J. Indian Inst. Sci., 65 (C), Sept. 1984, pp. 69-107

Microbial degradation of substituted benzoic acids

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Abstract

A brief note on the role of molecular oxygen in the degradation of aromatic compounds is given. Pathways for the degradation of benzoic acid, hydroxybenzoic acids, methoxybenzoic acids, aminobenzoic acids, alkylbenzoic acids, phthalic acids, halogenated benzoic acids are discussed in detail. The nature and role of the enzymes involved in the degradation of these compounds is discussed. A summary of the aromatic ring cleavage reactions occurring in microorganisms and the enzymes responsible for these reactions is given.

Key words: Microorganisms, degradation, benzoic acids, aromatic acids, ring cleavage, dioxygenase, monooxygenase, decarboxylase.

1. Introduction

Molecular oxygen, in addition to its major function as a terminal electron acceptor, plays a vital role in the metabolism of aromatic compounds. These reactions are catalyzed by oxygenases, which utilize molecular oxygen as one of the substrates and incorporate either one or both the atoms of oxygen into the organic substrate. Oxygenases are accordingly classified as monooxygenases and dioxygenases[1]. Monooxygenases while incorporating one atom of oxygen into the substrate reduce the other to water. These enzymes are further subdivided on the basis of the reductant involved in the reaction. Dioxygenases are classified as intramolecular or intermolecular dioxygenases depending on whether they incorporate both the atoms of oxygen into the same or different substrates. The classification of oxygenases as described by Hayaishi[1] is shown in Table I. The discovery of new enzymes catalyzing double hydroxylation reactions required the addition of two more subclasses to the classification proposed by Hayaishi[1]. Several double hydroxylation reactions in bacteria were shown to be catalyzed by dioxygenases requiring an external electron donor[2]. These enzymes were earlier classified as miscellaneous and may now be added as the fourth subclass to the class of intramolecular dioxygenases shown in Table I. Anthranilate hydroxylase from *Aspergillus niger* catalyzes the conversion of anthranilate to 2,3-dihydroxybenzoate and requires NADPH and iron as cofactors[3,4]. This enzyme has now been shown to be a double hydroxylating monooxygenase by $H_2{}^{18}O$ and $^{18}O_2$ studies[5-7]. This enzyme may now be included as a seventh subclass under the external monooxygenases described in Table I.

Studies on the microbial degradation of aromatic compounds and on the role of oxygenases in this process assume importance for the following reasons. A variety of aromatic

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Table I
Classification of oxygenases (Hayaishi, 1974[1])

Oxygenase	EC No.*
A. Dioxygenases	
1. Intramolecular dioxygenase	1.13.11
a. Hemoprotein	
b. Iron-sulfur protein	
c. Copper protein	
d. Ring hydroxylating dioxygenase+	
2. Intermolecular dioxygenase	1.14.11
3. Miscellaneous	1.13.99
B. Monooxygenases	
1. Internal monooxygenase	1.13.12
2. External monooxygenase	1.14
a. Pyridine nucleotide-linked flavoprotein	1.14.13
b. Flavin-linked hemoprotein	1.14.14
c. Iron-sulfur protein-linked hemoprotein	1.14.15
d. Pteridine-linked monooxygenase	1.14.16
e. Ascorbate-linked copper protein	1.14.17
f. With another substrate as reductant	1.14.18
g. Double hydroxylating monooxygenase+	1.14.19

* EC numbers refer to the new numbering system introduced in 1972 by the International Union of Biochemistry, Enzyme Nomenclature Commission.

+ Additions to the classification proposed by Hayaishi

compounds synthesized as secondary metabolites by plants enter the soil and need to be biodegraded to maintain ecological balance. In addition, several aromatic compounds are added to the soil either as industrial wastes or from the utilization of industrial products. Soil microorganisms possess versatile mechanisms to produce new enzymes to biodegrade these compounds into intermediates which can enter the major metabolic pathways. Occasionally, even this versatility is defeated by a few man-made chemicals and therefore an understanding of the pathways of the degradation of the aromatic compounds is important to ensure that sufficient caution is exercised before environmental pollution by these compounds becomes a severe health hazard. Although several excellent reviews of this area are available[8-15], rapid progress made in recent years prompted us to undertake this review, which deals with a relatively smaller group of aromatic compounds *viz.*, substituted benzoic acids in a greater detail.

2. Degradation of benzoic acid and its derivatives

Benzoic acid and its derivatives are components of several natural compounds and a few of them such as halogenated benzoic acids and phthalic acids are produced in large quantities for industrial purposes. For this reason the metabolism of these compounds has attracted the attention of several workers.

2.1 *Metabolism of benzoic acid*

The degradation of benzoic acid by microorganisms occurred most often by an initial double hydroxylation followed by ring cleavage[16-21], although in a few instances monohydroxy derivatives were also postulated as intermediates. Initially, catechol formation from benzoate was postulated to proceed through an epoxide intermediate[22]. However, Reiner and Hegeman[23] isolated (−) 3, 5−cyclohexadiene-1, 2-diol-1-carboxylic acid from a mutant of *Alcaligenes eutrophus*, blocked in benzoate catabolism and showed that the hydroxyl groups were in *cis*-configuration. This observation ruled out the formation of an epoxide intermediate which would yield on hydration a *trans*-diol rather than a *cis*-diol. Subsequently, the *cis*-diol was identified as an intermediate in the oxidation of benzoate by *Acinetobacter, Alcaligenes, Azotobacter* and *Pseudomonas* sp.[24,25].

Fewson *et al*[26] showed that benzoate 1,2-dioxygenase required Fe^{2+} and NADH for maximum activity. The benzoate oxygenase system of *Pseudomonas arvilla* was composed of an NADPH-cytochrome *c*-reductase and a benzoate oxygenase[27]. Yamaguchi and Fujisawa[28-31] purified these components and studied them in detail. The cytochrome *c*-reductase component was an iron-sulfur protein with a molecular weight (M_r) of 37,500 containing one FAD and one iron-sulfur cluster of 2Fe-2S type[28]. The colour of the enzyme was bleached with concomitant loss of NADPH-cytochrome *c*-reductase activity upon treatment with *p*-chloro-mercuriphenylsulfonic acid. The enzyme treated with this reagent contained one FAD per mol but no iron and labile sulfide[30]. The authors showed that the iron-sulfur cluster could be reconstituted with ferrous ions and sulfide in the presence of β-mercaptoethanol. Although the iron-sulfur depleted enzymes did not show any oxygenase reductase activity, it had ferricyanide and nitrobluetetraazolium (NBT) reductase activity. The oxygenase component of benzoate dioxygenase was also purified to apparent homogeneity. It did not contain heme or flavin but was shown to contain ten mol of iron and eight mol of labile sulfide per mol of the enzyme[29,31]. The enzyme had a M_r of 201,000 and was composed of two types of subunits α and β, present in equimolar ratios. The α-subunit had a M_r of 50,000 and accounted for all the iron and labile sulfide (2 mol of each per mol of subunit). β-subunit had a M_r of 20,000 indicating that the enzyme was a trimer. Both these components were necessary for the enzyme activity and the absence of any one of them resulted in complete loss of activity. In addition, both NADH and molecular oxygen were essential for oxygenation reaction. The electrons from NADH were transferred to the oxygenase component by the cytochrome *c*-reductase whose bound FAD shuttled between reduced and oxidized forms. The electrons were finally transferred to benzoate which was converted to *cis*-3,5-cyclohexadiene-1,2-dio-1-carboxylic acid with the incorporation of both the atoms of molecular oxygen. The *cis*-diol was dehydrogenated and simultaneously decarboxylated by a bihydrodiol dehydrogenase to yield catechol. The dehydrogenase purified from *A. eutrophus*, required NAD^+ specifically and had a M_r of 96,400 consisting of four subunits of M_r 24,000[32]. The conversion of benzoate to catechol and the functions of the components of benzoate dioxygenase are shown in fig. 1.

As indicated earlier, in a few instances benzoate was degraded by the initial formation of salicyclic acid or 3-hydroxybenzoic acid[33-38]. However, the hydrolases catalyzing these monohydroxylation reactions have not been obtained in cell-free systems.

