retention time. The ^{1}H NMR spectra were run in CCl_4 and PhH as solvents, and the results are given in Table I.

Stereochemistry of Enones 5c and 5d. The enones were prepared as previously described.[11] Inasmuch as preparative VPC using a 10-ft, 20% Carbowax 20-M on Chromosorb P column would not totally separate the isomers, ^{1}H NMR spectra were run on two enriched samples of **5c** and **5d**, respectively. Enone **5c** had the earlier retention time. The results are given in Table I.

Epoxidation of Cyclopropenes 3a and 3b. Analysis of Enones 5a–5d. The cyclopropenes were prepared as described previously.[11] An ~0.15 M solution of the cyclopropene in CCl_4 with dodecane as an internal standard was prepared. Less than 1 equiv of *m*-chloroperbenzoic acid was added, and the solution was stirred at 0 °C. Aliquots were analyzed by VPC (column B), and the areas of the enone peaks were measured by a planimeter. The ratio of enones did not change throughout the run from ~10 to 70% reaction. The enones were also independently shown to be stable to the reaction conditions.

Equilibration of Enones 5c and 5d. Two runs were done, one enriched in enone **5c** and the other in **5d**. An ~0.03 M solution of the enone mixture in Spectrograde $CHCl_3$ was refluxed with approximately 0.5 equiv of *p*-toluenesulfonic acid. The solution darkened almost immediately, but only a slow decomposition was detected by VPC. Equilibration of the enones occurred with a half-life of ~5 h with an overall decomposition rate which was ~7 times slower. VPC analysis (column B) showed that in one run, an 81:19 ratio of **5c**/**5d** equilibrated to a 56:44 ratio. In the other run, a 29:71 ratio of **5c**/**5d** equilibrated to a 55:45 ratio.

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Registry No. 3a, 64425-32-7; **3b**, 64425-34-9; **4a**, 73986-28-4; **4b**, 74034-35-8; **4c**, 73986-29-5; **4d**, 74034-36-9; **5a**, 64425-37-2; **5b**, 64425-35-0; **5c**, 64425-43-0; **5d**, 64425-41-8.

Kinetics and Mechanism of the Oxidation of L-Phenylalanine by Hydrogen Peroxide in the Presence of Ferrous Sulfate as a Catalyst

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The kinetics of oxidation of L-phenylalanine by hydrogen peroxide in the presence of Fe(II) ions have been studied. The reaction is first order with respect to both phenylalanine and Fe(II) ions but zero order with respect to hydrogen peroxide. The energy of activation has been calculated to be 11.45 ± 0.01 kcal mol^{-1}. A reaction mechanism has also been proposed.

Hydrogen peroxide is a common oxidizing agent for analytical and synthetic organic chemistry. The chemistry of metal ion catalyzed reactions of hydrogen peroxide commenced at the end of the last century, when Fenton[1] described the reaction of tartaric acid with hydrogen peroxide in the presence of ferrous sulfate. Thereafter, a free-radical mechanism was proposed for this reaction by a few investigators.[2-5] However, a few reports[6-10] described the oxidation of some amino acids by the Fenton reagent. These studies refer mainly to their oxidation products, but no work appears to have been done on the kinetics of the oxidation of amino acids by this reagent. Since the oxidative degradation of amino acids is important in the biological systems, the kinetics of oxidation of phenylalanine by hydrogen peroxide in the presence of Fe(II) ions as catalyst were studied in acidic medium and are reported in this paper.

Experimental Section

General Methods. All the chemicals used were of AnalaR (BDH) grade. The ionic strength of the reaction mixture was maintained by the addition of sodium nitrate solution. The concentration of hydrogen peroxide in kinetic runs was measured colorimetrically.[11]

After the reactants had reached thermostat temperature, the reaction was started by addition of the catalyst. The kinetics were followed by examining 5-mL aliquots of the reaction mixture for hydrogen peroxide content. The aliquots were added to 5 mL of titanium sulfate solution to stop the reaction. Optical density was measured at 420 nm, and the concentration of hydrogen peroxide was read from the calibration graph. All the reactions were studied in an open atmosphere. However, the dependence of the reaction on atmosphere was checked by studying two identical reaction mixtures, one under nitrogen and the other without nitrogen. The rate constants in both cases were almost equal.

