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BIO-MIMETIC OXYGENATION

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I. INTRODUCTION

Bioorganic chemistry includes various organic chemical approaches to the understanding of many kinds of biological phenomena. Among them, much attention has been focused on the mechanisms of enzymatic reactions. Methods for such approaches are dependent on how many informations are available from the side of enzymology for a target enzyme. For example, a large number of elegant model systems have been designed for certain hydrolases such as α-chymotrypsin and lysozyme, for which the complete sequence of amino acids and the three-dimensional structure in crystalline form were already established. On the other hand, there are still many unsolved problems for most enzymes, including oxygenases and related enzymes utilizing molecular oxygen or other dioxygen species, which are the subject matter of this report, so that nonenzymatic models designed so far for such enzymes appear to be considerably far from the actual enzyme systems. However, as a matter of fact, studies on the nonenzymatic oxygenation reactions as bio-mimics have contributed not only to the understanding of oxygenase-catalyzed reactions in providing valuable concepts for enzymologists, but also to synthetic organic chemistry in providing useful synthetic methods.

It may be appropriate to outline in the first place biological oxygenations and to discuss how one can approach the problem. Oxidation reactions in living organisms are catalyzed by several enzymes, an oxidoreductase system, which is classified into the following six enzymes; dehydrogenase, oxidase, oxygenase, peroxidase, catalase, and superoxide dismutase. (i) *Dehydrogenases* require a hydrogen or electron acceptor (A), such as NAD^+, $NADP^+$ and FAD, as cofactor, and dehydrogenate reversibly a substrate (SH_2) into a product (S) as eqn (1). (ii) *Oxidases* utilize molecular

$$SH_2 + A \rightleftharpoons S + AH_2 \qquad (1)$$

oxygen as hydrogen or electron acceptor and are stoichiometrically divided into three classes as eqns (2a, 2b and 2c), which involve one-, two- and four-electron

$$SH + O_2 \longrightarrow S + O_2^{\cdot-} + H^+ \qquad (2a)$$

$$SH_2 + O_2 \longrightarrow S + H_2O_2 \qquad (2b)$$

$$SH_2 + 1/2O_2 \longrightarrow S + H_2O \qquad (2c)$$

reduction of an oxygen molecule, respectively. The enzymes require a cofactor such as FMN, FAD, or transition metals (Fe, Cu, Mo, etc.), which plays a role in the reduction of oxygen. (iv) *Oxygenases* catalyze the incorporation of molecular oxygen into the product. This was found independently by Hayaishi[1] and Mason[2] from ^{18}O-labeled experiments. Dioxygenases catalyze the incorporation of two atoms of oxygen (eqn 3a), and monooxygenases (or mixed function oxidases), which require a hydrogen donor (AH_2) as cofactor such as NADH, NADPH, ascorbic acid, and an α-keto acid, catalyze the incorporation of one atom oxygen (eqn 3b).

$$S + O_2 \longrightarrow SO_2 \qquad (3a)$$

$$S + O_2 + AH_2 \longrightarrow SO + H_2O + A \qquad (3b)$$

Most of the enzymes contain a transition metal ion which plays undoubtedly an important role in activating molecular oxygen and/or a substrate.[3-7] (iv) *Peroxidases* and *catalases* catalyze the decomposition of organic peroxides and hydrogen peroxide, respectively, with the simultaneous dehydrogenation of a substrate (eqn 4), and

$$ROOH + SH_2 \longrightarrow ROH + H_2O + S \qquad (4)$$

they possess usually heme iron.[8] (v) *Superoxide dismutases*, which have been recently discovered by Fridovich,[9] catalyze the disproportionation of the superoxide radical (eqn 5) and contain transition metal ions.

$$2O_2^{\cdot-} + H^+ \longrightarrow O_2 + H_2O_2 \qquad (5)$$

Certain kinds of luciferases, which catalyze the bioluminescence of luciferins, may be regarded as an oxygenase. As eqn (6), such a luciferase catalyzes the conversion of luciferin (L) to an electronically excited product (P*) via a peroxide intermediate (LO_2).[10] Except dehydrogenases, these enzymes utilize molecular oxygen or dioxygen species as oxidant.

$$L + O_2 \longrightarrow LO_2 \longrightarrow P^* \qquad (6)$$

During the last two decades, the biological significance of these enzymes, especially those involving the action of molecular oxygen or oxygen species, has drawn much attention in many respects. Oxygenases, for example, play important roles in the biosynthesis and catabolism of various types of metabolites and also in the metabolic disposal of foreign compounds such as drugs and toxic substances.[6] Furthermore, peroxidases, catalases and

superoxide dismutases are believed to function detoxification of toxic oxygen species such as organic peroxides, hydrogen peroxide, and superoxides, respectively.[11,12] In addition, there are a large number of natural products, which have been suggested to be formed via metabolic pathways involving oxygenases although not characterized yet.

How can we approach oxygenase problems?

In general, the process of oxygenase-catalyzed reactions may be divided into three steps in organic chemical sense. The first step is the activation of molecular oxygen and/or a substrate. The second step is the formation of a reactive intermediate which would be a peroxidic compound in case of dioxygenases and an oxygenated compound such as an arene oxide in case of monooxygenases. The third step involves the transformation of such an intermediate into a final product. An instructive example is seen in mechanisms proposed for catechol dioxygenases.

By the action of pyrocatechase and metapyrocatechase, catechol (**1**; R = H) is oxygenated to give *cis,cis*-muconic acid (**2**) and an aldehydic acid **3**, respectively.[13] Labeling experiments with ^{18}O-enriched oxygen have shown that the two carbonyl groups of both products are labeled. Several other catechol dioxygenases of these types have been also characterized. Kinetic studies show that the enzyme reactions proceed via the mechanism of eqn (7),[13] which involves the successive formation of an enzyme-substrate complex (ES) and a ternary complex (ESO_2) followed by product formation. Based on ex-

$$E \rightleftharpoons ES \rightleftharpoons ESO_2 \longrightarrow E + \text{product} \qquad (7)$$

perimental evidences with these enzymes and 3,4-dihydroxyphenylacetate 2,3-dioxygenase, Senoh *et al.* have proposed a general mechanism involving a common peroxide intermediate **5** shown in Scheme 8.[14,15]

substrate are activated. The second step is coupling of **4** and O_2^- to form an α-ketohydroperoxide **5**, which in the final step is transformed into either the product **2** or **3** via a dioxetane intermediate **6** or **7**, respectively. Later, Hamilton proposed an alternative mechanism involving Baeyer–Villiger type rearrangements of **5** to **8** and **9** which are tautomers of the final product **2** and **3**, respectively.[16,17] In addition, a mechanism involving direct attack of a $[Fe{-}O_2]^{2+}$ complex to the catechol **1** cannot be excluded.

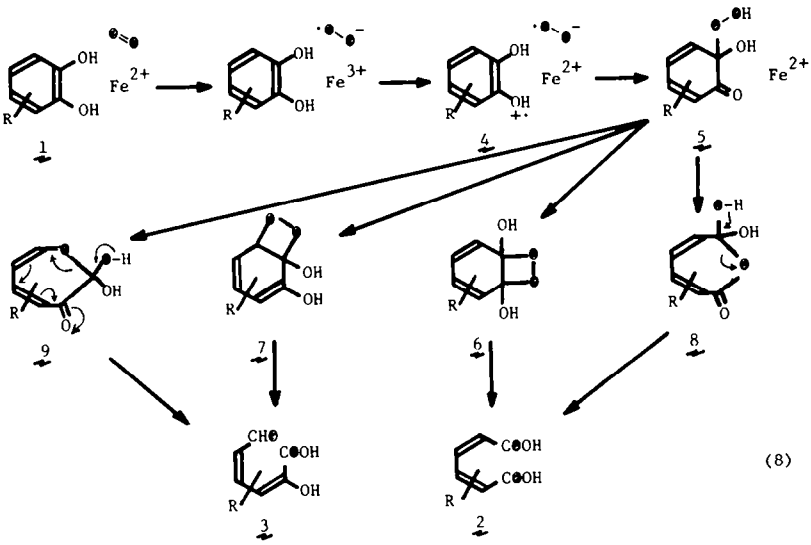
As shown in the above example, a number of different mechanisms can be taken into consideration for each step of an oxygenase-catalyzed reaction. However, such mechanisms are not always based on substantial chemical evidences. It will be essential therefore for organic chemists, who are approaching oxygenase problems, to study biomimetic oxygenation reactions with a mechanistic base but not with a superficial interpretation.

In this report the author surveys oxygenation reactions which have been reported as biomimics for oxygenase-catalyzed reactions and related enzymatic reactions and discusses in terms of the three principal steps possibly being involved in the enzymatic reactions: namely, (i) activation of molecular oxygen and/or a substrate, (ii) formation of an oxygenated intermediate and (iii) transformation of the intermediate into a product.

II. BIOMIMETIC DIOXYGENATION

Biosynthesis and metabolism of thyroxine

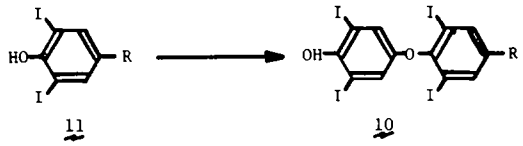
Thyroxine (**10a**) is known to be synthesized in the thyroid gland from its precursor, 3,5-di-iodotyrosine (**11a**), but enzymes involved in the biosynthesis and metabolism are not yet well characterized. Several biomimetic reactions for the *in vivo* reactions have been carried out by means of oxygenation techniques, implying possibilities that molecular oxygen or other oxygen species may involve *in vivo*.



The enzymes contain at least one nonheme iron atom which is known to act in a ferrous form. In the first step, molecular oxygen is reduced by ferrous ion to give the superoxide anion radical (O_2^-), and the ferric ion thus formed abstracts one electron from **1** to give a catechol cation radical **4**. For this step, both oxygen and the

The first model reactions was reported by von Mutzenbecher in 1939, who found that aerobic oxidation of a slightly alkaline solution of **11a** at 37° gave **10a** though only in 0.2% yield.[18] Later several workers showed that some structural modifications in the side chain of **11a** can improve the yield of thyroxine analogs **10** as shown in

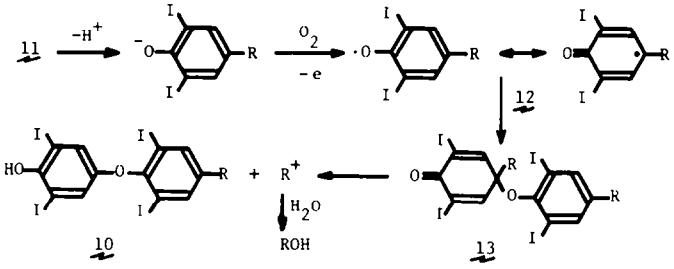
eqn (9).[19] The reaction involves autoxidation of an phenolate anion, since the phenolic group of **11** mostly dissociated at pH 7.5.



(9)

R: a, $CH_2CH(NH_2)COOH$ (*ca.* 0.2%);[18]
b, $CH_2CH(NHAc)COOH$ (2–3%);[20]
c, $CH_2CH(NHAc)CONH(CH_2)_4CH(NHAc)COOH$ (11%);[21]
d, CH_2COOH (6%);[22]
e, CH_2CH_2COOH (11%);[22]
f, $CH_2CH_2CH_2COOH$ (~ 1%);[22]
g, $CH_2CH(NHAc)COOEt$ (> 30%; in the presence of $MnSO_4$).[23]

The reaction has been interpreted by a free radical mechanism,[24] which was originally proposed by Johnson and Tewkesbury[25] and supported by Harrington.[26] As shown in eqn (10), the phenolate anion of **11** is first oxidized by molecular oxygen to give a phenoxy radical **12**. Two molecules of **12** couple to form a quinol ether intermediate **13** which loses a side chain, probably as R^+, to form thyroxine (**10a**) or an analog. The fate of a side chain eliminated during the reaction has been also examined and has been found in its hydroxylated form; namely the α-N-acetyl-lysine peptide of hydroxypyruvic acid from **11c**,[21] β-hydroxypropionic acid from **11e**, and serine peptide from iodinated polytyrosine.[27] These results are consistent with this mechanism. However, several experimental facts, for example, that oxidation of **11e** with one electron-transfer oxidizing agent such as alkaline ferricyanide gives no thyroxine analog but mostly polymers,[28] and that the coupling reaction is highly affected by the nature of the side chain of **11** (eqn 9), are inconsistent with the free radical coupling mechanism. There may be a possibility that the initial step involves the oxygenation at the side chain to give an oxygenated intermediate, similar to the second model reactions described below.



(10)

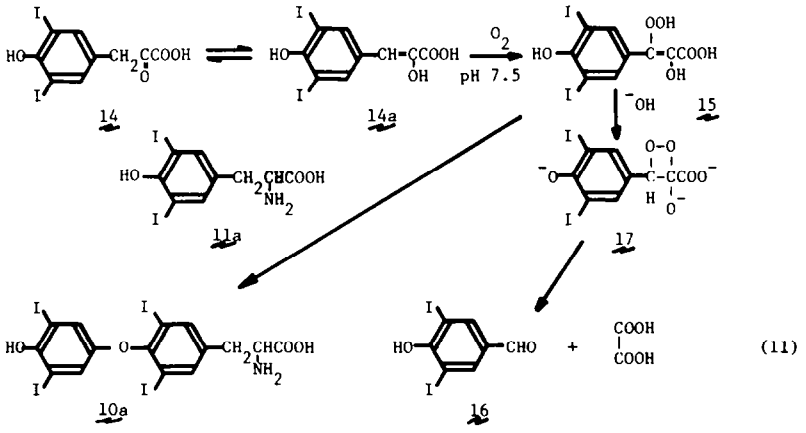
Although there have been reported several model reactions for the biosynthesis of thyroxine, such as the nonenzymatic or peroxidase-catalyzed iodination of peptides containing tyrosine residues,[29] the oxygenation reaction of 4 - hydroxy - 3,5 - di - iodophenylpyruvic acid (**14**; DIHPPA) with 3,5-di-iodotyrosine (**11a**) leading to thyroxine (**10a**) in fairly good yield is particularly interesting. This reaction was first reported by Hillmann to occur under anaerobic conditions.[30] Meltzer and Stanaback found that the reaction readily takes place under oxygen at room temperature to give thyroxine up to 30% yield, and that oxygen can be replaced by other oxidants such as iodate, hydrogen peroxide, and tert-butyl hydroperoxide albeit in a lower yield.[31]

This model reaction has been extensively studied at the laboratories of Cahnmann and the present author.[20,32–47] Their findings are summarized as follows. (i) Naturally, the phenolic and nonphenolic rings of thyroxine are originated from DIHPPA and di-iodotyrosine, respectively.[35] This provided a convenient synthetic method for specifically labeled thyroxine and its analogs.[47] (iii) Di-iodotyrosine can be replaced by various analogs[32] including di-iodotyrosine residues in a peptide chain,[38,39] but the yields of thyroxine analogs are not more than that (18%) of thyroxine itself: for example, under standardized conditions, **11a** (18%); **11d** (9%); **11e** (10%); **11f** (4%); 3,5-dibromophloretic acid (18%).[32] (iv) The reaction of various analogs of DIHPPA with di-iodotyrosine (**11a**) shows that the *p*-hydroxyphenylpyruvic acid structure and the presence of at least one halogen atom (I or Br) at position 3, are essential for the formation of thyroxine analogs.[33,41]

The detailed mechanistic studies of the model reaction with DIHPPA have been carried out by means of electron spin resonance (ESR)[40,42] and kinetic analysis.[43] Nishinaga *et al.* have found that the reaction takes place in two distinct phases (eqn 11); namely oxygenation of DIHPPA to give a peroxide intermediate **15** and its subsequent reaction with di-iodotyrosine (**11a**). In the first phase, DIHPPA, only in its enolic form (**14a**), which can be obtained in a borate buffer at pH 7.5, is oxygenated to a hydroperoxide **15**. Although **15** cannot be isolated in a pure form because of its instability, it has a lifetime sufficient to handling in solution. Under strong alkaline conditions, **15** decomposes easily to give 3,5-diiodobenzaldehyde (**16**) and oxalic acid as major products, possibly via, at least in part, a mechanism involving a dioxetane intermediate **17**. Chemiluminescence in the base-catalyzed oxygenation of DIHPPA has been recently reported, supporting the intermediary formation of **17**.[48] The hydroperoxide **15** reacts anaerobically with

di-iodotyrosine (**11a**) at pH 8.5 to form thyrosine (**10a**). When the reaction is carried out stepwise, the yield of thyroxine can be increased up to 40%.[43] It has been also shown that photooxygenation of DIHPPA either with or without sensitizing dye in organic solvents, in which its enolic form is predominant, gives the same hydroperoxide **15**.[45]

catechol **21a** followed by its dehydrogenation to an ortho quinone intermediate which is then cleaved by hydrolysis to give hydroxy-*p*-benzoquinone (**22**) and tyrosine (**23a**). In this connection, nonenzymatic oxygenation of 3'-hydroxythyropropionic acid (**21b**) have been examined in aqueous alkaline media at the author's laboratory.[50] Hydroxylation of **20b** to **21b** can be achieved with the

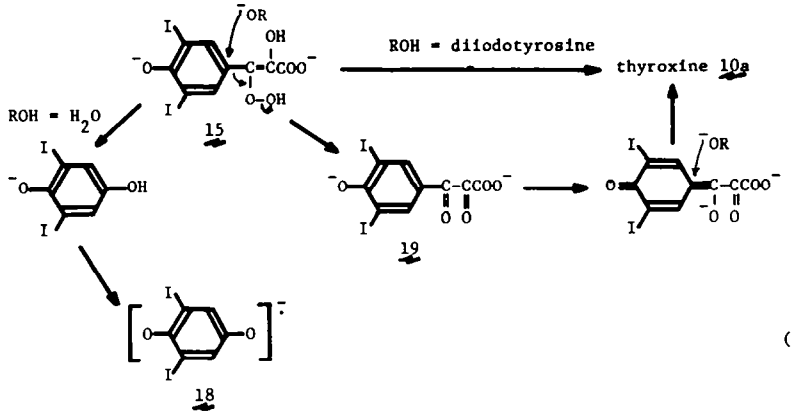


(11)

Although the mechanism of the coupling reaction between **15** and di-iodotyrosine is not fully understood, Nishinaga and Cahnmann have proposed a plausible mechanism (eqn 12).[44] Elimination of the hydroxy anion from **15** may lead to the formation of an electron deficient oxygen system which promotes the attack of the phenolate ion of di-iodotyrosine. The concomitant formation of the semiquinone radical **18** in the reaction, which increases at higher pH,[42] is ascribed to a competition reaction between the phenolate and hydroxy anions to the electron deficient oxygen system. An alternative mechanism involving a diketo acid **19** followed by its tautomerization to a quinone methide is less plausible, since the synthesized ethyl ester of **19** has been found to be unreactive toward di-iodotyrosine in basic media.[44]

hydroxyl radical generated by the photolysis of hydrogen peroxide in aqueous solution.[51] A facile cleavage of the diphenyl ether linkage of **21b** occurs at pH 7.6 and above under aerobic conditions to give phloretic acid (**23b**) in nearly quantitative yield. Based on the observation of ESR signals assignable to the semiquinone radicals of **21a** and 1,2,4-trihydroxybenzene during the reaction, a mechanism of eqn (14) involving a hydroxyquinol ether intermediate **24** has been postulated.

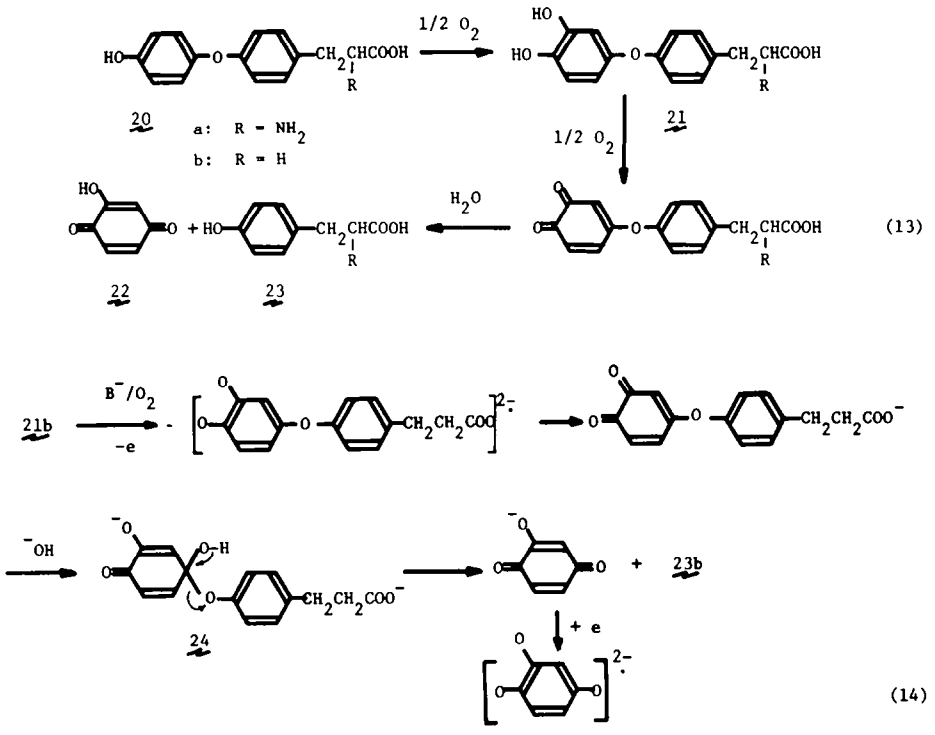
We have further demonstrated that the hydroxylation on the phenolic ring of thyronine derivatives is not a prerequisite of the oxidative cleavage of the diphenyl ether linkage under selected oxygenation conditions.[51-54] Thus, base-catalyzed oxygenation of 3,5-di-iodothyronine (**25a**) in dimethyl sulfoxide containing potassium *tert*-butoxide gives 3,5-di-iodotyrosine (**26a**) in 20% yield



(12)

Rupture of the diphenyl ether linkage of thyroxine (**10a**) leading to the formation of di-iodotyrosine is one of the possible pathways in its metabolism, being often accompanied by deiodination.[48] A mechanism for the oxidative cleavage of thyronine (**20a**) by polyphenol oxidase has been postulated as shown in eqn (13).[49] It involves an initial hydroxylation on the phenolic ring to a

being accompanied by the formation of the *p*-benzosemiquinone radical detected by ESR.[52] It should be noted that the reaction hardly occurs in protic solvents such as alcohols, and that thyroxine itself is unreactive under the oxygenation conditions. However, when oxygenation is carried out in methanol in the presence of Co(II)-salpr [bis(3-salicylideneaminopropyl)amineco-



(13)

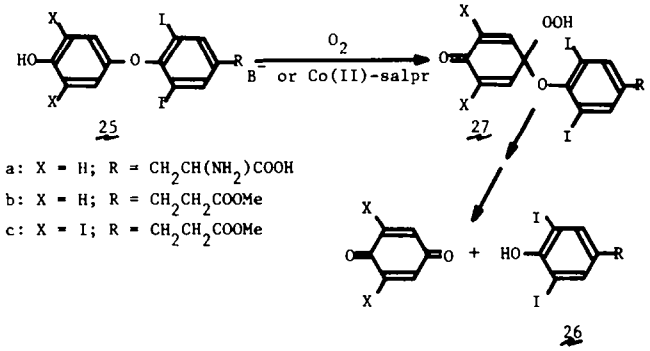
(14)

balt(II)], which is known to form predominantly a mononuclear Co(II)–O_2 complex, both of 3,5-di-iodo- (**25b**) and 3,5,3′,5′-tetraiodo- (**25c**) thyropropionic ester have been found to be cleaved to give the corresponding *p*-benzoquinone and 3,5-di-iodophloretic ester (**26**).[53] Considering the results obtained from our basic studies of base- and Co(II)-catalyzed oxygenations of phenols (see below), a hydroperoxyquinol ether **27** appears the most plausible intermediate (eqn 15), which may be reduced under the reaction conditions to give the corresponding hydroxyquinol ether of type **24** in eqn (14).[52,53] A similar mechanism has been also suggested for the cleavage of the diphenylether linkage of thyropropionic acid (**20b**) with the hydroxyl radical, which simultaneously occurs with the hydroxylation to **21a** (see above).[51] The above results imply that the rupture of the diphenyl ether linkage of thyroid hormones *in vivo* may be possible to proceed via a direct oxygenation process occurring at para position with respect to the phenolic group.

Regarding the biomimetic oxygenations for the biosynthesis and metabolism of thyroxine, the author wishes to state that these studies have provided us useful informations to designing the model reactions for biological oxygenations done at the later stage of our investigation.

