Microbial Transformation of Quinoline by a *Pseudomonas* sp.†

ONKAR P. SHUKLA

Division of Biochemistry, Central Drug Research Institute, Lucknow-226001, India

Received 11 March 1985/Accepted 22 November 1985

A *Pseudomonas* sp. isolated from sewage by enrichment culture on quinoline metabolized this substrate by a novel pathway involving 8-hydroxycoumarin. During early growth of the organism on quinoline, 2-hydroxyquinoline accumulated as the intermediate; 8-hydroxycoumarin accumulated as the major metabolite on further incubation. 2,8-Dihydroxyquinoline and 2,3-dihydroxyphenylpropionic acid were identified as the other intermediates. Inhibition of quinoline metabolism by 1 mM sodium arsenite led to the accumulation of pyruvate, whereas inhibition by 5 mM arsenite resulted in the accumulation of 2-hydroquinoline as the major metabolite and 2,8-dihydroxyquinoline as the minor metabolite. Coumarin was not utilized as a growth substrate by this bacterium, but quinoline-grown cells converted it to 2-hydroxyphenylpropionic acid, which was not further metabolized. Quinoline, 2-hydroxyquinoline, 8-hydroxycoumarin, and 2,3-dihydroxyphenylpropionic acid were rapidly oxidized by quinoline-adapted cells, whereas 2,8-dihydroxyquinoline was oxidized very slowly. Quinoline catabolism in this *Pseudomonas* sp. is therefore initiated by hydroxylation(s) of the molecule followed by cleavage of the pyridine ring to yield 8-hydroxycoumarin, which is further metabolized via 2,3-dihydroxyphenylpropionic acid.

Quinoline and its derivatives occur widely in coal tar, bone oil, oil shale (1, 21), and plant alkaloids (18) and serve as intermediates and solvents in the chemical industry (27). Quinoline derivatives have antimalarial, antiamoebic, antitumor, antifungal, antitubercular, and antiviral activities (27) and have also been shown to display toxic, mutagenic, and carcinogenic activities (27). Quinoline has been reported to serve as a source of nitrogen for oats and corn (15), probably owing to mineralization of nitrogen by microbial action (28). In recent years, quinoline has been found to be rapidly degraded in sewage (6), with a half-life of 2 h. A *Moraxella* sp. (17) and a *Pseudomonas* sp. (35) were isolated from soil by enrichment culture and were found to utilize quinoline as the sole source of carbon, nitrogen, and energy. The *Moraxella* sp. accumulated 2-hydroxyquinoline, and the involvement of 2,6-dihydroxyquinoline as intermediate was postulated owing to its oxidation by the quinoline-adapted cells. Naphthalene-grown cells of *Pseudomonas putida* have been reported to convert quinoline to o-aminophenyl-β-hydroxypropionic acid (22). Animal systems metabolize quinoline by hydroxylation and excretion of glucuronide and sulfate conjugates (42), and further ring cleavage has not been reported in animal systems. The degradation of the quinoline derivative kynurenic acid, an intermediate in tryptophan metabolism, occurs by dihydroxylation and meta-cleavage of the benzene ring (11, 38). The mode of degradation of quinoline itself in bacteria is still not known. The present paper reports the isolation of a *Pseudomonas* sp. which degrades quinoline and the characterization of 2-hydroxyquinoline, 2,8-dihydroxyquinoline, 8-hydroxycoumarin, and 2,3-dihydroxyphenylpropionic acid as intermediates of quinoline catabolism in this organism.

MATERIALS AND METHODS

Reagents and chemicals. Quinoline and 8-hydroxyquinoline were products of BDH, Poole, England; quinoline was further purified by distillation. The following compounds were synthesized by published methods: 2-hydroxyquinoline (7), 6-hydroxyquinoline (20), 2,8-dihydroxyquinoline (12), 8-hydroxycoumarin (8), phenylpropionic acid (40), melilotic acid, 2-hydroxyphenylpropionic acid (24), 2,3-dihydroxy-trans-cinnamic acid (5), and 2,3-dihydroxyphenylpropionic acid (4). Reagent-grade chemicals were used for the preparation of media and other biochemical studies.

| Cell-free broth | (i) Extract with ethyl acetate and (ii) acidify (phosphoric acid), saturate (NaCl), and extract with ethyl acetate |
| Ethyl acetate extract | 5% aqueous NaHCO3 |
| NaHCO3 extract | Ethyl acetate layer |
| Cool, acidify, and extract with ethyl acetate | 10% aqueous Na2CO3 |
| Na2CO3 extract | Ethyl acetate extract |
| Acidify and extract with ethyl acetate | 0.1 N aqueous H2SO4 |

FIG. 1. Fractionation of quinoline metabolites.

