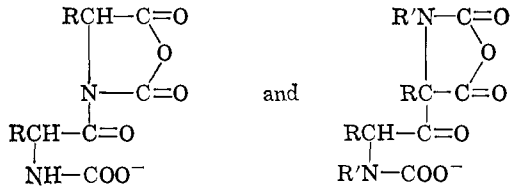
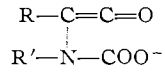
the nitrogen of the ring is more acidic than one attached to the α-carbon and is thus preferentially abstracted. If the NCA is N-substituted, the α-carbon will lose a proton in a Claisen-type reaction. The result in either case is the formation of strongly basic, negatively charged species (II and VIII) which attacks an additional molecule of the monomer at the 5-position, causing opening of the ring and formation of a carbamate group.



Alternatively, structure VIII may simply open to give a ketene–carbamate form which could then follow the propagation mechanism



The propagation reaction proceeds by the mechanism proposed by Idelson and Blout,[4] *i.e.*, formation of mixed anhydrides followed by loss of carbon dioxide, and regeneration of a carbamate group at the active chain end.

Acknowledgment.—We wish to thank the National Science Foundation for its generous support of this research under Grant No. GB 256.

COMMUNICATIONS TO THE EDITOR

Oxidation by Molecular Oxygen. I. Reactions of a Possible Model System for Mixed-Function Oxidases[1,2]

Sir:

In 1954, Udenfriend and co-workers[3] described a nonenzymatic system (composed of ascorbic acid, ferrous or ferric ions, and molecular oxygen) which caused hydroxylation of aromatic compounds. Several authors[4] have proposed that the hydroxylating agent in this system is the hydroxyl radical. However, a careful examination of the isomeric products formed clearly indicates that the hydroxyl radical is not the hydroxylating species when oxygen is the oxidant.[5-7]

The Udenfriend reaction has been considered as a possible model for phenylalanine hydroxylase which is a mixed-function oxidase.[8] Since other mixed-function oxidases with similar characteristics cause hydroxylation of saturated hydrocarbons and epoxidation of olefins,[8,9] we have looked at the reactions of the model system with a simple saturated hydrocarbon and an olefin.[10] The results are shown in Table I. The significant data in the table are the relative amounts of products formed under the varying conditions and not the absolute amounts since the solvent (acetone) can also react and ascorbic acid is oxidized by O_2 in the absence of other substrates.

TABLE I

OXIDATION OF CYCLOHEXANE AND CYCLOHEXENE

	Conditions[a]	Products from cyclohexane: Cyclohexanol, yield (mg.)[b]	Products from cyclohexane: Cyclohexanone, yield (mg.)[b]	Cyclohexene oxide, yield from cyclohexene (mg.)[b]
1	Undenfriend reaction,[c] 1.1 mmole of ascorbic acid, 0.04 mmole of Fe^{++}	2.1	0.7	1 to 2
2	Same as (1) but with 50 μg. of catalase[c]	2.4	0.5	1 to 2
3	Same as (1) but without O_2[d]	<0.1	<0.1	<0.1
4	Same as (3) plus 1.0 mmole of H_2O_2[d]	<0.1	<0.1	<0.1
5	Fenton reaction, 1.0 mmole of H_2O_2, 1.0 mmole of Fe^{++}	*ca.*2 (plus other products)	*ca.*2	2 to 3 (plus other products)

[a] All reactions were carried out in a heterogeneous mixture containing 31.5 ml. of 0.058 *M* acetate buffer (pH 4.5), 30 ml. of acetone, and 5 ml. of cyclohexane or cyclohexene. Separate experiments were performed to obtain the oxidation products of cyclohexane and cyclohexene. [b] Analyzed by gas chromatography. [c] Shaken under an atmosphere of air for 2 hr. [d] Shaken under an atmosphere of N_2 for 2 hr.

With the complete system (1, see Table I) both cyclohexanol and cyclohexanone are formed from cyclohexane, and cyclohexene oxide is formed from cyclohexene. Excess catalase has no effect on the amounts of products formed (2). If ascorbic acid is omitted from these experiments, then no products are formed. If O_2 is omitted (3) no products are formed. Also,

(1) Presented at the 146th National Meeting of the American Chemical Society, Denver, Colo., Jan., 1964; Abstracts of Papers, Division of Biological Chemistry, p. 13A.

(2) This investigation was supported by PHS research grant GM-09585 from the Division of General Medical Sciences, Public Health Service.

