Metabolism of Bicyclic Aza-arenes by *Pseudomonas putida* to Yield Vicinal *cis*-Dihydrodiols and Phenols

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Metabolism of the aza-arenes quinoline, isoquinoline, quinazoline, and quinoxaline by a mutant strain of the bacterium *Pseudomonas putida* resulted in attack at the carbocyclic ring (to yield stable *cis*-dihydrodiols and phenols) and at the heterocyclic ring (to yield phenols and ring cleavage products).

The metabolism of polycyclic aromatic hydrocarbons (PAHs) (**A**) in prokaryotic organisms (bacteria) has been shown to yield *cis*-dihydrodiols (**B**) as initial metabolites[1] whereas their metabolism in eukaryotic organisms (fungi, plants, and animals) was found to yield arene oxides (**C**) initially.[2] Members of the azapolycyclic aromatic hydrocarbon (APAH) series often appear to be more carcinogenic than their PAH analogues,[3] and thus detailed metabolism studies of the most abundant environmental aza-arene carcinogen, quinoline (**2A**), which forms *trans*-dihydrodiol (**D**) and arene oxide (**C**) intermediates in mammalian systems, have recently been undertaken.[4] The latter arene oxide metabolite proved to be a remarkably stable compound owing to the proximity of the fused heterocyclic ring. In general, arene oxide metabolites are aromatized spontaneously to yield phenols (**E**) or hydrated enzymatically to yield *trans*-dihydrodiols (**D**).

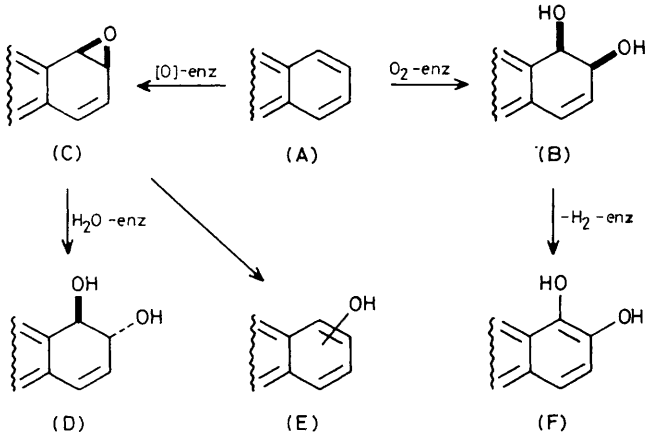
Despite the prevalence of APAHs in the environment their bacterial metabolism has not been investigated. Here a mutant strain of *Pseudomonas putida* (lacking the enzyme *cis*-glycol dehydrogenase),[5,6] which has been found to yield the *cis*-dihydrodiol metabolite of naphthalene (**1B**) exclusively in high yield,[7] has been used in biotransformations of the azanaphthalenes quinoline (**2A**), isoquinoline (**3A**), quinazoline (**4A**), and quinoxaline (**5A**).

Complete metabolism of the aza-arenes (**2A**—**5A**) yielded the corresponding *cis*-dihydrodiols (**2B**—**5B**) which were

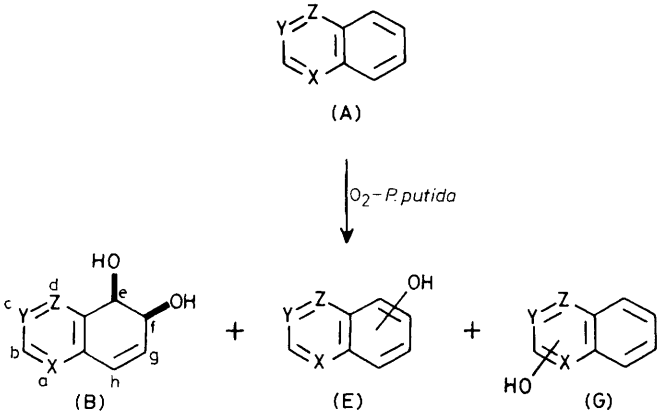
Table 1. Optical rotations, n.m.r. data, and yields of metabolites.

Substrate	*cis*-Dihydrodiol metabolites	Yield/%†	$[\alpha]_D$	$J_{e,f}$/Hz	n.O.e.	Phenol metabolites (% yield)†
(**2A**)	(**2B**)	33	+220°[a]	4.50	$H_d \longleftrightarrow H_e$	3-OH (13), 8-OH (27)
(**3A**)	(**3B**)	47	−2.5°[b]	4.90	$H_d \longleftrightarrow H_e$ $H_a \longleftrightarrow H_h$	5-OH(45)
(**4A**)	(**4B**)	55	−40°[b]	4.97	$H_d \longleftrightarrow H_e$	4-OH (10)
(**5A**)	(**5B**)	45[d]	+210°[c]	4.60		2-OH (20), 5-OH (40)

[a] Tetrahydrofuran (THF) solvent; [b] THF–MeOH solvent; [c] MeOH solvent; [d] Only one *cis*-dihydrodiol possible.



