. From this rather striking observation one can make the following conclusions: (a) the increase in the formation of benzyl radicals, processes 1, 8, and 11, is dependent on the ionization of the -COOH and $-NH_3^+$ groups in phenylalanine, particularly the $-NH_3^+$ group; (b) the photoionization processes 2 and 12, monitored as [e_{aq}^-], also increase on ionization of the $-NH_3^+$ group; and (c) the singlet excited states are probably not involved since the pK_a of ^1S(Phe) is expected[28] to be lower by a few pH units compared with ^0S(Phe).

Another absorption band with λ_{max} ~360 nm was observed on flash photolysis of Phe at pH 5.9 and 11.1, Figures 1 and 2. This transient was found to decay relatively fast, $\tau \sim 50$ μsec. It is tentatively suggested that this band may be the triplet–triplet absorption of Phe (see more below). In the presence of oxygen all these transients are not observed and instead a weak absorption is seen, Figure 2. This is in agreement with the finding that the absorption maxima of organic peroxy radicals $RO_2\cdot$ is at ~245 ± 5 nm, with a sharp-rising band (see ref 18a, 29, and 30).

The decay kinetics of the radicals observed are given in Table I.

Excited State Precursors. In order to identify the nature of the excited states involved in the formation of the transient species described above, the photolysis was carried out in the presence of small amounts of typical quenchers of triplet excited states. Nickel ions and ethyl pyruvate were chosen as quenchers. Figure 3 shows the results obtained. Under the conditions of the experiments (different cut-off filters were used) less than 5% of the exciting light was absorbed by the quenchers. A considerable decrease in O.D.$_{318}$ was observed using 2 × 10^{-4} *M* concentrations of $Ni(ClO_4)_2$ at pH 6.6 or of ethyl pyruvate at pH 11.3. The Stern–Volmer plots were not linear due to the formation of transient species from the quenchers, which also absorb at 318 nm. With Ni^{2+} the transient spectrum (Figure 3A) corresponds to the Ni^+ odd-valent ion which was observed[31] from the reaction of e_{aq}^- with Ni^{2+}. Its formation in the flash experiment could be due to either (a) energy transfer to Ni^{2+} followed by reduction, or, more likely, (b) quenching of the triplet excited Phe *via* an electron transfer process: $^1T(Phe) + Ni^{2+} \rightarrow Phe(oxid) + Ni^+$.

In the presence of ethyl pyruvate a different transient spectrum is observed (Figure 3C) which is identical with that produced on direct excitation of ethyl pyruvate. The triplet energy level E_T of ethyl pyruvate[32] is ~2.4 eV (65 kcal/mol). From the tangents to these Stern–Volmer plots, approximate quenching constants have been derived (Table II).

The lifetime of ^1S(Phe) is known[13–15] to be ~6.8 nsec. Assuming a rate constant as high as 5 × 10^{10} M^{-1} sec^{-1} for the quenching of the ^1S state by Ni^{2+} or ethyl pyruvate, the results obtained at quencher concentrations of ≥1 × 10^{-4} *M* could not be due to interaction with the singlet excited state. Quite clearly, a longer-lived excited state, probably the triplet state, must be involved.

The effect of light intensity (*I*) on the formation of the transient species was examined at pH 0.45, 5.8, and 11.1 at two different wavelengths, 318 and 360 nm. At 318 nm, a nonlinear dependence was found at pH 0.45 and 5.8 (Figure 4A), indicating a dependence on I^2. At pH 11.1, however, the transient absorption was directly proportional to *I*. The species absorbing at

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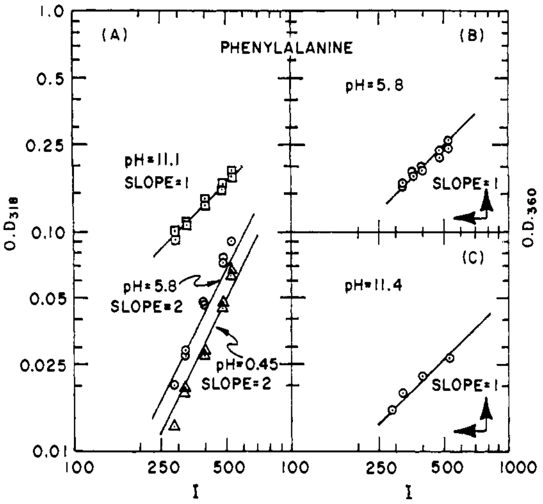


Figure 4. Dependence upon light intensity of the transient species produced on photolysis of 2 mM phenylalanine, N_2, at different pH values. Transients monitored at 318 and 360 nm. A 240-nm cut-off filter was used.

