Microbial Metabolism of Pyridine, Quinoline, Acridine, and Their Derivatives under Aerobic and Anaerobic Conditions

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INTRODUCTION

Two-thirds of known organic chemicals contain heterocyclic structures (70, 73). Heterocyclic aromatic compounds exist naturally in biological systems as electron carriers, nucleotides, energy storage molecules, mycotoxins, and alkaloids. Synthetic heterocyclic aromatic compounds are used as industrial solvents, dyes, explosives, pharmaceuticals, and pesticides. Waste materials containing large amounts of heterocyclic aromatic compounds are generated by the mining industry, coal tar- and oil shale-processing operations, wood-preserving facilities, and chemical manufacturing plants.

Because of their heterocyclic structure, these chemicals are more soluble in water than are their homocyclic analogs and can be more easily transported to groundwater. Despite the potentially serious consequences of such contaminants, insufficient research has been conducted to determine their fate in the environment (7, 8, 30). Therefore, it is important that we gain a clearer understanding of the processes by which they are degraded and the types of microorganisms involved.

Pyridine and its derivatives are important representatives of heterocyclic compounds. The pyridine ring is a major constituent of natural plant alkaloids, pyridoxyl derivatives, and coenzymes such as nicotinamides. Nicotinic, anabasine, and cadavrin are produced by plants and animals and were used as natural pesticides for many years. The herbicides paraquat, diquat, and picloram also contain the pyridine ring structure.

Microbial transformation of pyridine and its derivatives has been reviewed by Kost and Modyanova (68), Shukla (104), and Sims and O’Loughlin (112). In this review we present information currently available concerning the microbial metabolism of pyridine, quinoline, acridine, and their derivatives under aerobic and anaerobic conditions, with emphasis on metabolic pathways.

TRANSFORMATION OF PYRIDINE

Although research has shown that a number of organisms are capable of transforming heterocyclic aromatic compounds, the mechanism of cleavage of the pyridine ring remains unclear. In early work, Shukla (101) isolated from soil a Corynebacterium sp. and a Brevibacterium sp. capable of degrading pyridine. Cells grown on pyridine were unable to transform monohydroxylated pyridines. Similar observations were reported for Nocardia strain Z1. Hydroxylated pyridine derivatives were never observed during the transformation of pyridine (51). Further investigations with Corynebacterium sp. showed that formic acid and ammonium were formed and released into the culture medium during the degradation of pyridine and that in the presence of semicarbazide as an inhibitor, succinic acid semialdehyde accumulated as an intermediate product (108). Similar results were obtained for Brevibacterium sp. (109), with succinic acid semialdehyde and pyruvic acid accumulating in the presence of arsenate. Subsequently, Sims et al. (116) isolated from soil a Micrococcus luteus species which oxidized pyridine. Only aliphatic intermediates of pyridine metabolism were identified; therefore, the initial steps of ring cleavage remained uncertain.

Watson and Cain (140) investigated the metabolic pathways of pyridine biodegradation by Bacillus strain 4. When this spe-
cies was grown in the presence of semicarbazide as an inhibitor, several carbonyl compounds derived from pyridine, including succinic acid semialdehyde, accumulated. The products of Bacillus strain 4 were similar to those obtained during the transformation of pyridine by Corynebacterium and Brevibacterium spp. Mutants of Bacillus strain 4 deficient in succinic acid semialdehyde dehydrogenase were unable to grow on pyridine. In the presence of cyanide, further oxidation of formic acid (a by-product of pyridine metabolism) was inhibited, and formic acid accumulated in the culture broth. Experiments with [2-14C]pyridine showed that formic acid was derived from the carbon 2 atom of the heterocyclic ring. Formamide was not detected in the culture medium. The formation of succinic and formic acid from the carbon 2 atom of the pyridine ring indicates a ring fission between carbons 2 and 3. The metabolic steps in the transformation of pyridine to succinic acid semialdehyde by Bacillus strain 4 remain somewhat speculative but may follow the pathway suggested in Fig. 1.

Watson and Cain (140) also studied the transformation of pyridine by Nocardia strain Z1 and found that glutaric acid semialdehyde was produced as an intermediate product. The formation of an intermediate product with five carbon atoms indicates that the pyridine ring must be cleaved between the heteroatom and carbon 2. Therefore, the authors proposed a pathway for the metabolism of pyridine by Nocardia strain Z1 as outlined in Fig. 2.

Subsequently, Shukla and Kaul (110) isolated from soil a Nocardia sp. which was capable of transforming pyridine, pyridine-N-oxide, and 2-hydroxypyridine. In the presence of semicarbazide, transformation of pyridine resulted in the formation of succinic acid semialdehyde as an intermediate product. This Nocardia sp. followed a pyridine metabolic pathway similar to that reported by Watson and Cain (140) for Bacillus strain 4, including cleavage of the heterocyclic aromatic ring between carbons 2 and 3.

Ronen and Bollag (93) reported that an Alcaligenes sp. was able to rapidly mineralize pyridine under denitrifying conditions. The ratio of pyridine to nitrate strongly affected the pyridine metabolism. In further studies, the Alcaligenes sp. was reinoculated into a subsurface sediment and found to mineralize 10 µg of pyridine per g (94). Kaiser and Bollag (60) studied the transformation of pyridine by sewage sludge under aerobic, denitrifying, and sulfate-reducing conditions. Pyridine was degraded within 9 days under aerobic conditions and mineralized under denitrifying and sulfate-reducing conditions. The amounts of nitrate and sulfate that were utilized were 79 and 85% of the amounts predicted from stoichiometric equations, respectively.

**TRANSFORMATION OF HYDROXYLATED PYRIDINES**

Houghton and Cain (51) reported that several Achromobacter spp. can transform monohydroxylated pyridines. 2- and 3-hydroxypyridine were transformed to 2,5-dihydroxypyridine, whereas 4-hydroxypyridine was converted to 3,4-dihydroxypyridine. After a short lag period, isolated bacteria growing on 2-hydroxypyridine were also able to transform 3-hydroxypyridine and vice versa. The 4-hydroxypyridine-degrading organisms were unable to transform 2- or 3-hydroxypyridine. In a continuation of this work, Cain et al. (15) reported that when 2- and 3-hydroxypyridine were oxidized to 2,5-dihydroxypyridine, production of maleic acid occurred through ring cleavage. Cell extracts accumulated maleamic and formic acids and a small amount of ammonium, the latter probably formed by deamination of maleamic acid. The resulting maleic acid was further transformed to fumaric acid. Subsequently, Khanna and Shukla (62) reported that 2,5-dihydroxypyridine accumulated during the transformation of 3-hydroxypyridine by a gram-negative bacterium isolated from soil.

Gupta and Shukla (32) and Kolenbrander et al. (65) have described an Arthrobacter sp. which degrades 2-hydroxypyridine. During transformation of this pyridine derivative, a blue pigment, which was probably the result of 2,3,6-trihydroxypyridine condensation, appeared in the culture medium. Further degradation of 2,3,6-trihydroxypyridine involved the production of maleamic acid, maleic acid, and pyruvic acid as intermediate products. Similar observations were reported by Ensign and Rittenberg (19). The authors suggested that during the metabolism of 2-hydroxypyridine, a tripyridol was produced, and that the blue pigment was generated from its oxidation. At low substrate concentrations, no pigment formation occurred, indicating that the excess substrate was shunted to a side reaction leading to formation of the pigment.

Considering all of the foregoing observations together, we propose the pathway for transformation of 2-hydroxypyridine presented in Fig. 3. It is likely that 3-hydroxypyridine is metabolized in a similar manner.
Both dioxygenase and monooxygenase activities have been observed during the transformation of hydroxylated pyridines. The stoichiometry for the reaction between 2,5-dihydroxypyridine and oxygen in the presence of crude cell extracts of the *Achromobacter* sp. suggested that a dioxygenase was involved in the degradation process (15). However, degradation of 2-hydroxypyridine by cell extracts of various *Arthrobacter* spp. suggested monooxygenase activity (66). Enzymatic oxidation of 2-hydroxypyridine required molecular oxygen and NADH, each in equimolar amounts with respect to the substrate. The enzyme was stimulated by flavin mononucleotide but not flavin adenine dinucleotide (FAD) or riboflavin and was inhibited by the flavin analog quinacrine.

Kolenbrander and Weinberger (66) investigated the location of degradative genes for the transformation of 2-hydroxypyridine. In *Arthrobacter crystallopoietes*, the degradative gene appears to be carried by a plasmid; consequently, over time, the bacterium loses the ability to degrade 2-hydroxypyridine, because the plasmid is not always passed during propagation. However, in *A. pyridincola* and *A. viridescens*, the genes responsible for 2-hydroxypyridine metabolism are located on the chromosome.

