

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 metabolites¹ 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**).

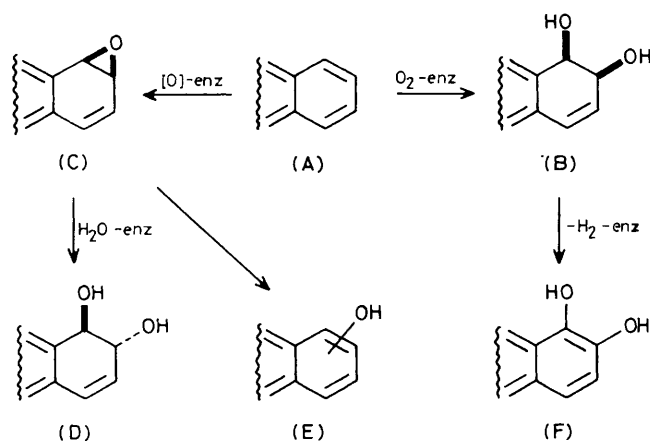
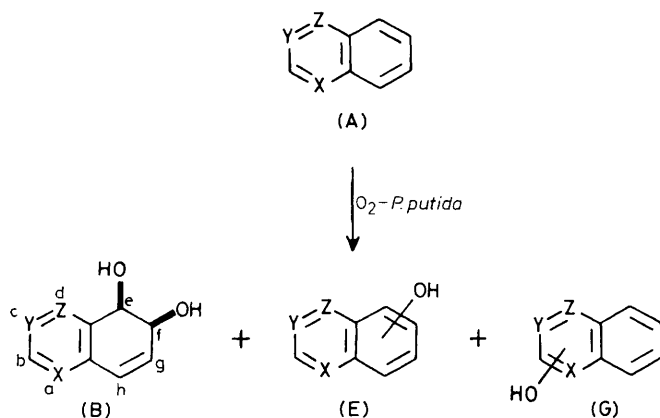
Despite the prevalence of APAHs in the environment their bacterial metabolism has not been investigated. Here a mutant strain of *Pseudomonas putida* (lacking the enzyme *cis*-glycol dehydrogenase),^{5,6} which has been found to yield the *cis*-dihydrodiol metabolite of naphthalene (**1B**) exclusively 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	<i>cis</i> -Dihydrodiol metabolites					Phenol metabolites (% yield) [†]
	(2B)	Yield/% [†]	[α] _D	$J_{e,f}$ /Hz	n.O.e.	
(2A)	(2B)	33	+220 ^a	4.50	H _d ↔ H _e	3-OH (13), 8-OH (27)
(3A)	(3B)	47	-2.5 ^b	4.90	H _d ↔ H _e	5-OH (45)
(4A)	(4B)	55	-40 ^b	4.97	H _a ↔ H _h	4-OH (10)
(5A)	(5B)	45 ^d	+210 ^c	4.60	H _d ↔ H _e	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-CHCl₃), and further purified by recrystallization. Structure elucidation was achieved using spectroscopic methods, (i.e., 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 [(CD₃)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 centre⁸), 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 (CD₃)₂CO and CF₃CO₂H (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-quinoline⁴). Bacterial metabolism of arenes to yield *cis*-dihydrodiols through dioxygenase enzyme oxidation was previously found to be accompanied by sulphur oxidation⁹ and aliphatic hydroxylation¹⁰ 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-hydroxy-quinoline (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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