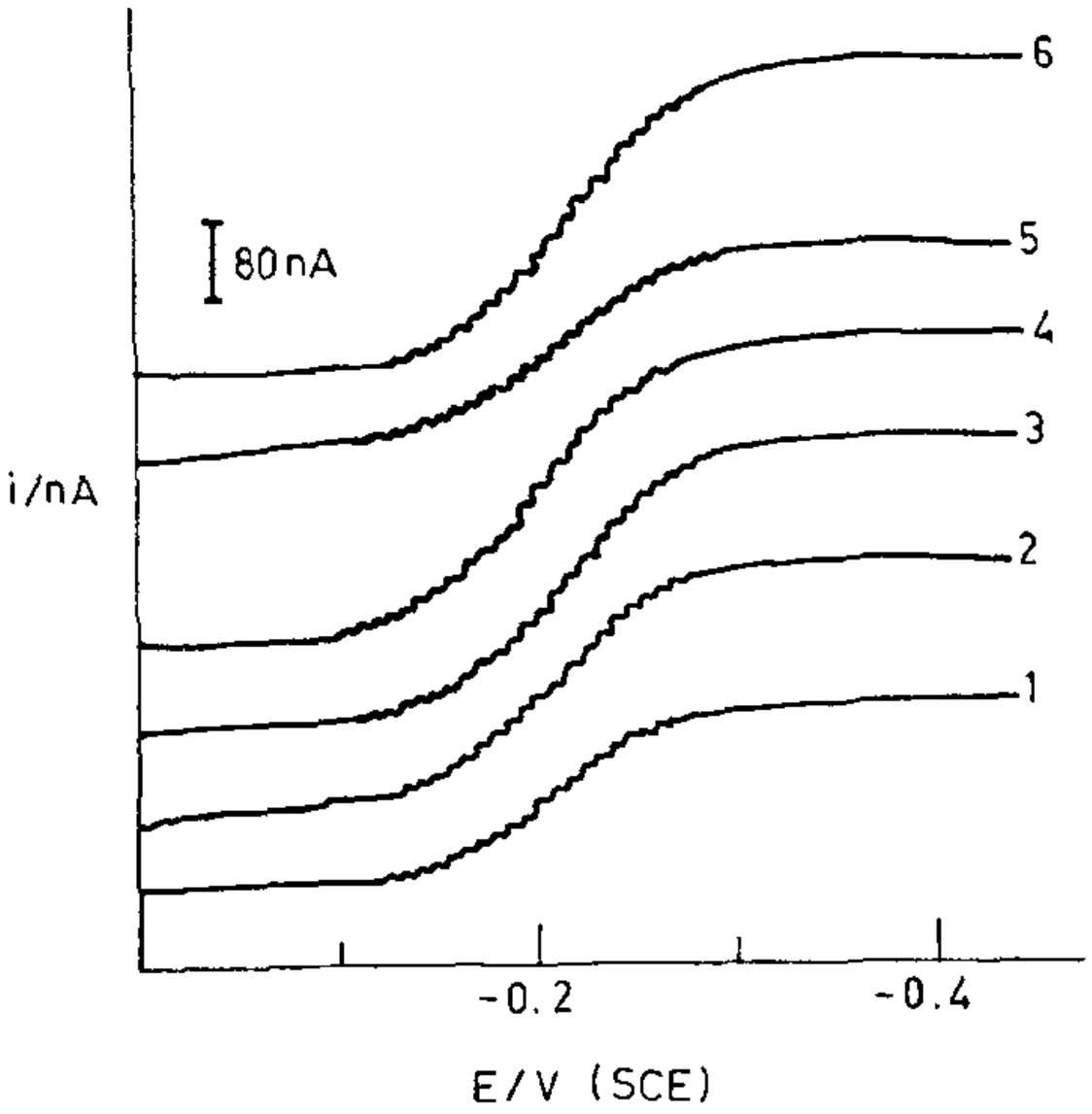

Figure 1. Polarograms of backstripped molybdenum equivalent to (1) 1.9 μg/l silicon; (2) 2.2 μg/l phosphorus; (3) 19.8 μg/l cerium; (4) 100 μg/l boron; (5) 14 μg/l thorium; (6) 60 μg/l nitrogen (as ammonium ion)

ation of trace amounts of silicon, phosphorus, cerium, boron, thorium and nitrogen obtained by the method outlined above. Incidentally, this method is all the more significant for the estimation of these six elements because in these cases not only their sensitivities are amplified by several folds but also this happens to be the only satisfactory polarographic method available for their estimation. The use of the charging current compensated dc (ccc.dc) polarograph[5] further improves the detection limit by effectively removing the dc charging current.

