Bacterial Metabolism of 6,7-Dihydro-5*H*-benzocycloheptene by *Pseudomonas putida*. Synthesis and Absolute Configuration of Benzylic Alcohol and *cis*-Diol Metabolites of 6,7-Dihydro-5*H*-benzocycloheptene

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Metabolism of 6,7-dihydro-5*H*-benzocycloheptene (**1**) by growing cultures of a mutant strain of *Pseudomonas putida* yielded benzylic monohydroxylation (6,7-dihydro-5-hydroxy-5*H*-benzocycloheptene) (**2**) and dihydroxylation (*cis*-6,7,8,9-tetrahydro-5,6-dihydroxy-5*H*-benzocycloheptene) (**3**) products. The monoalcohol (**2**) and diol (**3**) metabolites were found to be optically pure (≥98% enantiomeric excess) by chiral stationary phase HPLC analysis and ^{1}H NMR analysis of the 2-methoxy-2-phenyl-2-trifluoromethylacetyl (MTPA) derivatives.

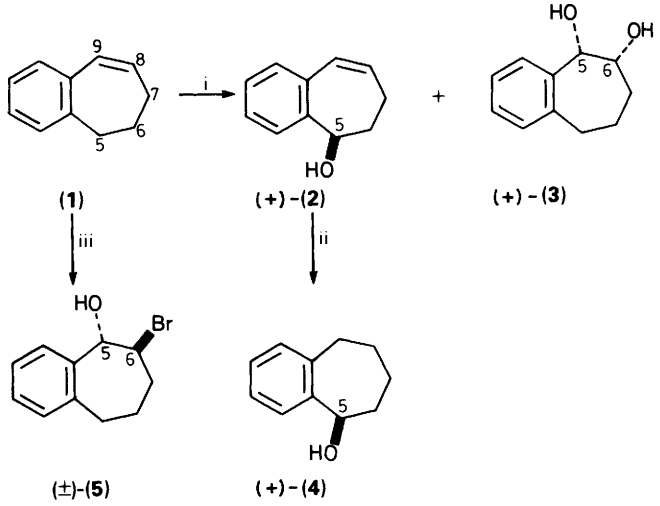
The absolute configurations of the (+)-(5*R*) alcohol (**2**) and (+)-(5*S*,6*R*)-diol (**3**) metabolites were assigned by stereochemical correlation methods to the (+)-(5*S*,6*S*)-*trans*-6-bromo-6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocycloheptene derivative (**6a**). Racemic bromohydrin (**5**) was resolved *via* the MTPA ester diastereoisomers. The absolute configuration of (−)-(5*R*,6*R*)-*trans*-6-bromo-6,7,8,9-tetrahydro-5-(2-methoxy-2-phenyl-2-trifluoromethylacetoxy)-5*H*-benzocycloheptene (**6b**) was unequivocally determined by X-ray crystallographic analysis.

Optically pure samples of the microbial metabolites (**2**), (**3**), and (**4**) have been chemically synthesised from the (+)-(5*S*,6*S*)-*trans*-bromohydrin (**5**) and stereochemically assigned.

The metabolism of arenes by bacteria to yield *cis*-dihydrodiol metabolites,[1–3] and their applications as synthetic precursors, is of current interest in many laboratories.[4–10] As part of a wider study[3] on the metabolism of bicyclic arenes by a mutant strain of *Pseudomonas putida* to yield both mono- and di-hydroxylated products, 6,7-dihydro-5*H*-benzocycloheptene (**1**) (1,2-benzosuberan) was selected as substrate. This compound afforded the opportunity for enzyme-catalysed monohydroxylation at aliphatic or aromatic carbon atoms and *cis*-diol formation at olefinic or aromatic double bonds. One problem associated with the benzylic monohydroxylation products obtained from indene[11,12] (inden-1-ol) and from 1,2-dihydronaphthalene[12] (1,2-dihydro-1-naphthol) using *P. putida* is their instability under basic and acidic conditions respectively and their tendency to form the corresponding decomposition products under the conditions of biotransformation. A preliminary report on the metabolism of the olefin (**1**) by *P. putida*[12] indicated that the monohydroxylation product (**2**) was formed as a relatively stable metabolite.

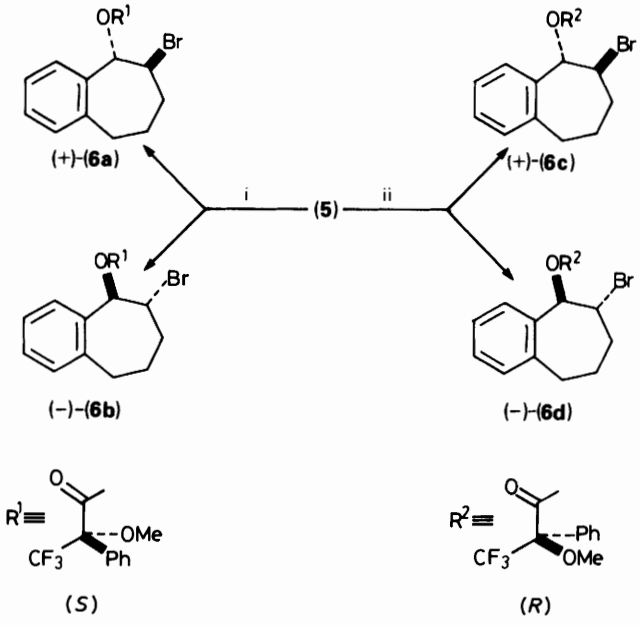
Biotransformations of compound (**1**) were carried out using cell suspensions of a mutant strain of *P. putida* (UV4) in a shake flask culture. Extraction of the centrifuged culture medium with dichloromethane yielded a mixture of two major products which were identified as the alcohol (**2**) and the *cis*-diol (**3**) (Scheme 1) by ^{1}H NMR analysis. The relative yields of the alcohol (**2**) (90%) and the diol (**3**) (10%) remained similar over the biotransformation period. When the olefin substrate (**1**) was found by HPLC analysis to have been fully utilised by the bacteria after 50 h, the products were isolated by dichloromethane extraction.