As noted above, 4-hydroxypyridine is transformed via 3,4-dihydroxypyridine (52). Studies with H$_{2}^{18}$O demonstrated that in the presence of NADP$^+$ or an artificial dye (methylene blue), the oxygen used for oxidation of 4-hydroxypyridine to 3,4-dihydroxypyridine by the enzyme 4-hydroxypyridine-3-hydroxylase was derived from water (141). Watson et al. (142) reported that cell extracts of an *Agrobacterium* sp. transformed 3,4-dihydroxypyridine into 3-formiminopyruvic acid, pyruvic acid, two molecules of formic acid, and ammonium. The identification of 3-formiminopyruvic acid as an intermediate restricted the possibility of ring cleavage to the bonds between carbons 2 and 3 or carbons 5 and 6. Since ring fission between unsubstituted atoms is rare, fission between carbons 2 and 3 is the most likely site. The ring cleavage mechanism we propose for the metabolism of 3,4-dihydroxy-pyridine by the *Agrobacterium* sp. is shown in Fig. 4. The lack of a requirement for NADH or other cofactors and the absolute requirement for molecular oxygen during the metabolism of 3,4-dihydroxypyridine suggested that the enzyme responsible for this reaction was a dioxygenase.

Metabolism of hydroxylated pyridines under anaerobic conditions has been studied only rarely. Kaiser and Bollag (60) investigated the degradation of monohydroxypyridines under denitrifying and sulfate-reducing conditions by a mixed culture obtained from sewage sludge.

3-Hydroxypyridine was metabolized after a lag period of 2 months, whereas 2- and 4-hydroxypyridines were not transformed. This finding suggests that 3-hydroxypyridine is metabolized by a pathway different from that for 2- and 4-hydroxypyridine under these conditions.

**TRANSFORMATION OF CARBOXYLATED PYRIDINES**

**2-Carboxypyridine**

The metabolism of 2-carboxypyridine (2-picolinic acid) transformation started with a hydroxylation at position 6 of the heterocyclic aromatic ring, leading to 6-hydroxypicolinic acid. This oxidation could occur anaerobically in the presence of methylene blue and was unaffected by pyridine nucleotides, indicating that the oxygen was supplied by water and not by molecular oxygen (16, 126).

In addition to 6-hydroxypicolinic acid, other metabolites such as 3,6-dihydroxypicolinic acid and 2,5-dihydroxypyridine were isolated during the metabolism of 2-picolinic acid by a *Bacillus* sp. (103). 6-Hydroxypicolinic acid and 2,5-dihydroxy-pyridine were also found in the culture medium during transformation of 2-picolinic acid by another unidentified gram-negative coccus isolated from soil (111).

*Bacillus* cells caused α-oxoglutaric acid to accumulate in the culture medium when inhibited with arsenite. However, when cell growth was only partially inhibited with arsenite, pyruvic acid was produced (107). Tate and Ensign (125, 126) reported that picolinic acid-grown resting cells of *Arthrobacter piscinophilus* oxidized 6-hydroxypicolinic acid to carbon dioxide, ammonium, and water. Ohsugi et al. (78) reported that a bacterium isolated from soil utilized 2-picolinic acid, producing dicarboxylic acids with two-, five-, six-, seven-, and nine-carbon chains. The authors suggested that these dicarboxylic acids were precursors in the biosynthesis of desthiobiotin and found that desthiobiotin was also produced during the transformation of 2-picolinic acid.

Figure 5 shows the proposed pathway for metabolism of 2-picolinic acid.
3-Carboxypyridine

3-Carboxypyridine (nicotinic acid) is the most frequently investigated of the pyridine derivatives. Hughes (52) reported that many strains of *Pseudomonas fluorescens* are able to oxidize nicotinic acid, with 6-hydroxynicotinic acid being the first intermediate product formed. Experiments with cell extracts demonstrated that during the transformation of nicotinic acid to 6-hydroxynicotinic acid, the oxygen of the hydroxyl group was derived from water and not from molecular oxygen (55, 59).

Further investigations into the oxidation of nicotinic acid by a strain of *P. fluorescens* were conducted by Behrman and Stanier (5). In agreement with Hughes (52), they showed that hydroxylation at carbon 6 was the first step in the pathway. The second step was an oxidative decarboxylation producing 2,5-dihydroxypyridine. The ring of 2,5-dihydroxypyridine was then cleaved oxidatively to yield maleamic and formic acids. Hydrolytic deamination of maleamic acid generated maleic acid, an isomer of fumaric acid. It was proposed that ring cleavage occurred between carbons 5 and 6, although the N-formylmaleamic acid was not detected. When appropriate concentrations of arsenite were present, pyruvic acid was present among the end products. All of the reactions except the initial hydroxylation step were catalyzed by soluble enzymes. Similar results were obtained by Gauthier and Rittenberg (25, 26), who investigated the 2,5-dihydroxypyridine oxygenase from *Pseudomonas putida* N9. In these experiments, 2,5-dihydroxypyridine was oxidized to equivalent amounts of maleamic acid and formic acid, with the consumption of 1 mol of oxygen. The enzyme did not require NADH, and the reaction did not proceed anaerobically in the presence of an electron acceptor. The incorporation of $^{18}$O$_2$ into maleamic acid and formic acid clearly implicated a dioxygenase in the ring cleavage step.

Gupta and Shukla (33) isolated from soil a *Sarcina* sp. which was capable of metabolizing nicotinic acid. Resting cells grown on nicotinic acid accumulated 6-hydroxynicotinic acid and pyruvic acid. Under restricted aeration, 2,5-dihydroxypyridine accumulated in the culture medium. Thus, this *Sarcina* sp. appeared to metabolize nicotinic acid via a pathway similar to that described for *P. fluorescens*. On the basis of intermediates formed during the transformation of nicotinic acid by *P. fluorescens*, the pathway presented in Fig. 6 was proposed (33).

Ensign and Rittenberg (20) isolated from soil a *Bacillus* sp. which oxidized nicotinic acid by a pathway different from that postulated for *Pseudomonas* spp. Resting cells of the organism oxidized nicotinic acid via 6-hydroxynicotinic acid and 2,6-dihydroxynicotinic acid. The latter compound was further transformed to 2,3,6-trihydroxyxypyridine via oxidative decarboxylation followed by ring cleavage and the formation of maleamic acid. A blue pigment was produced during the oxidation of nicotinic acid; it was formed via nonenzymatic oxidation of 2,3,6-trihydroxyxypyridine.

Hirschberg and Ensign (40) purified enzymes from the *Bacillus* sp. that hydroxylated nicotinic acid and 6-hydroxynicotinic acid to 2,6-dihydroxynicotinic acid, but it was not possible to separate the nicotinic acid-hydroxylating enzyme from the 6-hydroxynicotinic acid-hydroxylating enzyme. This indicated either that two distinct enzymes with very similar properties existed or that one multifunctional enzyme complex was responsible for both activities. Further investigations showed that both hydroxyl groups incorporated into 2,6-dihydroxynicotinic acid during the transformation of nicotinic acid were derived from water (41). The proposed pathway for the metabolism of nicotinic acid by the *Bacillus* sp. is shown in Fig. 7.

Additional experiments with 6-hydroxypyridine demonstrated that this compound served as an inducer of the enzyme for nicotinic acid metabolism. The lag period before the appearance of the hydroxylase activities was shorter when 6-hydroxynicotinic acid was added to the cells (42).

The transformation pathway for nicotinic acid under anaerobic conditions differs from that described above for *Pseudomonas* and *Bacillus* spp. under aerobic conditions. The first step in the degradation of nicotinic acid by both aerobic and anaerobic organisms appears to be the same, i.e., the forma-

**FIG. 5.** Proposed metabolism of 2-picolinic acid (107).

**FIG. 6.** Proposed pathway for the transformation of nicotinic acid by *Pseudomonas fluorescens* (33).

**FIG. 7.** Proposed pathway for the transformation of nicotinic acid by a *Bacillus* sp.
tion of 6-hydroxynicotinic acid, but thereafter the pathways diverge. Harary (36–38) reported that a Clostridium sp. transformed nicotinic acid to produce propionic acid, acetic acid, carbon dioxide, and ammonium. 6-Hydroxynicotinic acid was proposed as an intermediate product. Stoichiometric conversion of nicotinic acid and the oxidizing agent methylene blue led to 6-hydroxynicotinic acid and reduced methylene blue. The hydroxylation of nicotinic acid to 6-hydroxynicotinic acid was a reversible reaction catalyzed by an enzyme which appeared to be a FAD-containing nonheme iron protein that used triphosphopyridine nucleotide as the ultimate electron acceptor (47).

Tsai et al. (129), also working with Clostridium spp., reported that in the presence of pyruvic acid, several intermediate products accumulated in the medium, including 6-hydroxynicotinic acid, 1,4,5,6-tetrahydro-6-oxonicotinic acid, and α-methylene-glutaric acid. Pyruvic acid supplied the electrons for the further metabolism of 6-hydroxynicotinic acid. Subsequent experiments showed that nicotinic acid was converted to equimolar amounts of propionic acid, acetic acid, carbon dioxide, and ammonium and that the following compounds were sequential intermediates: 6-oxonicotinic acid, 1,4,5,6-tetrahydro-6-oxonicotinic acid, α-methylene glutaric acid, methylitaconic acid, dimethylmaleic acid, and pyruvic acid (121). An enzyme requiring reduced ferredoxin or reduced methyl viologen dye as the electron donor was partially purified from a Clostridium sp. and found to catalyze the reversible reduction of 6-hydroxynicotinic acid to 1,4,5,6-tetrahydro-6-oxonicotinic acid (48). Methylitaconic acid was identified as the intermediate in the conversion of α-methylene glutaric acid to dimethylmaleic acid, and the enzyme catalyzing this reaction was isolated and characterized as a vitamin B12-coenzyme-dependent α-methylene glutarate mutase (71). The formation of methylitaconic acid is therefore B12-dependent, accounting for the high levels of B12 compounds found in cells cultured on medium in which nicotinic acid serves as the major carbon, nitrogen, and energy source.