Table II. Quenching Constants of the Triplet Excited States of Some Aromatic Amino and Carboxylic Acids in Aqueous Solution by Ethyl Pyruvate

Aromatic acid	pH	Quenching constant, g^a
Phenylalanine	11.3	1.3×10^3
Phenylalanine	6.6	7.5×10^3 [b]
Phenylalanine amide	9.2	2.8×10^3
N-Acetylphenylalanine	1.55	2.5×10^3
N-Acetylphenylalanine	7.0	1.9×10^3
Phenylacetic acid[c]	1.5	1.3×10^3
Phenyl acetate[c]	6.8	9.0×10^2

[a] Constant derived from the initial slope of the Stern-Volmer yield *vs.* quencher concentration curve. [b] Using nickel perchlorate as quencher. [c] From ref 11.

360 nm was found to be produced linearly with the intensity I, both at pH 5.8 and 11.4 (Figure 4B,C).

These results indicate that: (a) the photodissociation processes 1 and 8 are biphotonic, while process 11 is monophotonic; (b) since the lifetime of the flash is $\tau_{1/2}$ ~10 μsec, long-lived triplet excited states are the precursors at pH 0.45 and pH 5.8; (c) since the quencher experiments mentioned above also demonstrate the involvement of a long-lived excited state at pH 11.1, the monophotonic nature of this process is not inconsistent with these results; (d) since the bond energy for rupture of $PhCH_2$-CR is not likely to be significantly dependent on the state of protonation of the $-NH_3^+$ and -COOH groups, one must assume that the triplet energy level of Phe at pH 11.1 is higher than those at pH 5.8 or 0.45; (e) the increased photodecomposition with increase in pH may be due to the inefficiency of the biphotonic mechanism for this process; (f) the lifetimes of the long-lived excited state precursors must be less than ~10 μsec, in order for them to absorb a second quanta of light; (g) the fact that the 360-nm band is monophotonic at pH 11.1 and 5.8 is consistent with the tentative suggestion that this fast-decaying transient may be the T-T absorption.

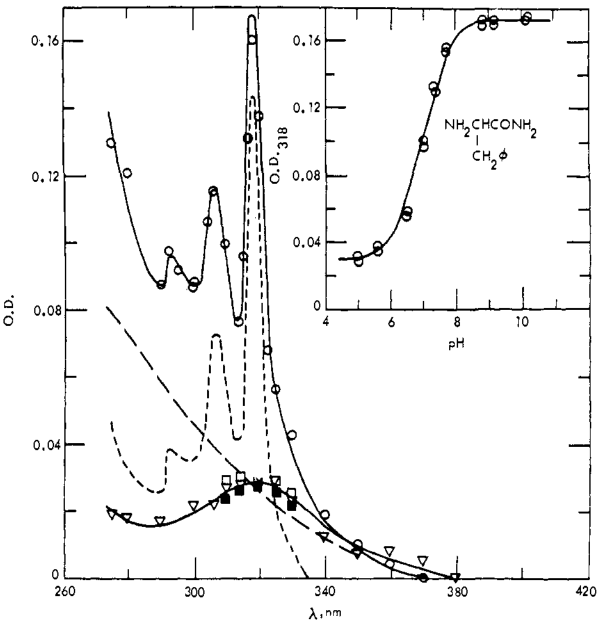


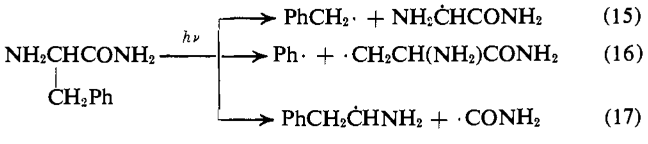
Figure 5. Transient spectra produced in the photolysis of 2 mM phenylalanine amide, N_2, (a) at pH 9.2 (○). Spectrum of the $PhCH_2\cdot$ (-----) and the $NH_2\dot{C}NCONH_2$ radicals (– – –) used to synthesize the experimental curve; (b) at pH 5.1 (▽), in the presence of 1.0 M t-BuOH (□), in presence of 1.0 M t-BuOH plus N_2O (■). A 240-nm cut-off filter was used. Insert: dependence upon pH of the absorbance at 318 nm.