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8-HYDROXYCOUMARIN: AN INTERMEDIATE IN THE MICROBIAL TRANSFORMATION OF QUINOLINE

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QUINOLINE and related heterocyclic compounds occur widely in nature[1] and are extensively used as solvents, intermediates, reagents and drugs[2, 3]; they display toxic[4], carcinogenic and mutagenic activity[5–7]. Rapid degradation of quinoline in sewage has been reported[8], and soil enrichments for quinoline-degrading bacteria have yielded a *Moraxella sp*[9] and a *Pseudomonas sp*[10]. The cleavage of quinoline ring in kynurenic acid, an intermediate of tryptophan catabolism, occurs *via* 7,8-dihydroxykynurenic acid and fission of the benzene ring[11, 12]; a similar sequence is likely for kynurine[13]. However, the microbial metabolism of quinoline molecule itself is not understood. 2-hydroxyquinoline was reported as an intermediate in *Moraxella sp*[9] but further metabolites were not detected. Microbial transformation of quinoline by naphthalene-adapted *Pseudomonas putida* yielded o-aminophenyl-β-hydroxyphenylpropionic acid[14]. The present paper reports the isolation and characterization of 8-hydroxycoumarin as an important metabolite of quinoline in a *Pseudomonas sp* isolated from sewage.

The quinoline-degrading bacterium was isolated from sewage by enrichment in phosphate salts medium[15] containing 0.03% quinoline. The organism was purified by streaking on nutrient-agar plates and phosphate agar-plates exposed to quinoline. The bacterium is gram-negative rod (1 μm × 2–3 μm), motile by a single polar flagellum; aerobic, catalase and oxidase positive; starch and gelatin not hydrolysed; sugars are not utilised as growth substrates; fluorescent pigments not produced and naphthalene not catabolised. The organism has been designated as *Pseudomonas sp*[16] but differs from *P. putida*[14], and the strain isolated earlier in this laboratory[10].

The organism was grown in 1 litre Erlenmeyer flasks containing 300 ml of phosphate salts medium and 0.03% quinoline, with shaking at 30° C. The formation of metabolites was monitored by recording the UV spectrum of cell-free fermentation broths, as well as using phenol reagent to detect phenolic intermediates. Transformation products were isolated from broth by saturation with sodium chloride and extraction with ethyl acetate. After concentration of the extract *in vacuo*, the products were analysed by TLC on silica gel

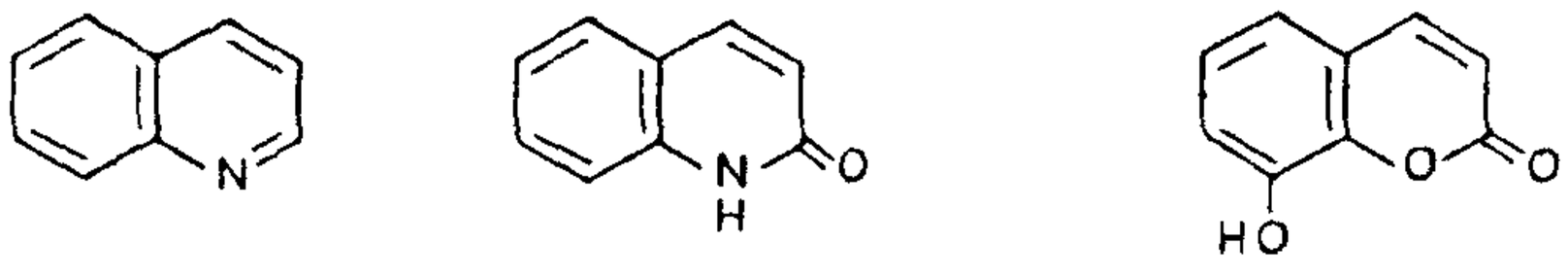

Figure 1. Quinoline and its metabolites in *Pseudomonas* sp.