† Central Drug Research Institute communication no. 3680.
FIG. 2. Growth of Pseudomonas sp. strain QPS-1 in phosphate salts medium containing 0.03% quinoline, disappearance of quinoline, and accumulation of phenolic (expressed as 8-hydroxycoumarin) intermediates.

Media. The phosphate salts medium for the growth of the organism and fermentation contained (in grams per liter) Na₂HPO₄, 4.26; KH₂PO₄, 2.65; MgSO₄ · 7H₂O, 0.2; MnSO₄ · H₂O, 0.02; FeSO₄ · 7H₂O, 0.05; CaCl₂, 0.02; and sodium molybdate, 0.001. The salts were dissolved separately (at a concentration 100 times that required in the medium), the pH was adjusted to 3 with 6 N H₂SO₄, and the solutions were filter sterilized and added to the medium just before use. Ammonium sulfate (1 g/liter) was added to this medium when growth on nonnitrogenous substrates was tested. The media were sterilized at a 15-lb/in² pressure of steam for 20 min.

Isolation of quinoline-degrading bacteria. Quinoline-degrading bacteria were isolated from sewage by enrichment culture in phosphate salts medium (30) of the above composition containing 0.03% quinoline. A 5-ml portion of sewage supernatant was inoculated in 100 ml of medium, and flasks were incubated on a rotary shaker at 30°C and 220 strokes per min (Emenevee Engineers, Poona, India). After 1 week of incubation, portions were inoculated into fresh medium, and incubation was continued. Two more transfers at 48-h intervals revealed bacterial growth accompanied by a red-brown coloration in the medium. The cultures were purified by being streaked on nutrient agar plates, followed by transfer of selected colonies on phosphate salt agar slants solidified by addition of 2% agar; quinoline was supplied by addition of 1 drop to the lower portion of cotton plugs. The organism was characterized by using Bergey's Manual of Determinative Bacteriology (13), and tests for characterization were done by the method of Stanier et al. (37). The organism was stained by the method of Conn et al. (9), and flagella were stained by the method of Leifson (23).

Isolation and characterization of transformation products. Erlenmeyer flasks (1 liter) containing 300 ml of phosphate salts medium and 0.03% quinoline were inoculated with a 5% inoculum of the pure culture organism grown in medium of the same composition. The flasks were incubated at 28 to 30°C with shaking. At different periods of growth, fermentation broths were separated from the cells by centrifugation, and the formation of metabolites was monitored by following the changes in the UV spectrum between 200 and 400 nm on a Beckman model S24 spectrophotometer; pheno-
nolic compounds were estimated by using Folin phenol reagent (36). The metabolites were extracted from the broth with ethyl acetate and analyzed by thin-layer chromatography (TLC) on silica gel G plates with hexane-ethyl acetate (1:1, vol/vol) (solvent A) and hexane-ethyl formate-propionic acid (26:14:3, vol/vol/vol) (solvent B) (29) as the solvents. Paper chromatography was done on Whatman no. 1 paper with 5% sodium formate-formic acid (200:1, vol/vol) (solvent C). Compounds were detected under UV light or after being sprayed with Folin phenol reagent followed by 10% aqueous sodium carbonate. High-pressure liquid chromatography was performed on a Waters Associates Liquid Chromatograph with a model 450 variable wavelength detector, model 6000A pump, and μBondapak phenyl column. The fractionation scheme outlined in Fig. 1 was adopted to achieve separation of acidic, phenolic, basic, and neutral metabolites. Extraction with other solvents including butanol or alternative fractionation schemes did not reveal additional metabolites. The metabolites were purified by preparative TLC, and separated components were eluted from developed plates with ethyl acetate, concentrated in vacuo to dryness, and crystallized. The metabolites were characterized by their UV, infrared (IR), mass, and nuclear magnetic resonance (NMR) spectra and by liquid chromatography. UV spectra were recorded on a Hitachi model 320
UV/visible spectrophotometer, IR spectra were recorded on a model 157 Perkin Elmer Infracord, NMR spectra were recorded on a Varian EM360 spectrometer (60 MHz) or Perkin Elmer R 32 spectrometer (90 MHz), and mass spectra were recorded on a JEOL model JMS-D300 spectrometer.