A few experiments were carried out using 2,4-hexadien-1-ol as a quencher. Using 1 mM of quencher, 5 mM Phe, pH 5.5, and a 260-nm cut-off filter, the absorption at 318 nm was considerably reduced. It was suspected, however, that $PhCH_2\cdot$ might be reacting with the quencher. To check this point, a pulse radiolysis experiment was set up.[18] The conditions were: O_2-free aqueous solutions of 5×10^{-2} M benzyl chloride, 3.0 M *tert*-butyl alcohol (to scavenge the OH radicals), pH 9.2, and 1-2.5 mM hexadienol. The benzyl radical was produced from the reaction

$$e_{aq}^- + PhCH_2Cl \longrightarrow PhCH_2\cdot + Cl^- \qquad (14)$$

The decay of $PhCH_2\cdot$ was monitored at 318 nm and was found to be pseudo first order in [hexadienol]. From this rate, $k(PhCH_2\cdot + \text{2,4-hexadionol}) = 2.0 \pm 0.4 \times 10^7\ M^{-1}\ sec^{-1}$ was derived. In view of this result, a word of caution is in order when using conjugated dienes as quenchers since they appear to react relatively fast with many free radicals.

Phenylalanine Amide. On flash photolysis of phenylalanine amide ($pK_a = 7.22$), Phe-NH_2, at pH 9.2 a transient optical spectrum is observed, Figure 5, which is somewhat similar to that obtained from Phe at pH 11.1. No absorption band at 360 nm is, however, observed. The following primary photolytic processes are suggested.



$$NH_2\underset{CH_2Ph}{CH}CONH_2 \xrightarrow{h\nu} PhCH_2\cdot + NH_2\dot{C}HCONH_2 \qquad (15)$$

$$\longrightarrow Ph\cdot + \cdot CH_2CH(NH_2)CONH_2 \qquad (16)$$

$$\longrightarrow PhCH_2\dot{C}HNH_2 + \cdot CONH_2 \qquad (17)$$

The experimental curve at pH 9.2 was synthesized and was found to be in excellent agreement with the formation of equimolar concentrations (0.81 μM) of $PhCH_2\cdot$ and $NH_2\dot{C}HCONH_2$. The latter radical has[33] a λ_{max} ~265 nm and ϵ_{265} $5.5 \times 10^3\ M^{-1}\ cm^{-1}$ and an ionization constant $pK_a = 4.35 \pm 0.1$.

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(33) P. S. Rao and E. Hayon, in preparation.

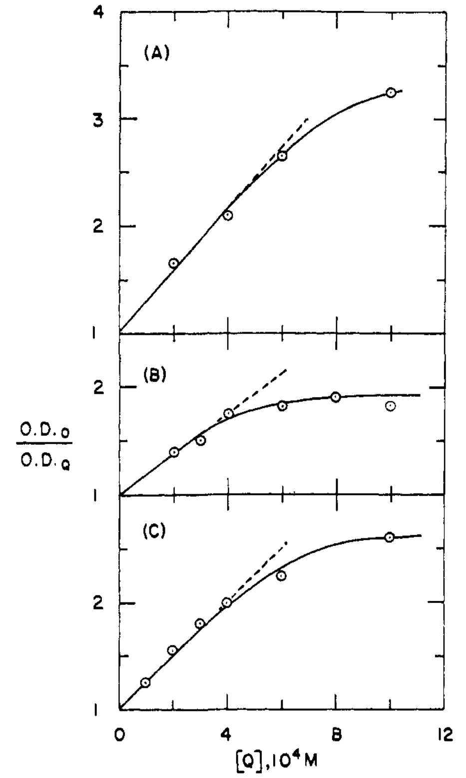


Figure 6. (A) Quenching of the excited state of phenylalanine amide (5 mM) by ethyl pyruvate at pH 9.2, monitored at 318 nm, using a 260-nm cut-off filter; (B) quenching of the excited state of N-acetylphenylalanine (5 mM) by ethyl pyruvate at pH 7.0, monitored at 318 nm, using a 250-nm cut-off filter; (C) quenching of the excited state of N-acetylphenylalanine (5 mM) by ethyl pyruvate at pH 1.5, monitored at 318 nm, using a 250-nm cut-off filter.