G using petroleum ether, ethylformate, propionic acid (26:14:3) as the solvent, and detected by UV and phenol reagent. Individual components were separated by *preparative TLC, eluted with ethylacetate*, concentrated to dryness, and crystallised from hot water. The purified metabolites were characterised by UV, IR, NMR, mass spectra and comparison with authentic samples. 8-Hydroxycoumarin was synthesized using the method of Cingolani[17].

The fermentation of quinoline by this bacterium resulted in gradual disappearance of characteristic peak of quinoline at 306 nm and formation of a new compound with λ_{max} 328 nm; the maximum accumulation of this metabolite occurred after 24 hr fermentation. Extraction of broth and purification yielded a compound that crystallised as long colourless needles, mp 199–200° C. λ_{max} 328 nm (log ϵ, 3.8) and 269 nm (log ϵ, 3.88). The compound analysed for C_9H_7ON, (analysis %, C, 74.0; H, 5; N, 10.07). The mp and mmp, infrared and ultraviolet spectra of the compound were identical to 2-hydroxyquinoline (figure 1), thereby establishing it to be the first metabolite, of quinoline catabolism in *Pseudomonas sp.* 2-Hydroxyquinoline does not give positive colour with phenol reagent. However, a weak phenol test was given by the fermentation broths of quinoline in the early stages, and the maximum accumulation of phenolic intermediates was detected around 36 hr. Ultraviolet spectrum of the