The alcohol (**2**) was isolated as a high R_f band by PLC. The structure assignment was based upon spectral comparison with an authentic synthetic sample and reported data.[13] The metabolite (**2**) was found to be optically active ($[\alpha]_D$ +162°) and of high optical purity [≥ 98% enantiomeric excess (e.e.)] by



Scheme 1. *Reagents:* i, *P. putida*, O_2; ii, H_2, Pd–C; iii, NBS, THF, H_2O.

HPLC analysis using a chiral stationary phase column (Chiralcel OB, α 1.20) and by catalytic reduction to yield the alcohol (**4**), $[\alpha]_D$ +29°. The alcohol (**4**) was found to be enantiomerically homogeneous by ^{1}H NMR analysis of the 2-methoxy-2-phenyl-2-trifluoromethylacetyl (MTPA) derivative. Alcohol (**4**) was previously[14,15] found as a metabolite ($[\alpha]_D$ −26.6°) from the microbial reduction of the corresponding ketone (benzosuberone). Alcohol (**2**) was also reported[13] as a chiral metabolite which was obtained by enantioselective hydrolysis of the corresponding racemic acetate (5-acetoxy-6,7-dihydro-5*H*-benzocycloheptene) using growing cultures of *Rhizopus nigricans*. In the latter case[13] the metabolite (**2**) appeared to be formed in lower optical yield ($[\alpha]_D$ +98.5, 62% e.e.).



Scheme 2. *Reagents:* i, (−)-MTPACl, pyridine; ii, (+)-MTPACl, pyridine.

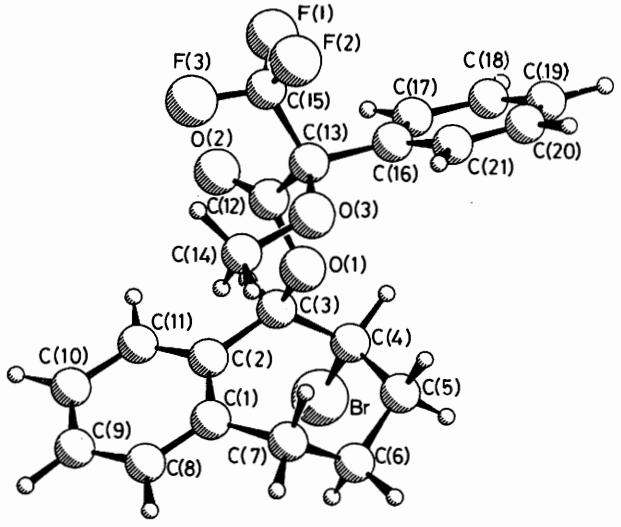


Figure. Crystal structure of the MTPA derivative (**6b**). The numbering system used here and in Tables 1 and 2 differs from that used in Schemes 1–3.

A second minor metabolite of the substrate (**1**) from *P. putida* was isolated as a low R_f component after PLC purification. Recrystallisation yielded a pure sample of the *cis*-diol (**3**), $[\alpha]_D$ +25.2°. Since this is a new metabolite of unknown optical purity and absolute configuration, assignment of its stereochemistry is required.

The previously reported configurational assignments of (−)-(5*S*) and (+)-(5*R*) to the alcohol metabolites (**4**)[14,15] and (**2**)[13] respectively were based upon conversion of the alcohol (**2**) to a dimethyl α-acetoxydicarboxylate ester[15] whose absolute configuration had been reported.[16] Since the latter absolute configuration was based solely upon an empirical comparison of signs of optical rotation of several acetonide derivatives, it was considered desirable in the present study to have an unequivocal method for determining the absolute configuration for all the chiral metabolites (**2**), (**3**), and (**4**) which were obtained from the three different types of microbial biotransformations (oxidation, reduction, and hydrolysis).

The synthesis required the initial formation of the racemic bromohydrin (**5**) from (**1**) using *N*-bromosuccinimide (NBS) in aqueous tetrahydrofuran (THF). Resolution of the bromohydrin (**5**) into enantiomers was attempted by fractional crystallisation of one diastereoisomer from a mixture of MTPA esters (**6a**)–(**6b**) (Scheme 2). Using the (−)-MTPA chloride, the less soluble bromo-MTPA diastereoisomer (**6b**), $[\alpha]_D$ −139.2°, was found to crystallise from methanol solution. Since the other diastereoisomer (**6a**) could not be obtained in pure form by fractional crystallisation the diastereomeric mixture (**6a**)–(**6b**) was separated by preparative HPLC (Zorbax ODS, MeOH–H_2O, 4:1) into an early crystalline fraction (**6b**) $[\alpha]_D$ −139.2°, and a late fraction which proved to be a viscous oil (**6a**), $[\alpha]_D$ +68°. Owing to the low α value (1.07) found in the latter HPLC separation of bromo-MTPA diastereoisomers (**6a**)–(**6b**) it was not a convenient method for obtaining large samples of the non-crystalline component (**6a**). Fortunately this difficulty was overcome by the use of (+)-MTPA chloride which yielded a mixture of diastereoisomers (**6c**)–(**6d**) from which the crystalline compound (**6c**), $[\alpha]_D$ +140.5°, could readily be obtained by fractional recrystallisation. Thus, by separately using both the (−)- and (+)-forms of MTPA chloride, it was possible to obtain sufficient quantities of the pure crystalline bromo-MTPA enantiomers (**6b**) and (**6c**).

