water (50 ml). The layers were separated, the organic phase was washed with H_2O (3 × 25 ml), and the combined aqueous layers were evaporated to dryness. The residue was taken up in H_2O and chromatographed on AG-50WX2 using 0.4 *M* pyridine acetate (pH 4.0) as eluent. In several cases the buffer was adjusted to higher pH in order to elute the product in a volume of 375–475 ml. The ninhydrin-positive fractions were pooled, lyophilized, and crystallized.

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Registry No.—Boron tribromide, 10294-33-4.

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Oxidation of Tyrosine and of NH_2-Terminal Tyrosine Peptides with the Cu^{2+}/H_2O_2 System

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The oxidations of tyrosine and of tyrosine-containing peptides to aminochromes have long been known as enzymatic reactions.[2] More recently, analogous chemical oxidations have been studied spectroscopically. Wilchek, *et al.*,[3] reported that, at room temperature, *N*-bromosuccinimide oxidation of tyrosine esters and of tyrosinamide (but not of free tyrosine) gave a product that was identified spectroscopically as an unstable red aminochrome, λ_{max} 480 and 320 nm. Dukler, *et al.*,[4] found that tyrosine methyl ester and di- and tripeptides with NH_2-terminal tyrosine were oxidized at room temperature by potassium nitrosodisulfonate (Fremy salt), forming a product with absorption maxima at 305 and 475 nm, characteristic for dopachrome (**2**, R = H). As in the case of the enzymatic reaction, oxidation by this reagent of peptides with COOH-terminal tyrosine resulted, not in an aminochrome, but in

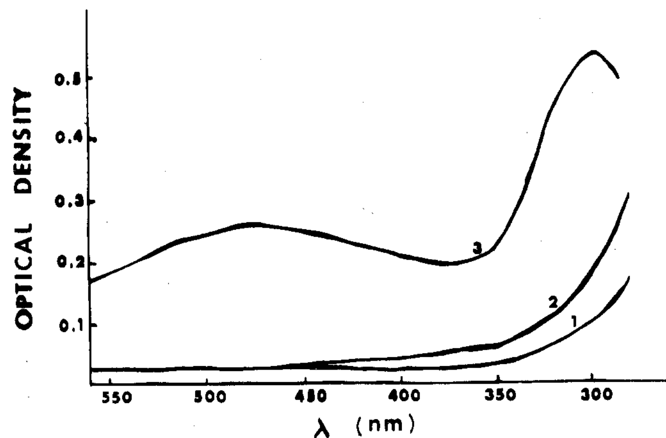


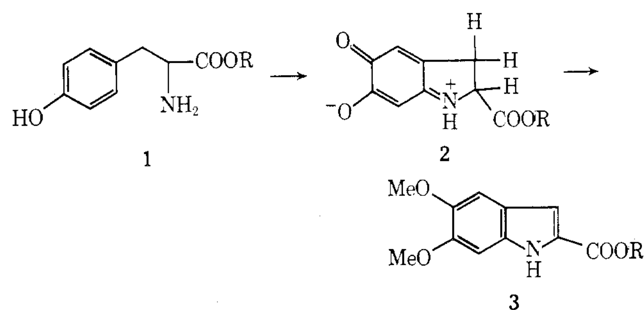
Figure 1. Oxidation of tyrosine by the Cu^{2+}/H_2O_2 system: curve 1, zero time; curve 2, after 16 hr at room temperature; curve 3, after 16 hr at room temperature, followed by addition of Pt black. No change in curve 3 was observed after 8 hr at room temperature.

dopaquinone, indicated by the characteristic *o*-quinone absorption at 390 nm. Dukler, *et al.*, did not report on the oxidation of tyrosine itself, but found that carbobenzoxy-L-tyrosine gave, on short-term treatment with Fremy salt followed by treatment with $Na_2S_2O_4$ and cleavage of the carbobenzoxy moiety, 3,4-dihydroxy-L-phenylalanine; longer term treatment with Fremy salt gave polymeric oxidation products of tyrosine.