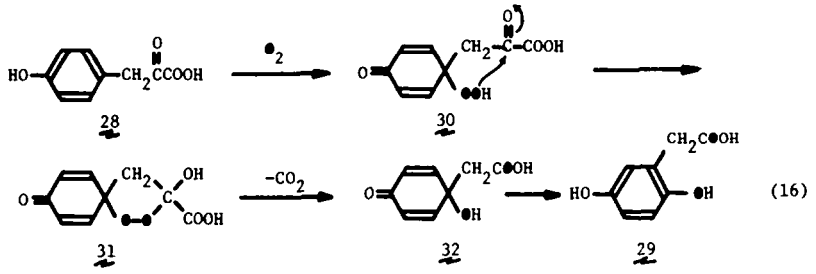
p-Hydroxyphenylpyruvate dioxygenase

The enzyme, which is also called *p*-hydroxyphenylpyruvate hydroxylase (*E.C.* 1.13.11.27), catalyzes the conversion of *p*-hydroxyphenylpyruvic acid (**28**; HPPA) to homogentisic acid (**29**). A mechanism of eqn (16), involving a peroxyquinol **30**, a cyclic peroxide **31**, and a



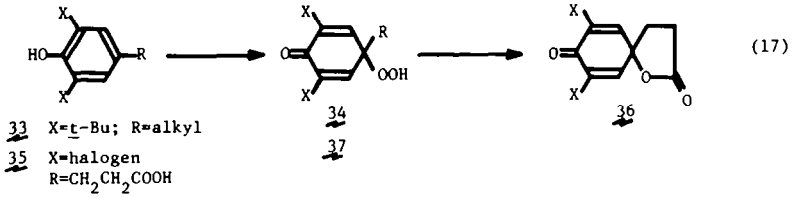
(15)

quinol **32**, has been proposed by Witkop and Goodwin based on a model reaction for the last step[55] and later substantiated by Lindblad *et al.* with ^{18}O-labeling experiments.[56] In order to gain further insight on the validity of this mechanism, which still lacks substantial chemical evidence, we investigated the dye-sensitized photooxygenation of **28** and its related compounds. It had been shown that the dye-sensitized photooxygenation of certain para-substituted phenols gives a peroxyquinol of type **30** or products believed to be formed from the peroxyquinol.[57-65] For example, 2,6 - di



(16)

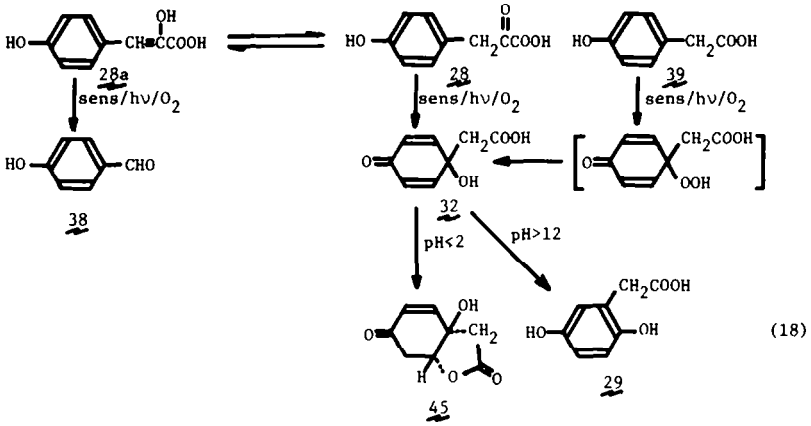
- *t* - butyl - *p* - alkylphenols **33** gives **34**[57,59] and 3,5-dihalogenophloretic acid (**35**) yields spirolactones **36** in aqueous media possibly via hydroperoxides **37** (eqn 17).[58]



(17)

The keto-enol tatomerization of **28**, which depends on solvent and additive as in case of DIHPPA (**14**), is an important factor for photooxygenation. Methylene blue-sensitized photooxygenation of the enol form (**28a**) in methanol results in the formation of *p*-hydroxybenzaldehyde (**38**) in 70% yield and oxalic acid. On the other hand, rose bengal-sensitized photooxygenation of a solution of **28** in phosphate buffer at pH 7.0, which is found to show a 8:2 keto-enol ratio after standing for 24 hr, gives quinol **32** in 18% yield besides **38** (12%) and *p*-hydroxyphenylacetic acid (**39**; 15%). The inhibitory effect of singlet oxygen quencher and the enhanced effect of D_2O solvent on this reaction show that the reaction involves singlet oxygen. Furthermore, the formation of **39** has been interpreted to result from the cleavage of **28** by hydrogen peroxide which is formed by the secondary decomposition of the intermediate peroxyquinol **30**. Treatment of quinol **32** with aqueous alkali gives homogentisic acid (**29**) in good yield.[66,67]

Although the hypothetical peroxide intermediates **30**



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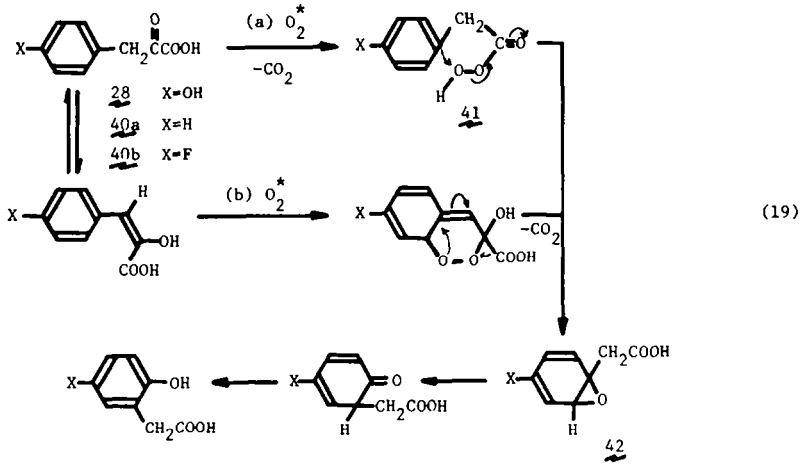


(19)

and **31** have not been detected in the photooxygenated mixture, a series of reactions to give **29** from **28** provide a biomimetic representation of the Witkop–Lindblad mechanism for *p*-hydroxyphenylpyruvate dioxygenase. It should be noted that this biomimetic dioxygenation does not mean that singlet oxygen is the actual reactive species in the enzymatic reaction, for which the mechanism of oxygen activation is not known.

It became interesting to examine whether or not quinol **32** can be incorporated into the enzymatic system to give homogentisic acid (**29**). The test has been done independently by two research groups.[68,69] Using a highly purified enzyme from bovine liver[68] or pig liver homogenate,[69] neither significant incorporation nor inhibition of **32** has been observed. These results lead to two explanations. (i) The quinol may not be released from the active site of the enzyme until the end product has been formed. Namely, the mechanism of eqn (16) is not necessarily disproved. (ii) The enzymatic reaction proceeds by a completely different mechanism.

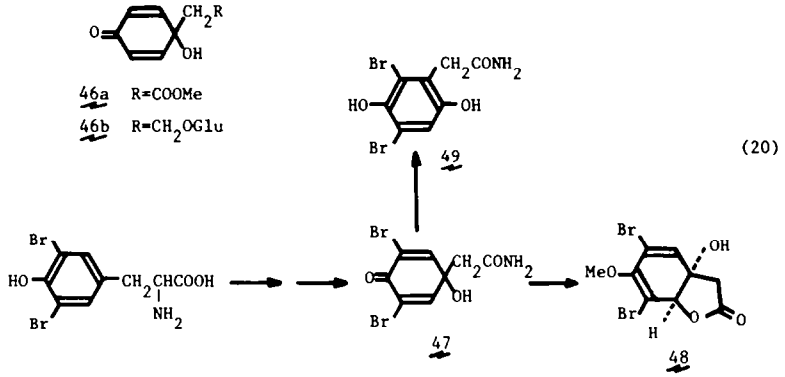
Based on experimental facts that nonphenolic phenylpyruvic acids such as **40a** and **40b** are also a substrate for this enzyme, albeit much less effective than **28**,[70] Hamilton has proposed a mechanism (path a of eqn 19) involving a peracid **41** and an epoxyphenylacetic acid **42**.[71] Saito *et al.* have proposed another mechanism (path b of eqn 19) involving a Diels–Alder addition of oxygen to the enolic form of **28** leading to the same intermediate **42**.[72] The idea of the latter mechanism has been derived from an analogous Diels–Alder reaction of singlet oxygen to styrene-type compounds,[73] and from the presence of the enzyme phenylpyruvate keto-enol tautomerase in the rat liver enzyme system.[56] Since experimental data reported so far neither prove nor exclude any of the above three mechanisms,[74] further studies are required to clarify the reaction mechanism.

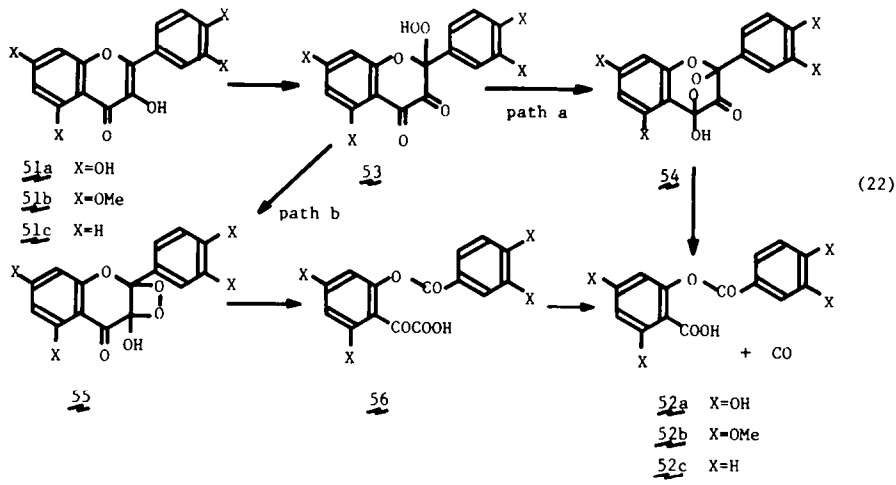
During the course of the study on the singlet oxygen reaction of **28**, we have obtained the following findings which are of interest in relation to the biogenesis of certain phenolic metabolites. In phosphate buffer at pH 8.5, photosensitized oxygenation of *p*-hydroxyphenylacetic acid (**39**) and phloretic acid (**43a**) gives the corresponding *p*-quinols **32** and **44a**, respectively, with a concomitant formation of hydrogen peroxide (eqns 18 and 21). And on mild acid treatment quinol **32** undergoes intramolecular Michael addition to yeild a hydroxylactone **45** in good yield (eqn 18).[66,67] During the past several years, natural products related to **29**, **32** and **45** were isolated: antitumoral compounds such as the methyl ester **46a** (jacaranone) of quinol **32**[75] and the glucoside **46b**, and a series of antibiotics including **47**, **48** and **49**,[76–80] which appear to be derived from 3,5-dibromotyrosine by similar sequence of reactions *in vivo* (eqn 20).

The photooxidative formation of quinol **32** and hydroxylactone **45** in aqueous media has been applied to tyrosol (**43b**) and N-acetyltyramine (**43c**), which give directly the corresponding hydroxylactone **50a** and **50b**, respectively, probably due to a more nucleophilic nature of their hydroxy or acetylamino group than the carboxylate group of **32**.[67]

Quercetinase

In the pathway of a microbial metabolism of rutin (quercetin 3-rhamnoglucoside) by *Asperigillus* or *Pullularia* species, quercetin (**51a**) is degraded into a depside **52a** and carbon monoxide (eqn 22).[81–84] Tracer experiments have shown that C-3 is liberated as carbon monoxide,[81] and that an oxygen molecule is incorporated into two carbonyl oxygens of **52a**.[83,84] Later, this enzyme was purified and characterized to have copper ion.[85] This particular reaction led the author to consider a hypo-





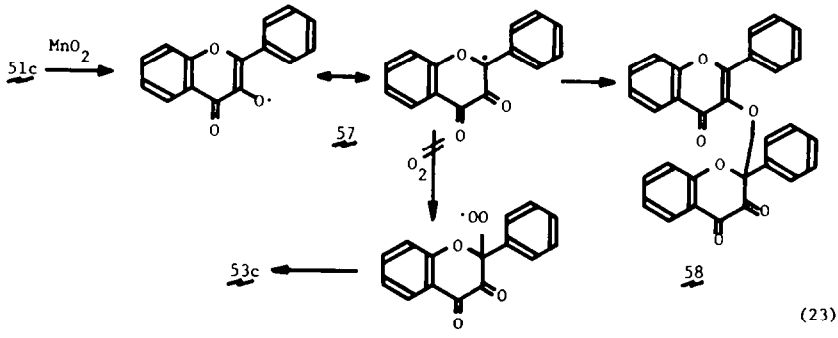
(22)

thetical mechanism of eqn (22). A ketohydroperoxide **53**, which would be formed initially from **51a**, can undergo rearrangement to a 5-membered cyclic peroxide **54** followed by decarbonylation leading to **52a** and carbon monoxide (path a). An alternative pathway (path b), which involves rearrangement of **53** to a dioxetane **55** followed by cleavage to **52a** via a keto acid **56**, cannot account the tracer experiments.

In view of the resemblance between the oxygenation of **51a** to **53** and the "ene" reaction of singlet oxygen to an olefin having allylic hydrogens, we have investigated the reaction of 3-hydroxyflavones with singlet oxygen, which is generated by dye-sensitized photooxygenation.[86,87] Under the conditions, **51a**, **51b** and **51c** give the corresponding depsides **52a** detected by TLC, **52b** and **52c** isolated as the methyl ester (44–77%), respectively, in addition to carbon monoxide and carbon dioxide. From the facts that carbon monoxide is stable under the reaction conditions, and that photosensitized oxygenation of *p*-methoxyphenylglyoxylic acid gives *p*-anisic acid and carbon dioxide in good yield, the formation of carbon dioxide has been explained by the oxidative decarboxylation of **56** which might form via path b in a competition with path a.

The formation of ketohydroperoxide **53** can be alternatively ascribed to a radical intermediate **57** which results from a Type I photosensitized oxygenation, possibly involving hydrogen abstraction of the 3-OH group by the triplet excited sensitizer. This possibility has been excluded by the following experiments. On oxidation with manganese dioxide, which is known to oxidize phenols into phenoxy radicals, **51c** gives only a dehydrodimer **58**[88] under either nitrogen or oxygen atmosphere (eqn 23). The results indicate that the radical **57**, even under oxygen, easily dimerizes to **58** rather than reacting with the ground-state oxygen.

The singlet oxygen reaction of the 3-hydroxyflavones was thought to be a good mimic for the quercetinase-catalyzed reaction and to be a possibility that the enzyme reaction might involve singlet oxygen. However, this was not conclusive, since other oxygenation methods have been found effective for the depside formation from 3-hydroxyflavones. Nishinaga and Matsuura have found that autoxidation of 3,4′-dihydroxyflavone (**59a**) in dimethylformamide (DMF) in the presence of potassium *t*-butoxide results in the formation of the corresponding depside (**60a**) and carbon monoxide in almost quantitative yield.[89] This idea was originated from the α-ketohydroperoxide formation in the base-catalyzed oxygenation of 3,5 - di - iodo - 4 - hydroxyphenylpyruvic acid in its enolic form (eqn 11), which has the same partial structure as in **59a** as shown by bold line in formula **59a**. The reaction also proceeds rapidly in dimethylsulfoxide containing the same base but slowly in MeONa–MeOH or NaOH–H_2O. 3-Hydroxyflavone itself undergoes very slow autoxidation even in *t*-BuOK–DMF. No oxygen uptake is apparently observed because of the liberation of the same volume of carbon monoxide. However, when the oxygenation is carried out with 3,4′,7-trihydroxyflavones in *t*-BuOK–DMF, oxygen absorption increases at the expense of a decrease of carbon monoxide liberated. For example, while 3,4′-dihydroxyflavone (**59a**) absorbs 1.0 mole of oxygen and liberates 0.98 mole of carbon monoxide, 3,7,4′-trihydroxyflavone (**59b**) and quercetin (**59c**) absorbed 1.4 and 1.5 mole of oxygen, respectively, and liberates 0.03–



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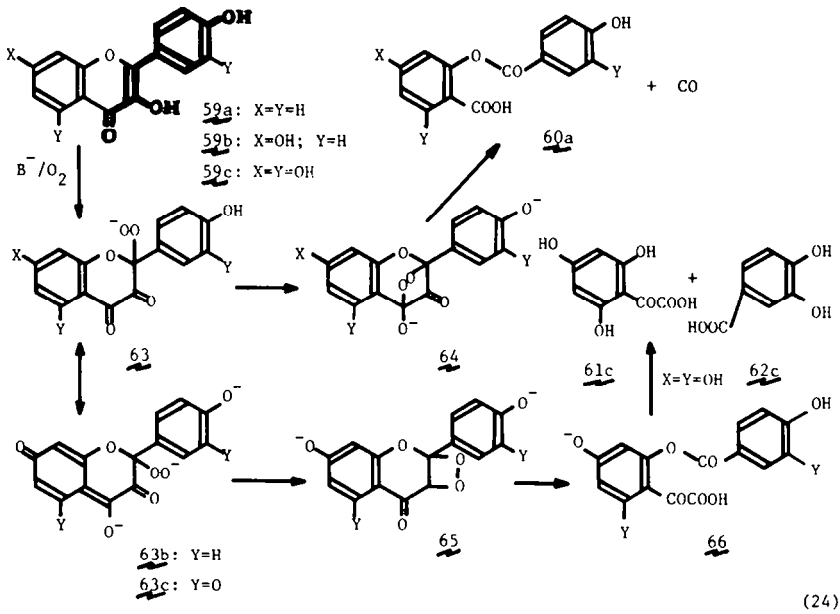

(24)

0.1 and 0.02–0.3 mole of carbon monoxide, respectively. In case of **59b**, the yield of depside **60b** decreases as 5–20%.

Nordstrom *et al.* have shown in their studies on autoxidation of 3-hydroxyflavones in aqueous alkali that a 4-hydroxy group is necessary for a high rate of oxidation,[90] and that quercetin (**59c**) gives 2,4,6 - trihydroxy - phenylglyoxylic acid (**61c**) and protocatechuic acid (**62c**) in addition to phloroglucinol and 2,4,6-trihydroxybenzoic acid.[91–93] From the above facts, Nishinaga and Matsuura have proposed a dual mechanism for the base-catalyzed oxygenation of 3,4′-dihydroxyflavones.[94] According to this mechanism (eqn 24), **59a** having no 7-hydroxyl undergoes exclusively cleavage to depsides **60a** via a hydroperoxy anion **63** and a 5-membered peroxide **64**, while **59b** and **59c**, having a 7-hydroxyl group, follow partly the same pathway in addition to a pathway via a dioxetane which is formed from a highly resonance-stabilized hydroperoxy anion, **63b** or **63c**, respectively.

In a series of biochemical studies by Simpson *et al.*, it was found that quercetinase is a copper-enzyme; a mechanism was proposed involving a ternary complex of substrate, copper, and oxygen which undergoes a sequence of intramolecular reactions to form depside **52a** (eqn 25).[84,85] If the mechanism is applicable to an nonenzymatic model system, a copper–3-hydroxyflavone complex might undergo a similar cleavage reaction in the presence of oxygen. When a copper(II)– or cobalt(II)–chelate **67** of 3-hydroxyflavone (**51c**) is treated with oxygen in various organic solvents (DMF, DMSO, pyridine, or CH_2Cl_2), no reaction takes place. However, addition of an excess of **51c** in the system causes oxygenation to give the corresponding depside **52c**,[94] indicating that in the nonenzymatic reaction such a ternary complex **67** may be capable of catalyzing the oxygenation of 3-hydroxyflavones but no internal reaction occurs as eqn (25).

Then Nishinaga *et al.* have successfully carried out the model oxygenation of various 3-hydroxyflavones using Co(II)-salen or Cu(II) acetate as catalyst.[94,95] The result is shown in Table 1. Gaseous products are found to be a mixture of carbon monoxide and carbon dioxide. The yield of the latter increases with increasing reaction time, since carbon monoxide is oxidized to carbon dioxide under the conditions. A comparison of the reactivities of the substrate with Co(II)–salen catalyst in DMF shows that substitution by a hydroxy or methoxy group at C-7 and C-4′ results in acceleration of the reaction rate. This effect is in fairly good agreement with that in the enzymatic reaction[85] and of the base-catalyzed oxygenation of 3-hydroxyflavones.[89,90] The result suggests that in these three oxygenation reactions, a donor–acceptor interaction involving 3-hydroxyflavone or its anion may be responsible for the rate-determining step.

The oxygenation catalyzed by transition metals is highly solvent dependent; the reaction occurs readily in DMSO as well as DMF, but slowly in methanol and not

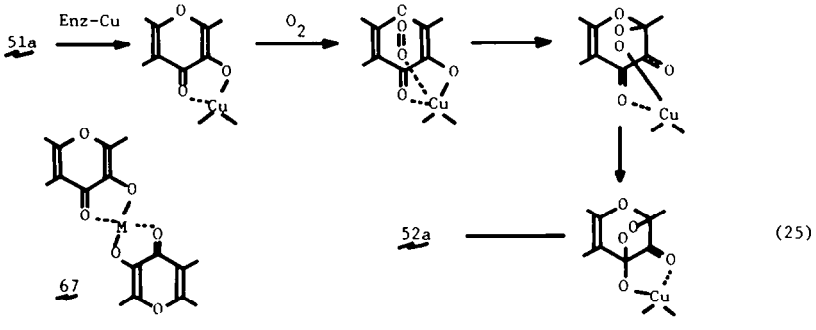

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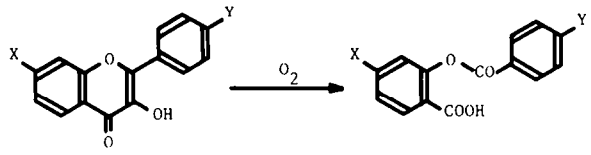
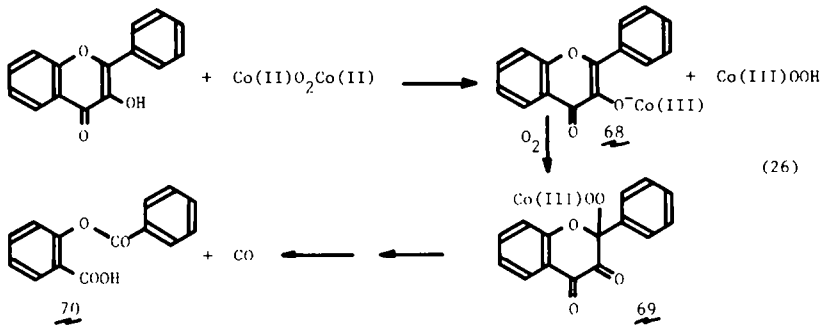
Table 1. Co(II)- and Cu(II)-catalyzed oxygenation of 3-hydroxyflavones



X	Y	Catalyst	Solvent	$t_{1/2}$(hr.)[a]	Yield (%)	Relative rate for the enzyme reaction[85]
H	H	Co(II)-salen	DMF	18	97	0.05
OMe	H	Co(II)-salen	DMF	15	98	--
OH	H	Co(II)-salen	DMF	9.3	97	0.9
H	OMe	Co(II)-salen	DMF	4.2	61	--
H	OH	Co(II)-salen	DMF	2.5	72	2.02
OH	OH	Co(II)-salen	DMF	1.5	36	23.9
H	H	$Cu(OAc)_2$	DMF	--	37	--
H	OH	$Cu(OAc)_2$	MeOH-NaOMe	--	95	--
H	OH	Co(II)-salen	MeOH-NaOMe	--	60	--

[a] Time required for half conversion of the starting material.



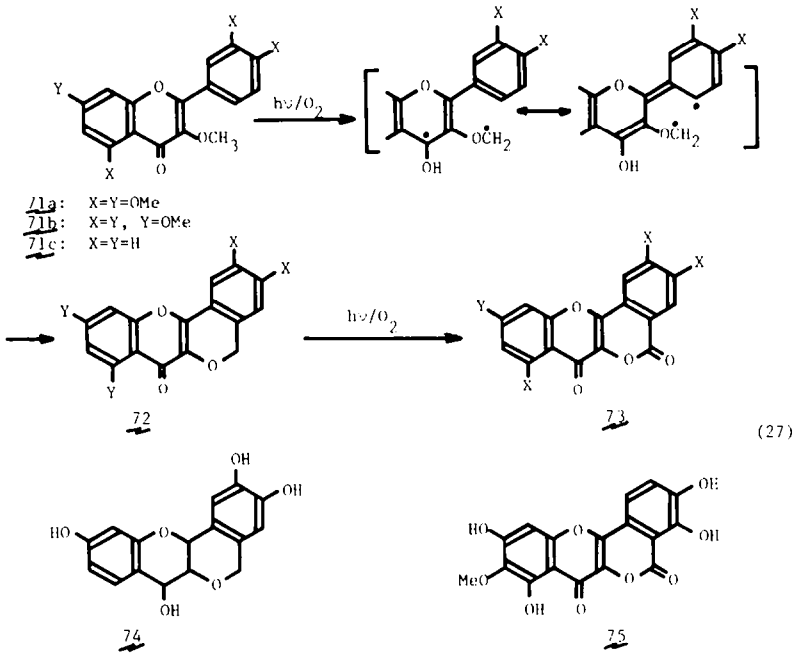
(26)

in tetrahydrofuran, acetic acid or methylene chloride. The latter group of solvents is known not to assist the formation of a 2:1 complex (Co_2O_2) of Co(II)–salen although it is formed in DMSO or DMF.[96-98] Co(II)–salpr, which is known to give a 1:1 complex with oxygen, is much less effective for the catalysis. From these experimental observations and the results obtained in the study of Co(II)–salen catalyzed oxygenation of phenols (see below), a scheme of eqn (26) has been suggested for the Co(II)-catalyzed oxygenation of 3-hydroxyflavones.[94,95] According to this scheme, the first step is a proton transfer equilibrium between the substrate and the Co_2O_2 complex followed by oxygen addition to a Co(III)-3-flavonoxyl complex **68** to form a cobalt-peroxy intermediate **69** which eventually gives rise to the depside **70** and carbon monoxide through a similar path to that of eqn (22), path a. However, one cannot rigorously eliminate an alternative mechanism involving an electron transfer between the substrate and the Co_2O_2 complex leading to a 3-flavonoxyl radical.

Apart from the quercetinnase problem, the photooxygenation of 3-methoxyflavones appears interesting in view of the biogenesis of certain flavonoids. Photolysis of quercetin pentamethyl ether (**71a**) under nitrogen has been found to yield **72a** as one of the photoproducts.[99] We have found that unsensitized photooxygenation of simple 3-methoxyflavones (**71b** and **71c**) gives further oxidation products **73** in addition to **72**.[100] The results represent possible mimics for the biogenesis of uncommon flavonoids such as peltogynol (**74**) and distimonanthin (**75**), which are supposed to be derived from a 3-methoxyflavone precursor *in vivo*. A pathway of eqn (27) has been suggested, involving an intramolecular hydrogen abstraction of the excited **71** followed by dehydrogenation to give **72** which may be photooxidized by a free radical chain mechanism to form **73**.[99,100]

Ring-cleavage of aromatic compounds

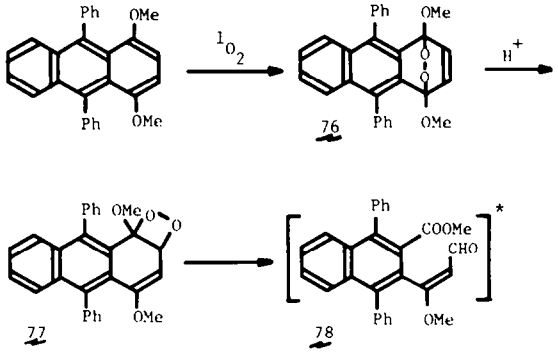
There are many natural products which appear to be formed via ring-cleavage of aromatic compounds *in vivo* possibly by the action of dioxygenases.[101,102] Substrates for the dioxygenases catalyzing aromatic ring-cleavage usually have a phenolic group. This implies that aromatic hydroxylation may be prerequisite in the catabolic pathway of aromatic compounds as seen in the early stage of the utilization of aromatic hydrocarbon by microorganisms.[103] Well-studied examples of dioxygenase-catalyzed aromatic ring-cleavage reaction are found in the metabolism of catechols (see eqn 8). Various attempts have been made for approaching to this problem, especially to know active dioxygen species involving the enzymatic reactions.