A crystal of the bromo-MTPA ester (**6b**), $[\alpha]_D$ −139.2° was selected for crystallographic analysis. Although there are two crystallographically independent molecules in the asymmetric unit, they do not differ significantly in any detail of conformation, even in the OMTPA side-chain. The cycloheptene ring adopts a chair conformation with axial bromine and *quasi*-axial-OMTPA substituents. The absolute configuration determined relative to the known absolute configuration of (*S*)-(−)-MTPA was thus established as (5*R*,6*R*). The atomic coordinates are listed in Table 1, significant bond lengths and angles in Table 2, and the torsion angles for the cycloheptene rings in Table 3. A projection of the molecule is shown in the Figure.

Treatment of the crystalline bromo-MTPA diastereoisomer (**6c**) with di-isobutylaluminium hydride (DIBAL) yielded the parent bromohydrin enantiomer (+)-(5*S*,6*S*)-(**5**), $[\alpha]_D$ +134.8° (Scheme 3). Acetylation of the latter bromohydrin using acetic anhydride in pyridine yielded the bromoacetate (+)-(5*S*,6*S*)-(**7**), $[\alpha]_D$ +164°, which was in turn reduced with lithium aluminium hydride to give the optically pure alcohol (+)-(5*R*)-(**4**), $[\alpha]_D$ +29.7°. The stereochemical correlation in Scheme 3 thus confirms that the sample of alcohol (**4**) ($[\alpha]_D$ −26.6°) previously isolated as a metabolite from *Cryptococcus macerans*[14,15] was largely composed of the (5*S*)-enantiomer.

Acetylation of the alcohol (**4**), $[\alpha]_D$ +29.7°, with acetic anhydride in pyridine gave the acetate (**8**), $[\alpha]_D$ +78°, as a low m.p. solid. Benzylic bromination of the acetate (**8**) using NBS in carbon tetrachloride gave the bromo-acetate (**9**) as an unstable mixture of *cis*–*trans*-isomers which decomposed during attempted chromatographic purification. The crude sample of bromoacetate (**9**) was thus treated with sodium methoxide in THF to yield a sample of the alcohol (**2**). Purification by PLC and HPLC methods yielded a crystalline sample of alcohol (**2**), $[\alpha]_D$ +157°, in low yield. This sample was found to be spectrally indistinguishable from the metabolite (**2**), $[\alpha]_D$ +162°, obtained as a biotransformation product of olefin (**1**) by *P. putida*. The low yield of alcohol (**2**) was due to competition from a transannular cyclization reaction to yield a cyclic ether.

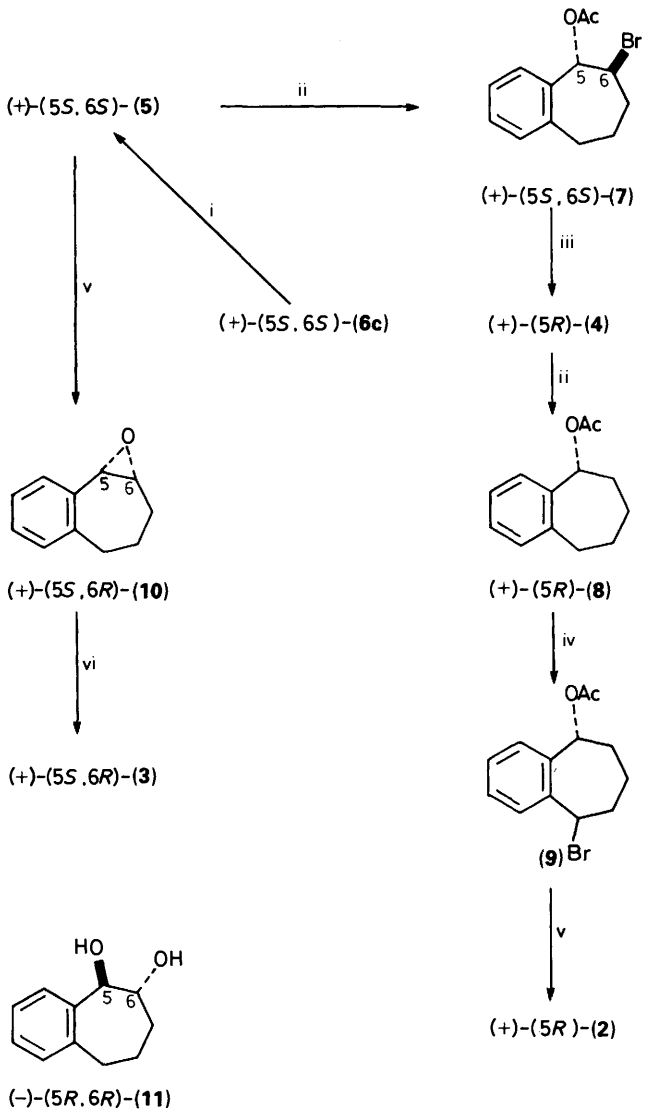
Using the (−)-(5*R*,6*R*)-diastereoisomer of the bromo-MTPA derivative (**6b**), $[\alpha]_D$ −139.2°, and identical methods, the other enantiomer of the alcohol (**4**), $[\alpha]_D$ −29.4°, and the bromohydrin (**5**), $[\alpha]_D$ −133°, were also obtained.

Table 1. Fractional atomic co-ordinates for (**6b**).