**Other analytical methods.** For manometric studies (39) or in vitro experiments with resting cell suspensions, cells were harvested during the logarithmic phase of growth by centrifugation at 10,000 × g for 10 min, washed with cold 0.05 M phosphate buffer (pH 7.0), and used immediately. Keto acids were estimated by the method of Friedman and Haugen (14) and characterized by TLC in solvent B (19, 33). Other analytical methods and sources of chemicals have been described previously (19, 30, 33).

**RESULTS**

Three bacterial strains degrading quinoline were isolated by using the enrichment culture described previously (31). One of the strains (QPS-2) produced a diffusible pink pigment (λ_max = 490 nm) in the liquid medium as well as in the agar slants and was identical to the culture isolated earlier by enrichment from soil (35); this strain produced only traces of phenol during quinoline degradation. The two other strains (QPS-1 and QPS-4) secreted brown diffusible pigments in the medium and released large amounts of phenolic metabolites during quinoline catabolism. Strain QPS-1 was selected for further investigations, which are reported below. The organism has been identified as a *Pseudomonas* sp. on the basis of the following characteristics: gram-negative rods (1 μm by 2 to 3 μm); motile by a single polar flagellum; aerobic, catalase and oxidase positive; accumulates poly-β-hydroxybutyrate; fluorescent pigments not produced; growth on arginine and betaine, arginine dihydrolase present; optimum growth temperature, 30 to 37°C, with growth at 41°C also; nitrate reduced to nitrite; starch and gelatin not hydrolyzed; glucose, fructose, sucrose, maltose, lactose, mannitol, sorbitol, ribose, arabinose, and xylose not utilized as growth substrates. However, several organic acids, i.e., acetate, succinate, fumarate, malate, lactate, β-hydroxybutyrate, etc., supported growth. The organism appears to be a strain of *P. pseudoalcaligenes* (13), except that it reduced nitrates to nitrite.

Quinoline served as the sole source of carbon, nitrogen, and energy for this organism. Growth of the organism occurred rapidly at 0.01 to 0.015% quinoline; growth occurred with a lag at 0.025 to 0.03% quinoline. Higher concentrations were toxic and completely abolished growth. The organism was rather selective in its choice of heterocyclic growth substrates, and pyridine, α-, β-, and γ-picolinines, isoquinoline, lepidine, and quinaldine were not utilized for growth. Among nonnitrogenous aromatic compounds, *p*-hydroxybenzoate and phenylacetate were utilized, while

**FIG. 7.** 1H NMR spectra of 2-hydroxyquinoline (CDCl₃) on a Varian Model EM 360 instrument.
benzoate, salicylate, phthalate, cinnamate, phenylpropionate, and coumarin were not utilized as growth substrates.

The growth of Pseudomonas sp. strain QPS-1 in quinoline media was preceded by a lag of 4 to 6 h and reached a maximum around 22 to 24 h; quinoline disappeared rapidly after the initial lag. Maximum accumulation of a metabolite with a $\lambda_{max}$ at 328 nm was noticed around 12 h, while maximum accumulation of phenolic compounds occurred around 16 h (Fig. 2). Characteristic changes in the UV spectra of fermentation broths were noticed at different periods of incubation (Fig. 3), indicating the formation of distinct quinoline metabolites. Further incubation resulted in the complete disappearance of all the metabolites. No accumulation of 2,6-dihydroxyquinoline ($\lambda_{max}$, 356 nm) was noticed at any stage of fermentation. When the cells of strain QPS-1 were inoculated into 0.03% quinoline-containing medium in Roux bottles and incubated with occasional shaking, growth proceeded slowly, reaching a maximum around 40 to 44 h. The initial phase of growth (20 h) resulted in the accumulation of 2-hydroxyquinoline, while maximum accumulation of phenols was detected around 30 to 32 h (data not shown). Fermentation of quinoline under these semiaerobic conditions resulted in less decomposition and better recovery of metabolites; these conditions were therefore used for accumulation, isolation, and characterization of transformation products.