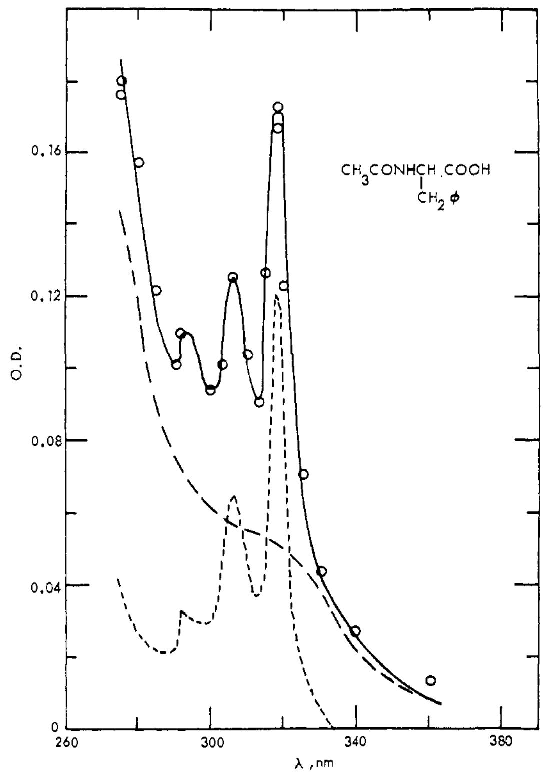
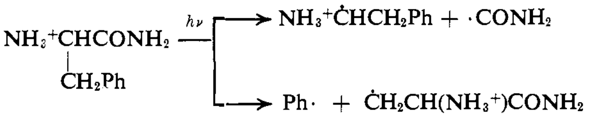


Figure 7. Transient spectrum produced in the photolysis of 2 mM N-acetylphenylalanine at pH 0.95 in the presence of N_2 (1 atm). Spectrum of the $PhCH_2\cdot$ (- - - -) and $CH_3CONH\dot{C}HCOOH$ (– – –) radicals used to synthesize the experimental curve. A 240-nm cut-off filter was used.

$$NH_3^+\dot{C}HCONH_2 \rightleftharpoons NH_2\dot{C}HCONH_2 + H^+ \qquad (18)$$

No evidence is presently available for process 16 (both radicals absorb weakly and have maxima below 270 nm) or process 17. The absorption of the $\cdot CONH_2$ radical has been reported[34] and λ_{max} ~320 nm and ϵ_{350} ~350 M^{-1} cm^{-1}.

A very much weaker and different spectrum is observed on flash photolysis of the Phe-NH_2 at pH 5.1, Figure 5. This spectrum remains unchanged in the presence of 1.0 M t-BuOH (an OH radical scavenger) and 1 atm of N_2O (an e_{aq}^- scavenger), indicating that the transient is not an e_{aq}^- or OH radical adduct. The spectrum is not unlike that of the $\cdot CONH_2$ radical,[34] and might be produced according to



$$\underset{CH_2Ph}{NH_3^+CHCONH_2} \xrightarrow{h\nu} \begin{cases} NH_3^+\dot{C}HCH_2Ph + \cdot CONH_2 & (19) \\ Ph\cdot + \dot{C}H_2CH(NH_3^+)CONH_2 & (20) \end{cases}$$

On monitoring at 318 nm the change in absorbance with pH, a titration-type curve is obtained (insert, Figure 5) from which a pK = 7.1 ± 0.1 can be derived, in good agreement with the pK_a = 7.22 of the ground state Phe-NH_2. These results indicate the strong effect the $-NH_3^+$ group has on the formation of the excited state precursors and the photodissociation process 15.