Atom	*x/a*	*y/b*	*z/c*
Br(1)	0.653 48(11)	0.872 50(0)	0.459 69(16)
F(1)	0.382 4(6)	1.203 1(10)	−0.123 8(8)
F(2)	0.390 7(7)	1.369 6(10)	−0.075 8(9)
F(3)	0.313 8(6)	1.269 0(9)	0.006 5(10)
O(1)	0.531 4(6)	1.134 1(7)	0.257 7(7)
O(2)	0.405 4(7)	1.081 8(9)	0.110 2(11)
O(3)	0.481 9(6)	1.339 5(7)	0.176 9(8)
C(1)	0.505 8(9)	1.104 2(11)	0.529 2(11)
C(2)	0.487 6(8)	1.033 8(10)	0.422 2(11)
C(3)	0.539 1(8)	1.032 2(11)	0.324 9(11)
C(4)	0.638 3(9)	1.008 9(11)	0.368 2(13)
C(5)	0.699 7(10)	1.094 6(13)	0.449 2(15)
C(6)	0.676 0(9)	1.127 8(14)	0.576 0(16)
C(7)	0.585 8(9)	1.179 6(12)	0.555 7(12)
C(8)	0.455 3(12)	1.098 5(14)	0.613 6(13)
C(9)	0.385 6(11)	1.027 3(14)	0.598 1(16)
C(10)	0.366 7(10)	0.959 8(14)	0.493 2(15)
C(11)	0.417 3(9)	0.965 8(12)	0.407 0(13)
C(12)	0.462 2(9)	1.146 1(11)	0.149 0(11)
C(13)	0.470 9(8)	1.253 7(11)	0.087 3(11)
C(14)	0.422 3(10)	1.344 3(12)	0.254 3(12)
C(15)	0.387 7(11)	1.273 6(17)	−0.028 3(18)
C(16)	0.551 2(9)	1.252 3(11)	0.036 6(12)
C(17)	0.601 4(10)	1.343 2(13)	0.034 0(14)
C(18)	0.670 2(10)	1.343 9(13)	−0.014 4(15)
C(19)	0.698 7(11)	1.252 7(17)	−0.061 3(14)
C(20)	0.651 8(11)	1.159 4(16)	−0.059 7(16)
C(21)	0.577 5(10)	1.161 4(14)	−0.014 1(12)
Br(1′)	−0.129 55(10)	0.924 61(16)	0.334 52(14)
F(1′)	0.129 2(5)	0.578 5(8)	−0.028 4(7)
F(2′)	0.101 9(6)	0.413 3(7)	−0.002 8(8)
F(3′)	0.189 0(6)	0.502 3(8)	0.151 0(9)
O(1′)	−0.011 7(6)	0.657 2(7)	0.239 3(7)
O(2′)	0.110 5(6)	0.701 9(9)	0.183 5(9)
O(3′)	0.012 8(6)	0.454 1(7)	0.174 9(8)
C(1′)	0.021 1(8)	0.695 3(10)	0.534 2(11)
C(2′)	0.038 2(8)	0.761 1(10)	0.439 5(10)
C(3′)	−0.016 4(8)	0.762 9(10)	0.300 2(9)
C(4′)	−0.115 2(9)	0.782 5(11)	0.271 0(10)
C(5′)	−0.172 7(9)	0.701 0(13)	0.314 4(15)
C(6′)	−0.147 0(9)	0.676 1(13)	0.460 8(12)
C(7′)	−0.057 3(8)	0.619 4(12)	0.508 5(13)
C(8′)	0.072 4(10)	0.704 0(14)	0.662 2(14)
C(9′)	0.147 4(10)	0.774 3(15)	0.698 1(14)
C(10′)	0.163 7(10)	0.837 5(14)	0.600 5(15)
C(11′)	0.110 7(9)	0.830 1(11)	0.475 2(13)
C(12′)	0.049 0(9)	0.642 8(12)	0.181 0(11)
C(13′)	0.034 7(8)	0.540 5(10)	0.102 3(10)
C(14′)	0.068 5(11)	0.437 2(13)	0.304 9(12)
C(15′)	0.115 2(11)	0.510 3(13)	0.061 2(15)
C(16′)	−0.047 1(8)	0.556 6(11)	−0.016 7(10)
C(17′)	−0.102 0(8)	0.469 9(12)	−0.067 1(12)
C(18′)	−0.173 8(11)	0.483 7(15)	−0.169 6(16)
C(19′)	−0.194 7(10)	0.582 6(15)	−0.228 6(12)
C(20′)	−0.141 7(12)	0.666 8(18)	−0.179 8(17)
C(21′)	−0.064 3(11)	0.652 7(14)	−0.072 0(14)

Table 2. Selected bond lengths (Å) and angles (°).

	Molecule A	Molecule B
Br–C(4)	1.957(14)	1.949(13)
C(1)–C(2)	1.411(17)	1.394(18)
C(2)–C(3)	1.489(20)	1.491(14)
C(3)–C(4)	1.521(19)	1.511(18)
C(4)–C(5)	1.538(20)	1.524(22)
C(5)–C(6)	1.564(25)	1.534(20)
C(6)–C(7)	1.517(21)	1.529(19)
C(7)–C(1)	1.533(20)	1.520(18)
C(1)–C(2)–C(3)	123.5(1.2)	124.1(1.1)
C(2)–C(3)–C(4)	120.2(1.0)	118.1(1.0)
C(3)–C(4)–C(5)	117.2(1.2)	119.3(1.1)
C(4)–C(5)–C(6)	114.3(1.3)	116.4(1.1)
C(5)–C(6)–C(7)	115.8(1.2)	112.5(1.2)
C(6)–C(7)–C(1)	116.2(1.2)	112.8(1.2)
C(7)–C(1)–C(2)	120.2(1.3)	123.8(1.0)



Scheme 3. *Reagents:* i, DIBAL; ii, Ac_2O, pyridine; iii, $LiAlH_4$; iv, NBS, CCl_4; v, NaOMe, THF; vi, H_2O–dioxane (pH 2.5).