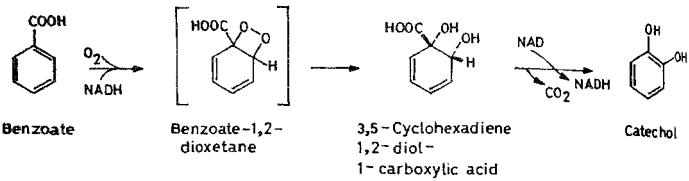

FIG. 1a. Mechanism for the conversion of benzoate to catechol.

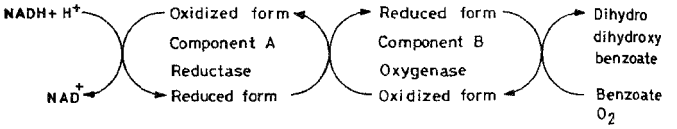

FIG. 1b. Components of benzoate dioxygenase.

Though double hydroxylation of benzoate to catechol was the common pathway in bacterial systems, monohydroxylation to *p*-hydroxybenzoic acid appeared to be the sole pathway for its degradation in fungi[37,39–46]. The hydroxylation of benzoate at the fourth position was also demonstrated in a soil *Pseudomonas*[47]. Benzoate 4-hydroxylase was isolated in our laboratory from both bacterial and fungal sources[48–50] and these enzymes required tetrahydropteridine, NADPH and ferrous iron for activity. The enzymology of benzoate dioxygenase and benzoate 4-monooxygenase has not been fully studied.

2.2 Metabolism of hydroxybenzoic acids

The various pathways for the metabolism of hydroxybenzoic acids are given in fig. 2. The metabolism of all the monohydroxybenzoic acids was initiated through the introduction of another hydroxyl function at the *ortho* or *para* position to the existing hydroxyl group.

Salicylate was oxidatively decarboxylated to catechol by several species of bacteria belonging to the genus *Pseudomonas*[33,51,52], *Mycobacterium fortuitum*[53], *Acinetobacter*[54] *Pullularia pullulans*[55] and some species of bacilli[56]. Salicylate 1-monooxygenase (decarboxylating) was the second flavoprotein monooxygenase to be discovered and the first example of external flavoprotein monooxygenases[57]. The enzyme from *Pseudomonas putida*[58] was a monomer with a M_r 57,000 and contained 1 mol of FAD per mol of enzyme, whereas the same enzyme from a soil bacterium was a dimer of M_r 91,000 and contained 2 mol of FAD and was composed of two subunits of M_r 46,000[59] Both the enzymes showed a strict requirement for NADH, while NADPH was ineffective as an electron donor. The monooxygenase nature of the reaction was established by $^{18}O_2$ studies[60]. Extensive studies carried out with the enzyme from both the sources led to the postulation of the mechanism shown in fig. 3[61]. According to this scheme the enzyme binds salicylate to form the ES complex, followed by the binding of NADH to give E-S-NADH complex. NAD^+ was dissociated from

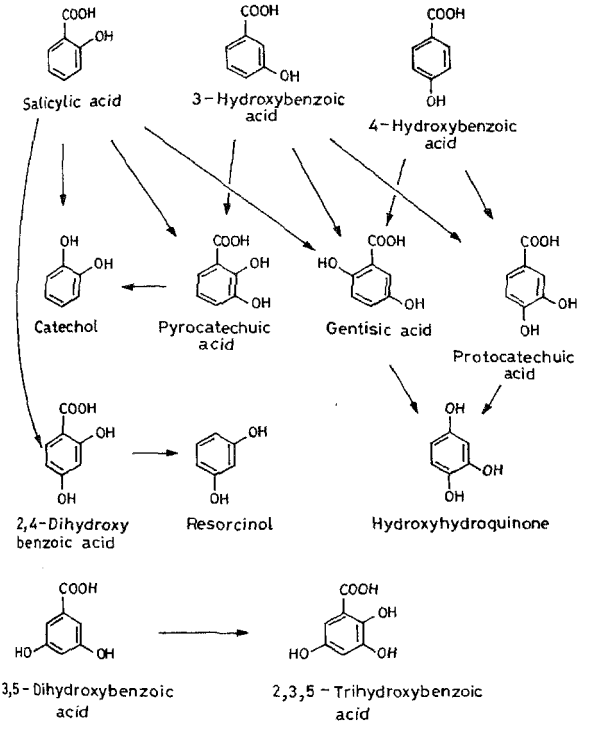

FIG. 2. Microbial metabolism of hydroxybenzoic acids.

the complex to give reduced enzyme-salicylate complex. The reduced enzyme-salicylate complex was also formed by the operation of a minor pathway involving the reduction of the free enzyme, followed by the binding of the substrate to the reduced enzyme. Reduced enzyme-salicylate complex then reacted with oxygen to form reduced enzyme-substrate-oxygen ternary complex which dissociated to yield the products and free enzyme. The reduced enzyme also reacted with oxygen to produce hydrogenperoxide and free enzyme. Salicylate hydroxylase reported by White-Stevens and Kamin[59] binds both benzoate and salicylate at the same site and also both these substrates stimulated the oxygen activation by the reduced enzyme. However, being an unsuitable substrate, benzoate decomposed the enzyme-benzoate-oxygen complex to H_2O_2, benzoate and free enzyme. Recently, Hamzah

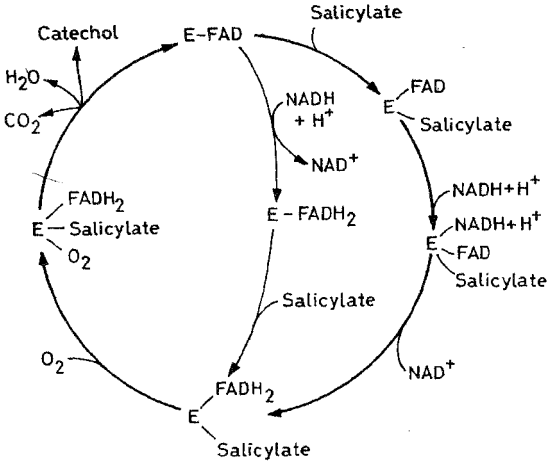

FIG. 3. Reactions of salicylate hydroxylase.

and Tu[62] by their elegant experiments using deuterated salicylate showed that the hydroxyl group was introduced at the carbon atom carrying the carboxyl group. It was found that the product catechol retained all the deuterium and no kinetic isotope effect was observed.

Chakrabarty[63] showed that enzymes of salicylate pathway were coded by plasmids in *P. putida* R_1. It was also demonstrated that this gene cluster named as SAL plasmid could be transferred by conjugation from *P. putida* to a variety of other Pseudomonads. *Pseudomonas* PMD_3 also contained a transmissible SAL plasmid of the size of 110 megadaltons of DNA[64]

Fungi in general appear to hydroxylate salicylate at the third position yielding 2,3-dihydroxybenzoic acid. Though this pathway was shown in *A. niger*[65], *A. nidulans*[66] and *Trichoderma lignorum*[67], the enzyme catalyzing this reaction was not obtained in cell-free systems. *A. nidulans*[68] hydroxylated salicylate to 2,4-dihydroxybenzoate. Salicylate was converted to gentisic acid in *Pseudomonas*[69], *Lignobacter*[70], *Nocardia*[71], *Trichoderma*[72] *Trichosporon*[73] and several species of bacilli[56]. While the enzyme synthesizing resorcylic acid was not obtained in a cell-free system, the enzyme leading to the formation of gentisic acid was obtained in cell-free extracts from a *Lignobacter*[70] and required NADH or NADPH as an electron donor.

The degradation of 3-hydroxybenzoic acid proceeded through the hydroxylation at the fourth or sixth positions yielding protocatechuate or gentisate, respectively. *P. dachunhae*[69], *P. testosteroni*[74], *A. japonicus*[75] and *A. niger*[76,77] hydroxylated 3-hydroxybenzoate to

protocatechuic acid, whereas *P. acidovorans*[35], *Streptomyces*[78], *P. fluorescens*[79], *P. aeruginosa*[80] and an unidentified *Pseudomonas*[81] yielded gentisic acid as the product. Both these pathways were shown in several species of bacilli[82] and soil bacteria[83]. Recently, a novel reaction was shown to occur in a mutant of *P. testosteroni*[84,85] While the wild type metabolized 3-hydroxybenzoate through protocatechuate, the mutant accumulated 2,3-dihydroxybenzoate, a dead-end metabolite in this organism. Surprisingly, 3-hydroxybenzoate-2-hydroxylase was not induced by its substrate but by the analog, benzoate.