The addition of ethyl pyruvate as a quencher reduces considerably the absorbance at 318 nm, pH 9.2, see Figure 6A. The Stern–Volmer plot is not linear, for the same reasons as described above. The quenching constant obtained from the tangent of Figure 6A is given in Table II. The relatively low concentrations used in this quenching experiment suggest the formation of a long-lived excited state as the precursor of process 15.

A nonlinear dependence upon the light intensity (I) was observed on flash photolysis of Phe-NH_2 at pH 5.1. The O.D.$_{318}$ was found to be proportional to I^2. At pH 9.2, the O.D.$_{318}$ was found to be proportional to I. These results parallel those found for phenylalanine which were biphotonic when the amino group is protonated and monophotonic when the amino group is deprotonated.

N-Acetylphenylalanine. The state of protonation of the α-amino group in Phe and in Phe-NH_2 was seen above to greatly influence the course of the photochemical decomposition, the quantum yield, and (by indirect conclusion) the triplet energy level of the excited state precursors. The N-acetyl derivative was therefore examined with the object of removing the "influence" of the free amino group and, at the same time, observing the effect of a peptide -CONH- group on the electronic transitions of the aromatic ring.

On flash photolysis of N-acetylphenylalanine (pK_a probably ~3.6 ± 0.1), Ac-Phe, at pH 0.96 a strong transient absorption is observed, see Figure 7. The experimental curve was very well synthesized taking into account the formation of only the $PhCH_2\cdot$ and $CH_3CONH\dot{C}HCOOH$ radicals, produced from process 21.

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(34) E. Hayon, T. Ibata, N. N. Lichtin, and M. Simic, *J. Amer. Chem. Soc.*, **92**, 3898 (1970).

$CH_3CONHC(CH_2Ph)HCOOH$

$$\xrightarrow{h\nu} PhCH_2\cdot + CH_3CONH\dot{C}HCOOH \quad (21)$$

$$\xrightarrow{h\nu} CH_3CONH\dot{C}HCH_2Ph + \cdot COOH \quad (22)$$

$$\xrightarrow{h\nu} Ph\cdot + \cdot CH_2CH(NHCOCH_3)COOH \quad (23)$$

The $CH_3CONH\dot{C}HCOOH$ radical[35] has a shoulder at 310 nm and a maximum at 265 nm, with ϵ values of 3,900 and 14,000 M^{-1} cm^{-1}, respectively. The radical has[35] a $pK_a = 4.6 \pm 0.1$.

$$CH_3CONH\dot{C}HCOOH \rightleftharpoons CH_3CONH\dot{C}HCOO^- + H^+ \quad (24)$$

The experimental curve does not appear to allow for the formation of $\cdot COOH$, process 22. The spectrum of this radical is known.[27] This result is in agreement with the conclusions reached[11] in the flash photolysis of $PhCH_2CH_2COOH$. No evidence for process 23 could be expected since the radicals formed would not be observed under these experimental conditions.

The photolysis of Ac-Phe at pH 8.5 produced a different spectrum with a pronounced absorption above 340 nm, Figure 8. The experimentally observed curve was synthesized on the basis of the absorption spectrum of three radicals.

$CH_3CONHC(CH_2Ph)HCOO^-$

$$\xrightarrow{h\nu} PhCH_2\cdot + CH_3CONH\dot{C}HCOO^- \quad (25)$$

$$\xrightarrow{h\nu} e_{aq}^- + CH_3CONH\dot{C}HCH_2Ph + CO_2 \quad (26)$$

$$\xrightarrow{h\nu} Ph\cdot + \cdot CH_2CH(NHCOCH_3)COO^- \quad (27)$$

The $CH_3CONH\dot{C}HCOO^-$ radical[35] has maxima at 320 and 255 nm and ϵ values of 4,800 and 14,000 M^{-1} cm^{-1}, respectively. The absorption of e_{aq}^- at 720 nm was also observed, see Table III. The absorption spectrum of the $CH_3CONH\dot{C}HCH_2Ph$ radical is not known but is expected to be very similar to that of the $CH_3CONH\dot{C}HCH_3$ radical[36] with λ_{max} 360 nm and ϵ_{360} 3.4×10^3 M^{-1} cm^{-1}. Based on this ϵ value, the $[e_{aq}^-] = [CH_3CONH\dot{C}HCH_2Ph]$ confirms the occurrence of process 26. One should point out that the 360-nm band is not due to the electron adduct to Ac-Phe, since this absorption was the same in the presence of N_2O and *t*-BuOH, see Figure 8.