In order to assign absolute stereochemistry to the *cis*-diol metabolite (**3**), $[\alpha]_D$ +25.2°, the stereochemical correlation sequence shown in Scheme 3, (**5**) ⟶ (**10**) ⟶ (**3**) was carried out. Treatment of the bromohydrin (**5**), $[\alpha]_D$ +134.8°, with sodium methoxide in THF yielded the epoxide (**10**), $[\alpha]_D$ +23°, as a viscous oil. Previous studies on the acid-catalysed hydrolysis of cyclic epoxides similar to compound (**10**) (*e.g.* indan-1,2-epoxide and tetralin-1,2-epoxide) found that the absolute configuration at the non-benzylic centre (C-6) remained unaltered. The acid-catalysed hydrolysis of the epoxide (**10**), $[\alpha]_D$ +23°, using perchloric acid in aqueous dioxane buffer (pH 2.5) yielded mainly (93%) the *trans*-diol (**11**), $[\alpha]_D$ −72°. Fractional crystallisation from chloroform removed most of the *trans*-diol (**11**). The minor (7%) component, the *cis*-diol (**3**), $[\alpha]_D$ +25.4°, was obtained in pure crystalline form by a combination

Table 3. Torsion angles for the cycloheptene rings.

	Molecule A	Molecule B
C(1)–C(2)–C(3)–C(4)	60.5	55.3
C(2)–C(3)–C(4)–C(5)	−70.5	−65.2
C(3)–C(4)–C(5)–C(6)	56.0	58.0
C(4)–C(5)–C(6)–C(7)	−63.5	−67.0
C(5)–C(6)–C(7)–C(1)	80.5	81.2
C(6)–C(7)–C(1)–C(2)	−57.8	−59.7
C(7)–C(1)–C(2)–C(3)	−4.1	−2.2

of PLC and recrystallisation. The diol (**3**) was found to be enantiomerically homogeneous by ^{1}H NMR analysis of the di-MTPA ester derivative and to be indistinguishable from the natural metabolite (**3**), $[\alpha]_D$ +25.2°.

Using the bromo-MPTA ester (**6b**), $[\alpha]_D$ −139.2°, and a similar sequence of reactions to that outlined in Scheme 3, the bromohydrin (**5**), $[\alpha]_D$ −133°, the epoxide (**10**), $[\alpha]_D$ −24°, the *trans*-diol (**11**), $[\alpha]_D$ +72°, and the *cis*-diol (**3**), $[\alpha]_D$ −25.6°, enantiomers of opposite chirality to those obtained from the bromo-MTPA ester (**6c**) were isolated.

It is noteworthy that the absolute configuration of the enzyme-catalysed monohydroxylation (**2**) and dihydroxylation (**3**) products at the benzylic chiral centre are identical (allowing for the change in substituent priorities in applying the sequence rule). In view of the previously reported ability of a dioxygenase enzyme to catalyse both benzylic monohydroxylation and *cis*-diol formation, and the stability of metabolites (**2**) and (**3**), compound (**1**) appears to be ideally suited for further study using pure enzymes.

Experimental

^{1}H NMR spectra were obtained using a General Electric QE300 instrument and tetramethylsilane as reference. The term 'petrol' refers to light petroleum, b.p. 40–60 °C. Analytical and preparative TLC separations were carried out using Kieselgel 60 $PF_{254+366}$. 6,7-Dihydro-5*H*-benzocycloheptene (**1**) was prepared by the reduction and subsequent dehydration of 1-benzosuberone. Both the latter compound and (+)- and (−)-MTPA acids were purchased from the Aldrich Chemical Company.

Biotransformation.—The distilled olefin (**1**) (2 g) was added to a culture of *P. putida* UV4 grown on a mineral salts medium[17] containing gluconate (12% w/v) and resuspended in the same medium but with pyruvate instead of gluconate. The biotransformation was carried out for 50 h in a two-litre Erlenmeyer flask shaken at 30 °C on an orbital shaker (400 rev/min).

Isolation and Identification of Metabolites.—The culture medium was centrifuged and the supernatant was saturated with sodium chloride prior to extraction with dichloromethane (5 × 100 ml). From the residue (0.250 g) obtained after concentration of the dichloromethane extract, two compounds (high R_f and low R_f) were isolated by PLC (petrol–diethyl ether, 1:2). The high R_f component crystallised from petrol as colourless plates (0.13 g), m.p. 56–58 °C; $[\alpha]_D$ +162° ($CHCl_3$) and was identified as 6,7-dihydro-5-hydroxy-5*H*-benzocycloheptene (**2**). The structure of compound (**2**) was deduced from a comparison of the ^{1}H NMR data with that of an authentic sample which was prepared by synthesis (see later) and the literature data.[13] The optical purity of the metabolite (**2**) was found to be ≥98% e.e. using a chiral stationary phase HPLC column (Daicel Chiralcel OB, 25 × 0.46 cm; propan-2-ol–hexane, 10:90; 0.5 ml/min; α 1.20).

Catalytic reduction (1 atm, H_2, Pd–C, 12 h) of the alcohol metabolite (**2**) (0.015 g, 0.094 mmol) in ethanol (10 ml) and removal of solvent yielded the alcohol (**4**) (0.013 g, 86%). Recrystallisation of the alcohol (**4**) from petrol gave crystals, m.p. 76–78 °C; $[\alpha]_D$ +29.0° ($CHCl_3$) (lit.,[14,15] $[\alpha]_D$ −26.6°).