When 1,4,5,6-tetrahydro-6-oxonicotinic acid was incubated with a crude enzyme extract of a Clostridium sp., 2-formylglutaric acid was produced and α-methylene glutaric acid was converted to dimethylmaleic acid (130). This reaction may involve cleavage of the bond between carbons 3 and 4 and attachment of carbon 3 to carbon 5. Taking into account the above-described intermediates produced during transformation of nicotinic acid by Clostridium spp., a pathway for nicotinic acid metabolism under anaerobic conditions was proposed (Fig. 8) (130).

Experiments with [2-14C]nicotinic acid indicated that the labeled carbon was almost equally distributed between the methyl groups of propionic and acetic acids. The isotope from either [6-14C]nicotinic acid or [7-14C]nicotinic acid was distributed almost equally between carbon dioxide and the carboxy group of propionic acid (72, 82). The distribution of radioactivity supported the proposed pathway for Clostridium spp., which involves cleavages between carbon 6 and the nitrogen atom, between carbons 3 and 4, and between carbon 2 and the nitrogen atom (Fig. 8), leading to the formation of four end products, propionic acid, acetic acid, carbon dioxide, and ammonium.

Imhoff-Stückle and Pfennig (58) isolated from marine mud sediment another anaerobic bacterium, characterized as Desulfococcus niacini sp. nov., which utilized nicotinic acid as an electron donor and carbon source. This bacterium used sulfur as well as thiosulfate and sulfite as the electron acceptor, and all of them were reduced to sulfide. In contrast to the Clostridium sp. reported above, Desulfococcus niacini oxidized nicotinic acid to carbon dioxide and ammonium, but neither propionic acid nor acetic acid was generated as an end product.

4-Carboxypyridine

The initial step in the transformation of 4-carboxypyridine (isonicotinic acid) appears to be hydroxylation at carbon 2. Gupta and Shukla (34) found that 2-hydroxyisonicotinic acid was produced during the metabolism of isonicotinic acid by a Sarcina sp. and suggested 2,6-dihydroxyisonicotinic acid (citrazinic acid) as a further intermediate product. Ensing and Rittenberg (21) showed that growing cultures of a soil bacterium produced 2,6-dihydroxyisonicotinic acid. Cells growing on isonicotinic acid were simultaneously adapted to growth on 2,6-dihydroxyisonicotinic acid, indicating that the metabolism of isonicotinic acid involved hydroxylation of the pyrrole ring at the two alpha positions. Dihydroxyisonicotinic acid formed a blue pigment by chemical oxidation, similar to that observed during the transformation of 2,6-dihydroxypyridine by the Pseudomonas sp. Singh and Shukla (119) observed that a Bacillus sp. isolated from soil was able to grow on isonicotinic acid. Incubation of resting cells with arsenite led to the accumulation of succinic acid semialdehyde during isonicotinic acid transformation. The pathway depicted in Fig. 9 was proposed for the metabolism of isonicotinic acid by various microorganisms.

The metabolism of isoniazid (isonicotinic acid hydrazide) evidently occurs in a similar way. Gupta and Shukla (35) re-
ported that a *Sarcina* sp. was able to utilize isoniazid as well as isonicotinic acid as sole source of carbon, nitrogen, and energy. It has been reported that in addition to a *Sarcina* sp., some *Bacillus* spp. have the ability to transform both isoniazid and isonicotinic acid (118).

Fishbain et al. (23) reported that *Mycobacterium smegmatis* transformed isoniazid to equivalent amounts of isonicotinic acid and hydrazine. Therefore, it was postulated that isoniazid was metabolized by these organisms through a pathway similar to that proposed for isonicotinic acid. The conversion of isoniazid to isonicotinic acid is shown in Fig. 10.

**Pyridine-2,6-Dicarboxylic Acid**

Arima and Kobayashi (4) demonstrated that an *Achromobacter* sp. oxidized pyridine-2,6-dicarboxylic acid (2,6-dipicolinic acid) to carbon dioxide, ammonium, and water. Further investigations showed that 3-hydroxydipicolinic acid was an intermediate product of 2,6-dipicolinic acid transformation, which was further degraded to α-ketoglutaric acid and oxalic acid (64). Oxalic acid is an end product of 2,6-dipicolinic acid and is directly produced from it. Incubation of resting cells of the *Achromobacter* sp. growing on α-ketoglutaric acid produced no oxalic acid, because α-ketoglutaric acid is not an educt of oxalic acid.

Phthalate-degrading bacteria isolated from marine sediments were reported to metabolize pyridinedicarboxylic acids (3, 127, 128). As well as *Achromobacter* sp. and the various phthalic acid-degrading marine bacteria, a *Bacillus brevis* strain has been reported to be able to grow on 2,6-dipicolinic acid (117). Strain CC9M partially oxidized 2,6-dipicolinic acid to produce 2,3-dihydroxypicolinic acid, which can be further degraded upon exposure to sunlight (3).

Seyfried and Schink (100) studied the anaerobic transformation of 2,6-dipicolinic acid by a defined coculture of two bacteria from marine sediment. 2,6-Dipicolinic acid was transformed to propionic acid, acetic acid, carbon dioxide, and ammonium. The dipicolinic acid-fermenting bacterium could be cultivated only in coculture with another bacterium which oxidized acetic acid to carbon dioxide. The aerobic and anaerobic pathways for transformation of 2,6-dipicolinic acid are shown in Fig. 11.

**TRANSFORMATION OF ALKYLPYRIDINES**

The initial step of microbial transformation of alkylpyridines may occur on the aromatic ring or the alkyl substituent. Oxidation of the heterocyclic ring results in the formation of pyridine-N-oxide or hydroxyalkylpyridines. Oxidation of the side chain leads to the formation of hydroxyalkyl pyridines.

Korosteleva et al. (67) found that *Pseudomonas* sp. strain KM-3 transformed 3-methylpyridine (3-picoline) to 3-hydroxymethylpyridine by oxidizing the alkyl group. In addition, nicotinic acid was identified as one of the metabolic products. The authors proposed a metabolic pathway for the further transformation of nicotinic acid by this *Pseudomonas* sp. similar to that described for *Pseudomonas fluorescens*, even though expected metabolites such as 2,5-dihydroxy pyridine and maleic acid were not detected. The proposed pathway for the transformation of 3-methylpyridine is presented in Fig. 12.

Several *Curvularia* spp., as well as *Cunninghamella blakesleena*, *Sporotrichum sulfureens*, and *Pseudomonas* sp. strain AM-1, were found to hydroxylate 2,6-dimethylpyridine to 2-methyl-6-hydroxymethylpyridine. *Sporotrichum sulfureens* further metabolized 2-methyl-6-hydroxymethylpyridine to form small quantities of 2,6-dihydroxymethylpyridine (18). No other metabolites have been reported for the subsequent transformation of 2,6-dihydroxymethylpyridine; however, it may be further oxidized to 2,6-dipicolinic acid, which can then be degraded by one of the proposed pathways for this compound.

Oxidation of the alkyl substituent has also been shown during the transformation of alkylpyridines by fungi. A group of Russian scientists proposed the use of a microbiological procedure instead of chemical methods to produce isomeric hydroxyalkylpyridines (75, 133). Various fungi grown in medium containing carbon sources other than alkylpyridines were able to catalyze the initial hydroxylation of alkylpyridines, but no further reactions took place. Modyanova et al. (75) found that fungi which hydroxylate dialkylbenzenes (*Beauveria bassiana* VKM F-2533, *Aspergillus sclerotiorum* JMM 56673, and *Aspergillus niger* NRRL 3228) and steroids (*Tolimella hyalospora*, *Absidia orchis* 6, *Rhizopus nigricans* 7, and *Trichothecium roseum* 27) also carried out side chain oxidation of methylpyridines and dimethylpyridines. 2-, 3-, and 4-methylpyridines were transformed to 2-, 3-, and 4-hydroxymethylpyridines, respectively, and only one methyl group of dimethylpyridines was hydroxylated. Vorob’eva et al. (133) studied the hydroxylation of ethylenpyridines by microscopic fungi. 2-Ethylpyridine was transformed to 2-(1-hydroxethyl)pyridine, 2-(2-hydroxyethyl)pyridine, and 2-ethylpyridine-N-oxide. *Beauveria bassiana* VKM F-2533, *Beauveria* sp. strain 12, and *Penicillium* sp. strain 13 transformed 2-ethylpyridine to the optically active 2-(1-hydroxyethyl)pyridine with yields of 59.7, 7.0, and 10%, respectively. *Aspergillus awamori* VNIIG VUD T-2 and *Scopulariopsis brevicaulis* formed 2-(2-hydroxyethyl)pyridine from 2-ethylpyridine with yields of 30 to 40%.

