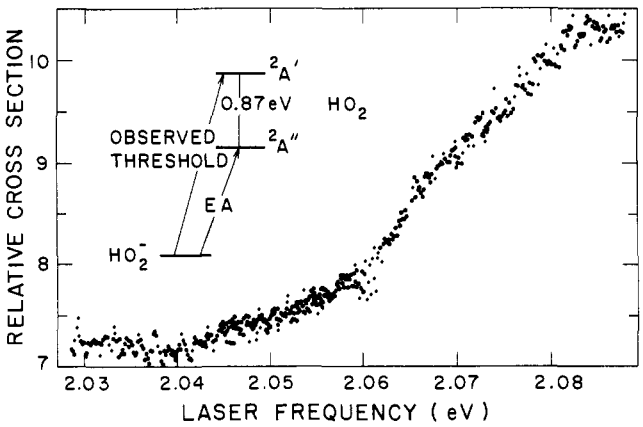

Figure 1. Relative photodetachment cross section for HO_2^- and energy level diagram showing ion and neutral states. The observed threshold of the $^2A'$ state of HO_2 is at 2.06 eV. Consequently the electron affinity (EA) of HO_2 is 1.19 eV.

photodetachment spectroscopy was possible. This technique, which has produced electron affinities for many atomic and molecular species, is described in detail elsewhere.[11] A mass analyzed beam of HO_2^- was crossed with a tunable dye laser beam. The ions were made by an electric discharge in O_2 and 2,3-dimethyl-1-butene under conditions which typically produce ions characterized[12,13] by a temperature of 1500 K. Electrons and neutrals generated by photodetachment were collected and counted to produce a plot of photodetachment cross section vs. photon energy. In order to observe a photodetachment threshold using convenient dye laser technology, a search was made for the onset of photodetachment to the $^2A'$ excited state of the neutral, which lies[14] 0.872 eV above the ground state.

The data, shown in Figure 1, display a strong onset near 2.06 eV for detachment to the excited state. Modeling[13,15] of the cross section in this region places the electron affinity of the excited state slightly above the onset, but the exact position is relatively insensitive (±0.003 eV) to the details of the model. The photodetachment cross section was featureless near 1000 and 1300 cm^{-1} both above and below this feature where other thresholds involving the lower frequency vibrations of the neutral or ion might appear. Involvement of the highest frequency vibration would be inconsistent with the flowing afterglow measurement, so the absence of other thresholds indicates that the threshold observed corresponds to the $HO_2^-(0,0,0) \rightarrow HO_2\ ^2A'(0,0,0)$ threshold. Photodetachment spectra down to photon energies of 1.75 eV showed little decrease in cross section. This observation alone indicates the EA to be substantially smaller than 1.75 eV, inconsistent with the current literature value[2] of 1.85 eV but supporting this determination of 1.19 eV.

Two different methods have been used to determine the EA of HO_2. By measuring forward and reverse rate constants with a flowing afterglow apparatus, the EA is found to be 1.16 ± 0.15 eV. Threshold photodetachment provides both a consistency check on this value and improves the precision of the determination, giving $EA(HO_2) = 1.19 \pm 0.01$ eV.

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Preparative Hydroxylation of Aromatic Compounds Catalyzed by Peroxidase

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Selective hydroxylation of aromatic compounds is a difficult task in preparative organic chemistry.[1] The problem is particularly severe when the compounds to be hydroxylated (or their products) are optically active and/or unstable, since in these instances the reaction should be conducted rapidly and under mild conditions in order to prevent racemization and decomposition. Therefore, such hydroxylations are often carried out either by microbiological means[2] or by circumventing the direct hydroxylation as exemplified by the catalytic asymmetric production of L-3,4-dihydroxyphenylalanine (L-DOPA).[3] Both of these approaches have serious shortcomings: the former is laborious, time consuming, and usually provides relatively low yields; the latter has only a limited applicability and employs extremely O_2-unstable catalysts.

Mason and co-workers have discovered[4,5] that horse radish peroxidase, in addition to its usual peroxidatic and catalytic activities, can also catalyze the hydroxylation of some aromatic compounds by molecular oxygen in the presence of dihydroxyfumaric acid as a hydrogen donor. However, the yields obtained were very low and the process lacked specificity, apparently due to considerable nonenzymatic hydroxylation. Therefore, the preparative potential of this reaction has never been explored.

