Structure and Stereochemistry of *cis*-Dihydro Diol and Phenol Metabolites of Bicyclic Azaarenes from *Pseudomonas putida* UV4

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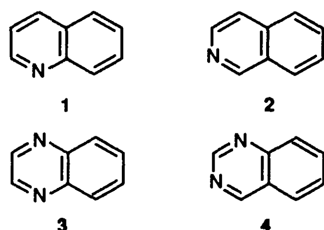
Biotransformation of quinoline, isoquinoline, quinoxaline and quinazoline using growing cultures of *Pseudomonas putida* UV4 yielded *cis*-dihydro diols from the oxidation of the carbocyclic aromatic ring. Aromatic hydroxylation was observed in both carbocyclic and heterocyclic rings. Ring cleavage of the quinoline skeleton to yield anthranilic acid, and *cis*-diol formation (with alkene bond reduction) to yield *cis*-5,6,7,8-tetrahydroquinazoline-5,6-diol from quinazoline were observed. The *cis*-dihydro diol metabolites of quinoline (5,6- and 7,8-) and quinoxaline (5,6-) were found to be optically pure, while metabolism of isoquinoline gave one homochiral (5,6-) and one racemic (7,8-) *cis*-dihydro diol product. The absolute configurations of the *cis*-dihydro diol metabolites have been determined using ^{1}H NMR analyses, stereochemical correlations and X-ray crystallography methods.

Azaarenes (*e.g.* quinoline **1** and isoquinoline **2**) are widely distributed throughout the global environment, mainly as a result of partial combustion of plant material and fossil fuels.[1] Quinoline **1**, the most abundant of the azaarenes in the environment, is both carcinogenic and mutagenic. The latter effects probably result from the derived metabolites of quinoline **1**. Known and potential metabolites of quinoline **1**[2] and isoquinoline **2**[3] have recently been synthesised as part of a programme of investigations into the biological effects of azaarenes.[4] Soil bacteria (such as *Pseudomonas putida*) play an important role in the biodegradation of polycyclic aromatic hydrocarbons including azaarenes. The total biodegradation of the arene ring of amino aromatic substrates by anaerobic *Pseudomonads* has also been reported.[5] The initial step in the bacterial metabolism of mono- and poly-cyclic aromatic hydrocarbons was shown to involve the formation of *cis*-dihydro diols by the use of blocked mutant strains of *P. putida* which lacked the appropriate dehydrogenase enzyme associated with catechol formation.[6]

cis-Dihydro diol metabolites of arenes produced by the dioxygenase enzyme systems of *P. putida* have been widely used in the synthesis of a range of natural products.[7,8] Chemoenzymatic studies are currently in progress in these laboratories exploring direct synthetic routes to chiral products by utilizing some of the new bicyclic azaarene *cis*-dihydro diol metabolites.

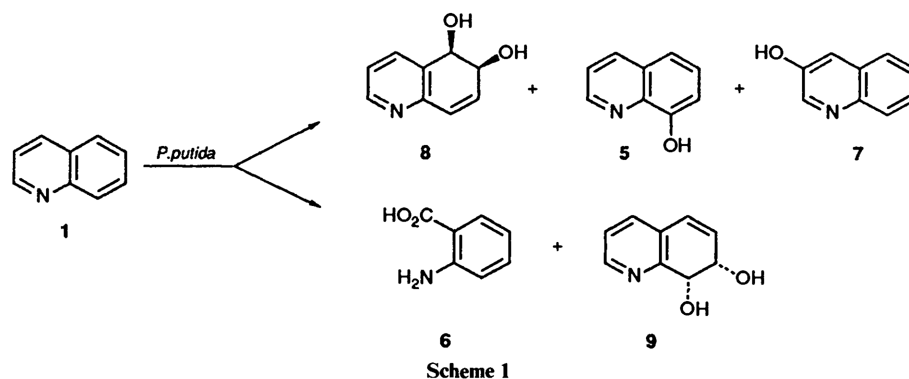
Results and Discussion

Initial results from these laboratories on the metabolism (*ca.* 0.1 g) of quinoline **1**, isoquinoline **2**, quinoxaline **3** and quinazoline **4** by *P. putida* UV4 were reported in a preliminary communication.[9] Biotransformation of quinoline **1** by *P. putida* UV4 and extraction of the resulting culture medium gave a semisolid mixture which was separated into four components by PLC on silica-gel. The less polar products were identified as quinolin-8-ol **5**, anthranilic acid **6**, and quinolin-3-ol **7** by chromatographic and spectral comparisons with authentic samples. The more polar component was identified as *cis*-5,6-dihydroquinoline-5,6-diol **8** from its ^{1}H NMR spectrum which showed an NOE between 4-H and 5-H and *cis*-coupling between 5-H and 6-H ($J_{5,6}$ 5.1 Hz). ^{1}H NMR analysis of the crude extract provided an estimate of the relative proportions of metabolites **5** (27%), **6** (27%), **7** (13%) and **8** (33%). When a larger scale (*ca.* 1.4 g) metabolism of quinoline was carried out at a later date, in addition to compounds **5–8**, a much less stable minor *cis*-dihydro diol **9** was also isolated (Scheme 1). The *cis*-dihydro diol **9** was found to have a slightly higher R_f value on silica-gel TLC plates and could be separated from the isomeric *cis*-dihydro diol **8**. Both the *cis*-dihydro diol metabolites of quinoline **8** and **9** showed characteristic coupling constants ($J_{7,8}$ 5.4 Hz, $J_{5,6}$ 5.1 Hz).

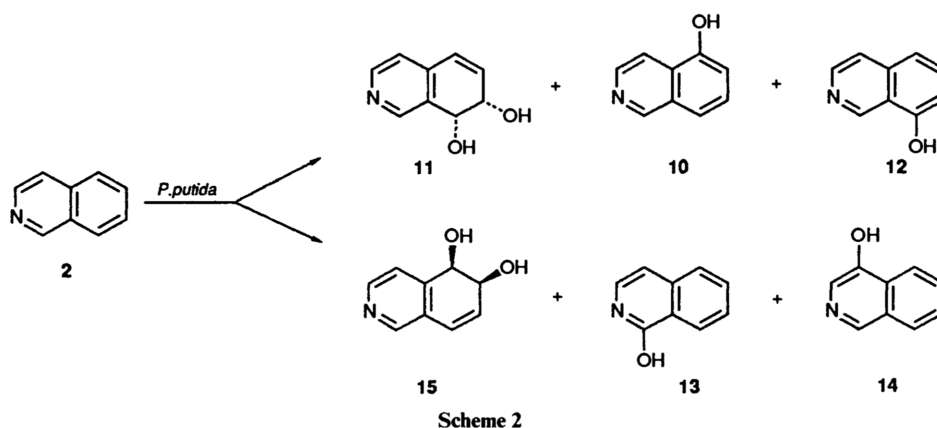


Biotransformation of isoquinoline,[9] **2**, gave isoquinolin-5-ol **10** and *cis*-7,8-dihydroisoquinoline-7,8-diol **11** (47% relative yield of each product) with little evidence of recovered substrate. Biotransformation of the isoquinoline **2** in the present study was conducted on a larger scale (*ca.* 7.7 g) and gave additional metabolites including the phenols **12–14**, and a relatively unstable *cis*-dihydro diol **15** (Scheme 2). The *cis*-dihydro diol metabolites **11** and **15** had identical R_f values on TLC and could not be separated by PLC or HPLC methods. However, preferential aromatization of the *cis*-dihydro diol **15** in the mixture of diols allowed a pure sample of the isomeric metabolite **11** to be obtained. The ^{1}H NMR spectra ($J_{7,8}$ 5.1 Hz, $J_{5,6}$ 5.4 Hz and NOE interactions) of the *cis*-dihydro diols **15** and **11** were consistent with the structures assigned. The mixture of the *cis*-dihydro diols **11** and **15** could only be resolved by means of capillary GC of the trimethylsilyl (TMS) ether derivatives. Characterization of the less stable isomer **15** was thus limited to GC–MS data of the di-TMS derivative and the ^{1}H NMR spectral information extracted from the mixture.

