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Structures and Stereochemical Assignments of Some Novel Chiral Synthons Derived from the Biotransformation of 2,3-Dihydrobenzofuran and Benzofuran by *Pseudomonas putida*

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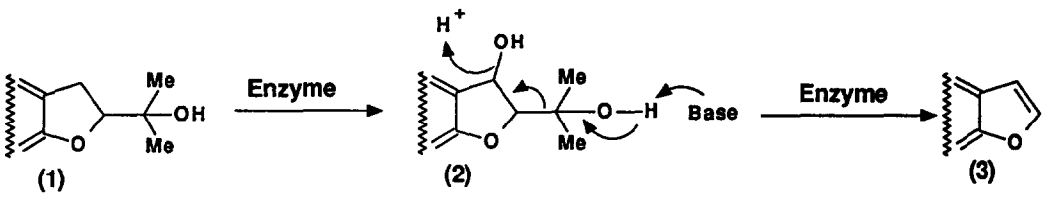
Abstract: Metabolism of 2,3-dihydrobenzofuran using intact cells of *Pseudomonas putida* UV4 gave mainly (3S)-3-hydroxy-2,3-dihydrobenzofuran which was in turn oxidized to an unstable intermediate, (3S,4R,5S)-3,4,5-trihydroxy-2,3,4,5-tetrahydrobenzofuran. Spontaneous dehydration of this *cis,cis*-triol occurred in the carbocyclic ring to give (3S)-3,5-dihydroxy 2,3-dihydrobenzofuran and in the heterocyclic ring to yield (4R,5S)-*cis*-4,5-dihydroxy-4,5-dihydrobenzofuran. Bacterial metabolism of benzofuran was found to occur in the carbocyclic ring to form (6S,7S)-*cis*-6,7-dihydroxy-6,7-dihydrobenzofuran and its dehydration product, 6-hydroxybenzofuran. Dioxygenase-catalysed *cis*-dihydrodiol formation in the heterocyclic ring of benzofuran (to give *cis*-2,3-dihydroxy-2,3-dihydrobenzofuran as a transient intermediate) is proposed to account for the appearance of (1R)-1,2-dihydroxy-1-(2'-hydroxyphenyl)ethane as a major metabolite of benzofuran. *cis*-4,5-Dihydroxy-4,5-dihydrobenzofuran and *cis*-6,7-dihydroxy-6,7-dihydrobenzofuran are potentially valuable chiral synthons which can be added to the small pool of bicyclic *cis*-dihydrodiol metabolites currently available for synthesis.

Introduction

The 2,3-dihydrobenzofuran and benzofuran type of ring structures occur extensively among natural products *e.g.* furanocoumarins and furanoquinoline alkaloids.[1] The enzyme-catalysed benzylic hydroxylation of aryl substituted 2,3-dihydrofurans bearing a 1-hydroxy-1-methylethyl group has been identified as a key step in the biosynthesis of the furan ring system (*e.g.* **1** --> **2** --> **3**).[2,3] The enzyme-catalysed oxidation of a dihydrofuran ring has also been implicated in the chemically-induced carcinogenesis associated with aflatoxins (*via* a transient epoxide intermediate) [4].



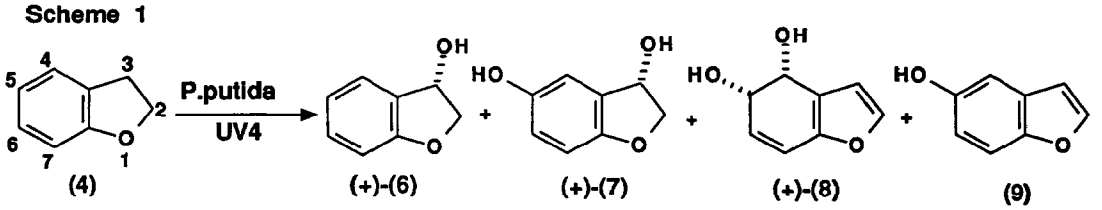
Enzyme-catalysed hydroxylation and epoxidation of arenes in mammalian, plant and fungal systems generally involve monooxygenase enzymes.[5] Oxidative metabolism of arenes can also be catalysed by

bacterial dioxygenase enzyme systems to yield both monohydroxylation and *cis*-dihydroxylation products.[6] In this paper is reported the oxidative metabolism of 2,3-dihydrobenzofuran (**4**) and benzofuran (**5**) by a mutant strain (UV4) of the soil bacterium *Pseudomonas putida* to yield a range of monol, diol and phenolic products[7] whose structures and stereochemistry (enantiomeric excess and absolute configuration) have been unambiguously determined

