REVIEW

BIOTRANSFORMATIONS OF FLUOROAROMATIC COMPOUNDS

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SUMMARY

Fluorochemicals are rare in Nature and fluoroaromatic compounds have not been described as natural products with respect to de novo formation of aryl-fluorine bonds. By contrast many synthetic fluoroaromatic compounds are substrates for several microbial enzymes, particularly oxygenases, and are transformed to previously undescribed fluorochemicals suitable for further modifications by other enzyme systems or by chemical means. These lead to interesting multi-functional molecules, monomers for novel chiral and achiral polymers, chiral intermediates and synthons for some heterocycles and α- amino acids. Wild-type and mutant microbial strains have been used to biotransform some fluoroaromatic compounds in near quantitative yields to novel fluorinated products, as well as other fluorophenolics already known by chemical synthesis. Some of the microbial transformations of mono-, di-, tri- and tetrafluoroaromatic compounds are described, and the vast potential of further biotransformations is indicated.

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INTRODUCTION

Fluorine chemistry is 100 years old [1]. In spite of the relative abundance of this most electronegative element in fluoro-minerals, ranking 13th in the Earths' crustal rocks, Nature has decided not to use this nucleus for the creation of biological molecules, but with some rare exceptions [1,2]. The biological formation of carbon-fluorine bonds is not at all understood [56]; however many, and possibly most fluorinated analogues of compounds normally metabolized (transformed) are suitable pseudo-substrates for biocatalysts (cells or enzymes) to give rare or novel products. We intend to illustrate the catalytic versatility some micro-organisms possess for the biotransformations of synthetic fluoroaromatic compounds to oxidized fluorinated chemicals.

Biological processing of fluorinated chemicals was brought to the fore by Sir Rudolf Peters's studies on the biochemical basis of fluoroacetate toxicity in animals [2]. Fluoroacetate, one of a handful of naturally occuring fluorochemicals, is a constituent of several plants, first noted in South Africa e.g. gifblaar (Dichapetalum cymosum) because of their toxicity to grazing cattle in the Transvaal, and later in the Antipodes (Acacia georginae and some Leguminosae), and South America (Rosaceae) [2]. After ingestion and absorption fluoroacetate is metabolized to the thio-ester of Coenzyme A (CoA) which is a substrate for the enzyme that normally condenses "acetyl units" with oxaloacetate to form citrate. Only one diastereoisomer of the chiral analogue fluorocitrate is formed, (-) erythro(2R,3R). This is the only isomer of fluorocitrate that is a powerful inhibitor of both aconitase activity (isomerization of citrate to isocitrate) and the transport of citrate across the mitochondrial membrane in eucaryotes [4,5]. The biological conversion of fluoroacetate to the actual toxic metabolite fluorocitrate was described as a lethal synthesis [2].

Synthetic fluoroaromatic compounds have been very useful analogue metabolites for studying biological processes. They have been used : (i) to help elucidate metabolic pathways [6]; (ii) as reporter compounds after their incorporation into macromolecules [7,8]; (iii) for studies of metabolic regulation

[9]; (iv) to investigate the mechanisms of many enzyme catalyzed reactions [10]; and (v) for the isolation of mutant strains of micro-organisms with lesions in metabolic pathways, their regulation or transport processes across membranes [11-14].

The potential of micro-organisms to act as chemists for the provision of novel and interesting fluorochemicals has not been examined systematically. Our experience over the last few years suggests that biotransformations of many synthetic fluoroaromatic compounds are rich sources of novelty fluorochemicals, and can easily provide meta-stable synthons for further chemical or enzymic modifications. Here we give examples of microbiological routes to many simple fluorochemicals with interesting and crowded functionalities. Many of these compounds would not be regarded as priority synthesis targets because of the difficulty of attaining most of the substitutions, in the particular regio- and stereoselective patterns obtained. Our experimental approach is the use of microbial mutants defective in oxygenative catabolic pathways to accumulate products in near equimolar amounts from the fluoro-substrates. We have rarely used isolated enzymes for these biological oxidations because of coenzyme regeneration problems and cost effectiveness. Several microbial oxidative reaction sequences give products which possess one or more chiral centres in high enantiomeric excess, while others provide synthons for fluorinated heterocycles and amino acids. Many of these compounds are suitable for sequential biotransformations using alternative microbial metabolic pathways. Chemical or biochemical modifications of many of these novel fluorinated analogue metabolites can lead to unique chiral polymers, novel analogue carbohydrates, other heterocycles and a variety of intermediates for chiral cyclo-addition reactions. There is no attempt here to provide a comprehensive review of fluoroaromatic biotransformations - it is intended to display the characteristics of microbial oxidation systems for aromatic compounds and how they may be exploited to augment and complement synthetic strategies in organofluorine chemistry. A summary of microbial aromatic compound catabolism, strategies and tactics evolved (and evolving), seems to be an essential prerequisite before considering fluoroaromatic compounds for biotransformations and predictions of likely fluoroanalogue products.

GENERAL REACTION PATHWAYS FOR AEROBIC CATABOLISM OF AROMATIC COMPOUNDS

Micro-organisms, principally bacteria, yeasts and fungi, possess many enzyme systems able to activate molecular oxygen and catalyze oxygen insertions into organic compounds. These reactions frequently initiate catabolic pathways, particularly those for aromatic compounds and hydrocarbons, which allow for their complete combustion to CO_2 and H_2O, and the provision of intermediates for biosynthesis and cell growth. Examples of oxygenative reaction sequences used for the bacterial oxidation of simple aromatic hydrocarbons and benzoic acids are shown in Figures 1-4.