We wish to report the effect of another oxidizing system, Cu^{2+}/H_2O_2 (3% unstabilized H_2O_2 containing trace amounts of Cu^{2+}), on tyrosine and on some NH_2-terminal and COOH-terminal tyrosine peptides, and the first direct nonenzymatic conversion of free tyrosine to an aminochrome. This metal-activated hydrogen peroxide system contains hydroxy and peroxy radicals, and oxidations by this system are considered to proceed by radical mechanisms.

Results and Discussion

Tyrosine. Tyrosine (**1**, R = H) was treated at room temperature with excess Cu^{2+}/H_2O_2 reagent, and the ultraviolet absorption spectrum was scanned at intervals against a Cu^{2+} blank of the same concentration. No evi-



dence for dopachrome formation was obtained, even after 16 hr at room temperature. A predominant end absorption at shorter wavelengths was observed; the reagent and unreacted tyrosine are known to absorb in this region (Figure 1, curves 1 and 2).

Addition of Pt black at the end of the 16-hr period caused the immediate development of two absorption maxima at 305 and 475 nm, characteristic of dopachrome (**2**, R = H) (Figure 1, curve 3). These maxima did not change with time or with addition of more H_2O_2. In the absence of Cu^{2+} from the peroxide system, Pt black did not show this effect.

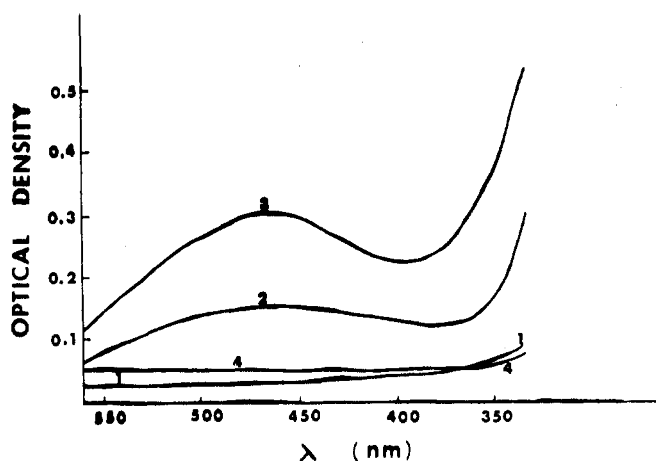


Figure 2. Oxidation of tyrosine peptides by the Cu^{2+}/H_2O_2 system: curve 1, L-tyrosyl-L-leucine at zero time; curve 2, L-tyrosyl-L-leucine after 45 min at room temperature; curve 3, L-tyrosyl-L-leucine after 3.75 hr at room temperature; curve 4, DL-leucyl-DL-tyrosine after 6 hr at room temperature.

Dukler, *et al.*,[4] reported that their dopachrome (2, R = CH_3), formed by the action of Fremy salt in a buffered solution (pH 8) on tyrosine methyl ester, on long standing in the presence of the reagents was converted into a dihydroxyindole. It is known that the rearrangement of dopachromes to dihydroxyindoles is catalyzed by acid and by alkali.[5,6] The rearrangement observed by Dukler, *et al.*, was therefore probably due to the alkalinity of the solution. A similar rearrangement was not observed in the present study when the reaction mixture containing the dopachrome was held at room temperature for 8 hr, since the pH of the solution was 5.0 and the dopachrome is known to be stable at this pH.[6]

The presence of dopachrome as a product of the reaction, indicated by the absorption maxima observed, was confirmed by its conversion to the known methyl 5,6-dimethoxyindole-2-carboxylate (3, R = Me) by the method of Dukler, *et al.*[4,7] The formation of dopachrome involves introduction of an oxygen ortho to the OH group, dehydrogenation, and intramolecular cyclization through a Michael-type addition reaction; this process evidently requires the hydrogen acceptor Pt black in the case of free tyrosine.

Spectroscopic Studies of the Oxidation of Tyrosine Peptides with the Cu^{2+}/H_2O_2 System. As is the case with other oxidizing agents, the effect of the Cu^{2+}/H_2O_2 system on tyrosine peptides depends on the position of the tyrosine moiety in the peptides.