3-Hydroxybenzoate 4-hydroxylase was purified from *A. niger*[77] and *P. testosteroni*[74]. The enzyme from both these sources was shown to be a flavoprotein requiring NADPH or NADH as an electron donor. The fungal enzyme exhibited a high degree of substrate specificity, whereas the bacterial enzyme hydroxylated 2,3-, 2,5- and 3,5-dihydroxybenzoates. Based on the inhibition by superoxide dismutase, the possible involvement of superoxide radicals (O_2^-) in this reaction was suggested. 3-Hydroxybenzoate-6-hydroxylase purified from *P. aeruginosa* was a flavoprotein requiring NADH or NADPH as an external electron donor[80]. It had a M_r of 85,000 and contained 1 mol of FAD per mol of enzyme. The reaction mechanism of these hydroxylases has not been worked out in detail. A comparative study of the 4-hydroxylase and 6-hydroxylase might yield interesting information on the structure function relationship of this class of enzymes, as both these enzymes use the same substrates but yield different products.

Most of the bacteria and fungi so far studied appear to degrade *p*-hydroxybenzoate through protocatechuic acid. *p*-Hydroxybenzoate-3-monooxygenase was purified from four different Pseudomonads[87,88]. All of them were found to be very similar in their requirement for NADPH, FAD content and the substrate specificity. However, the M_r of the enzyme isolated from different sources varied significantly *i.e.*, from 65,000 to 93,000[87]. The studies of Massey and his associates led to the postulation of mechanism shown in fig. 4[88-90]. The salient features of this mechanism may be summarized as follows. As in the case of other flavoproteins, the reaction catalyzed by this enzyme is composed of two discrete half reactions. The first half of the reaction was the reduction of enzyme-bound flavin by NADPH, the second half-reaction consisted of the interaction of molecular oxygen with the reduced enzyme-substrate complex. In the first half of the reaction, substrate also functioned as an effector by enhancing the reduction of the enzyme-bound flavin. Recently, Shoun *et al*[91,92] showed that the reduction rate was enhanced 2×10^5 times in the presence of the aromatic substrate. Studies on the NADPH interaction with the enzyme showed that the maximum reduction rate was pH independent in the presence and absence of the substrate, whereas the K_m for NADPH was pH dependent. The alterations of the pH profiles of the dissociation constant of the substrate indicated that the hydroxyl function on the aromatic substrate was important, although the interaction with the aromatic ring occurs at the same site.

In the second half-reaction, reduced enzyme substrate complex reacted with oxygen. By using stop flow kinetic measurements and an alternate substrate 2,4-dihydroxybenzoate, Massey's group detected three transient intermediates[88]. The first and third of these intermediates were identified as flavin C_{4a} hydroperoxide and C_{4a} hydroxide respectively. The nature of the second intermediate which showed a high extinction (1500 M^{-1} cm^{-1}) is not

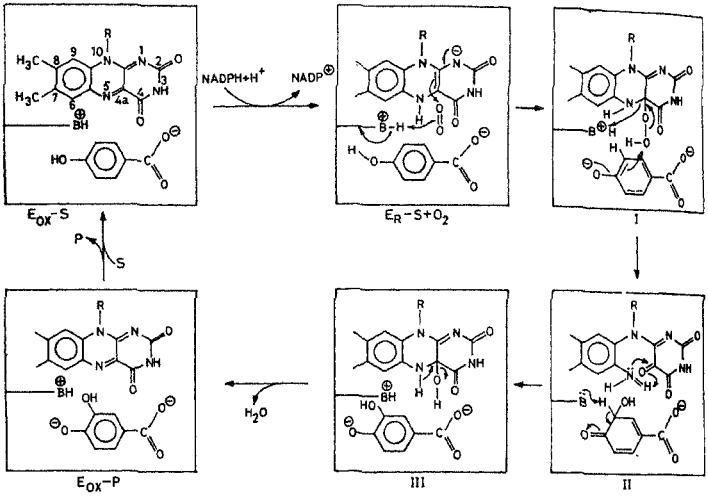

FIG. 4. Proposed mechanism of 4-hydroxybenzoate-3-hydroxylase.

yet established. Initially for the intermediate II, a C_{4a} ring-opened structure as shown in fig. 4 was proposed. This proposal was criticized by Hemmerich[93] as such an intermediate appeared improbable because it could be irreversibly autooxidized during the reaction and was not likely to have the observed high extinction. Similar objections could be raised against the intermediates proposed by Godard and Harding[94]. Alternatively, Hemmerich[93] suggested that a C_{10a} ring-opened structure could be identified as the intermediate II observed in rapid reaction kinetics. This proposal awaits experimental confirmation. Recently Massey's group adopted a new approach for the study of the flavin and enzyme interaction by reconstitution of apoenzyme with modified flavins such as 2-thio FAD[95], 1-deaza-FAD[96] and 8-mercaptoflavins[97,98]. While the studies provided information on the interaction of flavins with the enzyme, they failed to provide any new insight into the mechanism of hydroxylation[95-101]. Recently it was shown that when fluoro derivatives of *p*-hydroxybenzoate were used as substrates for the enzyme, it resulted in the release of fluoride with the consumption of two mol of NADPH instead of the normal equimolar consumption, indicating the formation of a quinonoid intermediate and its non-enzymatic reduction to protocatechuic acid[102]. These results indicated an electrophilic attack of oxygen on the substrate. Shoun *et al*[103,104] showed that the active site of *p*-hydroxybenzoate hydroxylase contained an arginine at the aromatic substrate binding site and a histidine at the NADPH binding site. The differential interaction of the -SH groups in the enzyme from *P. fluorescens* was shown recently using *p*-hydroxymercuribenzoate and N-ethylmaleiimide[105].

Conversion of *p*-hydroxybenzoate to gentisate was reported to occur in some *Bacillus* sp.[106,107]. Such a reaction obviously involved the migration of carboxyl function to the second position. The enzyme catalyzing this hydroxylation has not been purified and therefore the mechanistic possibilities have not been examined.

The pathways thus far discussed for the metabolism of hydroxybenzoic acids are the most frequently encountered ones, and the monohydroxylation of the aromatic compounds with an existing hydroxyl group leads to the formation of dihydroxy compounds which are oxygenatively cleaved. The reactions catalyzed by such ring cleaving dioxygenases are discussed at the end of this review. Some minor pathways were also reported for the metabolism of hydroxybenzoic acids. Several of the benzoic acid derivatives were non-oxidatively decarboxylated. These reactions are shown in Table II. These decarboxylases appeared not to require TPP or PALP, the usual cofactors for other decarboxylases. Recently, some interesting reactions were identified in the soil yeast, *Trichosporon cutaneum*[7,108,109] This organism lacked protocatechuate dioxygenase and gentisate dioxygenase but metabolized these compounds by further hydroxylation. While protocatechuate to

Table II
Decarboxylation of benzoic acid and its derivatives
(From Sugumaran, M. and Vaidyanathan, C.S. 1978[12])

Substrate	Product	Organism
Benzoic acid	Benzene	*Hypoxylum pruimatum*
Salicylic acid	Phenol	*Glomerella cingulata*
4-Hydroxybenzoic acid	Phenol	*Klebsiella aerogenes (Aerobacter aerogenes)*
Procatechuic acid	Catechol	*Glomerella cingulata* *Aspergillus niger* *Aspergillus nidulans* *Trichoderma lignorum*
2,4-Dihydroxybenzoic acid	Resorcinol	*Aspergillus nidulans*
Gentisic acid	Quinol	*Klebsiella aerogenes (Aerobacter aerogenes)*
Protocatechuic acid	Catechol	*Aspergillus* *Klebsiella aerogenes (Aerobacter aerogenes)* *Rhodopseudomonas*
6-Methylsalicylic acid	m-Cresol	*Penicillium patulum*
Orsellinic acid	Orcinol	*Gliocladium roseum* *Umbilicaria pustulata*
4,5-Dihydroxyphthalic acid	Protocatechuic acid	*Pseudomonas*
Gallic acid	Pyrogallol	*Klebsiella aerogenes (Aerobacter aerogenes)*

hydroxyhydroquinone conversion was catalyzed by a specific enzyme, gentisate was converted to hydroxyhydroquinone by the nonspecific salicylate hydroxylase. The insertion of a third hydroxyl group on the aromatic ring of substituted benzoic acids also occurred in the metabolism of 3,5-dihydroxybenzoic acid. This compound was converted to 2,3,5-trihydroxybenzoate by *Bacillus brevis*[110]. The hydroxylase required NADH or NADPH and the trihydroxybenzoate was further metabolized by a non-specific gentisate dioxygenase.