(27)

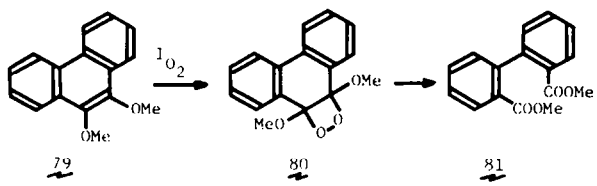
Singlet oxygen. Earlier biomimetic oxygenations were carried out using singlet oxygen which is one of the candidates for the active dioxygen species in catechol dioxygenases. Singlet oxygen reacts with condensed aromatic hydrocarbons to give 1,4-endoperoxides.[104,105] Baldwin *et al.* have found that an endoperoxide **76**, prepared by the singlet oxygen reaction of 1,4 - dimethoxy - 9,10 - diphenylanthracene, undergoes facile 1,2-bond cleavage to give **78**, most probably via acid-catalyzed rearrangement of a dioxetane **77** (eqn 28).[106]

A direct 1,2-addition of singlet oxygen to an electron-rich aromatic giving a dioxetane, followed by its 1,2-bond cleavage, appears to occur in cartain cases. Photosensitized oxygenation of 9,10-dimethoxyphenanthrene (**79**) at 220°K gives a dioxetane **80**, which undergoes acid-catalyzed cleavage of 9,10-bond to yield **81**.[111] In the course of our studies on the singlet oxygen reaction of electron-rich benzene derivatives substituted by methoxy and dimethylamino groups, which usually give oxidation products derived from spontaneous decomposition of an



(28)

The formation of the dioxetane intermediate has been supported by a mechanistic study of chemiluminescence occurring simultaneously.[107] Similar types of cleavage reactions have been observed by Rigaudy *et al.* with 1,4-dimethoxyanthracene and 1 - (N,N - dimethylamino) - 9,10 - diphenylanthracene 1,4-peroxides, the latter of which undergoes spontaneous decomposition.[108–110] initially formed 1,4-endoperoxide,[112–114] we have found that **82** produces selectively a 1,2-cleavage product **83** upon rose bengal-sensitized photooxygenation in methanol.[114] The product is apparently derived from a dioxetane **84**, which may be formed by direct 1,2-cycloaddition of singlet oxygen although a mechanism involving a methanol-assisted rearrangement of a 1,4-



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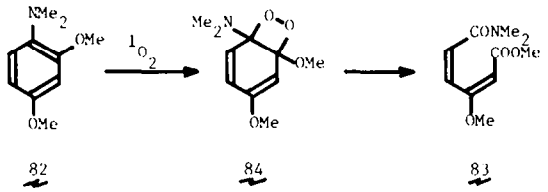
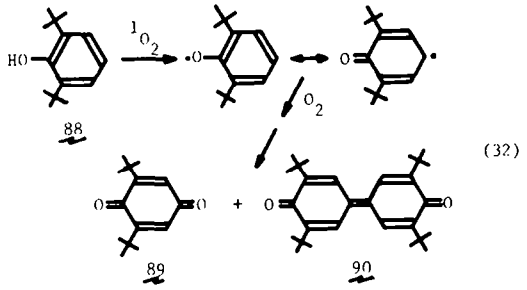


82 84 83 (30)

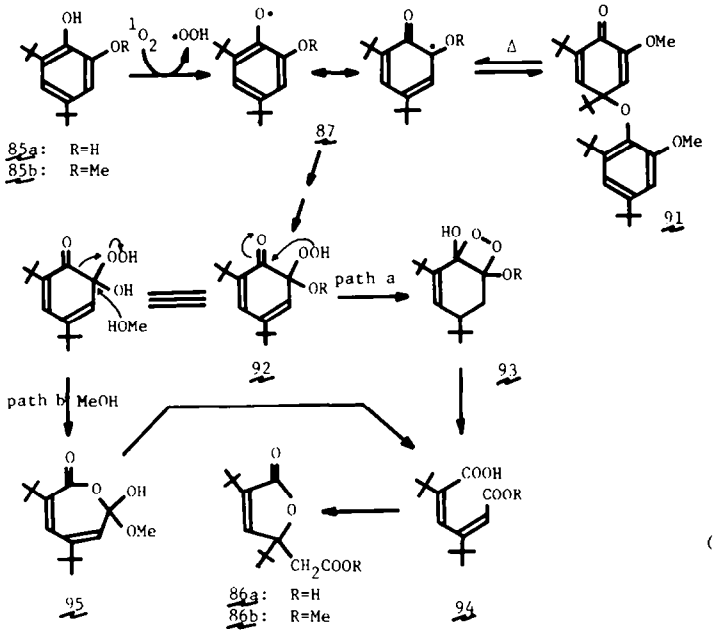
endoperoxide intermediate cannot be excluded. A number of reports have been published on the 1,2-cleavage of aromatic compounds with singlet oxygen,[104] but do not directly concern with biological oxygenations. Problems concerning the selectivity in 1,4- and 1,2-cycloaddition of singlet oxygen to an aromatic ring should await further investigated.

We have also shown that dye-sensitized photooxygenation of a catechol and its monomethyl ether yields ring-cleavage products.[115–117] Thus, 3,5-di-*t*-butylcatechol (**85a**) gives **86a** (11%) in pyridine and **86a** (16%) and **86b** (4%) in methanol, while the monomethyl ether **85b** gives only **86b** (22%) either in methanol or benzene (eqn 31). These reactions have been interpreted in terms of a phenoxy radical intermediate **87** which may be formed by the apparent hydrogen abstraction from **85** by singlet



88 89 90 (32)

possibly by an electron transfer between the reactants. The phenoxy radical **87** reacts either with the hydroperoxy radical or the ground-state molecular oxygen to give a peroxy-*o*-quinol **92**. An alternative mechanism

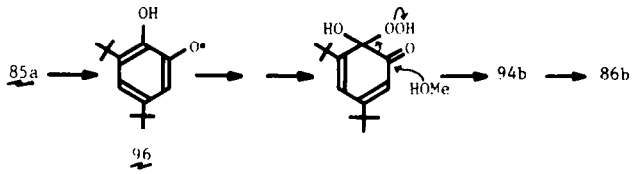


85a: R=H
85b: R=Me
87 91 92 93 95 86a: R=H 86b: R=Me 94 (31)

oxygen or the triplet excited sensitizer.[117] The radical mechanism is based on the facts that the photosensitized oxygenation of 2,6-di-*t*-butylphenol (**88**) gives 2,6-di-*t*-butyl-*p*-benzoquinone (**89**) and 3,5,3′,5′ - tetra - *t* - butyldiphenoquinone (**90**) which can be derived from the phenoxy radical of **88** (eqn 32),[118,119] and that the non-photochemical autoxidation of the radical **87b** generated from thermolysis of its dimer **91** gives the same ring-cleavage product **86b**.[117] Foote *et al.* have recently observed quenching of singlet oxygen by various phenols with a correlation between their donor character and the quenching rates[120] and detected phenoxy radicals as the initial product.[121]

The ring-cleavage reaction of **85** is now interpreted by a scheme of eqn (31).[117] Singlet oxygen generated by dye-sensitized photooxygenation reacts with phenol **85** to give phenoxy radical **87** and the hydroperoxy radical, involving a direct addition of singlet oxygen to **85**, however, cannot be excluded. Peroxy-*o*-quinol **92** is then converted to a dioxetane **93** via path a. Cleavage of **93** gives α,β′-*di*-*t*-butylmuconic acid (**94a**) or its methyl ester (**94b**) which in turn cyclizes to give **86a** or **86b**, respectively. The formation of the ester **86b** from **85a** in methanol can be rationalized either by path b involving a Baeyer–Villiger type rearrangement of **92** to **95** being accompanied by methanol addition or by assuming another phenoxy radical **96** which is transformed according to eqn (33).

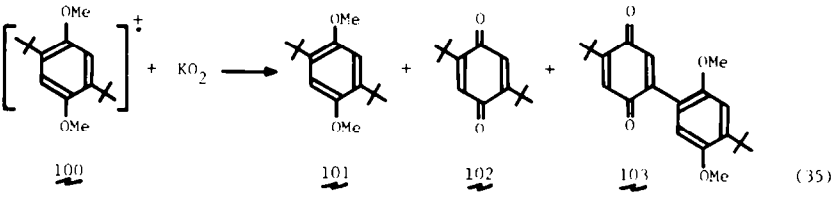
Although there has been no experimental evidence for the involvement of singlet oxygen in the enzymatic oxygenation using purified dioxygenases, it seems reasonable to assume the formation of singlet oxygen as a candidate of oxygen activation mechanisms, considering the following recent reports. Nakano *et al.* have



(33)

shown the formation of singlet oxygen in the lipid peroxidation with rat liver microsomes.[122-124] As shown in eqn (8), reaction of the superoxide radical with a cation radical derived from a substrate is a plausible mechanism for certain dioxygenases such as catechol dioxygenases and tryptophan 2,3-dioxygenase. Recently Mayeda and Bard have claimed that the reaction between the cation radical **97** of diphenylisobenzofuran (**98**) and the superoxide radical proceeds via an electron transfer giving **98** and singlet oxygen which couple to yield 1,2-dibenzoylbenzene (**99**) as the final product (eqn 34).[125] Nishinaga *et al.* have shown that the cation radical **100**[126] of 2,5 - di - *t* - butyl - 1,2 - dimethoxybenzene (**101**) reacts with potassium superoxide to undergo mainly electron transfer giving **101** (88%) besides 2,5 - di - *t* - butyl - *p* - benzoquinone (**102**) and **103**, both of which appear to be derived via minor pathway, namely direct coupling between **100** and the superoxide radical.[127] When the reaction is carried out in the presence of **98**, the singlet oxygen-reaction product **99** is obtained in 77% yield.[127]

Base-catalyzed oxygenation. 3,5 - Di - *t* - butylcatechol (**85a**) undergoes facile oxidative cleavage by base-catalyzed oxygenation. Thus, autoxidation of **85a** in aqueous and methanolic alkali was found to give **104** and **105**, respectively,[128,129] the latter of which is also formed by oxidation of 3,5 - di - *t* - butyl - *o* - benzoquinone (**106**) with hydrogen peroxide.[129] Alkaline autoxidation of 4,6 - di - *t* - butylpyrogallol (**107**) gives **104** and **108**.[130,131] In order to clarify the feature of these reactions, Nishinaga *et al.* have examined separately the *t*-butoxide-catalyzed oxygenation of **85a**, **104**, **105**, **106** and **107** in DMF, all of which gives **109** as the major product, and they have observed the transient formation of the corresponding semiquinone radicals by ESR in cases of **85a** and **107**.[132] *t*-Butoxide-catalyzed oxygenation of **85a** in various organic solvents has been found to give **105**, **109**, and a direct cleavage product **110**, the ratio of which



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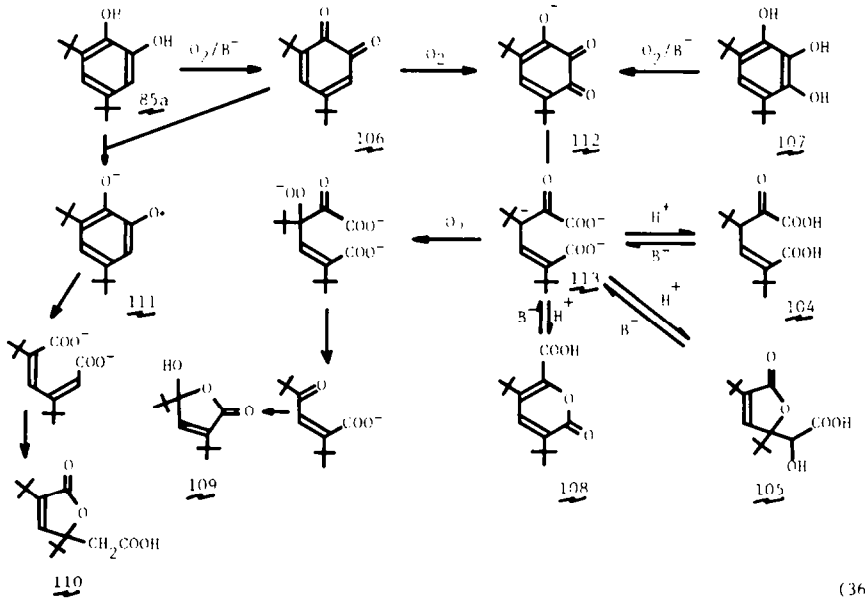


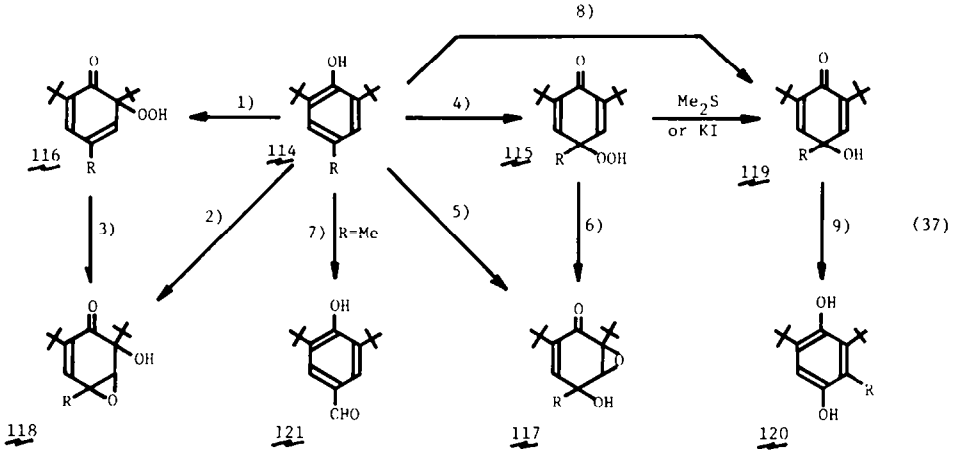
(36)

is solvent- and temperature-dependent.[133] A mechanism, involving the intermediary formation of 3,5 - di - *t* - butyl - *o* - benzosemiquinone (**111**), 3,5 - di - *t* - butyl - 6 - oxido - *o* - benzoquinone (**112**), and the 2,4 - di - *t* - butyl - 5 - oxo - 2 - hexen - 4 - idodioate anion **113**, has been proposed.[132]

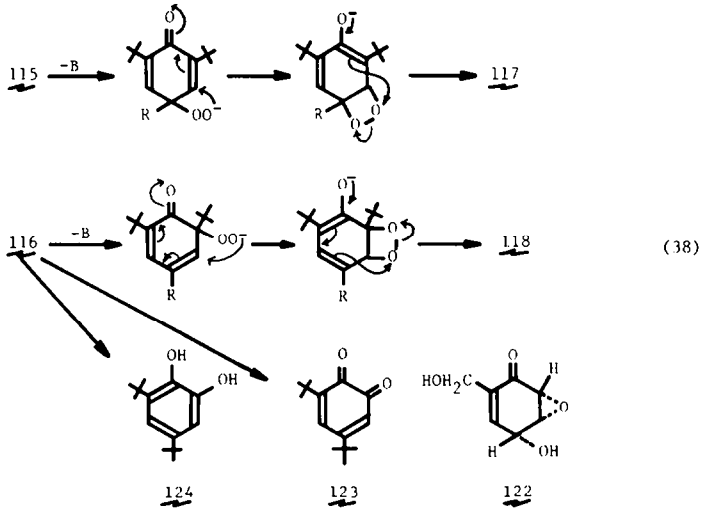
In view of the similarity of the base-catalyzed oxygenation of phenols to the biological transformation of natural phenolic compounds such as thyroxine, its precursors, and catechols, the base-catalyzed oxygenation of hindered phenols has been investigated at the author's laboratory.[134–139] The base-catalyzed oxygenation of monohydric hindered phenols in aqueous alkaline media was reported by several workers to give a variety of oxidation products, such as hydroperoxides, quinols, and quinones, depending on the structural features of the phenols and reaction conditions.[140] Nishinaga *et al.* have found that on base-catalyzed oxygenation 2,6 - di - *t* - butyl - 4 - alkylphenols **114** undergo very selective reactions depending on solvent and temperature. The principal results are summarized in eqn (37).

Under the conditions, **114** gives peroxyquinols **115** and **116**, which by the action of a base undergo rearrangement of epoxyquinols **117** and **118**, respectively, with a simultaneously occurring back-reaction to the parent phenol and the ground-state molecular oxygen. The formation of epoxy-*p*-quinol **117** is interesting in relation to the similarity of its structure to that of naturally occurring epoxy-*p*-quinol such as epoxidone (**122**).[141] The formation of these epoxyquinols from peroxyquinols **115** and **116** has been interpreted in terms of dioxetane intermediates as eqn (38).

Base-catalyzed rearrangement of *p*-quinols **119** gives hydroquinone derivatives **120**,[139] which represents a mimic for the NIH shift occurring during the enzymatic hydroxylation of phenols; for example, *p*-hydroxyphenyl-pyruvate dioxygenase (see above). When the R group of **114** has an α-hydrogen, oxygenation in the presence of a large excess of a base results in the selective oxidation at α-position of the R group as exemplified by the formation of **121** (eqn 37).[137] Peroxy-*o*-quinols **116**, which have been never well defined, are particular interesting compounds in connection with the hypothetical mechanism of enzymatic cleavage of phenolic compounds (see **5** of eqn 8). However, as shown in eqn (38), base-catalyzed reaction of **116** does not give any cleavage product. Furthermore, on acid treatment **116** undergoes de-*t*-butylation to give 3,5 - di -

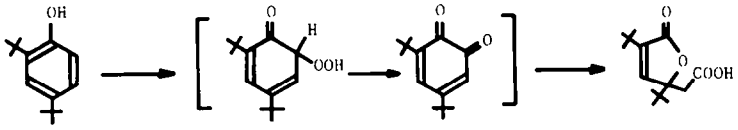


(1) O_2/*t*-BuOK/*t*-BuOH/0°;[138] (2, 3) O_2/*t*-BuOK/*t*-BuOH/room temp.;[134]
(4) O_2/KOH/EtOH;[140] (5, 6) O_2/*t*-BuOH/DMF (HMPA);[135]
(7) O_2/excess *t*-BuOK/DMF;[137] (8) O_2/$NaNH_2$/Et_2NH;[136]
(9) *t*-BuOK/DMF.[139]



t - butyl - *o* - benzoquinone (**123**) and on reduction it gives 3,5-di-*t*-butylcatechol (**124**) possibly via an unstable *o*-quinol.[138,142]

t-Butoxide-catalyzed oxygenation of 2,4-di-*t*-butylphenol, however, gives a 1,2-cleavage product, possibly via a peroxy-*p*-quinol followed by its conversion into 3,5 - di - *t* - butyl - *o* - benzoquinone (eqn 39).[135]



(39)

Two mechanisms, by which *t*-butylated phenols **114** give peroxyquinols **115** and **116** by base-catalyzed oxygenation, have been considered: an electron transfer between a phenolate anion and oxygen followed by a radical-chain autoxidation process (eqn 40a) (Russell mechanism)[143] and the one followed by cage-recombination of a phenoxyl radical and $O_2^{\cdot-}$ (eqn 40b).[135,144] In

$$ArO^- + O_2 \longrightarrow ArO^{\cdot} + O_2^{\cdot-}$$

$$ArO^{\cdot} + O_2 \longrightarrow O{=}ArOO^{\cdot}$$

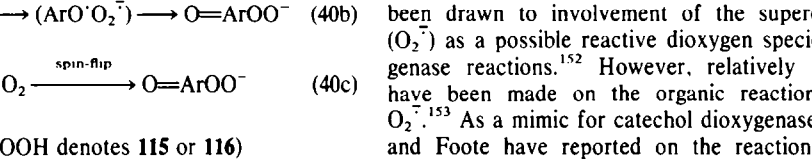
$$O{=}ArOO^{\cdot} + ArO^- \longrightarrow O{=}ArOO^- + ArO^{\cdot} \quad (40a)$$

$$ArO^- + O_2 \longrightarrow (ArO^{\cdot}O_2^{\cdot-}) \longrightarrow O{=}ArOO^- \quad (40b)$$

$$ArO^{\cdot-} + O_2 \xrightarrow{\text{spin-flip}} O{=}ArOO^- \quad (40c)$$

(O=ArOOH denotes **115** or **116**)

view that such mechanisms are considered possible to occur *in vivo*, Nishinaga *et al.* have examined the mechanism of the base-catalyzed oxygenation of 2,6 - di - *t* - butyl - 4 - R - phenols (**114**; R = *t*-Bu or Aryl) in some detail.[145] Reaction of 2,6 - di - *t* - butyl - 4 - R - phenoxyl radicals **125** with oxygen gives peroxide **126** independent on solvent but does not give peroxyquinols even in the presence of an excess of the parent phenol (eqn 41). Furthermore, the radicals react with potassium superoxide in various solvents not to give the corresponding peroxyquinols but to undergo electron transfer reverting back to the parent phenols and the ground-state oxygen. In the latter cases some peroxides such as **126** are obtained, which are most likely formed via coupling between the phenoxyl radical and the formed oxygen molecule. These results suggest a one-step ionic mechanism (eqn 40c), analogous to the recent proposal by Turro *et al.* that thermal reaction of a cyclic acetylene or a ketene with the ground-state oxygen produces a singlet oxygenoid being accompanied by spin-flip.[146] An interaction between the orbitals of an electron-donating phenolate anion and the ground-state oxygen may cause the perturbation of the degenerated π^*-orbitals of triplet oxygen to split the level followed by the occupation of the resulting elevated orbital by the anion.

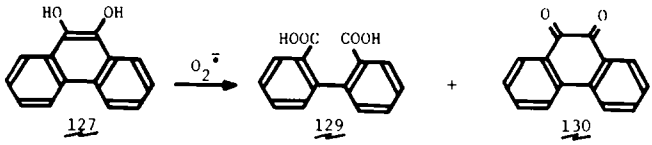


125 **126** (41)

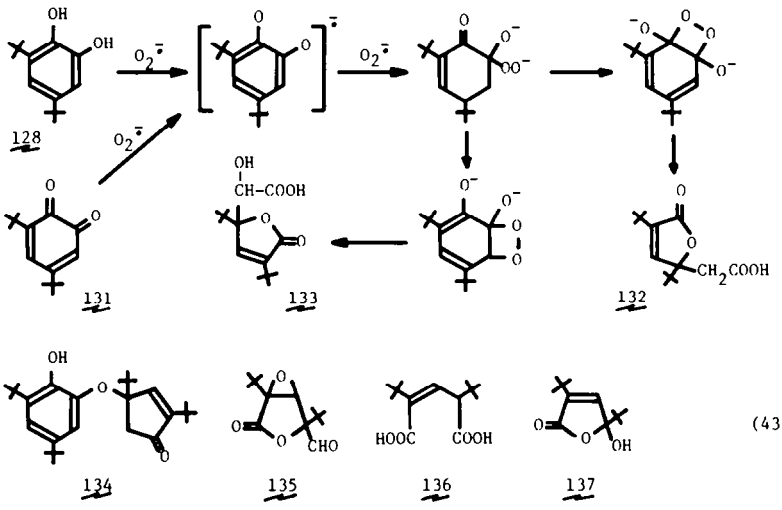
It may be added that Nishinaga *et al.* have developed new synthetic reactions using the base-catalyzed oxygeneration products during their studies: peroxy-*o*-quinols **116** and epoxy-*o*-quinols **118** undergo base-catalyzed transformation into cyclopentadienones and cyclopentenone derivatives,[147,148] and the acetates of peroxy-*p*-quinols **115** and *p*-quinols **119** undergo new types of rearrangement.[149,150] The base-catalyzed oxygenation of resorcinal derivatives has been extensively investigated in connection with the biogenesis of litmus dyes.[151,144]

Oxidation with superoxide anion. Since the discovery of superoxide dismutase (eqn 5), special attention has been drawn to involvement of the superoxide anion ($O_2^{\cdot-}$) as a possible reactive dioxygen species in dioxygenase reactions.[152] However, relatively few studies have been made on the organic reactions involving $O_2^{\cdot-}$.[153] As a mimic for catechol dioxygenases Moro-oka and Foote have reported on the reaction of 9,10-dihydroxyphenanthrene (**127**) and 3,5-di-*t*-butylcatechol (**128**) with potassium superoxide in nonpolar solvents.[154] Oxidation of **127** gives diphenic acid (**129**) as the main product with a concomitant formation of 9,10-phenanthrenequinone (**130**) (eqn 42), the yield of which increases under nitrogen atmosphere. In case of catechol **128**, the products consist of a mixture of **131**, **132**, **133**, **134**, **135**, **136** and **137** (eqn 43), most of which are ring-cleavage products obtained previously by singlet oxygen and base-catalyzed oxygenation of **128** (eqns 31 and 36). The same products are obtained from the reaction of *o*-quinone **131** with $O_2^{\cdot-}$. The ratio of the products varies depending on atmospheric conditions (under oxygen or nitrogen). They have proposed a mechanism involving the initial hydrogen transfer between $O_2^{\cdot-}$ and **128** or **131** followed by coupling between an *o*-semiquinone intermediate and $O_2^{\cdot}$ (eqn 43).

