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Oxidation of indoles

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Abstract

Chemical, auto and biological oxidations of indole and related compounds are reviewed. In biological oxidation both ring cleaving and ring hydroxylating oxidations are discussed in detail.

Key words: Indoles, oxidation, biological oxidation, autoxidation, metabolism of indoles, degradation of indoles, hydroxylation.

1. Introduction

Indolic compounds have generated enormous interest because of their biological activity. While pure chemists have contributed their mite, particularly with regard to chemical oxidation, only recently biochemists have come to realize the importance of these studies in explaining mechanisms of biological oxidation of indolic compounds. The present review has attempted to review chemical, biological and autoxidation of indoles.

2. Chemical oxidation of indoles

Various oxidizing agents oxidize indole to a variety of products, the degree and extent of oxidation being dependent on the particular reagent and experimental conditions used. Very often in these reactions indoxyl is observed as an intermediate. Indoxyl A_1 and indigo A_2 are formed when oxidation is carried out by using hydrogen peroxide

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or perbenzoic acid and a small amount of indirubin A_4 is also formed presumably by condensation of indoxyl with isatin A_3, another by-product of this oxidation[1]. In contrast to this reaction is the oxidation of indole by sodium perborate, which gives a mixture of indoxyl and leuco indigo A_5[2]. On oxidation with alkaline persulfate, indole forms indoxyl-O-sulfate A_6[3]. The oxidative cleavage of indole ring to 2-formamido-benzoic acid A_7 by manganese dioxide is also known[4].

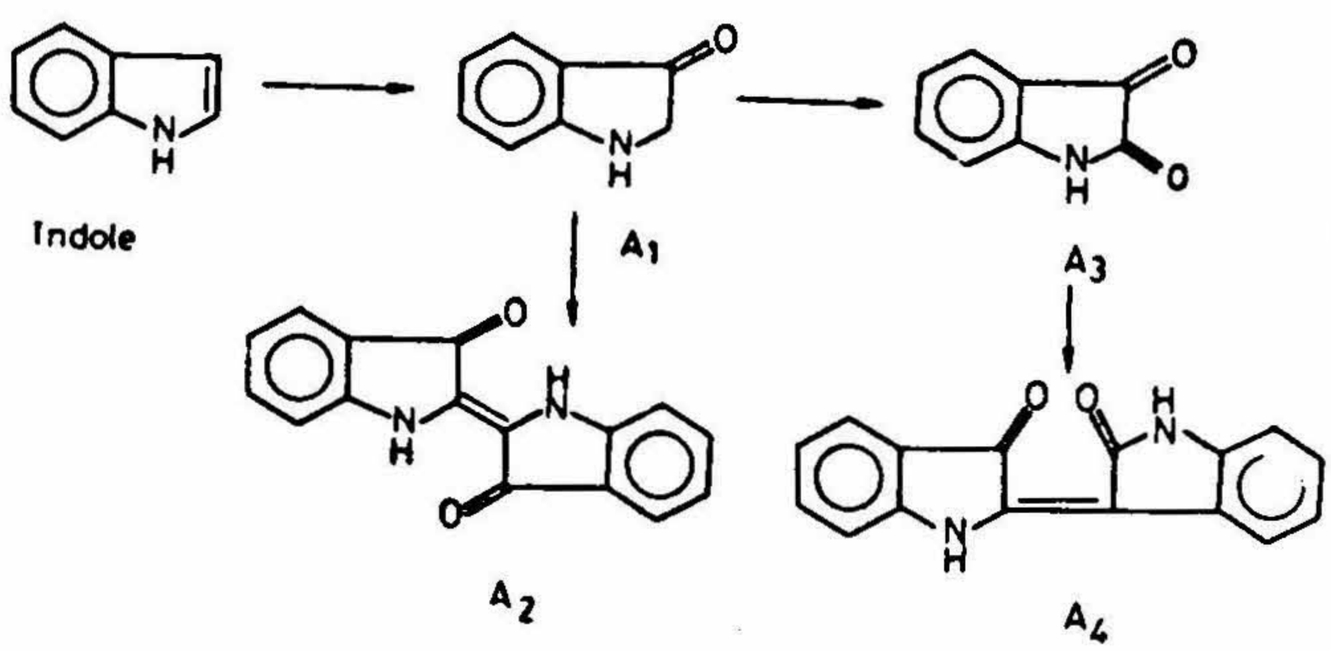


Chart I

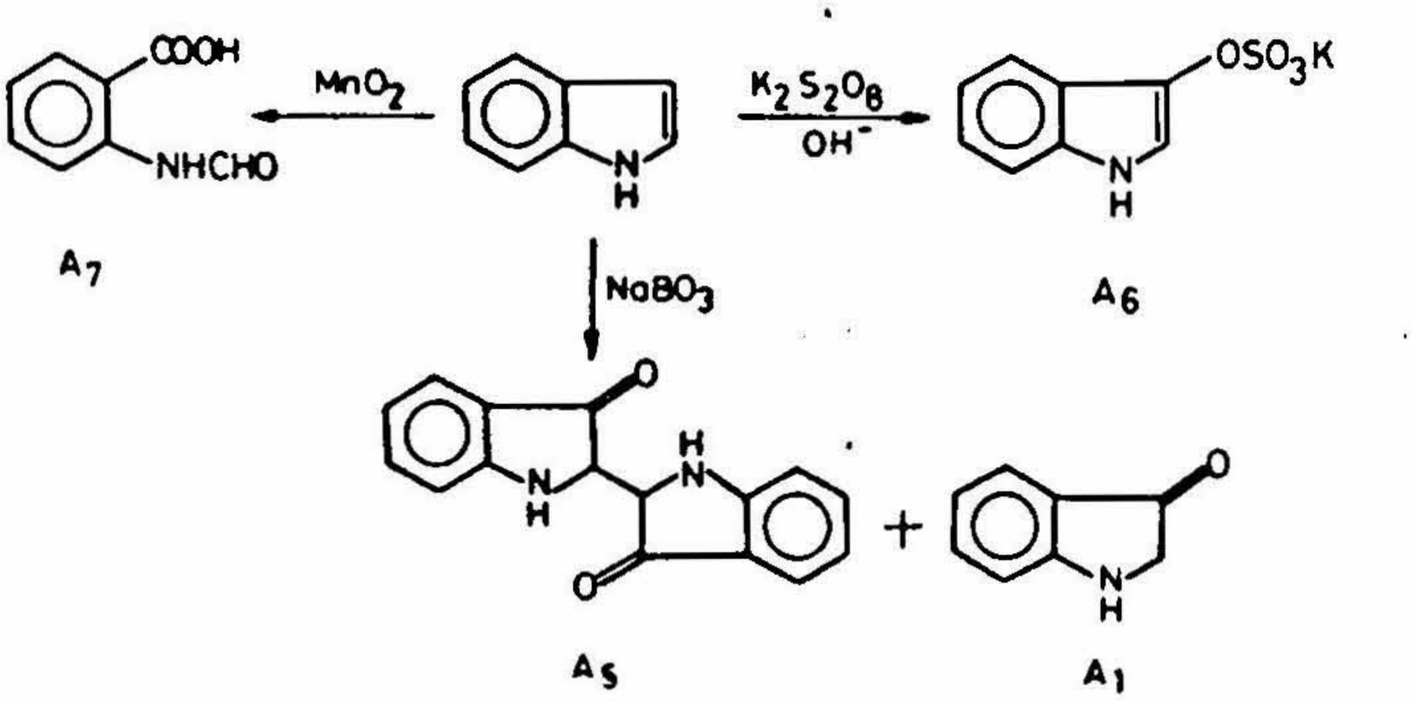
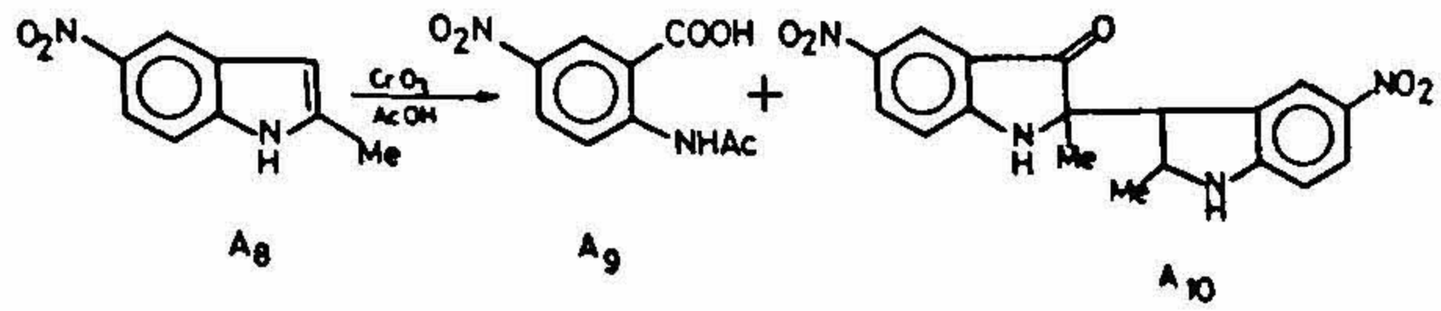


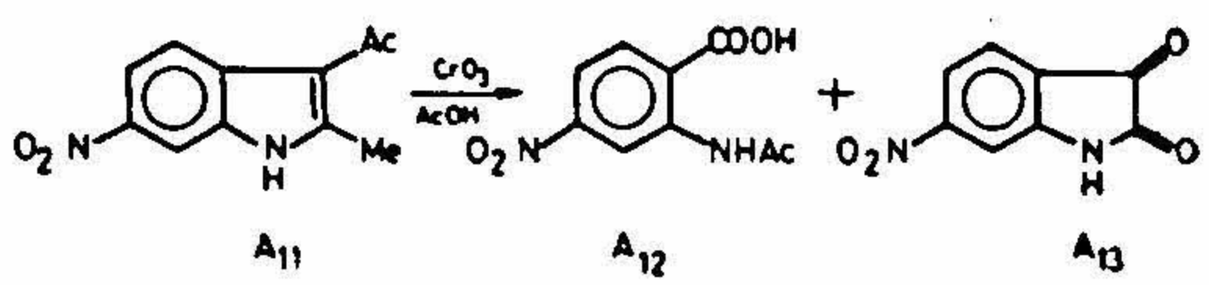
Chart II

It is important to mention here the cleavage of indole ring by other oxidants like chromium trioxide, potassium permanganate and periodate. Chromium trioxide in acetic acid can effect the cleavage of indoles between C_2 and C_3. When A_8 is oxidized by chromium trioxide A_{10} is the main product while A_9 is a by-product[5]. Oxidation of 2-methyl-3-acetyl-6-nitroindole A_{11} yields 6-nitroisatin A_{13} as a by-product[5]. Another reaction which has important commercial application is the preparation of benzo-diazopinone A_{16} by oxidation of indole derivative A_{14} with chromium trioxide in acetic acid. This reaction proceeds *via* the intermediate formation of ketoamide A_{15} by the oxidative cleavage of indole 2,3-double bond[6,7]. Recently, Shigeho *et al*[8] have reported

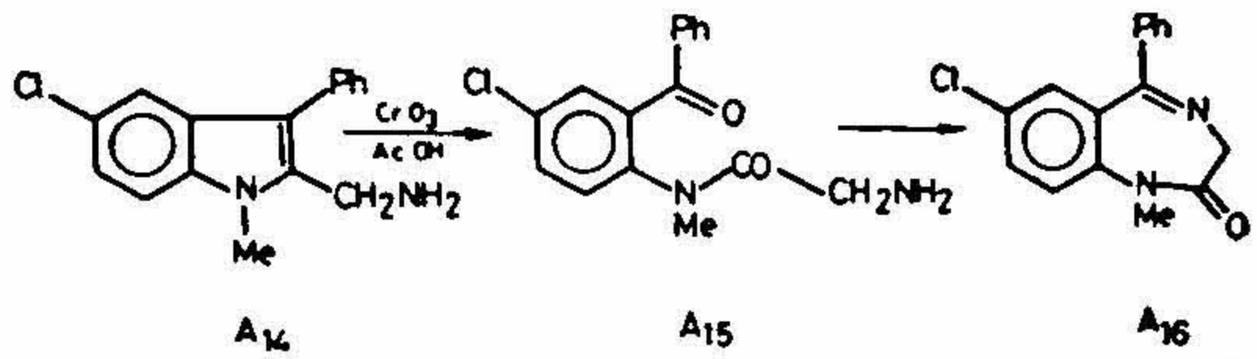
the oxidation of an indole derivative A_{17} with chromium trioxide to an isatin derivative A_{18}.



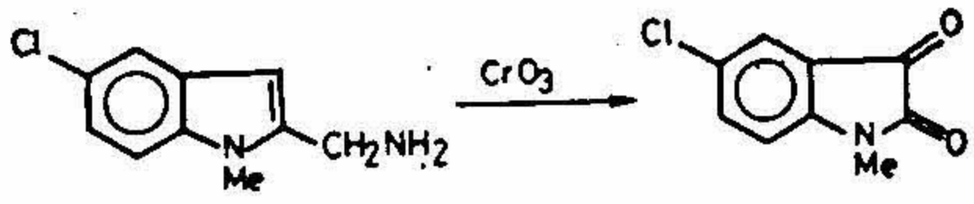
A_8 A_9 A_{10}



A_{11} A_{12} A_{13}



A_{14} A_{15} A_{16}



A_{17} A_{18}

Chart III

There are many reports on the oxidative cleavage of the indole ring by potassium permanganate. When 2-methylindole is oxidized to N-acetylanthranilic acid A_{19} by potassium permanganate the yield of the reaction is 40%; however, when the benzene ring is substituted by methyl groups, the substituents are oxidized to carboxyl groups[9]. The yield of such reactions is below 20%. This resistance of substituted alkyl group to oxidation has found application in determination of structure of 2-methylindoles substituted with additional methyl groups in the benzene ring. Thus, the dimethylindoles are subjected to oxidation to N-acetylanthranilic acids by alkaline permanganate. For example, 2,5-dimethylindole A_{20} afforded N-acetyl-4-methylanthranilic acid A_{21}, though small amount of dicarboxylic acid A_{22} is formed[9]. The susceptibility of the pyrrole ring to oxidative attack is remarkably diminished by the presence of nitro groups. Thus, the oxidation of 2-methyl-3,4,6-trinitroindole A_{23} with alkaline permanganate leaves the indole ring intact, yielding A_{24}[5].