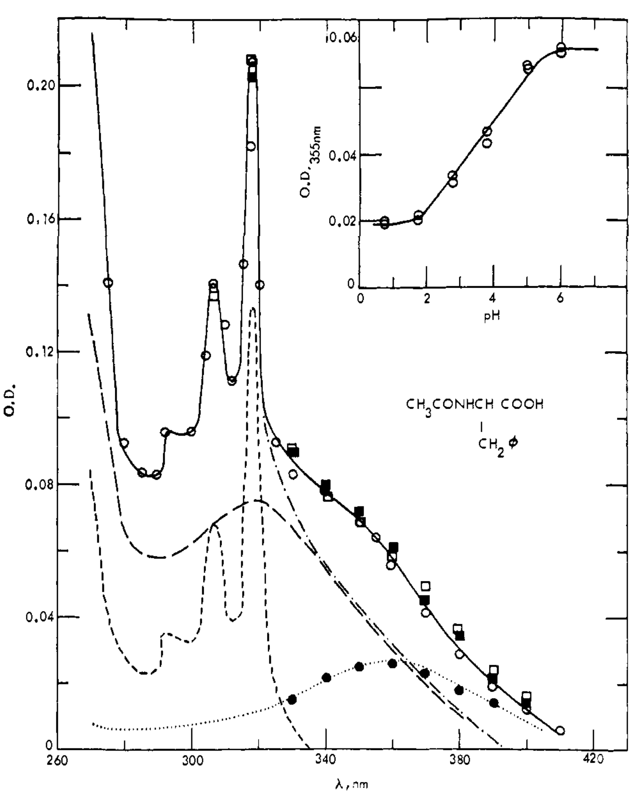

Figure 8. Transient spectrum produced in the photolysis of 2 mM N-acetylphenylalanine at pH 8.5 in the presence of N_2 (○), N_2 plus 1.0 M *t*-BuOH (□), N_2O plus 1.0 M *t*-BuOH (■). Spectrum of $PhCH_2\cdot$ (- - - - -) and $CH_3CONH\dot{C}HCOO^-$ (— —), and the sum of $PhCH_2\cdot$ plus $CH_3CONH\dot{C}HCOO^-$ (-·-·-) radicals. The spectrum of $CH_3CONH\dot{C}H_2$ (. . . . .) radicals used to synthesize the experimental curve. Insert: dependence of the absorbance at 355 nm upon pH. A 240-nm cut-off filter was used.

Table III. Comparison of the Apparent Quantum Yields for Photoionization of Aromatic Amino and Carboxylic Acids in Aqueous Solution

Compd	pH	Ionic structure	$[e_{aq}^-]$,[a] μM/flash
Phenylalanine	7.3	$NH_3^+CHCOO^-$ CH_2Ph	0.22
Phenylalanine	11.2	NH_2CHCOO^- CH_2Ph	0.45
N-Acetylphenylalanine	8.5	$CH_3CONHCHCOO^-$ CH_2Ph	0.40
α-Aminophenylbutyric acid	6.9	$NH_3^+CHCOO^-$ CH_2CH_2Ph	0.30
α-Aminophenylbutyric acid	11.1	NH_2CHCOO^- CH_2CH_2Ph	0.30
Phenyl acetate	9.2	$PhCH_2COO^-$	0.17[b]
Phenyl propionate	9.2	$PhCH_2CH_2COO^-$	0.20[b]
Phenyl butyrate	9.2	$PhCH_2CH_2CH_2COO^-$	0.24[b]

[a] Determined from flash photolysis of 2×10^{-4} M concentrations of the aromatic compounds, using a water cut-off filter ($\lambda_{exc} >$ 200 nm), assuming similar overall absorption of light intensity. Absorbance extrapolated to "zero time" after the flash, and e_{aq}^- concentration calculated taking ϵ_{700} 1.8×10^4 M^{-1} cm^{-1} for e_{aq}^-; these e_{aq}^- concentrations need not reflect a true comparison, since the triplet energy levels of these molecules differ and hence the absorption of a second quantum of light is not equally effective under these experimental conditions. [b] From ref 11.