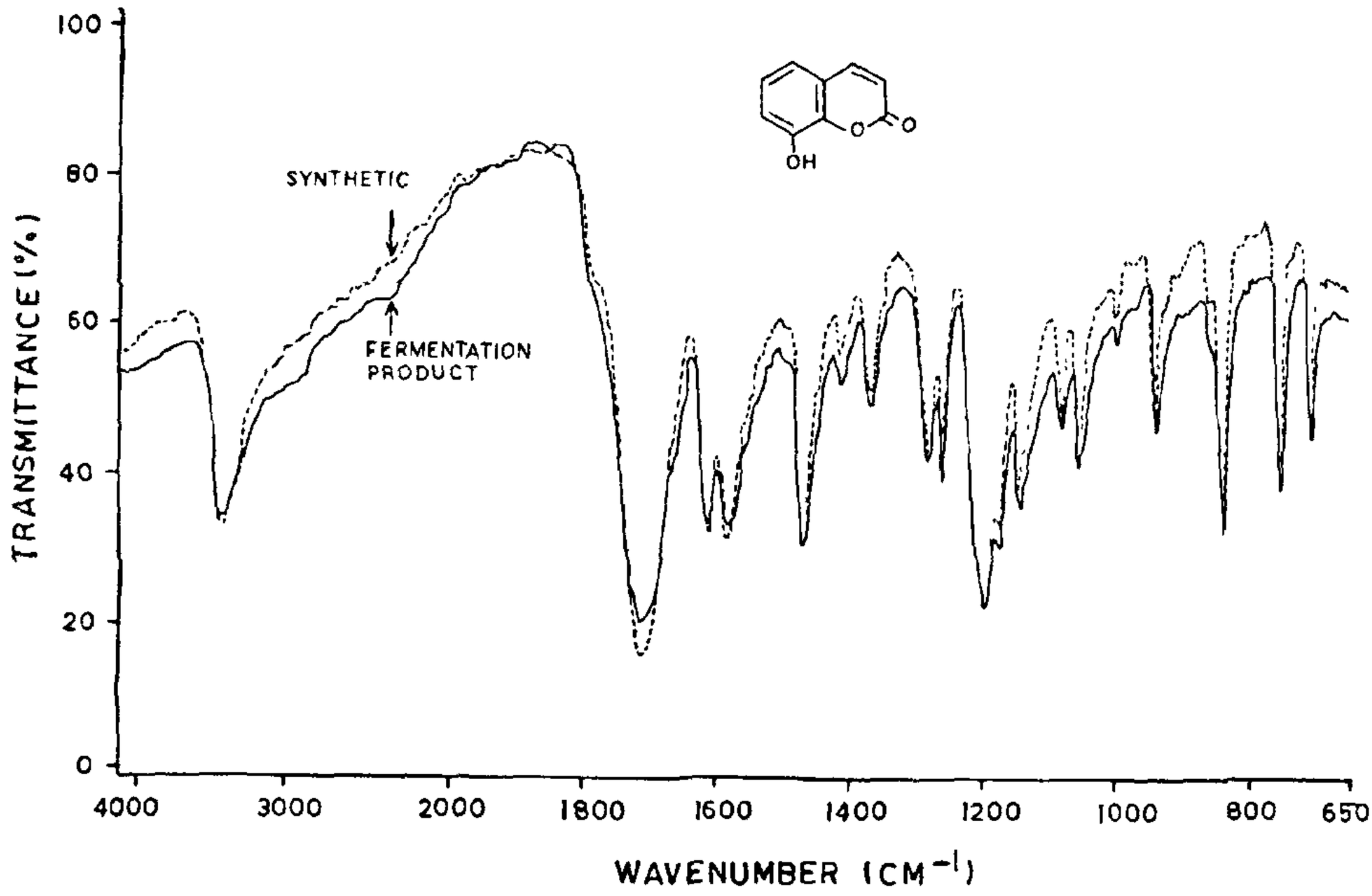

Figure 2. Infrared spectra of authentic 8-hydroxycoumarin and the fermentation product.

broth did not have absorption maxima around 357 nm corresponding to 2,6-dihydroxyquinoline[18]. The phenolic metabolite was purified by preparative TLC and crystallised from water to yield colourless needles, mp 159–161°C, UV. λ_{max} at 290 (log ∈ 4.08) and 255 nm (log ∈, 3.82). The compound analysed for $C_9H_6O_3$ (analysis C, 66.4; H, 4.8%; nitrogen, absent). The mass spectrum had characteristic peaks at m/e 162 (M^+, 43%), 134 (M-28, 33.69%), 105, 78. The NMR spectrum had proton signals at τ2.3 (1H, doublet, ζ, 5 cps); τ3.7 (1H, doublet, $\zeta = 5$ cps), and τ3.0 (3H, multiplet). The infrared spectrum of the metabolite had bands at 3380, 1715, 1200 cm^{-1}, and was superimposable with the IR spectrum of authentic 8-hydroxycoumarin (figure 2), thereby establishing the formation of 8-hydroxycoumarin during microbial transformation of quinoline in this bacterium. Both 2-hydroxyquinoline and 8-hydroxycumarin were further metabolised and disappeared from the broth on continued incubation: These two metabolites were also oxidised readily by quinoline-adapted cells, further supporting their intermediary role. Formation of 8-hydroxycoumarin during quinoline catabolism in this bacterium indicates a preferential cleavage of pyridine, rather than the benzene ring of the quinoline molecule.

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LATENT PERIOD FOR DYSPLASIA AND CARCINOMA OF CERVIX IN MICE DURING CHEMICAL CARCINOGENESIS

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THE influence of exogenous hormonal stress on prenatal, neonatal and adult stage for induction of cervical cancer in animals is well documented[1–3]. Since ovarian hormones play an important role in the development of cancer cervix[4], the effects of these hormones need be well elucidated. An attempt has therefore been made to investigate the latent period for dysplasia and carcinoma of cervix in virgin female mice using two techniques of chemical carcinogenic stress.

This study is concerned with 460 virgin Swiss female mice of 6–8 weeks age. They were divided into two groups. In group I, the animals remained intact with ovary whereas, in group II, the animals were subjected to extirpation of ovary. Chemical induction of cervical tumour was carried out using local application of 20-methylcholanthrene dissolved in 1% acetone and thread impregnation method of Murphy[5] in a ratio of