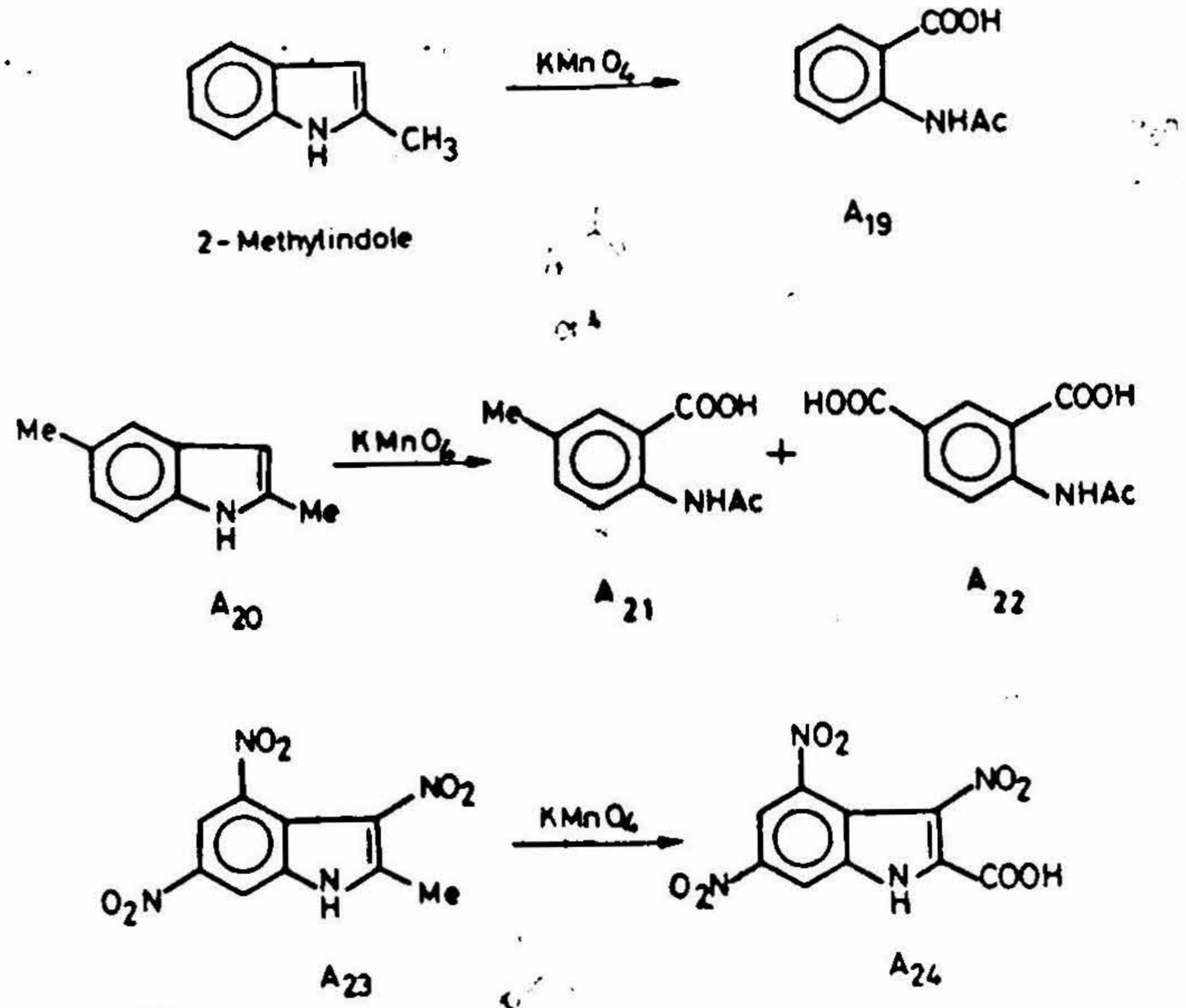


Chart IV

Oxidations of indole derivatives by sodium periodate and periodic acid are interesting because of the diverse nature of the cleavage by these two oxidants. While sodium periodate cleaves the 2,3-double bond of indole ring, periodic acid oxidizes effectively the substituent at 2-position leaving the ring intact. Thus, 2,3-dimethylindole A_{25}

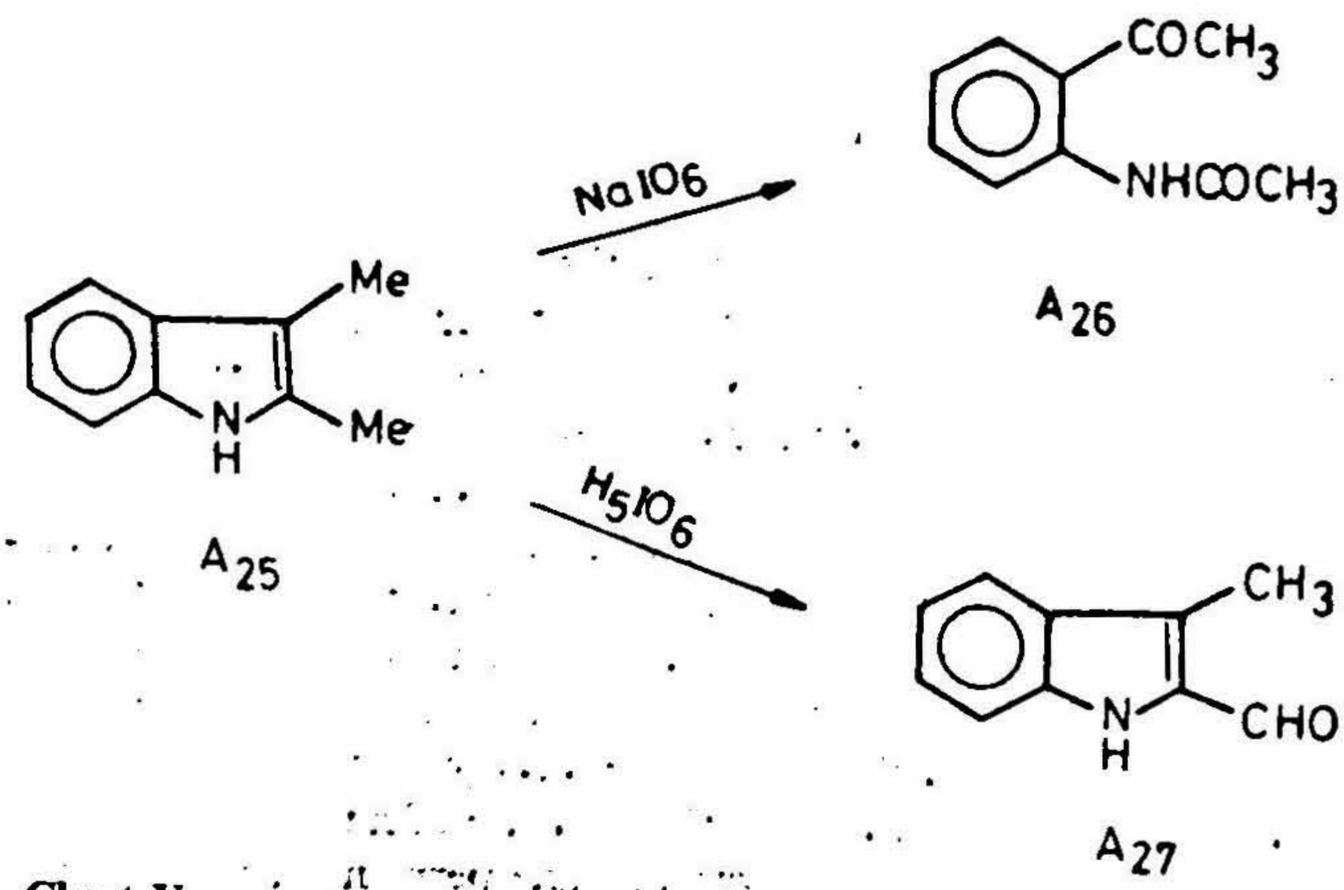


Chart V

yields *o*-acetamino acetophenone A_{26} and 2-formyl-3-methylindole A_{27} on oxidation with sodium periodate and periodic acid respectively[10,11].

When 3-methylindole is subjected to oxidation by peracetic acid 3-methyloxindole A_{28} is formed, while perbenzoic acid oxidizes it to 2-formamidoacetophenone A_{29}[12]. Sodium acetate and potassium persulfate oxidize 3-methylindole to 3-methyloxindole[13] while ozone yields A_{29}[14]. Perbenzoic acid oxidizes yohimbone and tetrahydroisoyobrin to compounds similar to A_{29}[14], while the alkaloid cinchonamine yields 3-hydroxyindolamine on oxidation with peracetic acid[15].

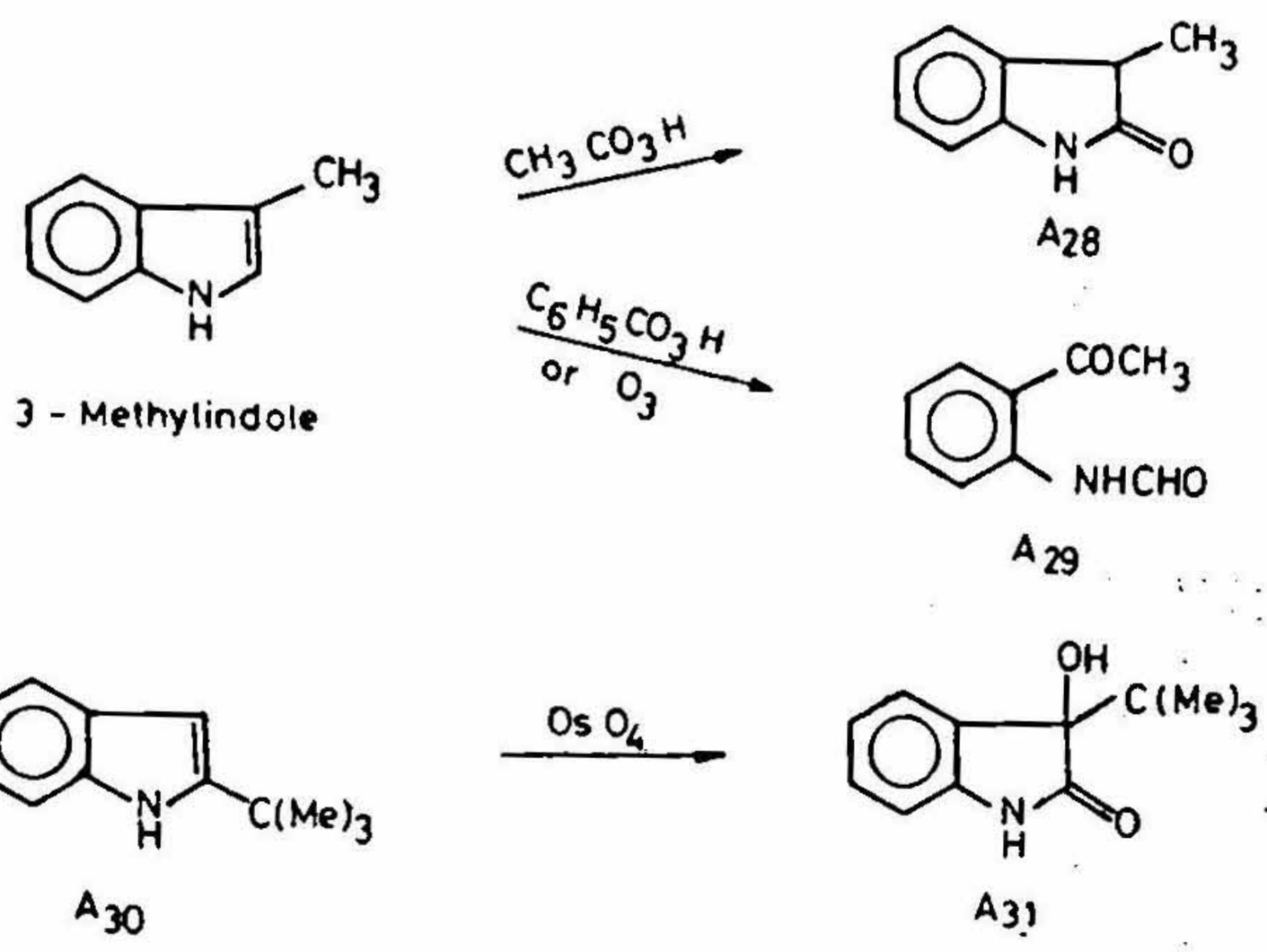


Chart VI

Osmium tetroxide and ozone normally act on olefines where the former reagent yields a glycol and the latter gives aldehydes or ketones. Osmium tetroxide usually converts indole to 2,3-dihydroxyindoline. However, such glycols could be isolated only from N-substituted indoles[16]. On oxidation with osmium tetroxide followed by treatment with base 2-*t*-butylindoles A_{30} yield 3-*t*-butyldioxindoles A_{31}[17]. This is due to a subsequent rearrangement of the glycol formed on treatment with osmium tetroxide. However, the base catalysed rearrangement of such glycols results in the formation of 2,2-disubstituted-3-indolinones[18,19].

The C_2–C_3 double bond is labile to ozonolysis and could be selectively cleaved[20–22]. Many workers have studied the ozonolysis of indoles and isolated quite stable ozonides[16,20,23–26]. The chemistry of the ozonide obtained from 2-phenyl-3-methylindole was extensively studied by Witkop *et al*[27,28]. 2-Phenyl-3-methylindole ozonide A_{32} undergoes decomposition to form A_{33} by heat or acid while it reacts with acetic anhydride to give A_{34}. Also, the same ozonide is reported to form *o*-benzaminophenol A_{35} on refluxing by a radical reaction[26].

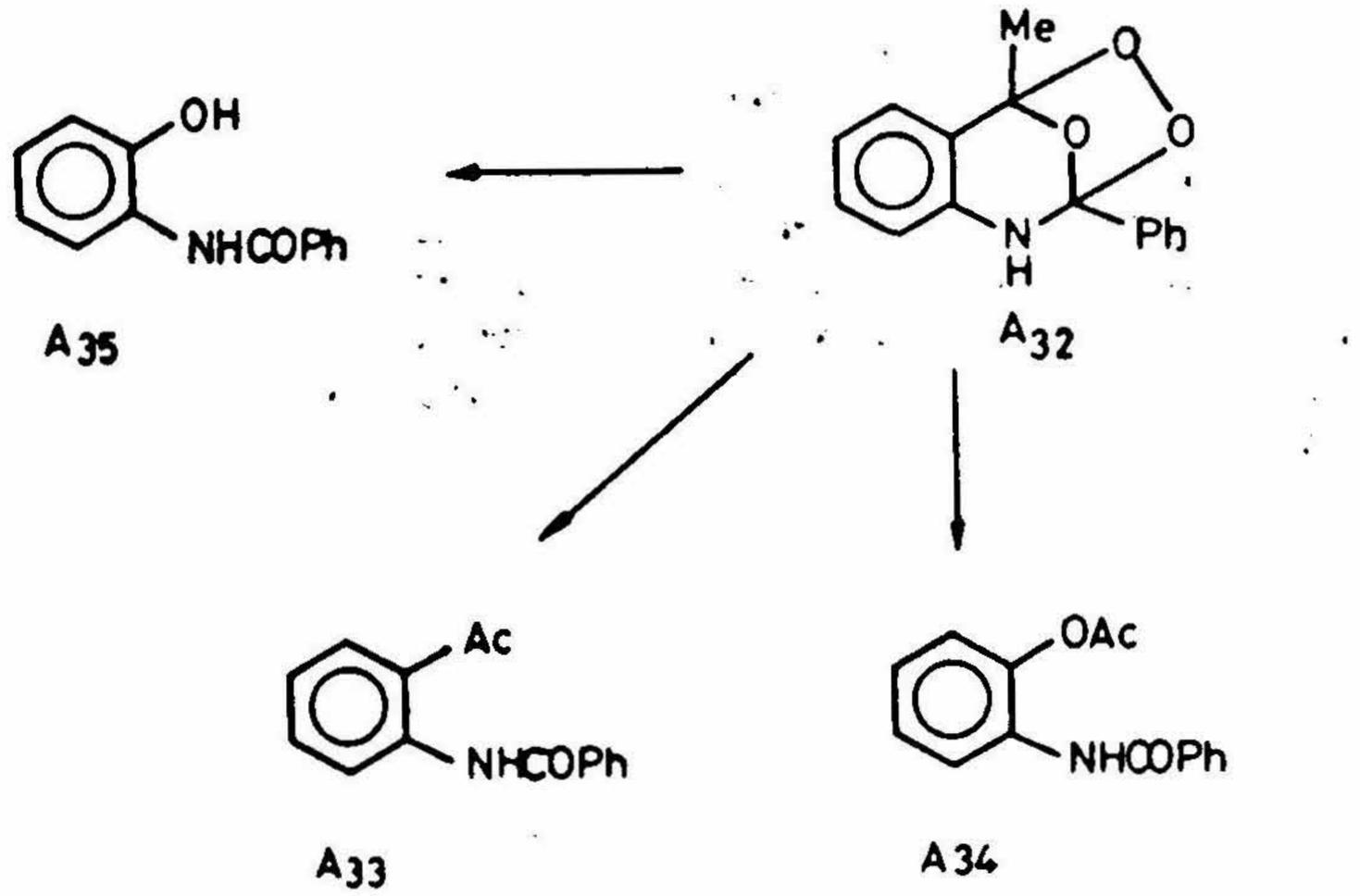


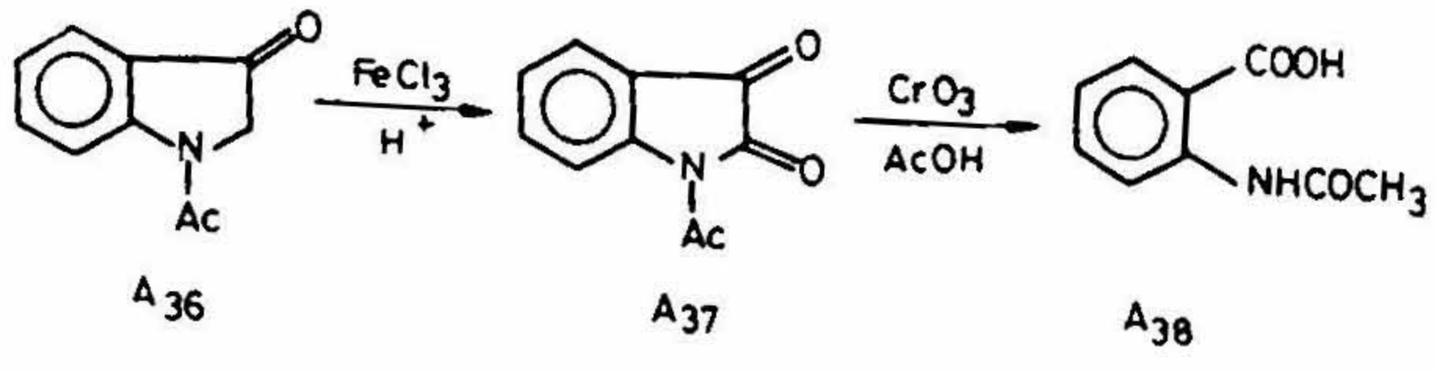
Chart VII

N-acetylindoxyl A_{36} yields N-acetyl anthranilic acid A_{38} on oxidation with potassium permanganate, while acidic ferric chloride converts it to isatin A_{39}[29]. In both the conversions, N-acetylisatin A_{37} is a likely intermediate. This hypothesis is supported by the conversion of A_{37} to A_{38} by chromic acid[30], while the product of oxidation of isatin by chromic acid is isatoic anhydride A_{40}. Alkaline peroxide converts isatin to anthranilic acid A_{41}[31]. Ferric chloride introduced a hydroxyl group at 2-position into ethyl indoxyl-2-carboxylate ($A_{42} \rightarrow A_{43}$)[32].