Identification of Products. For identification of the products the following reaction mixture was prepared and heated to 70 °C for about 2 h: 20 mL of 0.5 M phenylalanine, 5 mL of 0.01 M ferrous sulfate, 20 mL of 1 M perchloric acid, 15 mL of water mixed with 40 mL of 0.5 M H_2O_2. The gaseous products formed were passed through a freshly prepared lime-water solution, which turned milky and thus confirmed the presence of carbon dioxide. The formation of formic acid in the reaction mixture was tested by its reduction with a known procedure. The formaldehyde thus obtained was confirmed by chromotropic acid, which gave a violet-pink color.[12] Its formation was further confirmed by reaction

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Table I. First-Order Dependence of the Fe(II) Ion Catalyzed Oxidation Rate of Phenylalanine on Hydrogen Peroxide Concentration[a]

$10^3[H_2O_2]$, M	10^2k_{obsd}, mol L^{-1} min^{-1}	$10^3[H_2O_2]$, M	10^2k_{obsd}, mol L^{-1} min^{-1}
1.25	2.01	5.00	1.98
2.50	1.95	6.25	1.92
3.25	1.87		

[a] [Phenylalanine] = 0.10 M; [$FeSO_4$] = 5.00 × 10^{-4} M; [$HClO_4$] = 0.20 M; μ = 0.202 M; temperature 45 ± 0.5 °C.

Table II. First-Order Dependence on the Rate of Oxidation of Phenylalanine on Ferrous Sulfate Concentration[a]

$10^4[FeSO_4]$, M	10^5k_{obsd}, mol L^{-1} min^{-1}	$10^4[FeSO_4]$, M	10^5k_{obsd}, mol L^{-1} min^{-1}
2.50	1.20	10.00	3.20
5.00	1.75	12.50	4.55
7.50	2.60		

[a] [Phenylalanine] = 0.10 M; [H_2O_2] = 2.50 × 10^{-3} M; [$HClO_4$] = 0.20 M; μ = 0.20 M; temperature 45.0 ± 0.5 °C. A plot of these values gives a straight line.

with mercuric chloride solution. The mercurous chloride produced was treated with ammonia, which yielded grayish black color.[13] When the reaction mixture was made alkaline with sodium hydroxide solution and heated to 70 °C, the characteristic smell of ammonia was noted from the issuing gases. The ammonia evolved was confirmed by reaction with the Nessler reagent. The reaction mixture was treated with acidified 2,4-dinitrophenylhydrazine solution, which yielded a hydrazone. The IR spectrum of this hydrazone superimposed on the spectrum of a corresponding hydrazone of a standard sample of benzaldehyde. Moreover, the mixture melting point of the hydrazone did not show any depression.

Another reaction mixture, in which the concentrations of all the reagents were 10 times those reported above, was heated at about 60 °C for 4 h and then distilled. The aqueous distillate was extracted with ether, the ethereal extract dried over anhydrous magnesium sulfate, and the solvent removed by distillation on a water bath. The characteristic odor of benzaldehyde was distinct in the residue. The IR spectrum of this residue was superimposable on the IR spectrum of the standard sample of benzaldehyde. Even the air oxidation of the residue provided a few crystals having an IR spectrum and melting point corresponding to those benzoic acid, and their mixture melting point with an authentic sample of benzoic acid did not show any depression. Moreover, the melting point and IR spectrum of the semicarbazone prepared from some of the residue were identical with the semicarbazone prepared from standard benzaldehyde. The UV spectrum of the residue was run on a Unicam SP 8000 ultraviolet recording spectrometer and compared with the UV spectrum of the standard benzaldehyde. These UV spectra were also identical. From the residue of an identical, concentrated reaction mixture 2,4-dinitrophenylhydrazone was prepared, filtered, and washed with sodium carbonate solution followed by washings with water. The yield of the dried hydrazone of benzaldehyde was about 78%. The sodium carbonate extract was acidified with hydrochloric acid, and no organic product was obtained.