Extraction of broths with ethyl acetate and TLC of products in solvent B revealed the accumulation of four metab-
olites with $R_f$ values of 0.50, 0.51, 0.36, and 0.27. The metabolite with an $R_f$ of 0.5 gave UV fluorescence but a negative color reaction with phenol reagent, while the other three compounds gave a positive color reaction with phenol reagent. The formation of only four metabolites was also detected by high-pressure liquid chromatography (Fig. 4). The fermentation products were extracted with ethyl acetate, fractionated as shown in Fig. 1, further purified by preparative TLC, crystallized, and examined spectroscopically.

2-Hydroxyquinoline. The neutral fraction from fermentation products contained only one component ($R_f$, 0.5). Crystallization of this metabolite from hot water gave long, colorless needles with a melting point of 199 to 200°C (mixed melting point, 198 to 200°C) and UV $\lambda_{max}$ of 230, 269 (logs, 3.88), and 328 nm (logs, 3.84). The compound analyzed for C$_9$H$_7$NO. Found: C, 74.00; H, 5.00; N, 10.07. Theoretical: C, 74.48; H, 4.82; N, 9.65. This indicated the incorporation of one oxygen atom in the quinoline molecule. The IR spectrum had bands at 2,600 to 3,200 cm$^{-1}$ ($\nu$N-H), 1,640 ($\nu$C=O), 1,600, and 1,430 cm$^{-1}$ (Fig. 5). The mass spectrum had prominent peaks at $m$/z 145, 100% ($M^+$); 117, 38% (M

![FIG. 11. NMR spectra of 8-hydroxycoumarin (CDCl$_3$) on a 90-MHz instrument.](image)

![FIG. 12. UV spectra of 2,8-dihydroxyquinoline.](image)

![FIG. 13. Mass spectra of 2,8-dihydroxyquinoline.](image)
The compound occasionally showed a molecular ion at 144 (M - 1) or 146 (M + 1), but other ions were identical. The NMR spectrum had proton signals at δ 7.75 (d, 1H, H-3), 6.65 (d, 1H, H-4), and 7 to 7.5 (m, 4H, H-5,6,7,8) (Fig. 7). The NMR spectrum of quinoline has a proton signal at δ 8.8 corresponding to H-2, but this was absent in this metabolite, suggesting that it is 2-hydroxyquinoline (2-quinolinone). Other properties of the metabolite were identical to those of 2-hydroxyquinoline.

The presence of the O-H band in the IR spectrum and the negative color reaction with phenol reagent also indicate the presence of the oxygen atom as the keto group. The IR, NMR, and mass spectra of the metabolite were identical with those of 2-hydroxyquinoline (Fig. 5 through 7).

8-Hydroxycoumarin. 8-Hydroxycoumarin was purified from the phenolic fraction by preparative TLC (Rf, 0.51; solvent B) and gave a green-blue color on TLC plates with phenol reagent. Crystallization of the product from hot water yielded white needles with a melting point of 159 to 161°C (mixed melting point, 158 to 160°C). The compound analyzed for C₉H₆O₃. Found: C, 66.5; H, 3.62; N, absent. Theoretical, C, 66.66; H, 3.70. UV λmax, 255 (loge, 3.93) and 290 nm (loge, 4.08). In the presence of alkali, a major peak at 272 nm and minor peaks at 234 and 308 nm were detected (Fig. 8). The IR spectrum had bands at 3,480 cm⁻¹ (O-H), 1,700 to 1,720 (lactone), 1,610, 1,580, 1,462, and 1,200 cm⁻¹ (Fig. 9). The mass spectrum had a prominent molecular ion at m/z 162, 100% (M⁺) and other peaks at 134, 77% (M - 28); 105,
MICROBIAL TRANSFORMATION OF QUINOLINE