Scheme 1



Scheme 2. (**1**) X = Y = Z = CH; (**2**) X = N, Y = Z = CH; (**3**) Y = N, Z = X = CH; (**4**) Y = X = N, Z = CH; (**5**) Z = X = N, Y = CH.

found to be present as the major components (33—55%).† In each case they were separated by a combination of flash and preparative thin layer chromatography (silica-gel, MeOH–$CHCl_3$), and further purified by recrystallization. Structure elucidation was achieved using spectroscopic methods, (i.r., n.m.r., mass spec., g.l.c.–mass spec., and accurate molecular weight determination). The *cis*-dihydrodiol configuration was deduced from the δ values [H_e (4.7—4.8), H_f (4.3—4.6), H_g (6.2—6.6), H_h (6.5—6.6)] and from the vicinal coupling constant J_{ef} (4.5—5.0 Hz), since *trans*-dihydrodiols normally have larger J_{ef} values (*ca.* 10 Hz). The position of the vicinal-diol moiety was deduced from nuclear Overhauser enhancement (n.O.e.) difference spectroscopy [$(CD_3)_2CO$, 400 MHz] since protons at C_d and C_e (**2B**, **3B**, **4B**) or C_a and C_h (**3B**) were found to be spatially proximate (Table 1). The *cis*-dihydrodiols (**2B**—**5B**) were all found to be optically active (Table 1) although the optical purity and absolute stereochemistry are presently unknown.

While the formation of chiral *cis*-dihydrodiol metabolites from the APAHs (**2A**—**5A**) appears to be analogous to the bacterial metabolism of PAHs (in all cases found to be optically pure and having an (*R*)-configuration at the benzylic chiral centre[8]), the isolated diols (**2B**—**5B**) proved to be more stable than their PAH analogue (**1B**). Thus, in order to aromatize the *cis*-dihydrodiol of quinoline (**2B**) it proved necessary to heat to *ca.* 150 °C. Furthermore, diol (**2B**) was recovered unchanged after dissolution in a mixture of $(CD_3)_2CO$ and CF_3CO_2H (1:1) for three weeks. The increased stability of *cis*-diols (**2B**-**5B**) towards acids [in comparison with (**1B**)[7] and the *cis*-diol metabolites of benzene and toluene[1,5,6]] may again be due to the presence of the fused heterocyclic rings (pyridine, pyrazine, and pyrimidine) and thus compounds (**2B**—**5B**) may have increased potential value as chiral synthons.

The formation of carbocyclic phenol metabolites of (**2A**) [8-hydroxyquinoline, (**2E**)], (**3A**) [5-hydroxyisoquinoline, (**3E**)], and (**5A**) [5-hydroxyquinoxaline, (**5E**)] was unexpected since mono-phenolic products have not previously been detected as bacterial metabolites of PAHs (Table 1). The stability of the isolated *cis*-dihydrodiols (**2B**—**5B**) suggests that formation of the carbocyclic ring phenols (**2E**), (**3E**), and (**5E**) *via* dehydration of the diols is unlikely and the possibility of phenols being produced *via* mono-oxygenase-catalysed formation of arene oxide intermediates cannot be excluded (aromatization of quinoline 7,8-oxide has been found to yield 8-hydroxy-quinoline[4]). Bacterial metabolism of arenes to yield *cis*-dihydrodiols through dioxygenase enzyme oxidation was previously found to be accompanied by sulphur oxidation[9] and aliphatic hydroxylation[10] of the same substrate molecule. Although the present strain of *P. putida* does not appear to contain the specific enzyme toluene mono-oxygenase, enzyme-catalysed sulphoxidation has been found.[7] The concomitant formation of *cis*-dihydrodiols (**A**) and arene oxides (**B**) as initial arene metabolites by a single organism has not been observed and this possibility is currently under investigation with *P. putida*.

Enzymatic hydroxylation of the heterocyclic rings of the APAHs (**2A**), (**4A**), and (**5A**) in *P. putida* yielded 3-hydroxyquinoline (**2G**), 4-hydroxyquinazoline (**4G**), and 5-hydroxyquinoxaline (**5G**), respectively (Table 1). The formation of an unstable *cis*-dihydrodiol metabolite on the heterocyclic ring,

† This value was estimated from n.m.r. analysis of the crude product mixture which contained no traces of substrate and is expressed as a % of the total stable metabolites detected.

followed by spontaneous dehydration (as proposed in the metabolism of indole to indoxyl and indigo by *P. putida*)[11] could account for the formation of 3-hydroxyquinoline.

The detection of anthranilic acid as a significant metabolite (27%) of quinoline (**2A**) in the present study is noteworthy. Anthranilic acid is clearly established as the normal biosynthetic precursor of a wide range of quinoline ring systems in plants and micro-organisms.[12] The reverse process has now been found to occur *in vivo*. Further biodegradation of anthranilic acid by *Pseudomonads* (to carbon dioxide and ammonia) has recently been reported.[13]

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