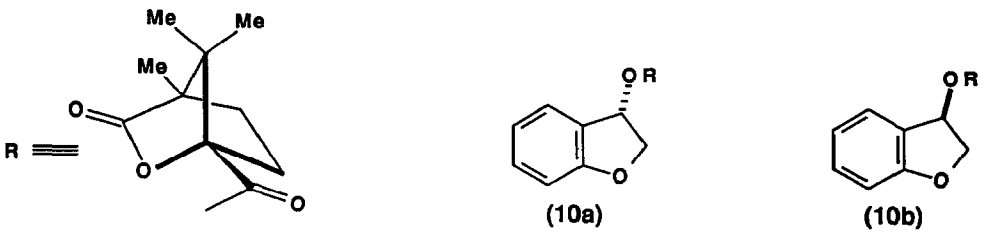
Results and Discussion

Biotransformation of 2,3-dihydrobenzofuran (**4**) by growing cultures of *P. putida* UV4 using the previously reported method,[8] followed by extraction with EtOAc, yielded the crude mixture of products (**6-9**) which was separated by preparative TLC (Scheme 1).

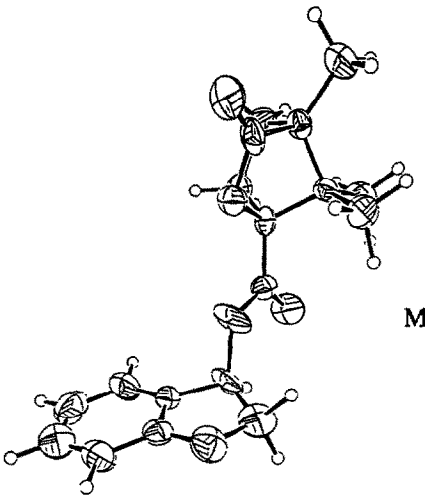
Scheme 1



The least polar compound turned out to be achiral, and was identified as 5-hydroxybenzofuran (**9**) by spectral methods. The major metabolite proved to be both chiral and more polar, and was identified as 3-hydroxy-2,3-dihydrobenzofuran (**6**) by spectral comparison with an authentic sample. This metabolite, $[\alpha]_D$ + 47 , was found to have an enantiomeric excess (e.e.) of *ca.*73% by chiral stationary phase (CSP) HPLC analysis and $[\alpha]_D$ comparison with an optically pure sample of alcohol (**6**) obtained by chemical resolution *via* the camphanate diastereoisomers. Thus, formation of the camphanate esters (**10b**), $[\alpha]_D$ -109 and (**10a**), $[\alpha]_D$ + 55 , separation by fractional crystallization, and subsequent alkaline hydrolysis yielded the individual enantiomers, (-)-(**6**), $[\alpha]_D$ -67° , and (+)-(**6**), $[\alpha]_D$ + 67° respectively .



An X-ray crystal structure analysis of the camphanate ester (**10b**), $[\alpha]_D$ -109 showed that, relative to the (1'S) camphanate group, alcohol (-)-(**6**) had the (3R) configuration and thus the bacterial metabolite (+)-(**6**) was predominantly of the (3S) configuration (Figure 1).