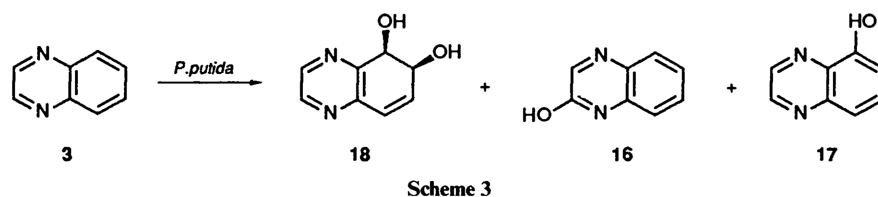
Quinoxaline **3** in common with the azaarenes **1** and **2** has also been metabolized by *P. putida* UV4.[9] The metabolites identified were quinoxalin-2-ol **16** (20%) quinoxalin-5-ol **17** (40%) and *cis*-



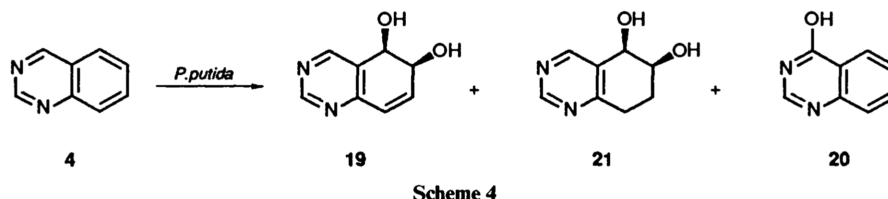
Scheme 1



Scheme 2



Scheme 3



Scheme 4

5,6-dihydroquinoxaline-5,6-diol **18** (45%) in the relative yields indicated (from ^{1}H NMR and GC–MS analyses) (Scheme 3). The dihydro diol **18** again showed the expected ^{1}H NMR spectral characteristics of a *cis*-diol ($J_{5,6}$ 4.0 Hz).

In the first report of the present study,[9] metabolism of quinazoline **4** was found to give a *cis*-dihydro diol **19** and a phenol **20**. Further investigations of the metabolites of quinazoline **4**, by *P. putida* UV4 showed that in addition to quinazolin-4-ol **20** and *cis*-5,6-dihydroquinazoline-5,6-diol **19**, a further minor *cis*-diol **21** along with several unidentified phenols was present (Scheme 4). Attempts to separate the mixture of *cis*-diols **19** and **21** did not succeed and thus the minor *cis*-diol **21** was tentatively identified as *cis*-5,6,7,8-tetrahydroquinazoline-5,6-diol **21** on the basis of the ^{1}H-NMR spectral data (**19**, $J_{5,6}$ 5.0 Hz; **21**, $J_{5,6}$ 3.1 Hz) and GC–MS analysis of the mixture.

Enantiomeric Excess (% e.e.) and Absolute Configuration Determination of Azaarene cis-*Dihydro Diol Metabolites*.—The *cis*-dihydro diol metabolites of quinoline (**8**, $[\alpha]_D$ +220); **9**, $[\alpha]_D$ +45), isoquinoline (**11**, $[\alpha]_D$ −2.5; mixture of **11** and **15**, $[\alpha]_D$ +49), quinoxaline (**18**, $[\alpha]_D$ +210) and quinazoline (mixture of **19** and **21**, $[\alpha]_D$ −40) appeared to be optically active. In order to determine the % e.e. of the *cis*-dihydro diol metabolites by the lanthanide-induced shift (LIS) method, ^{1}H NMR spectra were obtained using [$^2H_3$]acetonitrile as the solvent with increasing concentrations of the chiral LIS reagent, tris[3-heptafluoropropylhydroxymethylene)(−)camphorato]europium(III) [Eu(hfc)$_3$]. Chemically synthesised samples of racemic *cis*-1,2-dihydronaphthalene-1,2-diol and racemic *cis*-5,6-dihydroquinoline-5,6-diol **8** thus showed splitting of the allylic proton signals due to the presence of each enantiomer. No such splitting was found in any of the ^{1}H NMR signals when Eu(hfc)$_3$ was added to the metabolically produced *cis*-dihydro diols of quinoline **8**, **9**, isoquinoline **15** and quinoxaline **18**.

The LIS results are consistent with a high optical purity of the *cis*-dihydro diol metabolites **8**, **9**, **15** and **18**. In contrast, 7,8-dihydroisoquinoline-7,8-diol **11**, obtained as a bacterial metabolite of isoquinoline **2**, showed baseline splitting of the 7-H signals in the ^{1}H NMR spectrum in the presence of Eu(hfc)$_3$. This observation also confirmed that the *cis*-dihydro diol **11** ($[\alpha]_D$ −2.5) was essentially racemic, since the peak areas associated with each enantiomer appeared to be almost equal. The validity of the Eu(hfc)$_3$ method for % e.e.