(3) S. Udenfriend, C. T. Clark, J. Axelrod, and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).

(4) See, for example, R. Breslow and L. N. Lukens, *ibid.*, **235**, 292 (1960), and R. R. Grinstead, *J. Am. Chem. Soc.*, **82**, 3472 (1960).

(5) R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.*, 138 (1962).

(6) G. A. Hamilton and J. P. Friedman, *J. Am. Chem. Soc.*, **85**, 1008 (1963), and unpublished results.

(7) It is important to distinguish between the Udenfriend reaction with O_2 as the oxidant and the reaction with H_2O_2 as the oxidant. The original workers[3] concluded that the two systems gave similar products but later work has shown that when H_2O_2 is the oxidant the hydroxyl radical is the hydroxylating agent, but some different species is involved when O_2 is the oxidant.[5,6]

(8) H. S. Mason, *Advan. Enzymol.*, **19**, 128 (1957).

(9) For a recent review see M. Hayano and J. W. Foster in "Oxygenases," O. Hayaishi, Ed., Academic Press, Inc., New York, N. Y., 1962, pp. 181, 241.

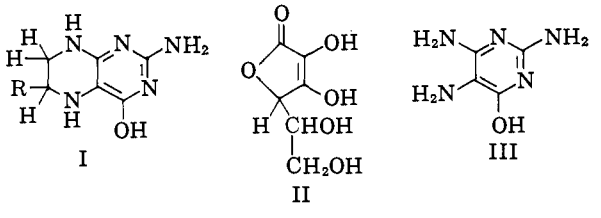
(10) Other investigators[11,12] have studied the reaction of the Udenfriend system with aliphatic carbon–hydrogen bonds but the systems studied were complex and insufficient controls were done to delineate the course of the reaction.

(11) M. Chvapil and J. Hurych, *Nature*, **184**, 1145 (1959).

(12) A. Cier, C. Nofre, and A. Revol, *Compt. rend.*, **247**, 542, 2486 (1958); *ibid.*, **250**, 2638 (1960).

if H_2O_2 (approximately equivalent to the amount which might be formed if ascorbic acid reacted with O_2 to give H_2O_2 and dehydroascorbic acid) is added with no O_2 present then again no products are formed (4). The results of these experiments thus clearly indicate that H_2O_2 is not an intermediate in the oxidation with molecular oxygen. Line 5 of Table I shows the amounts of products formed when the hydroxyl radical (generated by the Fenton reaction)[13] is the oxidizing agent. This reaction did give some of the same products observed with the Udenfriend reaction. However, gas chromatographic analysis revealed at least five other products in the reaction of HO· with cyclohexane and two others with cyclohexene. Under the Udenfriend conditions these products were not observed. Consequently it appears that the Udenfriend reaction on cyclohexane or cyclohexene does not involve the hydroxyl radical as the oxidizing agent. Further evidence for this is obtained if cyclohexene oxide is added at the beginning of the run; it is not significantly reacted under the Udenfriend conditions but under the Fenton conditions it is largely decomposed. If cyclohexanol is added under the Udenfriend conditions then a small amount of it is oxidized to cyclohexanone, and presumably some, if not all, of the cyclohexanone observed under these conditions arises by oxidation of the cyclohexanol formed in the reaction. Cyclohexanone added at the beginning of the run does not appear to be affected under either conditions.

Recently, Kaufman[14] has identified the cofactor for phenylalanine hydroxylase as a tetrahydropteridine of general structure I. Compound I contains a partial structure analogous to the enediol structure of ascorbic acid (II) which is a necessary reagent in the Udenfriend model system. If the enzymatic and model systems react by similar mechanisms then compounds more closely related to I should also react in the model system. Consequently, the hydroxylation of anisole by the model system has been investigated with the ascorbic acid replaced by 2,4,5-triamino-6-hydroxypyrimidine (III). The hydroxylated products obtained are compared in Table II. The similarity of the products formed with II and III is suggestive that the two reactions proceed by related mechanisms. Furthermore, the obvious close similarity of III to the natural cofactor I suggests that the mechanisms of the enzymatic and model hydroxylations are related. A mechanism for the model and enzymatic hydroxylations is considered in the following communication.[15]



I II III

TABLE II

THE HYDROXYLATION OF ANISOLE BY MOLECULAR OXYGEN

Conditions[a]	% yield of methoxyphenols[b]	Isomer distribution *ortho*	*meta*	*para*
Udenfriend system with II	8	43	18	39
Udenfriend system with III	4	49	13	38

[a] The reactions were carried out in 61.5 ml. of water saturated with anisole, buffered with acetate at pH 4.5, and contained 0.04 mmole of $Fe(ClO_4)_2$ and 1 mmole of ascorbate or pyrimidine. The reactions were shaken overnight under an atmosphere of air. [b] Yield based on the initial amount of ascorbate or pyrimidine. The products were analyzed by gas chromatography.