2.3 Metabolism of methoxybenzoic acids

Methoxybenzoates are formed during the biodegradation of lignins, hence the interest in the metabolism of these compounds. The two major and distinct routes for the catabolism of these compounds are: i) the initial O-demethylation occurs most often in bacterial systems and ii) initial oxidative decarboxylation appears to be favoured by fungi.

p-Anisic acid (*p*-methoxybenzoic acid) was O-demethylated to *p*-hydroxybenzoic acid in *Pseudomonas*[111] and *Nocardia* sp. DSM 1069[112], whereas it was oxidatively decarboxylated to *p*-methoxyphenol by some *Aspergillus* sp.[113]. In contrast, *Nocardia carolina*[114] metabolized *p*-anisic acid through an initial hydroxylation to isovanillic acid, which was further degraded through protocatechuic acid. *m*-Methoxybenzoate was first demethylated and then hydroxylated to gentise acid by *Nocardia*[112].

Veratric acid (3,4-dimethoxybenzoic acid) degradation proceeded through O-demethylation to a mixture of vanillic acid and isovanillic acid in *Nocardia*[112]. Further metabolism of vanillate occurred through at least three distinct routes. It was converted to protocatechuate in several bacterial systems[111, 115–117] and *A. japonicus*[75]. The O-demethylase and protocatechuate dioxygenase were separated by ultracentrifugation[118]. The O-demethylase was composed of two protein components, an iron-sulfur protein and an iron containing flavoprotein[119]. In addition to molecular oxygen, the enzyme required NADPH for demethylation.

Polyporus dichrous, a lignin destroying fungus was shown to oxidatively decarboxylate vanillate to methoxyhydroquinone[120]. Subsequently, the occurrence of such a pathway was shown in *Phanerocrate chrysosporium*[121], *Sporotrichum pulventrum*[122] and in several white-rot and brown-rot fungi[123]. In contrast to these observations, soft-rot fungi did not possess vanillate hydroxylase but contained protocatechuate dioxygenase[123]. Using $-O^{14}CH_3$ vanillate it was demonstrated that decarboxylation occurred before ring cleavage prior to the release of $^{14}CO_2$[122]. It was suggested that further hydroxylation of methoxyhydroquinone occurred before ring cleavage. Vanillate hydroxylase[124], hydroxyquinol dioxygenase[125] and an intracellular quinone reductase[126] activities were demonstrated in *S. pulventrum*. Vanillate hydroxylase was purified and the reaction catalyzed by this enzyme required NADPH and FAD in addition to molecular oxygen[127]. The enzyme had a M_r of 65,000 and exhibited broad substrate specificity. In addition to vanillate it also oxidatively decarboxylated protocatechuate, *p*-hydroxybenzoate, gallate, 3-O-methylgallate and 2,4,6-trihydroxybenzoate. Interestingly, horse radish peroxidase also catalyzed oxidative decarboxylation of vanillate and syringate to methoxyhydroquinone and 2,6-dimethoxyhydroquinone, respectively[128]. These compounds were subsequently oxidized by the same enzyme to their respective *p*-benzoquinones. A novel transformation of vanillate was demonstrated in several *Bacillus* sp.[129] and in a *Streptomyces* sp.[78], which

nonoxidatively decarboxylated it to guiacol and demethylated guiacol yielding catechol. Piperonylic acid (3,4-methylenedioxy-benzoic acid) was found to be metabolized to protocatechuic acid *via* vanillic acid[130, 131]. The various pathways for the metabolism of methoxybenzoic acids are shown in fig. 5.

2.4 Metabolism of aminobenzoic acids

Aminobenzoic acids are extensively produced in several industries and are also formed by reductive metabolism of nitrobenzoic acids[132]. Metabolically, anthranilic acid (*o*-aminobenzoic acid) is produced during the catabolism of tryptophan and its unique properties have focussed attention both on its biosynthesis and degradation.

Anthranilic acid was metabolized through catechol in several microorganisms[133–138]. Using [2-^{14}C]-anthranilic acid, Taniuchi *et al*[139] showed that carbon atom 2 of anthranilic acid was one of the carbon atoms hydroxylated and that neither *cis*-nor *trans*-benzenglycol

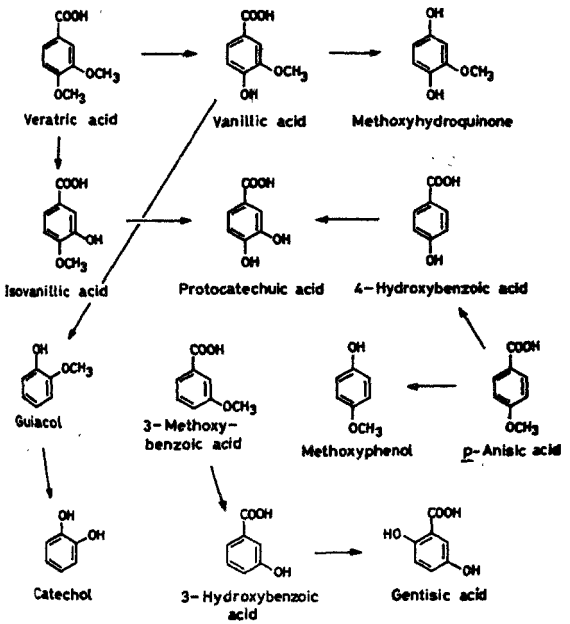

FIG. 5. Metabolism of methoxybenzoic acids.

was a dissociable intermediate in the reaction. Anthranilate oxidase from *P. aeruginosa* required molecular oxygen, NADPH and GSH for the activity[140–142]. Anthranilate oxidase from *P. fluorescens* was composed of two protein components. Ichihara *et al*[22] proposed an arene oxide intermediate in the formation of catechol from anthranilic acid. According to this scheme the arene oxide on addition of water yielded a trans-diol which was decarboxylated and deaminated to produce catechol. However, using $^{18}O_2$, Kobayashi *et al*[143] showed that both the atoms of molecular oxygen were incorporated into catechol by anthranilate hydroxylase from a *Pseudomonas* indicating the dioxygenase nature of the reaction. Hayaishi[144] proposed a mechanism involving a cyclic peroxide intermediate in the reaction catalyzed by anthranilate hydroxylase. According to this mechanism two atoms of molecular oxygen were added to the double bond between C_1 and C_2 of anthranilic acid and the cyclic peroxide intermediate thus formed released ammonia and carbon-dioxide yielding catechol in the presence of reducing equivalents. In contrast to these observations, *N. opaca* and *Achromobacter* hydroxylated anthranilate to 5-hydroxyanthranilate, a reaction which did not involve either deamination or decarboxylation[145–147].

On the other hand, fungi metabolized anthranilate through the formation of 2,3-dihydroxybenzoic acid. Thus, *A. niger*[148], *Neurospora crassa*[6] and *Claviceps paspali*[149] double hydroxylated anthranilate to pyrocatechuic acid which was subsequently converted to catechol by a separate decarboxylase[150–152]. With *Claviceps* enzyme, Floss *et al*[5] observed the conversion of anthranilate to pyrocatechuate in the presence of $H_2{}^{18}O$. The product catechol contained one atom of oxygen from the solvent indicating the monooxygenase nature of the enzyme reaction. Anthranilate hydroxylase from *A. niger* was purified in our laboratory and studied extensively[3,4,6,153–156]. This enzyme catalyzed the conversion of anthranilate to pyrocatechuic acid in the presence of NADPH and GSH. The enzyme was induced by tryptophan, kynurenine, anthranilic acid and 3-hydroxyanthranilic acid. The involvement of Fe^{2+} in the reaction was shown by activity and ESR studies. The enzyme had a M_r of 89,000 with identical subunits and reduced NBT. Kinetic studies indicated the reaction mechanism as Bi Uni/Uni Bi ping pong. $^{18}O_2$ studies showed that only one atom of molecular oxygen was incorporated into the product confirming the earlier observation of Floss *et al*[5]. Based on the above results a mechanism for the fungal hydroxylation of anthranilic acid was proposed. Figure 6 shows the bacterial and fungal pathways of anthranilate metabolism.