Molecular structure of compound (**10b**)

Figure 1

The two more polar metabolites of 2,3-dihydrobenzofuran (**4**), isolated as very minor products, were tentatively identified as the dihydroxy compounds (**7**) and (**8**) on the basis of limited chromatographic and spectral data. Compounds (**7-9**) were however isolated in larger quantities from the metabolism of the alcohol (**6**). Addition of the racemic alcohol (**6**) as substrate gave varying yields of metabolites (**7-9**) and recovered substrate . Thus a large scale biotransformation of substrate (**6**) (7.0 g) gave metabolites (**7-9**) in a total yield of 16% (recovered substrate in 42% yield and of < 5% e.e.), while a small scale biotransformation gave a higher yield of metabolites (*ca.* 25%) and a very low recovery of substrate (**6**) (2% yield and 60% e.e. of the 3S enantiomer). Past studies[9] on racemic benzylic alcohol derivatives of benzocycloalkene substrates with *P. putida* UV4 showed varying degrees (0-100%) of preferential metabolism of one enantiomer. The optically active metabolite (**7**) was unequivocally identified as 3,5-dihydroxy-2,3-dihydrobenzofuran, $[\alpha]_D$ + 14.3 , from spectral analysis of the metabolite and its derivatives. CSP-HPLC separation of metabolite (**7**) into enantiomers indicated that it was of 70% e.e. ^{1}H-N.m.r. spectral analysis of its di-MTPA esters confirmed the % e.e. value. Phenolic alcohol (**7**) was assigned a (3S) configuration by c.d. spectral comparison with (+)- (3S)-3-hydroxy-2,3-dihydrobenzofuran (**6**) (Figure 2).

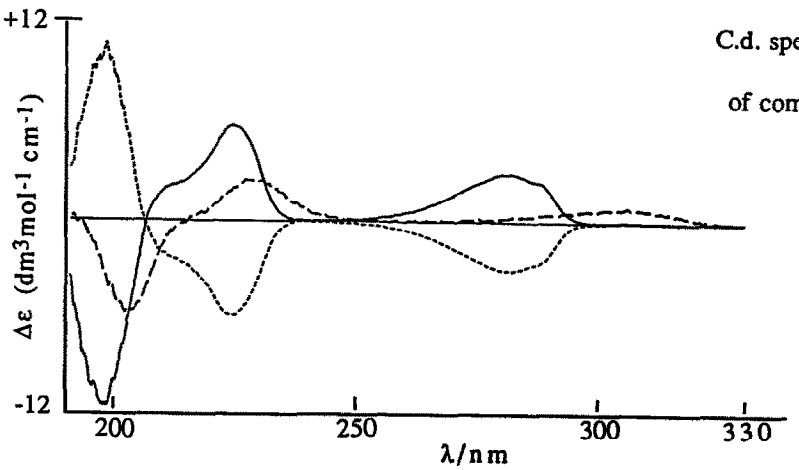
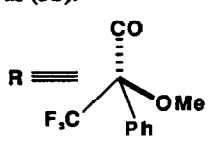
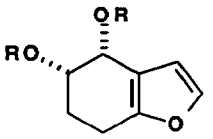


Figure 2

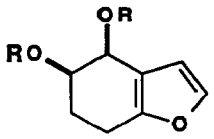
When the (+) and (-)-enantiomers of 3-hydroxy-2,3-dihydrobenzofuran (**6**), synthesised by the literature method[10] and separated by CSP-HPLC, were each used as substrate with *P. putida* UV4, the enantiomerically homogeneous phenolic alcohol metabolites (+)-(**7**), $[\alpha]_D$ +22.5 , and (-)-(**7**) respectively were produced (Scheme 2). The yield of metabolite (-)-(**7**)[and (**9**)] obtained using substrate (-)-(**6**) was very low(< 5%) and an accurate $[\alpha]_D$ value could not be obtained. Use of the pure enantiomers of alcohol (**6**) of known absolute configurations as bacterial substrates allowed the absolute configuration of metabolite (+)-(**7**) to be confirmed as (3S).