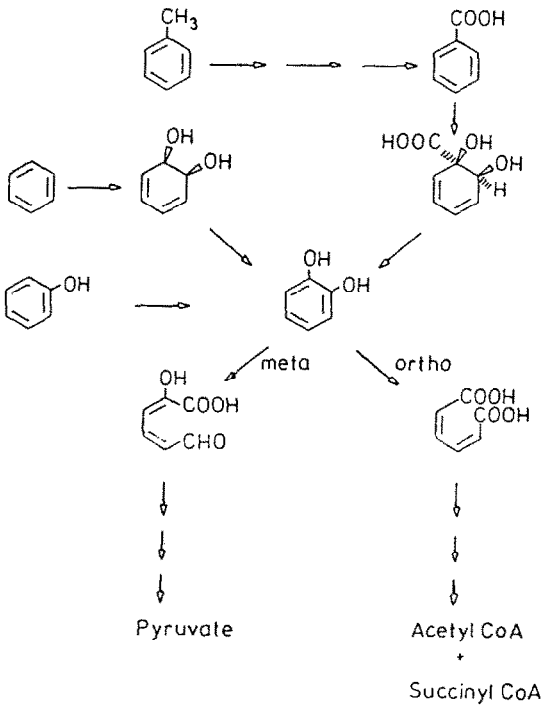

Fig. 1. Central role that catechol can be as a substrate for ortho- and meta-ring cleavage reactions.

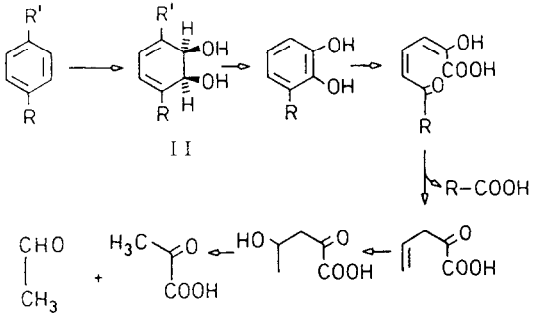

Fig. 2. Oxidation of 1- and 1,4-substitued benzenes initiated by ring hydroxylations.

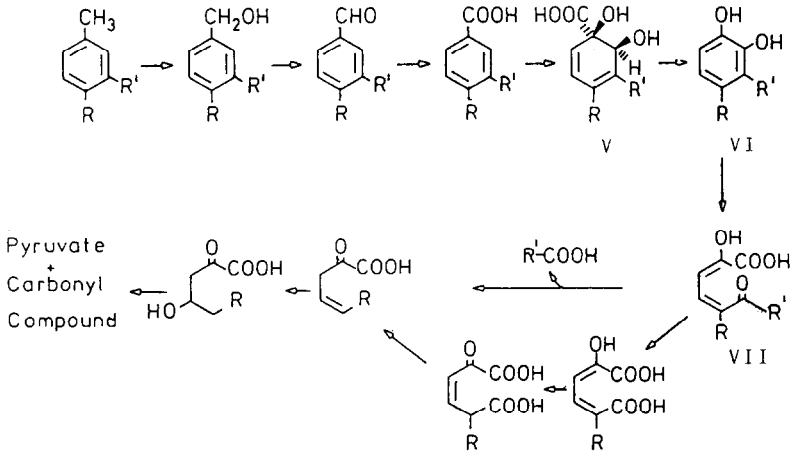

Fig. 3. Oxidation of 3-, 4- and 3,4- substituted toluenes initiated by hydroxylation of the unactivated methyl group.

The metabolic strategies that have evolved are similar, namely the formation of catechols in two enzymic steps by: (i) dioxygenation to give 3,5-cyclohexadiene-cis-1,2-diols (with two new adjacent asymmetric centres) and (ii) the dehydrogenation and consequent rearomatization of these diols (sometimes with decarboxylation, Figure 3) providing catechols. This may occur with or without prior enzymic modifications of ring substituents, e.g. oxidation of methyl to carboxyl (Figures 1,3,4).

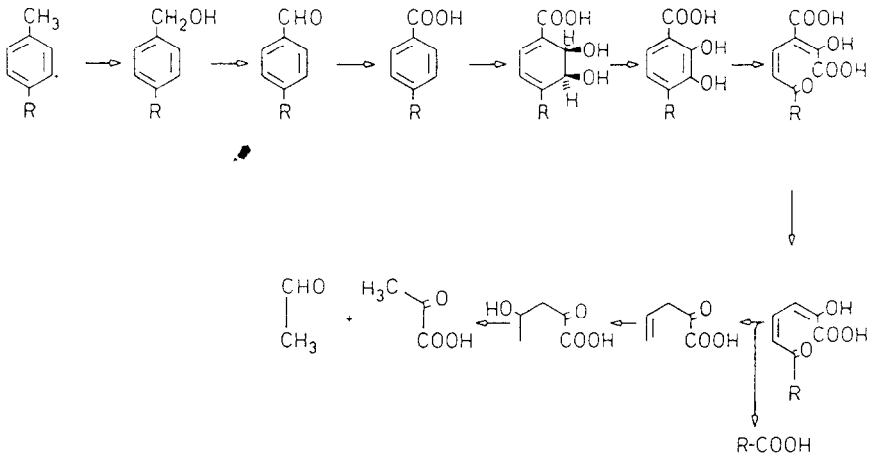

Fig.4. An alternative route for the oxidation of 4-substituted toluenes by yielding 2,3-dihydroxybenzoates as ring cleavage substrates.

The catechols formed are substrates for other regio-specific oxygen insertion enzymes, ring-cleavage dioxygenases, which are of two general types: ortho- cleavage enzymes which catalyze intra-diol ring opening to produce cis,cis-muconates, and (ii) meta-cleavage enzymes (extra-diol ring opening) which generate 2-hydroxymuconic semialdehydes (Figure 1). Subsequent catabolites formed in the ortho-cleavage pathways include chiral lactones and 3-oxoadipate (Figure 5). The meta-cleavage pathways yield 2-oxo-acids directly and these are processed by hydrolytic or oxidative routes to form pyruvate as a common intermediate from chiral aldolase substrates (4-hydroxy-2-oxoalkanoates) (Figures 2-4).

For aromatic compounds which already possess a phenolic function catabolism is usually initiated by mono-oxygenases to give either a catechol, e.g. p-hydroxybenzoate to protocatechuate (Figure 5) or a quinol e.g. cresols and xylenols to give gentisates (Figure 6) [15, 16] as substrates for ring opening dioxygenases. 1,3-Diphenolic substrates are further hydroxylated to hydroxyquinols or pyrogallols before the benzene ring can be opened to yield aliphatic compounds (Figure 7) [17,18].