The low R_f component was recrystallised from dichloromethane–pentane as fibrous needles (0.01 g), m.p. 142–143 °C, $[\alpha]_D$ +25.2° ($CHCl_3$) and was identified as (+)-*cis*-6,7,8,9-tetrahydro-5,6-dihydroxy-5*H*-benzocycloheptene (**3**). The structure of the metabolite (**3**) was confirmed by direct comparison with an authentic sample, m.p. 137–138 °C (lit.,[18] 133 °C), obtained by osmium tetroxide oxidation of olefin (**1**).

The optical purity of both the monoalcohol (**2**), $[\alpha]_D$ +162°, and the diol (**3**), $[\alpha]_D$ +25°, metabolites from *P. putida* was determined by formation of mono- and di-MTPA esters respectively. The catalytic (Pd–C) hydrogenation product (**4**), $[\alpha]_D$ +29°, obtained from alcohol (**2**) was treated with (−)-MTPA chloride (1.1 equiv.) in pyridine solvent at room temperature. Purification by PLC (diethyl ether–petrol, 1:9) yielded a high b.p. oil which was identified as the MTPA ester of alcohol (**4**) (Found: M^+, 378.144 46. $C_{21}H_{21}F_3O_3$ requires M, 378.144 26); δ($CDCl_3$) 1.50–2.16 (6 H, m, 6-, 7-, and 8-H), 2.54–2.96 (2 H, m, 9-H), 3.58 (3 H, s, OMe), 6.17 (1 H, d, $J_{5,6}$ 7.5 Hz, 5-H), and 7.00–7.50 (9 H, m, aryl H). The MTPA ester derivative of racemic alcohol (**4**) showed two clearly distinguishable singlets (δ 3.49 and 3.58) for the MeO signals of the two diastereoisomers, confirming that both the alcohol (**4**), $[\alpha]_D$ +29°, and its precursor olefin (**2**), $[\alpha]_D$ +162°, were virtually optically pure.

Similar treatment of the diol (**3**), $[\alpha]_D$ +25°, with (−)-MTPA chloride and pyridine followed by a similar purification yielded the di-MTPA ester of the diol (**3**) as a viscous oil (Found: M^+, 610.178 08. $C_{31}H_{28}F_6O_6$ requires M, 610.179 002); δ($CDCl_3$) 1.40–2.40 (6 H, m, 7-, 8-, and 9-H), 3.28 (3 H, s, OMe), 3.51 (3 H, s, OMe), 5.33 (1 H, m, 6-H), 6.07 (1 H, s, 5-H), and 6.95–7.60 (14 H, m, aryl H). A sample of the di-MTPA esters of the racemic diol (**3**) was found to have additional OMe signals at δ 3.34 and 3.48 indicating that the enzymatically formed diol (**3**), $[\alpha]_D$ +25°, was essentially optically pure.

(±) *trans*-6-Bromo-6,7,8,9-tetrahydro-5-hydroxy-5*H*-benzocycloheptene (**5**) was synthesised in 80% yield by treatment of the olefin (**1**) with *N*-bromosuccinimide in aqueous THF according to the literature method.[19]

(−)-(5R,6R)-(**6b**) *and* (+)-(5S,6S)-trans-6-*Bromo*-6,7,8,9-*tetrahydro*-5-(2-*methoxy*-2-*phenyl*-2-*trifluoromethylacetoxy*)-5H-*benzocycloheptene* (**6a**).—Treatment of the racemic bromohydrin (**5**) (5.0 g, 20.7 mmol) with (−)-MTPA chloride (5.8 g, 23 mmol) in pyridine (10 ml) at room temperature yielded the diastereoisomers (**6a**)–(**6b**) (9.0 g, 95%). Fractional recrystallisation of the mixture from methanol yielded the less soluble isomer (**6b**) (2.6 g). A small sample (0.2 g) of the residual mixture of the diastereoisomers (**6a**)–(**6b**) was separated by semi-preparative HPLC (Du Pont ODS, 25 × 0.94 cm; methanol–water, 4:1; 4 ml/min; α 1.11). The more polar diastereoisomer (**6a**) was eluted in early fractions and upon concentration was obtained as a viscous high b.p. oil in ≥98% diastereoisomeric excess, $[\alpha]_D$ +68.3° ($CHCl_3$) (Found: M^+ 456.055 73. $C_{21}H_{20}BrF_3O_3$ requires M, 456.054 83); δ($CDCl_3$) 1.62–2.40 (4 H, m, 7- and 8-H), 2.57–2.75 (2 H, m, 5-H), 3.54 (3 H, s, OMe), 4.60 (1 H, m, 6-H), 6.24 (1 H, d, $J_{5,6}$ 6.5 Hz, 5-H), and 7.03–7.40 (9 H, m, aryl H). The later fractions gave the less polar *diastereoisomer* (**6b**), which was recrystallised from methanol, m.p. 82–83 °C; $[\alpha]_D$ −139.2° ($CHCl_3$) (Found: C, 54.8; H, 4.4. $C_{21}H_{20}BrF_3O_3$ requires C, 55.2; H, 4.4%); δ($CDCl_3$) 1.65–2.33 (4 H, m, 7- and 8-H), 2.58–2.96 (2 H, m, 5-H), 3.41 (3 H, s, OMe), 4.58 (1 H, m, 6-H), 6.27 (1 H, d, $J_{5,6}$ 6.2 Hz, 5-H), and 7.10–7.40 (9 H, m, aryl H).

Similar treatment of the racemic bromohydrin (**5**) with (+)-MTPA chloride in pyridine followed by recrystallisation of the

product yielded the *bromoester* (**6c**), m.p. 82–83 °C; $[\alpha]_D$ +140.5° ($CHCl_3$) (Found: C, 55.1; H, 4.6. $C_{21}H_{20}BrF_3O_3$ requires C, 55.2; H, 4.4%). The spectral characteristics of the bromo-MTPA esters (**6c**) and (**6b**) were identical.