The superoxide anion is a dioxygen species acting as reducing, oxidizing, nucleophilic, or radical reagent.[153] It



127 **129** **130** (42)



(43)

disproportionates nonenzymatically in the presence of proton into hydrogen peroxide and molecular oxygen (eqn 44),[9] the latter of which is generated partly as singlet oxygen.[155] Hydrogen peroxide can also react with $O_2^{\cdot-}$ in the presence of proton to give the hydroxyl radical and singlet oxygen (eqn 45).[156] Therefore, it

$$O_2^{\cdot-} + 2H^+ \longrightarrow H_2O_2 + O_2 \qquad (44)$$

$$O_2^{\cdot-} + H_2O_2 + H^+ \longrightarrow \cdot OH + H_2O + {}^1O_2 \qquad (45)$$

should be considered that, in a reaction system involving $O_2^{\cdot-}$, not only $O_2^{\cdot-}$ but also the ground-state triplet oxygen, singlet oxygen, hydrogen peroxide, and the hydroxyl radical will participate as active oxygen species.

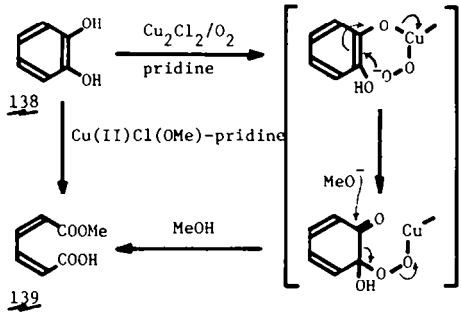
Transition metal-catalyzed oxygenation. As described before, catechol dioxygenases possess ferrous or ferric ion as a cofactor, and involvement of a iron-oxygen complex in the enzymatic reactions is quite plausible. In this connection, exploration of model systems for the dioxygenases is an attractive problem. However, oxygenation systems involving ferrous ion usually give rise to monooxygenation of organic substrates; for example, an aromatic compound gives phenolic products but does not give a ring cleavage product (see below). For this reason, biomimetic oxygenation systems using copper and cobalt ions have been so far designed for the ring cleavage of aromatic compounds.

The first model reaction using transition metal for catechol dioxygenases has been reported by Grinstead.[129] 3,5 Di-*t*-butylcatechol (**128**) is autoxidized in the presence of a transition metal catalyst such as Mn(II), Co(II), etc. to a cleavage product **133** via the corresponding *o*-quinone **131** (see eqn 43).[129] He has suggested that electron transfer is involved in the formation of the quinone, and that hydrogen peroxide may act as oxidizing agent for the cleavage of the quinone (eqn 46).

$$\text{Catechol} + O_2 \xrightarrow{\text{Mn(II)}} o\text{-Quinone} + H_2O_2 \qquad (46)$$

The transition metal-catalyzed oxidation of catechols to *o*-quinones has been investigated by Tyson and Martell.[157] The Mn(II) and Co(II) chelates of 4-nitrocatechol in the presence of oxygen catalyzes oxidation of 3,5-di-*t*-butylcatechol (**128**) to the corresponding *o*-quinone **131** in addition to hydrogen peroxide and water, respectively. The reactions provide possible mimics for oxidases such as *o*-diphenol oxidase (eqns 2b and 2c).

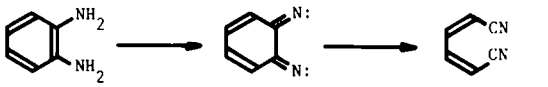
Tsuji and Takayanagi have found that catechol (**138**) itself can be cleaved by oxygenation catalyzed by cuprous chloride-pyridine in the presence of methanol to give methyl muconate (**139**) in good yield (eqn 47).[158] They have also found that phenol also gives **139** under the same conditions.[159,160] The use of a higher alcohol instead of methanol reduces the yield of the product. From tracer experiments with $^{18}O_2$, which has been shown to be incorporated into the carboxylic acid of **139**, they have presumed a mechanism of eqn (47) involving



(47)

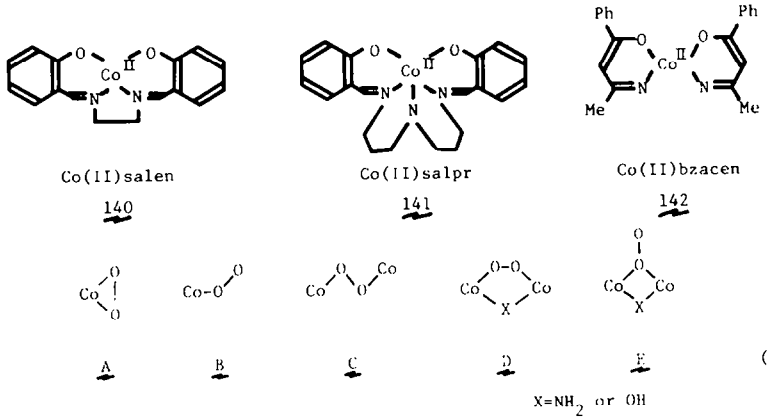
attack of a Cu_2Cl_2–O_2 complex to catechol.[160] However, Rojić *et al.* have recently shown that a Cu(II)Cl(OMe) pyridine complex reacts with catechol in aqueous methanolic pyridine, even in the absence of oxygen, to give **139** in good yield, and that 4 - *t* - butyl - *o* - benzoquinone and phenol give the corresponding methyl muconates in the absence and presence of oxygen, respectively.[161] The stoichiometry of these reactions indicates that the oxidation of catechol and the benzoquinone with the Cu(II)-complex are actually four electron and two electron transfer reactions, respectively, and that the oxygen in this reaction appears to reoxidize Cu(I) formed from the Cu(II)-complex, implying an alternative explanation to previous hypothesis involving activation of molecular oxygen for the enzymatic cleavage of catechols.

In relation to the oxidative cleavage of catechol, Tsuji *et al.* have reported the cuprous chloride-catalyzed oxygenation of *o*-phenylenediamine leading to muconononitrile, for which an electron transfer mechanism has been postulated (eqn 48).[162]



(48)

As described in earlier chapters of this report (eqns 15 and 26 and Table 1), Co(II)–Schiff base complexes such as Co(II)salen (**140**) and Co(II)salpr (**141**) act as a good catalyst for dioxygenation of relatively electron-rich aromatic and heterocyclic compounds having a dissociable hydrogen. The oxygenation reactions of hindered phenols using such Co(II) complexes as catalyst were developed by Nishinaga *et al.*, as mimics for activation of oxygen by transition metal-required dioxygenases.[163] Cobalt complexes containing nitrogen bases as ligands are known to form several types of Co-dioxygen complexes of eqn (49).[164–166]



Co(II)salen **140** Co(II)salpr **141** Co(II)bzacen **142**

A B C D E (49)

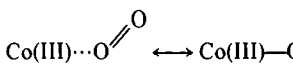
X=NH_2 or OH

The uptake of oxygen by Co(II)–Schiff base complexes occurs reversively to give a 1:1 complex (Co–O_2) and/or a 2:1 complex (Co_2O_2) depending on ligand properties (eqn 50). Crystallographic and ESR spectroscopic

$$L—Co(II) + O_2 \rightleftharpoons L—Co(II)—O_2$$

$$L—Co(II)—O_2 + L—Co(II) \rightleftharpoons L—Co(II)O_2—Co(II)—L \quad (50)$$

evidence show that, for example, Co(II)bzacene-pyridine (**142**) and Co(II)salpr (**141**) complexes form a paramagnetic 1:1 complex of type **B**. Their electronic structure can be represented by a resonance hybrid as shown by eqn (51a), in which an unpaired electron is delocalized on the superoxide-like oxygen. On the other hand, the electronic structure of diamagnetic 2:1 complexes such as [Co(II)salen·DMF]$_2O_2$ is approximated to a peroxide complex as shown by eqn (51b). In fact, the O–O bond

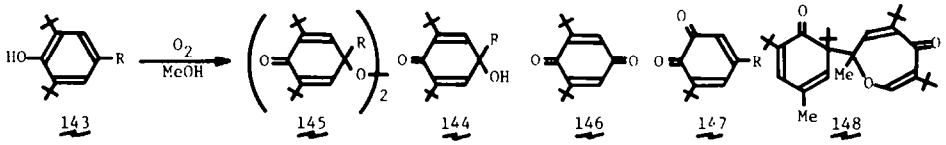


10–20% 80–90% (51a)

$$Co(II)—O_2—Co(II) \longleftrightarrow Co(III)^-O—O^-Co(III) \quad (51b)$$

lengths in the Co(II)bzacen-pyridine-O_2 and [Co(II)salen·DMF]$_2O_2$ complexes are 1.26 Å[167] and 1.34 Å,[168] respectively, which are considerably longer than that of the ground-state molecular oxygen (1.21 Å) and close to those of O_2^- (1.28 Å) and O_2^{2-} (1.49 Å), respectively. However, Co(II)salen having a strong donor ligand such as pyridine and imidazole at an axial position, forms rather a 1:1 complex of eqn (51a), and it gives no oxygen complex in inert solvents such as methylene chloride which cannot be coordinated as a ligand.

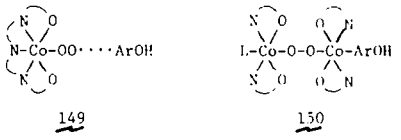
Nishinaga *et al.* have shown that Co(II)-catalyzed oxygenation of 4 - alkyl - 2,6 - di - *t* - butylphenols **143** in methanol proceeds via different courses depending on catalyst; namely the main product is a *p*-quinol **144** and a peroxide **145** with Co(II)salpr and Co(II)salen catalysts, respectively, being accompanied by byproducts **146**, **147** and **148** (eqn 52).[169–171] When the oxygenation is carried out with Co(II)salen in the presence of a good π-donor such as pyridines or imidazoles, the product distribution becomes similar to that in the oxygenation with Co(II)salpr, indicating that the product selectivity is largely dependent of the nature of Co(II)–O_2 complexes formed under the reaction conditions.[171]



143 **145** **144** **146** **147** **148** (52)

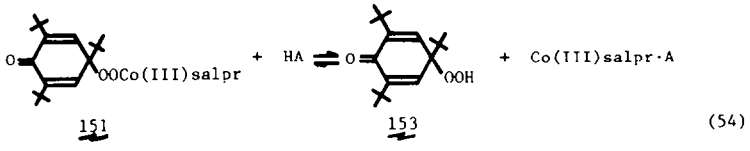
143a R=t-Bu	Co(II)salen	47%	--	34	12	--
	Co(II)salpr	10	60	8	6	--
143b R=i-Pr	Co(II)salen	44	8	45	--	--
	Co(II)salpr	12	80	8	--	--
143c R=Me	Co(II)salen	--	8	14	--	35
	Co(II)salpr	--	96	--	--	--

The nature of interaction between the Co(II)–O_2 complex and the phenol appears different with different types of catalyst. As a possible explanation, it has been suggested that while the 1:1 Co(II)–O_2 complex may interact with the phenol at the superoxide-like oxygen (eqn 53; **149**), the phenol may coordinate on a cobalt atom in case of the 2:1 $Co(II)_2O_2$ complex (eqn 53; **150**).[170]



149 **150** (53)

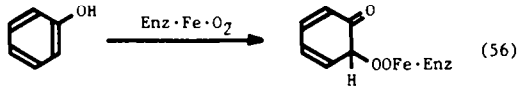
The Co(II)salpr-catalyzed oxygenation of 2,4,6 - tri - *t* - butylphenol (**143a**) has been investigated in detail, and it has been found that **143a** gives a crystalline cobalt-peroxy complex **151** in methylene chloride at room temperature or in methanol at 0°, after absorbing 1.25 mole equivalent of oxygen.[170,173] A solution of Co(II)salpr in methylene chloride under oxygen shows an ESR signal due to Co(III)–O_2^-, which decays by the addition of **143a** under intercepting the solution from oxygen and instead a new signal due to the 2,4,6 - tri - *t* - butylphenoxyl radical **152** appears. Further oxygenation of the solution shows a disappearance of the second signal with a reappearance of the signal due to Co(III)–O_2^-.[172] They have also observed an acid-base equilibrium between the Co-peroxy complex **151** and a peroxy-*p*-quinol **153** (eqn 54), giving pKa = 8.8 for **153** and $K = 6 \times 10^{-8}$ for HA=MeOH.[173] Furthermore, peroxy-*p*-quinol has been found to be decomposed in the presence of Co(III)salpr in methanol giving rise to *p*-quinol **144** with the concomitant formation of a small amount of byproducts including 2,6 - di - *t* - butyl - *p* - benzoquinone (**144**). Based on these experimental facts, Nishinaga *et al.* have suggested a mechanistic pathway of eqn (55) for the Co(II)-catalyzed oxygenation of 2,4,6 - tri - *t* - butylphenol (**143a**).[163,173]



151 **153** (54)

According to this scheme, at the initial step, **143a** is converted into the phenoxyl radical **152**, which, in the Co(II)salpr-catalyzed oxygenation, reacts with Co(II) and oxygen to form the Co-peroxy complex **151**. In the Co(II)salen-catalyzed oxygenation, the radical **152** may react rather with molecular oxygen to form peroxide **145a**. The peroxy-*p*-quinol **153** formed from **151** is decomposed by Co(II)salpr to give a quinol radical **154** which is reduced by methanol to form the *p*-quinol **144a** or is transformed into the *p*-benzoquinone **146** by *β*-scission.

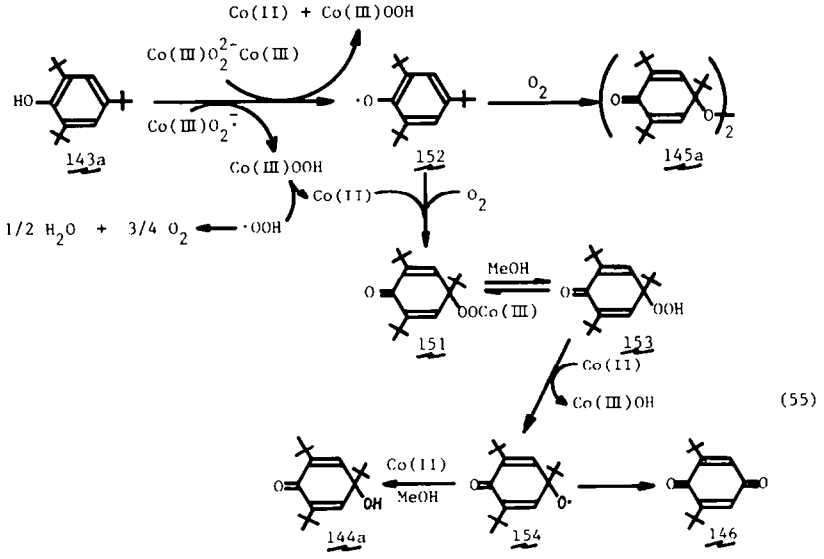
The Co-peroxy complex of type **151** is particular interesting in relation to the enzymatic dioxygenation of phenols catalyzed by Fe(II)-containing dioxygenases such as catechol dioxygenases (see eqn 8). As one of possible mechanisms for such dioxygenation, Hamilton has assumed involvement of a Fe-peroxy complex of eqn (56) as the key intermediate.[17] Nisinaga *et al.* have succeeded isolation of a similar type of Co-peroxy complexes **156** from 4 - aryl - 2,6 - di - *t* - butylphenols **155**. Oxygenation of **155** in the presence of Co(II)salpr in methylene chloride gives quantitatively **156** (eqn 57), which, under acidic conditions, is converted into an *o*-benzoquinone **158** via a peroxy-*p*-quinol **157**.[174,175] As has been described earlier (eqn 37), attempts to biomimetic ring-cleavage of peroxy-*p*-quinols are still unsuccessful.



(56)

Tryptophan 2,3-dioxygenase and indoleamine 2,3-dioxygenase

Tryptophan 2,3-dioxygenase, which is also known as tryptophan pyrrolase, catalyzes the 2,3-bond cleavage of



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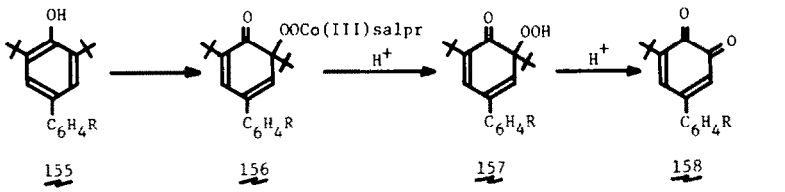
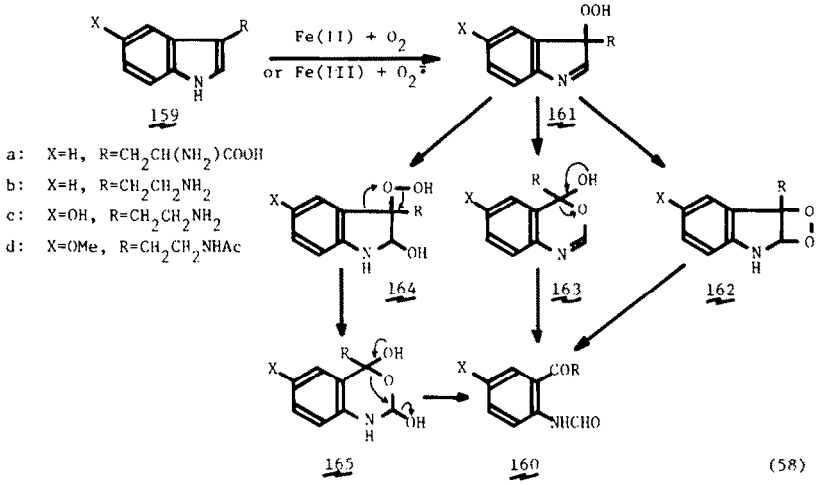


R=4-MeO, 3-MeO, 2-Me, 3-Me, 4-Me, H, etc. (57)

tryptophan (**159a**) into formylkynurenine (**160a**) (eqn 58) and is characterized as a heme protein containing ferrous ion.[176] Oxygen-18 has been shown to be incorporated into the two carbonyl groups of **160a**. Recently, β-indoleethylamine derivatives such as tryptophan (**159a**), tryptamine (**159b**), serotonin (**159c**), and melatonin (**159d**) have been found to be converted to the analogous 2,3-cleavage products (**160**) by the action of indoleamine 2,3-dioxygenase, which is also a heme protein containing ferric ion and utilizes the superoxide radical as oxygen source.[177,178] The stoichiometry involved in both enzymatic reactions is formally the same, $Fe(II) + O_2 \rightleftarrows Fe(III) + O_2^-$, although such an equilibrium is not substantiated. The key intermediate has been long considered to be an indolenine hydroperoxide **161**. It has been suggested that the reaction proceeds via cyclization of **161** to a dioxetane **162**,[17,179,180] via Baeyer–Villiger type of rearrangement of **161** to an oxazine **164**,[17,181] or via hydration of **161** to **164** followed by its rearrangement to **165** similar to that of **3**→**5** (eqn 1),[180,182] based on the known chemical behaviors of 2,3-disubstituted 3-hydroperoxyindolenines. However, there has been no substantial evidence for any of these intermediates in both enzymatic and chemical oxygenations.

Oxygenation with singlet oxygen. The dye-sensitized photooxygenation of tryptophan has drawn particular attention in relation to the photodynamic action (the damaging effects on a biological system occurring in the presence of sensitizing dyes, light and oxygen) to proteins and paptides,[187–189] in which tryptophan is one of the degradable amino-acid residues, as well as in relation to discoloration of irradiated fabrics such as silk and wool.[190] The photosensitized oxygenation of tryptophan (**159a**) in aqueous media usually gives a complex mixture of extensively degraded products,[191,192] except certain cases: for example, in formic acid **159a** gives **160a** in good yield.[193] The complexity is probably due to the secondary reactions of the primary and intermediary products such as **160a** and some peroxidic intermediates. This led us to investigate photooxygenation of indole derivatives in organic solvents, in which singlet oxygen is most probably involved as a reactive species, under carefully controlled conditions.[192]

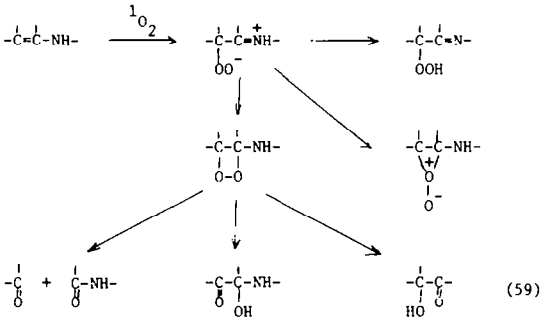
The dye-sensitized photooxygenation of enamines is usually interpreted by a mechanism involving addition of singlet oxygen to give a dioxetane, which in some cases can be isolated[193] and is often decomposed to give two carbonyl fragments,[194] undergoes C–N bond cleavage,[195]



(58)

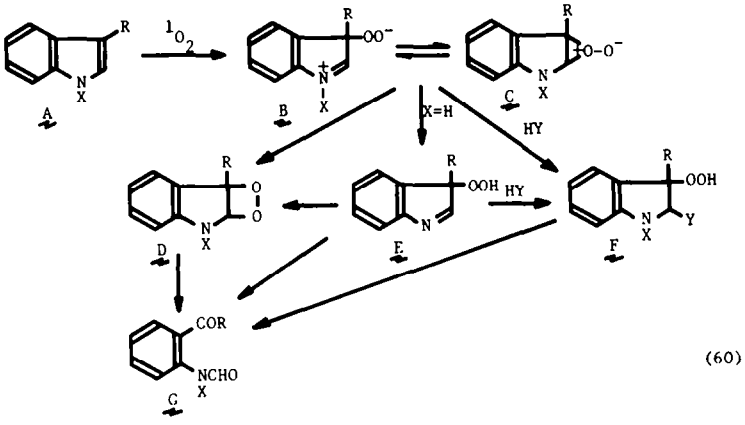
Earlier approaches to this problem have been done by several workers. Witkop has shown that ozonolysis of **159a** gives rise to formylkynurenine (**160a**)[183] which is now applied to the selective degradation of tryptophan residues in peptides.[184] Base-catalyzed oxygenation of **159a** has been found by Tabone *et al.* to give *o*-aminoacetophenone possibly via **160a**.[185] Yoshida and Kato have reported photosensitized oxygenation of **159a** in aqueous media identifying kynurenine and hydroxykynurenines among the products.[186] For the latter two cases, mechanisms involving dioxetane **162** has been assumed. There have been also a large number of papers concerning autoxidation of indole derivatives.[180]

or rearranges to a ketol when the enamine has an olefinic β-hydrogen (eqn 59).[193] According to a recent calculation by Dewar and Thiel,[196] the initial product from an enamine and singlet oxygen is a zwitterionic peroxide which is then transformed into a dioxetane or an iminohydroperoxide (eqn 59). If Dewar's scheme is applied to 3-substituted indoles (**A**; N-unsubstituted or N-substituted), a scheme (eqn 60) can be written in which reaction of singlet oxygen first gives a zwitterionic peroxide **B** which may be interconvertible with a perepoxide **C** and will be transformed into **D** or, when X=H, into **E**. If a nucleophile HY is present in the reaction system, **B** will give a hydroperoxide **F**. These peroxides



(59)

peroxide moiety could be possible. This idea has been substantiated in the photosensitized oxygenation of tryptophol, tryptamine, and tryptophan derivatives. Saito *et al.* have demonstrated that photooxygenation of N-methyltryptophol (**172a**) and N^b-methoxycarbonyl-N^a-methyltryptamine (**172b**) at $-70°$ in methanol gives exclusively hydroperoxides **173a** and **173b**, respectively, while at room temperature gives the corresponding 2,3-cleavage products **174a** and **174b**, respectively (eqn 63).[200,201] The dichotomous behavior of the reaction is also affected by solvent polarity; namely the formation of **173** tends to increase with decreasing the polarity of the solvent. It should be noted that in some cases an



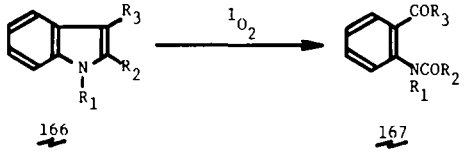
(60)

intermediates, **D**, **E** and **F**, can give finally a formylkynurenine-type of product **G** via pathways shown in eqn (60). Pathways involving direct addition of singlet oxygen to **A** giving **D** and **E** cannot be excluded.

Recently, detailed studies on the dye-sensitized photooxygenation of indole derivatives have been carried out by us[197-202] and by Nakagawa and Hino's group.[203-208] Saito *et al.* have shown that rose bengal- or methylene blue-sensitized photooxygenation of substituted indoles **166** in organic solvents or aqueous media at room temperature gives the corresponding 2-acylaminophenyl ketones **167** in good yield (eqn 61),[198,199] and that singlet oxygen generated by microwave discharge also cleaves

alcohol **175** is obtained instead of **173**, and that **173** is not transformed into **174** under the reaction conditions but thermally gives **175**.