15%; and 78, 43% (Fig. 10). The NMR spectrum had proton signals corresponding to the coumarin nucleus at δ 7.7 (d, 1H, H-3), 6.25 (d, 1H, H-4), and 6.8 to 7.2 (m, 3H, H-5,6,7) (Fig. 11). These data indicate that the metabolite may be a hydroxycoumarin. 8-Hydroxycoumarin has been reported to have a melting point of 160°C (8), and the UV, IR, NMR, and mass spectra of the metabolite were identical to those of 8-hydroxycoumarin (Fig. 8 through 11).

2,8-Dihydroxyquinoline. 2,8-Dihydroxyquinoline constituted the minor component of the phenolic fraction (Rf, 0.27; solvent B). It was purified by preparative TLC in solvents B and A. The compound gave a blue color on TLC plates with phenol reagent. An amorphous yellow powder was obtained on attempted crystallization from hot water. The product had a melting point of >260°C with decomposition (the melting point was undepressed when the product was mixed with an authentic sample). UV λ\text{max}, 258 nm (logε, 4.315); other peaks, 286 (logε, 3.83) and 230 nm (Fig. 12). On
addition of alkali the major peak shifted to 273 nm, while the intensity of the other bands decreased and a new shoulder appeared at 235 nm. The mass spectrum had peaks at m/z 161, 100% (M+); 133, 18.75% (M−28); 115, 11% (Fig. 13). The IR spectrum had broad absorption bands at 2,600 to 3,500 cm⁻¹ (O−H, N−H) and at 1,640 (C=O), 1,600, and 1,285 cm⁻¹ (Fig. 14). The metabolite and authentic 2,8-dihydroxyquinoline had the same mobility on TLC and identical UV, IR, and mass spectra (Fig. 12 through 14).

2,3-Dihydroxyphenylpropionic acid. TLC of the acidic fraction in solvent B revealed the presence of only one major component (Rf, 0.36), which gave a deep-blue color on being sprayed with phenol reagent. The metabolite was unstable and underwent oxidation on the plates in the presence of air, yielding a blue-green color. Very little of this metabolite was detected in fermentation flasks incubated with shaking, but fermentation in Roux bottles gave a better yield. For recovery of undegraded metabolite, the TLC plates were dried in vacuo and the band corresponding to the compound was quickly eluted and concentrated in vacuo to yield an oily material that solidified on cooling in a vacuum desiccator. The dried product had a melting point of 123 to 124°C (mixed melting point, 123 to 128°C, in agreement with the reported value of 125 to 127°C [4]). The compound analyzed for C₁₀H₁₁O₂. Found: C, 58.6; H, 5.56. Theoretical: C, 59.34; H, 5.5. UV λmax, 277 nm. The IR spectrum had a broad absorption band around 2,600 to 3,500 cm⁻¹ (O−H, COO−H), 1,720, and 1,690 (C=O) (Fig. 15). The mass spectrum (Fig. 16) gave fragments at m/z 182, 23% (M⁺); 164, 55% (M−18); 136, 57%; and 122, 100%. In some batches the fragment of m/z 182 was absent, but other fragments were identical. The UV, IR, and mass spectra of the metabolite were identical to those of the authentic 2,3-dihydroxyphenylpropionic acid (Fig. 15 and 16). The mobility of another likely metabolite, 2,3-dihydroxyxycinnamic acid, in TLC in solvent B was the same as for this metabolite, but the two compounds resolved on paper chromatography in solvent C. Dihydroxyxycinnamic acid had a different UV spectrum, with λmax of 223 and 287 nm. Paper chromatography of the total acidic fraction from quinoline fermentation revealed no spots corresponding to 2,3-dihydroxyxycinnamic acid.