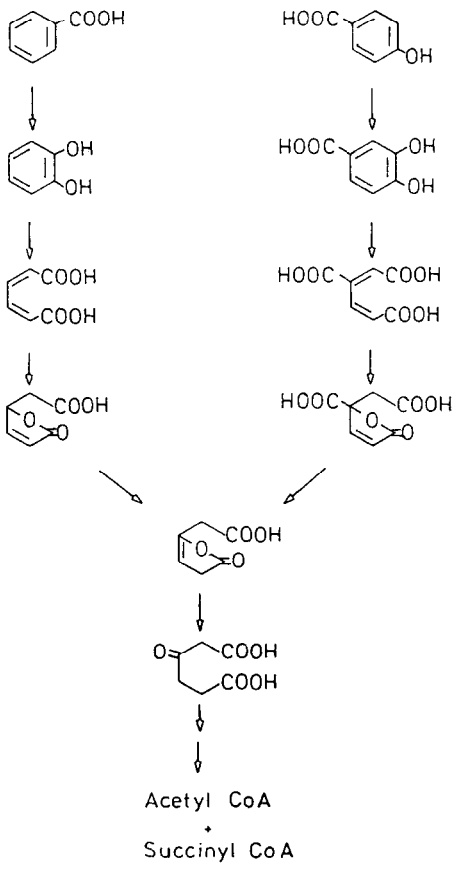

Fig. 5. The β-ketoadipate pathways leading from the ring fission of catechol and protocatechuate and converging at the enol-lactone of β-ketoadipate.

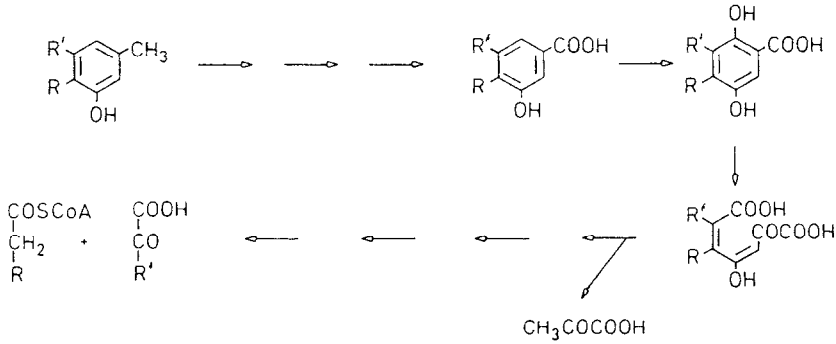

Fig. 6. Cresols and xylenols frequently yield substituted gentisates as ring-cleavage substrates by bacterial oxidations.

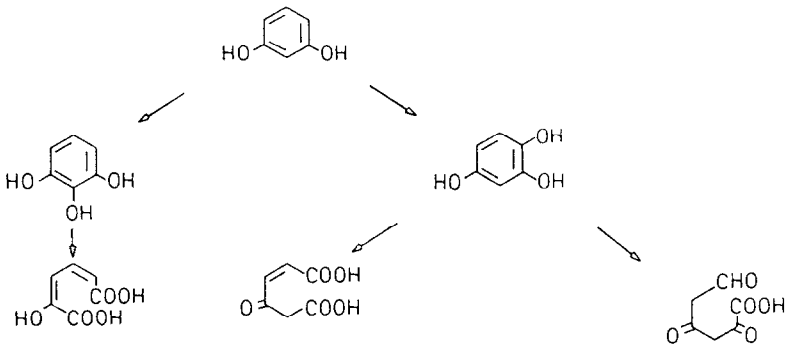

Fig. 7. Resorcinylic compounds require further hydroxylation of the ring to give hydroxyquinols or pyrogallols before ring-cleavage can occur.

FLUOROAROMATIC COMPOUNDS FOR BIOTRANSFORMATIONS

Hydrogen and hydroxyl substituents of many substrates and metabolites can be replaced by fluorine and this can confer remarkable enhanced or diminished biological properties - which amounts to subtle differences in molecular recognition of the analogues by macromolecules. Thus provision of synthetic fluorinated hydrocarbons and benzoic acids to microbes which possess the metabolic pathways depicted in Figures 1-7, or the isolated enzyme systems has a number of potential consequences, few of which can always be predicted to occur. Thus fluoro-analogues could be: (i) inert; (ii) catabolized completely with fluoride elimination (iii) partially transformed with retention of fluorine to a "dead-end" product or mixture of products; (iv) inhibiting to an enzyme in a metabolic reaction sequence; or (v) toxic to the cells and provide positive selections for mutant strains with catabolic defects.

Ideally for the production of new fluorochemicals by biotransformations, the reactions should occur in a controlled manner to single desired products in near 100% yield, and in high enantiomeric excess where relevant. This can be obtained frequently by the use of mutant strains deficient in one or more of the enzymes of the metabolic reaction sequences. In the biotransformation examples which follow frequent use of mutants is made to obtain novel chemicals in high yields.

MONOFLUORO HYDROCARBONS AND BENZOIC ACIDS

The initial dioxygenase of the benzene/alkylbenzene pathway in *P. putida* catalyzes the formation of 3,5-cyclohexadiene-3-fluoro-*cis*-1,2-diol (Compound II, R=F, R^1=H, Figure 2) from fluorobenzene, and its 6-methylanalogue (R=F, R^1=CH_3) from 4-fluorotoluene, in high EE, unlike the other 4-halotoluenes which give racemic mixtures. The absolute configuration of these products has not been determined but is deduced by analogy of the known stereochemistry of the equivalent toluene diol (R=CH_3,R^1=H) as 1*S*,2*R* [19].