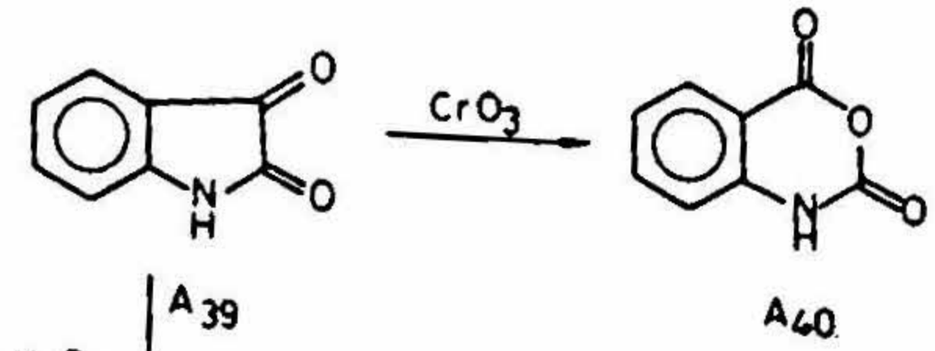
In nitration of indole-3-carboxaldehydes with nitric acid in acetic acid at 80° C, one of the principal side reactions is the nitration at C_3 accompanied by cleavage of the carboxaldehyde group[33]. In several instances, nitroisatins and nitroanthranilic acids were observed as by-products of nitration. For example, 1-methylindole-3-carboxaldehyde A_{44} afforded small amounts of 1-methyl-6-nitroisatin A_{45}, and N-methyl-5-anthranilic acid A_{46}[33].

Fremy's salt (potassium nitrosodisulfonate) effectively oxidizes hydroxyindoles to indoloquinones, though indoles without hydroxyl group are susceptible for hydroxylation in certain instances[34,35]. Thus 5-hydroxyindoles are formed from 3-methylindole and 2-phenylindole[35] by Fremy's salt.. 5-Hydroxyindoles yield 4,5-quinones, while 4-hydroxyindoles gave a mixture of 4,5-quinones and 4,7-quinones.

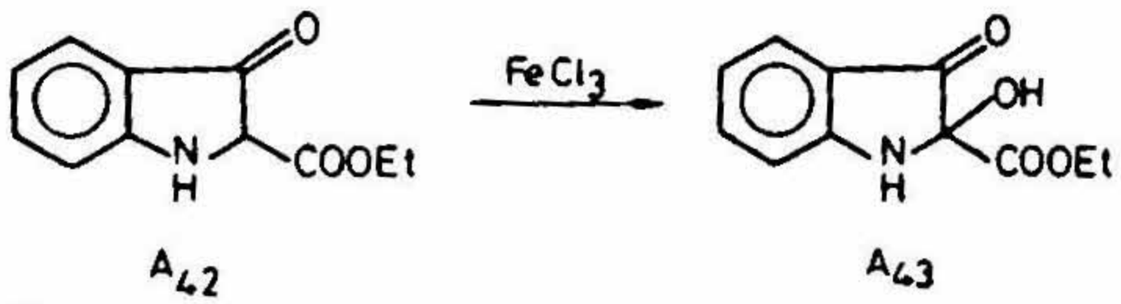
Recently, Yukimasa *et al*[36] have investigated the oxidation of indoles in pyridine containing Cu_2Cl_2 in the presence of oxygen. Thus, 3-alkylindole A_{47} and 2-methylindole gave C_2-C_3 cleavage products A_{48} and N-acetylanthranilic acid A_{49}. Oxidation of



A_{36} A_{37} A_{38}

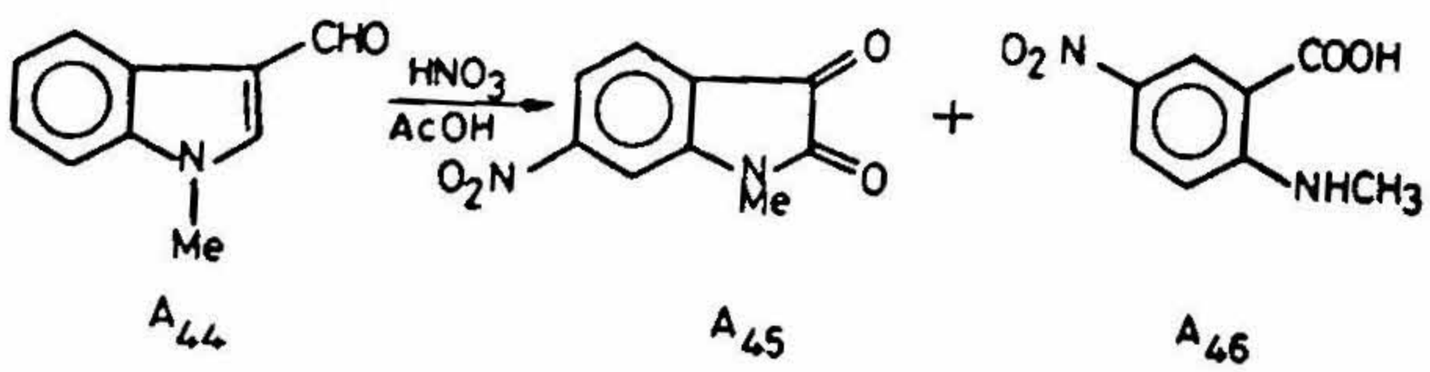


A_{40} A_{41}



A_{42} A_{43}

Chart VIII



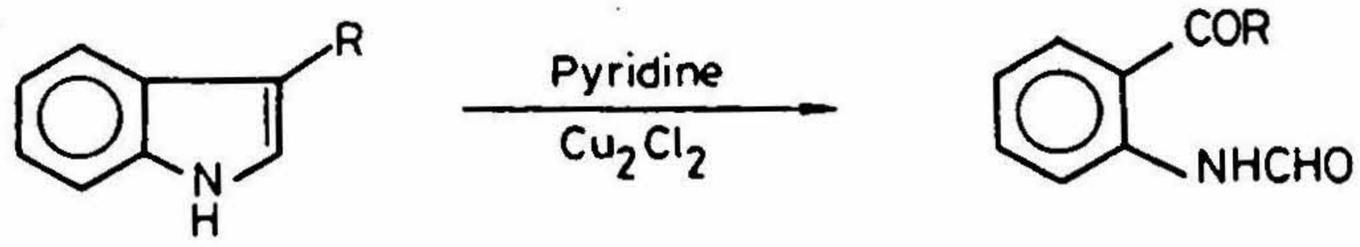
A_{44} A_{45} A_{46}

Chart IX

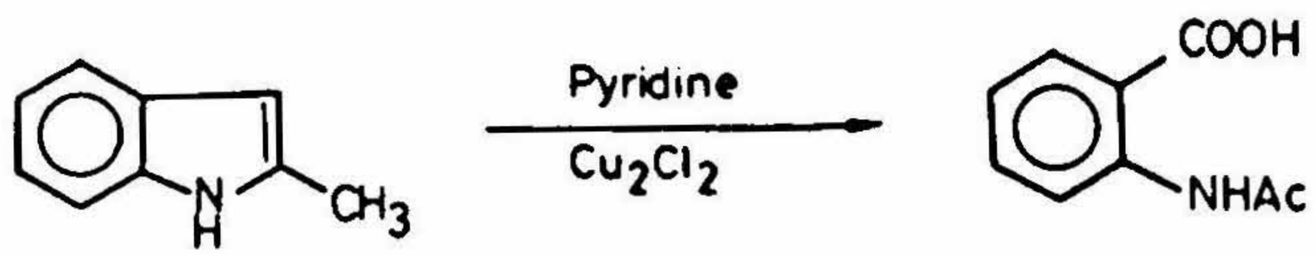
indoles in the presence of Co and Cu porphyrins gave ketoamides. For example, oxidation of 3-methylindole yielded A_{50}[37].

Kaneko *et al*[38] have very recently reported a novel indole trimer A_{51}, which can be obtained by oxidation of indole with $TiCl_3$ and H_2O_2. This oxidation proceeds by a free radical mechanism.

Oxidation of indoles to corresponding oxindoles is achieved by N-bromosuccinimide treatment. Thus, when indole-3-propionic acid A_{52} in acetic acid is treated with N-



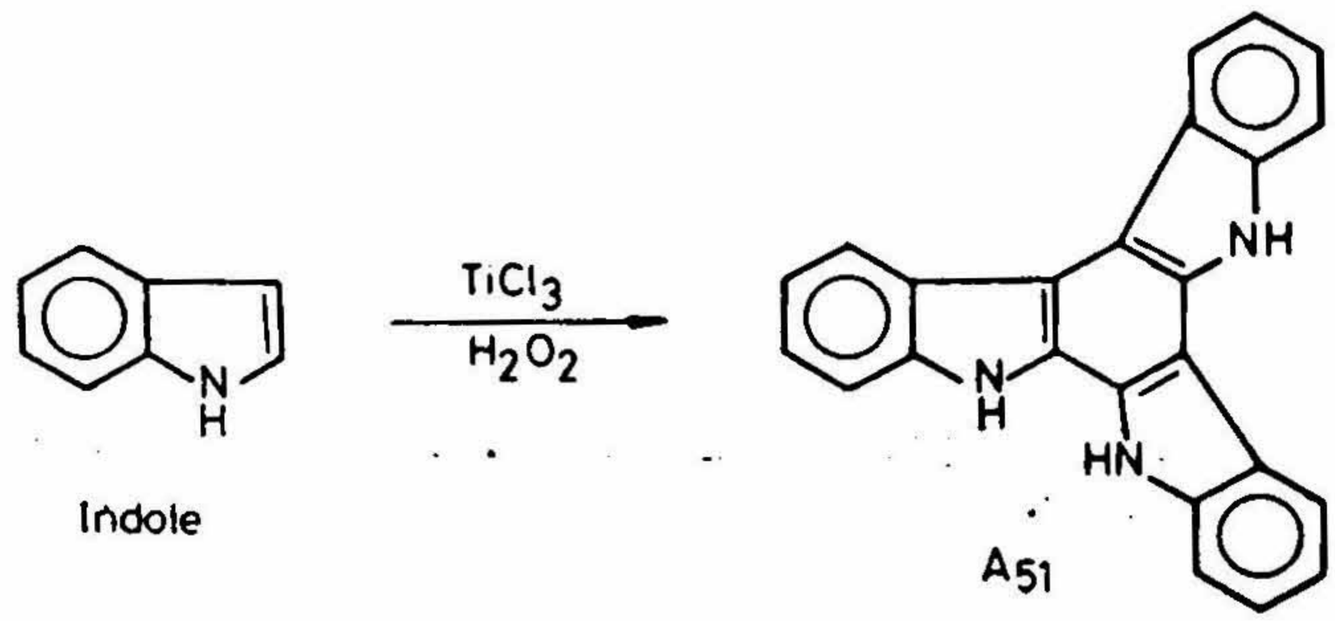
A_{47} A_{48}



2 - Methylindole A_{49}

3 - Methylindole A_{50}

Chart X



Indole A_{51}

Chart XI

bromosuccinimide and hydrogenolysed over palladium, oxindole-3 propionic acid A_{54} is obtained[39]. This oxidation proceeds *via* intermediate formation of the bromolactone A_{53}. Further hydrogenolysis of the C–Br and the benzylic C–O bond yields A_{54}.

N-bromosuccinimide in acetic acid converts 3-methylindole to 5-bromo-3-methyloxindole A_{56}[40]. A_{56} could have formed by hydrolysis of 2-bromo-3-methylindole A_{55} and subsequent bromination. 2-Haloindoles very easily undergo hydrolyses yielding oxindoles[41]. 3-Substituted indoles A_{57} are oxidized to oxindoles A_{58} by treatment of equimolar N-bromosuccinimide followed by hydrolysis. Further treatment with N-bromosuccinimide yields 3-bromooxindoles A_{59}[42].

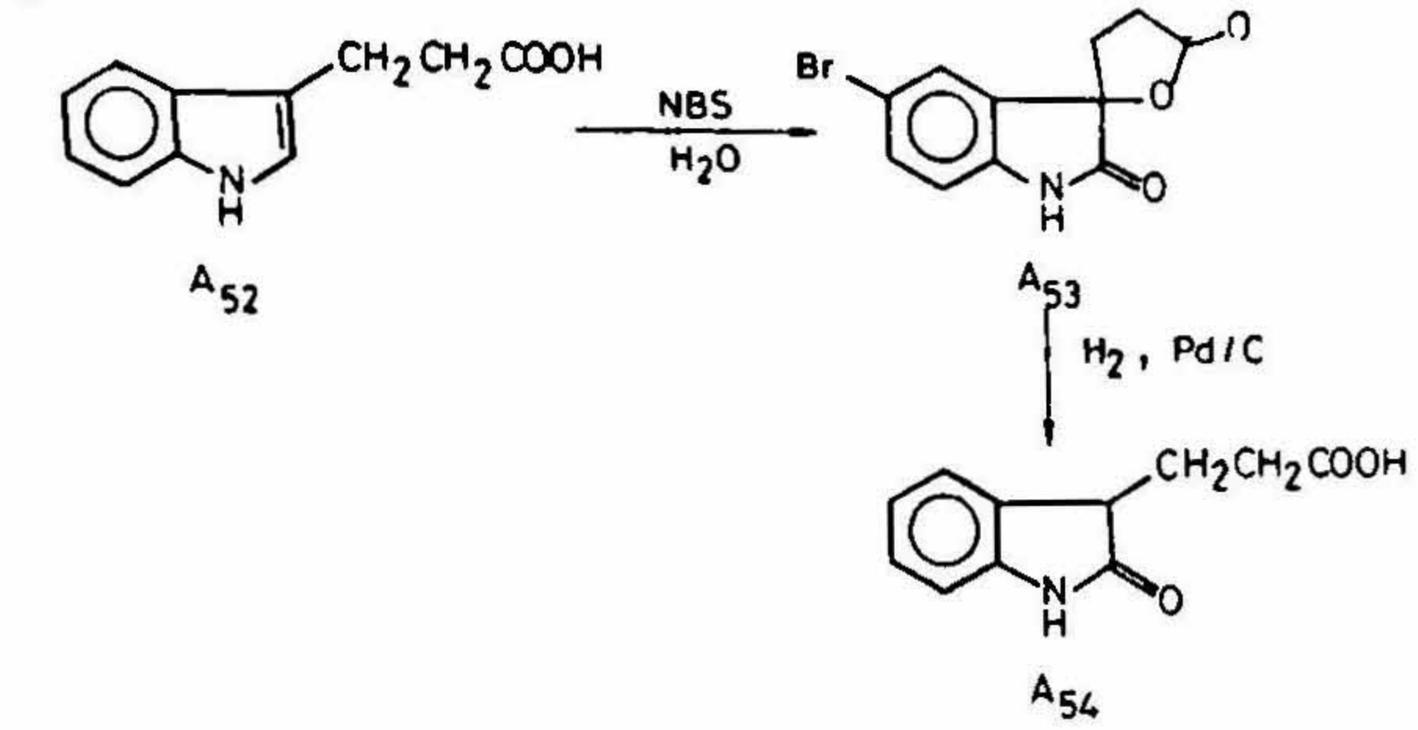
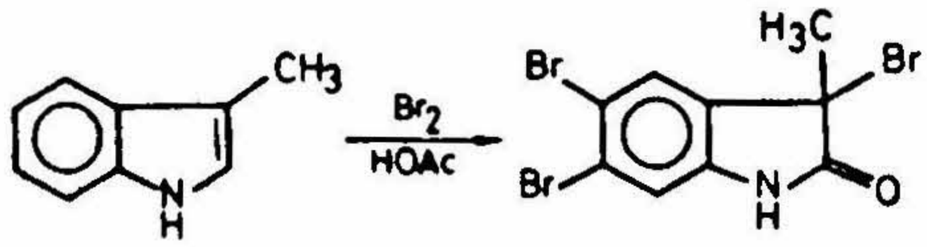


Chart XII

Da Settimo *et al*[43] have investigated the oxidation of indoles with bromine in acetic acid. Thus, indole is oxidized to bromooxindoles A_{60} and A_{61}, while skatole is oxidized to bromo-3-methyl-oxindole A_{62}.