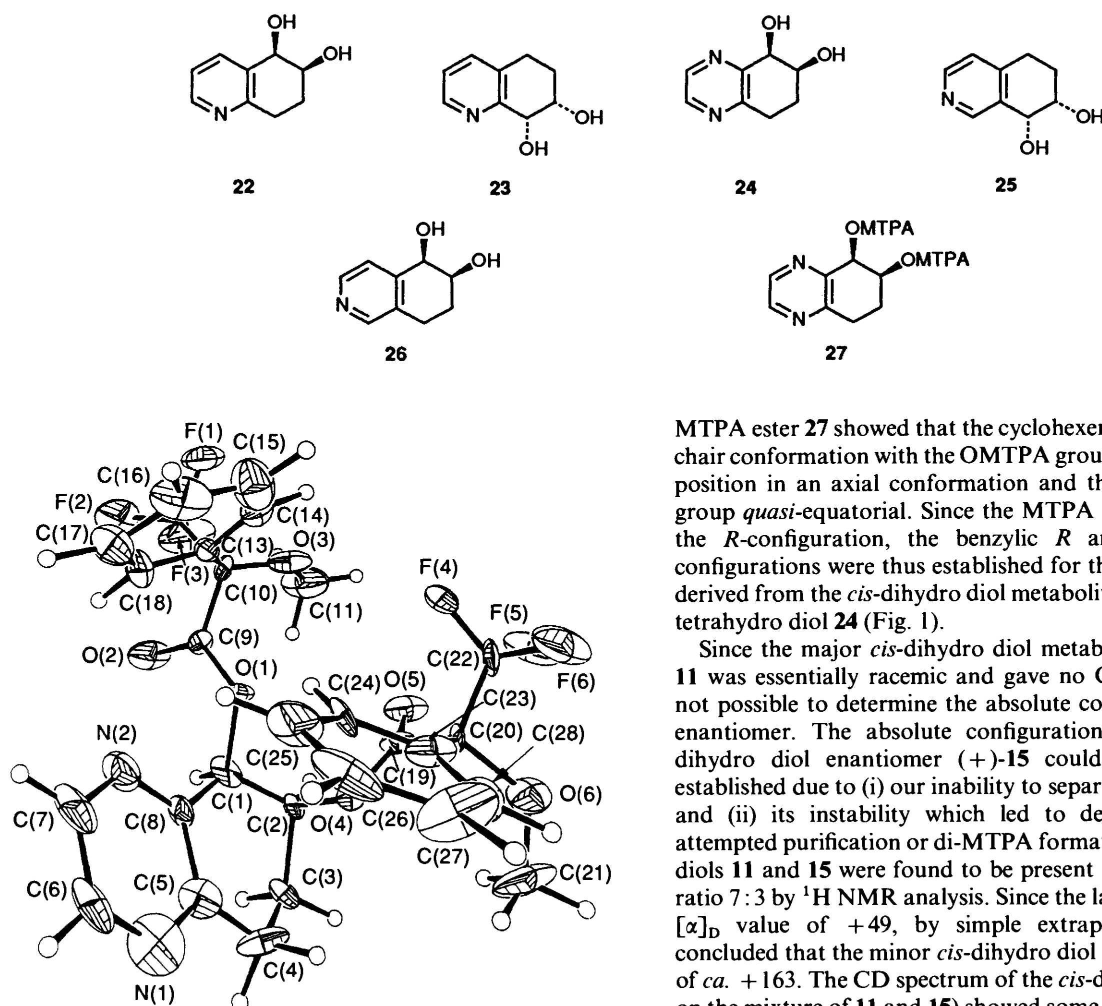


Fig. 1

determination of the *cis*-dihydro diols of azaarenes was confirmed by catalytic hydrogenation of *cis*-diol metabolites (+)-**8**, (+)-**9** and (+)-**18** to yield the *cis*-tetrahydro diols (−)-**22**, (−)-**23** and (−)-**24**, respectively, which were subsequently converted into the corresponding diMTPA esters. All of these diMTPA esters proved to be single diastereoisomers (>98%) by ^{1}H NMR analysis. Unfortunately, the *cis*-tetrahydro diols **25** and **26** obtained by catalytic hydrogenation of the corresponding *cis*-dihydro diol metabolites of isoquinoline (**11** and **15**) decomposed during attempts to synthesise the corresponding diMTPA esters. The % e.e. of the *cis*-dihydro diol metabolite of quinazoline **19** could not be determined due to overlap of the ^{1}H NMR signals with those of the *cis*-tetrahydro diol metabolite **21**.

The absolute configurations of the *cis*-dihydro diol metabolites (+)-**8**, (+)-**9** and (+)-**18** were determined by stereochemical correlation to the corresponding *cis*-tetrahydro diols (−)-**22**, (−)-**23**, and (−)-**24**. Previous reports from these laboratories[10,11] have shown that (−)-*cis*-5,6,7,8-tetrahydroquinoline-5,6-diol **22** and (−)-*cis*-5,6,7,8-tetrahydroquinoline-7,8-diol **23** had the 5*R*,6*S* and 8*R*,7*S* configurations. Thus, the *cis*-dihydro diol derivatives of quinoline (**8** and **9**) have also been assigned 5*R*,6*S* and 8*R*,7*S* configurations.

The *cis*-tetrahydroquinoxalinediol **24** ($[\alpha]_D$ − 20) obtained by catalytic hydrogenation of the corresponding *cis*-dihydro diol **18** ($[\alpha]_D$ +210), was converted into the corresponding diMTPA ester **27** ($[\alpha]_D$ −4.8) using (+)-MTPA chloride in pyridine. X-Ray crystallographic analysis on the latter di-MTPA ester **27** showed that the cyclohexene ring adopts a half-chair conformation with the OMTPA group at the non-benzylic position in an axial conformation and the benzylic OMTPA group *quasi*-equatorial. Since the MTPA groups were both of the *R*-configuration, the benzylic *R* and non-benzylic *S*-configurations were thus established for the di-MTPA ester **27** derived from the *cis*-dihydro diol metabolite **18** through the *cis*-tetrahydro diol **24** (Fig. 1).

Since the major *cis*-dihydro diol metabolite of isoquinoline **11** was essentially racemic and gave no CD spectrum, it was not possible to determine the absolute configuration of either enantiomer. The absolute configuration of the minor *cis*-dihydro diol enantiomer (+)-**15** could not be rigorously established due to (i) our inability to separate it from isomer **11** and (ii) its instability which led to decomposition during attempted purification or di-MTPA formation. The *cis*-dihydro diols **11** and **15** were found to be present in the mixture in the ratio 7:3 by ^{1}H NMR analysis. Since the latter mixture gave an $[\alpha]_D$ value of +49, by simple extrapolation, it may be concluded that the minor *cis*-dihydro diol **15** had an $[\alpha]_D$ value of *ca.* +163. The CD spectrum of the *cis*-diol (+)-**15** (obtained on the mixture of **11** and **15**) showed some similarity to those of *cis*-diols (+)-**8** and (+)-**9**, having a benzylic *R* and allylic *S* configuration. This evidence is, however, insufficient to provide an unequivocal assignment of absolute configuration to the (+) enantiomer of *cis*-diol **15**.

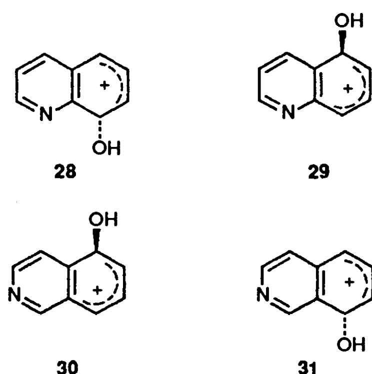
The preliminary report of this work[9] had indicated that the *cis*-dihydro diol metabolite of quinazoline **19** was laevorotatory ($[\alpha]_D$ −40). The latter $[\alpha]_D$ measurement did not, however, take account of a significant proportion of a further metabolite (*ca.* 30%) now identified as the corresponding *cis*-tetrahydro diol **21**. Our inability to separate the mixture of *cis*-diols **19** and **21** again precluded the determination of absolute configuration of either metabolite.