Many novel fluorochemicals have been generated from 2-,3-and 4-fluorobenzoates by wild type and mutant bacterial strains [20-28]. They include chiral cis-hydrodiols, catechols and cis,cis-muconates and lactones. Thus all four isomers of monofluoro-3,5-cyclohexadiene-1,2-diol-1-carboxylates, which differ by the position of fluorine, have been isolated [20] (Figure 8) using the mutant strain B9 of Alcaligenes eutrophus defective in the diol dehydrogenase enzyme.

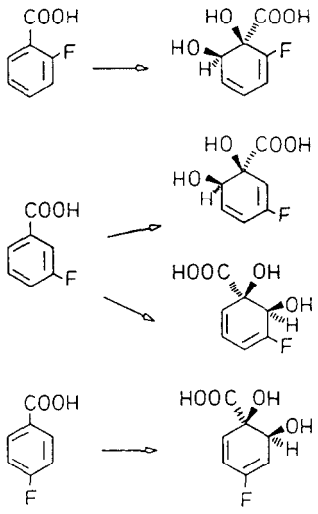

Fig. 8. Dihydroxylations of the three isomers of mono-fluorobenzoate by a mutant of Alcaligenes eutrophus.

The absolute stereochemistry of these compounds has not been determined. It is noteworthy that both 2- and 3-fluorobenzoates are hydroxylated in the 1,2-and 1,6-positions. 2-Fluorobenzoate gives only one fluorinated product by the 1,6-dihydroxylation which cannot be metabolized further because of the dehydrogenase lesion; the putative 1,2-hydroxylation product from 2-fluorobenzoate spontaneously eliminates fluoride ion with catechol formation so fortuitously overcoming the metabolic defect. About 20% of 2-fluorobenzoate is converted to the non-

toxic 6-fluoro-3,5-cyclohexadiene-1,2-diol-1-carboxylate and 80% of the fluorine is eliminated by the 1,2-hydroxylation route.

Pseudomonas sp. B13, utilizes benzoate, 3-chloro- and 3-bromobenzoates, but not 2-, 3-, or 4-fluorobenzoates, as sole sources of carbon and energy [22], although adaptation to use of the 4-fluoro-isomer [22] occurs within days. 2-Fluorobenzoate can be used as carbon source by mutants of strain B13 which have an altered regioselectivity of hydroxylation [23]. The wild type strain B13 oxidizes 2-fluorobenzoate with 75% of the organically bound fluorine released as fluoride and 25% retention into the toxic metabolite 3-fluorocatechol, and the dead-end metabolite 2-fluoro-cis, cis-muconate, the latter being a non-productive substrate for the cycloisomerases [22]. By contrast the mutant strain B13-2 selected for faster growth on 2-fluorobenzoate has altered regio-selectivity for the hydroxylation such that 97.5% of the fluorine is eliminated (1,2-dioxygenation) and only 2.5% of the substrate was metabolized via the 6-fluoro-1,6-diol and further co-metabolized to 3-fluorocatechol and the dead-end metabolite 2-fluoromuconate.

3-Fluorobenzoate does not serve as a growth substrate for either Alcaligenes sp. strain A7-2 or Pseudomonas B13, but they can co-metabolize this isomer in high yields (80-87%) to 2-fluoro-cis, cis-muconate. This only occurs after the variant catechol-1,2-dioxygenase II has been induced by prior exposure of the cells to 3-chlorobenzoate. The regio-selectivity for hydroxylation of 3-fluorobenzoate is similar to that for 2-fluorobenzoate, i.e. 85% 1,2-dioxygenation, but with the former substrate 3-fluorocatechol is generated and finally 2-fluoro-cis, cis-muconate which is not metabolized further by any of the cycloisomerases induced in these strains. It is evident that a cycloisomerase from the yeast Trichosporon cutaneum will lactonize 2-fluoro-cis, cis-muconate [29] so that direct routes to chiral fluorobutenolides are available.

4-Fluorobenzoate which is a growth substrate for some pseudomonads [22,27], uses the benzoate ortho-cleavage pathway enzymes to generate sequentially the 4-fluoro-1,2-diol, 4-fluorocatechol and 3-fluoro-cis, cis-muconate (Figure 9).

Fluoride ion is eliminated on lactonization to give the diene lactone of 5-oxo-furan-2-ylacetate.

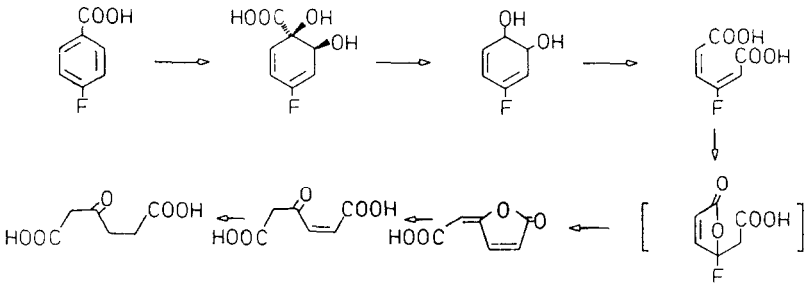

Fig. 9. Pathway for the utilization of 4-fluorobenzoate by Pseudomonas sp.

During a study of 4-nitrobenzoate oxidation by Nocardia erythropolis it was observed that 2-fluoro-4-nitrobenzoate was a substrate which gave rise to small quantities of 2-fluoroprotocatechuate (also formed from 2-fluoro-4-hydroxybenzoate by a Pseudomonas enzyme, Figure 11) [33]. Another oxidation product was tentatively identified as 2-fluoro-4-hydroxybenzoate. Similarly 5-fluoroprotocatechuate is probably formed from 3-fluoro-4-nitrobenzoate [30].

3-FLUOROPHTHALIC ACID

Some fluorescent and non-fluorescent pseudomonads can grow with o-phthalate as sole carbon source [31-33]. They metabolize it to protocatechuate, via 4,5-dihydro-4,5-dihydroxyphthalate and 4,5-dihydroxyphthalate. When mutants of P.testosteroni [32], which are individually defective in the enzymes for the catabolism of these intermediates are exposed to 3-fluorophthalate, fluorinated analogues of the metabolites were formed in moderate to high yields [34]. Thus four novel fluoro-chemicals were isolated and identified (Figure 10). It is not known if the initial hydroxylase generating the 3,5-cyclohexadiene-1,2-diol can discriminate the 3-fluoro-substitution to give the compound in high enantiomeric excess.