It is interesting to note that $[CH_3CONH\dot{C}HCH_2Ph] = 0.4$ μM and $[PhCH_2\cdot] = 0.75$ μM at pH 8.5 indicates that $\phi_{(25)}/\phi_{(26)} \sim 1.8$. This compares with $\phi_{(11)}/\phi_{(12)} \sim 1.0$. Processes 21 and 25 are produced with the same quantum yield at pH 0.95 and 8.5, $\phi_{(21)} \simeq \phi_{(25)}$. No evidence for process 27 is available.

Since the ϕ for formation of the benzyl radical is independent of pH (the actual difference observed at 318 nm in Figures 7 and 8 is due to the different λ_{max} and ϵ of the parent radicals, processes 21 and 25), the dependence of process 26 upon pH was monitored at 355 nm. It can be seen (insert, Fig. 8) that the formation of the $CH_3CONH\dot{C}HCH_2Ph$ radical has a $pK = 3.6 \pm 0.1$, very close to the expected pK_a of the -COOH group in Ac-Phe.

The addition of ~1 mM 2,4-hexadien-1-ol to 5 mM Ac-Phe, using a 260 nm cut-off filter, reduced the $O.D._{318} > 75\%$ at both pH 0.8 and 6.5. However, as mentioned above, this quenching could also be due to the reaction of $PhCH_2\cdot$ with the quencher.

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(35) M. Simic, P. Neta, and E. Hayon, *J. Amer. Chem. Soc.*, **92**, 4763 (1970).

(36) L. J. Mittal, J. P. Mittal, and E. Hayon, *Photochem. Photobiol.*, in press.

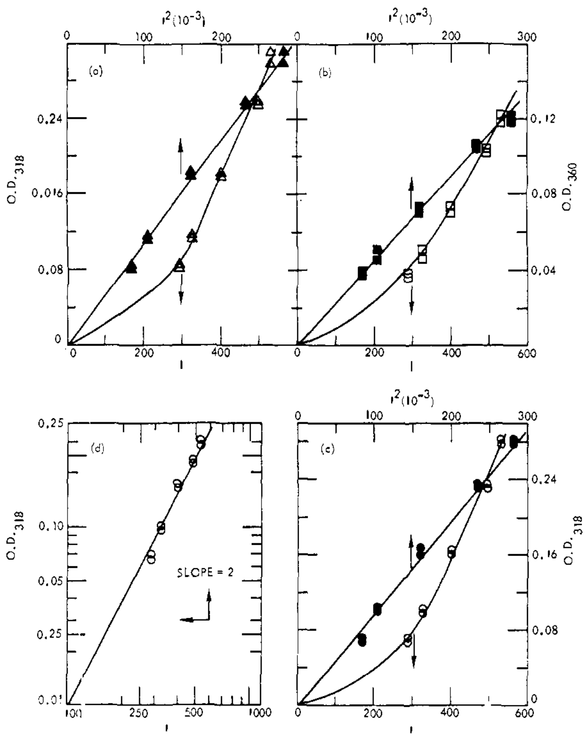

Figure 9. Dependence upon the intensity of the exciting light of the concentration of intermediates produced in the flash photolysis of 2 m*M* *N*-acetylphenylalanine, N_2: (a) O.D.$_{318}$ at pH 8.5, (b) O.D.$_{360}$ at pH 8.5, (c) O.D.$_{318}$ at pH 0.95, and (d) O.D.$_{318}$ at pH 0.95 presented on a log-log scale.

The absorbance at 318 nm was found to be dependent upon the square of the light intensity, I^2, at both pH 0.95 and 8.5, Figure 9. This result indicates that processes 21 and 25 are biphotonic and is consistent with the observation that $\phi_{(21)} \sim \phi_{(25)}$. It is interesting to point out that some *N*-acetyl derivatives of aliphatic amino acids also undergo[36] a biphotonic photodissociation reaction. The absorbance at 360 nm and at pH 8.5 on flash photolysis of Ac-Phe is also found, Figure 9b, to be dependent on I^2. This rather important observation shows that the photoionization process 26 is also biphotonic, in agreement with the results obtained[11] in the photolysis of aromatic carboxylic acids.