2.5 Metabolism of alkylbenzoic acids

Alkylbenzoic acids are formed as intermediates during the microbial degradation of xylenes and *p*-cymene, *m*-and *p*-Toluic acids were shown to be the intermediates in the catabolism of *m*- and *p*-xylene, respectively[157–162]. Davis *et al* [160] showed that *p*-toluic acid was converted to 4-methylcatechol by a dioxygenase. Davey and Gibson[162] showed that *Pseudomonas* grown on *p*-xylene as sole source of carbon also oxidized toluene, *p*-methylbenzyl alcohol, *p*-tolualdehyde and *p*-toluic acid. These authors isolated mutants of *Pseudomonas* which accumulated *p*-toluic acid and 4-methylcatechol when grown on *p*-xylene and 3-methylcatechol when grown on *m*-xylene. The oxidation of toluic acids to methylcatechols apparently involved double hydroxylation reactions leading to the formation of dihydrodiols. However, Omori and Yamada[163] demonstrated the conversion of *p*-toluic acid to *p*-cresol followed by side chain oxidation leading to the formation of *p*-hydroxybenzoic acid.

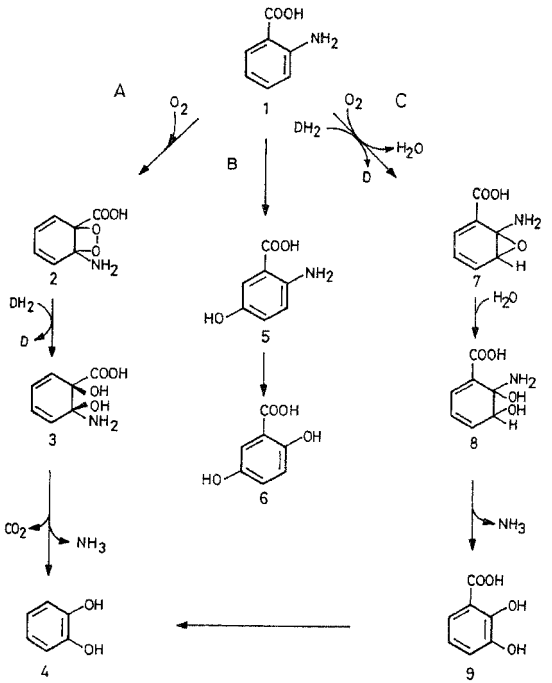

FIG. 6. Microbiol metabolism of anthranilic acid. (A) *Pseudomonas;* (B) *Nocardia opaca;* (C) *Aspergillus niger.*

The genes for the oxidation of toluic acids were present in a transmissible plasmid in a *Pseudomonas*[157,164,165]. The dioxygenase which converted toluic acids to their respective dihydrodiols was apparently coded by the plasmids and the loss of plasmid resulted in the loss of ability to degrade these compounds[166,167]. Cumic acid which was formed from *p*-cymene by side chain oxidation was metabolized to 2,3-dihydroxy *p*-cumic acid by a PL strain of *Pseudomonas*[168,169]. Initially, 3-hydroxy *p*-cumic acid was reported as an intermediate in the formation of 2,3-dihydroxy *p*-cumic acid[168]. DeFrank and Ribbons[170,171] isolated a mutant blocked in *p*-cumic acid metabolism and showed that it accumulated 2,3-dihydro-2,3-dihydroxy *p*-cumic acid. Using $^{18}O_2$ these authors showed that both the hydroxyl groups in the dihydrodiol were derived from molecular oxygen[171]. In the presence of acid this dihydrodiol was rapidly dehydrated to 3-hydroxy-*p*-cumic acid, which led to the

postulation that 3-hydroxy *p*-cumic acid was an intermediate in the catabolism of *p*-cumic acid. The dihydrodiol dehydrogenase which catalyzed the formation of 2,3-dihydroxy *p*-cumic acid from *p*-cumate 2,3-dihydrodiol was present in the crude extracts of cells grown on *p*-cymene[171] The pathways for the metabolism of toluic acids and *p*-cumic acid are shown in fig. 7.

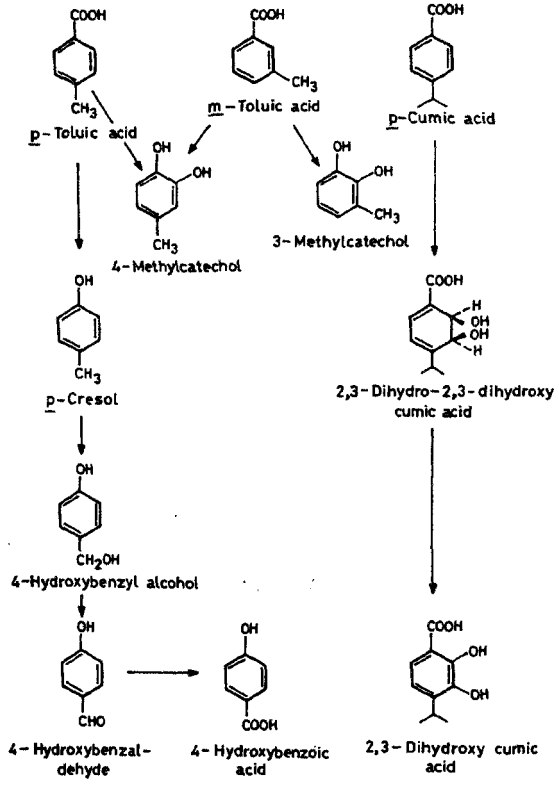

FIG. 7. Metabolism of alkylbenzoic acids.

2.6 *Metabolism of phthalic acids*

Monocarboxyl derivatives of benzoic acid *viz.*, phthalic acids are produced extensively in various industries. Phthalate (*o*-phthalic acid) esters constitute the most important group of plasticizers for vinyl and cellulose plastics. Terephthalic acid (*p*-phthalic acid) is an essential component of synthetic fibers, whereas copolymers of isophthalate (*m*-phthalic acid) and terephthalate are extensively used as transparent polyamides. Isophthalic acid was isolated from *Iris versicolor*[172] and 4,5-dihydroxyisophthalic acid was excreted into the culture medium by a *Streptomyces* sp.[173]. It was also suggested that some of the phthalate esters present in the soil and river waters may also be products of natural origin[174]. Though the metabolism of phthalic acid by a soil *Pseudomonas*[175] was reported as early as 1960, until recently little attention was paid to the biodegradation of these compounds. Phthalate esters are environmental contaminants and toxic to animal systems[176,178]. The presence of these esters was detected in milk[179,180], bovine tissues[181,182], sea jelly fish[183], and in soil[184]. This recent realization of the environmental toxicity of these compounds has revived the interest on the biodegradation of these compounds. Several bacteria belonging to the genera *Pseudomonas, Nocardia* and some soil bacteria[185–190] were shown to utilize phthalate esters as carbon source and the initial reaction in all these cases appeared to be the hydrolysis of the esters to free acid and alcohol. Diverse catabolic routes were found to be operative for subsequent metabolism of phthalic acid. In a soil *Pseudomonas*[175] and *P. testosteroni*[191], phthalic acid was degraded *via* the intermediate formation of 4,5-dihydroxyphthalic acid and protocatechuic acid. The initial reaction was apparently catalyzed by a dioxygenase to yield 4,5-dihydro-4,5-dihydroxyphthalic acid. *o*-Phthalate dioxygenase was purified and the dioxygenase nature of the reaction was confirmed by $^{18}O_2$ studies[192]. Similar pathways for the degradation of phthalate were reported in *Nocardia*[193] and *Aeromonas*[194]. 4,5-Dihydroxyphthalate decarboxylase was purified from *P. testosteroni*[195] and was found to be a tetramer of M_r 1,52,000. The purified enzyme did not require externally added cofactors and also decarboxylated 4-hydroxyphthalic acid to 3-hydroxybenzoic acid at a much lower efficiency. Recently this decarboxylase was also purified from *P. fluorescens* PHK by affinity chromatography. This was a hexamer of M_r 4,20,000[196]. Two novel routes for the catabolism of phthalic acid were reported. Aftring *et al*[197] isolated several bacterial strains from sea mud which utilized all the three phthalic acid isomers. A *Bacillus* sp. isolated by this group metabolized phthalate both by aerobic and anaerobic routes[198]. The initial reaction in the aerobic degradation was the nonoxidative decarboxylation to benzoic acid which was successively hydroxylated at third and sixth positions to yield gentisic acid, a substrate for ring cleavage[38,198]. Eaton and Ribbons[199–202] isolated several *Micrococcus* sp. capable of utilizing phthalate esters as carbon source. The initial reaction in these organisms was shown to be double hydroxylation at 3,4-positions rather than at 4,5-position. Using substrate analogue phthaldehydate, Eaton and Ribbons[201] isolated, 3,4-dihydroxy-phthaldehydate, which was not further metabolized in *Micrococcus* 12 B. Dioxygenase nature of this reaction was also confirmed by $^{18}O_2$ studies. While the phthalate 3,4-dioxygenase and protocatechuate dioxygenase were found to be inducible, 3,4-dihydroxyphthalate decarboxylase was shown to be a constitutive enzyme. Some of these *Micrococcus* strains decarboxylated 3,4-dihydroxyphthalate to yield 2,3-dihydroxybenzoic acid, whereas most of the strains yielded protocatechuic acid as the product of 3,4-dihydroxyphthalate decarboxylation[202]. Both *ortho*- and *meta*-cleavage pathways for protocatechuate degradation were operating