In this work we have found that under certain conditions the reaction in Scheme I, catalyzed by peroxidase,[6] can be used for fast, convenient, and selective hydroxylations which afford yields up to 70%. Three important drugs have been produced as examples using this enzymatic hydroxylation: L-DOPA[7] from L-tyrosine (I), D-(−)-3,4-dihydroxyphenylglycine[8] from D-(−)-*p*-

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(3) Instead of direct hydroxylation of L-tyrosine to form L-DOPA (Sih, C. J.; Foss, P.; Rosazza, J.; Lemberger, M. *J. Am. Chem. Soc.* **1969**, *91*, 6204. Florent, J.; Renaut, J. German Patent 2 102 793, 1970), this elegant process involves the condensation of 3,4-dihydroxybenzaldehyde with *N*-acetylglycine, followed by asymmetric hydrogenation catalyzed by rhodium complexes and subsequent hydrolysis of the resultant *N*-acetyl-L-DOPA (Knowles, W. S.; Sabacky, M. J.; Vineyard, B. D. U.S. Patent 4 005 127, 1977. For a general review, see: Merrill, R. E. *CHEMTECH* **1981**, *11*, 118–127). Clearly, this approach, while excellent for L-DOPA, cannot be used for the production of a number of hydroxylated aromatic compounds, e.g., dihydroxyphenylglycine and adrenaline prepared in this work.
(4) Mason, H. S.; Onopryenko, I.; Buhler, D. *Biochim. Biophys. Acta* **1957**, *24*, 225–226. Mason, H. S. *Proc. Int. Symp. Enzyme Chem.* **1958**, 220.
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(6) It is noteworthy that both peroxidases (EC 1.11.1.7) used in this study, horse radish peroxidase and lactoperoxidase from cow milk, are readily available from most commercial suppliers of biochemicals; they are relatively inexpensive and stable during storage and operation. The particular preparations of the horse radish peroxidase and lactoperoxidase used in this work were obtained from Sigma and had a specific activity of 175 and 80 purpurogallin units/mg, respectively.
(7) L-DOPA is widely used for the treatment of Parkinson's disease: Barbeau, A., Ed. "L-DOPA and Parkinsonism"; F. A. Davis: Philadelphia, 1970. Stern, D., Ed. "The Clinical Uses of L-DOPA"; Lankaster Medical and Technical Publishing: London, 1975. Recently, it has been found that L-DOPA and its derivatives also possess an antitumor activity: Wick, M. M.; Byers, L.; Frei, E.; *Science (Washington, DC)* **1977**, *197*, 468. Wick, M. M. *Nature (London)* **1977**, *269*, 512–513.
(8) Should this compound be readily available, it has the potential to become an important intermediate in the synthesis of semisynthetic antibiotics, including the cephalosporin-type antibiotics.

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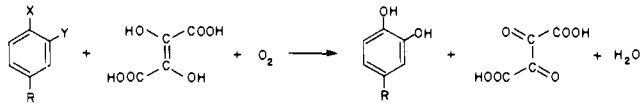
Scheme I. Peroxidase-Catalyzed Hydroxylation of L-Tyrosine (I), D-(-)-*p*-Hydroxyphenylglycine (II), and L-Phenylephrine (III) in the Presence of Oxygen and Dihydroxyfumaric Acid



I: R = $CH_2CH(NH_2)COOH$, X = OH, Y = H

II: R = $CH(NH_2)COOH$, X = OH, Y = H

III: R = $CH(OH)CH_2NHCH_3$, X = H, Y = OH

hydroxyphenylglycine (II), and L-epinephrine (adrenaline)[9] from L-(−)-phenylephrine (III).

An Erlenmeyer flask was filled with 1 L of a 2 mM solution of I, II, or III in 60 mM of acetate buffer (pH 5.0). This system was cooled to 0 °C and then 4 mmol of dihydroxyfumaric acid and 0.5 mg of horse radish peroxidase were added. Oxygen was bubbled through the solution and the reaction was carried out at 0 °C with vigorous stirring. Four millimoles of dihydroxyfumaric acid were added after 1 h and again after another hour; 3 h after beginning the process the reaction was terminated by acidification (in the case of III, dihydroxyfumaric acid was added every 30 min and the reaction was terminated after 1.5 h). Both consumption of I, II, or III and production of their dihydroxylated successors were measured simultaneously during the syntheses by using specific colorimetric assays.[10] After 3 h, 1.4–1.5 mmol of L-DOPA and D-(−)-3,4-dihydroxyphenylglycine were produced and 0.5–0.6 mmoles of I and II, respectively, remained. In the case of epinephrine, 0.8–1.0 mmol were produced after 1.5 h and 1.0–1.2 mmoles of unreacted III remained.

In each of the syntheses, the sum of the amounts of the substrate and the product was, within experimental error, equal to the initial amount of the substrate. This implies that no byproducts were formed. In agreement with this conclusion, no other aromatic products were detected by paper chromatography for the first two syntheses and by silica gel chromatography for the third one.