(13) For a review see N. Uri, *Chem. Rev.*, **50**, 375 (1952).

(14) S. Kaufman, *Proc. Natl. Acad. Sci. U. S.*, **50**, 1085 (1963); *J. Biol. Chem.*, **239**, 332 (1964); *ibid.*, **237**, PC2712 (1962).

(15) G. A. Hamilton, *J. Am. Chem. Soc.*, **86**, 3391 (1964).

FRICK CHEMICAL LABORATORY
PRINCETON UNIVERSITY
PRINCETON, NEW JERSEY

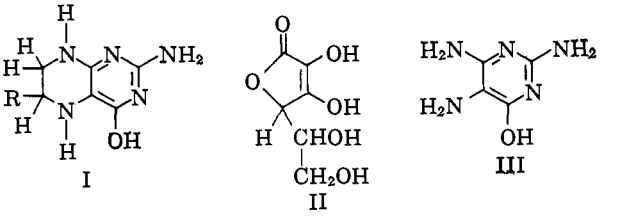
GORDON A. HAMILTON
ROBERT J. WORKMAN
LAURA WOO

RECEIVED APRIL 4, 1964

Oxidation by Molecular Oxygen. II. The Oxygen Atom Transfer Mechanism for Mixed-Function Oxidases and the Model for Mixed-Function Oxidases[1,2]

Sir:

The Udenfriend oxidation[3] by molecular oxygen has been considered a model for aromatic hydroxylases and other mixed-function oxidases.[4] It has now been shown that H_2O_2 and the hydroxyl radical are not intermediates in the model oxidation, and that the model system oxidizes aromatic compounds to phenols, saturated aliphatic compounds to alcohols, and olefins to epoxides.[4-6] Such reactions are reminiscent of carbene reactions[7,8] and the implication is that an oxygen species with six electrons and similar to a carbene or carbenoid species is responsible for the oxidations. Other information relevant to a consideration of the mechanism of the model and enzymatic mixed function oxidases is the following: the tetrahydropteridine cofactor (I), required by phenylalanine hydroxylase,[9] has structural features similar to ascorbic acid (II) and 2,4,5-triamino-6-hydroxypyrimidine (III) which



I II III

are necessary for the model oxidations[4]; both the model and enzymatic mixed-function oxidases appear

(1) Presented at the 146th National Meeting of the American Chemical Society, Denver, Colo., Jan., 1964; Abstracts of Papers, Division of Biological Chemistry, p. 13A.

(2) This investigation was supported by PHS research grant GM-09585 from the Division of General Medical Sciences, Public Health Service.

(3) S. Udenfriend, C. T. Clark, J. Axelrod, and B. B. Brodie, *J. Biol. Chem.*, **208**, 731 (1954).

(4) G. A. Hamilton, R. J. Workman, and L. Woo, *J. Am. Chem. Soc.*, **86**, 3390 (1964), and references therein.

(5) R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.*, 138 (1962).

(6) G. A. Hamilton and J. P. Friedman, *J. Am. Chem. Soc.*, **85**, 1008 (1963).

(7) (a) J. Hine, "Divalent Carbon," Ronald Press, New York, N. Y., 1964; (b) E. Chinopores, *Chem. Rev.*, **63**, 235 (1963).

(8) The reaction of a carbene with anisole has been studied by H. Meerwein, H. Disselnkötter, F. Rappen, H. U. Rintelen, and H. Van de Vloed [*Ann.*, **604**, 151 (1957)], and it was observed that the isomer distribution of methylanisoles was *ortho*:*meta*:*para*, 34:35:31, which is not unlike the isomer distribution of phenols formed on hydroxylation of anisole by the model system[4] (*ortho*:*meta*:*para*, 43:18:39). The striking result is that a large amount of *meta* isomer is formed in both cases.

(9) S. Kaufman, *Proc. Natl. Acad. Sci. U. S.*, **50**, 1085 (1963); *J. Biol. Chem.*, **239**, 332 (1964); *ibid.*, **237**, PC2712 (1962).