(−)-(5R,6R)- *and* (+)-(5S,6S)-trans-6-*Bromo*-6,7,8,9-*tetrahydro*-5-*hydroxy*-5H-*benzocycloheptene* (**5**).—Reduction of the bromo-MTPA ester (**6c**), $[\alpha]_D$ +140.5° (2 g, 4.4 mmol), with an excess of DIBAL (1M solution in hexane; 15 ml) in diethyl ether solution (40 ml) at ambient temperature for 12 h gave the bromohydrin (**5**) (0.95 g, 90%) which was recrystallised from petrol, m.p. 64–65 °C [lit.,[19] 89–91 °C (racemic)]; $[\alpha]_D$ +134.8° ($CHCl_3$).

Similar treatment of the bromo-MTPA ester (**6b**), $[\alpha]_D$ −139.2°, gave the bromohydrin (**5**), m.p. 64–65 °C; $[\alpha]_D$ −133° ($CHCl_3$). Both enantiomers of the bromohydrin (**5**) were spectrally indistinguishable from the racemic sample.

(−)-(5R,6S) *and* (+)-(5S,6R)-5,6-*Epoxy*-6,7,8,9-*tetrahydro*-5H-*benzocycloheptene* (**10**).—Cyclisation of the bromohydrin (+)-(**5**), $[\alpha]_D$ +134.8° (0.5 g, 2.07 mmol) was carried out at ambient temperature (3 h) using sodium methoxide (1.0 g) in diethyl ether (50 ml) to yield the epoxide (**10**) (0.3 g, 90%) as an oil, b.p. 50–52 °C at 0.01 mmHg (lit.,[19] 80 °C at 0.4 mmHg); $[\alpha]_D$ +23.0° ($CHCl_3$); δ($CDCl_3$) 1.51–2.24 (4 H, m, 7- and 8-H), 2.63–3.00 (2 H, m, 9-H), 3.40 (1 H, m, 6-H), 4.02 (1 H, d, $J_{5,6}$ 4.3 Hz, 5-H), and 7.06–7.51 (4 H, m, aryl H).

When the bromohydrin (−)-(**5**), $[\alpha]_D$ −133°, was treated in an identical manner the epoxide (**10**) with $[\alpha]_D$ −24.0° was obtained. The spectral data for the (+)- and (−)-enantiomers of the epoxide (**10**) were identical.

(−)-(5R,6S)- and (+)-(5S,6R)-cis-6,7,8,9-*Tetrahydro*-5,6-*dihydroxy*-5H-*benzocycloheptene* (**3**) *and* (+)-(5S,6S)- *and* (−)-(5R,6R)-trans-6,7,8,9-*Tetrahydro*-5,6-*dihydroxy*-5H-*benzocycloheptene* (**11**).—Acid-catalysed hydrolysis of the epoxide (**10**), $[\alpha]_D$ +23.0° (0.25 g, 1.56 mmol) was achieved by stirring in 40% aqueous dioxane solution (330 ml; pH 2.5) at ambient temperature for 18 h. Recrystallisation of the crude product mixture from chloroform yielded the pure *trans*-diol (**11**). The *cis*-diol (**3**) was separated from the residual *trans*-diol (**11**) by PLC (CH_2Cl_2–MeOH, 96:4; multi-elution).

The (−)-(5R,6R)-*diol* (**11**) was obtained in 81% yield (0.225 g), m.p. 129–130 °C (racemic m.p. 130–131 °C); $[\alpha]_D$ −72° (THF) (Found: C, 74.1; H, 8.2. $C_{11}H_{14}O_2$ requires C, 74.1; H, 7.9%); δ($CDCl_3$) 1.32–2.37 (4 H, m, 7- and 8-H), 1.63 (1 H, br s, OH), 2.73 (2 H, m, 9-H), 3.14 (1 H, br s, OH), 3.42 (1 H, m, 6-H), 4.72 (1 H, d, $J_{5,6}$ 9.0 Hz, 5-H), 4.75 and 7.07—7.69 (4 H, m, aryl H). The (+)-(5*S*,6*R*)-diol (**3**) was obtained in 5% yield (0.014 g), m.p. 142—143 °C [lit.,[18] 133 °C (racemic)]; $[\alpha]_D$ +25.4° ($CHCl_3$); δ($CDCl_3$) 1.55–2.24 (4 H, m, 7- and 8-H), 1.85 (1 H, br s, OH), 2.35 (1 H, br s, OH), 2.65 (1 H, m, 9-H), 3.02 (1 H, m, 9-H), 3.96 (1 H, m, 6-H), 4.94 (1 H, s, 5-H), and 7.08–7.38 (4 H, m, aryl H).

Acid-catalysed hydrolysis of the (−)-(5*R*,6*S*)-enantiomer of the epoxide (**10**) yielded the (+)-(5*S*,6*S*)-*trans*-diol (**11**), $[\alpha]_D$ +70° (THF), and the (−)-(5*R*,6*S*)-*cis*-diol (**3**), $[\alpha]_D$ −25.6° ($CHCl_3$).

(+)-(5S,6S)-trans-5-*Acetoxy*-6-*bromo*-6,7,8,9-*tetrahydro*-5H-*benzocycloheptene* (**7**).—Acetylation of the bromohydrin (**5**), $[\alpha]_D$ +134.8° (0.24 g, 1.0 mmol), using an excess of acetic anhydride in dry pyridine at ambient temperature gave the bromoacetate as a viscous high b.p. oil (0.25 g, 90%), $[\alpha]_D$ + 164° ($CHCl_3$) (Found: M^+, 282.024 94. $C_{13}H_{15}BrO_2$ requires M, 282.025 57); δ($CDCl_3$) 1.72–2.53 (4 H, m, 7- and 8-H), 2.13 (3 H, s, OAc), 2.78 (1 H, m, 9-H), 3.11 (1 H, m, 9-H), 4.58 (1 H, m, 6-H), 6.06 (1 H, d, $J_{5,6}$ 6.7 Hz, 5-H), and 7.12–7.30 (4 H, m, aryl H).