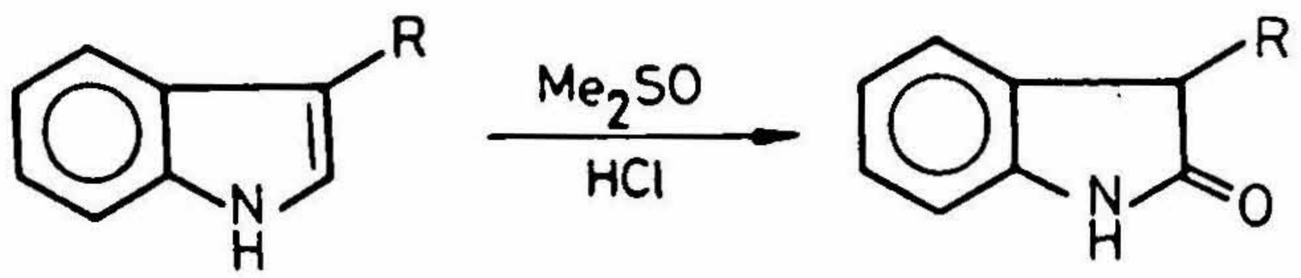
Indole A_{60} A_{61}



3-Methylindole A_{62}

Chart XIII

Oxindoles are also obtained by bromination of 3-substituted indoles in pyridine and subsequent hydrolysis[44]. A general method for the oxidation of indoles to oxindoles has been reported recently. Thus, oxindoles A_{63} were obtained in 51–82% yield by oxidizing indoles with Me_2SO–HCl[45].



3-Alkylindole A_{63}

Chart XIV

Halogenation by N,N-dichlorourethane, a source of positive halogen, has been investigated by Foglia and Swern[46]. 3,3,5-Trichlorooxindole A_{66} is formed from indole, indole-3-carboxylic acid A_{64} and indole-2-carboxylic acid A_{65} on reaction with dichlorourethane in aqueous solution. But, when methyl esters were subjected to oxidation, the carboxyl groups were not lost ($A_{67} \rightarrow A_{70}$).

Another method of oxidation of indoles to oxindoles involves treatment of 3-methylindole[8] and other products[47] with potassium persulfate. But, this method is of little application in synthesis.

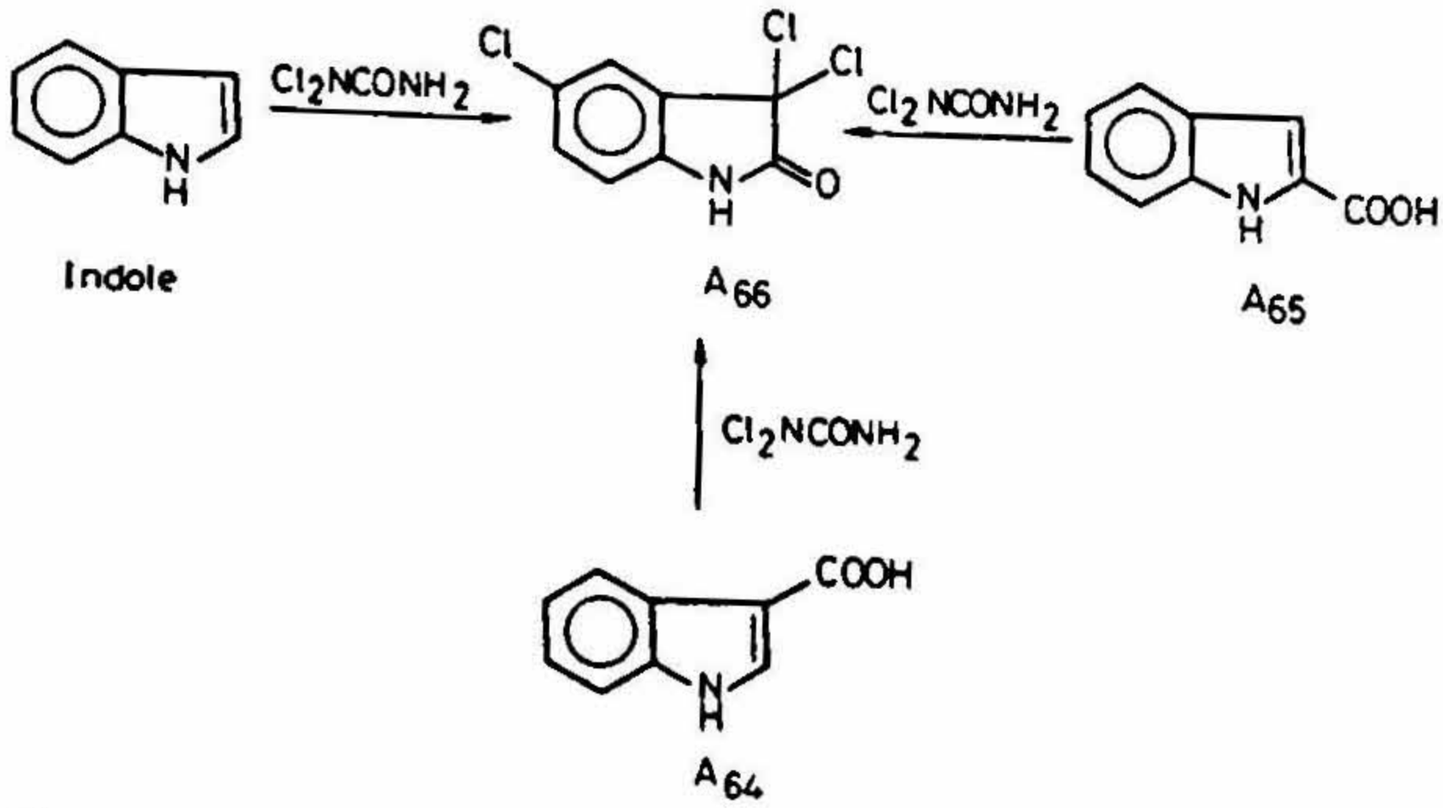
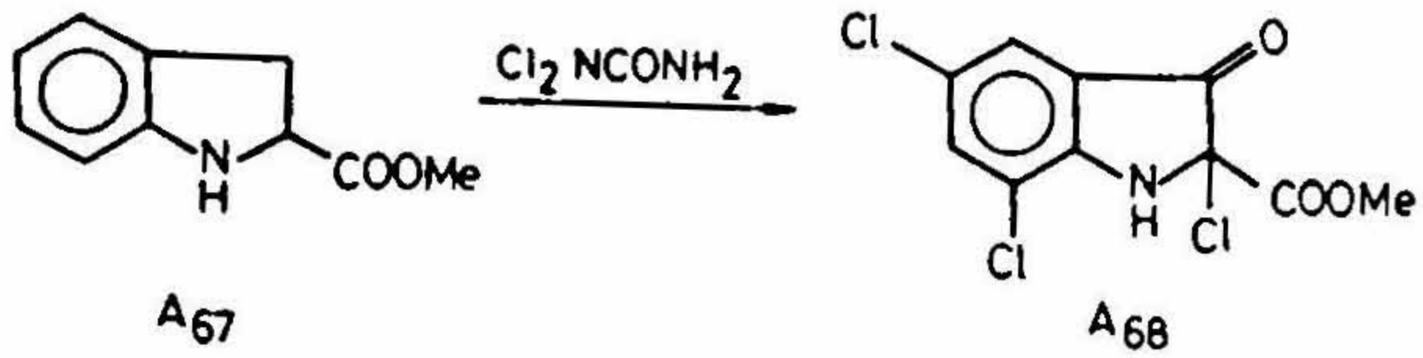


Chart XV



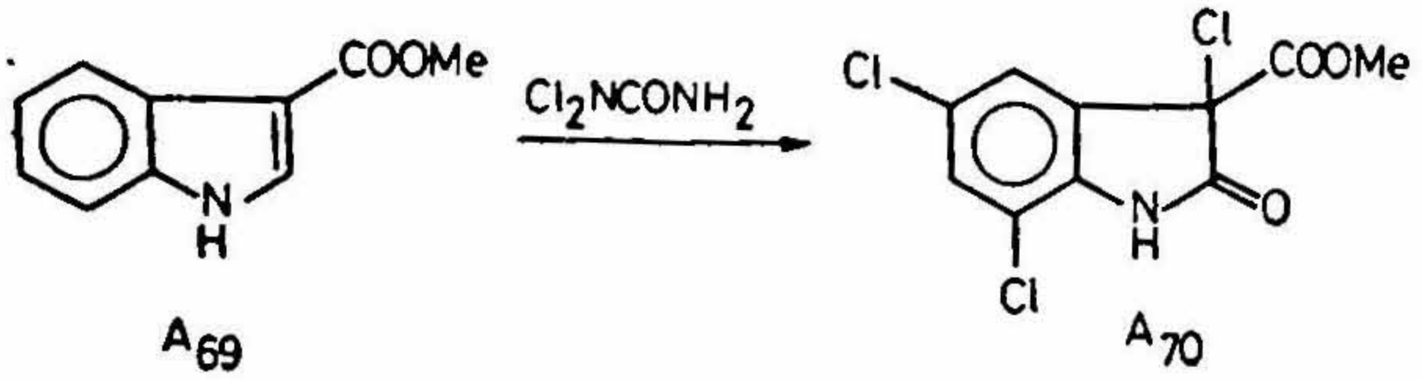
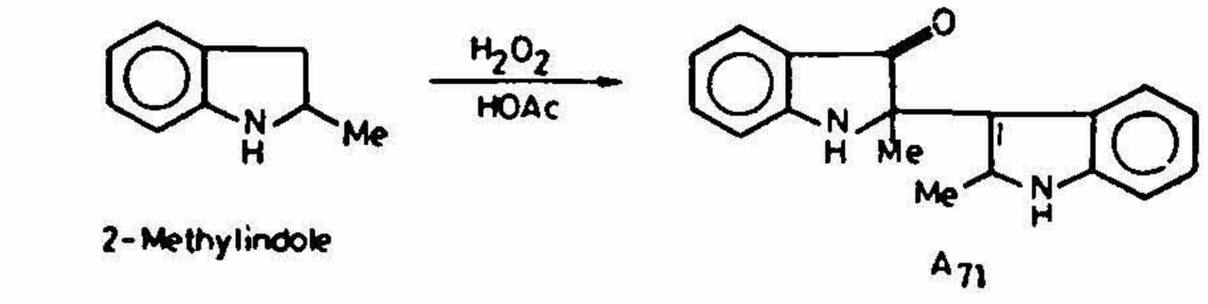


Chart XVI

2-Methylindole readily gets oxidized by a variety of oxidizing agents. Two molecules of 2-methylindole readily combine to form A_{71} in the presence of air and peracetic acid. Same reaction is carried out by hydrogen peroxide[29,48]. Indole yields 2,2-di-3-indolyl-3-indolinone under similar conditions. 2-Alkylindoles are oxidized by hydrogen peroxide in acetic acid to compounds analogous to A_{71} in concentrated solutions, but in dilute solutions the symmetric coupling products A_{75} are formed. This compound A_{75} might be formed from A_{73} by a subsequent oxidation or from A_{74}[49].

Another reaction that has immense value in degradative structure elucidations is oxidation with hydrogen peroxide[50,51]. In the presence of ammonium molybdate, hydrogen peroxide selectively cleaves the C_2–C_3 bond of indole ring[52,53]. 2-Substituted indoles yield 2-acylanthranilic acids and 2,3-disubstituted indoles give *o*-aminophenyl ketones.



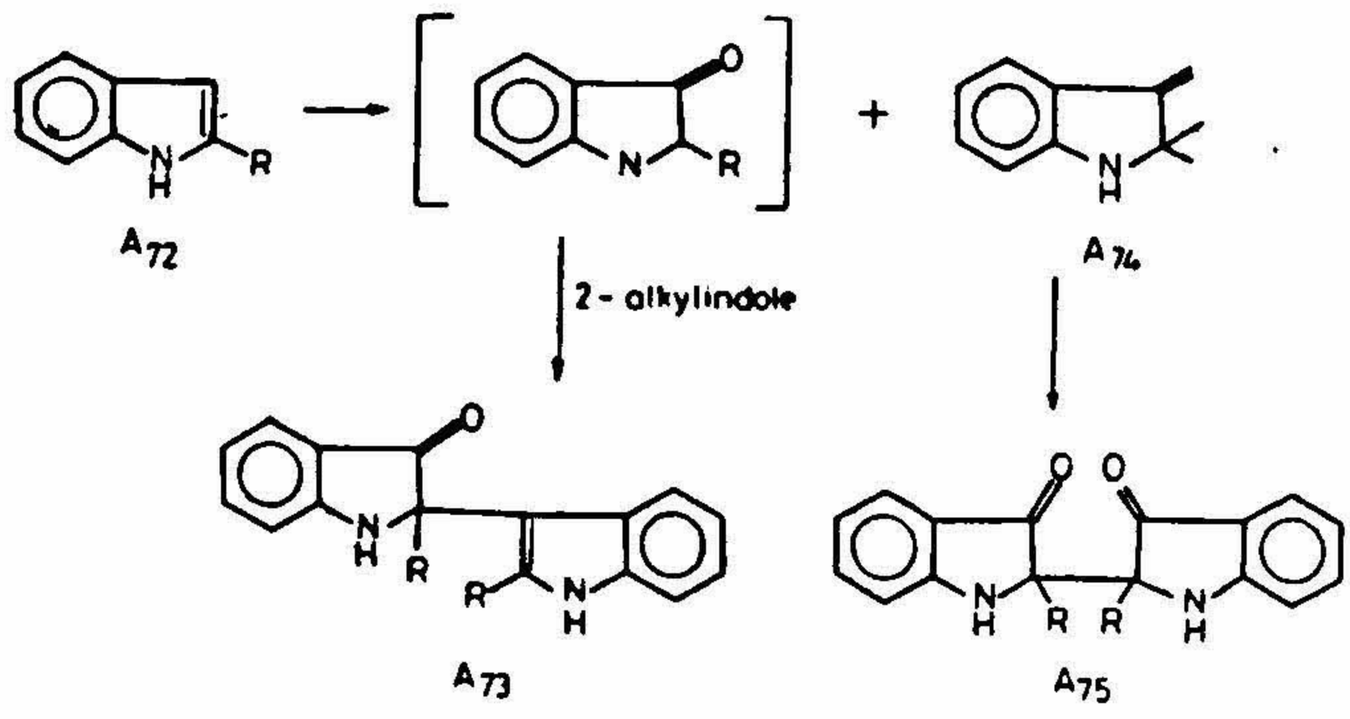


Chart XVII

Oxidation of Grignard derivatives of indoles with hydrogen peroxide also needs a special mention here, though this study is normally without definite results[54,55]. Oxidation of Grignard derivative of indole with *p*-nitro perbenzoic acid gives 3-bromoindole[56].

3. Autoxidation

The determination of the structure of the hydroperoxides formed from oxygen and tetrahydrocarbazoles by Beer *et al*[57] and a series of papers by Witkop and coworkers[58–62] which considered the paths and mechanisms of oxidation for a number of indole derivatives served as a firm basis to understand the nature of reaction between oxygen and indole. Early work involved the isolation of crystalline peroxides and their structure elucidation. Thus, the structure of hydroperoxide was shown to be B_2. These hydroperoxides are the primary products of autoxidation of indole in the presence of oxygen which undergo subsequent reactions to yield different products. However, preparation of hydroperoxides from simple indoles like 2-methyl-3-phenylindole and 2,3-diphenylindole met with little success. It was, hence, believed that these indoles are resistant to oxygen[63], which was proved wrong by autoxidation of 2-benzyl-3-phenylindole to 3-phenyldioxindole[64].

The peroxides are susceptible to reduction. Thus, they are reduced to alcohol B_3 by dithionite[57]. On treatment with acid or alkali B_3 gives B_4[55,65]. B_2 is converted to the lactam B_5 under slightly acidic or neutral conditions[55,58]. The probable mechanism for the formation of B_5 is *via* B_6 ($B_2 \rightarrow B_6 \rightarrow B_5$).