α-Aminophenylbutyric Acid. The photolysis of α-amino phenylbutyric acid, $Ph(CH_2)_2CH(NH_3^+)COOH$ was examined in order to observe the effect of an additional CH_2 group between the benzyl and amino acid groups. A striking difference in the transient spectrum is observed. No benzyl radical is produced, but the photoionization yield is still substantial (see Table III). The transient with λ_{max} ~320 nm decays very slowly (in the seconds range) giving rise to a strong permanent product in the uv region. The formation of this intermediate was found to be biphotonic at pH 11.1. It was not possible to identify the transient(s) giving rise to this absorption.

Conclusion

The absorption and fluorescence spectra of simple phenylalkylcarboxylic acids in aqueous solution at room temperature have been interpreted[37] in terms of intramolecular charge-transfer interactions. These exhibit an enhancement of phosphorescence yields, low fluorescence yields, an increase in the intersystem crossing process k_{isc}, and an enhancement in spin-orbit coupling and some electronic overlap. The electronic transitions of the aromatic amino acids examined here are expected to exhibit some of these properties.

The results obtained and presented above show quite clearly for the first time that a long-lived excited state, probably the triplet state, is involved in all the photolytic processes observed on flash photolysis of aqueous solutions of phenylalanine, phenylalanine amide, and *N*-acetylphenylalanine. This includes both photodissociation and photoionization processes. Similar results and conclusions were drawn from the photolysis of phenolic compounds[9,10] (phenol, *p*-cresol, tyrosine) and of various aromatic alkyl carboxylic acids.[11]

One of the main photolytic reactions of Phe is the liberation of carbon dioxide by a photoionization process 12. Since all the energy is initially absorbed by the benzene ring, it is reasonable to conclude that some strong interaction takes place even in the presence of a number of methylene groups $(-CH_2-)_n$ (n = 3 for α-aminophenylbutyric acid), considered hitherto to act as insulating groups. The results presented for the phenylalanine derivatives, and for the aromatic alkylcarboxylic acids,[11] show that the ejected electron in the photoionization of these aromatic compounds comes from the ionized carboxyl group $-COO^-$.

From the dependence upon light intensity, the photodissociation processes leading to the formation of benzyl radicals were found to be biphotonic in neutral solution for Phe, $Phe-NH_2$, and Ac-Phe. The photoionization processes were also found to be biphotonic, *e.g.*, see results for acetylphenylalanine. The latter observations can be rationalized since the ionization potential of aromatic compounds[38] in the gaseous phase is ~8 eV and the triplet energy level of phenylalanine and derivatives is 340 nm (0,0). The absorption of a second quantum plus ~1 eV energy of solvation can produce the required energy for ionization. The rupture of a -C-C- bond, however, requires only ~3.5 eV and it is not clear why a second quantum is needed to bring about the photodissociation reaction.

The following photophysical processes are suggested for Phe, $Phe-NH_2$, and Ac-Phe.

$$^0S(Phe) \xrightarrow{h\nu_1} {}^1S(Phe)$$

$$^1S(Phe) \longrightarrow {}^0S(Phe) + h\nu_F$$

$$^1S(Phe) \longrightarrow {}^1T(Phe)$$

$$^1T(Phe) \longrightarrow {}^0S(Phe)$$

$$^1T(Phe) \longrightarrow {}^0S(Phe) + h\nu_P$$

$$^1T(Phe) \xrightarrow{h\nu_2} {}^*T(Phe)$$

$$^*T(Phe) \longrightarrow \text{dissociation and electron ejection}$$

The dependence of the quantum yield of processes 1, 11, 15, and 19 on the pH and the state of protonation of the α-amino group shows (a) the strong effect the amino group has on the nature and formation of the *T states and (b) that the triplet and not the singlet excited state is involved, based on the quenching experiments and on the fact that the observed pK_a is the same as pK_a[^{0}S(Phe)], in accord[28] with the pK_a of the triplet state being close to that of the ground state.

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(37) J. Tournon and M. A. El-Bayoumi, *J. Amer. Chem. Soc.*, **93**, 6396 (1971); *J. Chem. Phys.*, **56**, 5128 (1972).

(38) A. Terenin and F. Vilesov, *Advan. Photochem.*, **2**, (385 1964).