Several studies indicate that the initial transformation of alkylpyridines takes place on the pyridine ring. Kost et al. (69) investigated the microbial transformation of 2,6-dimethylpyridine by bacteria, fungi, and yeasts. 2,6-Dimethylpyridine was transformed to 2,6-dimethylpyridine-N-oxide. The low concentration of 2,6-dimethylpyridine-N-oxide which accumulated in the culture media indicated that this product was further transformed by the microorganisms involved.

Feng et al. (22) investigated the transformation of ethylpyridines by a mixed culture of aerobic bacteria. Oxidation of the pyridine was found to have occurred, since 6-ethyl-2(1H)-pyridone and 4-ethyl-2(1H)-pyridone were determined to be intermediate products of 2- and 4-ethylpyridine degradation, respectively. The presence of 4-ethyl-2-pyridone suggests that reduction of the pyridine ring also occurred.

Shukla (102) reported that resting cells of an *Arthrobacter* sp. accumulated succinic acid semialdehyde and pyruvic acid during the transformation of 2-methylpyridine (2-picoline) in the presence of an inhibitor (semicarbazide). Because no other intermediate products were reported, the metabolic path for transformation of 2-picoline remains unknown. A few researchers studied the transformation of alkylpyridines under anaerobic conditions. Transformation of 3- and 4-picoline by a microbial population under sulfate-reducing conditions was investigated by Kaiser et al. (61). In the presence of sulfate, 3- and 4-picoline (0.4 mM) were transformed

![FIG. 10. Transformation of isoniazid to isonicotinic acid.](image-url)
within 30 days. A metabolite identified as 2-hydroxy-4-picoline accumulated during the transformation of 4-picoline. The 3- and 4-picoline-degrading culture could transform benzoic acid; however, 2-picoline, dimethylpyridines, and trimethylpyridines were not degraded.

Rogers et al. (91) investigated the transformation of various alkylpyridines in the presence of a soil inoculum under both aerobic and anaerobic conditions. Biodegradation rates under aerobic but not anaerobic conditions were greatly affected by the specific ring substitution of structural isomers. Unfortunately, no information was provided about possible intermediate products formed during the metabolism of these alkylpyridines.

**TRANSFORMATION OF CHLORINATED PYRIDINES**

Behrman and Stanier (6) investigated the transformation of halogenated nicotinic acids. 2-Fluoro-, 5-fluoro-, and 5-chloronicotinic acid were transformed to their 6-hydroxy derivatives by whole cells of *Pseudomonas fluorescens* N-9, but 5-bromonicotinic acid could not be oxidized. The findings for 2-fluoro- and 5-fluoronicotinic acid are in agreement with those of Hughes (53).

Two products were isolated from the culture medium after the transformation of 5-fluoronicotinic acid by *P. fluorescens* N-9. These were probably fluorine-substituted intermediates which could not be further metabolized because the site of enzyme action was blocked by the fluorine atom. Behrman and Stanier (6) suggested that the two compounds were fluorocitric acid and acetic acid.

Chlorpyrifos [O,O-diethyl-O-(3,5,6-trichloro-2-pyridyl)phosphorothioate] is a pyridine-based herbicide. The pathway of chlorpyrifos degradation in soil has been reported to involve both chemical and microbiological processes (87). The major products were the hydrolysis products, 3,5,6-trichloro-2-pyridinol and 3,5,6-trichloro-2-methoxypyridine, and carbon dioxide. In soils in which 3,5,6-trichloro-2-pyridinol did not accumulate, large quantities of carbon dioxide were produced, suggesting that chlorpyrifos metabolism involved cleavage of the heterocyclic aromatic ring (86). However, no reports were available concerning a ring cleavage mechanism for 3,5,6-trichloro-2-pyridinol. Racke et al. (86) used the most-probable-number technique to study the microorganisms that utilize 3,5,6-trichloro-2-pyridinol as the sole carbon and energy sources. However, when soils that rapidly degrade 3,5,6-trichloro-2-pyridinol were used as an inoculum, no microorganisms were isolated, suggesting that transformation of 3,5,6-trichloro-2-pyridinol occurs through a cometabolic process.

**TRANSFORMATION OF NICOTINE**

Early work by Frankenburg and Vaitekunas (24) with microorganisms isolated from the surface of tobacco seeds indicated that nicotine is transformed by various pathways, with both the pyridine and pyrrolidine rings being attacked. This finding was subsequently confirmed by several investigators working with a variety of different organisms. The pathways are depicted in Fig. 13 and 14.

Hochstein and Rittenberg studied the transformation of nicotine by strain PP-34 (45, 46). They found that 6-hydroxynicotine was formed and further transformed to 6-hydroxy-N-methylpyrrolidine by dehydrogenation of the pyrrolidine ring (Fig. 13). The hydroxylation of nicotine to 6-hydroxynicotine required 0.5 mol of oxygen per mol of nicotine; however, the
product contained 1 mol of oxygen, indicating that the oxygen originated from water rather than from molecular oxygen (43).

Maeda et al. (74) reported that another bacterial strain, JTS-0006, followed a pathway for the transformation of nicotine similar to that reported by Hochstein and Rittenberg (46). During nicotine transformation, 6-hydroxynicotine, 6-hydroxy-N-methylmyosmine, and 6-hydroxypseudooxynicotine were detected in the culture medium (Fig. 13).

Hochstein and Dalton (44) isolated from *Arthrobacter oxydans* nicotine oxidase which incorporated oxygen from water into nicotine to form 6-hydroxynicotine. This nicotine oxidase was conceived as a two-headed enzyme carrying out the dissimilar functions of hydration followed by oxidation. 6-Hydroxypseudooxynicotine was isolated after a one-step oxidation reaction of 6-hydroxynicotine (46). 6-Hydroxypseudooxynicotine was further oxidized to 2,6-dihydroxypseudooxynicotine (88, 89). Further transformation of 2,6-dihydroxypseudooxynicotine yielded 2,6-dihydroxy-pyridine and γ-methylaminobutyric acid (27) (Fig. 13). A monoxygenase catalyzed the transformation of 2,6-dihydroxy-pyridine to 2,3,6-trihydroxy-pyridine (50). In the presence of oxygen and the absence of a catalyzing enzyme, this trihydroxy-pyridine showed a rapid spontaneous oxidation and formed a blue pigment. In the presence of crude cell extracts of *A. oxydans*, this pigment was not formed and 2,3,6-trihydroxy-pyridine was converted by a cleavage enzyme to maleamic acid. The purification of this inducible oxidase that catalyzed the oxidation of 2,6-dihydroxy-pyridine required reduced pyridine nucleotide; this indicated that the enzyme was a monoxygenase (49). The authors suggested that the conversion of 2,6-dihydroxy-pyridine to maleamic acid involved at least two enzymes, the oxidase and the enzyme cleaving the aromatic ring.

It had been reported that 2,6-dihydroxypseudooxynicotine, an unstable intermediate product of 6-hydroxypseudooxynicotine, was further transformed to 2,6-dihydroxy-N-methylmyosmine by a nonenzymatic reaction in the absence of a proper enzyme fraction (27, 88, 89). 2,6-Dihydroxy-N-methylmyosmine was not further metabolized and appeared to be a metabolic artifact in the main pathway of nicotine degradation by *A. oxydans*, arising only because of the incomplete enzyme system used (88, 89).

The *Pseudomonas* sp. studied by Wada (134, 135) can metabolize both nicotine and nornicotine. Nornicotine and nicotine differ only with respect to substituents in position 1 of the pyrrolidine ring (-H for nornicotine and -CH₃ for nicotine). During the transformation of nornicotine, 6-hydroxymyosmine and 3-succinoyl-6-hydroxy-pyridine were detected in the culture medium. The formation of 6-hydroxymyosmine indicated that this *Pseudomonas* sp. used a metabolic pathway which involved a hydration of the heterocyclic aromatic ring prior to transformation to pseudooxynicotine. The same bacterium used a different pathway to degrade nicotine.

Several microorganisms were reported to transform nicotine by first modifying the pyrrolidine ring (Fig. 14). Gherna and Rittenberg (28) reported that a *Pseudomonas* sp. was able to oxidize nicotine, N-methylmyosmine, pseudooxynicotinic, 3-succinoylpyridine, and 6-hydroxy-3-succinoylpyridine. Cells of this *Pseudomonas* sp. adapted to nicotine were simultaneously adapted to the oxidation of 2,5-dihydroxy-pyridine (28). Thus, the authors suggested a cleavage at the 3' position of 6-hydroxy-3-succinoylpyridine with the formation of 2,5-dihydroxy-pyridine and succinic acid.

Wada and Yamasaki (136) reported that during the transformation of nicotine by bacteria isolated from soil, a different
set of products, N-methylmyosmine, pseudooxynicotinic, and 3-succinoylpyridine, could be detected. The *Pseudomonas* sp. which metabolized both nicotine and nornicotine transformed nicotine by attacking the pyrrolidinone ring first; pseudooxynicotinic, 3-succinoylpyridine, and 3-succinolyl-6-hydroxypyridine were found as metabolites of nicotine degradation.

*Achromobacter nicotinophagum* metabolized nicotine via two different pathways (56). During the exponential growth phase, nicotine was metabolized to 6-hydroxynicotinic and then further metabolized to aliphatic products. When the cells stopped dividing, the metabolism of nicotine to aliphatic products was inhibited and nicotine was transformed by an alternate pathway via pseudooxynicotinic and 3-succinopyridine to 6-hydroxy-3-succinopyridine. No further transformation of 6-hydroxy-3-succinopyridine could be detected.