Results and Discussion

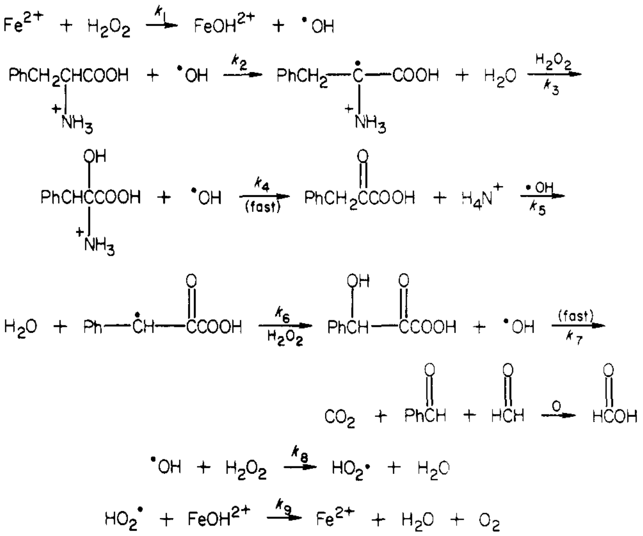
Trial and error plots of the rate data for runs with excess L-phenylalanine indicate that the reaction is zero order in hydrogen peroxide. The plot of concentration of hydrogen peroxide vs. time is a straight line, and its slope yields k_{obsd}. The rate constants were found to be independent of the concentration of hydrogen peroxide. The results are summarized in Table I.

Table III. First-Order Dependence of the Fe(II) Ion Catalyzed Oxidation Rate of Phenylalanine on Its Concentration

10^2[phenylalanine], M	10^2k_{obsd}, mol L^{-1} min^{-1}	10^2[phenylalanine], M	10^2k_{obsd}, mol L^{-1} min^{-1}
5.00	1.05	12.50	2.29
7.50	1.31	15.00	2.97
10.00	1.98		

[a] [H_2O_2] = 2.50 × 10^{-3} M; [$FeSO_4$] = 5.00 × 10^{-4}]; [$HClO_4$] = 0.20 M; μ = 0.202 M; temperature 45 ± 0.5 °C.

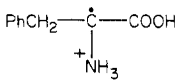
Scheme I. Proposed Reaction Scheme for the Oxidation of L-Phenylalanine by the Fenton Reagent



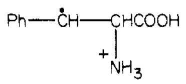
The value of k_{obsd} increases directly with the increase in ferrous sulfate concentration (Table II). This shows first-order dependence of the rate on the catalyst concentration. The order of the reaction with respect to L-phenylalanine was determined by changing its concentration and keeping other variables constant. The observed pseudo-zero-order rate constants, k_{obsd}, when divided by the L-phenylalanine concentration, gave a constant value (Table III). This shows that the order with respect to L-phenylalanine is unity.

All the experiments have been carried out in the presence of aqueous perchloric acid. The concentration of this acid used in all experiments was ≥0.2 M which was sufficient to prevent the hydrolysis of Fe(II) ions. It is fairly well established[5] that hydrogen peroxide in the presence of Fe(II) ions produces the hydroxyl free radical (·OH) which will very likely attack the amino acid. A probable mechanism of the oxidation of L-phenylalanine involving this free radical is represented in Scheme I.

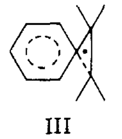
The hydroxyl free radical, ·OH, generated in step 1 can attack a carbon which is α or β to the carboxylic group of the phenylalanine, giving free radicals I and II. The former is likely to stabilize through a bridgehead, III, and the latter through direct resonance with the aromatic ring. The bridged-head free radical may yield an α-keto acid as shown in Scheme I, whereas the benzylic free radical may



I



II



III

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Table IV. Variation of the Oxidation Rate of Phenylalanine with Ionic Strength of the Medium

μ, M	$10^2 k_{obsd}$, mol L^{-1} min^{-1}	μ, M	$10^2 k_{obsd}$, mol L^{-1} min^{-1}
0.202	1.98	0.400	2.03
0.300	1.87	0.650	1.78
0.350	1.99		