simultaneously in these organisms[199]. Harada and Koiwa[203] isolated several bacteria of genera *Alcaligenes, Corynebacterium* and *Arthrobacter,* capable of growing on phthalic acids. But no single strain was found to be capable of growth on all the three phthalic isomers. These authors found that *Corynebacterium* cells grown on isophthalic acid caused the accumulation of 3-hydroxyphthalic acid and protocatechuate when incubated in medium containing phthalic acid. Based on this observation a pathway involving 3-hydroxyphthalate, 3-hydroxybenzoate and protocatechuate as intermediates was proposed for phthalic acid degradation. However, the presence of these enzymes or simultaneous adaptation of the organism to these compounds was not shown. 3-Hydroxybenzoate could be formed by a non-enzymic dehydration reaction of 3,4-dihydrodiol generated during the dioxygenase reaction on phthalate. However, this postulation needs to be examined critically. The various pathways for the catabolism of phthalate are shown in fig. 8.

In comparison to the wealth of information available on phthalate degradation very little is known about the metabolism of isophthalate and terephthalate. Though bacteria of genera *Arthrobacter, Corynebacterium*[203] and *Nocardia*[193] utilized isophthalate and terephthalate, the pathways for the degradation of these compounds were not established. Based on oxygen uptake experiments, Elmorsi and Hopper[204] suggested that monohydroxy derivatives may be intermediates in the degradation of isophthalate and terephthalate. However, these compounds were not isolated from the culture medium, nor were the enzymes responsible for such conversions demonstrated in the cell-free systems. Among the monohydroxyph-

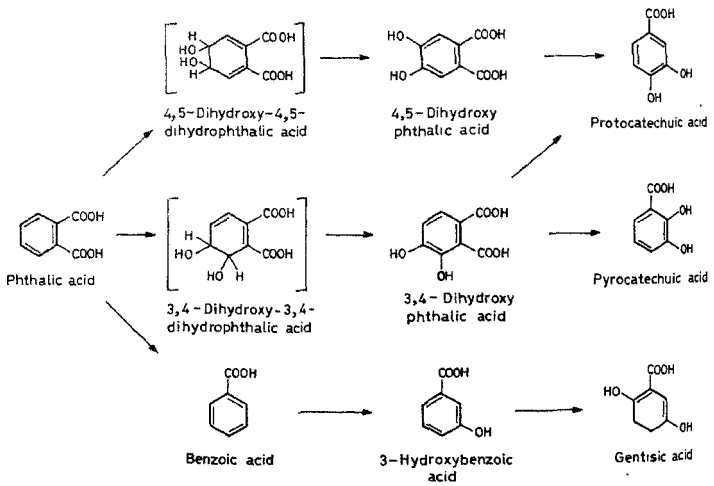

FIG. 8. Microbial degradation of phthalic acids.

thalic acids, 4-hydroxyisophthalic acid an intermediate in 2,4-xylenol metabolism by *P. putida* was oxidatively decarboxylated to protocatechuic acid[205]. 4-Hydroxyisophthalate hydroxylase was purified and shown to be a flavoprotein requiring NADPH or NADH as an external electron donor[206]. The enzyme was a dimer of M_r 105,000 and exhibited a high degree of substrate specificity. 5-Hydroxyisophthalate was degraded through 4,5-dihydroxyisophthalate and protocatechuate in a soil bacterium. The enzymes responsible for the above conversions were demonstrated in cell-free systems[207] Pathways for the metabolism of isophthalic acid, terephthalic acid and hydroxyisophthalic acids are shown in fig. 9.

2.7 Metabolism of halogenated benzoic acids

Halogenated aromatic compounds are widely used as insecticides and are produced in large quantities. These compounds are quite recalcitrant to microbial degradation. However, recent studies revealed that these compounds are biodegradable[8]. Two distinct routes of catabolism of halogenated benzoic acids were observed. These are dehalogenation before the cleavage of aromatic ring and the cleavage of aromatic ring without dehalogenation. In the latter case it was often observed that such a metabolism leads to dead-end intermediates. It was also observed that exposure of *Pseudomonas* cells to halogenated aromatic compounds resulted in the enrichment of mutants defective in catabolic pathways for other aromatic compounds[208]. This observation partly explains the recalcitrance of these compounds to biodegradation.

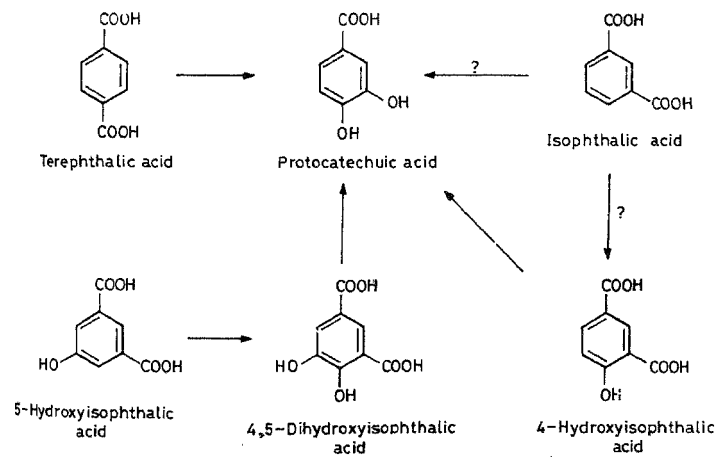

FIG. 9. Microbial metabolism of terephthalic acid, isophthalic acid and hydroxyisophthalic acids.

Dehalogenation before ring cleavage was demonstrated in several bacterial and fungal strains. While 3-chlorobenzoate was converted to 3-hydroxybenzoate by a soil *Pseudomonas*[209], 4-chlorobenzoate was converted to *p*-hydroxybenzoate in an *Arthrobacter*[210] and in a *Nocardia* sp.[211]. A similar pathway was observed in *A. niger*[212]. The mechanism of dehalogenation remains uncertain. NIH-shift was not observed and these organisms did not metabolize 3-chloro-4-hydroxybenzoate[210], thus an arene oxide intermediate appears highly unlikely. The cometabolism of 3-chlorobenzoate and 3-fluorobenzoate was demonstrated in *Vibrio* sp.[213], *N. erythropolis*[214] and in several mixed strains of organisms in the sewage[215,216]. However, the major route for the metabolism of halogenated benzoic acids appears to be by double hydroxylation to respective catechols. Thus, the oxidation of fluorobenzoates led to the formation of mixture of fluorocatechols in *Pseudomonas* sp.[217], *A. eutrophus*[218] and *Acinetobacter calcoaceticus* [219]. Along with fluorocatechols, catechol was also formed in *Pseudomonas* sp. B-13[220] and *Acinetobacter*[219]. Higher levels of benzoate dioxygenase, dihydrodiol dehydrogenase and catechol dioxygenase was observed. This probably prevented the accumulation of toxic 3-fluorocatechol in *Pseudomonas* sp. B-13 grown on 4-fluorobenzoate[220]. Double hydroxylation of chlorobenzoates was reported in *Arthrobacter*[221] and *Pseudomonas*[222]. Reineke and Knackmuss[223–225] studied the effect of various substituents in the aromatic ring on the catabolism of these compounds. Their results showed that electron attracting substituents like halogens, decreased the reaction rates of benzoate dioxygenase. In *Pseudomonas putida* mt-2 the rate of benzoate 1,2-dioxygenase catalyzed reaction was not affected by substituents, whereas in *Pseudomonas* sp. B-13 and *Alcaligenes* the rates decreased drastically due to steric effects[223]. These authors also isolated several dihydrodiols from the culture medium of organisms grown on chlorobenzoic acids and showed their *cis*-configuration by acetonide formation[226].