The results have been interpreted in terms of a stepwise mechanism involving a zwitterion **176** (or peroxide of type C in eqn 60). It has been incidentally found that hydroperoxides **173** undergo facile acid-catalyzed reaction in methanol to give a 2,3-dihydrobenzoxazines **177**.[200] This Baeyer–Villiger type of reaction has been proved to be a general process occurring from type **F** of hydroperoxides in the singlet oxygen reaction of indoles under acidic conditions:[199,202,204] it occurs also intramolecularly as shown in eqn (64).[202]



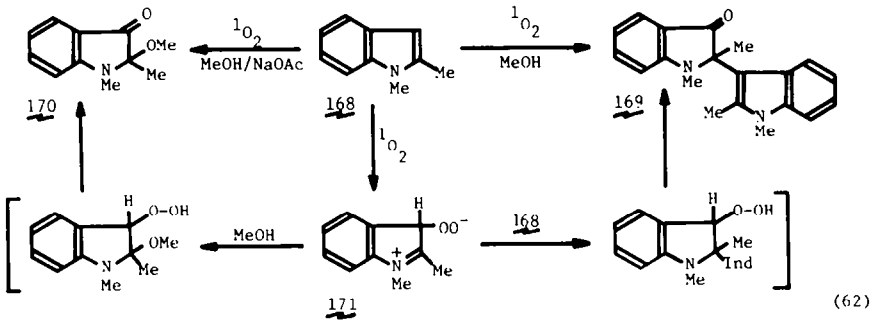
(61)

R_1=H,Me: R_2=H,Me,Ph: R_3=Me,CH_2COOH,COPh,CH_2CH_2COOH, etc.

indoles in the same manner.[202] However, 1,2-dimethylindole (**168**) behaves quite differently to photooxygenation giving a dimeric product **169** in methanol and a solvent-added product **170**.[197] As shown in eqn (62), the formation of these products is now reasonably explained by considering an intermolecular trapping of a zwitterionic peroxide **171** of type **B** (or C) in eqn (60) by the substrate or the solvent.[60] A similar explanation has been applied to the reaction of N-substituted tetrahydrocarbazoles with singlet oxygen.[198]

If a peroxide intermediate **B**, **C**, or **D** is involved in the photooxygenation of indoles having a nucleophilic group in the 3-side chain, an intramolecular trapping of the

The formation of type **F** of hydroperoxide has been observed also by Nakagawa *et al.* in the dye-sensitized photooxygenation of NH-free tryptamine and tryptophan derivatives. Thus, N^b-methyltryptamine (**178**) gives **179** and **180** possibly formed via a 3a-hydroperoxypyrroloindole **181**, although not isolated (eqn 65).[203] On the other hand, Rose Bengal-sensitized photooxygenation of N^b-methoxycarbonyltryptamine (**182a**) in a polar solvent at room temperature gives an alcohol **183a**, a cleavage product **184a**, and a novel transformylated product **185a**,[205] while at low temperature with filtered light a hydroperoxide **186a** is successfully obtained (eqn 66).[206] Treatment with silica gel or thermolysis of **186a** gives the



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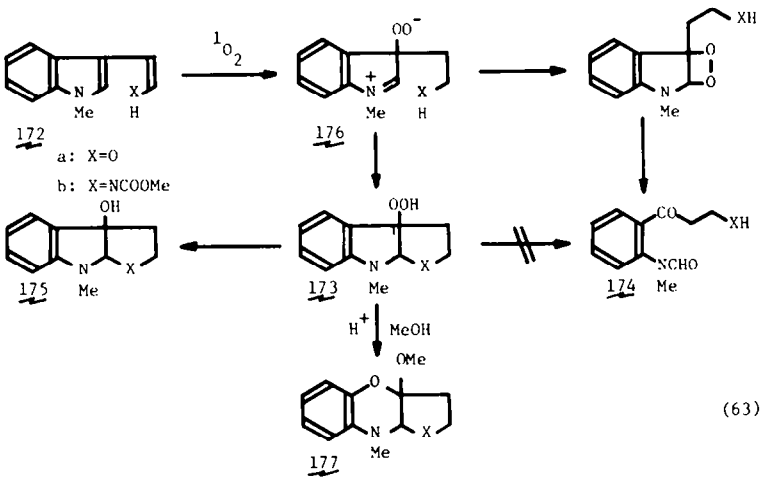
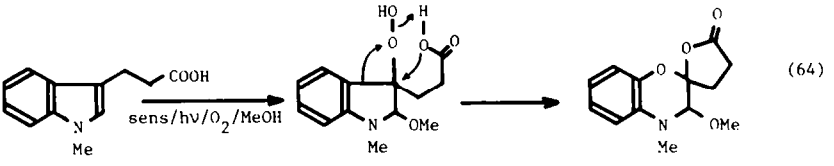


(63)



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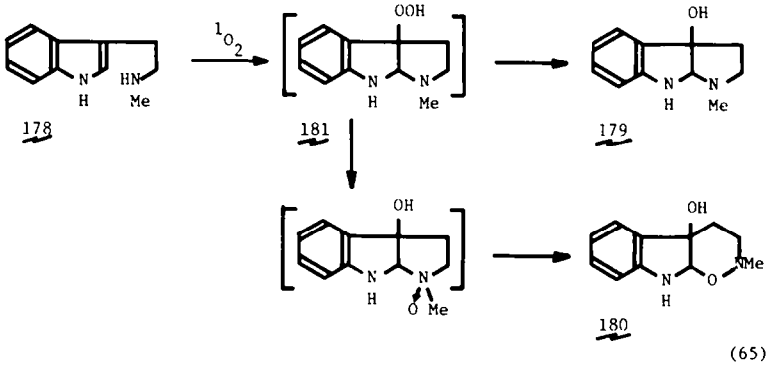
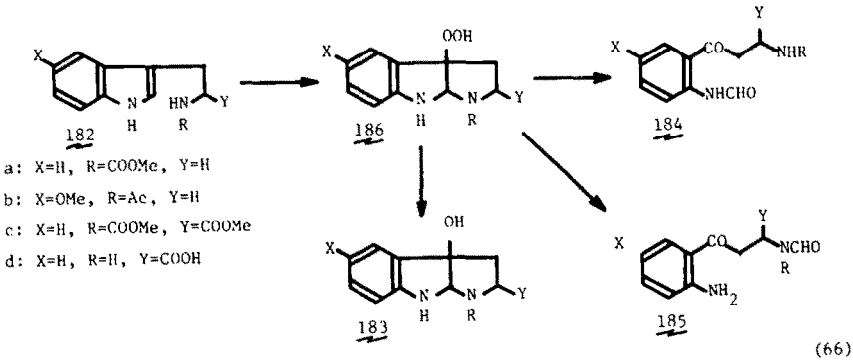


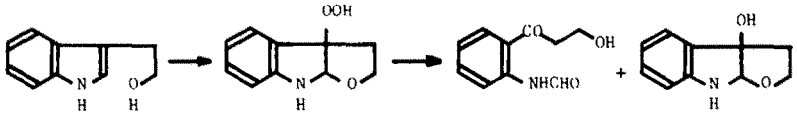
(65)

three products **183a**, **184a** and **185a**.[206] A similar result is observed in the photosensitized oxygenation of melatonin (**182b**),[207] but attempts to isolate the corresponding hydroperoxide **186b** are unsuccessful. Tryptophan itself in aqueous ethanol gives rise to an unstable peroxide which on reduction yields **183d**.[207] For the formation of hydroperoxides **186**, Nakagawa *et al.* have also suggested a mechanism analogous to that of eqn (60).

The formation of the 2,3-cleavage products such as **184** from hydroperoxide **186** is the most significant point for the above biomimetic dioxygenation of indoles having a nucleophilic group on the side chain, since such a process may be possible also in the enzymatic reaction catalyzed by tryptophan 2,3-dioxygenase or indoleamine 2,3-dioxygenase. The detailed study on this point has been carried out by Saito *et al.*[209] Rose Bengal-sensitized photooxygenation of tryptophol (**187**) at low temperature gives a stable hydroperoxide **188**, which undergoes transformation into a formylkynurenine-type product **189** and the corresponding hydroxy compound **190** under various conditions (eqn 67),[209] as in case of **186** of eqn (66).



182 186 184 183 185 (66)



187 188 189 190

	189	190
hν (>300nm)/N_2/MeOH	33%	30%
Refluxing in toluene	30	53
$FeSO_4$/MeOH	25	20
CuCl/MeOH	30	30
NaOH/MeOH	—	80

(67)

No chemiluminescence is observed in the thermolysis of **188** in toluene–carbon tetrachloride in the presence of 9,10-dibromoanthracene as a fluorescer. The result seems to exclude a mechanism involving retrocyclization of **188** to a hydroperoxyindolenine **191** followed by an alternative cyclization to a dioxetane **192** (eqn 68).

decomposition of a hydroperoxide of type **186a** into the alkoxy radical of type **193**.

Co(II)-*catalyzed oxygenation.* 3-Substituted indoles usually undergo non-catalytic oxygenation only with difficulty, but are oxygenated in the presence of Co(II)salen in methanol to form formylkynurenine-type

188 191 192 189* (68)

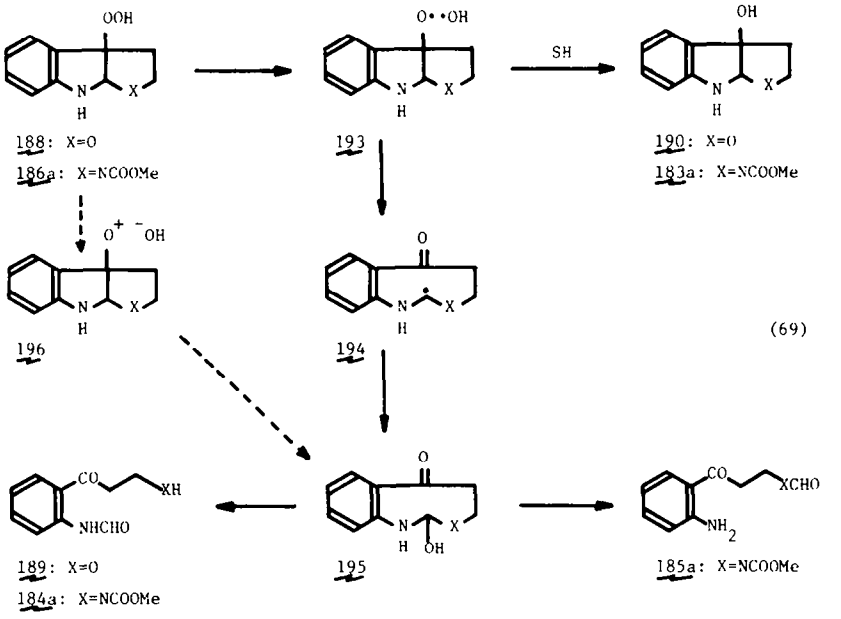
The results shown in eqn (67) and particularly the fact that the redox reaction of **188** with ferrous or cuprous ion in aqueous methanol occurs instantaneously, indicate that a radical mechanism of eqn (69) may be ascribed, at least in part, for the formation of **189** and **190**. The mechanism is also applicable to the reaction of eqn (66), including the transformylated product **185**.[209] The alkoxy radical **193** may be formed from hydroperoxide **188** or **186a** by a homolytic cleavage of the peroxide bond caused by thermolysis, photolysis, or redox reaction with a transition metal ion. The radical **193** may abstract hydrogen from the solvent or the starting hydroperoxide to give **190** or **183a**, or undergo β-scission to give **194**. The carbon radical **194** will either recombine with the hydroxyl radical or undergo a radical chain decomposition of the hydroperoxide **188** (or **186a**) to give a common intermediate **195** leading to the normal cleavage product **189** (or **184a**) or the transformylated product **185a**. An ionic mechanism involving a cationic intermediate **196** may not necessarily be excluded, since treatment of **186a** with acetic anhydride in pyridine gives only the monoacetate of **185a**.[207] It may be reasonable to assume that a similar mechanism is operative for the enzymatic 2,3-cleavage of tryptamine derivatives. If so, heme iron may play an important role in the redox

products in good yield. Nishinaga has carried out the oxygenation reaction with several indole derivatives **197** giving **198** shown in eqn (70).[210] Indole-3-acetic ester (**197b**) gives an unexpected product **199**, and indole itself gives a dimeric product.

The reaction proceeds faster in a solvent having a less electron-donating property; namely CH_2Cl_2 > MeOH > DMF, and the relative rates of **197** in CH_2Cl_2 (**197a** > **197d** > **197c** > **197b** > **197e**) show a fairly good linear relation with the constants of charge transfer-complex formation of **197** with trinitrobenzene. Based on these observations and the fact that Co(II)salen does not form an oxygen complex in CH_2Cl_2 but form a 2:1 complex in DMF,[164,165] Nishinaga has proposed a mechanism of eqn (71),[211] involving a substrate-Co(II)-oxygen complex **200** which is transformed into a Co(III)-peroxy complex **201** via an indolyl radical by an internal electron transfer of **200**.

Platinum-catalyzed oxygenation of 2,3-disubstituted indoles also give 2,3-cleavage product via hydroperoxyindolenines.[212,213]

Indole oxygenation related to indole alkaloid biosynthesis. In the biogenetic pathways of indole alkaloids, mono-oxygenation of geissoschizine (**202**) into a 3-hydroxyindolenine **203** followed by its hydration to give



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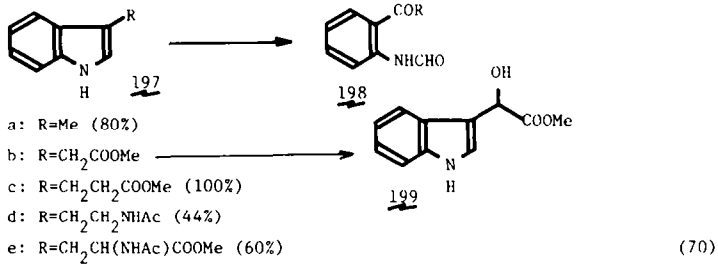

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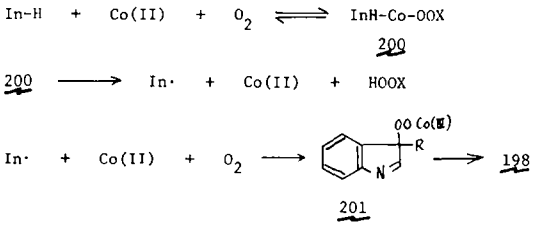


(71)

204 is considered important as one of the key steps leading to strychnine, and Iboga and Aspidosperma alkaloids (eqn 72).[214] The dioxygenation of **202** to a hydroperoxyindolenine **205** or its equivalent peroxide such as a zwitterionic peroxide **B** in eqn (60), may be a reasonable intermediate, which can undergo 2,3-bond cleavage to camptothecin-type alkaloids.

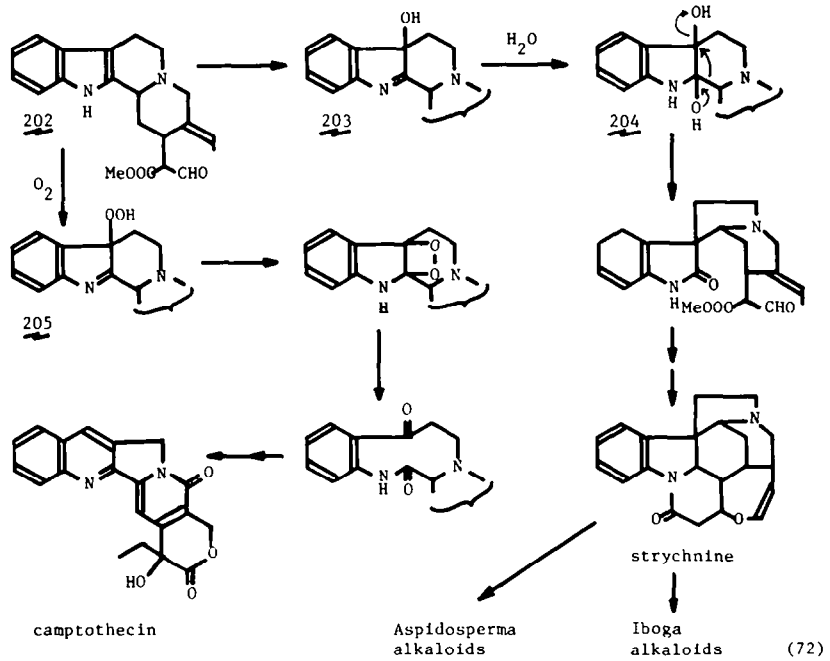
Winterfelt has shown that *t*-butoxide-catalyzed oxygenation of ajmalicin (**206**) in DMF gives a camptothecin-type product **207** (eqn 73).[215] The formation of **180** from **178** (eqn 65) implies that a similar oxygenation pathway may be possible for the biosynthesis of geneserine (**208**).[216] Nakagawa *et al.* have reported dye-sensitized photooxygenation of **209** followed by reduction with dimethyl sulfide to give **210** as a mimic for the biogenesis of brevianamide **E** (**211**).[217] Saito *et al.* have shown that reaction of N-acetyltetrahydrocarbazole (**212**) with singlet oxygen in aqueous media gives a dihydroxy compound **213** (eqn 74), which has a similar partial structure to that of the hypothetical intermediate **204** of eqn (71).[198]

Other biomimetic dioxygenations

Dioxygenation of unsaturated fatty acids has attracted particular attention because of its importance in the biosynthesis of prostaglandins.[218] Lipoxygenases, which occur widely in the plant kingdom, catalyzes the dioxygenation of unsaturated fatty acid containing a *cis,cis*-1,4-diene system to give *cis,trans*-diene hydroperoxides: for example, linoleic acid (**214**) gives 13 - hydroperoxy - *cis* - 9,*trans* - 11 - octadecadienoic acid (**215**) or 9 - hydroperoxy - *trans* - 10,*cis* - 12 - isomer **216** (eqn 75). The mode of reaction is analogous to that of "ene" reaction occurring between singlet oxygen and an olefin having allylic hydrogen.[65] Dye-sensitized photooxygenation of **214** has been shown to result in the nonselective peroxidation giving a complex mixture of hydroperoxides.[219]

Chan has found that in the presence of linoleic acid soybean lipoxygenase can catalyze oxygenation of **217** and **218**, which are known as good singlet oxygen acceptors, to give **219** and **220**, respectively, suggesting that an active oxygen species similar to singlet oxygen may be involved in the enzymatic reaction with the lipoxygenase (eqn 76).[220] However, reaction of **218** with singlet oxygen is known to give **221** but not **220**.

The isolation of prostaglandin endoperoxide (PGG_2; **222a**),[221] has made the biosynthetic pathway of prostaglandins starting from arachidonic acid (**223a**) more



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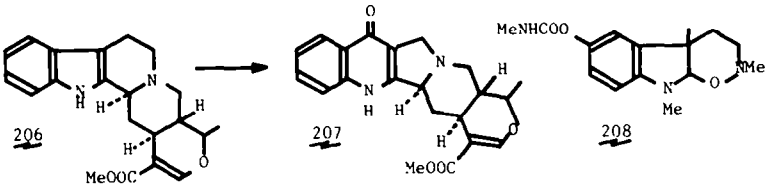

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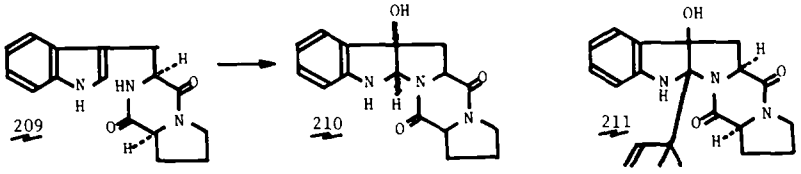

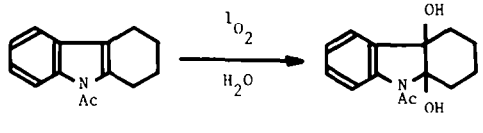

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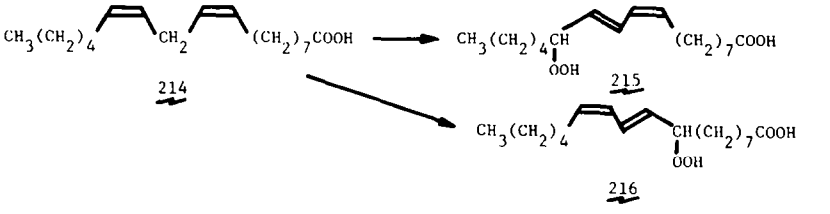
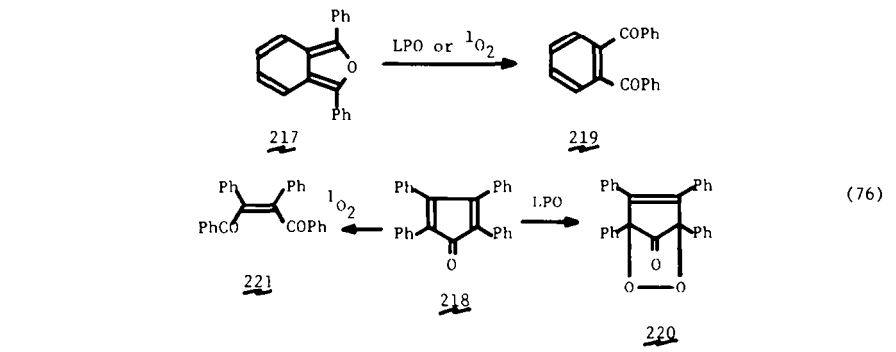


(75)

clear. In the first step, dioxygenation of **223** gives **222**, which undergoes several enzymatic transformations, for example leading to PGE_2(**224a**), thromboxane A_2 (**225a**), or cleavage to malondialdehyde (**226**) and an olefin **227a** (eqn 77). Because of its instability, the chemical properties of the endoperoxide (**222a**) are not well characterized. Coughlin and Salomon have recently reported a biomimetic reaction for the formation of **226** and **227** from **222**.[222] Thermolysis of an analog **228**, which can be synthesized by singlet oxygen reaction of a cyclopentadiene followed by diimide reduction, gives dibenzoylmethane (**229**) and ethylene in 10% yield (eqn 78). It has been suggested that the reaction occurs via a homolytic cleavage of the O—O bond followed by two



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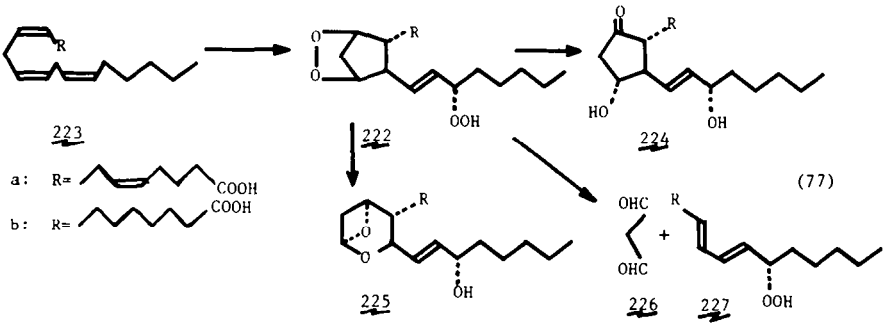

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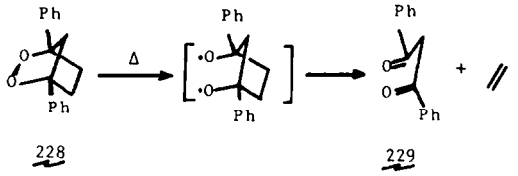
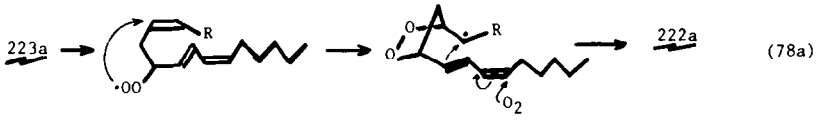


(78)

β-scission but not via a concerted [2+2+2] reaction, and that the transformation of **222** to **226** and **227** may be a catalytic process considering from a relatively high activation energy for the thermolysis of **228**. Pikes *et al.* have reported the chemical synthesis of **222b** and **224b** by the oxidation of linolenic acid (**223b**) with nascent oxygen (probably singlet oxygen),[223] although the yields are presumably very low.

As a mimic for a hypothetical mechanism of the formation of **222a** from **223a**, involving cyclization of an allylic peroxy radical followed by further attack of oxygen (eqn 78a), Porter *et al.* have reported reaction of a γ-hydroperoxy olefin with the *t*-butoxy radical (generated from di-*t*-butylperoxyoxalate at 25°) under oxygen giving an analog of **222**, possibly formed via a radical chain mechanism of eqn (78b).[224a] They have also carried out radical initiated oxygenation of γ-linolenic acid (**223b**). One of the products appears to be $PGF_{1\alpha}$ (α-OH instead of carbonyl in **224b**) based on the mass spectral analysis of trimethylsilylated products.[224b] Pryor and Stanley have also reported autoxidation of methyl linolenate initiated by ozone or nitrogen dioxide giving products positive to the tests characteristic of prostaglandin.[224c]

There are a large number of natural products which are considered to be biosynthesized from their precursors by the action of dioxygenases, although such enzymes are not yet characterized. Among them, various terpenoids have been synthesized using dye-sensitized photooxygenation from their possible *in vivo* precur-



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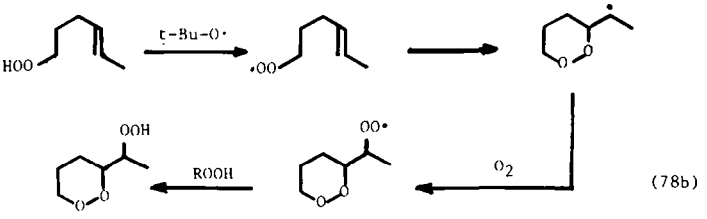


(78b)

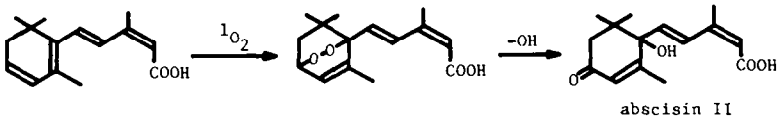
sor.[65,225] Typical examples shown below include the synthesis of abscisin II (eqn 79),[226,227] ergosterol peroxides (eqn 80),[228] mayurone (eqn 81),[229] dihydroactinidiolide,[230] loliolide,[231] and grasshopper ketone[231] (eqn 82). β-Carotene, which is known as an excellent quencher of singlet oxygen, is slowly oxidized by sensitized photooxygenation to give β-ionone and its further degradation products[232] or an oxygenated carotenoid **230** having an allene alcohol moiety analogous to neoxanthin (eqn 83).[233]

An enzymatic oxidation of β-carotene is known to give β-ionone and several unidentified products.[234] These examples of "biogenetic-type synthesis" imply that similar modes of oxygenation may occur in the biosynthesis of these natural products.

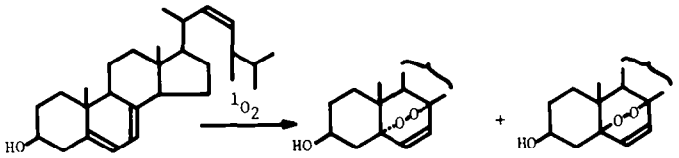
Bioluminescence. As shown in eqn (6), certain kinds of luciferases are regarded as dioxygenases. A large number of attempts have been made towards organic chemical approaches to this problem in recent years.[235] These concern with the formation of dioxetanes and other types of peroxide intermediate and their decomposition leading to an excited-state molecule.[236] Because of the availability of many reviews, the author does not include bioluminescence problems in this Report.