Of the *cis*-dihydro diol metabolites of azaarenes which have been stereochemically assigned during this study, compounds (+)-**8**, (+)-**9**, (+)-**15** and (+)-**18** were found to be enantiomerically homogeneous (>98% e.e.) and of the same absolute configuration (benzylic *R*:allylic *S*). Similar % e.e.'s and absolute configurations were previously recorded for the bacterial metabolism of the polycyclic aromatic hydrocarbons naphthalene,[12] anthracene,[13] phenanthrene[14] and benz[*a*]-anthracene.[15] The observation that one of the *cis*-dihydro diol metabolites of isoquinoline **11** was racemic is thus noteworthy for being an exception to this earlier established trend. The latter result also indicates the importance of having reliable methods for determination of % e.e. and absolute configuration of *cis*-dihydro diol metabolites of arenes and azaarenes.

Formation of Phenolic Metabolites of Azaarenes.—The bacterial metabolism of polycyclic aromatic hydrocarbons[12–14] and of the bicyclic azaarenes **1–4** was similar in the production of *cis*-dihydro diol products but some important differences

were, however, noted. Thus, yields of metabolite obtained from the bicyclic arene naphthalene were much higher (> 80%) than those found using bicyclic azaarene substrates (**1**–**4**, < 30%). Furthermore, phenolic products were always obtained from azaarene metabolism, while substrates which were carbocyclic members of the PAH series appeared to yield *cis*-dihydro diols only. The possibility of spontaneous aromatization of the azaarene *cis*-dihydro diols during metabolism should thus be considered.

The *cis*-dihydro diol metabolites of quinoline **8** and **9** were found to be of widely differing stability. For example, an NMR sample of *cis*-5,6-dihydroquinoline-5,6-diol **8** remained unchanged after storage for 3 weeks at ambient temperature in a mixture of trifluoroacetic acid–chloroform (1:1). By contrast, storage of a sample of *cis*-7,8-dihydroquinoline-7,8-diol **9** for 1 week in chloroform solution at room temperature resulted in total aromatization to yield quinolin-8-ol **5**. This behaviour might be explained in terms of the greater stability of the allylic carbocation intermediate **28** which would be preferentially formed from acid treatment of the *cis*-dihydro diol **9**. Kinetic studies on the aromatization of the 5,6- and 7,8-arene oxides of quinoline to yield mainly quinolin-5-ol and -8-ol, respectively, have recently been rationalized in terms of the relative stabilities of the derived allylic carbocations **28** and **29**.[16,17] Formation of quinolin-6-ol as the major product when the *cis*-dihydro diol **5** is heated (unpublished results) suggests, however, that other factors may also be involved.



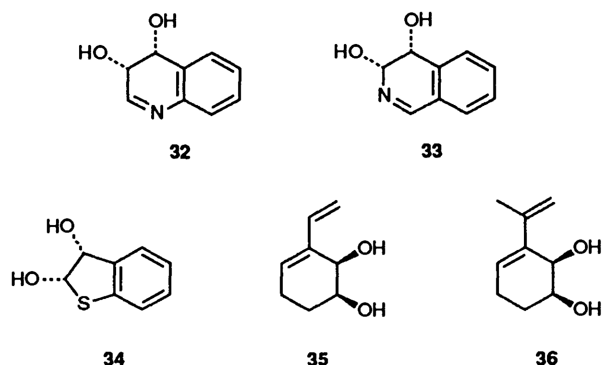
Metabolites resulting from oxidation of isoquinoline, **2**, in mammalian liver systems (arene oxides, *trans*-dihydro diols)[3] and in *P. putida* UV4 (*cis*-dihydro diols **11** and **15**) were consistently found to be more readily aromatized than the corresponding quinoline metabolites. A time–course study of metabolites produced by the action of *P. putida* UV4 on isoquinoline **2** (Scheme 2), indicated that the *cis*-diols **11** and **15** (major metabolites) and isoquinolin-5-ol **10** were present during the early stages of metabolism (4 h). After 24 h the only metabolites observed were the phenolic products isoquinolin-5-ol **10**, -8-ol **12**, -4-ol **14** and -1-ol **13**. The phenolic metabolites derived from the carbocyclic ring of the quinoline **5**, the isoquinolines **10** and **12** and the quinoxaline **17** can all be formed by aromatization of the corresponding *cis*-dihydro diol precursors.

The allylic carbocation intermediates **30** and **31** derived from the corresponding *cis*-dihydro diols **15** and **11** could, in principle, account for the formation of the phenolic metabolites **10** and **12** (in either the culture medium or on silica-gel TLC). This does not, however, explain the exclusive formation of isoquinolin-7-ol on thermal dehydration of the *cis*-dihydro diol **11** (unpublished data) and again suggests that other factors may also be involved during aromatization.

Formation of the phenolic metabolites in the heterocyclic rings of quinoline **7** and isoquinoline **14** could also result from the formation and subsequent dehydration of unstable *cis*-dihydro diols **32** and **33**. The formation of anthranilic acid **6** as a metabolite of quinoline could also be accounted for by hydrolysis of the imino bond of the *cis*-diol **32**, followed by oxidative cleavage of the residual three-carbon atom chain. The first example of *cis*-dihydro diol formation in a heterocyclic ring **34** has recently been found when benzothiophene was metabolized by *P. putida* UV4.[18] The formation of anthranilic acid as a major bacterial metabolite of quinoline is noteworthy, since this bioconversion may form part of the nitrogen cycle in nature. Thus, anthranilic acid, after utilization as a precursor of plant alkaloids along with other organonitrogen compounds, may be converted into quinoline during partial combustion of plant material and fossil fuels.[1] Metabolism of quinoline by soil bacteria (*e.g. P. putida*) may, in turn, liberate anthranilic acid which is then available as a substrate for further biodegradation by soil *Pseudomonads* (to yield CO_2 and NH_3)[5] or may be directly assimilated by the plant.

The formation of the heterocyclic phenols **13**, **16** and **20** from isoquinoline **2**, quinoxaline **3** and quinazoline **4**, respectively, cannot be accounted for by a similar mechanism to that postulated for carbocyclic phenols **5**, **10**, **12** and **17**, *i.e. cis*-dihydro diol formation at a C=C bond. Aromatic hydroxylations at the carbon atom adjacent to a nitrogen atom have previously been reported for bicyclic azaarenes. Amine oxidase enzymes are reported[19,20] to be responsible for this type of oxidation.

The unexpected biotransformation of quinazoline **4** to yield a product which was tentatively identified as the *cis*-tetrahydro diol **21**, appeared to involve both an oxidation and a reduction step. The formation of a *cis*-dihydro diol moiety (oxidation) and hydrogenation of a C=C bond (reduction) during bacterial metabolism of arene substrates has previously been reported.[21,22] Thus, the *cis*-tetrahydro diols **35** and **36** were isolated as metabolites from styrene and α-methyl styrene, respectively, using *P. putida*.[21,22] A satisfactory mechanistic explanation for the formation of the *cis*-tetrahydro diol metabolites **21**, **35** and **36** is currently unavailable.