Accumulation of quinoline metabolites in the presence of sodium arsenite. Inhibition of quinoline metabolism by resting cells in the presence of 1 mM sodium arsenite led to the accumulation of keto acids. The keto acids were converted to their dinitrophenylhydrazones and separated by TLC. On purification the major fraction (Rf, 0.31; solvent B) yielded a product with a melting point of 210 to 212°C, and the IR spectrum of the compound was superimposable on that of authentic pyruvate dinitrophenylhydrazone. Inhibition of quinoline metabolism by 5 mM arsenite yielded 2-hydroxyquinoline as the major product and 2,8-dihydroxyquinoline as the minor metabolite; other metabolites were not detected.

Microbial conversion of coumarin. Coumarin was not utilized as a growth substrate by this bacterium, but incubation of coumarin with quinoline-grown cells resulted in the formation of an acidic metabolite with a melting point of 82 to 83°C and UV λmax of 273 and 220 nm, identical to melilotic acid. The IR spectrum had bands at 3,400 (O−H), 2,500 to 3,000 (COO−H), 1,690 (C=O), 1,610, 1,450, and 1,320 cm⁻¹ (Fig. 17). The mass spectrum had prominent fragments at m/z 166, 33% (M⁺); 148, 64% (M−H₂O); and 120, 100% (loss of CO, H₂O) (Fig. 18). The IR and mass spectra of the product were identical with those of melilotic acid (Fig. 17 and 18).

FIG. 19. Oxidation of substrates and intermediates by quinoline-grown cells of Pseudomonas sp. strain QPS-1. Warburg flasks (capacity, 15 ml) contained (in a final volume of 2.8 ml) phosphate buffer, 100 μmol; cells, 2 mg; and substrates, 5 μmol. KOH (20%; 0.2 ml) was added in the central well, and flasks were incubated with shaking at 37°C. Symbols: O, quinoline; ●, 2-hydroxyquinoline; △, 8-hydroxycoumarin; ▲, 2,8-dihydroxyquinoline; □, 2,3-dihydroxyphenylpropionic acid; ●, endogenous, 8-hydroxyquinoline; 6-hydroxyquinoline.

FIG. 20. Pathway for the degradation of quinoline in Pseudomonas sp. I, Quinoline; II, 2-hydroxyquinoline; III, 2,8-dihydroxyquinoline; IV, 8-hydroxycoumarin; V, 2,3-dihydroxyphenylpropionic acid; VI, coumarin; VII, melilotic acid.
Oxidation of substrates and intermediates. Quinoline was rapidly oxidized by quinoline-adapted cells of *Pseudomonas* sp., but succinate-grown cells failed to oxidize quinoline. 2-Hydroxyquinoline, 8-hydroxycoumarin, and 2,3-dihydroxyphenylpropionic acid were also rapidly oxidized by quinoline-adapted cells; 2,8-dihydroxyquinoline was oxidized very slowly (Fig. 19). 8-Hydroxyquinoline and 6-hydroxyquinoline were not oxidized.

Incubation of quinoline, 2-hydroxyquinoline, and 8-hydroxycoumarin with quinoline-grown cells resulted in the complete disappearance of these substrates; 2,8-dihydroxyquinoline is insoluble and yielded a suspension when added to the incubation mixtures. Incomplete recovery (75%) of 2,8-dihydroxyquinoline was obtained at the end of the incubation.

DISCUSSION

Benzenoid derivatives are generally degraded via introduction of two hydroxyl groups either ortho or para to each other, followed by oxidative cleavage of the resulting diol to yield cis,cis-muconic acid or muconic semialdehyde derivatives as the intermediates (10, 16). Hydroxypyridines and pyridine carboxylic acid are also degraded via di- or trihydroxy pyridines as intermediates (32). Pyridine, α-picoline, N-methylisonicotinate, and isonicotinate use alternative pathways in which the ring is probably reduced to the di- or tetrahydropyridine state followed by oxidative cleavage to acyclic intermediates which, on hydrolysis, generate succinic semialdehyde as a key intermediate (30, 32, 34, 41); glutaric semialdehyde has been detected as the intermediate in *Nocardia* sp. strain Z1, suggesting that hydrolytic cleavage of the partially reduced pyridine ring has occurred (41).