OXIDATION OF INDOLES

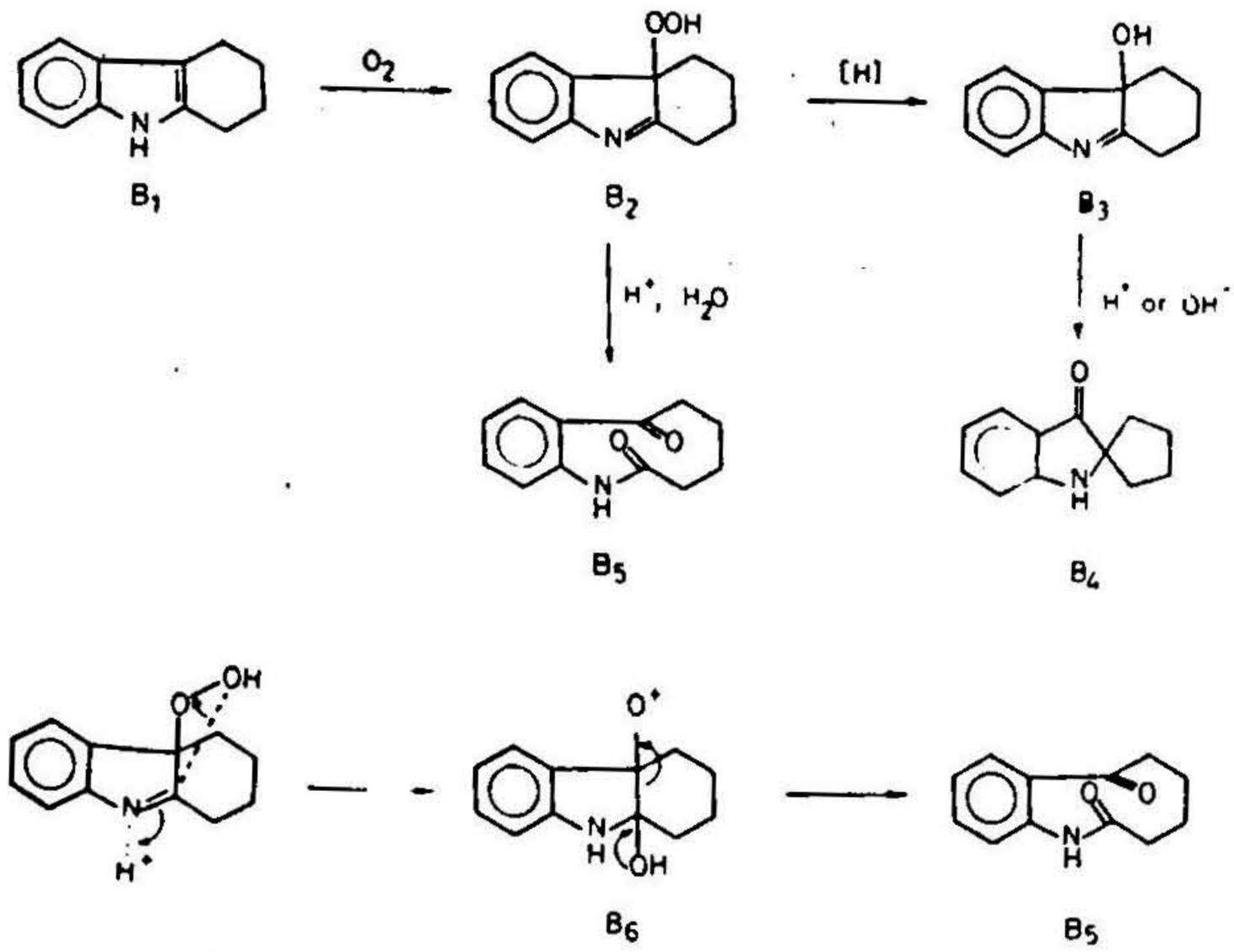


Chart XVIII

Indoxyl B_7 is formed on autoxidation of indole in the presence of light which subsequently forms either indigo B_8 or B_{11}[66,67]. B_{11} is very likely a condensation product of indoxyl red B_{10} and indole[68]. Indoxyl red is formed from indoxyl B_7 *via* leucoindoxy red B_9.

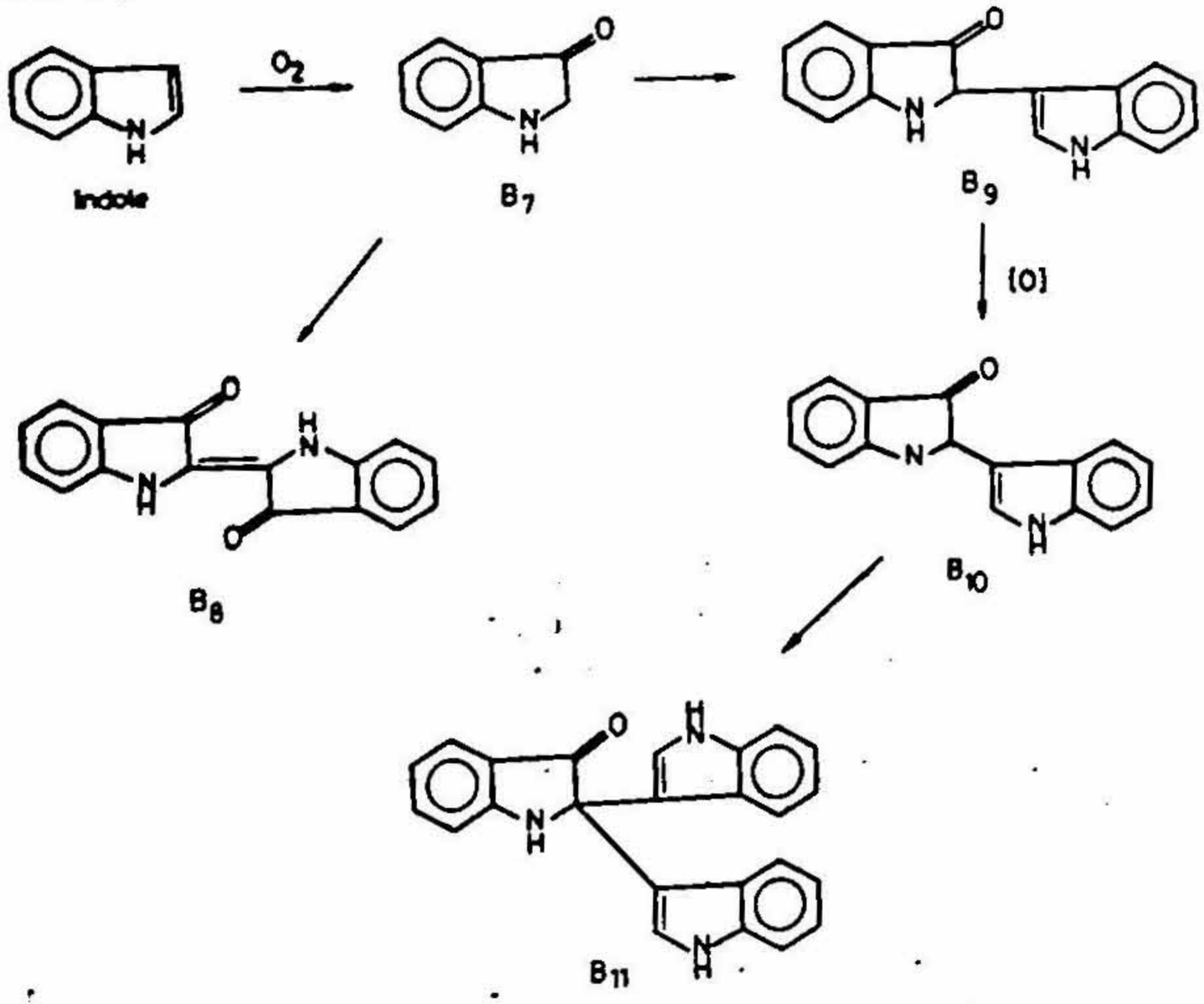


Chart XIX

2-Acetyl-3-ethylindole B_{13} is formed on autoxidation of 2,3-diethylindole B_{12}[69]. But autoxidation of 2,3-dimethylindole B_{14} results in the formation of small amounts of 2-formyl-3-methylindole B_{15} in addition to the major product, B_{16}[70]. The initiation of this oxidation is through generation of hydroperoxide B_{17} by the attack of oxygen at 3-position[69]. This was supported by isolation of the hydroperoxide of B_{12} and its conversion to B_{13} on heating. These reactions can be explained by a mechanism proposed by Taylor[70]. Thus B_{17} and B_{18} are in equilibrium, while the enamine form B_{18} is favoured by alkyl substitution. B_{18} undergoes allylic rearrangement to yield B_{19}, which ultimately decomposes to B_{20}.

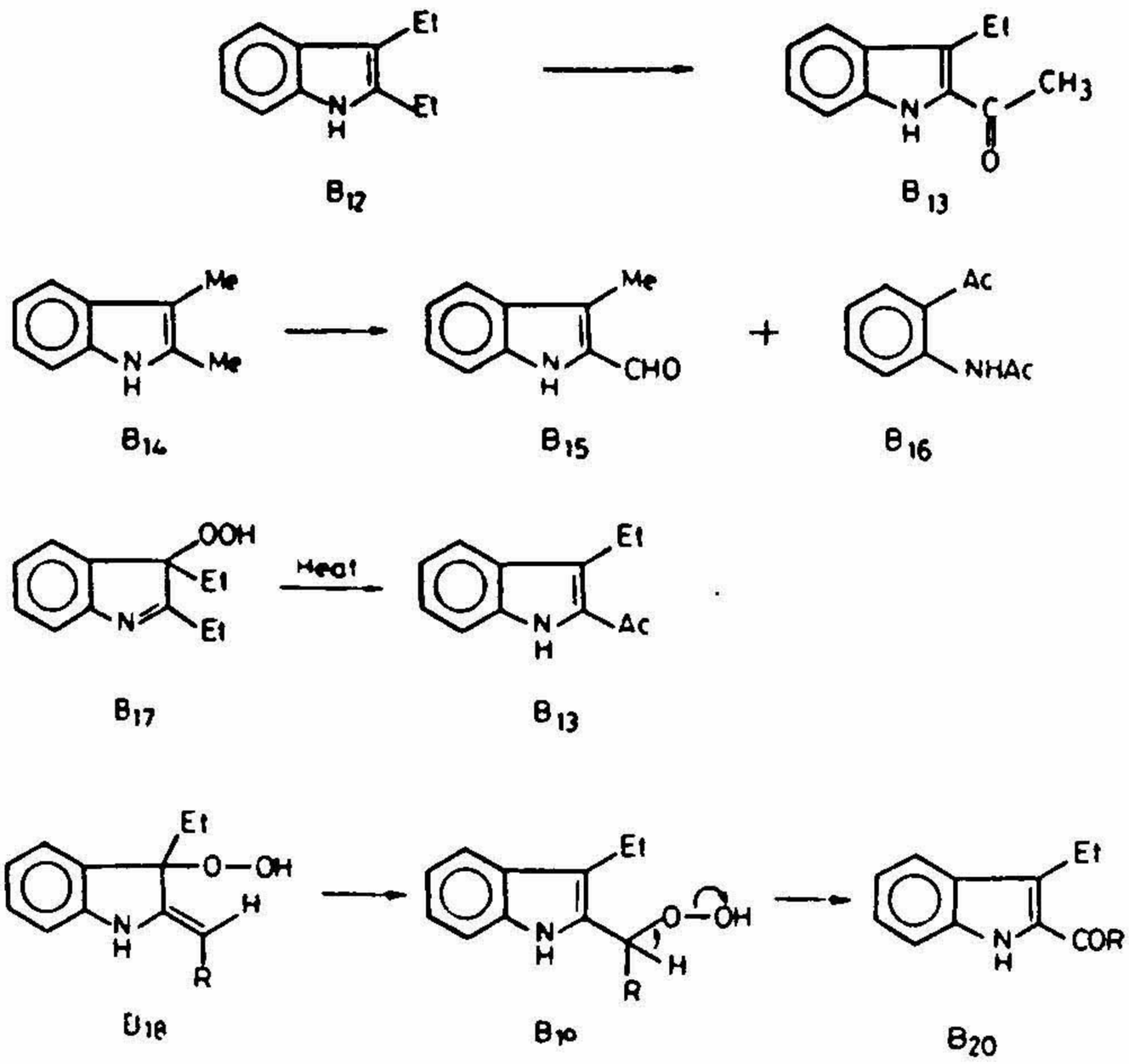


Chart XX

When oxygen was bubbled into a hexane solution of 2-isopropyl-3-methylindole B_{21} in the presence of azobisisobutyronitrile, the initial product formed was indolenine hydroperoxide B_{22}. Longer exposure to oxygen yielded the oxindole B_{24}. The third product isolated from the reaction mixture was the hydroxy acylamino acetophenone derivative B_{25}. These products formed the basis for another mechanism, in which the hydroperoxide rearranged to dihydroxyindolenone B_{23}. Addition of a hydroperoxide molecule to B_{23} followed by fragmentation yields B_{24} or B_{25}[71].

Chen and Leete[64] have reported the oxidation of 2-benzyl-3-phenylindole B_{26} to 3-phenyldioxindole B_{27}. But, on catalytic oxidation B_{26} yielded 2-benzoyl-3-phenylindole

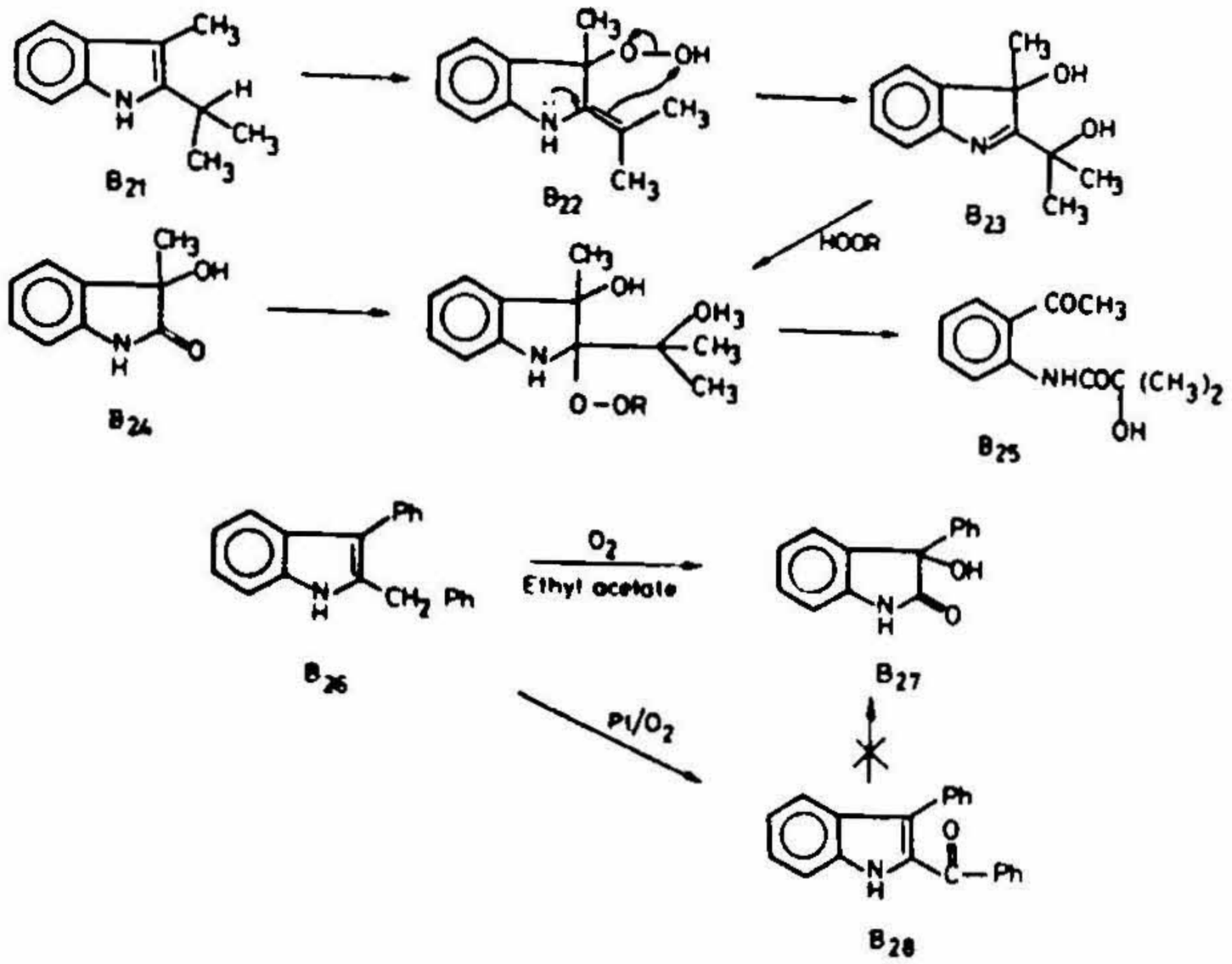


Chart XXI

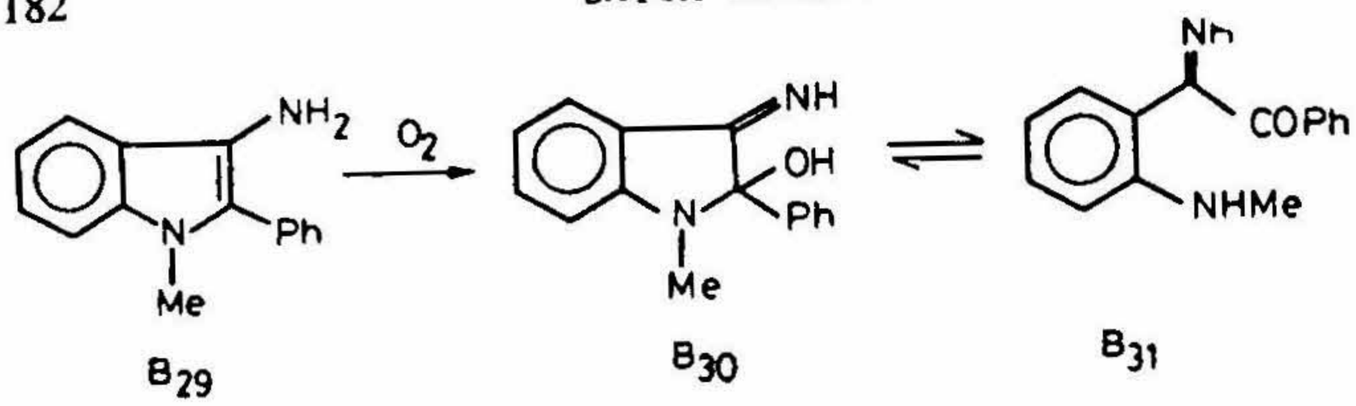
B_{28}. These two oxidation products of B_{25} are apparently formed by two distinct routes since B_{28} was not converted to B_{27} on exposure to air.