III. BIOMIMETIC MONO-OXYGENATION

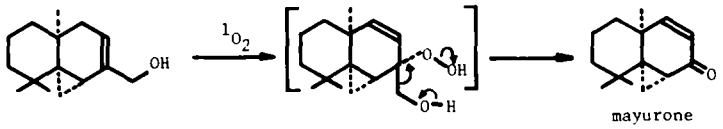
Several reviews have appeared on biomimetic mono-oxygenation as well as di-oxygenation.[16,17,163,237,238,243] As shown in eqn (3b), mono-oxygenases require a hydrogen or electron donor and catalyze the incor-



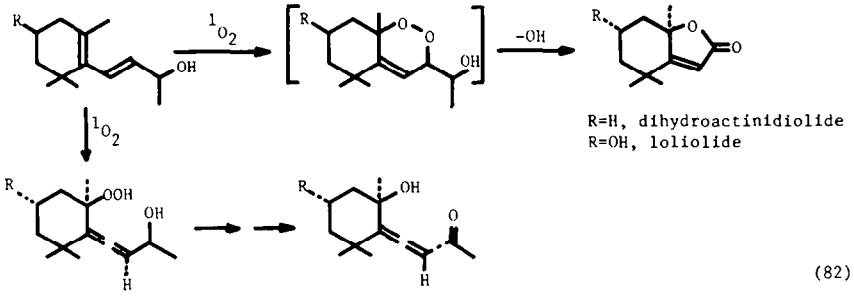
abscisin II (79)



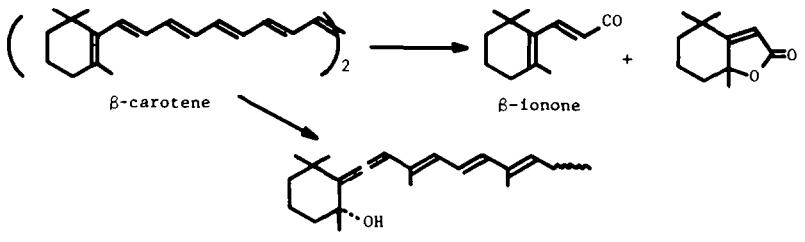
ergosterol (80)



mayurone (81)

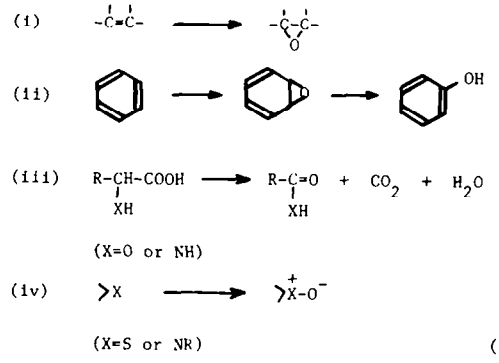


grasshopper ketone (82)



230 (83)

poration of one atom of molecular oxygen into a product. The major types of reaction are, (i) epoxidation of an olefinic bond (squalene epoxidase) and an aromatic ring, (ii) hydroxylation on an aliphatic carbon (steroid hydroxylase) and an aromatic ring (phenylalanine hydroxylase), the latter of which is considered to be preceded by aromatic epoxidation, (iii) oxidative decarboxylation of an α-amino and α-hydroxy acids (lysine mono-oxygenase), and (iv) mono-oxygenation on a heteroatom such as sulfur and nitrogen (eqn 84).



(84)

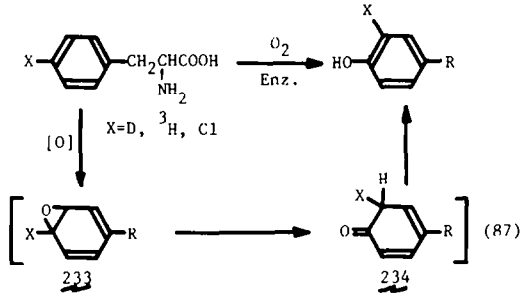
NAD(P)H, ascorbic acid, and α-keto acids usually act as hydrogen donor. Cofactors such as flavins and transition metals are involved. There may be two kinds of pathways for the enzymatic reactions; first, dioxygenation followed by reduction of a formed peroxide and secondly, direct mono-oxygenation by reactive mono-oxygen species.

α-Keto acid coupled oxygenase. A typical example of the first type is α-keto acid coupled oxygenases, such as γ-butyrobetaine hydroxylase, collagen proline hydroxylase, and thymine 7-hydroxylase, which are classified also as dioxygenase.[239] Lindblad *et al.* have found that one mole of α-ketoglurate is decarboxylated per mole of hydroxylated product to give succinic acid. They have suggested a mechanism of eqn (85) (for example, for betaine hydroxylation) involving an intermediate complex **231** between a hydroperoxide and α-ketoglutarate.[240] In fact, *t*-butyl hydroperoxide reacts with α-ketoglutaric acid to give *t*-butyl alcohol and succinic acid. It should be mentioned that *p*-hydroxyphenylpyruvate dioxygenase reaction (see eqn 16) involves an intramolecular trapping of the intermediate hydroperoxide by the α-keto acid side chain.

Hydroxylation and oxenoid mechanisms

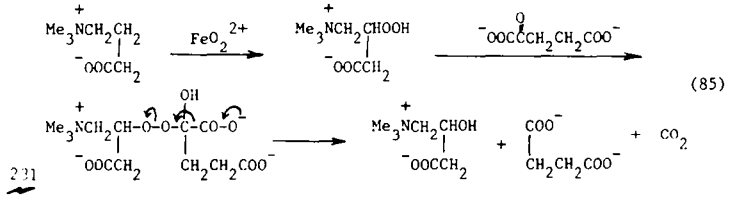
Much attention has been focused on aliphatic and aromatic hydroxylations catalyzed by certain mono-oxygenases. These reactions are considered to occur via the second type of pathway involving active mono-oxygen species, which can be formed by reduction of molecular oxygen. A number of mono-oxygen species such as the hydroxyl radical, the hydroxyl cation, atomic oxygen, metal–oxygen complexes and so on, have been previously considered as possible species. Mono-oxygen species equivalent to atomic oxygen are called "oxenoid", which was named by Hamilton,[242] and various model oxidation methods for enzymatic hydroxylations have been explored during the past two decades.[16,17,163,237,238]

NIH *shift.*[244–247] In the course of studies on phenylalanine hydroxylase from liver, NIH workers have found that the labeled hydrogen of 4-^{3}H-phenylalanine undergoes 1,2-shift to give 3-^{3}H-tyrosine in more than 90% yield, and that the chlorine atom of 4-chlorophenylalanine also undergoes 1,2-shift to give 3-chlorotyrosine (eqn 87).[248] For these reactions, a mechanism involving an arene oxide intermediate **233** has been proposed, which rearranges to **234**, a tautomer of the product phenol. In fact, it has been found that under physiological conditions arene oxides undergo rearrangement into phenols with simultaneous NIH shift.[249–252] The mechanism for oxygen activation by phenylalanine hydroxylase, which requires ferrous ion as cofactor and a tetrahydropteridine as hydrogen donor, is not yet established, although a tetrahydropteridine-Fe(II)-O_2 complex[253] or a tetrahydropteridine - 4a - hydroperoxide has been suggested as active intermediate (see bleow).[254]



(87)

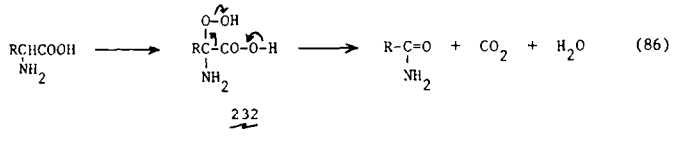
NIH shift has been employed for a diagnostic method for model oxenoid reagents to see whether or not NIH



(85)

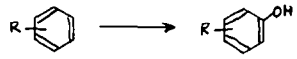
The oxidative decarboxylation of α-amino and α-hydroxy acids appears to involve a similar mechanism. Assuming an α-hydroperoxy intermediate **232**, which has been suggested in case of L-lysine monooxygenase,[241] a pathway of eqn (86) may account for the reaction.

shift occurs when they are used for model aromatic hydroxylation. However, it may be noted that enzymatic aromatic hydroxylation is not always accompanied by NIH shift; in other words, direct hydroxylation by a mono-oxygen species such as insertion into a C–H bond



(86)

Table 2. Aromatic hydroxylation models and NIH shift



Reagent	NIH shift	Ref.
$Fe(II)/H_2O_2$ (Fenton)	no	249
$Fe(II)/O_2$/ascorbic acid/EDTA (Udenfriend)	no	255
$Fe(II)/O_2$/reduced pteridine (Viscontini)	no	249
$Fe(III)/H_2O_2$/catechol (Hamilton)	no	249
$N_2O/Hg/h\upsilon$ [$O(^3P)$]	yes	256a
pyridine N-oxide/$h\upsilon$	yes	256
t-BuOOH/$Mo(CO)_6$	yes	287
9-Diazofluorene/$h\upsilon/O_2$	yes	293
CF_3CO_3H	yes	257
$CoO_2(OAc)_2$	yes	258
Reduced flavin/O_2	no	259

may occur in certain enzymatic reactions.[247]

Various reagents used in model reactions for the enzymatic aromatic hydroxylation have been tested for NIH shift. The results, which are summarized in Table 2, will be separately discussed in the subsequent chapters.

Hydroxyl radical. The hydroxyl radical, which was earlier considered as a possible mono-oxygen species, is formed by three-electron reduction of molecular oxygen or by one-electron reduction of hydrogen peroxide (eqn 88). Therefore, if it is involved in mono-oxygenases, a three-electron process should occur with molecular oxygen. Aromatic hydroxylation has been done by the

$$O_2 + e^- + H^+ \longrightarrow \cdot OOH$$

$$\cdot OOH + e^- + H^+ \longrightarrow HOOH$$

$$HOOH + e^- + H^+ \longrightarrow \cdot OH + H_2O \qquad (88)$$

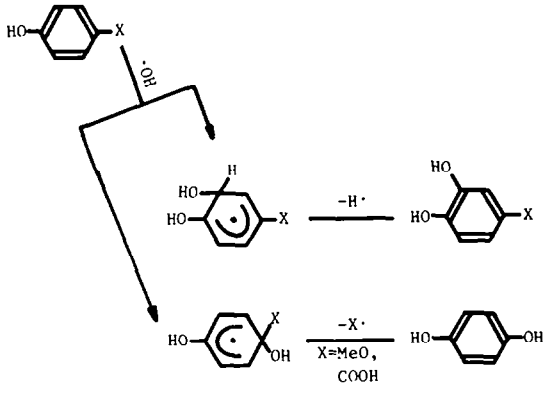
hydroxyl radical generated by the Fenton reagent ($Fe(II)/H_2O_2$)[262-264] or photolysis of hydrogen peroxide.[265-267] A characteristic difference has been observed between the hydroxyl radical and the Udenfriend reagent (see below) in the distribution of hydroxylated products of monosubstituted benzenes (eqn 89).[262]

	X=Cl	X=OMe	$X=NO_2$
o-%	42 (62)	84 (61)	24
m-%	29 (16)	0 (9)	30
p-%	29 (22)	16 (30)	46

(89)

In parenthesis: Udenfriend reagent.

When phenols are hydroxylated with hydroxyl radicals generated by photolysis of hydrogen peroxide, only ortho- and para-hydroxylations occur but not meta.[265-267] *p*-Methoxy- and *p*-carboxy-substituents in phenols are replaced by a hydroxy group forming hydroquinone with a concomitant formation of the corresponding catechols. The reactions have been interpreted in terms of a radical addition mechanism of eqn (90). A model mono-oxygenation reagent, reduced flavin and oxygen has been claimed to generate the hydroxyl radical (see below).

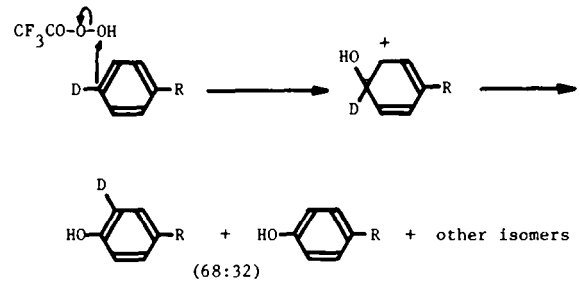


(90)

In summary, involvement of the hydroxyl radical in the enzymatic hydroxylation appears less likely, since no NIH shift is observed in the hydroxylation of *p*-^{3}H-acetanilide with the Fenton reagent[249] and the reduction of molecular oxygen into a hydroxyl radical by three-electron transfer (eqn 88) has no substantial evidence in the enzymatic reaction.

Hydroxyl cation. The hydroxyl cation (OH^+) has been often considered as a possible active species in enzymatic hydroxylations. Trifluoroperacetic acid, which is usually interpreted as a source of OH^+, is a good reagent for aliphatic and aromatic hydroxylation. Thus, 2-methylbutane gives four alcohols with predominant formation of a tertiary alcohol (>90%),[243] and substituted benzenes including acetanilide[268] and 4-deuteriotoluene[257,268] and hydroxylated into the corresponding phenols (eqn 91). Based on the observation of NIH shift (Table 2), a cationic intermediate has been proposed.

Tobinaga *et al.* has shown that potassium ferricyanide-H_2O_2 system can hydroxylate camphor and benzoic acid (eqn 92). They have suggested a hydroxyl cation in a complexed form may be a responsible oxygen species.[269]



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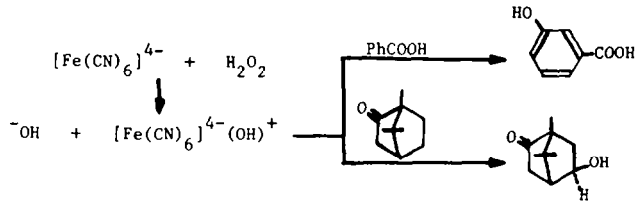


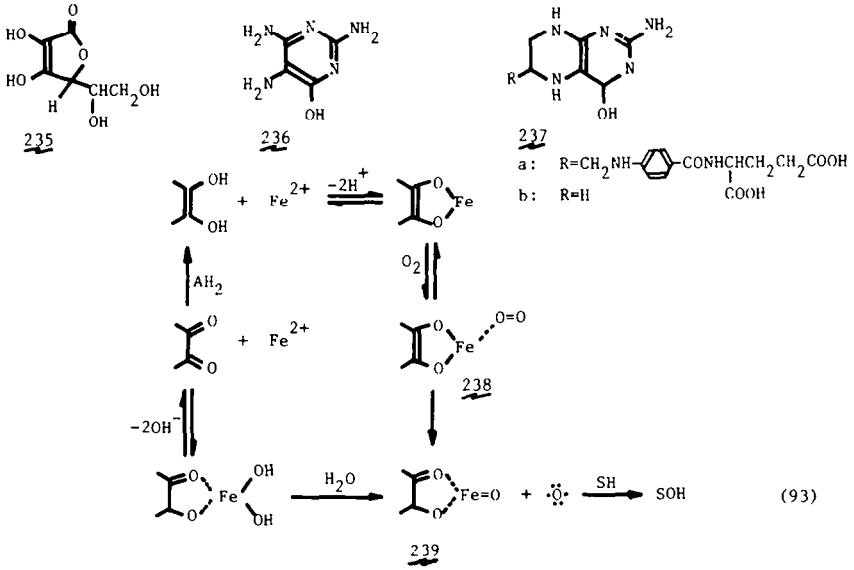
(92)

The hydroxyl cation is equivalent to the two-electron reduction stage of molecular oxygen and regarded as a protonated atomic oxygen. Although the hydroxyl cation or its complexed form appears to be one of the plausible active species in certain mono-oxygenase reactions, there has been no substantial chemical evidence for its existence and it still remains to be clarified.

Transition metal–oxygen systems. The first model system, using a transition metal ion, for enzymatic hydroxylation has been reported by Udenfriend *et al.* who have found that Fe^{2+}/EDTA/ascorbic acid/oxygen system (Udenfriend system) can hydroxylate aromatic nuclei; for example, tyrosine to 3,4-dihydroxy-phenylalanine and salicylic acid to dihydroxybenzoic acids.[270] Although the reagent was considered to generate the hydroxyl radical,[271,272] this has been disproved based on the facts that the product distribution in the hydroxylation of monosubstituted benzenes (eqn 89)[262] and cyclohexane[253,273] is quite different from that with the hydroxyl radical.

Hamilton *et al.* have found that for the Udenfriend system ascorbic acid (**235**) can be replaced by a diaminopurine **236**, a model compound for tetrahydrofolic acid (**237**), which is cofactor of phenylalanine hydroxylase. The product ratio ($o:m:p = 49:13:38$) of methoxyphenols from anisole is essentially the same as that (43:18:39) with the Udenfriend system.[253] The result shows that both systems are virtually the same in nature. Hamilton has proposed a mechanism involving the intermediary formation of a perferryl ion complex **238** from an enediol- (or enediamine-) Fe^{2+} complex and oxygen followed by its conversion into oxene (atomic oxygen) which oxidizes a substrate (SH) to a hydroxylated product (SOH) (eqn 93).[242] In the enzymatic reaction, the counterpart **239** of oxene will be finally reduced by a coupled reduction enzyme to accomplish a cycle.

A similar system has been reported by Viscontini who demonstrates that tetrahydropteridine **237b**/Fe^{2+}/EDTA/O_2 system can hydroxylate phenylalanine.[274] He has also shown that Fe^{2+} is replaced by Fe^{3+} and suggested a mechanism of eqn (94), where a complex **240** formally produces a hydroxyl radical.[275] Blair and Pearson have shown that Fe^{2+}/tetrahydrobiopterine/O_2 system also oxidizes phenylalanine into tyrosine with observing almost no NIH shift, and have suggested involvement of the perferryl ion (FeO_2^{2+}).[276]



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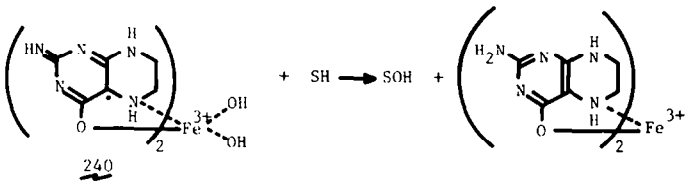
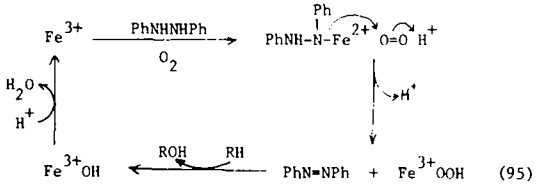


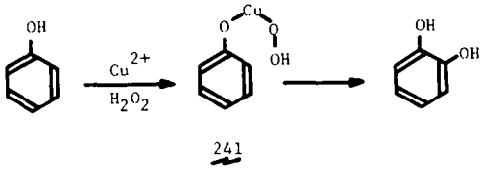
240 (94)

Lindsay-Smith *et al.* have reported a system, Fe^{3+}/N - benzyl - 1,4 - dihydronicotinamide (NBNH)/O_2, capable of aromatic hydroxylation and suggested a ternary complex NBNH–FeO_2^{3+} may be the active species.[277] Recently, Mimoun and de Roch have shown that a modified Udenfriend system consisting of $FeCl_2$, hydrazobenzene (or *o*-phenylenediamine), benzoic acid, and oxygen, can oxidize hydrocarbons such as cyclohexane giving cyclohexanol and cyclohexanone and toluene giving benzyl alcohol and cresols.[278] In the latter case, the product distribution is dependent on the nature of hydrogen donors: the ratio, benzyl alcohol:*o*-:*m*-:*p*-cresol is 38.5:30.5:29.8:1.2 with hydrazobenzene and 11.1:10.8:77.3:0.8 with *o*-phenylenediamine. They have suggested a mechanism involving a $Fe^{3+}OOH$ as active species (eqn 95). Other transition metal salts such as $FeCl_3$, $MnCl_2$, VCl_3, $CuCl_2$ and $NiCl_2$ are also effective.



(95)

Hydrogen peroxide also acts as oxidizing agent instead of molecular oxygen for aromatic hydroxylation in the presence of a transition metal ion having a higher valency. Brackman and Havinga have shown that either Cu^{2+}/H_2O_2 or Cu^{2+}/amine/O_2 system reacts with phenol to result in selective ortho-hydroxylation.[279] In the latter case the initial reduction of oxygen into hydrogen peroxide has been claimed, providing a mimic for tyrosinase which contains copper ions. An intramolecular hydroxylation of a ternary complex **241** has been suggested (eqn 96). Hamilton *et al.* have shown that Fe^{3+}/catechol/H_2O_2 system is used for aromatic hydroxylation.[280,281] Based on the facts that the nature of the reaction differs from that of the hydroxyl radical, they have suggested a mechanism involving a complex **242** which is transformed to an oxenoid species $Fe^{+} = O$ (eqn 97). The complex **242** is a species equivalent to $(FeOOH)^{2+}$ which is known to be formed from ferric ion and hydrogen peroxide.[282,283]

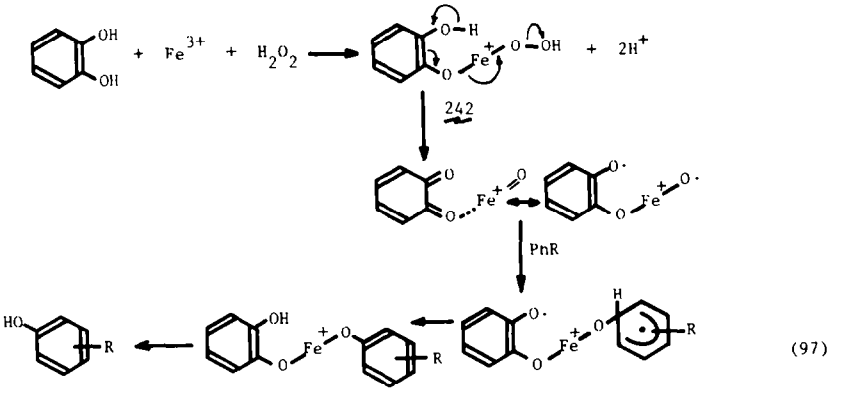


241 (96)

In the absence of a hydrogen donor, transition metal ions can also catalyze oxygenation of hydrocarbons leading to hydroxylation.[273,277,284,285] Staudinger and Ullrich have shown that acetophenetidine undergoes *o*-, *m*- and *p*-hydroxylation by oxygenating in the presence of Fe^{2+}-diphophonate (*o*:*m*:*p* = 39:29:32), Ti^{3+} (23:24:53), Cu^{+} and Sn^{2+}-phosphonate and suggested that these systems are essentially the same as the Udenfriend system (39:45:22).[273,285] They have also shown that Fe^{2}/2-mercaptobenzoate/O_2 system can hydroxylate aliphatic and aromatic hydrocarbons with a similar selectivities to that in rat liver microsome.[286] Lindsay-Smith *et al.* have carried out hydroxylation of various benzene derivatives with the same metal ions without a hydrogen donor.[277] Based on the facts that the ratio of *o*-, *m*- and *p*-hydroxylations is dependent on the nature of metal ions used, that the rate increases with increased concentration of a metal ion, and that no hydrogen peroxide is detected in the reaction system, they have proposed a mechanism involving perferryl ion (FeO_2^{2+}) at low metal concentrations or a $(FeO_2Fe)^{4+}$ complex at high concentrations, which adds to a benzene nucleus (eqn 98).

As described eqn (55), Nishinaga *et al.* have shown that Co(II)salpr–O_2 complex reacts with hindered phenols to give *p*- and *o*-peroxy cobalt complexes which have an analogy to the intermediate of eqn (98). The formation of the peroxy cobalt complexes served to mimic also enzymatic aromatic hydroxylation, if we assume that reduction with a hydrogen donor occurs after the formation of such a peroxy iron complex.

For the above biomimetic aromatic hydroxylations



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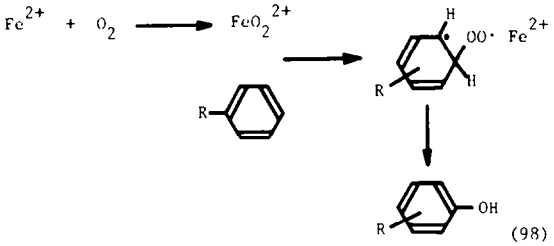
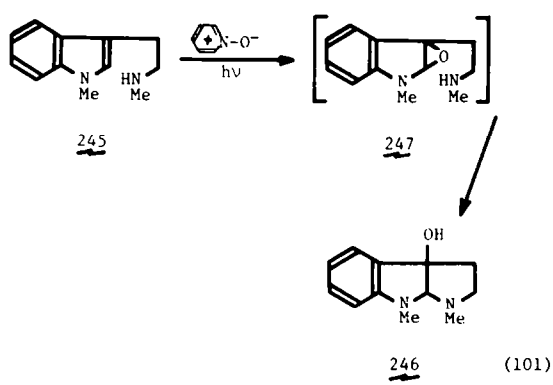


(98)

using transition metal catalysis, appreciable NIH shift has never been observed. This led to search for other model mono-oxygen species. Several model systems, which cause NIH shift (Table 2), have been developed. Sharpless and Flood have found that a chromyl complex **243** can oxidize 1-^{3}H-naphthalene to give 1,4-naphthoquinone retaining ^{3}H, probably via α-naphthol (eqn 99).[258] Based on this observation, they have suggested that a mono-oxygen species having a Fe=O partial structure such as **244a**, **244b** and **244c** may be responsible for the enzymatic hydroxylation, where an iron ion involved as cofactor. A hydroxylating reagent, *t*-butyl hydroperoxide/Mo(CO)$_6$ (Table 2),[287] has been also found to hydroxylate an aromatic ring with NIH shift.[256] However, the actual active species involved in this reaction is unknown.