Strain JTS-0006 was reported to be able to use nicotine-N-oxide as the sole carbon and nitrogen source (74). Pseudooxynicotinic and 3-succinopyridine were detected as intermediate products in the growing-cell culture. The formation of these metabolites indicated that the bacterial strain JTS-0006 transformed nicotine-N-oxide through a pathway similar to that described for the metabolism of nicotine by *Pseudomonas* spp.

**TRANSFORMATION OF OTHER PYRIDINE DERIVATIVES**

**Nitrogen Substituents**

Vaughan et al. (132) reported that 3-cyanopyridine (3-pyridine-carbonitrile) was hydrolyzed to nicotinic acid and ammonium by *Nocardia rhodochrous*. Ammonium was utilized for growth, whereas nicotinic acid was not further metabolized. An *Escherichia coli* strain transformed nicotinamide in a similar manner (131), producing nicotinic acid and ammonium.

**N-Substituted Derivatives**

As discussed in the section on transformation of hydroxylated pyridines (above), Shukla and Kaul (110) described a *Nocardia* sp. which was able to transform pyridine, pyridine-N-oxide, and 2-hydroxypyridine. Transformation of pyridine-N-oxide under limited aeration resulted in the accumulation of 2-hydroxypyridine. In the presence of arsenite, pyruvic acid was produced during the transformation of pyridine-N-oxide and 2-hydroxypyridine. However, 2-hydroxypyridine was never produced during the degradation of pyridine. The authors suggested, therefore, that pyridine transformation follows a pathway different from that of pyridine-N-oxide, with pyridine being transformed via the pathway described for *Bacillus* strain 4 and pyridine-N-oxide being oxidized via 2-hydroxypyridine to 2,5-dihydroxy pyridine. The formation of a blue pigment (probably the condensation of 2,3,6-trihydroxy pyridine) indicated that a trihydroxy pyridine derivative was produced prior to ring fission by the *Nocardia* sp. For the complete transformation of pyridine-N-oxide, a pathway similar to that described above for degradation of 2-hydroxy pyridine (Fig. 3) was proposed.

Several studies have also been undertaken to investigate the transformation of 4-carboxy-1-methylpyridinium chloride, a photolytic product of paraquat. Wright and Cain (143) reported the isolation of *Achromobacter* strain D, which utilized 4-carboxy-1-methylpyridinium chloride as the sole source of carbon and nitrogen. This *Achromobacter* sp. cleaved the pyridine ring by a multienzyme reaction sequence and liberated methylamine, which was further oxidized to ammonium and carbon dioxide. Later investigations on the degradation of 4-carboxy-1-methylpyridinium chloride showed that in addition to methylamine and carbon dioxide, succinic acid and formic acid were produced as end products (144, 145). The oxygen-requiring step consumed 1 mol of oxygen per mol of 4-carboxyl-1-methylpyridinium chloride, consistent with direct oxidative cleavage (by a dioxygenase) of the partially reduced pyridinium ring. Radioisotopic experiments indicated that ring cleavage must occur between carbons 2 and 3 of the heterocyclic ring. The N-formyl group was hydrolyzed to formic acid, while γ-(N-formyl-N-methylamino)vinylacetaldheyde was hydrolyzed to its corresponding acid, γ-(N-methylamino)vinylacetic acid. These reactions were followed by another hydrolysis step, leading to the formation of succinic acid semialdehyde and methylamine. Experiments with cell extracts of the *Achromobacter* sp. and 14C-labeled 4-carboxy-1-methylpyridinium chloride showed that the carbon dioxide was derived from the 4-carboxyl group and the methylamine was derived from the N-methyl group of the substrate (146). Succinic acid most probably originated from the carbon 3 to carbon 6 atoms of the heterocyclic ring, whereas formic acid originated from carbon 2. According to these intermediate products, a pathway was proposed for the transformation of 4-carboxyl-1-methylpyridinium chloride by *Achromobacter* strain D as shown in Fig. 15.

Orpin et al. (79, 81), studying *Achromobacter* strain 4C1, proposed a different pathway for the transformation of N-methyl-4-carboxoxyridine (N-methylisonicotinic acid) (Fig. 16). In contrast to *Achromobacter* strain D, this bacterium appeared to remove the methyl group before ring fission occurred. The authors suggested that N-methyl-4-carboxoxyridine was hydroxylated at position 2 to form 2-hydroxy-N-methylisonicotinic acid, which was then demethylated to 2-hydroxy-4-carboxoxyridine. These two steps were not demonstrated by enzyme assays, but 2-hydroxy-4-carboxoxyridine was hydroxylated by the crude cell extract to 2,6-dihydroxy-4-carboxoxyridine. Maleamic acid was deaminated and maleic acid was isomerized to fumaric acid by soluble enzyme systems. The cells of this soil bacterium were grown on 2-hydroxisonicotinic acid with an oxygen consumption of 0.5 mol per mol of substrate transformed. This indicated that the oxygen source for this hydroxylation reaction was water.

Orpin et al. (80) also studied a gram-negative rod-shaped
bacterium that transformed pyridine-2-carboxamide (picolineamide), a photolytic product of diquat. During the transformation of pyridine-2-carboxamide, 2,5-dihydroxypyridine was released into the culture medium. Cultures inhibited by arsenite accumulated 6-hydroxypicolinic acid and pyruvic acid. Cell extracts of the bacterium transformed picolineamide into picolinic acid, picolinic acid to 6-hydroxypicolinic acid, 2,5-dihydroxypyridine to maleamic and formic acids, and maleamic acid to fumaric acid (Fig. 17).

Mimosine, \(\beta\)-(\(N\)-(3-hydroxy-4-oxopyridyl)]-\(\alpha\)-aminopropionic acid, is a nonprotein amino acid which is found in tropical plants and induces toxic effects in ruminants and monogastric animals (17). Bacteria isolated from the rumen of sheep have been found to transform mimosine to 3-hydroxy-4(1H)pyridone and 2,3-dihydroxypyridine, which were further degraded by a wide range of ruminal bacteria (17). In addition, Allison et al. (2) identified and characterized four strains of obligate anaerobic bacteria that degraded 3-hydroxy-4(1H)pyridone and 2,3-dihydroxypyridine. These isolates did not fit into any existing taxon; therefore, a new genus and species, \(\textit{Synergistes jonesii}\), were proposed.

**Pyridoxine**

Pyridoxine (vitamin B\(_6\)) was metabolized by two strains of \(\textit{Pseudomonas}\) strains IA and MA-1, by related but different pathways. \(\textit{Pseudomonas}\) strain IA oxidized pyridoxine via isopyridoxal and 5-pyridoxic acid (or its lactone) to pyriiconic acid \(\rightarrow\) pyridoxal-5′-[\(\text{N}\)-acetylaminomethylene]succinic acid and further to carbon dioxide and ammonium (13, 57, 90). The transformation of pyridoxine to isopyridoxal was catalyzed by a FAD-dependent enzyme isolated from \(\textit{Pseudomonas}\) strain IA. This enzyme used 2,6-dichloroindophenol but not oxygen as the hydrogen acceptor (122). Sparrow et al. (120) found that the transformation of 5-pyridoxic acid to pyriiconic acid was catalyzed by an oxygenase in the presence of a reductant. The open-chain product (pyriiconic acid) could be obtained only with purified oxygenase preparations; in crude cell extracts of \(\textit{Pseudomonas}\) strain IA, this product was rapidly converted to succinic acid semialdehyde, acetic acid, carbon dioxide, and ammonium. The proposed pathway for transformation of pyriiconic acid by \(\textit{Pseudomonas}\) strain IA is given in Fig. 18.

\(\textit{Pseudomonas}\) strain MA-1 oxidized pyridoxine by a pathway with analogous reaction mechanisms to those described above; however, the intermediate products formed were different. Pyridoxal was oxidized via pyriodoxal, 4-pyridoxic acid lactone, 4-pyridoxic acid, 2-methyl-3-hydroxy-5-formylpyridine-4-carboxylic acid, 2-methyl-3-hydroxy-5-carboxylic acid, 2,5-dihydroxypyridine-4,5-dicarboxylic acid, 2-methyl-3-hydroxypyridine-5-carboxylic acid, \(\alpha\)-(\(N\)-acetylaminomethylene)succinic acid, succinic acid semialdehyde, acetic acid, carbon dioxide, and ammonium (13), as shown in Fig. 19. The transformation of pyridoxine to 4-pyridoxic acid was catalyzed by a FAD-dependent enzyme isolated from \(\textit{Pseudomonas}\) strain MA-1. This pyridoxine-4-dehydrogenase utilized either oxygen or 2,6-dichloroindophenol as the hydrogen acceptor (122). A DPN+ (diphosphopyridine nucleotide)-specific pyridoxal dehydrogenase from \(\textit{Pseudomonas}\) strain MA-1 oxidized pyriodoxal to 4-pyridoxolactone (14), which in turn was cleaved to 4-pyridoxic acid by a second enzyme, 4-pyridoxolactonase. The ring cleavage of 2-methyl-3-hydroxypyr-
dine-5-carboxylic acid was catalyzed by a DPNH-dependent oxygenase (120). The metabolite α-(N-acetylaminomethylene) succinic acid accumulated only in the presence of purified oxygenase preparations (77). The two *Pseudomonas* oxygenases, FAD-dependent pyridoxine-4-dehydrogenase and DPNH-dependent pyridoxal dehydrogenase, responsible for the ring cleavage showed similar mechanisms, although they differed in their specificity toward the reductant and substrate.