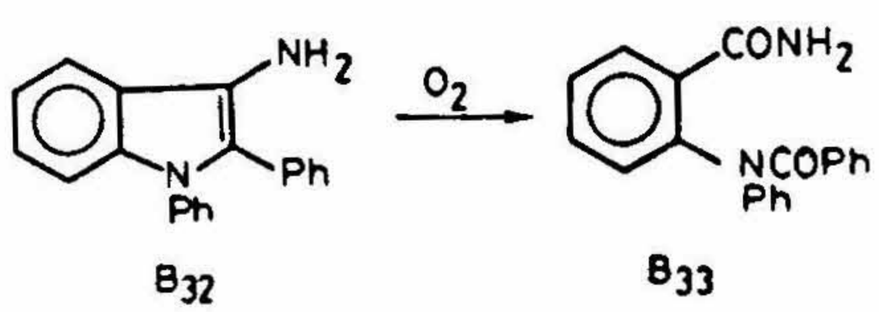
Effect of substituents on the susceptibility of the indole ring to autoxidation was also investigated. Electron releasing substituents such as amino, alkyl, at C_2 and C_3 were found to activate the indole nucleus and hence facilitate the autoxidation. Thus, 3-aminoindoles undergo autoxidation very easily. 3-Amino-1-methyl-2-phenylindole B_{29} on exposure to air gives B_{30} or its ring-chain tautomer B_{31}, while 3-amino-1,2-diphenylindole B_{32} yields B_{33}[72]. These reactions suggest that the initial attack of oxygen is at C-2 of the indole ring. In contrast to this, when the electron releasing substituent is at C-2, the active site of oxidation is C-3. Thus, ethoxy and ethylthio groups at C-2 of 3-methylindolenines B_{34} give corresponding 3-hydroxyindolenines B_{35}[73].

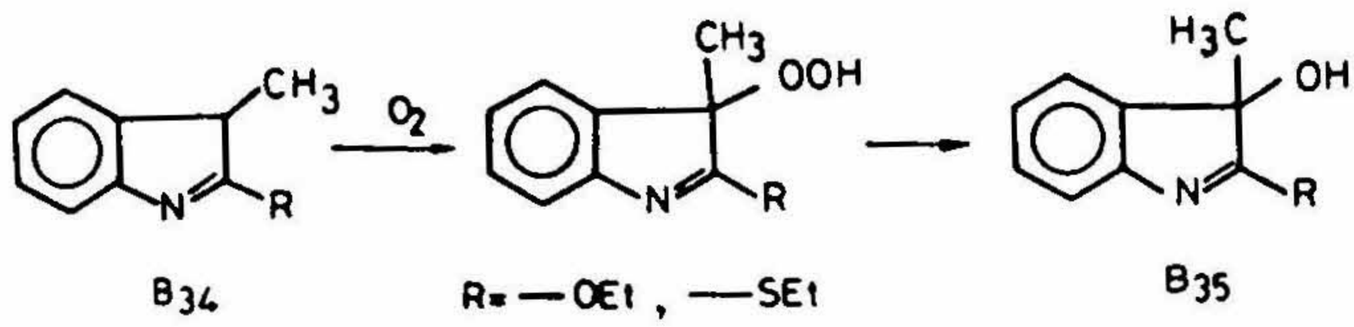
2-Methylindole undergoes to autoxidation to yield an unsymmetric dimer B_{35}[74], while 1-hydroxy-2-phenylindole B_{37} reacts with oxygen to give a symmetric dimer B_{38}[75].

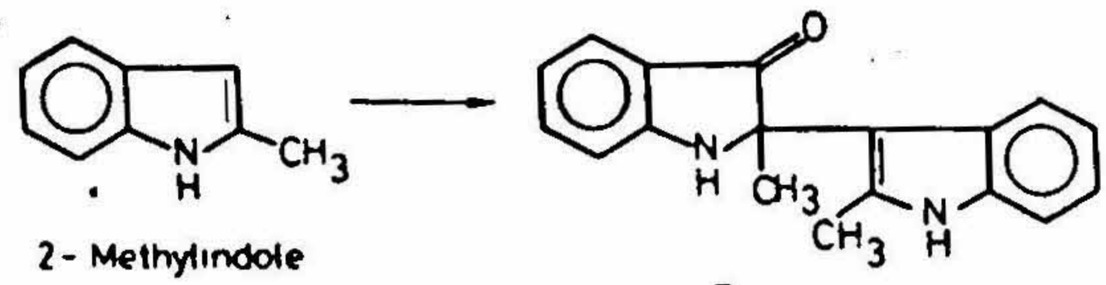
4. Biological oxidation

Owing to the physiological importance of the indole ring, considerable interest has been generated in the bio-transformations of indole and its derivatives. Particularly tryptophan and tryptamine have attracted much attention because of the possibility that abnormal matabolism of tryptamines may be involved in certain mental disorders[76].









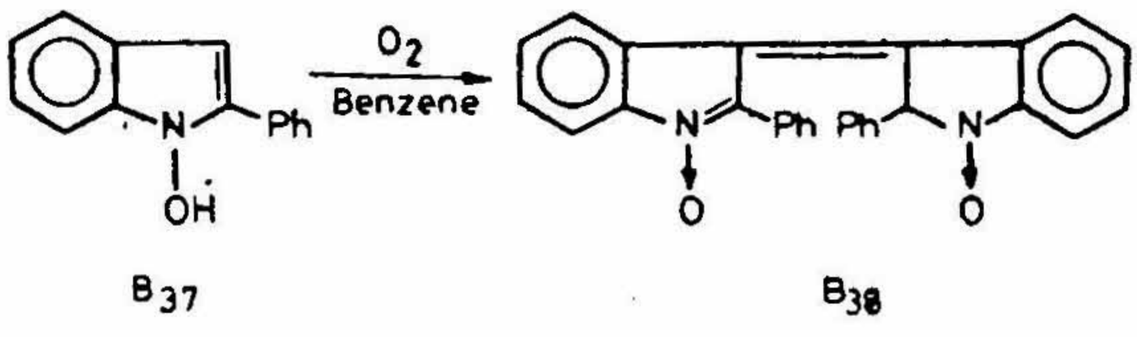


Chart XXII

Abnormal matabolism of indole has been reported in Hartnup disease[77] and schizophrenia[78,79].

For the sake of convenience the oxidation of indoles in biological systems can be divided into ring cleaving oxidations and hydroxylations that leave the indole ring intact. In biological systems it is known that the ring cleavage is often facilitated if the carbon atoms between which cleavage occurs are hydroxylated. This section deals with such metabolic transformations and pathways.

4.1. *Ring cleaving oxidation*

Prior to enzymatic studies of indole metabolism, there was an active interest in establishing the metabolic pathways by isolation of intermediates. First choice of convenience for such studies were micro-organisms wherein it proved fairly simple and easy to establish the pathway for the degradation of tryptophan.

Using the technique of simultaneous adaptation, Suda *et al*[80] elucidated the pathway for the metabolism of tryptophan in an unidentified strain of *Pseudomonas*[81] which was adapted to grow on a tryptophan medium. These cells were able to oxidize kynurenine, anthranilic and catechol but not other theoretically possible intermediates. The cells could not utilize D-tryptophan. Stanier and Tsuchida[82] carried out similar studies with another unidentified strain of *Pseudomonas*. They found that both isomers of tryptophan were attacked by these cells. The cells were also adapted to utilize kynurenine and kynurenic acid but not anthranilic acid. These observations showed the existence of two distinct routes for the metabolism of tryptophan (Fig. 1).

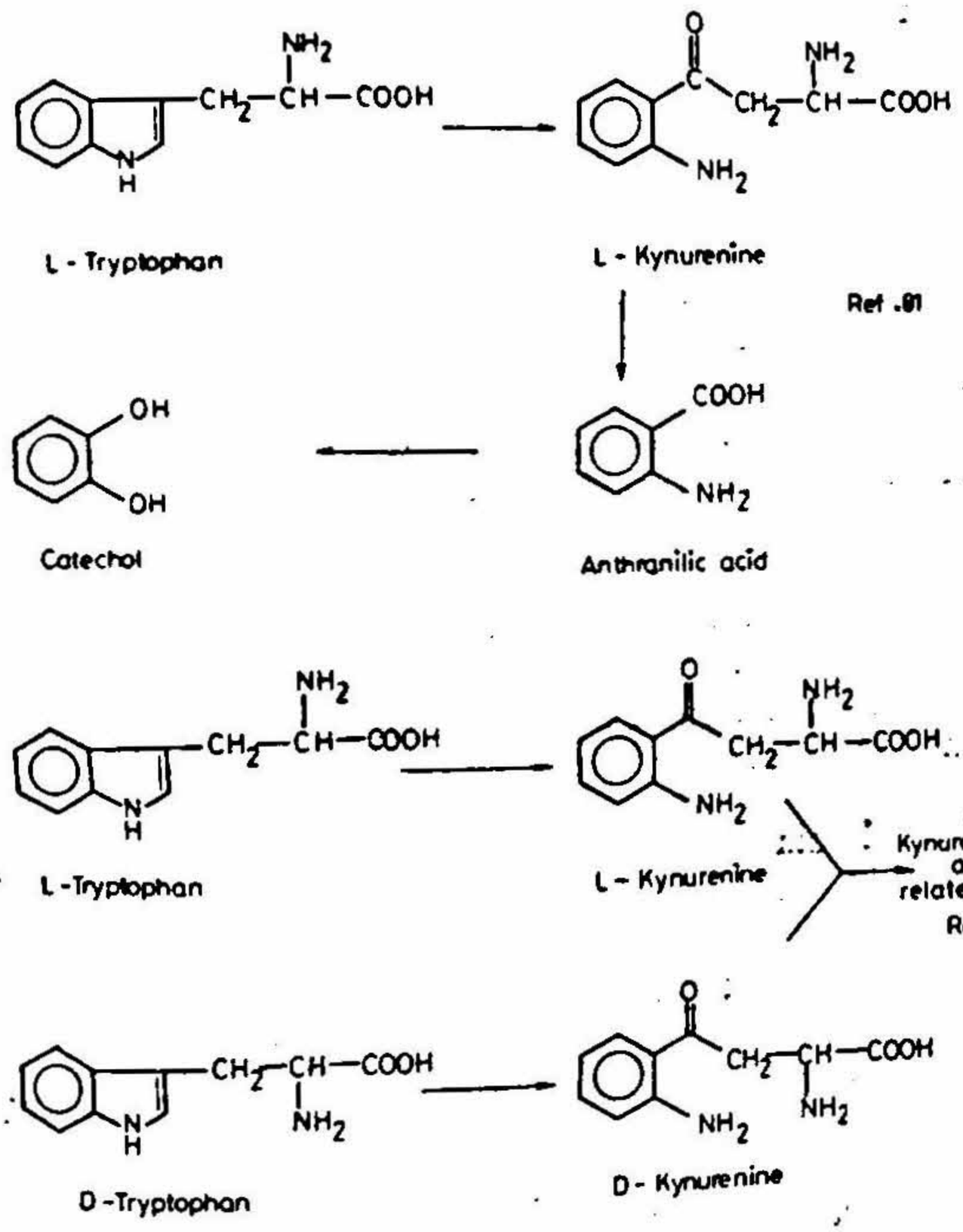


FIG. 1. Degradation of tryptophan.

The oxidation of tryptophan through the intermediate formation of anthranilic acid is known as the aromatic pathway and the alternative route is known as the quinoline pathway.

A detailed study of the aromatic pathway of tryptophan-adapted cells was carried out by Hayaishi and Stanier[83]. They employed spectrophotometric and oxygen uptake techniques with cell suspensions to establish the pathway. Since tryptophan, kynurenine and anthranilic acid have sharp and distinct absorption in the region between 250 nm and 360 nm, the speotrophotometric method proved to be ideal for the identification and estimation of these compounds.

In certain metabolically aberrant strains of *Pseudomonas*[83] an abnormality in tryptophan oxidation was observed. These strains excreted anthranilic acid into the medium and studies with cell suspension showed that tryptophan and kynurenine were rapidly converted to anthranilic acid which was isolated from the medium and characterised by comparing its properties with those of the authentic sample. Anthranilic acid was oxidized only slowly by these cells, whereas catechol was oxidized at a normal rate. This pointed out that the abnormality in the metabolism was caused by an enzyme deficiency.

Stanier and Hayaishi[84] studied the conversion of tryptophan with extracts of tryptophan-grown cells and such extracts were found to be capable of converting tryptophan to β-ketoadipic acid *via* kynurenine, anthranilic acid and catechol (Fig. 2). They were able to show the conversion of tryptophan to kynurenine and then to anthranilic acid, and tne oxidation of both anthranilic acid and catechol to β-ketoadipic acid. The inducible nature of these enzymes was shown by parallel studies, with adapted and non-adapted cells. These studies clearly showed that non-adapted cells did not contain enzymes active in tryptophan metabolism, specifically tryptophan peroxidase and kynureninase.

Some of the enzymes of the tryptophan oxidation system in *Pseudomonas* were found to be nighly unstable. If freshly prepared extracts with a high endogenous respiration were used at a high concentration, the oxidation of tryptophan, kynurenine, anthranilic acid and catechol could be demonstrated[85].