By contrast the decarboxylase clearly allows productive binding of 3-fluoro-4,5-dihydroxyphthalate in two orientations since both 2-fluoro- and 5-fluoroprotocatechuates are formed (Figure 10). 3-Fluoro-4-hydroxyphthalate was obtained by chemical dehydration of the dihydrodiol.

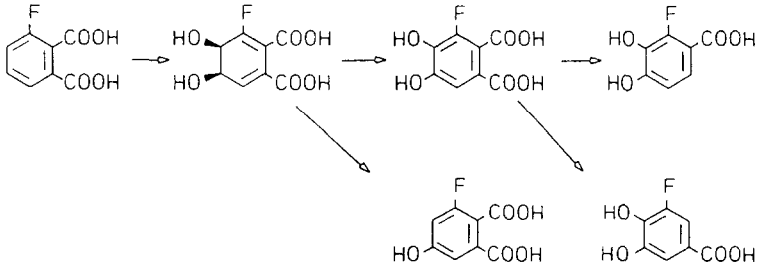

Fig. 10. 3-Fluorophthalate is oxidized to 3-fluoro-4,5-dihydroxyphthalate before decarboxylation to give two isomers of fluoroprotocatechuate. 3-Fluoro-4,5-dihydro-4,5-dihydroxyphthalate is an intermediate which decomposes in acid to 3-fluoro-5-hydroxyphthalate.

Micrococcus sp. strain 12B, isolated with the dibutyl ester of phthalate as carbon source, initiates catabolism of phthalate differently by hydroxylation of the ring in the 3,4-position before forming protocatechuate as the ring fission substrate (33a, b). After incubation of phthalate grown cells with 3-fluorophthalate, 3-fluoro-6-hydroxyphthalate was identified as an oxidation product. This compound presumably arose from the initial dihydroxylated intermediate, 3-fluoro-5,6-dihydro-5,6-dihydroxyphthalate, by a non-enzymic acid catalyzed dehydration during product isolation. Since both Micrococcus sp and P. testosteroni metabolize protocatechuate by the 4,5-meta ring cleavage route, these strains are able to provide several synthons for heterocyclic compounds e.g. for 3- and 5-fluoro-2,4-lutidinic acids (cf Figure 15 for an example of pyridine formation).

FLUOROPHENOLIC COMPOUNDS AS SUBSTRATES FOR ENZYMIC HYDROXYLATIONS

Fluorophenolic compounds are readily obtained by dehydration of the various fluorinated cis-diols accumulated by biotransformations. Fluorophenolics can also be formed by several

flavoprotein hydroxylases (mono-oxygenases) from bacteria and yeasts which hydroxylate fluoro-analogue substrates with either retention or elimination of the fluorine atom depending upon its position in relation to the site of hydroxylation. There is little indication that any of these enzymes catalyze the hydroxylation induced migration of fluorine to an adjacent carbon on the aromatic ring which is observed with other halogens, ^{2}H,^{3}H and CH_3 (the NIH shift) [35].

4-Hydroxybenzoate hydroxylase catalyzes the formation of protocatechuate by oxygenation of the 3 position of 4-hydroxybenzoate. Several fluorinated analogues of the substrate are also poor to good substrates for the enzyme [36]. 2-Fluoro-4-hydroxybenzoate is oxidized to 2-fluoroprotocatechuate, a product also formed by the oxidation of 3-fluorophthalate by P. testosteroni [34] and of 2-fluoro-4-nitrobenzoate by N. erythropolis [30]. 3-Fluoro- and 3,5-difluoro-4-hydroxybenzoates are hydroxylated to 5-fluoroprotocatechuate with equimolar release of fluoride ion from the latter substrate and only a fraction from the former. Perfluoro-4-hydroxybenzoate, the poorest substrate of the four fluoro-analogues was transformed to 2,3,5-trifluoro-4,5-dihydroxybenzoate (2,5,6-trifluoroprotocatechuate) with fluoride release (Figure 11). These mono-oxygenase reactions that result in fluoride release are expensive in that the ratios of substrate:O_2: NADPH consumptions become 1:1:2 compared with 1:1:1 for substrates that do not possess fluorine or allow its retention.

3-Hydroxybenzoate-4-hydroxylase from P.testosteroni catalyzes the formation 2-fluoroprotocatechuate from 2-fluoro-5-hydroxybenzoate in about 80% yield [37]. By contrast the 3-hydroxybenzoate-6-hydroxylase from P. aeruginosa converts 4-fluoro-3-hydroxybenzoate to 4-fluoro-2,5-dihydroxybenzoate [38]. 2,3,5-Trifluorocatechol can be produced from 2,3,4,5-tetrafluorophenol by the phenol hydroxylase of Trichosporon cutaneum [39] (Figure 11).

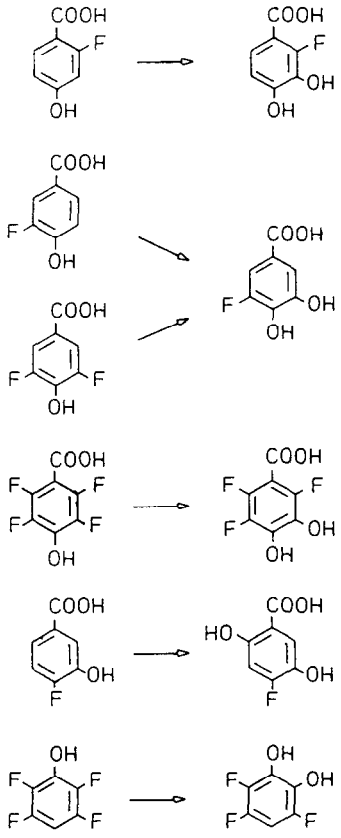

Fig. 11. Generation of some novel fluorophenols by microbial oxidations.