The genes for the enzymes of 3-chlorobenzoate degradation were shown to be present on a plasmid PAC 25 in *P. putida*[227]. This plasmid had a mean M_r of 68×10^6 daltons and was transmissible to a number of *Pseudomonas* sp.[227]. The PAC 25 coded for a dioxygenase specific for chlorocatechol and this enzyme did not act on catechol. In contrast to benzoate degradation which proceeded by the ortho-cleavage of catechol, the plasmid specified pathway for chlorocatechol proceeded through maleylacetate as an intermediate[228,229]. Recently a new strategy was adopted for construction of bacteria degrading halogenated aromatic compounds[225,230–234]. This was achieved by conjugation of strains with the ability to convert chlorobenzoates to chlorocatechols, but not capable of further metabolism of substituted catechols, with strains having the ability to degrade chlorocatechols but are unable to hydroxylate chlorobenzoates. This type of hybrid organisms have been employed to understand the degradation of halogenated aromatic compounds. The various pathways for the metabolism of halogenated benzoic acids are shown in fig. 10.

3. Pathways for the cleavage of aromatic ring

From the review of literature presented in the preceding pages, it is apparent that catechol and other dihydroxybenzoic acids are formed as terminal aromatic intermediates during the microbial degradation of substituted benzoic acids. Aromatic ring cleavage catalyzed by dioxygenases is the most critical step in the mineralization of aromatic compounds, as the aliphatic compounds formed by the action of dioxygenases enter the tricarboxylic acid cycle by simple decarboxylation, hydrolysis and isomerization reactions. All the dioxygenases

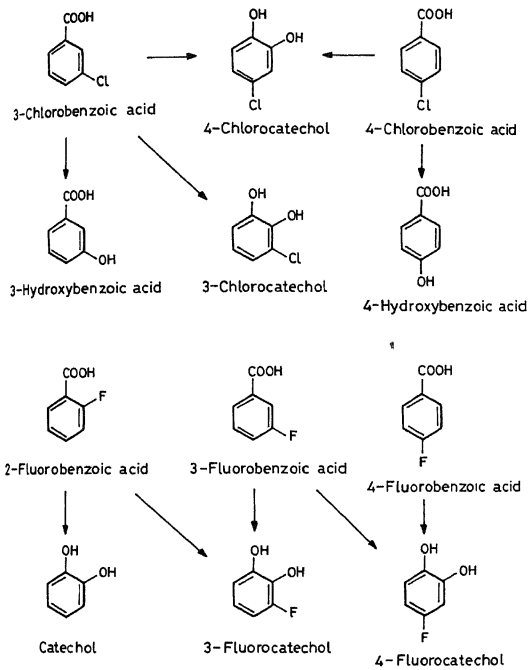

FIG. 10. Metabolism of halogenated benzoic acids.

acting on phenolic compounds that have thus far been purified and characterized contain nonheme iron as the sole cofactor except for the homoprotocatechuate-2,3-dioxygenase from *B. brevis* which was shown to contain manganese II[235]. Recently a model system for the Mn(II) dioxygenase was also demonstrated[236]. A brief summary of the ring cleavage of catechols and other hydroxybenzoic acids encountered in the metabolism of substituted benzoic acids is pertinent.

3.1 *Ring cleavage of catechol*

Catechol 1,2-dioxygenase also known as pyrocatechase, catalyzes the intradiol ring cleavage of catechol to *cis-cis*-muconic acid. It was first isolated by Hayaishi and Hashimoto[237] in 1950 and subsequently Hayaishi *et al*[238] using $^{18}O_2$ showed the dioxygenase nature of the reaction. Catechol dioxygenase was purified from *P. putida*[239], *P. fluorescens*[240], *A. cal-*

coaceticus[241] and *Brevibacterium fuscum*[242]. The enzyme from *Pseudomonas* had a M_r of 90,000 and contained 2 g atoms of iron per mol of enzyme[239]. However, catechol dioxygenase from *Brevibacterium* had a M_r of 64,000 and contained only 1 g atom iron per mol of enzyme. The enzyme from *Brevibacterium* exhibited narrower substrate specificity than the *Pseudomonas* enzymes. *Pseudomonas* enzyme catalyzed the extradiol cleavage of 3-methylcatechol, whereas the *Brevibacterium* enzyme cleaved this substrate only by the intradiol cleavage[243]. Recently, Que[244] showed that pyrocatechase oxidized *o*-aminophenol by an extradiol mechanism to an extent of 95 per cent.

Pyrocatechase has pronounced red colour with a broad absorption band between 390 and 650 nm. Treatment with acid or dithionite resulted in the loss of red colour and the enzyme activity. On reconstitution of the apoenzyme with Fe^{2+} and reoxidation of reduced enzyme, the enzyme activity as well as colour were restored. Pyrocatechase showed a sharp electron spin resonance (ESR) signal at $g = 4.28$ which was characteristic of Fe^{3+}. This signal decreased upon the addition of sodium dithionite under anaerobic conditions and reappeared when air was let in[245].

Dorn and Knackmuss[246] detected two pyrocatechases in a *Pseudomonas* grown on 3-chlorobenzoate, whereas cells grown on benzoate contained only one of these enzymes. Pyrocatechase II observed in 3-chlorobenzoate grown cells had a M_r of 67,000 and showed higher activities with 3-chloro-and 4-chlorocatechols in comparison to the pyrocatechase I found in benzoate grown cells. Pyrocatechase I had a M_r of 82,000 and chlorocatechols were poorer substrates for this enzyme. Further metabolism of chloromuconic acids proceeded through maleylacetate instead of β-ketoadipate, the normal intermediate in the catechol orthocleavage pathway[228, 229]

Catechol 2,3-dioxygenase also known as metapyrocatechase, catalyzes the conversion of catechol to 2-hydroxymuconic semi-aldehyde[247]. It was the first dioxygenase to be obtained in a crystalline form[247]. It had a M_r of 140,000 and was made up of three or four subunits, each having one g atom of iron[248]. The cleavage of catechol, methyl and chlorosubstituted catechols is depicted in fig. 11.

3.2 Gentisic acid degradation

Gentisate 1,2-dioxygenase catalyzes the conversion of gentisate to maleylpyruvate. The enzyme was purified from a *Pseudomonas* sp. by Lack[249] and Sugiyama *et al*[250] and required Fe^{2+} for activity. Crawford *et al*[251] isolated gentisate dioxygenase from *Morexella osloensis* in a homogeneous form and the enzyme appeared to be a tetramer made up of identical subunits of M_r 40,000. In addition to gentisate, a number of substituted gentisic acids served as substrates for this enzyme, but with less efficiency. Maleylpyruvate was further metabolized to fumarate and pyruvate by a GSH-dependent isomerization reaction and the subsequent hydrolysis of fumarylpyruvate. Some organisms contained a GSH-independent enzyme and others hydrolyzed maleylpyruvate directly to maleate and pyruvate. Crawford and Frick[252] developed a rapid spectrophotometric procedure for the identification of these pathways. Recently, Crawford *et al*[253] found that a *Bacillus* sp. cleaved 5-chlorosalicylate between carbon atoms 1 and 2 and this enzyme did not show any gentisate dioxygenase acitivity. Figure 12 shows the pathways for the oxidation of gentisic acid.