With the NH_2-terminal tyrosine peptide, L-tyrosyl-L-leucine (1, R = leucine moiety), the Cu^{2+}/H_2O_2 system gave an absorption spectrum (Figure 2, curves 1, 2, and 3) similar to that found by Dukler, *et al.*,[4] when tyrosylglycylglycine was oxidized by potassium nitrosodisulfonate (maximum at 475 nm). Dukler, *et al.*, have attributed the spectrum to the fact that the N-terminal peptide was oxidized by a dopachrome mechanism, forming an aminochrome; analogously, the present product may be regarded as an aminochrome (2, R = leucine moiety). At room temperature and with excess Cu^{2+}/H_2O_2 the aminochrome absorption at 475 nm increased up to 4 hr, and then slowly began to decline. With neither potassium nitrosodisulfonate nor the present Cu^{2+}/H_2O_2 system was treatment with Pt black required for aminochrome formation from NH_2-terminal tyrosine peptides. Addition of Pt black produced no significant changes in the spectrum other than reduction of the end absorption at shorter wavelengths.

With the COOH-terminal tyrosine peptide DL-leucyl-DL-tyrosine, the absorption spectrum obtained on addition of excess Cu^{2+}/H_2O_2 gave no indication of formation of an aminochrome (maxima at 305 and 475 nm) or of an *o*-quinone (maximum at 390 nm) after 6 hr at room temperature (Figure 2, curve 4). Similar results were obtained with another COOH-terminal tyrosine peptide, glycyl-L-tyrosine. Even after 14 hr at room temperature, followed by treatment with Pt black, no spectral evidence that the COOH-terminal tyrosine peptides were oxidized by either an aminochrome mechanism or a dopaquinone pattern was obtained. The dopaquinone pattern of oxidation of COOH-terminal tyrosine peptides occurs on enzymatic oxidation[2] and on oxidation with potassium nitrosodisulfonate.[4]

Experimental Section

L-Tyrosyl-L-leucine, glycyl-L-tyrosine, and DL-leucyl-DL-tyrosine were obtained from Nutritional Biochemicals Corp., Cleveland, Ohio, and tyrosine from Matheson Coleman and Bell, Cincinnati, Ohio. The 3% H_2O_2 was prepared by dilution of 30% unstabilized H_2O_2 (Fisher Scientific Co.).

Tyrosine or the tyrosine peptide (0.3 mmol) was added to 50 ml of freshly prepared $CuSO_4$ (5×10^{-4} *M*)/H_2O_2 (3% unstabilized) (44.1 mmol of H_2O_2), and the mixture was allowed to stand at room temperature. At intervals aliquots were withdrawn from the solutions, and their absorption spectra were determined after dilution with distilled water (one part reaction mixture to 35 parts water for the spectra shown in Figure 1, and 1:3 for the spectra shown in Figure 2), using a Beckman DB spectrophotometer and reading against a $CuSO_4$ blank of the same concentration as the diluted solution. When Pt black was added to the diluted solution, the mixture was centrifuged to remove the metal after catalytic decomposition of the peroxide was complete, and the absorption spectrum of the supernatant was determined.

For confirmation of the identity of dopachrome produced in the oxidation of tyrosine, the reaction mixture on a preparative scale, after 4 hr at room temperature followed by treatment with Pt black, was allowed to stand overnight at room temperature with $Na_2S_2O_4$ and extracted with ethyl acetate, and the product obtained was converted by ethereal diazomethane to 3, R = Me, mp 117-119°.

Anal. Calcd for $C_{12}H_{13}NO_4$: N, 5.96. Found: 5.99.

Registry No.—1 (R = H), 60-18-4; 1 (R = leucine moiety), 17355-10-1; 3 (R = Me), 28059-24-7; $CuSO_4$, 10124-44-7.

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Hydrolysis and Alcoholysis of Orthothio Esters

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Recently we reported a convenient oxidation of aldehydes to esters and acids *via* 1,3-dithiane derivatives.[1] The hydrolysis to produce the carboxylic acids proceeded less efficiently and in poorer yield than alcoholysis and, unlike the latter, has now been found to give several neu-