One aspect of the metabolism of azaarenes **1**–**4** by *P. putida* UV4, which distinguished it from other arene biotransformations, was the change in both metabolic profile and yields of products observed over a period of *ca.* 12 months. Thus, during the preliminary studies,[9] the yields of *cis*-dihydro diols and phenolic products isolated from quinoline **1**, isoquinoline **2**, quinoxaline **3** and quinazoline **4** were generally in the range 20–30% and the substrates appeared to have been totally metabolized. When these studies were repeated as part of the present work, it was found that the total yield of isolated metabolites was consistently lower (< 10%), that a wider range of *cis*-dihydro diols and phenolic metabolites was present, and that a major proportion of the substrate was recovered unchanged. The less stable diols **9** and **11** isolated for the first time during the present study were probably aromatized during

the preliminary extraction procedure.[9] All attempts to improve the total yield of metabolites to that originally found 1 year earlier with *P. putida* UV4[9] were unsuccessful. During this intervening period the activity towards certain azaarenes appears to have decreased and one can only surmise that either uptake of azaarenes had been impaired or that the specific activity towards them has decreased. Changes in the yields of bioproducts from other arene substrates over this period however were not generally observed.

Experimental

^1H NMR spectra were obtained using Bruker (WH250) and General Electric (QE-300 and GN Ω-500) instruments and tetramethylsilane as internal reference. Coupling constants are given in Hz. Mass spectra were recorded at 70 eV on an AEI-MS902 instrument updated by V.G. instruments. GLC–MS analyses were carried out with a VG12-250 instrument linked to a PDP11/23 PLUS data system and at 25 m BP1 capillary column. Values of $[\alpha]_D$ are recorded in units of 10^{-1} deg cm^2 g^{-1}.

Biotransformations using *P. putida* UV4 were carried out as reported previously[23] and the treatment/storage of *P. putida* organism prior to its use in bioconversions is now summarized. Cells used for biotransformation studies were subcultured on a weekly basis on a mineral salts (MS) agar containing 5 g dm^{-3} sodium pyruvate as carbon source (PMS agar). Prior to use in a particular biotransformation a colony was streaked into L-agar containing 1 g dm^{-3} glucose and grown overnight before resulting colonies were transferred to PMS agar containing 0.16 g dm^{-3} indole. Only those colonies showing a strong blue colour (owing to the production of indigo catalysed by the toluene dioxygenase) were selected for toluene dioxygenase assay. Whole-cell toluene dioxygenase was assayed in shake flasks by transfer of a colony from a replica of the indole plate, showing strong indigo formation, to PMS medium and grown overnight at 30 °C with shaking. The culture was then centrifuged and the pellet resuspended in 0.2 mol dm^{-3} phosphate buffer pH 7.2 in duplicate flasks containing 2 cm^3 of toluene in the centre well. The flasks were then closed with a Suba Seal and 0.100 cm^3 aliquots of the aqueous phase removed and added to 0.900 cm^3 water for determination of toluene *cis*-dihydro diol formation over a 3 h period in a 1 cm cuvette. Only cells exhibiting a $O.D_{265}/O.D_{600}$ h^{-1} of greater than 90 were used in the biotransformation. Cells were maintained in this way for over 6 years during which time the activity has steadily increased (from around 20 then to over 100 now). The decrease in activity toward the azaarenes **1–4** over a period of *ca.* 1 year during the present study was thus unexpected in view of the increase in activity using toluene as substrate.

Quinoline **1** *Metabolism*.—Metabolism of quinoline (1.4 g) by *P. putida* UV4 and extraction with dichloromethane gave a high portion of unmetabolized substrate (>90%) and a relatively low yield of metabolites (<10%). GC–MS analysis on a 25 m BP1 column of the silylated products (BSA/pyridine, 100 °C/min, 100–200 °C at 2 °C min^{-1}) showed the presence of *cis*-5,6-dihydroquinoline-5,6-diol (**8**, 33.2 min), quinolin-3-ol (**7**, 26.5 min), quinolin-8-ol (**5**, 21.3 min) and anthranilic acid (**6**, 19.5 min). Purification of the crude extract on a silica-gel column (chloroform → 15% methanol in chloroform) to remove a large proportion of unchanged substrate followed by preparative TLC on silica gel using 10% methanol–chloroform as eluent allowed the metabolites to be separated.

cis-5,6-Dihydroquinoline-5,6-diol **8** (0.032 g), R_f 0.2, m.p. 153–155 °C (ethyl acetate), $[\alpha]_D$ +220 (THF) (Found: C, 65.7; H, 5.5; N, 8.6. $C_9H_9NO_2$ requires C, 66.3; H, 5.5; N, 8.6); δ_H(400 MHz; [$^2H_6$]acetone), 2.92 (2 H, br s, 2 × OH), 4.32 (1 H, m, 6-H), 4.69 (1 H, d, $J_{5,6}$ 5.1, 5-H), 6.33 (1 H, dd, $J_{7,6}$ 4.6, $J_{7,8}$ 9.9, 7-H), 6.59 (1 H, d, $J_{8,7}$ 9.9, 8-H), 7.19 (1 H, dd, $J_{3,2}$ 4.9, $J_{3,4}$ 7.6, 3-H), 7.82 (1 H, m, 4-H) and 8.37 (1 H, m, 2-H).

Other metabolites were identified as quinolin-3-ol (**7**, 0.011 g, R_f 0.5), quinolin-8-ol (**5**, 0.023 g, R_f 0.7) and anthranilic acid, (**6**, 0.022 g, R_f 0.5) by spectral comparison with reference compounds.[16,17] Using PLC and multiple elution with diethyl ether, quinolin-3-ol (**7**, R_f 0.4) and anthranilic acid (**6**, R_f 0.6) were also found to be separable.

Later biotransformations of quinoline **1** yielded a second, less-polar, diol product **9**, (R_f 0.3) which was isolated in low yield after PLC purification on silica gel (10% MeOH in $CHCl_3$ as eluent). The *cis*-dihydro diol **9** was found to be very unstable and showed evidence of dehydration to yield quinolin-8-ol **5** during purification by PLC. This low-melting unstable compound was identified as *cis*-7,8-dihydroquinoline-7,8-diol **9**, $[\alpha]_D$ +45 (MeOH) (Found: M, 163.0628. $C_9H_9NO_2$ requires *M*, 163.0633); δ_H(300 MHz; [$^2H_6$]acetone) 4.40 (1 H, m, 7-H), 4.58 (1 H, d, $J_{8,7}$ 4.9, 8-H), 6.17 (1 H, dd, $J_{6,5}$ 9.7, $J_{6,7}$ 4.8, 6-H), 6.60 (1 H, d, $J_{5,6}$ 9.7, 5-H), 7.27 (1 H, dd, $J_{3,2}$ 5.0, $J_{3,4}$ 7.5, 3-H), 7.53 (1 H, d, $J_{4,3}$ 7.5, 4-H) and 8.37 (1 H, d, $J_{2,3}$ 5.0, 2-H).