Sakamoto *et al*[86] who studied the decomposition of indole in a tap water bacterium detected isatin, formylanthranilic acid, anthranilic acid, salicylic acid and catechol in the culture filtrate. Based on these findings the following pathway was proposed for the degradation of indole : Indole → Indoxyl → dihydroxyindole → isatin → formyl-anthranilic acid → anthranilic acid → catechol (Fig. 4). Fuzioka and Wada[87] isolated a soil microorganism which utilized indole as sole source of carbon and nitrogen. Dihydroxyindole was detected as the intermediate in the metabolism of indole. Direct evidence for the formation of dihydroxyindole as intermediate was not obtained since indole was oxidized to anthranilic acid without the accumulation of dihydroxyindole. However, when skatole was incubated with indole-grown cells, the compound was oxi-

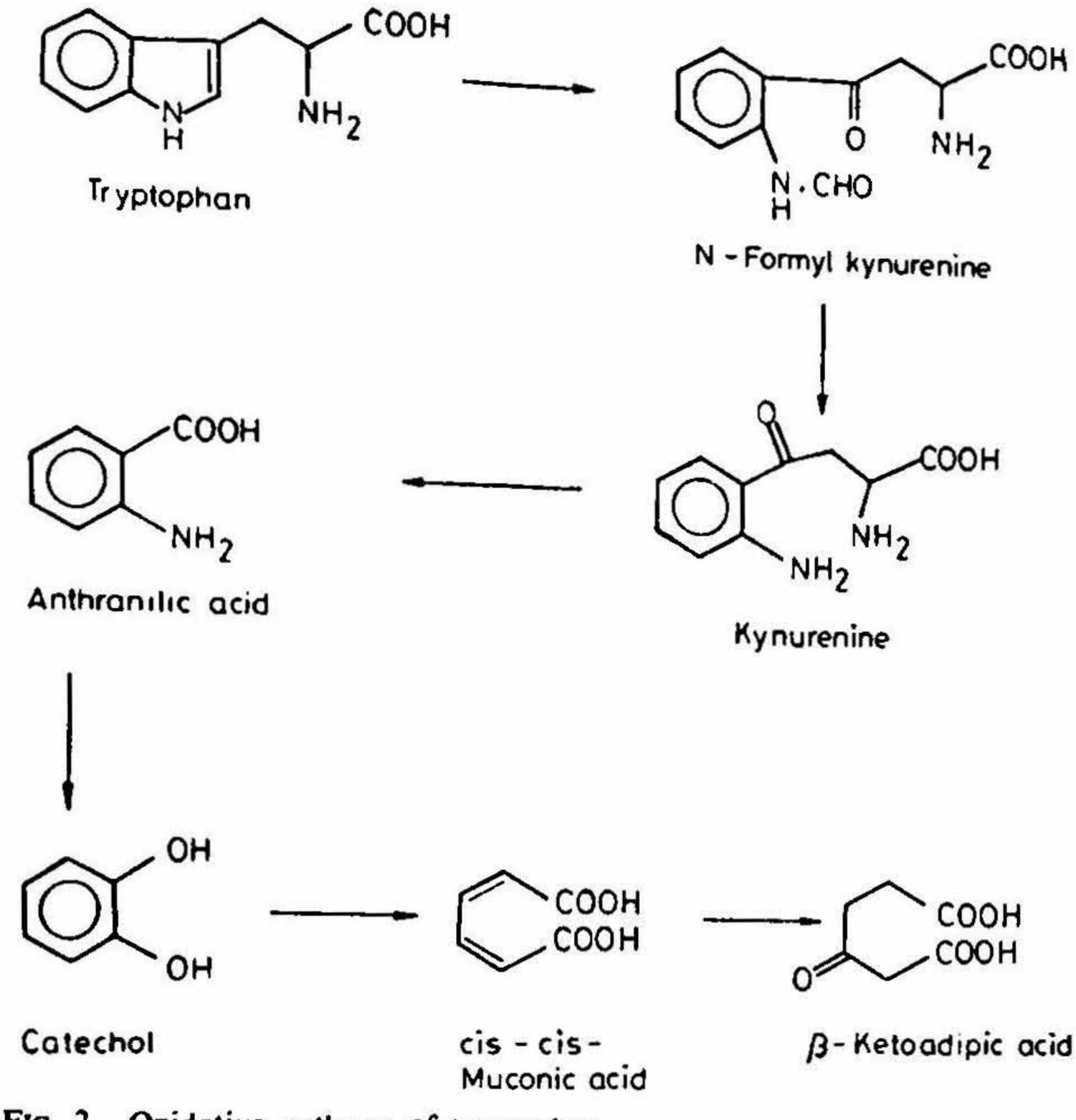


FIG. 2. Oxidative pathway of tryptophan.

dized with the consumption of one mole of oxygen per mole of the substrate to (+)-2-oxo-3-methyl-3-hydroxyindoline. This compound was not further metabolized by the organism. Skatole does not have a β-hydrogen atom and hence enolization of the intermediate formed is not possible. Therefore, ketol accumulates in the medium. The formation of 2-oxo-3-methyl-3-hydroxyindolinine from skatole and the induction of dihydroxyindole oxygenase by indole strongly suggest that indole is metabolized to anthranilic acid *via* dihydroxyindole (Fig. 4). Indole oxygenase was also partially purified from the soil organism[87].

The enzyme catalysing the oxidation of indole to dihydroxyindole could not be solubilized. The activity was found to be associated with the cellular debris. Though an epoxide mechanism has been proposed by the authors, a cyclic peroxide intermediate is more likely as the formation of 2-oxo-3-methyl-3-hydroxyindolenine from skatole could be better explained with the cyclic peroxide intermediate rather than epoxide intermediate[88] (Fig. 3).

The enzymes responsible for the ring cleaving oxidation of indole derivatives have been purified and characterized in certain instances. The indole nucleus of tryptophan

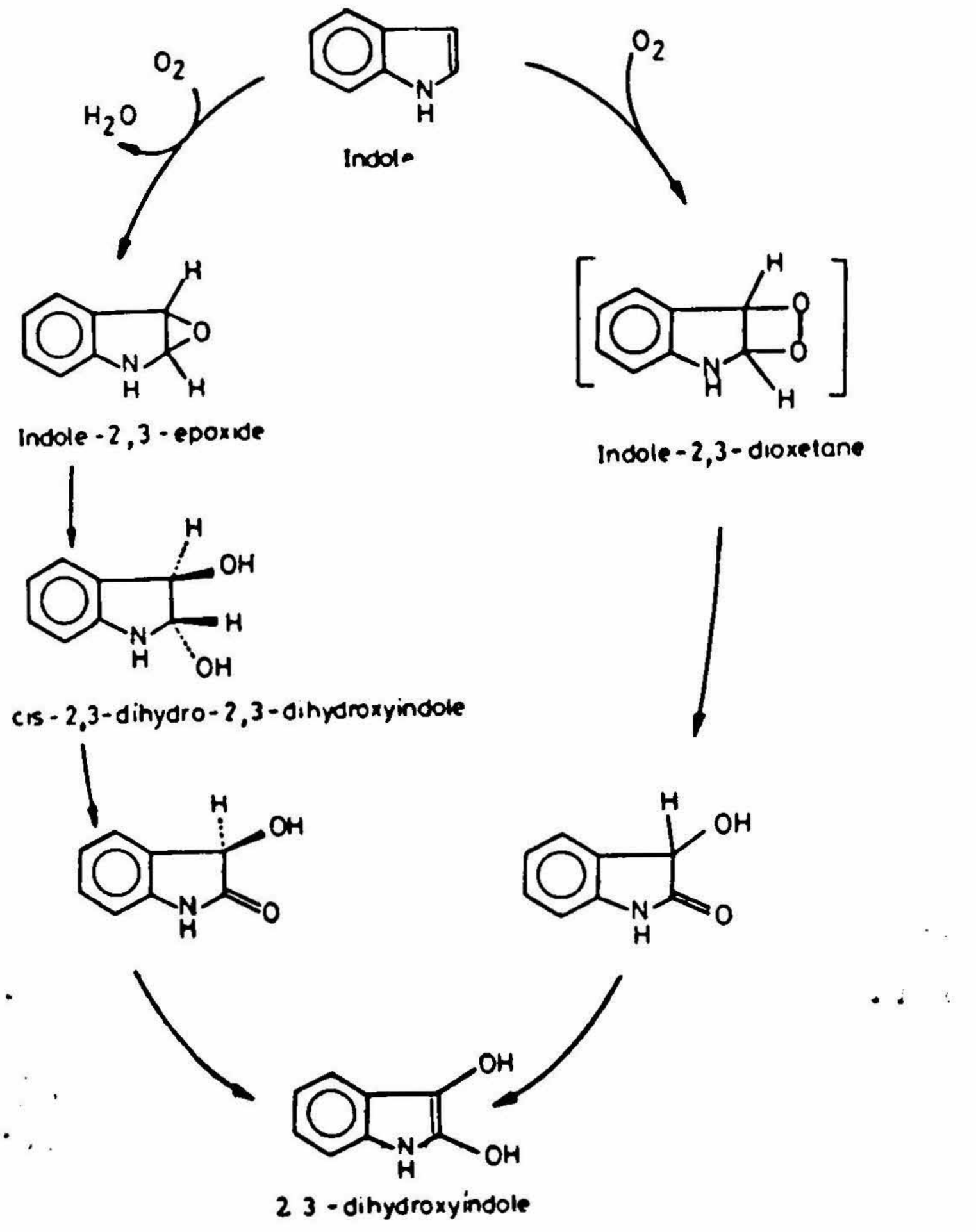


FIG. 3. Oxidation of indole by bacteria.

is cleaved between C_2 and C_3, yielding N-formylkynurenine. This reaction is catalysed by tryptophan-2,3-dioxigenase which contains a ferroporphyrin[89] and probably copper[90]. This reaction might procced *via* the intermediate formation of 3H-indolyl-hydroperoxides. A number of reviews are available on this oxidation catalysed by tryptophan-2,3-dioxygenase[91–96].

Hayaishi and his co-workers have made extensive studies on indoleamine-2,3-dioxygenase, hemoprotein which catalyses the oxidative ring cleavage of tryptophan and

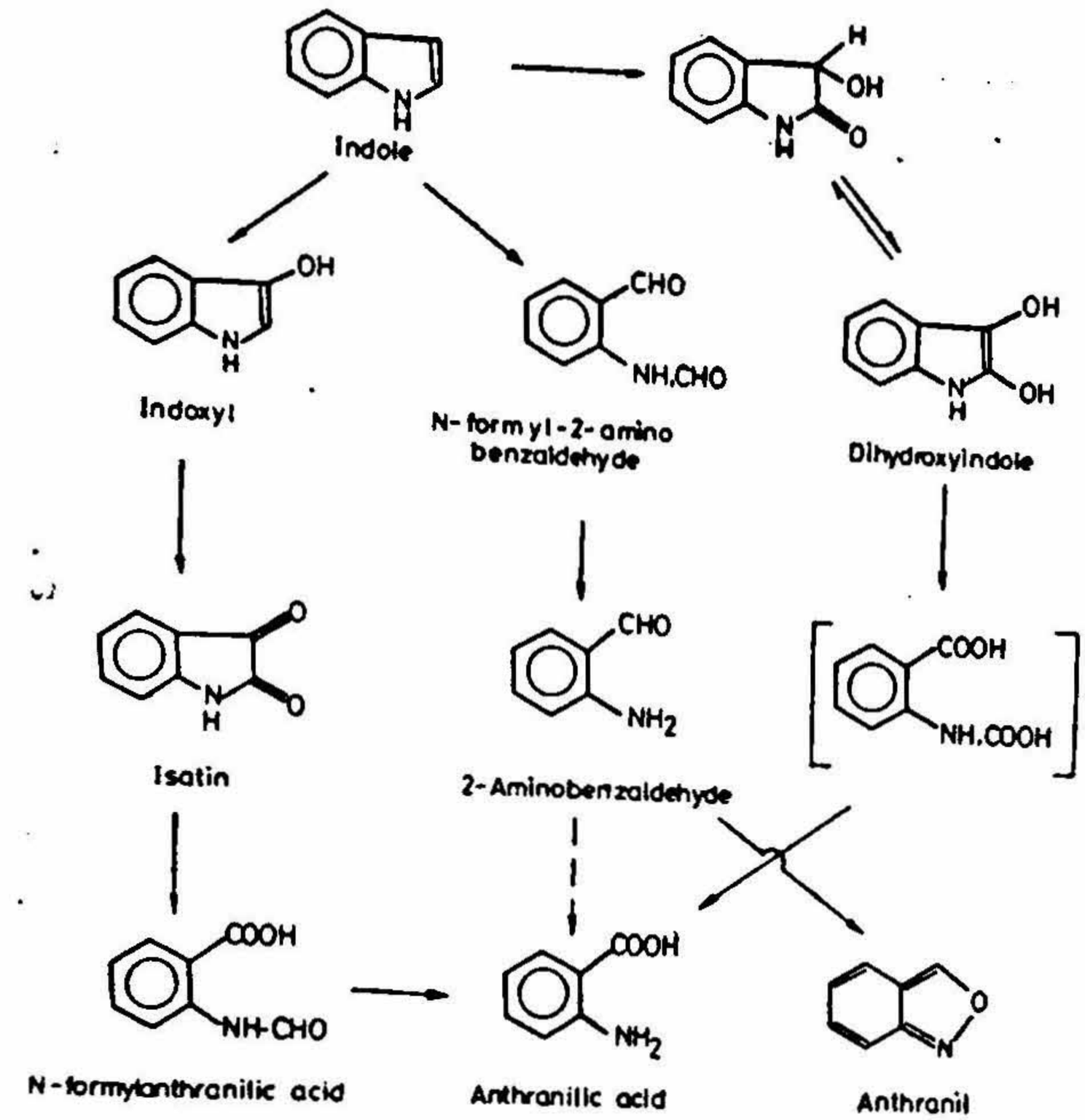


FIG. 4. Metabolism of indole in plants and in bacteria.

several other indoleamine derivatives[97,98]. This enzyme is interesting because there is clear-cut evidence for O_2^- (superoxide anion) participation in the catalytic process. The enzyme activity was drastically inhibited by superoxide dismutase[99], while catalase does not inhibit the activity. But catalase is necessary[100] for the enzyme activity for protecting the enzyme from H_2O_2 produced from O_2^-. Because of the absolute necessity of O_2^- for the enzyme activity, Sono and Hayaishi have classified this into a new category of enzymes. However, there are many unexplained problems regarding how the enzyme utilizes O_2^- for cleaving the indole ring. This enzyme has been discussed in detail in their recent review[101].

Comparatively more attention was paid to the oxidation of indole in higher plants than in microorganisms. A powerful indole oxidising system which converts indole to anthranil, by a 2,3-dioxygenase type of reaction, was isolated in 1964[102]. The partially purified enzyme from fresh, mature leaves of *Tecoma stans*, oxidized indole with the consumption of three atoms of molecular oxygen. By trapping experiments, the immediate ring cleaved product was identified as N-formylaminobenzaldehyde. Based

on chemical and enzymological studies, the reaction sequence shown in fig. 4 has been established to be one which is involved in the conversion of indole to anthranil.

The enzyme system showed a pH optimum of 5·0 and was found to be remarkably stable at acidic pH. It was highly susceptible to pH changes in neutral and alkaline condi ions. There was a sudden drop in activity on the pH range 5·8-6·2; almost 60% of the activity being lost over the range of 0·4 pH unit.

Among various metal ions tested only Hg^{+2}, Fe^{+2}, and Fe^{+3} were inhibitory to the reaction. Metal chelating agents such as 8-hydroxyquinoline, diethyldithiocarbamate and salicylaldoxime inhibited the *Tecoma* enzyme to varying degrees. The inhibition caused by the latter two reagents could be reversed only by Cu^{+2} ion, and not by any other metal ions. These studies, taken together with the reconstitution of enzymes activity in the dialysed preparations by Cu^{+2} ion, showed that indole oxidase is a cupro-protein. Unlike tryptophan-2,3-dioxygenase[91] and indoleamine-2,3-dioxygenase[98] *Tecoma* enzyme did not possess heme cofactor.

In addition to Cu^{+2}, the indole oxidase system also required FAD. Atebrin inhibited the reaction drastically and the inhibitions could be reversed by the addition of FAD The dialysed enzyme, which is inactive, could be reactivated by the addition of both Cu^{++} and FAD, thereby showing the flavin requirement for the reaction. Though it is possible to explain the FAD requirement for the second oxidation step, in view of the finding that pyridine dioxygenase requires flavin to show full activity[102], it can be speculated that flavins are involved in the indole dioxygenase reaction as well. However, further purification and fractionation of the individual enzymes are necessary to answer this problem.

Studies on the effect of sulfhydryl reagents and sulfhydryl compounds revealed that a sulfhydryl-cupric ion complex at the active site is essential for the reaction[109].

An enzyme activity which brings about a rapid indole disappearance has been detected in cell-free extracts of maize (*Zea mays*) leaves[104]. The indole utilization by this enzyme system is not dependent on serine and pyridoxal phosphate. It does not result in the incorporation of radioactive indole or serine into tryptophan. The products of indole oxidation were characterized as anthranilic acid and anthranil (Fig. 4). The enzyme activity is strongly inhibited by dithionite and diethyldithiocarbamate. The inhibition by the latter could be specifically removed by Cu^{+2}. The activity of dialysed enzyme could be restored by addition of Cu^{+2} and FAD. The activity of the indole oxidizing system was two to three times higher in normal maize varieties (Ganga-2 and Ganga-5) than in Opaque-2.

Divakar *et al*[105] have purified an indole oxygenase from the leaves of *Jasminum grandiflorum*. This enzyme is also a cupro flavoprotein as evidenced by inhibition of the reaction by atebrin and diethyldithiocarbamate. This inhibition could be reversed by FAD and Cu^{+2}. The enzyme activity is completely lost on dialysis which could be

completely restored by the addition of FAD and Cu^{+2}. The approximate molecular weight of the enzyme is 40,000. Both thiol compounds and thiol reagents drastically inhibited the enzyme activity.

The purified indole oxygenase was yellow in colour and the visible spectrum of the enzyme showed absorption maxima at 370 and 440 nm. The fluorescence spectrum of the enzyme was typical of a flavoprotein with an excitation maximum at 380 nm and 460 nm and an emission maximum at 520 nm. Approximately 0·75 g atoms of copper are present per mole of the enzyme. The enzyme activity was inhibited by substrate and this inhibition was abolished by addition of copper. The enzyme is highly specific for indole with a km value of 16 μM. The products of the reaction were identified as anthranilic acid and hydrogen peroxide[105].