The resulting mixtures of the substrate, product, and other components in each of the three syntheses were separated by chromatography on a Dowex-50-4X (200–400 mesh) H^+ form column, followed by elution with 0.7 N HCl. The L-DOPA, D-(−)-3,4-dihydroxyphenylglycine and L-epinephrine produced were positively identified by comparison of their ultraviolet spectra, R_f values in paper and silica gel chromatography,[11] and retention times in ion-exchange chromatography with those of the authentic samples. The specific optical rotations of the compounds in 0.7 N HCl were −11, −99, and −47° as compared with −11 and −50° for the authentic samples of the first and third compounds, respectively (for the second one it is not known). That is, no racemization has occurred during the enzymatic hydroxylation.

It is critical that the enzymatic synthesis be carried out at 0 °C since at room temperature in the case of I some other hydroxylated tyrosines in addition to L-DOPA are formed. We have found that at 0 °C in the absence of peroxidase no hydroxylation of tyrosine takes place. However, at room temperature this is not the case: dihydroxyfumaric acid and O_2 hydroxylate tyrosine with a rate which is as high as 30–50% of that in the presence of the enzyme. It is conceivable that this nonenzymatic (and therefore probably nonspecific) hydroxylation may result in the formation of several hydroxylated species.

Using the peroxidase-catalyzed hydroxylation of L-tyrosine we have found that (i) replacement of O_2 with air results in slowing down of the reaction, (ii) an increase in the reaction time (beyond 3 h) or the concentration of the enzyme or dihydroxyfumaric acid does not appreciably increase the rate of the enzymatic process, (iii) a reduction in the dihydroxyfumaric acid concentration results in a decreased rate of L-DOPA formation, and (iv) the pH optimum of the enzymatic reaction is at pH 5.

We have discovered that two other peroxidases, lactoperoxidase from cow milk and cytochrome *c* peroxidase from yeast,[12] are also capable of hydroxylating L-tyrosine to L-DOPA in accordance with Scheme I.

It is hoped that the simple and efficient enzymatic hyroxylation described herein could be useful for preparative transformations of various pharmaceuticals and fine chemicals.

(9) For various clinical uses of epinephrine, see: McLean, J. R. In "Encyclopedia of Chemical Technology", 3rd ed.; Wiley: New York, 1980; Vol. 9, pp 241–250.

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(11) Kirchner, J. G. "Thin-Layer Chromatography", 2nd ed.; Wiley: New York, 1978.

(12) A sample of yeast cytochrome *c* peroxidase, purified to homogeneity and crystallized, was kindly provided by Dr. Thomas Poulos from Professor Joseph Kraut's laboratory at the University of California, San Diego.

Anomalous Dipole Moments of Photoreactive Triplet States

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The photoreduction of carbonyl compounds is among the most extensively studied photoreactions.[1] These reactions are believed to proceed primarily via a $\pi^* \leftarrow n$ excitation whereupon the electron density at the carbonyl oxygen is depleted.[2] In aromatic carbonyls the rates of intersystem crossing and internal conversion are fast compared to the rate of reaction; consequently, the reactive state is the lowest triplet state.[2,3] The reactivity of systems having a lowest triplet state, T_1, which is $\pi\pi^*$ in character has been attributed to the presence of a triplet $n\pi^*$ state, T_2, at a slightly higher energy.[4] 2,4,5-Trimethylbenzaldehyde (TMBA) is thought to belong to this class of molecules.[5]

Many spectroscopic studies aimed at locating and characterizing the triplet states of photochemically active aromatic carbonyls have been of solid-phase samples at low temperature, while the reactions of these molecules have been studied in liquid solution at room temperature. TMBA dissolved in durene crystals (D), however, can be photoreduced[6] under conditions similar to those required for high-resolution spectroscopic investigation. In order to gain more detailed information about the triplet states of aromatic carbonyl compounds, we have obtained the laser-induced phosphorescence excitation spectrum of TMBA isolated in a durene single crystal (TMBA/D) at 25 K and have measured the effect of an electric field (Stark splitting) on several of the bands with the sample at 4.2 K. We have found, as have others for TMBA and similar compounds,[7] that the relative intensities of the bands in the excitation spectrum indicate the presence of a triplet $n\pi^*$ state near T_1, but the results of the electric field

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(2) Walling, C.; Gibian, M. J. *J. Am. Chem. Soc.* **1965**, *87*, 3361.

(3) Wilkinson, F. "Organic Molecular Photophysics"; Birks, J. B., Ed.; Wiley: New York, 1975; Vol. 2; pp 144–145.

(4) (a) Wagner, P. J. *Acc. Chem. Res.* **1971**, *4*, 168. (b) Mao, S. W.; Hirota, N. *Mol. Phys.* **1974**, *27*, 327.

(5) Migirdicyan, E. *Chem. Phys. Lett.* **1972**, *12*, 473 and cited references.

(6) Migirdicyan, E. *J. Chem. Phys.* **1971**, *55*, 1861.

(7) (a) Chakrabarti, A.; Sheng, S. J., unpublished results. (b) Hayashi, H.; Nagakura, S. *Mol. Phys.* **1974**, *27*, 969.

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