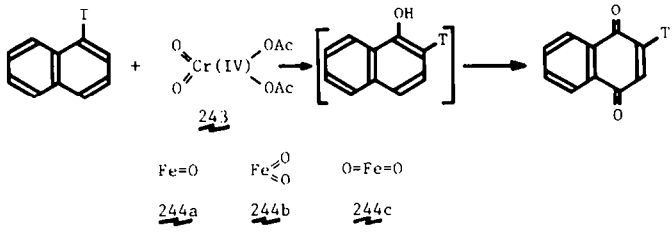


(101)

containing compounds as oxenoids, where a zwitterionic form X$^+$–O$^-$ contributes and X is relatively stable in either oxidized or reduced form making a mono-oxygenation reaction of eqn (102) exothermic.[17] Among

$$X{=}O + S \longrightarrow SO + X \qquad (102)$$

many candidates, sulfoxides, sulfones, arsine oxides, and amine oxides give no oxygen transfer at elevated temperatures.[291] However, several oxenoid reagents have been developed, including the chromyl complex **243**,



(99)

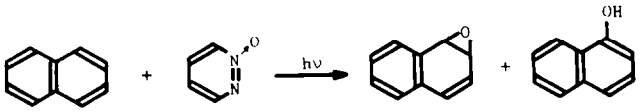
Other oxenoids. The simplest oxenoid so far known is the triplet oxygen atom [O(^{3}P)], which is generated by mercury-sensitized vapor phase photolysis of nitrous oxide. This species has been shown to oxidize aliphatic and aromatic hydrocarbons[273,288] to cause NIH shift in vapor phase aromatic hydroxylation.[293] Certain kinds of N-heteroaromatic N-oxides such as pyridine and pyridazine N-oxides, are believed to release atomic oxygen and have been used as hydroxylating or epoxidating reagents.[289] Jerina *et al.* have found that NIH shift is observable in the hydroxylation of 4-deuterioanisole by pyridine N-oxide photolysis (Table 2), and that photolysis of pyridazine N-oxide in the presence of naphthalene gives α-naphthol in addition to naphthalene 1,2-oxide (eqn 100), which is only an example of the isolation of an arene oxide by direct oxygenation process, albeit in poor yield.

N-heteroaromatic N-oxides, iodosobenzene, and ketone oxides.

Ullrich has shown that iodosobenzene (Ph–I=O) can act as an oxygen source in the hydroxylation by microsomal mono-oxygenase coupled with cytochrome P 450, suggesting that Fe^{3+}O may be the active species.[292] Hamilton *et al.* have reported two biomimetic mono-oxygenation reactions where a ketone oxide ($R_2C{=}O^+$–O$^-$) may play an important role.

Photolysis of diphenyldiazomethane in the presence of oxygen and cyclohexane gives cyclohexanol and cyclohexanone (eqn 103). A mechanism has been proposed to involve hydrogen abstraction by a ketone oxide intermediate **248**,[293] which has been later confirmed by its trapping with an aldehyde to give an ozonide **249**.[294]

Murray *et al.* have reported that Rose Bengal-sensitized oxygenation of the same diazo compound in the



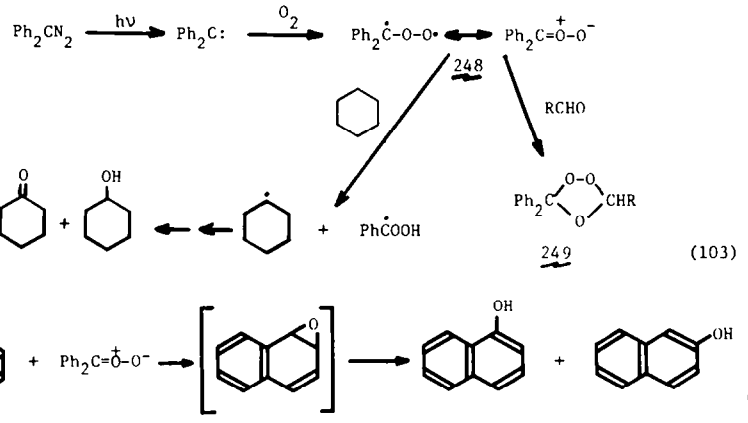
(100)

Nakagawa *et al.* have shown that photolysis of pyridine N-oxide in the presence of N-methyltryptamine (**245**) gives a pyrroloindole **246**, possibly via an unstable 2,3-epoxide **247**, providing a biogenetic synthesis of an indole alkaloid skeleton (eqn 101).[290,179]

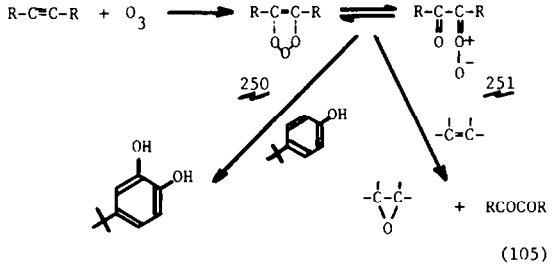
In searching for new oxenoid reagents, Hamilton has suggested the potential usefulness of X=O type oxygen-

presence of naphthalene gives a mixture of α- and β-naphthols (85:15) in 14% yield (eqn 104).[295] Naphthalene 1,2-oxide has been considered as intermediate,[295] since the product ratio is similar to that (88:12) from the decomposition of naphthalene 1,2-oxide in methylene chloride.[249]

The second type of ketone oxide generating system



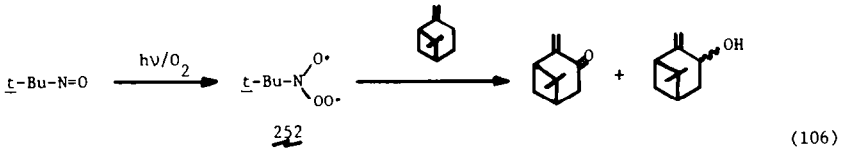
may be acetylene ozonides. An acetylene-ozone adduct **250**, which is tautomeric with a ketone oxide **251**, has been found by Keay and Hamilton to react with olefins and *p*-*t*-butylphenol to give epoxides and 4-*t*-butylcatechol, respectively (eqn 105).[296] A somewhat similar



type of mono-oxygenation system has been applied to oxidation of pinene using photooxygenation of a nitroso compound (eqn 106), for which an N-peroxy intermediate **252** is suggested.[297]

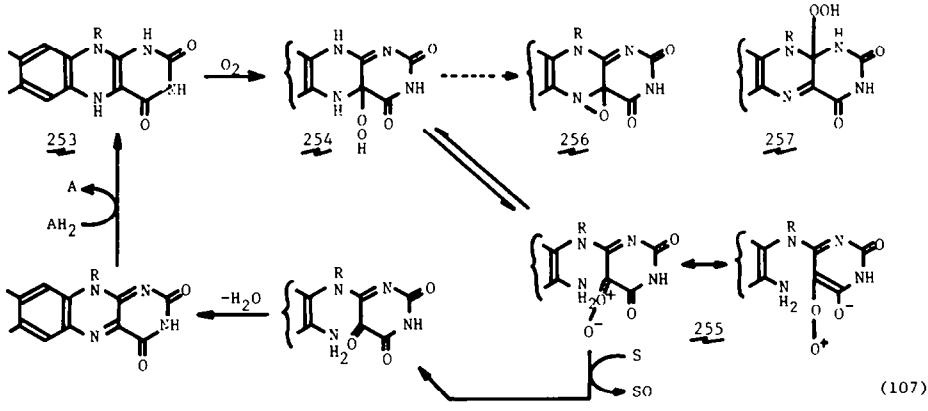
According to this mechanism, a reduced flavin **253** is first oxygenated to form 4a-hydroperoxide **254** followed by its tautomerization into a ketone oxide **255** which transfers an oxygen atom to a substrate (eqn 107). On the other hand, Orf and Dolphin have suggested an oxaziridine intermediate **256** as an oxenoid, based on MO calculations and assuming an oxaziridine intermediate in the photolytic deoxygenation of pyridine N-oxide (see eqn 101).[298]

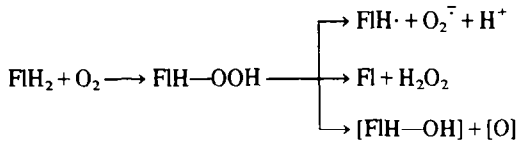
Reduced flavins are known to be very easily autoxidized without catalyst to give the parent flavins and hydrogen peroxide. Among extensive studies on the autoxidation reaction, Massay *et al.*[299,300] have suggested by kinetic studies that the first step of the autoxidation is the formation of a hydroperoxide (FlH–OOH) which follows three decomposition processes (eqn 108); namely, superoxide formation, direct decomposition into flavin (Fl) and hydrogen peroxide, and oxenoid formation in the presence of a substrate.[300] For the hydroperoxide, Mager and Berends suggest a 10a-hydroperoxy structure **257**,[301] while Hemmerich suggest 4a-hydroperoxy struc-



The ketone oxide mechanism has been suggested by Hamilton also for the hydroxylation catalyzed by flavin-requiring nonmetallic mono-oxygenases, such as salicylate hydroxylase and imidazole-acetate hydroxylase.[17]

ture **254**.[302] Although the latter hydroperoxide has been recently synthesized by chemical means,[303] the structure of the actual hydroperoxide intermediate in reduced flavin autoxidation still remains to be elucidated.





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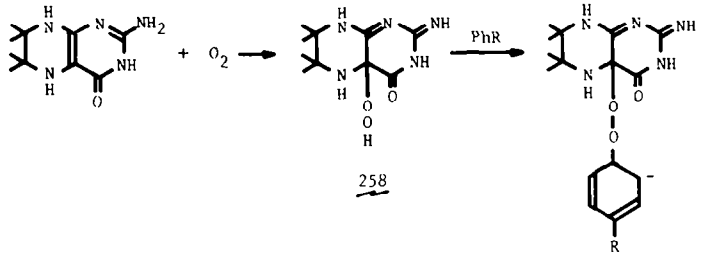

(109)

Mager and Behrends have reported that oxygenation of a reduced flavin (1,3,10-trimethyl-5,10-dihydroalloxazine) in the presence of phenylalanine gives tyrosine.[304] From kinetic studies of this reaction, they have proposed that the hydroxyl radical generated from a hydroperoxide **257** is the active axidizing species for the aromatic hydroxylation. Recently, Lindsay-Smith *et al.* have shown that the reduced 1,3,10 - trimethyl - 5,10 - dihydroalloxazine- or FMN-mediated oxygenation of phenylalanine to tyrosine does not cause appreciable NIH shift (Table 2).[259]

Tetrahydropteridines also behave like reduced flavins: thus their oxygenation in the presence of phenylalanine gives tyrosine.[304,305] A similar hydroperoxide intermediate **258** has been suggested to act as oxenoid in the nonenzymatic reaction as well as the enzymatic reaction such as phenylalanine hydroxylase (eqn 109).[306]

Acknowledgements—I would like to take this opportunity to express my gratitude to my collaborators whose names appear in this Report. It has been a pleasure to work with them.

REFERENCES

[1]O. Hayaishi, M. Katagiri and S. Rothberg, *J. Am. Chem. Soc.* **77**, 5450 (1955).
[2]H. S. Mason, W. L. Fowlks and E. Peterson, *Ibid.* **77**, 2914 (1955).
[3]O. Hayaishi, Ed., *Oxygenases.* Academic Press, New York (1962).
[4]O. Hayaishi and M. Nozaki, Eds., *Oxygenases.* University of Tokyo Press, Tokyo (1973).
[5]K. Bloch and O. Hayaishi, Eds., *Biological and Chemical Aspects of Oxygenases.* Maruzen, Tokyo (1966).
[6]O. Hayaishi, Ed., *Molecular Mechanisms of Oxygen Activation.* Academic Press, New York (1972).
[7]K. T. Yasunobu, H. F. Mower and O. Hayaishi, Eds., *Iron and Copper Proteins.* Plenum Press, New York (1976).
[8]I. Yamazaki, Ref. 6, p. 535.
[9]I. Fridovich, *Accounts Chem. Res.* **5**, 321 (1972); Ref. 6, p. 453.
[10]M. J. Cormier, J. E. Wampler and K. Hori, *Fortsch. Chem. org. Naturstoffe* **30**, 1 (1973).
[11]I. Fridovich, *Advan. Enzymol.* **41**, 35 (1975).
[12]I. Fridovich, *Ann. Rev. Biochem.* **44**, 147 (1975).
[13]M. Nozaki, Ref. 6, p. 135; and references cited therein.
[14]S. Senoh, H. Kita and M. Kamimoto, Ref. 6, p. 378.
[15]S. Senoh, *Seminar on Organic, Polymer, and Metal-Complex Reactions Related to Biological Reactions*, Nov. 1971, Kyoto; Abstracts of Papers, p. 23.
[16]G. A. Hamilton, In *Progress in Bioorganic Chemistry* (Edited by E. T. Kaiser and F. T. Kezdy), Vol. 1, p. 83. Wiley, New York (1971).
[17]G. A. Hamilton, Ref. 6, p. 405.
[18]P. von Mutzenbecher, *Z. Physiol. Chem.* **261**, 253 (1939).
[19]T. Matsuura and A. Nishinaga, *Nippon Kagaku Zasshi* **86**, 282 (1965).
[20]R. Pitt-Rivers, *Biochem. J.* **43**, 223 (1943).
[21]R. Pitt-Rivers and A. T. James, *Ibid.* **70**. 173 (1958).
[22]T. Matsuura and H. J. Cahnmann, *Biochim. Biophys. Acta* **29**, 216 (1958); *J. Am. Chem. Soc.* **81**, 871 (1959).
[23]*U.S. Pat.*, 2 889 363 (2 June 1959).
[24]H. J. Cahnmann and T. Matsuura, *J. Am. Chem. Soc.* **82**, 2050 (1960).
[25]T. B. Johnson and L. B. Tewkesbury, *Proc. Natl. Acad. Sci. U.S.A.* **28**, 73 (1942).
[26]C. R. Harrington, *J. Chem. Soc.* 193 (1944).
[27]M. Sela and S. Sarid, *Nature* **178**, 540 (1956).
[28]T. Matsuura, A. Nishinaga, K. Matsuo, K. Omura and Y. Oishi, *J. Org. Chem.* **32**, 3457 (1967).
[29]A. Taurog, *Recent Progress in Hormone Research* **26**, 189 (1970).
[30]G. Hillmann, *Z. Naturforsch.* **11b**, 424 (1956).
[31]R. I. Meltzer and R. J. Stanaback, *J. Org. Chem.* **26**, 1977 (1961).
[32]A. Nishinaga and T. Matsuura, *Ibid.* **29**, 1812 (1964).
[33]T. Shiba, H. J. Cahnmann, T. Matsuura, A. Nishinaga and H. Sakamoto, *Ibid.* **29**, 3061 (1964).
[34]T. Shiba and H. J. Cahnmann, *Ibid.* **27**, 1773 (1962).
[35]T. Shiba and H. J. Cahnmann, *Biochim. Biophys. Acta* **58**, 609 (1962).
[36]T. Shiba and H. J. Cahnmann, *J. Org. Chem.* **29**, 3171 (1964).
[37]T. Shiba and H. J. Cahnmann, *Ibid.* **29**, 1652 (1964).
[38]T. Shiba and H. J. Cahnmann, *Ibid.* **29**, 3063 (1964).
[39]K. Toi, G. Salvatore and H. J. Cahnmann, *Biochim. Biophys. Acta* **78**, 805 (1963); **97**, 523 (1965).
[40]T. Matsuura, H. Kon and H. J. Cahnmann, *J. Org. Chem.* **29**, 3058 (1964).
[41]H. J. Cahnmann and K. Funakoshi, *Biochemistry* **9**, 90 (1970).
[42]A. Nishinaga, H. Kon, H. J. Cahnmann and T. Matsuura, *J. Org. Chem.* **33**, 157 (1968).
[43]A. Nishinaga, H. J. Cahnmann, H. Kon and T. Matsuura, *Biochemistry* **7**, 388 (1968).
[44]A. Nishinaga and H. J. Cahnmann, *J. Chem. Soc. Perkin Transaction 1* 757 (1973).
[45]T. Matsuura, K. Omura and A. Nishinaga, *Chem. Commun.* 366 (1969); *Bull. Chem. Soc. Japan* **45**, 2866 (1972).
[46]H. J. Cahnmann and T. Shiba, *Biochem. Preparations* **10**, 176 (1963).
[47]G. Cilento, M. Nakano, H. Fukuyama, K. Suwa and I. Kamiya, *Biochem. Biophys. Res. Commun.* **58**, 296 (1974).
[48]J. E. Rall, J. Robbins and C. G. Lewallen, In *The Hormones* (Edited by G. Pincus *et al.*), Vol. V, p. 246. Academic Press, New York (1964).
[49]S. Lissitzky, M.-T. Bénévent, J. Nunez, C. Jacquemin and J. Roche, *Compt. Rend. Soc. Biol.* **154**, 267 (1966); and references cited therein.

[50]T. Matsuura, T. Nagamachi, A. Nishinaga, H. Kon and H. J. Cahnmann, *J. Org. Chem.* **34**, 2554 (1969).
[51]T. Matsuura, T. Nagamachi and A. Nishinaga, *Ibid.* **36**, 2016 (1971).
[52]A. Nishinaga, T. Nagamachi and T. Matsuura, *Chem. Commun.* 888 (1969).
[53]T. Nagamachi, A. Nishinaga and T. Matsuura, *Chemistry Letters* 111 (1972).
[54]T. Nagamachi, Ph.D. Thesis, Kyoto University (1970).
[55]S. Goodwin and B. Witkop, *J. Am. Chem. Soc.* **79**, 179 (1957).
[56]B. Lindblad, G. Lindstedt and S. Lindstedt, *Ibid.* **92**, 7446 (1970).
[57]T. Matsuura, K. Omura and R. Nakashima, *Bull. Chem. Soc. Japan* **38**, 1358 (1965).
[58]T. Matsuura, A. Nishinaga, K. Matsuo, K. Omura and Y. Oishi, *J. Org. Chem.* **32**, 3457 (1967).
[59]C. S. Foote, In *Free Radicals in Biology* (Edited by W. A. Pryor), Vol. 2. Academic Press, New York (1976).
[60]M. Thomas and C. S. Foote, unpublished data; M. Thomas, Ph.D. Thesis, UCLA.
[61]I. Saito, S. Kato and T. Matsuura, *Tetrahedron Letters* 239 (1970).
[62]I. Saito, N. Yoshimura, T. Arai, K. Omura, A. Nishinaga and T. Matsuura, *Tetrahedron* **28**, 513 (1972).
[63]T. Matsuura, H. Matsushima, S. Kato and I. Saito, *Ibid.* **28**, 5119 (1972).
[64]G. N. Grams, K. Eskins and G. Inglett, *J. Am. Chem. Soc.* **94**, 866 (1972).
[65]R. W. Denny and A. Nickon, *Organic Reactions* **20**, 133 (1973).
[66]I. Saito, M. Yamane, H. Shimazu, T. Matsuura and H. J. Cahnmann, *Tetrahedron Letters* 64 (1975).
[67]I. Saito, Y. Chujo, H. Shimazu, M. Yamane, T. Matsuura and H. J. Cahnmann, *J. Am. Chem. Soc.* **97**, 5272 (1975).
[68]C. Nakai, M. Nozaki, O. Hayaishi, I. Saito and T. Matsuura, *Biochem. Biophys. Res. Commun.* **67**, 590 (1975).
[69]J. Schweizer, R. Lattrell and E. Hecker, *Experientia* 31, 1267 (1975).
[70]K. Taniguchi, T. Kappe and M. D. Armstrong, *J. Biol. Chem.* **239**, 3389 (1964).
[71]G. A. Hamilton, In *Progress in Bioorganic Chemistry* (Edited by E. T. Kaiser and F. J. Kezdy), Vol. 1, p. 83. Wiley, New York (1971).
[72]I. Saito, H. Shimazu, T. Matsuura, C. Nakai, M. Nozaki, O. Hayaishi and H. J. Cahnmann, *4th Symposium on Organic Reaction with Enzyme Functions*, Tokyo (1976).
[73]For example, see: C. S. Foote, S. Mazur, P. A. Burns and D. Lerdal, *J. Am. Chem. Soc.* **95**, 586 (1973).
[74]An mechanism involving a 1,4-endoperoxide has also been proposed, but it is less likely because of the lack of exchange of the ^{18}O-labeled phenolic function with water during enzymatic reaction: A. H. Soloway, *J. Theor. Biochem.* **13**, 100 (1966); A. S. Widman, A. H. Soloway, R. L. Stern and M. M. Bursey, *Bioorganic Chem.* **2**, 176 (1973).
[75]M. Ogura, G. A. Cordell and N. R. Farnsworth, *Chem. Ber.* **109**, 2017 (1976).
[76]G. M. Sharma and P. R. Burkholder, *Tetrahedron Letters* 4147 (1967).
[77]G. M. Sharma, B. Vig and P. R. Burkholder, *J. Org. Chem.* **35**, 2823 (1970).
[78]L. Minale and G. Sodano, *J. C. S. Chem. Commun.* 674 (1972).
[79]R. J. Andersen and D. J. Folkner, *Tetrahedron Letters* 1175 (1973).
[80]G. E. Krejcarek, R. M. White, L. R. Hager, W. O. McClure, R. D. Johnson, K. L. Reinhart, Jr., P. D. Shaw and R. C. Brusca, *Ibid.* 507 (1975).
[81]D. W. S. Westlake, G. Talbot, E. R. Blakely and F. J. Simpson, *Can. J. Microbiol.* **5**, 62 (1959); F. J. Simpson, G. Talbot and D. W. S. Westlake, *Biochem. Biophys. Res. Commun.* **2**, 621 (1959).
[82]S. Hattori and I. Noguchi, *Nature* **184**, 1145 (1959).
[83]H. Sakamoto, *Seikagaku (J. Japan Biochem. Soc.)* **35**, 633 (1963).
[84]T. Oka, H. G. Krishnamurty, J. Child and F. J. Simpson, Abstracts of *19th Annual Meeting, Canadian Society of Microbiology, Ottawa*, p. 34 (1964).
[85]T. Oka, H. G. Krishnamurty and F. J. Simpson, *Can. J. Microbiol.* **18**, 893 (1972).
[86]T. Matsuura, H. Matsushima and H. Sakamoto, *J. Am. Chem. Soc.* **89**, 6370 (1967).
[87]T. Matsuura, H. Matsushima and R. Nakashima, *Tetrahedron* **26**, 435 (1970).
[88]H. D. Becker, *J. Org. Chem.* **30**, 989 (1965).
[89]A. Nishinaga and T. Matsuura, *J. C. S. Chem. Commun.* 9 (1973).
[90]C. G. Nordstrom and C. Majani, *Suomen Kem.* **B38**, 239 (1965).
[91]L. Jurd and R. M. Horowitz, *J. Org. Chem.* **22**, 1618 (1957).
[92]C. G. Nordstrom, *Suomen Kem.* **B41**, 351 (1968).
[93]C. G. Nordstrom, R. Hakalax and L. J. Karumaa, *Ibid.* **B36**, 102 (1963).
[94]A. Nishinaga, T. Tojo, H. Tomita and T. Matsuura, *18th Symposium on the Chemistry of Natural Products*, Kyoto (1974), Abstracts of Papers, p. 241.
[95]A. Nishinaga, T. Tojo and T. Matsuura, *J. C. S. Chem. Commun.* 896 (1974).
[96]C. Floriani and F. Calderazzo, *J. Chem. Soc.* (A), 946 (1969).
[97]S. Koda, A. Misono and Y. Uchida, *Bull. Chem. Soc. Japan* **43**, 3143 (1970).
[98]H. Kon and K. B. Sharpless, *Spectroscopy Letters* **1**, 49 (1968); E. W. Abel, J. M. Pratt and R. Whelan, *Chem. Commun.* 449 (1971); G. N. Schrauzer and L. P. Lee, *J. Am. Chem. Soc.* **92**, 155 (1970); D. V. Stynes, H. C. Stynes and J. A. Ibers, *Ibid.* **95**, 1142 (1973).
[99]A. C. Waiss, Jr. and J. Corse, *Ibid.* **87**, 2068 (1965); A. C. Waiss, Jr., R. E. Lundin, A. Lee and J. Corse, *Ibid.* **89**, 6213 (1967).
[100]T. Matsuura and H. Matsushima, *Tetrahedron* **24**, 6615 (1968).
[101]K. Kieslich, *Microbial Transformations of Non-Steroid Cyclic Compounds*. Georg Thime Publ., Stuttgart (1976).
[102]S. Senoh, *Nippon Kagaku Zasshi* **86**, 1087 (1965).
[103]O. Hayaishi, *Bacteriol. Rev.* **30**, 720 (1966); *Ann. Rev. Biochem.* **38**, 21 (1969).
[104]I. Saito and T. Matsuura, In *The Chemistry of Singlet Oxygen* (Edited by R. Murray and H. H. Wasserman). Academic Press, New York (1977).
[105]K. Gollnick and G. O. Schenck, In *1,4-Cycloaddition Reaction* (Edited by J. Hamer), p. 255. Academic Press, New York (1967).
[106]J. E. Baldwin, H. H. Basson and H. Krauss, Jr., *Chem. Commun.* 964 (1968).
[107]T. Wilson, *Photochem. Photobiol.* **10**, 441 (1969).
[108]J. Rigaudy, *Pure Appl. Chem.* **16**, 169 (1968).
[109]J. Rigaudy, C. Deletang, D. Sparfel and N. K. Cuong, *Compt. Rend. Acad. Sci.* **267**, C, 1714 (1968); **276**, C, 1215 (1973).
[110]J. Rigaudy, A. Defoin and N. K. Cuong, *Ibid.* **271**, C, 1258 (1970).
[111]G. Rio and J. Berthelot, *Bull. Soc. Chim. Fr.* 827 (1972).
[112]I. Saito and T. Matsuura, *Tetrahedron Letters* 4987 (1970); I. Saito, M. Imuta and T. Matsuura, *Tetrahedron* **28**, 5307 (1972).
[113]I. Saito, M. Imuta and T. Matsuura, *Ibid.* **28**, 5313 (1972).
[114]I. Saito, S. Abe, Y. Takahashi and T. Matsuura, *Tetrahedron Letters* 4001 (1974).
[115]T. Matsuura, A. Nishinaga, N. Yoshimura, T. Arai, K. Omura, H. Matsushima, S. Kato and I. Saito, *Ibid.* 1673 (1969).
[116]I. Saito, S. Kato and T. Matsuura, *Ibid.* 239 (1970).
[117]T. Matsuura, H. Matsushima, S. Kato and I. Saito, *Tetrahedron* **28**, 5119 (1972).
[118]T. Matsuura, N. Yoshimura, A. Nishinaga and I. Saito, *Tetrahedron Letters* 1669 (1969).
[119]T. Matsuura, N. Yoshimura, A. Nishinaga and I. Saito, *Tetrahedron* **28**, 4933 (1972).
[120]C. S. Foote, In *Free Radicals in Biology* (Edited by W. A. Pryor), Vol. 2. Academic Press, New York (1976).
[121]M. Thomas and C. S. Foote, unpublished data; M. Thomas, Ph.D. Thesis, UCLA.
[122]M. Nakano, T. Noguchi, K. Sugioka, H. Fukuyama, M. Sata, Y. Shimizu, Y. Tsuji and H. Inaba, *J. Biol. Chem.* **250**, 2404 (1975).
[123]K. Sugioka and M. Nakano, *Biochim. Biophys. Acta* **423**, 203 (1976).