^1H NMR spectra of *cis*-5,6-dihydroquinoline-5,6-diol **8** ($[\alpha]_D$ +220) [and *cis*-7,8-dihydroquinoline-7,8-diol **9** ($[\alpha]_D$ +45)] in [$^2H_3$]acetonitrile solvent containing increasing proportions of $Eu(hfc)_3$ failed to show evidence of more than one enantiomer. The ^1H NMR spectrum of a chemically synthesised racemic sample of *cis*-5,6-dihydroquinoline-5,6-diol[24] **8** in [$^2H_3$]acetonitrile containing $Eu(hfc)_3$ under identical conditions showed baseline splitting of the 6-H signal. On the basis of these observations, it is concluded that both the *cis*-dihydro diol metabolites of quinoline **8** and **9** were homochiral. This conclusion was confirmed by ^1H NMR analysis of the di-MTPA esters[11] (see later), formed from the catalytical hydrogenation product of the *cis*-dihydro diol metabolites **8** and **9**.

The absolute configurations of the *cis*-dihydro diols (+)-**8** and (+)-**9** were determined as 5*R*,6*S* and 8*R*,7*S* respectively, from the ^1H NMR spectral characteristics of the di-MTPA esters[11] and stereochemical correlations.[10,24] A solution of (+)-*cis*-5,6-dihydroquinoline-5,6-diol (**8**, 0.02 g, $[\alpha]_D$ +220) dissolved in ethanol (10 cm^3) was hydrogenated at atmospheric pressure using a Pd on carbon catalyst (10% Pd/C, 0.002 g). The hydrogenation was found to be complete after 72 h. The catalyst was filtered off, the filtrate evaporated under reduced pressure and the product recrystallized to yield (−)-(5*R*,6*S*)-*cis*-5,6,7,8-tetrahydroquinoline-5,6-diol **22** (0.016 g, 80%), m.p. 153–154 °C; $[\alpha]_D$ −7.2 (MeOH) (Found: C, 65.6; H, 6.6; N, 8.4. $C_9H_{11}NO_2$ requires C, 65.45; H, 6.7; N, 8.5%); δ_H(300 MHz; $CDCl_3$) 2.01 and 2.22 (1 H each, m, 7-H and 7′-H), 2.95 and 3.16 (1 H each, m, 8-H and 8′-H), 4.17 (1 H, m, 6-H), 4.74 (1 H, d, $J_{5,6}$ 3.7, 5-H), 7.2 (1 H, dd, $J_{3,4}$ 7.7 and $J_{3,2}$ 4.8, 3-H), 7.84 (1 H, d, $J_{4,3}$ 7.6, 4-H) and 8.47 (1 H, d, $J_{2,3}$ 4.7, 2-H). The absolute configuration of (−)-(5*R*,6*S*)-*cis*-5,6,7,8-tetrahydroquinoline-5,6-diol **22** has been stereochemically correlated with (−)-(5*R*,6*R*)-*trans*-6-bromo-5,6,7,8-tetrahydroquinolin-5-ol of known configuration.[10]

A similar catalytic hydrogenation procedure on (+)-*cis*-7,8-dihydroquinoline-7,8-diol (**9**, 0.005 g, $[\alpha]_D$ +45) yielded (−)-(8*R*,7*S*)-*cis*-5,6,7,8-tetrahydroquinoline-7,8-diol **23** (0.004 g, 79%), m.p. 138–140 °C (EtOAc), $[\alpha]_D$ −72 (MeOH) (Found: C, 65.6; H, 6.6; N, 8.4. $C_9H_{11}NO_2$ requires C, 65.45; H, 6.7; N, 8.5%); δ_H(250 MHz, $CDCl_3$) 1.94 and 2.20 (1 H each, m, 6-H and 6′-H), 2.74 and 3.18 (1 H, each, m, 5-H and 5′-H), 4.31 (1 H, m, 7-H), 4.90 (1 H, d, $J_{8,7}$ 3.3, 8-H), 7.16 (1 H, dd, $J_{3,2}$ 4.7, $J_{3,4}$ 7.6, 3-H), 7.45 (1 H, d, $J_{4,3}$ 7.6, 4-H) and 8.42 (1 H, d, $J_{2,3}$ 4.6, 2-H).

The absolute configuration of (−)-(8*R*,7*S*)-*cis*-5,6,7,8-tetrahydroquinoline-7,8-diol **23** was unequivocally established by stereochemical correlation with (+)-(7*S*,8*S*)-*trans*-7-bromo-

(2-methoxy-2-phenyl-2-trifluoromethylacetoxy)-5,6,7,8-tetrahydroquinoline.[24]

Isoquinoline **2** *Metabolism*.—Metabolism of isoquinoline **2** using growing cultures of *P. putida* UV4, followed by extraction of metabolites, was found to yield a high proportion (90–95%) of recovered substrate in addition to *cis*-dihydro diol (2–3%) and phenol (2–7%) metabolites. In a typical biotransformation, isoquinoline **2** (7.7 g) yielded *ca.* 95% of recovered starting material, a mixture of the *cis*-dihydro diols **11** and **15** (0.21 g) and isoquinolin-5-ol **10** (0.154 g) after extraction and purification by flash chromatography on silica gel using methanol (2 → 7%) in chloroform as eluent. An earlier biotransformation of isoquinoline **2** (2.0 g) yielded only *cis*-7,8-dihydroisoquinoline-7,8-diol **11** (0.043 g), isoquinolin-5-ol **10** (0.041 g) and isoquinolin-8-ol **12** (0.005 g). From the latter experiment a pure sample of *cis*-7,8-dihydroisoquinoline-7,8-diol **11** was obtained, m.p. 174–175 °C (ethyl acetate); $[\alpha]_D$ −2.5 (THF–MeOH, 1:1) (Found: M, 163.0634. $C_9H_9NO_2$ requires 163.0633); δ_H(500 MHz, $CDCl_3$) 4.46 (1 H, m, 7-H), 4.75 (1 H, $J_{8,7}$ 5.1, 8-H), 6.29 (1 H, dd, $J_{6,5}$ 9.9, $J_{6,7}$ 3.9, 6-H), 6.49 (1 H, d, $J_{5,6}$ 9.9, 5-H), 7.02 (1 H, d, $J_{4,3}$ 4.8, 4-H), 8.50 (1 H, d, $J_{3,4}$ 4.8, 3-H) and 8.63 (1 H, s, 1-H).

The mixture of *cis*-dihydro diols **11** and **15** was found to be in the ratio 70:30, respectively by ^1H NMR analysis. The optical rotation of the mixture ($[\alpha]_D$ +49 in MeOH–THF, 1:1) was considered to be mainly due to the *cis*-dihydro diol **15** and thus a minimum corrected $[\alpha]_D$ value was estimated to be *ca.* +163. ^1H NMR analysis (500 MHz, CD_3CN) of the mixture of the *cis*-dihydro diols in the presence of Eu(hfc)$_3$ showed that the *cis*-dihydro diol **11** was essentially racemic (< 5% e.e.). The ^1H NMR signals corresponding to the *cis*-dihydro diol **15** were assigned by analysis of the spectrum obtained from a mixture of the diols **11** and **15**.

cis-5,6-Dihydroisoquinoline-5,6-diol **15**: δ_H(500 MHz, $CDCl_3$) 4.33 (1 H, m, 6-H), 4.72 (1 H, d, $J_{5,6}$ 5.4, 5-H), 6.27 (1 H, dd, $J_{7,6}$ 5.4, $J_{7,8}$ 9.6, 7-H), 6.63 (1 H, d, $J_{8,7}$ 9.6, 8-H), 7.56 (1 H, d, $J_{4,3}$ 5.1, 4-H), 8.32 (1 H, s, 1-H) and 8.50 (1 H, d, $J_{3,4}$ 5.1, 3-H).