**Comparative Transformation Rates of Pyridine Derivatives**

The fate of heterocycles in nature is dependent on their structure, concentration, and chemical and physical properties, as well as the physical, chemical, and microbiological properties of the environment (31). Experiments were conducted with batch cultures and soil suspensions, using various pyridine derivatives as substrates. These studies showed that pyridinecarboxylic acids had the highest transformation rate, followed by monohydroxyxypyrindines, methylpyridines, aminopyridines, and chloropyridines (114, 115). Carboxyl substituents at any position of the pyridine ring stimulated decomposition more than did any other substituent, whereas chlorosubstituted pyridines showed the lowest degradation rate (76).

**TRANSFORMATION OF QUINOLINE AND ITS DERIVATIVES**

Quinoline can be transformed by microorganisms under both aerobic and anaerobic conditions (1, 12, 29, 99). Although many bacteria are capable of transforming quinolines, most quinoline-degrading organisms are *Pseudomonas* spp. There are several possible pathways for the transformation of quinoline.

Grant and Al-Najjar (29) isolated from garden soil a bacterium which could use quinoline as the sole carbon and nitrogen source. The authors suggested that the initial step in the transformation of quinoline by whole cells involved a hydroxylation at position 2 of the heterocyclic aromatic ring, leading to 2-hydroxyquinoline. Further transformation included additional hydroxylation steps leading to 2,6-dihydroxyquinoline and a trihydroxyquinoline (probably 2,5,6-trihydroxyquinoline).

The degradation of quinoline by *Pseudomonas aeruginosa* OP and *P. putida* QP also occurred via hydroxyquinolines (1). Moreover, a limited number of methylquinolines were hydroxylated by these two species but no further transformation of the hydroxylated products occurred. 2-Methylquinoline was not metabolized, since the methyl group at position 2 apparently blocked the formation of the hydroxyquinoline. However, the authors isolated a new strain of *Pseudomonas* (MQP) which was able to transform 2-methylquinoline, leading to the formation of hydroxylated methylquinolines and other unidentified metabolites.

Shukla (105) isolated from sewage an aerobic gram-negative motile bacterium which was identified as a *Pseudomonas* sp. and was found to degrade quinoline by an alternate pathway. 2-Hydroxyquinoline and 8-hydroxycoumarin were found in the culture medium and were further metabolized. Quinoline-adapted cells were also able to transform these two compounds without a lag phase, providing additional support for their intermediate role in the metabolism of quinoline. Further research demonstrated that 2,8-dihydroxyquinoline and 2,3-dihydroxyphenylpropionic acid were additional intermediate products (106). Arsenate-inhibited cells accumulated 2-hydroxyquinoline and pyruvic acid in the culture medium. The proposed pathway for metabolism of quinoline by this *Pseudomonas* sp. is presented in Fig. 20.

Boyd et al. (11) investigated the transformation of azaredes such as quinoline, isoquinoline, quinazoline, and quinoxaline by a mutant strain of *Pseudomonas putida*, which transformed naphthalene to form its cis-dihydriodiol metabolite. This bacterium transformed both the homocyclic and the heterocyclic moieties of quinolines. When the attack occurred on the homocyclic ring, the corresponding cis-hydriodiol derivatives were found in the culture medium, along with monohydroxylated derivatives such as 8-hydroxyquinoline or 5-hydroxyisoquinoline. The authors proposed that oxidation by a dioxygenase resulted in the formation of cis-hydriodiol derivatives and oxidation by a monoxygenase resulted in the formation of monohydroxylated derivatives via the areneoxide intermediates. *P. putida* also hydroxylated the heterocyclic ring to yield 3-hydroxyquinoline, 4-hydroxquinazoline, and 5-hydroxyquinoloxine. Hydroxylation of the heterocyclic ring was suggested by the following evidence: (i) an unstable cis-dihydriodiol metabolite formed on the heterocyclic ring; and (ii) a spontaneous dehydration of this cis-dihydriodiol metabolite re-
sulted in the formation of 3-hydroxyquinoline, which led to anthranilic acid (2-aminobenzoic acid). Detection of anthranilic acid as a metabolite in the culture medium during the anthranilic acid (2-aminobenzoic acid). Detection of anthranilic acid (2-aminobenzoic acid) resulted in the formation of 3-hydroxyquinoline, which led to 494 KAISER ET AL. MICROBIOL.REV.

anaerobic conditions and involved hydroxylation at position 2 of the heterocyclic ring. This indicated that these anaerobic microorganisms used a pathway for the transformation of quinoline similar to that described above for aerobic bacteria. Studies with H$_2$$_{18}$O showed that the oxygen for the hydroxylation reaction was derived from water (83).

Wang et al. (138, 139) demonstrated that a methanogenic consortium transformed quinoline in an anaerobic filter. After an acclimation period of 6 months, methane production was 85% of the expected amount, indicating that quinoline could be completely transformed under anaerobic conditions. Unfortunately, no further intermediates were reported, and the pathway of quinoline metabolism under anaerobic conditions remains unknown. Brockman et al. (12) isolated two gram-negative bacteria from deep subsurface sediments. The growing cell cultures of both organisms mineralized quinoline under aerobic conditions and transformed quinoline to soluble intermediates under anaerobic conditions. Both organisms contained four plasmids of identical size, ranging from 50 to 440 kb.

As previously discussed, most quinoline-degrading organisms are *Pseudomonas* spp. These bacteria are also able to transform the quinoline derivative kynurenic acid (2-carboxy-4-hydroxyquinoline). Hayashi et al. (39) reported that a partially purified enzyme preparation from a tryptophan-adapted *Pseudomonas* sp. produced l-glutamic acid and acetic acid from kynurenic acid. Further investigations showed that an enzyme derived from this *Pseudomonas* sp. catalyzed the transformation of kynurenic acid to 7,8-dihydroxykynurenic acid (124).

In a separate report, Taniuchi and Hayashi (123) indicated that a cell extract of *Pseudomonas fluorescens* degraded kynurenic acid in the presence of NAD. l-Glutamic acid, l-alanine, acetic acid, and carbon dioxide were formed as the major end products. A compound identical to 7,8-dihydroxykynurenic acid-7,8-diol was also formed. This compound

\[
\begin{align*}
\text{Quinoline} & \quad \rightarrow \quad 2\text{-oxo-1,2-dihydroquinoline} & & N\text{-formylanthranilic acid, anthranilic acid, and catechol (10).} \\
& \quad \rightarrow \quad 6\text{-hydroxy-2-oxo-1,2-dihydroquinoline,} & & 7\text{-8-dihydroxy-4-methyl-2-oxo-1,2-dihydroquinoline,} \\
& \quad \rightarrow \quad 6\text{-hydroxy-5-(2-carboxyethenyl)-4-methyl-1H-2-pyridone.} & & 6\text{-hydroxy-5-(2-carboxyethenyl)-4-methyl-1H-2-pyridone.} \\
& \quad \rightarrow \quad 5\text{-hydroxy-6-dihydroquinoline,} & & \text{Ring cleavage occurred between C-7 and C-8.} \\
& \quad \rightarrow \quad 6\text{-hydroxy-5-(2-carboxyethenyl)-4-methyl-1H-2-pyridone.} & & \text{The proposed degradation pathway of 4-methylquinoline is different from that of quinoline by the same organism, since the quinoline ring structure was cleaved between C-5 and C-6.} \\
\end{align*}
\]

**Fig. 21. Proposed pathway for the transformation of quinoline by Rhodococcus strain B1 (98).**
and carbazole were found in an aquifer contaminated by wood and the breakdown of aliphatic products.

The common degradative pathway for oxidized aromatic compounds is either the benzene or the pyridine ring and thereafter followed a common degradation pathway for oxidized aromatic compounds: oxidation, reduction, decarboxylation, ring cleavage, and the breakdown of aliphatic products.

Kynurenic acid was transformed by an Aerococcus sp. in a manner similar to that of the Pseudomonas sp. described above (16); i.e., 5-(\(\gamma\)-carboxy-\(\gamma\)-oxopropenyl)-4,6-dihydroxypicolinic acid and 5-\(\beta\)-(carboxyethyl)4,6-dihydroxypicolinic acid were isolated from the culture medium. Transformation of 5-\(\beta\)-(carboxyethyl)-4,6-dihydroxy-picolinic acid by an Aerococcus cell extract resulted in the formation of \(\alpha\)-ketoglutaric acid, glutamate, aspartate, and pyruvic acid.