3-Fluorocatechol is required for some pharmaceutical applications and is produced in tens of kg quantities from fluorobenzene by a mutant lacking the ring opening enzyme. An alternative route uses a combination of bacterial and chemical transformations. Fluorobenzene gives the 3-fluoro-cis-1,2-diol mentioned before with a bacterial mutant; this is oxidized chemically, rearomatizing to 3-fluorocatechol [55].

4-FLUOROPHENYLACETIC ACID

During growth of a Pseudomonas sp. on 4-fluorophenylacetic acid several fluorinated metabolites were excreted into the medium [40,41] which included both 2-and 3-hydroxylated derivatives of 4-fluorophenylacetic acid. These could have arisen as dehydration products of 4-fluoro-2,3-dihydro-2,3 dihydroxyphenylacetate. The metabolic route to three fluoroaliphatic intermediates, trans-3-fluorohex-3-ene-dioic acid, and the chiral compounds (-)-4-carboxymethyl-4-fluorobutanolide and D-(+)-fluorosuccinic acid is difficult to comprehend, although all their interconversions can be readily accounted for by reactions analogous to those catalyzed by ortho-cleavage pathway enzymes.

1-FLUORONAPHTHALENE

Although many bacteria are able to use naphthalene as their sole source of carbon, neither of the two isomers of fluoronaphthalene have been shown to be metabolized though it may be inferred that this occurs [13]. By contrast the fungus Cunninghamella elegans, which does not grow with naphthalene, oxidizes this compound and the analogue 1-fluoronaphthalene. Five metabolites of 1-fluoronaphthalene were identified. These, shown in Figure 12, were trans-3,4-dihydro-3,4-dihydroxy-1-fluoronaphthalene, trans-5,6-dihydro-5,6-dihydroxy-1-fluoronaphthalene, 4-hydroxy-1-fluoronaphthalene, 5-hydroxy-1-fluoronaphthalene and 8-hydroxy-1-fluoro-5-tetralone [53]. The major enantiomer of both trans - dihydrodiols formed had S,S absolute configurations (S,S:R,R ratios of about 60:40 were observed). The major enantiomer of the trans-5,6-dihydrodiol (S,S) formed by C. elegans has the opposite stereochemistry to that formed from 1-fluoronaphthalene by rat liver microsomes (R,R) [53]. The two phenols isolated were possibly formed by rearrangements of the arene oxide intermediates, the putative

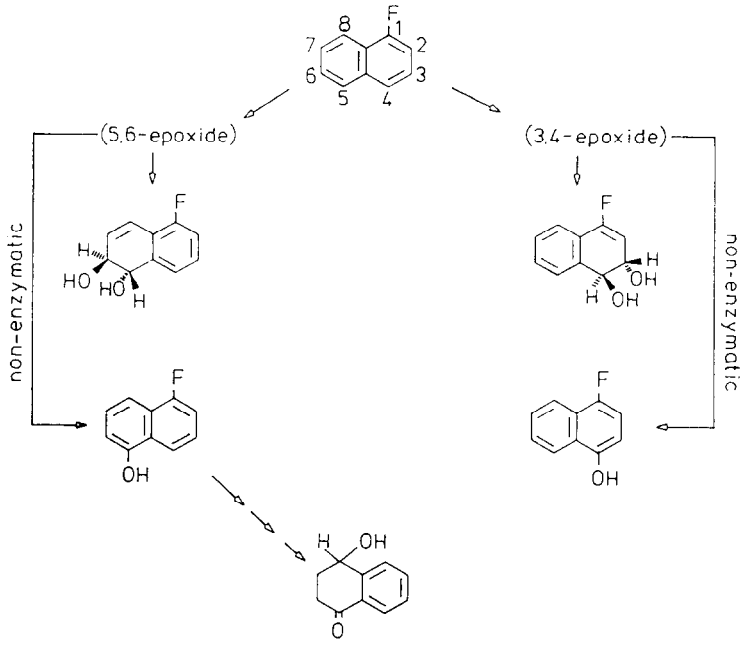

Fig. 12. Fungal oxidations of 1-fluoronaphthalene with non-enzymatic rearrangements.

precursors of the trans - dihydrodiols. The tetralone intermediate is consistent with the non-fluorinated compound produced from naphthalene by C. elegans. These results are of interest because they lend further support to another major metabolic divergence between procaryotes and eucaryotes; the initial oxidations of hydrocarbons by eucaryotes are catalyzed by monooxygenases to give arene oxides, while procaryotes use dioxygenases to form cis-dihydrodiols. Additionally the fungal and hepatic systems provide fluorinated trans-dihydrodiols with predominantly opposite chiralities.

DIFLUOROBENZOIC ACIDS

Recently we have shown that the six isomers of difluorobenzoate undergo biotransformation by a mutant strain of Pseudomonas putida (JT103) which is deficient in 3,5-cyclohexadiene-1,2-diol-1-carboxylic (NAD) dehydrogenase (decarboxylating) of the benzoate pathway (Fig. 1) [42,43]. These biotransformations were monitored by ^{19}F-NMR. With difluorobenzoates substituted with fluorine in the C-2 position the dioxygenation occurs preferentially across the C-1,2 bond giving rise to the spontaneous non-enzymic elimination of fluoride and decarboxylation, resulting in the formation fluorocatechols. One example, for 2,5-difluorobenzoate oxidation, is shown in Figure 13. The fluorocatechols can then undergo further metabolism since the mutation has been effectively by-passed.