[124]M. Nakano, K. Takayama, Y. Shimizu, Y. Tsuji, H. Inaba and T. Migita, *J. Am. Chem. Soc.* **98**, 1974 (1976).
[125]E. A. Mayeda and A. J. Bard, *Ibid.* **95**, 6223 (1973).
[126]A. Nishinaga, P. Ziemek and T. Matsuura, *J. Chem. Soc.* C, 2613 (1970); A. Nishinaga, H. Hayashi and T. Matsuura, *Chemistry Letters* 141 (1973); A. Nishinaga, H. Hayashi and T. Matsuura, *13th Symposium on Organic Free Radical Reactions*, Kyoto, 1972, Abstracts of Papers, p. 47.
[127]A. Nishinaga, T. Shimizu, H. Tomita and T. Matsuura, unpublished data.
[128]V. H. Schulze and W. Flaig, *Liebig's Ann. Chem.* **573**, 231 (1952).
[129]R. R. Grinstead, *Biochemistry* **3**, 1308 (1964).
[130]T. W. Campbell, *J. Am. Chem. Soc.* **73**, 4190 (1951).
[131]F. Stitt, G. F. Bailey, G. B. Coppinger and T. W. Campbell, *Ibid.* **76**, 3642 (1954).
[132]A. Nishinaga, T. Itahara and T. Matsuura, *Bull. Chem. Soc. Japan* **47**, 1811 (1974).
[133]A. Nishinaga, T. Itahara and T. Matsuura, *Ibid.* **49**, 3353 (1976).
[134]A. Nishinaga, T. Itahara and T. Matsuura, *Chemistry Letters* 667 (1974).
[135]A. Nishinaga, T. Itahara and T. Matsuura, *Tetrahedron Letters* 4481 (1974).
[136]A. Nishinaga, T. Itahara and T. Matsuura, *Angew. Chem.* **87**, 386 (1975).
[137]A. Nishinaga, T. Itahara and T. Matsuura, *Bull. Chem. Soc. Japan* **48**, 1683 (1975).
[138]A. Nishinaga, T. Itahara, T. Shimizu and T. Matsuura, *Tetrahedron Letters* 2467 (1976).
[139]A. Nishinaga, T. Itahara, T. Matsuura, S. Berger, G. Henes and A. Rieker, *Chem. Ber.* **109**, 1530 (1976).
[140]For example, M. S. Kharasch and B. S. Joshi, *J. Org. Chem.* **22**, 1439 (1957); H. R. Gersman and A. F. Bickel, *J. Chem. Soc.* 2711 (1959); 2356 (1962); J. E. Dunbar, M. W. Lansford, R. L. Redrotti, F. M. Scheidt, F. G. Lee and E. C. Smith, *J. Org. Chem.* **24**, 1251 (1959); A. Fairbourn and E. A. C. Lecken, *Proc. Chem. Soc.* 67 (1960); J. J. Conradi and G. A. McLarew, *J. Am. Chem. Soc.* **82**, 4745 (1960); and references cited therein.
[141]A. Closse, R. Mauli and H. P. Sigg, *Helv. Chim. Acta* **49**, 204 (1966); G. Read and L. C. Vining, *Chem. Ind.* 1239 (1962); S. Marumo, K. Sasaki and S. Suzuki, *J. Am. Chem. Soc.* **86**, 4507 (1964).
[142]A. Nishinaga, K. Nishizawa, H. Tomita and T. Matsuura, *Synthesis* 270 (1977).
[143]G. A. Russell, A. G. Bemis, E. J. Geels, E. G. Janzen and A. J. Moqe, *Advan. Chem. Ser.* **75**, 174 (1968).
[144]R. K. Haynes and H. Musso, *Chem. Ber.* **107**, 3723 (1974).
[145]A. Nishinaga, T. Shimizu and T. Matsuura, *Chemistry Letters* 547 (1977).
[146]N. J. Turro, V. Ramamurthy, D.-C. Liu, A. Krebs and R. Kemper, *J. Am. Chem. Soc.* **98**, 6758 (1976); N. J. Turro, Y. Ito, M.-F. Chow and W. Adam, unpublished data.
[147]A. Nishinaga, T. Itahara, T. Matsuura, A. Rieker and D. Koch, *Angew. Chem.* **88**, 154 (1976); A. Nishinaga, T. Itahara and T. Matsuura, *Synthesis* 604 (1976).
[148]A. Nishinaga and A. Rieker, *J. Am. Chem. Soc.* **98**, 4667 (1976).
[149]A. Nishinaga, T. Itahara, M. Hibi and T. Matsuura, *Synthesis* 553 (1976).
[150]A. Nishinaga, K. Nakamura, K. Yoshida, S. Hara and T. Matsuura, unpublished data.
[151]H. Musso, *Angew. Chem.* **75**, 965 (1963); H. Musso, In *Oxidative Coupling of Phenols* (Edited by W. I. Taylor and A. R. Battersby), p. 1. Dekker, New York (1967); H. Musso, *Liebig's Ann. Chem.* **689**, (1965); H. Musso and D. Bormann, *Chem. Ber.* **98**, 2774 (1965); R. Zinker and H. Musso, *Liebig's Ann. Chem.* **717**, 73 (1968).
[152]See references cited in Refs. 9, 11 and 12.
[153]T. Matsuura, *Kagaku* **28**, 1106 (1973); also see references cited in Ref. 154.
[154]Y. Moro-oka and C. S. Foote, *J. Am. Chem. Soc.* **98**, 1510 (1976).
[155]A. U. Khan, *Science* **168** (1970).
[156]J. W. Peters and C. S. Foote, *J. Am. Chem. Soc.* **98**, 873 (1976); and references cited therein.
[157]C. A. Tyson and A. E. Martell, *J. Am. Chem. Soc.* **90**, 3379 (1968); **94**, 936 (1972).
[158]J. Tsuji and H. Takayanagi, *Ibid.* **96**, 7349 (1974).
[159]J. Tsuji, H. Takayanagi and I. Sakai, *Tetrahedron Letters* 1245 (1975).
[160]J. Tsuji and H. Takayanagi, *Ibid.* 1365 (1976).
[161]M. M. Rogić, T. R. Demnin and W. B. Hammond, *J. Am. Chem. Soc.* **98**, 7441 (1976).
[162]H. Takahashi, T. Kajimoto and J. Tsuji, *Synthetic Commun.* **2**, 181 (1972).
[163]For reviews, see: A. Nishinaga, J. Tsuji and T. Matsuura, In *Enzyme-Simulated Organic Reactions II* (Edited by Z. Yoshida and W. Tagaki), (Kagaku no Ryoiki Zokan 113), Nankodo, Tokyo, p. 27 (1976); A. Nishinaga, *J. Synth. Org. Chem. Japan* **34**, 856 (1975).
[164]L. H. Vogt, Jr., H. M. Faigenbaum and S. E. Wiberley, *Chem. Rev.* **63**, 269 (1963); R. G. Wilkins, *Advan. Chem. Ser.* **100**, 111 (1971).
[165]A. Nishinaga, In *Bioinorganic Chemistry* (Kagaku Zokan 61), p. 179. Kagaku Dojin, Kyoto (1974).
[166]H. G. Olive and S. Olive, *Angew. Chem.* **86**, 1 (1974).
[167]B. M. Hoffman, D. L. Diemente and F. Basolo, *Chem. Commun.* 467 (1970).
[168]R. Nakon and A. E. Martell, *Inorg. Chem.* **11**, 1002 (1972); G. N. Schrauzer and L. P. Lee, *J. Am. Chem. Soc.* **92**, 1551 (1970).
[169]T. Matsuura, K. Watanabe and A. Nishinaga, *Chem. Commun* 163 (1970).
[170]A. Nishinaga, K. Watanabe and T. Matsuura, *Tetrahedron Letters* 1291 (1974).
[171]A. Nishinaga, T. Nakajima and T. Matsuura, *27th Meeting of Chem. Soc. Japan*, 1972, Abstracts of Papers I, p. 422.
[172]Cf. Ref. 174.
[173]A. Nishinaga, K. Shimizu, H. Tomita and T. Matsuura, unpublished data.
[174]A. Nishinaga, K. Nishizawa, H. Tomita and T. Matsuura, *J. Am. Chem. Soc.* **99**, 1278 (1977).
[175]A. Nishinaga, K. Nishizawa, H. Tomita and T. Matsuura, *Synthesis* 270 (1977).
[176]P. Feigelson and F. O. Brady, Ref. 6, p. 87.
[177]F. Hirata, O. Hayaishi, T. Tokuyama and S. Senoh, *J. Biol. Chem.* **249**, 1311 (1974).
[178]F. Hirata and O. Hayaishi, *Ibid.* **250**, 5960 (1975); and references cited therein.
[179]A. Ek, H. Kissman, J. B. Patrick and B. Witkop, *Experientia* **8**, 36 (1952).
[180]R. J. Sundberg, *Chemistry of Indoles*, p. 282. Academic Press, New York (1970).
[181]G. A. Hamilton, *Advan. Enzymol.* **32**, 55 (1969).
[182]G. A. Hamilton, private communication.
[183]B. Witkop, *Liebig's Ann. Chem.* **558**, 103 (1947).
[184]F. Sakiyama and N. Masuda, *Chemistry Letters* 949 (1973).
[185]J. Tabone, N. Mamounas and D. Robert, *Bull. Soc. Chim. Biol.* **33**, 1560 (1951).
[186]Z. Yoshida and M. Kato, *J. Am. Chem. Soc.* **76**, 311 (1954); *Nippon Kagaku Zasshi* **75**, 106, 109, 112 (1954).
[187]U. Gallo and L. Santamaria, Eds., *Research Progress in Organic, Biological, and Medicinal Chemistry*, Vol. II. American Elsevier, New York (1972).
[188]J. D. Spikes and R. Livigton, *Advan. Radiation Biol.* **3**, 29 (1969).
[189]J. D. Spikes and M. L. MacKnight, *Ann. N.Y. Acad. Sci.* **171**, 149 (1970).
[190]I. H. Leaver and G. Caird Ramsay, *Photochem. Photobiol.* **9**, 531 (1969).
[191]For a review, see: T. Matsuura and I. Saito, In *Photochemistry of Heterocyclic Compounds* (Edited by O. Buchardt), p. 456. Wiley, New York (1976).
[192]I. Saito, T. Matsuura, M. Nakagawa and T. Hino, *Accounts Chem. Res.* in press.
[193]For example, see: H. H. Wasserman and S. Terao, *Tetrahedron Letters* 1735 (1975).
[194]For example, see: C. S. Foote, A. A. Dzakpasu and J. W.-P. Lin, *Tetrahedron Letters* 1247 (1975); T. Matsuura and I. Saito, *Ibid.* 3273 (1968).

[195]W. Ando, T. Saiki and T. Migita, *J. Am. Chem. Soc.* **97**, 5028 (1975).
[196]M. J. S. Dewar and W. Thiel, *Ibid.* **97**, 3978 (1975).
[197]I. Saito, M. Imuta and T. Matsuura, *Chemistry Letters* 1173 (1972).
[198]I. Saito, M. Imuta and T. Matsuura, *Ibid.* 1197 (1972).
[199]I. Saito, M. Imuta, S. Matsugo, H. Yamamoto and T. Matsuura, *Synthesis* 225 (1976).
[200]I. Saito, M. Imuta, S. Matsugo and T. Matsuura, *J. Am. Chem. Soc.* **97**, 7191 (1975).
[201]I. Saito, M. Imuta, S. Matsugo, Y. Takahashi and T. Matsuura, *Ibid.* **99**, 2005 (1977).
[202]I. Saito, Y. Takahashi, M. Imuta, S. Matsugo, H. Kaguchi and T. Matsuura, *Heterocycles* **5**, 53 (1976).
[203]M. Nakagawa, T. Kaneko, K. Yoshikawa and T. Hino, *J. Am. Chem. Soc.* **96**, 624 (1974).
[204]M. Nakagawa, K. Yoshikawa and T. Hino, *Ibid.* **97**, 6496 (1975).
[205]M. Nakagawa, H. Okajima and T. Hino, *Ibid.* **98**, 635 (1976).
[206]M. Nakagawa, H. Okajima and T. Hino, *Ibid.* **99**, 4424 (1977).
[207]M. Nakagawa, H. Okajima, J. Chiba, H. Watanabe and T. Hino, unpublished data.
[208]M. Nakagawa and T. Hino, *J. Synth. Org. Chem. Japan* **35**, 2 (1977).
[209]I. Saito, A. Nakata, M. Imuta and T. Matsuura, 39*th Meeting of Chem. Soc.* Japan, Osaka (1977).
[210]A. Nishinaga, *Chemistry Letters* 273 (1975).
[211]A. Nishinaga, *Shokubai* **17**, 133 (1975).
[212]B. Witkop, J. B. Patrick and M. Rosenblum, *J. Am. Chem. Soc.* **73**, 2641 (1951).
[213]B. Witkop, J. B. Patrick and H. M. Kissman, *Chem. Ber.* **85**, 949 (1952).
[214]For a review, see: A. I. Scott, *Accounts Chem. Res.* **3**, 151 (1970).
[215]E. Winterfelt, *Liebig's Ann. Chem.* **745**, 23 (1971).
[216]C. Hootelé, *Tetrahedron Letters* 2713 (1969).
[217]T. Hino, J. Saegusa and M. Nakagawa, 96*th Meeting of Pharm. Soc. Japan*, Nagoya, 1976, Abstracts of Papers II, p. 82.
[218]For reviews, see: M. Hamberg, B. Samuelsson, T. Björkhem and H. Danielsson, Ref. 6, p. 30; P. Crabbé, *Chem. in Britain* **11**, 132 (1975).
[219]D. Cobern, J. S. Hobbs, R. A. Lucas and D. J. Mackenzie, Jr., *J. Chem. Soc.* **C**, 1897 (1966); G. E. Hall and D. G. Roberts, *Ibid.* **B**, 1109 (1966).
[220]H. W. S. Chan, *J. Am. Chem. Soc.* **93**, 2357, 4632 (1971). His earlier claims on the isolation of compound **220** are incorrect [J. E. Baldwin, J. C. Swallow and H. W. S. Chan, *Chem. Commun.* 1407 (1971)].
[221]M. Hamberg, J. Svensson and B. Samuelsson, *Proc. Nat. Acad. Sci., U.S.A.* **72**, 2994 (1975).
[222]D. J. Coughlin and R. G. Salomon, *J. Am. Chem. Soc.* **99**, 655 (1977).
[223]J. E. Pike and W. P. Schneider, *S. Africa Pat.* 6803391; *Chem. Abstr.* **72**, 66480z (1970).
[224a]M. Funk, R. Isaac and N. A. Porter, *J. Am. Chem. Soc.* **97**, 1281 (1975); [b]N. A. Porter and M. O. Funk, *J. Org. Chem.* **40**, 3614 (1975); [c]W. A. Pryor and J. P. Stanley, *Ibid.* **40**, 3615 (1975).
[225]P. G. Sammes, *Synthesis*, 636 (1970); T. Matsuura, *J. Synth. Org. Chem. Japan* **26**, 217 (1968); M. Matsumoto and K. Kondo, *Ibid.* **35**, 188 (1977); S. Isoe, *Ibid.* **33**, 460 (1975); G. Ohloff, *Pure Appl. Chem.* **43**, 481 (1975).
[226]M. Mousseron-Canet, J. C. Mani, J. L. Olive and J. P. Dalle, *Compt. Rend.* **262**, 1397, 1725 (1966).
[227]J. W. Cornforth, R. Mallaby and G. Ryback, *J. Chem. Soc.* **C**, 1565 (1968).
[228]J. Arditti, R. E. Fisch and B. H. Flick, *Chem. Commun.* 1217 (1972).
[229]H. Takeshita, T. Sato, T. Muroi and S. Itô, *Tetrahedron Letters* 3095 (1969).
[230]S. Isoe, S. B. Hyeon, H. Ichikawa, S. Katsumura and T. Sakan, *Ibid.* 5561 (1968).
[231]S. Isoe, S. Katsumura and S. B. Hyeon, *Ibid.* 1089 (1971).
[232]S. Isoe, S. B. Hyeon and T. Sakan, *Ibid.* 279 (1967).
[233]K. Tsukida, S. C. Chô and M. Yokota, *Chem. Pharm. Bull.* **17**, 1755 (1969).
[234]S. W. Sanderson, H. Co and I. G. Gonzalez, *J. Food. Sci.* **36**, 231 (1971).
[235]M. M. Cormier, J. E. Wampler and K. Hori, *Fortschr. Chem. Org. Naturstoffe* **30**, 1 (1973); J. Lee, *Photochem. Photobiol.* **20**, 535 (1974); J. W. Hasting and T. Wilson, *Ibid.* **23**, 46 (1976).
[236]N. J. Turro, P. Lechtken, N. E. Schore, G. Schuster and H.-C. Yekta, *Accounts Chem. Res.* **7**, 97 (1974); W. Adam, *J. Chem. Educ.* **52**, 138 (1975); *Angew. Chem. Internatl. Ed. Engl.* **13**, 619 (1974).
[237]T. Matsuura, In *Bioorganic Chemistry* (Edited by S. Oae, T. Mukaiyama and Z. Yoshida), (Kagaku Zokan, No. 58), p. 189. Kagakudojin, Kyoto (1972).
[238]T. Matsuura, Ref. 4, p. 286.
[239]M. T. Abbott and S. Udenfriend, Ref. 6, p. 168.
[240]B. Lindblad, G. Lindstedt, M. Tofft and S. Lindstedt, *J. Am. Chem. Soc.* **91**, 4604 (1969).
[241]O. Hayaishi and H. Hirata, *J. Biol. Chem.* **241**, 2733 (1966).
[242]G. A. Hamilton, *J. Am. Chem. Soc.* **86**, 3391 (1964).
[243]V. Ullrich, *Angew. Chem.* **84**, 689 (1972).
[244]B. Witkop, *Kagaku no Ryoiki* **23**, 949 (1968); *Experientia* **28**, 1129 (1972).
[245]S. Senoh, Ref. 4, p. 263.
[246]D. M. Jerina, *Chem. Technol.* **4**, 120 (1973).
[247]D. M. Jerina and J. W. Daly, *Oxidation at Carbon in Drug Metabolism* (Edited by D. V. Parke and R. L. Smith). Taylor & Francis, London, (1977).
[248]G. Guroff, J. W. Daly, D. M. Jerina, J. Renson, B. Witkop and S. Udenfriend, *Science* **157**, 1524 (1967).
[249]D. M. Jerina, J. W. Daly, B. Witkop, P. Zaltzman-Nirenberg and S. Udenfriend, *J. Am. Chem. Soc.* **90**, 6525 (1968); *Biochemistry* **9**, 147 (1970).
[250]E. A. Fehnel, *J. Am. Chem. Soc.* **94**, 3961 (1972).
[251]H. Yagi, D. M. Jerina, G. J. Kasperek and T. C. Bruice, *Proc. Natl. Acad. Sci., U.S.A.* **69**, 1985 (1972).
[252]For a review see: T. C. Bruice and P. Y. Bruice, *Accounts Chem. Res.* **9**, 378 (1976).
[253]G. A. Hamilton, R. J. Workman and L. Woo, *J. Am. Chem. Soc.* **86**, 3390 (1964).
[254]S. Kaufman, Ref. 7, p. 90.
[255]D. M. Jerina, J. W. Daly, W. Landin, B. Witkop and S. Udenfriend, *J. Am. Chem. Soc.* **88**, 3347 (1969).
[256]D. M. Jerina, D. R. Boyd and J. W. Daly, *Tetrahedron Letters* 457 (1970).
[256a]R. J. Cvetanovic, *Advan. Photochem.* **1**, 115 (1963).
[257]D. M. Jerina, J. W. Daly and B. Witkop, *Biochemistry* **10**, 366 (1971).
[258]K. B. Sharpless and T. C. Flood, *J. Am. Chem. Soc.* **93**, 2316 (1971).
[259]J. R. Lindsay-Smith, D. M. Jerina, S. Kaufman and S. Milstein, *J. C. S. Chem. Commun.* 881 (1975).
[260]H. Loeble, G. Stein and J. Weiss, *J. Chem. Soc.* 2704 (1950); G. Stein and J. Weiss, *Ibid.* 3265 (1951); G. R. A. Johnson, G. Stein and J. Weiss, *Ibid.* 3275 (1951).
[261]E. Boyland and P. Sim, *Ibid.* 2966 (1953).
[262]R. O. C. Norman and G. K. Radda, *Proc. Chem. Soc.* 138 (1962).
[263]J. R. Lindsay-Smith and R. O. C. Norman, *J. Chem. Soc.* 2897 (1963).
[264]Hj. Staudinger and V. Ullrich, *Z. Naturforsch.* **19b**, 409, 877 (1964).
[265]K. Omura and T. Matsuura, *Tetrahedron* **24**, 3475 (1968).
[266]K. Omura and T. Matsuura, *Ibid.* **26**, 255 (1970).
[267]T. Matsuura and K. Omura, *Synthesis* 173 (1974).
[268]V. Ullrich, J. Wolff, E. Amadori and Hj. Staudinger, *Z. Physiol. Chem.* **349**, 85 (1968).
[269]Y. Tobinaga, T. Chiba and F. Okazaki, 6*th Symposium on Chemistry of Natural Products*, Sapporo, 1962, Abstracts of Papers, p. 70.
[270]S. Udenfriend, C. T. Clark, J. Axelrod and B. Brodie, *J. Biol. Chem.* **208**, 731 (1954).
[271]R. Breslow and L. N. Lukens, *Ibid.* **235**, 292 (1960).
[272]R. R. Grinstead, *J. Am. Chem. Soc.* **82**, 3472 (1960).

[273]V. Ullrich and Hj. Staudinger, Ref. 5, p. 235.
[274]A. Bobst and M. Viscontini, *Helv. Chim. Acta* **49**, 884 (1966).
[275]M. Viscontini, *Angew. Chem.* **80**, 492 (1968); *Fortschr. Chem. Forsch.* **9**, 605 (1968).
[276]J. A. Blair and A. J. Pearson, *J. C. S., Perkin II* 245 (1975).
[277]M. B. Dearden, C. R. E. Jefcoate and J. R. Lindsay-Smith, *Advan. Chem. Ser.* **77**, III, 2260 (1968).
[278]H. Mimoun and I. S. de Roch, *Tetrahedron* **31**, 777 (1975).
[279]W. Brackman and E. Havinga, *Rec. Trav. Chim.* **74**, 1021, 2070, 1100, 1107 (1955).
[280]G. A. Hamilton, J. P. Friedman and P. M. Campbell, *J. Am. Chem. Soc.* **88**, 5266 (1966).
[281]G. A. Hamilton, J. W. Hanifin, Jr. and J. P. Friedman, *Ibid.* **88**, 5269 (1966).
[282]M. G. Evans, P. George and N. Uri, *Trans. Faraday Soc.* **45**, 230 (1949).
[283]J. A. Christiansen, *Acta Chem. Scand.* **9**, 272 (1955).
[284]C. Nofre, A. Cier and A. Lefier, *Bull. Soc. Chim. Fr.* 530 (1961).
[285]V. Ullrich and Hj. Staudinger, *Z. Naturforsch.* **24b**, 583 (1969).
[286]V. Ullrich, *Ibid.* **24b**, 699 (1969); U. Frommer and V. Ullrich, *Ibid.* **26b**, 322 (1971).
[287]M. N. Sheng and J. G. Zajecek, *Advan. Chem. Ser.* **76**, 418 (1968).
[288]F. Grovenstein, Jr. and A. J. Mosher, *J. Am. Chem. Soc.* **92**, 3810 (1970).
[289]T. Tsuchiya, H. Arai and H. Igeta, *Tetrahedron Letters* 2747 (1969); 2213 (1970); J. Streith, B. Danner and C. Sigwalt, *Chem. Commun.* 979 (1967).
[291]J. R. Giacin and G. A. Hamilton, cited in Ref. 17.
[292]V. Ullrich, unpublished data.
[293]G. A. Hamilton and J. R. Giacin, *J. Am. Chem. Soc.* **88**, 1584 (1966).
[294]R. W. Murray and A. Suzuki, *Ibid.* **93**, 4963 (1971); **95**, 3343 (1973); R. W. Murray and D. P. Higley, *Ibid.* **95**, 7886 (1973); D. P. Higley and R. W. Murray, *Ibid.* **96**, 3330 (1974).
[295]S. K. Chaudhary, R. A. Hoyt and R. W. Murray, *Tetrahedron Letters* 4235 (1976).
[296]R. E. Keay and G. A. Hamilton, *J. Am. Chem. Soc.* **97**, 6876 (1975).
[297]J. A. Maasen and Th. J. De Boer, *Rec. Trav. Chim.* **91**, 1329 (1972).
[298]H. W. Orf and D. Dolphine, *Proc. Natl. Acad. Sci., U.S.A.* **71**, 2646 (1974).
[299]P. Hemmerich, *Vitamins and Hormones* **28**, 467 (1970).
[300]V. Massey, G. Palmer and D. Ballou, In *Flavins and Flavoproteins* (Edited by H. Kamin), p. 349. Butterworths, London (1971).
[301]H. I. X. Mager and W. Berends, *Rec. Trav. Chim.* **83**, 1329 (1966); *Biochem. Biophys. Acta* **118**, 440 (1966).
[302]W. H. Walker, P. Hemmerich and V. Massey, *Helv. Chim. Acta* **50**, 2269 (1967).
[303]C. Kemal and T. C. Bruice, *Proc. Natl. Acad. Sci., U.S.A.* **73**, 995 (1976).
[304]H. I. X. Mager and W. Berends, *Rec. Trav. Chim.* **91**, 611 (1972); *Tetrahedron*, **30**, 917 (1974); and references cited therein.
[305]S. Kaufman, Ref. 7, p. 91.