**TRANSFORMATION OF ACRIDINE AND ITS DERIVATIVES**

Acridine, a three-ring nitrogen heterocycle, has many properties similar to those of pyridine and quinoline. Knezovich et al. (63) studied anaerobic transformation of acridine in a laboratory microcosm by using three different inocula: a stabilized mixed culture growing on ferulic acid originally from anaerobic sewage sludge, and sulfate-reducing and methanogenic aquifer materials from two sites in a groundwater aquifer contaminated by landfill leachate. Acridine (1 to 6 mg/ml) was degraded under denitrifying, sulfate-reducing, and methanogenic conditions in 1 to 3 weeks. Benzoic acid was one of the key aromatic intermediates. A tentative pathway of anaerobic acridine transformation was proposed. It began with oxidation of either the benzene or the pyridine ring and thereafter followed the common degradation pathway for oxidized aromatic compounds: oxidation, reduction, decarboxylation, ring cleavage, and the breakdown of aliphatic products.

Pereira et al. (84) reported that acridine, 7,8-bezoquinoline, and carbazole were found in an aquifer contaminated by wood and treatment chemicals. Although quinolines at this site were transformed by the indigenous microbial population, acridine and benzoquinoline were not.

**CONCLUSIONS**

A large variety of organisms including eubacteria, yeasts, and fungi are capable of metabolizing and cleaving homocyclic and heterocyclic aromatic compounds (137). As summarized in this review, many different microbial species are capable of degrading pyridinic and aza-arenic compounds under aerobic and anaerobic conditions.

Pyridine is a benzene-like compound with one carbon atom replaced by a nitrogen atom. The pyridine ring is susceptible to reduction, as demonstrated by the functions of pyridine nucleotides in metabolism (104). Unlike its homocyclic analog, the mechanism of pyridine ring cleavage is not fully understood. The initial step in the transformation of quinoline derivatives has been reported. Ring fission of benzene proceeds with the introduction of two hydroxyl groups ortho to each other, followed by ortho or meta cleavage. Formation of hydroxyquinoline, however, has not been observed during pyridine transformation. The metabolism of hydroxylated and carboxylated pyridines seems to be initiated by hydroxylation: degradation of hydroxylated pyridines occurs via \(d\)- and \(t\)-hydroxyquinoline derivatives before ring fission.

Carboxylated pyridines have been the most frequently investigated pyridine derivatives. Transformation of pyridines carboxylated at position 2 seems to occur through hydroxylation at positions 3 and 6 followed by decarboxylation to yield a dihydroxyquinoline derivative prior to ring cleavage. Similar observations have been reported for the metabolism of 4-carboxyquinoline (isonicotinic acid). For 3-carboxyquinoline (nicotinic acid), the events leading to ring cleavage follow one of three pathways: (i) transformation to a \(d\)- or \(t\)-hydroxyquinoline with the carboxyl group being replaced by a hydroxyl group, (ii) initial hydroxylation at position 6 followed by reduction of the heterocyclic ring, or (iii) initial hydroxylation at position 6 following by formation of 2,5-dihydroxyquinoline and \(N\)-formylmaleamic acid.

Alkyl- and chloropyridines represent two of the largest classes of pyridines present in the environment (112). Transformation of methylated pyridine derivatives begins with hydroxylation of the substituent to form a methoxyquinoline derivative; the substituent is then further oxidized to a carboxyl group, thus forming a carboxylated pyridine derivative. However, laboratory experiments with 4-ethylquinoline under aerobic conditions indicated another mechanism of ring cleavage. The compound may be initially hydroxylated at position 2 to form 4-ethyl-2(1H)pyridine. Prior to oxidation of the side chain, the ring may be cleaved between the heteroatom and the carbon atom in position 2 (22).

Nicotine transformation can occur by various pathways. Both the pyridine ring and the pyrrolidine ring of nicotine can be attacked, but attack of the pyrrolidine ring leading to a di- or trihydroxyquinoline occurs most frequently.

The initial step in the transformation of quinoline derivatives can occur on either the heterocyclic or the homocyclic ring. In one reported study, quinoline transformation by a *Pseudomonas* sp. started with transformation at position 2 followed by a second hydroxylation at position 8, leading to 2,8-dihydroxyquinoline. This compound was transformed to 8-hydroxycoumarin prior to ring fission. On the other hand, transformation of kynurenic acid by *Pseudomonas fluorescens* was initiated by hydroxylations at the homocyclic aromatic ring leading to 7,8-dihydroxykynurenic acid. Fission of the homo-
cyclic ring then occurred, leading to 5-(γ-carboxy-γ-oxoprope-nyl)-4,6-dihydroticlopyricolic acid.

It should be noted that under aerobic conditions, the oxygen atom of the hydroxyl group incorporated into the pyridine and quinoline metabolites is often derived from water, whereas in the case of homocyclic aromatics, the oxygen atom is derived from molecular oxygen. In addition, reduction of the pyridine ring during transformation was frequently observed.

During the transformation of pyridine derivatives, the production of some pigments has been observed. A blue pigment appeared when trihydroxypyridine was metabolized; this pigment was characterized as 4,5,4-tetrahydroxypyridine-1(2H)-pyridyl)-4,6-dihydroxypicolinic acid.

Many bacteria have been described which degrade pyridine and its derivatives, but little research has been done, e.g., on aromatic compounds and their derivatives under anaerobic conditions. Crit. Rev. Environ. Control 37:251–281.

Bott, G., M. Schmidt, T. O. Rommel, and F. Lingens. 1989. Microbial degradation of quinoline and methylquinolines. Appl. Environ. Microbiol. 56:345–351.

Allison, M. J., W. R. Mayberry, C. S. McSweeney, and D. A. Stahl. 1990. Microbial degradation of quinoline and methylquinolines. Appl. Environ. Microbiol. 56:352–356.

Amador, J. A., and B. F. Taylor. 1990. Coupled metabolic and photolytic pathway for degradation of pyridinecarboxylic acids, especially dipicolinic acid. Appl. Environ. Microbiol. 56:1352–1356.

Arima, K., and Y. Kobayashi. 1962. Bacterial oxidation of dipicolinic acid. I. Isolation of microorganisms, their culture conditions, and end products. J. Bacteriol. 85:665–676.

Behrman, E. J., and R. Y. Stanier. 1957. The bacterial oxidation of nicotinic acid. J. Biol. Chem. 228:923–945.

Behrman, E. J., and R. Y. Stanier. 1957. Observations on the oxidation of halogenated nicotinic acids. J. Biol. Chem. 238:947–953.

Berry, D. F., A. J. Francies, and J. M. Bollag. 1967. Microbial metabolism of homocyclic and heterocyclic aromatic compounds under anaerobic conditions. Microbiol. Rev. 51:43–59.

Bott, G., J.-M. Grill, and J. P. Kaiser. 1991. The transformation of heterocyclic aromatic compounds and their derivatives under anaerobic conditions. Crit. Rev. Environ. Control 21:297–329.

Bott, G., and F. Lingens. 1991. Microbial metabolism of quinoline and related compounds. IX. Degradation of 1H-4-oxoquinoline and quinoline by Pseudomonas diminuta 31/1 Fa1 and Bacillus circulans 31/2 A1. Biol. Chem. Hoppe-Seyler 372:381–383.

Bott, G., M. Schmidt, T. O. Rommel, and F. Lingens. 1990. Microbial metabolism of quinoline and related compounds. V. Degradation of 1H-4-oxoquinoline by Pseudomonas putida 33/1. Biol. Chem. Hoppe-Seyler 371:999–1003.

Boyd, D. R., R. Austin, S. McMordie, H. P. Porter, H. Dalton, R. O. Jenkins, and O. W. Howarth. 1957. Metabolism of bicyclic aza-arenes by Pseudomonas putida to yield vicinal cis-dihydrodiols and phenols. J. Chem. Soc. 22:1722–1724.

Brockman, F. J., B. A. Donovan, R. J. Hicks, and J. K. Fredrickson. 1989. Isolation and characterization of quinoline-degrading bacteria from subsurface sediment. Appl. Environ. Microbiol. 55:1029–1032.

Burg, R. W., W. V. Rodwell, and E. E. Snell. 1960. Bacterial oxidation of vitamin B12. J. Biol. Chem. 235:1164–1169.

Burg, R. W., and E. E. Snell. 1969. The bacterial oxidation of vitamin B12. J. Bacteriol. 121–128.

Cain, R. B., C. Houghton, and K. A. Wright. 1974. Microbial metabolism of the pyridine ring. Metabolism of 2- and 3-hydroxypyridines by the male-male pathway in Achromobacter sp. Biochem. J. 140:293–300.

Cagney, S., and P. A. Johnson. 1963. Microbial oxidation of kynurenine, xanthurenic and picolinic acids. Biochem. Biophys. Acta 78:577–587.

Dominguez-Bello, M. G., and C. S. Stewart. 1990. Degradation of dimosine, 2,3-dihydroxypropyline and 3-hydroxy-4(1H)-pyridine by bacteria from the rumen of sheep in Venezuela. FEBS Microbiol. Ecol. 73:283–290.

Egorov, N. S., L. I. Vorob’eva, E. V. Dovgilevich, L. V. Modyanova, P. B. Ten’ telev, O. K. Shilhikina, N. S. Kulikov, and O. V. Messina. 1985. Hydroxylation of 2,6-dimethylpyridine and its analogs by microorganisms. Appl. Biochem. Microbiol. 21:277–281.