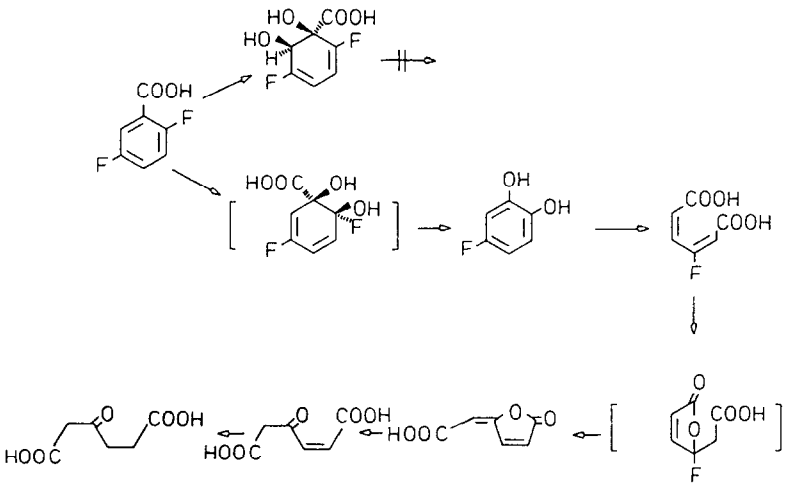

Fig. 13. Bacterial oxidation of 2,5-difluorobenzoate. Two regio isomers of the first intermediate are formed, one eliminates F^- spontaneously, so fortuitously by-passes the dehydrogenase lesion.

3,4-Difluorobenzoate undergoes dioxygenation to give two regioisomers of difluoro-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid in the ratio of 4:1 (Figure 14), while 3,5-difluorobenzoate gives the only possible product 3,5-difluoro-3,5-cyclohexadiene-1,2-diol-1-carboxylic acid (Figure 14).

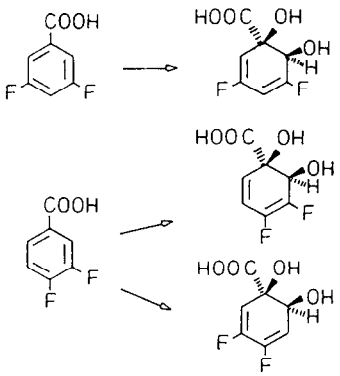

Fig. 14. Oxidation of 3,5-and 3,4-difluorobenzoate by a mutant of Pseudomonas putida U-JT103 defective in the diol dehydrogenase.

This clearly demonstrates how micro-organisms can be used to produce novel chiral fluorochemicals that are inaccessible by current methods of organic systhesis, just as was demonstrated for the biotransformations of the monofluorobenzoates (Figure 8).

BIOTRANSFORMATIONS OF TRIFLUOROMETHYL AROMATIC COMPOUNDS

The p-cymene pathway in several strains of P. putida (Figure 4) is remarkably versatile with respect to substrates and intermediates in that the 4-substituent can be very variable. Several 3,4-di-substituted toluenes and benzoates are also partially or wholly metabolized [44, 45]. Biotransformation of 4-trifluoromethylbenzoate played a key role in establishing the correct reaction sequence shown in Figure 4 [44]. Thus p-cumate induced cells of a mutant of P. putida JT107 unable to grow with p-cymene (or p-cumate), metabolized 4-trifluoromethylbenzoate quantitatively to the 2, 3-dihydro-2,3-dihydroxy-4-

trifluoromethylbenzoate (Compound V, R=CF_3 Figures 4,15) [44,46]; this derivative was much more stable to acid than the corresponding alkyl compounds accumulated by the mutant, thus allowing its isolation and identification.

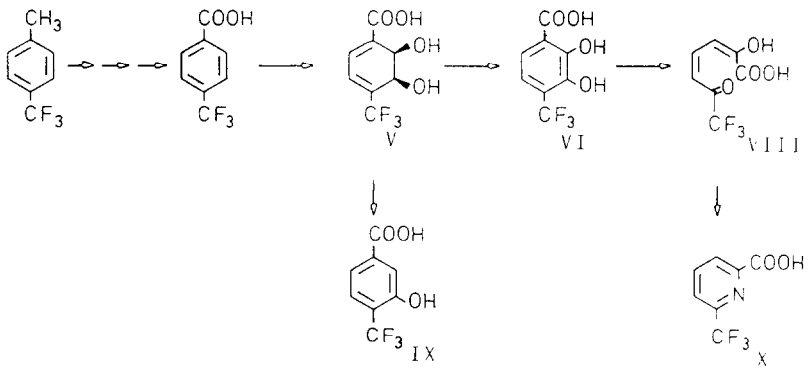

Fig. 15. Abbreviated reaction sequence for the oxidation of 4-trifluromethyltoluene and 4-trifluoromethylbenzoate by Pseudomonas putida. The vertical transformations are non-enzymic.

When the wild type strain of P. putida JT101 is exposed to 4-trifluoromethylbenzoate, three metabolites were detected and identified as Compounds V, VI and VIII (Figure 15) [44,46]. Products V and VIII gave previously undescribed derivatives e.g. 3-hydroxy-4-trifluoromethylbenzoate (IX) and 6-trifluoromethyl-2-picolinate (X) respectively in high yields. 2-Trifluoromethyl- and 3-trifluoromethylbenzoates were only slowly metabolized by JT101 strain and no products were identified [46].

3-Trifluoromethylbenzoate, but not the 2- and 4-isomers, is a substrate for the enzymes of the toluene-xylene catabolic pathway encoded by genes carried on the plasmid (designated TOL) of Pseudomonas putida mt-2 (Figure 3,R^1=CF_3). Oxidation of several substituted toluenes to their benzoates initiates the pathway, e.g. m-xylene gives m-toluate prior to ring hydroxylation to form 3-methylcatechol and dioxygenative cleavage between the carbons bearing the methyl and a hydroxyl group. Two products accumulated during the oxidation of 3-trifluoromethylbenzoate by P. putida mt-2 [47], and they were identified as cis-1,2-diol of 3,5-cyclohexadiene-3-trifluoromethyl-1-carboxylate and 7,7,7-trifluoro-2-hydroxy-6-

oxohepta-2,4-dienoate (Compounds V and VII, $R^1=CF_3$,Figure 3); the former is also a product with A. eutrophus biocatalyst [23]. From the cyclohexadiene diol two derivatives were easily obtained, 3-trifluoromethylsalicylate by acid dehydration and 3-trifluoromethylcatechol after (VI) enzymic dehydrogenation. The meta- ring cleavage metabolite (VII, Figure 3) was easily converted to 6-trifluoromethyl-2-picolinate by exposure to ammonia [47]. The yield of the two metabolites accounted for about 10 per cent of the 3-trifluoromethylbenzoate utilized in the ratio of 3 (ring cleavage product): 2 (diol product) [47].