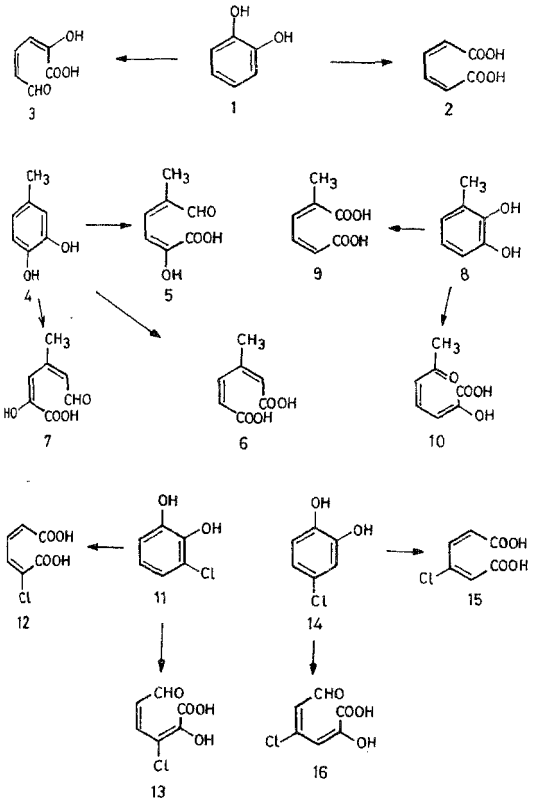

FIG. 11. Ring cleavage of catechol, methyl- and chlorocatechols.

3.3 *Cleavage of pyrocatechuic acid*

Ring cleavage of pyrocatechuic acid in *P. fluorescens* was first reported by Ribbons[254]. Subsequently, Madhyastha *et al*[169] reported pyrocatechuate ring cleavage between 1 and 2 positions by a soil pseudomonad grown on *p*-cymene. However, DeFrank and Ribbons[255] carefully studied the reactions and concluded that the ring cleavage occurred between 3 and 4 positions. From our laboratory, we reported the intradiol cleavage of pyrocatechuic acid in a

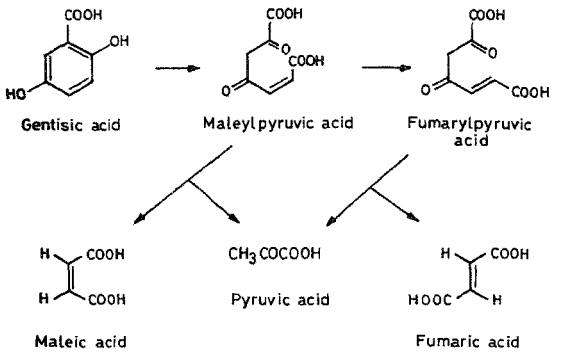

FIG. 12. Metabolism of gentisic acid.

higher plant *Tecoma stans*[256]. Interestingly, this enzyme required copper and not iron for activity. Oxidation of pyrocatechuate and its derivatives are shown in fig. 13.

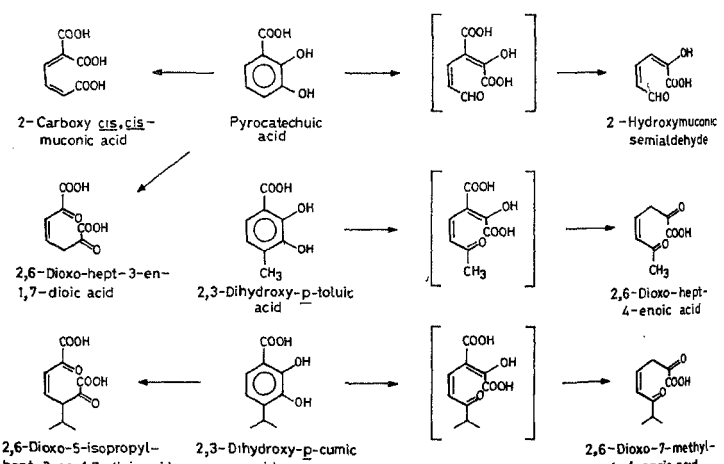

FIG. 13. Oxidation of pyrocatechuic acid and derivatives.

4. Ring cleavage of protocatechuic acid

Protocatechuate 3,4-dioxygenase catalyzes the conversion of protocatechuic acid to β-carboxy *cis-cis*-muconic acid and probably represents the most thoroughly studied intradiol cleaving enzymes. The enzyme was reported to be present in an unidentified soil *bacterium* [257], *Pseudomonas* sp.[258-260], *A. calcoaceticus*[261], *Nocardia*[262], *Neurospora*[116] and *Micrococcus*[263]. The enzymes from *P. aeruginosa*[264], *Acinetobacter*[261] and *P. putida*[259] were purified to homogeneity and that from *P. aeruginosa* was crystallized[265]. The *P. aeruginosa* enzyme had a M_r of 70,000 and was composed of 16 α (M_r 22,500) and 16 β (M_r 26,500) polypeptides. The enzyme was an octomer of $\alpha_2\,\beta_2\,Fe^{3+}$ type subunits. Though initially a M_r of 420,000 was reported for the *P. putida* enzyme, recent studies by Bull and Ballou[259] revealed that this enzyme was a tetramer of M_r 200,000 and the iron to subunit stoichiometry was found to be one g atom of iron per subunit (α,β)[259]. α-Subunit of protocatechuate dioxygenase was sequenced[266,267]. The enzyme was red in colour and showed broad absorption from 400 to 650 nm. Reduction bleached the colour and on reoxidation the colour reappeared. It had a sharp ESR signal at $g = 4.31$ which diminished on addition of substrate under anaerobic condition. These results were attributed to the presence of ferric iron whose ligand field was affected by the addition of substrate[264].

Protocatechuate 4,5-dioxygenase catalyzed the conversion of protocatechuate to 2-hydroxy-4-carboxymuconic semialdehyde. It was the first meta-cleaving dioxygenase to be characterized[268]. It was purified from *P. testosteroni* and was shown to attack gallate and 3-*o*-methylgallate also[269,270]. It had a M_r of 140,000 and was shown to contain 4 g atoms of iron per mol of enzyme. This enzyme from a *Pseudomonas* was also purified to near homogeneity. It had one mol of iron per mol of enzyme and M_r of 150,000[271]. The enzyme was rapidly inactivated during catalysis which was prevented by L-cystiene[269]. Such inactivation was attributed to the removal of iron, as external addition of ferrous iron fully reactivated the enzyme[271].

Protocatechuate 2,3-dioxygenase catalyzed the conversion of protocatechuate to 5-carboxy, 2-hydroxymuconic semialdehyde. The enzyme isolated from a *Bacillus* sp.[272,273] was shown to act on methyl and ethyl esters of protocatechuate also. However, only 2-hydroxymuconic semialdehyde was isolated, as the intermediate of ring cleavage product was very labile. All the three modes of ring cleavage of protocatechuate and the cleavage of gallate and *o*-methylgallate are shown in fig. 14.

The survey of literature clearly points out the existence of several diverse pathways for metabolism and biodegradation of aromatic compounds. These pathways are essentially aerobic and the activation of oxygen is a prerequisite to biodegradation. The detailed molecular mechanism of oxygen activation is still far from clear, but initial steps involve electron additions from metal ions, pteridine, flavin or pyridine nucleotide coenzymes. In addition, it is apparent that the metabolism of the same compound might occur by entirely different routes in different organisms.

A detailed study has to be done on the genetic aspects of the degradation of these compounds especially the halogenated aromatics and it would form a separate review by itself.

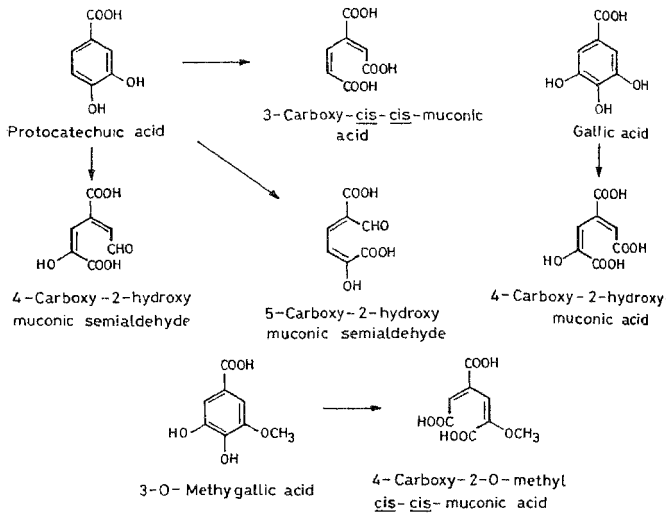

FIG. 14. Ring cleavage of protocatechuic, gallic and 3-O-methyl gallic acids.

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