(−)-(5S)- *and* (+)-(5R)-6,7,8,9-*Tetrahydro*-5-*hydroxy*-5H-*benzocycloheptene* (**4**).—Treatment of the bromo-MTPA diastereoisomer (**6b**), $[\alpha]_D$ −139.2° (0.25 g, 0.55 mmol), using an excess of $LiAlH_4$ (0.25 g, 6.6 mmol) in dry diethyl ether (25 ml) at reflux temperature for 20 h gave the alcohol (−)-(**4**) (0.054 g, 60%), m.p. 76–78 °C (petrol); $[\alpha]_D$ −29.4° ($CHCl_3$) [lit.,[20] m.p. 102–103 °C (racemic)], $[\alpha]_D$ −26.6°, 98% e.e.[14,15] The ^1H NMR spectrum was indistinguishable from that of the racemic sample.

Similar treatment of the (+)-bromoacetate (**7**), $[\alpha]_D$ +164°, gave the alcohol (**4**), $[\alpha]_D$ +29.7° ($CHCl_3$), in 70% yield.

(+)-(5R)-5-*Acetoxy*-6,7,8,9-*tetrahydro*-5H-*benzocycloheptene* (**8**).—Treatment of the alcohol (**4**), $[\alpha]_D$ +29.7° (0.2 g, 1.23 mmol), with acetic anhydride in pyridine yielded the *acetate* (**8**) (0.225 g, 90%), m.p. 23–24 °C (petrol); $[\alpha]_D$ +78.0° ($CHCl_3$) (Found: C, 75.9; H, 8.0. $C_{13}H_{16}O_2$ requires C, 76.4; H, 7.9%).

(+)-(5R)-5-*Hydroxy*-6,7,8,9-*tetrahydro*-5H-*benzocycloheptene* (**2**).—The acetate (**8**), $[\alpha]_D$ +78° (0.2 g, 0.98 mmol), was treated with NBS (0.192 g, 1.08 mmol) in refluxing CCl_4 (15 ml) for 0.5 h in the presence of azo isobutyronitrile (0.005 g) to give a crude yield of bromoacetate (**9**) isomers of *ca.* 90% as a viscous oil (M^+, *m/z* 283); δ($CDCl_3$) 1.68–2.53 (12 H, m, 6-, 6′-, 7-, 7′-, 8-, and 8′-H), 2.10 (3 H, s, OAc), 2.20 (3 H, s, OAc′), 5.50 (2 H, m, 9- and 9′-H), 5.96 (1 H, d, $J_{5,6}$ 6.2 Hz, 5-H), 6.42 (1 H, d, $J_{5',6'}$ 10.7 Hz, 5′-H), and 7.16–7.45 (8 H, m, aryl H). All attempts at chromatographic purification of this mixture of bromoacetates (**9**) resulted in decomposition and thus it was used as the crude product mixture in the following step. The bromoacetates (**9**) (0.15 g, 0.58 mmol) were thus converted into the alcohol product (**2**) by stirring in THF solution (10 ml) in the presence of sodium methoxide (0.2 g, 3.7 mmol) at ambient temperature for 4 h. The crude product mixture was purified by PLC (diethyl ether–petrol, 1:1) and subsequently by reverse phase HPLC (Du Pont ODS, 25 × 0.46 cm, MeOH–H_2O, 85:15; 0.9 ml/min) to yield crystals of the alcohol (**2**) (0.01 g, 12%), m.p. 56–58 °C (petrol), $[\alpha]_D$ +157° ($CHCl_3$). The synthetic sample of the alcohol (**2**), $[\alpha]_D$ +157° was found to be identical both chromatographically and spectroscopically with the metabolite (**2**), $[\alpha]_D$ +162°.

Crystal Data for (**6b**).—$C_{21}H_{20}O_3F_3Br$, M = 457.3. Monoclinic, a = 15.697(16), b = 12.585(13), c = 10.705(11) Å, β = 106.7(1)°. U = 2 025.4 Å^3, space group $P2_1$, Z = 4, D_c = 1.50 g cm^{-3}, $F(000)$ = 928, λ(Mo-K_α) = 0.710 69 Å, thin colourless hexagonal prisms, crystal dimensions 1.8 × 0.9 × 0.35 mm, μ(Mo-K_α) = 20.0 cm^{-1}.

Data Collection, Analysis, and Refinement.—Stöe STADI2 diffractometer, graphite-monochromated Mo-K_α radiation, ω-scan mode, scan width 2.0°, scan speed 4.0° min^{-1}, 2 941 unique reflections measured, $3 \leqslant \theta \leqslant 30°$. Direct methods and difference Fourier syntheses (SHELX); least-squares refinement, non-hydrogen atoms anisotropic; hydrogens included at calculated positions (C–H 1.08 Å) with common isotropic thermal parameters for methyl, methylene, tertiary CH, and phenyl-type hydrogens which refined to values of 0.08(2), 0.061(1), 0.03(3), and 0.08(1) Å^2 respectively. In the final cycles the 2 213 data with $F > 4\sigma(F)$ yielded an R value of 0.069, R_w = 0.068. The weighting scheme was $w = 1.56/[\sigma^2(F) + 0.0028\,F^2]$. Atomic co-ordinates are in Table 1, selected bond lengths and angles in Table 2, and torsion angles in Table 3.

Tables of bond lengths and angles, anisotropic thermal parameters, and hydrogen atom co-ordinates are deposited with the Cambridge Crystallographic Data Centre.*

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