The phenolic isomers of isoquinoline **10**, **12–14** were identified by comparison with GC–MS data from the literature,[25] ^1H NMR analysis and direct spectral comparison with authentic samples. The relative retention times (min) of the corresponding trimethylsilyl ether derivatives were **10** (10.38), **12** (10.40), **13** (6.42) and **14** (10.45). Isoquinolin-5-ol **10** and isoquinolin-8-ol **12** were formed by aromatization of the *cis*-dihydro diols **15** (on silica gel) and **11** (in the aqueous culture medium) respectively.

Although the quinolinols **10** and **12** have previously been reported in the literature,[23] the ^1H NMR spectral data found in the present study has been included. δ_H(300 MHz, $CDCl_3$): Isoquinolin-5-ol **10**, 7.07 (1 H, d, $J_{6,7}$ 7.4, 6-H), 7.46 (2 H, m, 7-H and 8-H), 8.05 (1 H, d, $J_{4,3}$ 5.9, 4-H), 8.51 (1 H, d, $J_{3,4}$ 5.9, 3-H) and 9.22 (1 H, s, 1 H). Isoquinolin-8-ol **12**, 7.24 (1 H, dd, $J_{7,5}$ 0.9, $J_{7,6}$ 8.2, 7-H), 7.66 (2 H, m, 5-H, 6-H), 8.29 (1 H, d, $J_{4,3}$ 6.2, 4-H), 8.41 (1 H, d, $J_{3,4}$ 5.7, 3-H) and 9.34 (1 H, s, 1-H).

Quinoxaline **3** *Metabolism*.—^1H NMR and GC–MS analysis of the crude extract obtained from the metabolism of quinoxaline **3** (2.5 g) indicated the presence of a *cis*-dihydro diol **18** (55%), quinoxalin-5-ol **17** (40%) and quinoxalin-2-ol **16** (20%) in the relative yields indicated. PLC purification on silica gel (15% MeOH in $CHCl_3$) yielded a pure sample of *cis*-5,6-dihydroquinoxaline-5,6-diol **18** (0.062 g), quinoxalin-2-ol **16** (0.012 g) and quinoxalin-5-ol **17** (0.059 g) with the major portion being unchanged substrate **3**. *cis*-5,6-Dihydroquinoxaline-5,6-diol **18**, m.p. 92–94 °C (from ethyl acetate); $[\alpha]_D$ + 210 (MeOH) (Found: M^+, 164.0591. $C_8H_8N_2O_2$ requires 164.0586); δ_H(250 MHz; $[^2H_6]$acetone) 4.58 (1 H, m, 6-H), 4.71 (1 H, d, $J_{5,6}$ 4.6, 5-H), 6.44 (1 H, dd, $J_{7,6}$ 4.6, $J_{7,8}$ 10.0, 7-H), 6.63 (1 H, d, $J_{8,7}$ 9.9, 8-H), 8.36 (1 H, d, $J_{3,2}$ 2.4, 3-H) and 8.44 (1 H, d, $J_{2,3}$ 2.2, 2-H).

Catalytic hydrogenation of *cis*-5,6-dihydroquinoxaline-5,6-diol **18** (0.015 g, $[\alpha]_D$ +210) using identical conditions to those described for the *cis*-dihydro diol **8** yielded *cis*-5,6,7,8-tetrahydroquinoxaline-5,6-diol **24** (0.013 g, 86%), m.p. 74–76 °C (EtOAc–hexane), $[\alpha]_D$ −20 (MeOH) (Found: M, 160.0730. $C_8H_{10}N_2O_2$ requires 166.0742); δ_H(300 MHz, $[^2H_6]$acetone) 2.00 and 2.18 (2 H, m, 7-H and 7′-H), 2.88 and 3.10 (2 H, m, 8-H and 8′-H), 4.18 (1 H, m, 6-H), 4.68 (1 H, d, $J_{5,6}$ 3.4, 5-H) and 8.41 (2 H, m, 2-H and 3-H). Treatment of the *cis*-tetrahydro diol **24** ($[\alpha]_D$ −20) with (+)-MTPA-chloride in pyridine solvent containing a trace of DMAP yielded the diMTPA ester derivative **27**, m.p. 131–132 °C ($CHCl_3$–hexane); $[\alpha]_D$ −4.8 ($CHCl_3$) (Found: C, 56.2; H, 4.0; N, 4.6. $C_{28}H_{24}F_6N_2O_6$ requires C, 56.2; H, 4.0; N, 4.7%); δ_H(300 MHz, $CDCl_3$) 2.33 and 2.44 (1 H each, m, 7-H and 7′-H), 3.12 (2 H, m, 8-H and 8′-H), 3.37 and 3.45 (3 H each, s, 2 × OMe), 5.74 (1 H, m, 6-H), 6.56 (1 H, d, *J* 3.2, 5-H), 7.26–7.51 (10 H, m, ArH), 8.38 (1 H, d, $J_{3,2}$ 2.4, 3-H) and 8.45 (1 H, d, $J_{2,3}$ 2.4, 2-H).

Crystal Data for the di-MTPA Ester **27**.—$C_{28}H_{24}F_6N_2O_6$, M = 598.5, orthorhombic, space group $P2_12_12_1$ (No. 19), a = 10.577(3), b = 11.249(3), c = 23.310(6) Å, U = 2773(1) Å^3, Z = 4, μ(Mo-Kα) = 0.85 cm^{-1}, D_c = 1.43 g cm^{-3}, F(000) = 1232.

Data Collection, Analysis and Refinement.—Siemens P3/V2000 diffractometer, scan width 1.2°, $\theta/2\theta$ scan, range $3 < 2\theta < 55°$; 3567 unique reflections measured; direct methods solution (SHELXS86), full-matrix least squares refinement (SHELX76); anisotropic vibration parameters for non-hydrogen atoms; hydrogens included at geometrically-calculated positions with common isotropic temperature factors for methyl-, methylene-, tertiary CH and benzene-type hydrogens which refined to U = 0.08(1), 0.06(1), 0.28(3) and 0.14(1) Å^2, respectively. 1810 data with $I > 3\sigma(I)$ gave R = 0.13 with $w = 8.18/[\sigma^2(Fo) + 0.001\,37\ F_o^2]$; maximum residual electron density 0.37 e Å^{-3}. Although this R factor is high, this does not affect the absolute stereochemical assignment which is based on the known internal absolute configuration of the (R)-MTPA group. An ORTEP picture of the molecule is shown in Fig. 1. Tables of atomic coordinates, temperature factors, bond lengths and angles have been deposited with the Cambridge Crystallographic Data Centre.*

