Enzymic Oxidation of *o*-Aminophenols

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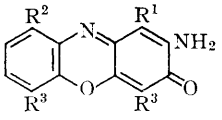
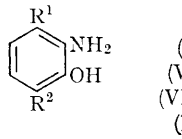
THE phenoxazone nucleus (I) is widespread in Nature:[1] some wood-rotting fungi contain cinnabarin (II) and/or cinnabaric acid (III),[2] and phenoloxidases of the laccase type are thought to play a role in the biosynthesis of actinomycins by *Streptomyces* species.[3] The oxidation of some *o*-aminophenols catalysed by a laccase obtained from the wood-rotting fungus *Polyporus versicolor*[4] has now been investigated.

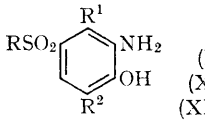
The rate of increase in light absorption at 435 mμ (characteristic of 3-aminophenoxazones[5]) of solutions of the *o*-aminophenols [(V)—(IX), buffered to pH 5 and containing *ca.* 0·5 μmole/ml.] is almost immeasurable in the absence of laccase but is rapid in its presence. By this method the initial rate of production of phenoxazone (IV) from (VIII) has been measured and a first-order rate dependence on enzyme concentration found. Measurements in



(I; $R^1=R^2=R^3=H$, $NH_2=H$)
(II; $R^1=CO_2H$, $R^2=CH_2OH$, $R^3=H$)
(III; $R^1=R^2=CO_2H$, $R^3=H$)
(IV; $R^1=R^2=H$, $R^3=Me$)



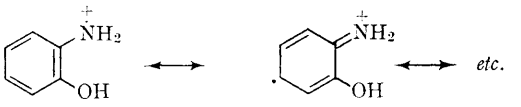
(V; $R^1=CO_2H$, $R^2=H$)
(VI; $R^1=CO_2Me$, $R^2=H$)
(VII; $R^1=Me$, $R^2=H$)
(VIII; $R^1=H$, $R^2=Me$)
(IX. $R^1=R^2=Me$)



(X; $R^1=CO_2Me$, $R^2=H$, R=n-octyl)
(XI; $R^1=Me$, $R^2=H$, R=n-octyl)
(XII; $R^1=Me$, $R^2=H$, R=n-heptyl)
(XIII; $R^1=H$, $R^2=Me$, R=n-heptyl)
(XIV; $R^1=R^2=Me$, R=n-heptyl)

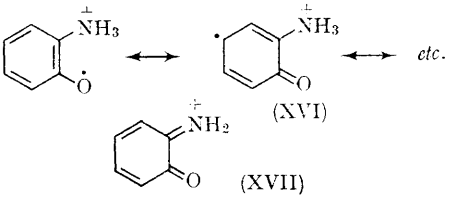
the substrate concentration range 0·02—0·8 μmole/ml. show Michaelis–Menten kinetics at 29·8°.

When laccase-catalysed oxidation of the aminophenols [(VI)—(IX)] is carried out in solutions containing sodium alkylsulphinates, phenoxazone formation is inhibited and sulphones [(X)—(XIV)] are produced. Examination of the n.m.r. and u.v. spectra of these sulphones shows that the sulphonyl link is *meta* to the amino-group and *para* to the hydroxy-group. The sulphones, (XII), (XIII), and (XIV), have been obtained also by oxidation of the corresponding aminophenols with acidic ferric chloride solution in the presence of sodium heptylsulphinate.



(XV)

These results indicate either that, in contrast to the relative oxidation potentials of aromatic amines and phenols in neutral solution,[6] the primary oxidation product of both the chemical and the laccase-catalysed oxidation of *o*-aminophenols is the protonated arylimino-radical (XV) rather than the protonated aryloxy-radical (XVI), or if, as seems very unlikely, such radical ions are incapable of reacting rapidly with a sulphinate ion, that the protonated iminoquinone (XVII) is an intermediate.



(XVI) (XVII)

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[1] W. Schäfer, *Progr. Org. Chem.*, 1964, **6**, 135.
[2] J. Gripenberg, *Acta Chem. Scand.*, 1958, **12**, 603; G. W. K. Cavill, P. S. Clezy, J. R. Tetaz, and R. L. Werner, *Tetrahedron*, 1959, **5**, 275.
[3] V. Ševčik, *Nature*, 1957, **179**, 1191; *Folia Biol.*, 1957, **3**, 212; 1958, **4**, 156.
[4] G. Benfield, S. M. Bocks, K. Bromley, and B. R. Brown, *Phytochemistry*, 1964, **3**, 79.
[5] H. Musso and H.-G. Matthies, *Chem. Ber.*, 1957, **90**, 1814.
[6] L. F. Fieser, *J. Amer. Chem. Soc.*, 1930, **52**, 5204.