Ensign, J. C., and S. C. Rittenberg. 1963. A crystalline pigment produced from 2-hydroxypropyline by Arthrobacter crystallinisoli n. sp. Arch. Microbiol. 47:137–155.

Ensign, J. C., and S. C. Rittenberg. 1964. The pathway of nicotinic acid oxidation by a Bacillus species. J. Biol. Chem. 239:2285–2291.

Ensign, J. C., and S. C. Rittenberg. 1965. The formation of a blue pigment in the bacterial oxidation of isonicotinic acid. Arch. Microbiol. 51:384–392.

Ensign, J. C., J. P. Kaiser, and J. M. Bollag. 1994. Microbial transformation of ethyldiphenyls. Biochimie 51:121–128.

Fishbain, D., G. Ling, and D. J. Kushner. 1972. Isoniazid metabolism and binding by sensitive and resistant strains of Mycobacterium smegmatis. Can. J. Microbiol. 18:783–792.

Frankenburger, W. G., and A. A. Vaitiekunes. 1955. Chemical studies on nicotine degradation by microorganisms derived from the surface of tobacco seeds. Arch. Biochem. Biophys. 58:309–512.

Gherna, R. L., and S. C. Rittenberg. 1957. The bacterial oxidation of vitamin B6. J. Biol. Chem. 232:923–945.

Gherna, R. L., and S. C. Rittenberg. 1960. Bacterial oxidation of monooxygenase and isonicotinic acid hydrazide. J. Biol. Chem. 235:783–792.

Gherna, R. L., and S. C. Rittenberg. 1962. Alternate pathways in nicotine degradation. Bacteriol. Proc. 1962. p. 27.

Grant, D. J., W. H. and S. C. Rittenberg. 1960. The bacterial oxidation of nicotine. VI. The metabolism of 2,6-dihydroxypropyline. J. Biol. Chem. 240:3639–3641.

Grant, D. J., W. H. and S. C. Rittenberg. 1965. The bacterial oxidation of nicotine. VII. The degradation of pyridine and its carbon source; it was identified as riboflavin and was not directly synthesized from pyridine (113).

Despite the large number of studies conducted so far, our knowledge of this important class of chemicals is still sparse. Many bacteria have been described which degrade pyridine and its derivatives, but little research has been done, e.g., on the genetics of pyridine metabolism. More studies must also be conducted to elucidate the transformation of these chemicals in the environment.
Purification and properties of a nicotinic acid hydroxylase. J. Biol. Chem. 244:1194–1203.

Holenberg, J. S., and I. Tsai. 1969. Nicotinic acid metabolism. IV. Ferredoxin-dependent reduction of 6-hydroxonicotinic acid to 6-ono-1,4,5,6-tetrahydroxy nicotinic acid. J. Biol. Chem. 244:1204–1211.

Houghton, C., and R. B. Cain. 1972. Microbial metabolism of the pyridine ring. Biochem. J. 130:879–893.

Hughes, D. E. 1952. 6-Hydroxonicotinic acid as an intermediate in the oxidation of nicotinic acid by Pseudomonas fluorescens. Biochim. Biophys. Acta 9:226–227.

Hughes, D. E. 1955. 6-Hydroxonicotinic acid as an intermediate in the oxidation of nicotinic acid by Pseudomonas fluorescens. Biochim. J. 60:303–307.

Hund, H.-K., A. de Beyer, and F. Lingens. 1990. Microbial metabolism of quinoline and related compounds. VI. Degradation of quinoline by Arthrobacter sp. Biol. Chem. Hoppe-Seyler 371:1005–1008.

Hunt, A. L., D. E. Hughes, and J. M. Lowenstein. 1958. The hydroxylation of nicotinic acid by Pseudomonas fluorescens. Biochim. J. 69:170–173.

Hylin, J. W. 1959. The microbial degradation of nicotine. II. The mode of action of Achromobacter nicotinophagum. Arch. Biochem. 83:528–537.

Ihawa, M., V. W. Rodwell, and E. E. Snell. 1958. Bacterial oxidation of vitamin B1. II. Structure of “260 compound.” J. Biol. Chem. 233:1555–1559.

Imhoff-Stuckle, D., and N. Pfennig. 1983. Isolation and characterization of a nicotinic acid-degrading sulfite-reducing bacterium, Desulfococcus niacinii sp. nov. Arch. Microbiol. 136:194–198.

Jones, M. V., and D. E. Hughes. 1972. The oxidation of nicotinic acid by Pseudomonas ovalis dalvii. Biochim. J. 139:755–761.

Kaiser, J.-P., and J.-M. Bollag. 1993. Metabolism of pyridine and 3-hydroxypyridine under aerobic, denitrifying and sulfate-reducing conditions. Experientia 47:292–296.

Kaiser, J.-P., R. D. Minard, and J.-M. Bollag. 1993. Transformation of 3- and 4-picolinate under sulfate-reducing conditions. Appl. Environ. Microbiol. 59:791–795.

Khanna, M., and O. P. Shukla. 1981. Microbial metabolism of 3-hydroxypyridine. Indian J. Biochem. Biophys. 14:301–302.

Knezovich, J. P., D. J. Bishop, T. J. Kulp, D. Grieben-Galic, and J. Dewitt. 1990. Anaerobic microbial degradation of acrydine and the application of remote fiber spectroscopy to monitor the transformation process. Environ. Toxicol. Chem. 9:1235–1243.

Kohayashi, Y., and K. Arima. 1962. Bacterial oxidation of dipicolinic acid. II. Identification of α-ketoglutartic acid and 3-hydroxypicolinic acid and some properties of cell-free extracts. J. Bacteriol. 84:765–771.

Kolenbrander, P. E., N. Lotong, and J. C. Ensign. 1983. Isolation and characterization of a pyridine-degrading Desulfococcus niacinii sp. nov. Arch. Microbiol. 136:194–198.

Korenstevia, L. A., A. N. Kost, I. L. Voroboiv, L. V. Modyanova, P. B. Terent’ev, and N. S. Kulikov. 1981. Microbiological degradation of pyridine and 3-methylpyridine. Appl. Microbiol. Biochem. 110:239–245.

Kolenbrander, P. E., and M. Weinerberger. 1977. 2-Hydroxypyridine metabolism and pigment formation in three Arthrobacter species. J. Bacteriol. 131:725–729.

Kost, N. A., L. V. Voroboiv, P. B. Terent’ev, L. V. Modyanova, O. K. Shibilkinia, and L. A. Korenstevia. 1977. Microbiological transformation of 2,6-dimethylpyridine. Appl. Biochem. Microbiol. 13:541–546.

Kuhn, E. P., and J. M. Sultita. 1989. Microbial degradation of nitrogen, oxygen and sulfur heterocyclic compounds under anaerobic conditions. Studies with aquifer samples. Environ. Toxicol. Chem. 8:1149–1158.

Kung, H.-F., and L. Tsai. 1971. Nicotinic acid metabolism. VII. Mechanisms of action of 6-oxo-1,4,5,6-tetrahydroxy nicotinic acid mutase (H2-dependent) and methylitaconate isomerase. J. Biol. Chem. 246:6436–6443.

Kung, H.-F., L. Tsai, and T. C. Stadtman. 1971. Nicotinic acid metabolism. VIII. Tracer studies on the intermediary roles of α-methylene glutarate, methylitaconate, dimethylmaleate, and pyruvate. J. Biol. Chem. 246:6444–6451.

Lettau, H. 1980. Chemie der Heterocyclen. Deutscher Verlag für Grund-stoffindustrie, Leipzig, Germany.

Maeda, S., S. Kishida, and T. Kishita. 1978. Microbial degradation of nicotinic acid-2-oxide degradation products. Agric. Biol. Chem. 42:1455–1460.

Modyanova, L. V., L. I. Voroboiv, O. K. Shibilkinia, E. V. Dovgilevich, and P. B. Terent’ev. 1990. Microbiological transformations of nitrogen-containing heterocyclic compounds. I. Hydroxylation of isonicotinic methyl- and dimethylpyridines by certain microscopic fungi. Sov. Botanich. 32:24–27.

Naik, M. N., R. B. Jackson, J. Stokes, and R. J. Swaby. 1972. Microbial degradation and phytotoxicity of picloram and other substituted pyridines. Soil Biol. Biochem. 4:313–323.

Nyns, E. J., D. Zach, and E. E. Snell. 1969. The bacterial oxidation of vitamin B6. VIII. Enzymatic breakdown of α-(N-acetylaminomethylene)-succinic acid. J. Biol. Chem. 244:2601–2605.

Ohsugi, M., Y. Inoue, K. Takami, and M. Namikawa. 1981. Microbial degradation of N-picolinic acid, 2-pyridinocarboxylic acid. Agric. Biol. Chem. 45:1879–1880.

Orpin, C. G., M. Knight, and W. C. Evans. 1971. The bacterial oxidation of N-methylisonicotinic acid. Biochem. J. 122:58P.

Orpin, C. G., M. Knight, and W. C. Evans. 1972. The bacterial oxidation of N-methylisonicotinic acid, a photolytic product of paraquat. Biochem. J. 127:833–844.

Pasan, L. L. Tsai, and E. R. Stadtman. 1964. Nicotinic acid metabolism. I. Distribution of isotope in fermentation products of labeled nicotinic acid. J. Biol. Chem. 239:902–906.