[a] $[H_2O_2] = 2.50 \times 10^{-3}$ M; $[FeSO_4] = 5.00 \times 10^{-4}$ M; $[HClO_4] = 0.20$ M; [phenylalanine] = 0.10 M; temperature 45 ± 0.5 °C.

form a β-keto acid through successive attack on the benzylic carbon followed by dehydration. Unfortunately neither of these intermediates could be isolated under our experimental conditions. However, the suggested formation of an α-keto acid (phenylpyruvic acid) as an intermediate in the proposed scheme is in agreement with Johnson et al.,[9] who obtained a poor yield of pyruvic acid from the oxidation of alanine by the Fenton reagent. The similar formation of an α-keto acid has also been established by the breakdown of α-amino acids by animal tissues.[14] In analogy with these observations the proposed formation of phenylpyruvic acid in the given scheme appears to be more probable. Consequently this intermediate converts to benzaldehyde as an aromatic product. This suggestion is in agreement with the reported work[8,15,16] on the oxidative degradation of amino acids by the Fenton reagent which are known to give aldehydes.

The rate of the reaction in the given scheme is given by eq 1. Applying steady-state approximations for the concentrations of $\cdot$OH, $PhCH_2\text{-}\dot{C}(^+NH_3)\text{-}COOH$, and $Ph\text{-}\dot{C}H\text{-}C(O)COOH$ in the scheme, we obtain eq 2. Substituting the concentration of $\cdot$OH from eq 2 into eq 1, we get eq 3 and 4.

$$-d[\text{phenylalanine}]/dt = k_2[\cdot OH][\text{phenylalanine}] \quad (1)$$

$$[\cdot OH] = k_1[Fe^{2+}]/k_8 \quad (2)$$

$$-\frac{d[\text{phenylalanine}]}{dt} = \frac{k_1 k_2 [Fe^{2+}][\text{phenylalanine}]}{k_8} \quad (3)$$

$$k_{obsd} = k_1 k_2 [Fe^{2+}][\text{phenylalanine}]/k_8 \quad (4)$$

Table V. Dependence of the Rate of Oxidation of Phenylalanine on Temperature

T, °C	$10^5 k_{obsd}$, mol L^{-1} min^{-1}	T, °C	$10^5 k_{obsd}$, mol L^{-1} min^{-1}
24.5	1.58	45.0	8.41
30.0	2.95	50.0	13.12
40.0	5.75		

[a] [Phenylalanine] = 0.10 M; $[H_2O_2] = 2.5 \times 10^{-3}$ M; $HClO_4] = 0.2$ M; $[FeSO_4] = 5.00 \times 10^{-4}$ M; $\mu = 0.202$ M.

The rate law obtained from the scheme which is given by eq 3 clearly explains the order of the reaction with respect to the phenylalanine, Fe(II) ion, and hydrogen peroxide concentrations.

The ionic strength of the reaction mixture was varied from 0.202 to 0.650 M by the addition of sodium nitrate solution. No significant change in the rate of the reaction was observed (Table IV). These observations indicate that the reaction does not follow an ionic mechanism. Moreover, it may also be inferred that nitrate ions do not have any specific effect, other than maintaining the ionic strength. However, the proposed free-radical mechanism was further confirmed by the addition of allyl acetate and acrylamide, which are effective scavengers[17-19] for free radicals. The reaction was almost completely arrested by the addition of ca. 0.3 M acrylamide or allyl acetate. Thus, any possibility of a nonchain reaction is excluded.

The reaction was studied at five different temperatures from 24.5 to 50 °C (±0.1 °C), and the results are given in Table V. The plot of log k_{obsd} against reciprocal temperature gives a straight line, and the energy of activation calculated from the slope of this plot is 11.45 ± 0.01 kcal mol^{-1}.

Registry No. L-Phenylalanine, 63-91-2; hydrogen peroxide, 7722-84-1; ferrous sulfate, 16547-58-3; benzaldehyde hydrazone, 5281-18-5.

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