Quinazoline **4** *Metabolism*.—Biotransformation of quinazoline **4** (1.2 g) using growing cultures of *P. putida* UV4, followed by dichloromethane extraction, yielded a crude mixture of products which was analysed by GC–MS analysis of the TMS derivatives (BP1 column, conditions identical with those used for metabolites of quinoline **1**). The metabolites were identified as quinazolin-4-ol **20** (20.5 min), *cis*-5,6-dihydroquinazoline-5,6-diol **19** (32.3 min), *cis*-5,6,7,8-tetrahydroquinazoline-5,6-diol **21** (34.1 min) and two unidentified quinazolinol isomers (20.1 and 20.5 min). Purifcation of the metabolites by preparative TLC (15% methanol–chloroform) gave a low R_f inseparable mixture of the *cis*-diols (0.052 g) **19** and **21** and a higher R_f mixture of phenols (0.043 g), including quinazolin-4-ol **20**).

cis-*5,6-Dihydroquinazoline*-5,6-*diol* **19**, δ_H(400 MHz, $[^2H_6]$-acetone) 4.42 (1 H, m, 6-H), 4.80 (1 H, d, $J_{5,6}$ 5.0, 5-H), 6.54 (1 H, d, $J_{8,7}$ 9.9, 8-H), 6.61 (1 H, dd, $J_{7,6}$ 4.1, $J_{7,8}$ 9.9, 7-H), 8.72 (1 H, s, 4-H) and 8.96 (1 H, s, 2-H). NOE difference ^1H-NMR spectroscopy confirmed the proximity of protons 4-H and 5-H as expected for structure **19**.

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* Details of the deposition scheme are available in 'Instructions for Authors,' issue 1, *J. Chem. Soc., Perkin Trans. 1*, 1993.

cis-5,6,7,8-*Tetrahydroquinazoline*-5,6-*diols* **21**, δ_H(400 MHz; [$^2H_6$]acetone) 1.9–2.2 (2 H, m, 7-H, 7'-H), 2.75 (1 H, m, 8-H), 3.09 (1 H, m, 8'-H), 4.1 (1 H, m, 6-H), 4.75 (1 H, d, $J_{5,6}$ 3.1, 5-H), 8.72 (1 H, s, 4-H) and 8.89 (1 H, s, 2-H).

The above spectral data was obtained from a mixture of *cis*-diols **19** and **21**, $[\alpha]_D$ +210 (MeOH).

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References

1 M. Dong, I. Schmeltz, E. J. La Voie and D. Hoffmann, in *Carcinogenesis*, vol. 3, *Polynuclear Aromatic Hydrocarbons*, Raven Press, New York, 1978, 97.
2 D. R. Boyd, R. J. H. Davies, L. Hamilton, J. J. McCullough and H. P. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1991, 2189.
3 D. R. Boyd, R. J. H. Davies, L. Hamilton and J. J. McCullough, *J. Chem. Soc., Perkin Trans. 1*, 1992, 31.
4 M. I. Willems, G. Dubois, D. R. Boyd, R. J. H. Davies, L. Hamilton, J. J. McCullough and P. J. van Bladeren, *Mutation Research*, 1992, **278**, 227 and references therein.
5 K. Braun and D. T. Gibson, *Appl. Environ. Microbiol.*, 1984, **48**, 102.
6 D. T. Gibson, V. Subramanian, B. D. Ensley, W. Reineke and S. F. Safe in *Microbial Degradation of Organic Compounds*, ed. D. T. Gibson, Marcel Dekker, New York, 1984, ch. 7, pp. 181–252.
7 H. A. J. Carless, *Tetrahedron Asymmetry*, 1992, **3**, 795.
8 G. N. Sheldrake in *Chirality in Industry: The Commercial Manufacture and Applications of Optically Active Compounds*, eds. A. N. Collins, G. N. Sheldrake and J. Crosby, Wiley-Interscience, New York, 1992, ch. 6.
9 D. R. Boyd, R. A. S. McMordie, H. P. Porter, H. Dalton, R. O. Jenkins and O. W. Howarth, *J. Chem. Soc., Chem. Commun.*, 1987, 1722.
10 D. R. Boyd, D. R. Bushman, R. J. H. Davies, M. R. J. Dorrity, L. Hamilton, D. M. Jerina, W. Levin, J. J. McCullough, R. A. S. McMordie, J. F. Malone and H. P. Porter, *Tetrahedron Lett.*, 1991, **32**, 2963.
11 D. R. Boyd, N. D. Sharma, R. Boyle, R. A. S. McMordie, J. Chima and H. Dalton, *Tetrahedron Lett.*, 1992, **33**, 1241.
12 A. M. Jeffery, H. J. C. Yeh, D. M. Jerina, T. R. Patel, J. F. Davey and D. T. Gibson, *Biochemistry*, 1975, **14**, 575.
13 M. N. Akhtar, D. R. Boyd, N. J. Thompson, M. Koreeda, D. T. Gibson, V. Mahadevan and D. M. Jerina, *J. Chem. Soc., Perkin Trans. 1*, 1975, 2506.
14 M. Koreeda, M. N. Akhtar, D. R. Boyd, J. D. Neill, D. T. Gibson and D. M. Jerina, *J. Org. Chem.*, 1978, **43**, 1023.
15 D. M. Jerina, P. J. van Bladeren, H. Yagi, D. T. Gibson, V. Mahadevan, A. S. Neese, M. Koreeda, N. D. Sharma and D. R. Boyd, *J. Org. Chem.*, 1984, **49**, 3621.
16 S. K. Agarwal, D. R. Boyd, R. J. H. Davies, L. Hamilton, D. M. Jerina, J. J. McCullough and H. P. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1990, 1969.
17 D. R. Bushman, J. M. Sayer, D. R. Boyd and D. M. Jerina, *J. Am. Chem. Soc.*, 1989, **111**, 2688.
18 D. R. Boyd, N. D. Sharma, R. Boyle, B. T. McMurray, T. A. Evans, J. F. Malone, H. Dalton and J. Chima, *J. Chem. Soc., Chem. Commun.*, 1993, 49.
19 J. J. McCormack, B. A. Allen and C. N. Hodnett, *J. Heterocycl. Chem.*, 1978, **15**, 1249.
20 C. Stubley, J. G. P. Stell and D. W. Mathieson, *Xenobiotica*, 1979, **9**, 475.
21 G. Bestetti, E. Galli, C. Benigni, F. Orsini and F. Pelizzoni, *Appl. Microbiol. Biotechnol.*, 1989, **30**, 252.
22 T. Omori, Y. Jigami and Y. Minoda, *Agr. Biol. Chem.*, 1974, **38**, 409.
23 D. R. Boyd, M. R. J. Dorrity, J. F. Malone, R. A. S. McMordie, N. D. Sharma, H. Dalton and P. Williams, *J. Chem. Soc., Perkin Trans. 1*, 1990, 489.
24 D. R. Boyd, N. D. Sharma, L. Hamilton and R. A. S. McMordie, manuscript in preparation.
25 E. J. La Voie, E. A. Adams, A. Shigematsu and D. Hoffmann, *Carcinogenesis*, 1983, **4**, 1169.

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