The piperidine ring differs from the benzene ring in having a lone pair of electrons on the nitrogen, which causes the ring to assume a positive charge, making it less susceptible to electrophilic attack (3). Pyridine is thus used as the solvent for the oxidation of other organic compounds with CrO₃ (26). The pyridine ring of the quinoline molecule is also less susceptible to oxidative cleavage (25). The quinoline ring in kynurenine has been shown to be degraded via the 7,8-diol, suggesting the preferential cleavage of the benzene ring (11, 38). However, the mechanism of cleavage of the ring in quinoline per se in bacteria is not known. Grant and Al-Najjar (17) isolated and characterized 2-hydroxyquinoline as a major metabolic product of quinoline in *Moraxella* sp. They implicated 2,6-dihydroxyquinoline as an intermediate on the basis of its oxidation by quinoline-adapted cells. Degradation of kynurenic acid (4-hydroxyquinoline) in *Pseudomonas* sp. was reported to proceed via 4,7,8-trihydroxyquinoline, and a putative pink cleavage product was postulated to arise from meta cleavage of the triol (2). Degradation of quinoline by a *Pseudomonas* sp. (QPS-2) and a *Nocardia* sp. proceeded via 2-hydroxyquinoline and 2,6-dihydroxyquinoline (31). However the details of these pathways are still not known.

The results presented in this paper indicate the existence of an alternative pathway for quinoline catabolism in microbes (Fig. 20); 2-hydroxyquinoline is the first intermediate of this pathway also. However, 2,8-dihydroxyquinoline, 8-hydroxycoumarin, and 2,3-dihydroxyphenylpropionic acid have been characterized as the other intermediates of this pathway. 2-Hydroxyquinoline and 2,8-dihydroxyquinoline accumulated as the exclusive products when quinoline was metabolized in the presence of 5 mM arsenite. Quinolinogrown cells rapidly oxidized 2-hydroxyquinoline, 8-hydroxycoumarin, and 2,3-dihydroxyphenylpropionic acid, supporting their role as intermediates; 2,8-dihydroxyquinoline was oxidized very slowly with overall consumption of only 0.5 mol of oxygen per mol of substrate. It also did not disappear significantly when incubated with resting cells for 24 h or more, suggesting that it may not be a true intermediate; no formation of 8-hydroxycoumarin was detected under these incubation conditions. 2,8-Dihydroxyquinoline is, however, highly insoluble and exists in suspension when added to the cells. The solubility and permeability of the compound may be limiting factor(s) in demonstrating its role as an intermediate of quinoline catabolism.

8-Hydroxycoumarin is the intermediate that has been isolated and characterized rigorously by spectroscopic methods as well as comparison with chemically synthesized substrate. A metabolite with the spectroscopic characteristics of 8-hydroxycoumarin also accumulated in broths during fermentation (16 h) (Fig. 3). It can be argued that the true intermediate of quinoline catabolism may be cis,3,4-dihydroxycinnamic acid which cyclizes under acidic conditions of processing of broths and extraction with ethyl acetate. However, extraction of broth with acidic acidification and concentration without application of heat also yielded 8-hydroxycoumarin. Incubation of 8-hydroxycoumarin with resting cells led to its complete disappearance, while the parent compound, coumarin, was not degraded beyond mellitolic acid. The reactions of quinoline metabolism seem to be very specific, and hydroxylation of coumarin to 8-hydroxy derivative does not occur in this bacterium. Small quantities of 2,3-dihydroxyphenylpropionic acid were detected during aerobic fermentation of quinoline, but larger amounts accumulated in semi-aerobic fermentation in Roux bottles. This compound was oxidized by quinoline-adapted cells. Closely related 2,3-dihydroxycinnamic acid was not detected spectroscopically and was not oxidized by the quinoline-adapted cells ruling its participation. The present study therefore establishes 2-hydroxyquinoline, 8-hydroxycoumarin, and 2,3-dihydroxyphenylpropionic acid as intermediates of quinoline catabolism in this organism.

LITERATURE CITED

10. Dagley, S. 1971. Catabolism of aromatic compounds by micro-
15. Funchess, M. J. 1917. The nitrification of pyridine, quinoline, guanidine carbonate etc. in soils, p. 65–82. In Alabama Agricultural Experiment Station Bulletin no. 196 (Technical Bulletin no. 3).