Several N-alkyl or N,N-dialkyl derivatives of 2,6-dinitro-4-(trifluoromethyl)-benzeneamine are extensively used as herbicides. Metabolism of these usually proceeds by alkyl modification or dealkylation. The resultant parent structure I (Figure 16) is metabolized to several products by a Streptomyces sp. isolated from soil [54]. Thus 3-nitro-5-(trifluoromethyl)-benzeneamine (II), N-[2-amino-5-(trifluoromethyl) phenyl]-methanesulphinamide (IV) were formed from I. Additionally a diphenyldiazene and a diphenyldiazene N-oxide were isolated, presumably formed by oxidation of II (Figure 16) [54].

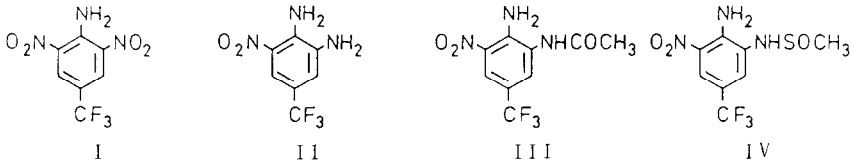

Fig. 16. Products identified after exposure of 2,6-dinitro-4 trifluoromethyl benzeneamine by Streptomyces sp.

DISCUSSION

Interest in new fluorochemicals continues to increase because of the potential biological , chemical and physical properties of these compounds. In 1982 approximately 600 fluorochemicals were available for purchase and this increased to 1500 by 1985 (Sources used were the catalogues of Aldrich, Fluorochem, Koch-Light and Yarsley). A third of these are

fluoroaromatic chemicals of which less than thirty are fluorophenolics. Synthetic routes to many fluoroaromatic compounds are difficult, but some of these can be achieved by biotransformations with the use of specific oxygen insertion reactions under mild reaction conditions. Some compounds produced are meta-stable synthons for further combinations of chemical and microbial modifications.

Here we have outlined the biotransformation reaction sequences for the production of some known and previously undescribed fluorochemicals from fluoroaromatic precursors. Many of the microbial products are interesting molecules in themselves and suitable for further extensive exploratory chemistry. Thus fluoroaromatics that do not possess a phenolic function are usually dioxygenated by bacteria to 3,5-cyclohexadiene-1,2-diols, doubly chiral building blocks, potential precursors for fluorinated phenols, other chiral intermediates, extension of chiralities by for example Sharpless epoxidations, cyclo-addition reactions, carbohydrates, heterocycles and many novel polymers and co-polymers. Microbial oxidations of these 1,2-*cis*-diols lead to fluorocatechols and fluorocatechol carboxylates, which are precursors for further microbial metabolism to unstable synthons for other heterocyclic compounds, diene-dicarboxylates, α-amino acids. Additionally the aldolase enzymes of the *meta*-cleavage pathways which catalyze pyruvate formation from chiral 2-oxo-4-hydroxyalkanoates (Figures 2-4) could be exploited for the stereoselective formation of carbon-carbon bonds using fluorinated substrates as these enzymes accept a variety of aldehydes as the electrophilic reactants.

At present the biocatalysts used in organic synthesis are obtained from two main sources. One is the commercially available enzyme or cell preparations, produced initially for reasons other than aids in organic synthesis. The other is the small and large culture collections maintained in specialized laboratories. With some notable exceptions random screening of these collections for the required reactions and for the best kinetics and yields is not undertaken. So far little attention has been given to the rational selection of well-defined oxygenative catabolic pathways and the isolation of mutants able

to accumulate intermediates in near quantitative yields. For these to be used in rational and systematic ways, chemists and biologists need to find areas of common interest.

Micro-organisms have evolved efficient enzymes for utilizing dioxygen and under mild conditions they insert oxygen atoms into natural and synthetic organic compounds with high regio- and stereoselectivity. This produces families of molecules with: (i) unusual and crowded substitution patterns (illustrated here with mono-,di-and tri-fluoro products); and (ii) abilities to act as precursors to a wide variety of other ring systems and polymers. After only very limited studies of fluoroaromatic compound biotransformations, it is clear that there is enormous scope for the easy generation of many novel fluorochemicals; we have identified over three hundred available fluoroaromatics as good candidates for microbiological modifications of the types described in this article. Many of these can be expected to yield one to a dozen products with appropriate mutants. Furthermore microbial metabolic evolution is a continuing process, and can be directed in the laboratory to generate variations in substrate specificities in mutant strains. One example of this was given for 2-fluorobenzoate biotransformation by selection of a mutant with altered regio-selectivity of hydroxylation [23]. The enzymes of many of these oxygenative catabolic pathways are present in cells only after growth in the presence of the natural substrate (or some analogues). Therefore it is necessary to understand not only the enzyme specificities, but also those for the regulatory elements that govern the expression of the genes that code for the enzymes in order to exploit fully the catalytic potential of microbial enzyme systems for synthesis. With the field of biocatalysts in organic synthesis expanding so rapidly [48-51], the addition of new enzymes and micro-organisms (especially mutant strains) must continue to increase. The scope, versatility and potential of aromatic oxygenases to oxidize selectively unactivated methyne, methylene and methyl carbons or other atoms has not been fully realized for use in organic synthesis. Additionally, well-known compounds but with specific isotopic labelling patterns, can be readily obtained from unlikely chemical precursors by

biotransformations. Because there is no single chemical process which permits the direct and specific oxidation of benzenoid compounds to synthetically useful substances, our colleagues used the microbial oxidation product of benzene, cis-1,2-dihydroxycyclohexa-3,5-diene, as an intermediate for a four step synthesis to both enantiomers of the natural product, (+)-pinitol, a polyhydroxy cyclohexane with six chiral centres [52]. Biotransformations provide opportunities for the direct preparation of fluorinated analogues of many natural products and bio-active molecules, or suitable precursors.

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