We have recently reported a new indole oxygenase from the leaves of *Tecoma stans*, which catalyses the conversion of indole to anthranilic acid[106]. It is optimally active at pH 5·2 and 30° C. Two moles of oxygen are consumed and one mole of anthranilic acid is formed for every mole of indole oxidized. Neither thiol compounds nor thiol reagents inhibited the enzyme activity. The oxygenase also attacks apart from indole 5-hydroxyindole, 5-bromoindole and 5-methylindole. It is not inhibited by copper specific chelators or non-heme iron specific chelators. Atebrin did not inhibit the enzyme activity suggesting that it is not a flavoprotein, unlike other indole oxidases and oxygenases. Dialysis resulted in complete loss of enzyme activity. The inactive enzyme could not be reactivated by addition of various cofactors including FAD and Cu^{++}. This enzyme converts *o*-aminobenzaldehyde, one of the proposed intermediates, to anthranilic acid. The scheme for the oxidation of indole is shown in fig. 4. Thus, two enzymes exist in *Tecoma stans* acting on the same substrate leading to two different pathways of indole[102,106].

4.2. *Hydroxylation of indoles*

Hydroxylation of indoles in higher organisms is important for it forms the basis of detoxifying mechanisms. As it has been pointed out in chemical oxidation of indoles, the most reactive position in indole is C_3. In many biological systems also the preferred site of oxidation is 3-carbon atom[107–109]. However, if this highly reactive position is already substituted then the preferred region of hydroxylation is C_6.

Indole was oxidized by slices or homogenates of rat or rabbit liver; the oxidation was not inhibited by CN^-, NaF, α,α'-dipyridyl or H_2O_2[110]. *In vivo* experiments with liver, kidney, muscle and brain of rabbits have shown that all are active in converting indole to indoxyl. The kidney is more active[109]. Eiichi Tahata[111] has reported the conversion of indole to isatin, indican, anthranilic acid and urochrome in the isolated rabbit liver. The decomposition of indole in rabbit liver is enhanced by Vitamin C and B_{12}, which indicates that these vitamins may take part in this reaction[112].

Indole injected into the portal vein of a Chloralose dog appears in the hepatic vein as indoxyl. Indole was brought in contact *in vitro* with washed tissue of liver, kidney, lung and brain. Only liver and lung perform the oxidation of indole to indoxyl, and it may be that the lung contained blood which caused the change[113].

In mammals, including humans, there are reports of hydroxylation at 6-carbon atom of the indole ring[114]. Hydroxylation at 5-carbon atom of oxindoles in rats, guinea pigs and rabbits has been reported[115]. Cell-free preparations of rat liver could also carry out the rather unusual hydroxylation at C_5 of indole[116]. Recently, when indole-2-^{14}C was used for metabolic studies in rat, the radioactive label could be traced to indoxyl oxindole, isatin, N-formyl anthranilic acid and derivatives of 5-hydroxyindoles[116, 117].

Kluyver has reported the oxidation of indole to indigo by bacteria[118]. Indole is oxidized at the 3-position by a strain of *Claviceps purpurea* to 2,2-bis (3-indolyl) indoxyl[119]. Ohima *et al*[120] have shown that two moles of indole were oxidized to form 1 mole of indigotin in *Pseudomonas indoloxidans* with indoxyl being detected as an intermediate in the reaction.

Earlier work by Sebek and Jager[121] reveals divergent pathways of indole metabolism in *Chromobacterium violaceum*. This organism metabolizes L-tryptophan to indole by the reaction of tryptophanase. Growing and washed cells synthesize violacein from indole. Since 5-hydroxyindole and related compounds are not converted to violacein by the organism, hydroxylation must occur at a later stage in synthesis. Rapid lyophilization of washed cells inactivates enzymes of the violacein pathway and indole is metabolized to indigo *via* indoxyl.

From IR and mass spectral data, recently Sheinkman *et al*[122] have reported the oxidation of indole in soil to give mainly 2,2-bis (3-indolyl) indoxyl B_{11} along with small amounts of indirubin, and indigo. 2-Methylindole is transformed into B_{36}.

According to Corbett and Chipko[123] indole is oxidized by H_2O_2 in the presence of chloroperoxidase to give oxindole as the major product. Under most conditions, oxindole was the only product formed, and under optimal conditions the conversion was quantitative. 2-Methylindole was not affected by H_2O_2 and chloroperoxidase, but was a strong inhibitor of indole oxidation. 1-Methylindole was a poor substrate and a weak inhibitor of indole oxidation. However, oxidation of indole with H_2O_2 and horse radish peroxidase yields 2,2-bis (3-indolyl)-indoxyl and other products[124]. H_2O_2-peroxidase oxidation of skatole and 3-chloroindole does not yield indoxyl, nor does 3-chloroindole yield chloride.

The microsomes of pea seeds catalyse the hydroxylation of several compounds depending on the specific hydroperoxide. The hydroxylation of indole as the model substrate was studied in detail by Ishimaru and Yamazaki[125]. It appears that an equimolar

amount of hydroperoxide is reduced to alcohol during the hydroxylation of indole. A trivial name, peroxygenase, is proposed for this type of enzymes.

Horvath[126] has shown that much more 4-hydroxyindole than 5-hydroxyindole was produced when homogenized, etiolated pumpkin (*Cucurbita pepo*) seedlings were incubated with indole. Exposure to light for 5 hr decreased the degree of indole hydroxylation in 3- and 6-day old seedlings. For light grown seedlings, much more 4-hydroxyindole was produced by the roots than by the shoot.

Infiltration of pedunculate oak (*Quercus robur*), horse chestnut (*Aesculus hippocastanum*) and sumac (*Rhus trichocarpa*) leaves with indole followed by 24 hr incubation gave a product identified as 2,2-bis-(3-indolyl) indoxyl[127]. Hydroxylation at the 5 and 6 positions of indole by leaf and stem tissues of *Tradescantia albiflora*, *T. venezuelensis*, *T. fluminensis*, *T. blossfelidiana*, *T. sillamontana*, *Zebrina pendula*, *Z. purpurii* and *Seterasia purpurea* was detected by Horvath[128,129].

An important biological compound containing indole ring is indole-3-acetic acid, a plant growth hormone. This compound is oxidized to 3-methyleneoxindole by horse radish peroxidase[130]. This transformation is quite unusual compared to other modes of oxidation. A mechanism was proposed *via* the intermediate formation of 3-hydroperoxyindolenine and its decomposition by intramolecular participation of the acetic acid side chain (fig. 5). The spectral changes associated with this reaction have also been studied[131].

Light dependent oxidation of indoleacetic acid and its analogs occurred in Triton-X-100 solubilized chloroplasts[132]. Solubilized chloroplasts showed high rates of light dependent oxygen uptake when indoleacetic acid was present. This reaction is mediated by chlorophyll and does not require the participation of enzymes. The chlorophyll dependent oxidation of various indoles was also observed.

Hydroxylation of indoleamines is generally at 5-carbon atom. The physiologically important amine serotonin is not a product of direct hydroxylation of tryptamine but it is formed by 5-hydroxylation and decarboxylation of tryptophan as shown in fig. 6[133,134]. Direct hydroxylation of tryptamine and related compounds is also achieved. Similar to indole, they are hydroxylated at 6-carbon by liver microsomes[135]. Generally amines are known to be metabolized by oxidative deamination[136]. However, 3-(2-acetoxyethyl)-5-methoxyindole is metabolized after hydroxylation at 7-carbon atom[137]. But not much is known about the enzymes involved in hydroxylation[138].

In contrast to the general trend of hydroxylation at 6-carbon atom, in many biological systems[139-142] and with enzyme preparations[143,144] tryptophan is hydroxylated at 5-position. During this reaction a proton from C_5 shifts to C_4. This is comparable to NIH shift reported in non-indolic aromatic hydroxylations[145]. Thus, there is a possibility of arene oxide formation as an intermediate in aromatic hydroxylation in bio-

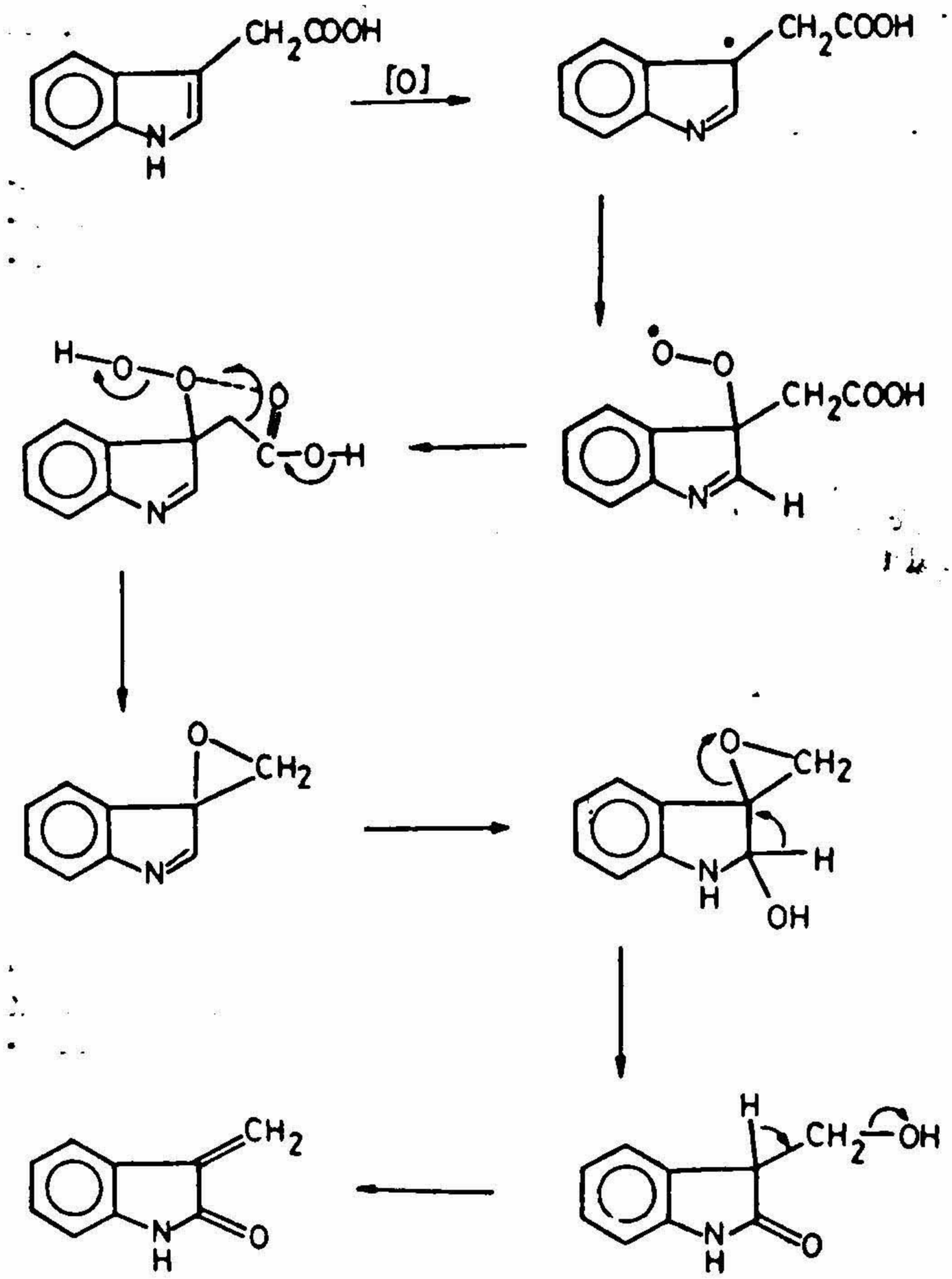


FIG. 5. Oxidation of indole acetic acid.

logical systems. Such an intermediate can explain the preferential hydroxylation at 5-carbon atom of tryptophan.

5. Concluding remarks

A comparison of the various types of oxidations discussed in this review may give us an insight into the probable mechanisms operating in biological systems that bring about metabolic transformations of indoles, whether they be hydroxylations or ring cleaving type of oxygenation. In many cases, metabolic pathways have been reported

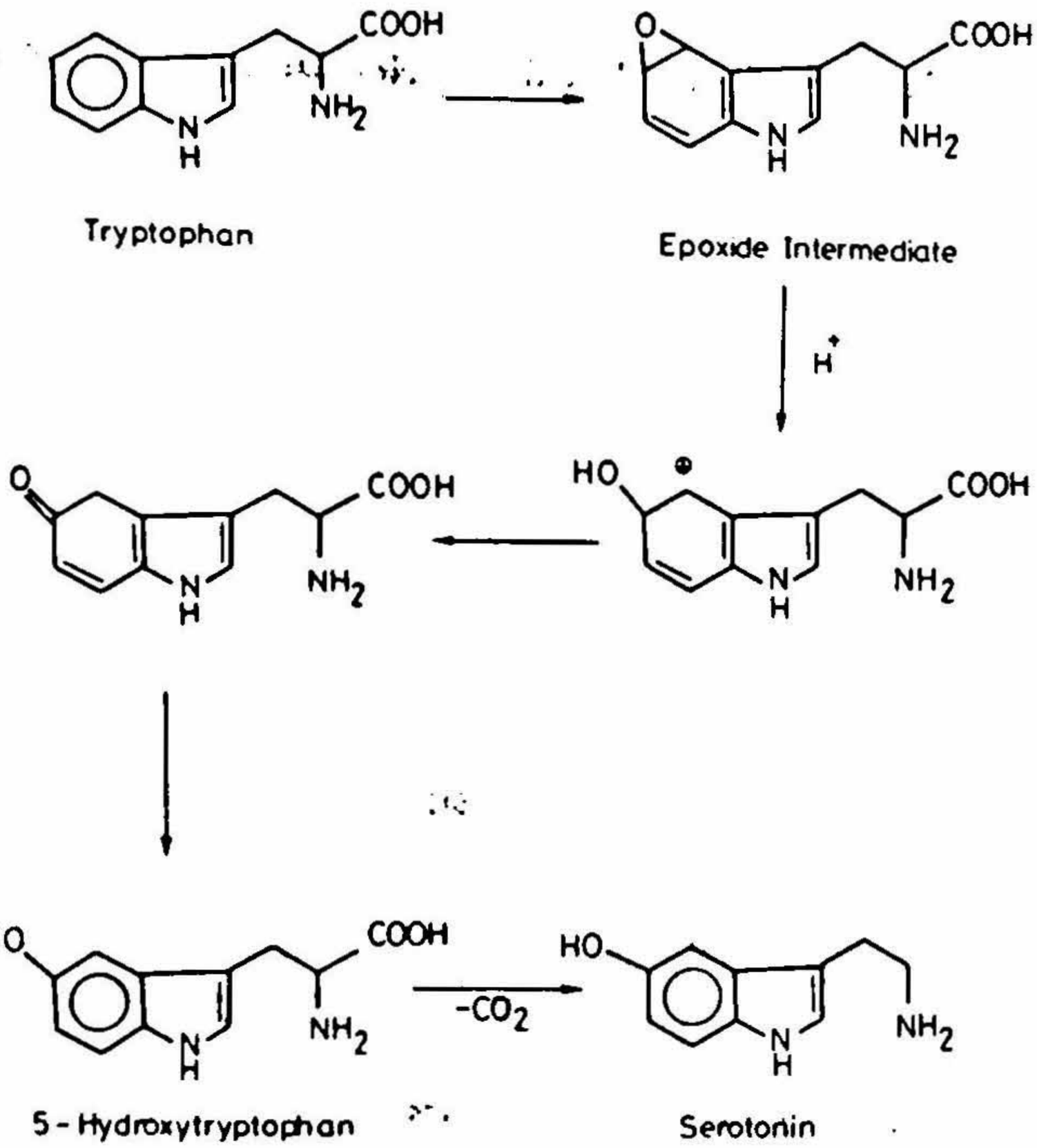


FIG. 6. Biosynthesis of serotonin.

based only on isolation of various intermediates and further research should centre on the isolation of the enzymes for the proposed pathways. In a few cases where the enzymes have been purified not much work has been done to establish the mechanisms of the catalysed reactions. Since chemical oxidations have yielded a variety of products and intermediates which have been isolated and well characterised, attempts can now be made to utilise these intermediates to elucidate the mechanism of action of purified enzymes. For example, it could be tested whether intermediates of chemical oxidation are also reactive intermediates in catalytic conversions by enzymes. In chemical oxidations of indoles there are no reports of hydroxylations. Thus, hydroxylations of indoles may be unique to biological systems serving either as detoxifying mechanisms or forming some physiologically important compounds like 5-hydroxytryptamine. In the same way, ring cleaving enzymatic oxidations of indoles may play a key role in controlling levels of serotonin and thus further studies on these lines may help in understanding the chemical basis of personality disorders like schizophrenia.

Acknowledgement

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