MTPA



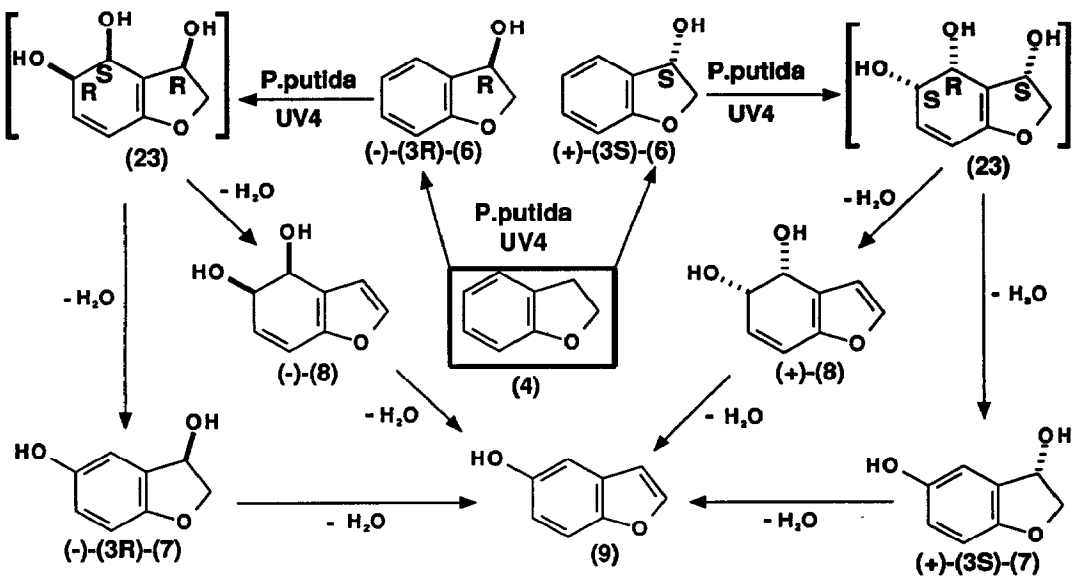
(12a)



(12b)

The most polar metabolite (R_f 0.36), isolated when racemic and (+) forms of 3-hydroxy-2,3-dihydrobenzofuran (**6**) were used as substrates with *P.putida* UV4, was identified as (+)-*cis*-4,5-dihydroxy-4,5-dihydrobenzofuran (**8**) (Scheme 2). This structural assignment was primarily based upon ^{1}H-n.m.r. spectral data which showed the characteristic *cis*- coupling constant ($J_{4,5}$ 5.9 Hz) and an NOE interaction between the protons at C-3 and C-4 (C<u>H</u>-OH).

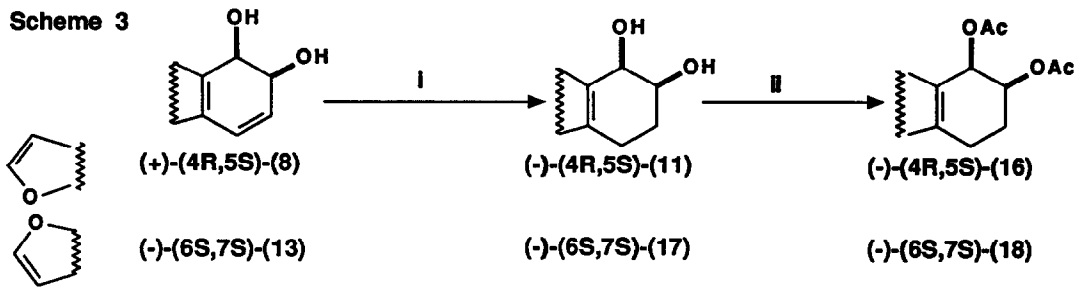
Scheme 2



The e.e. value and absolute configuration of the *cis*-dihydrodiol metabolite (+)-(**8**) were determined *via* catalytic hydrogenation to yield a *cis*-tetrahydrodiol (-)-(**11**) (Scheme 3). Esterification using the acid chloride derivative of (+)-(R)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) acid yielded a mixture of diastereoisomers (**12a,12b**) in the ratio 93:7 (from the ^{1}H-n.m.r. spectral analysis). This ratio represents an e.e. value of 86% for the *cis*-dihydrodiol metabolite (**8**), $[\alpha]_D$ +16 , derived from the racemic substrate (**6**). The *cis*-

dihydrodiol metabolite (+)-(8) derived from enantiomerically homogeneous (+)-3-hydroxy-2,3-dihydrobenzofuran (6) was, however, a single enantiomer (from ^{1}H-n.m.r. analysis of its di-MTPA ester)[9].

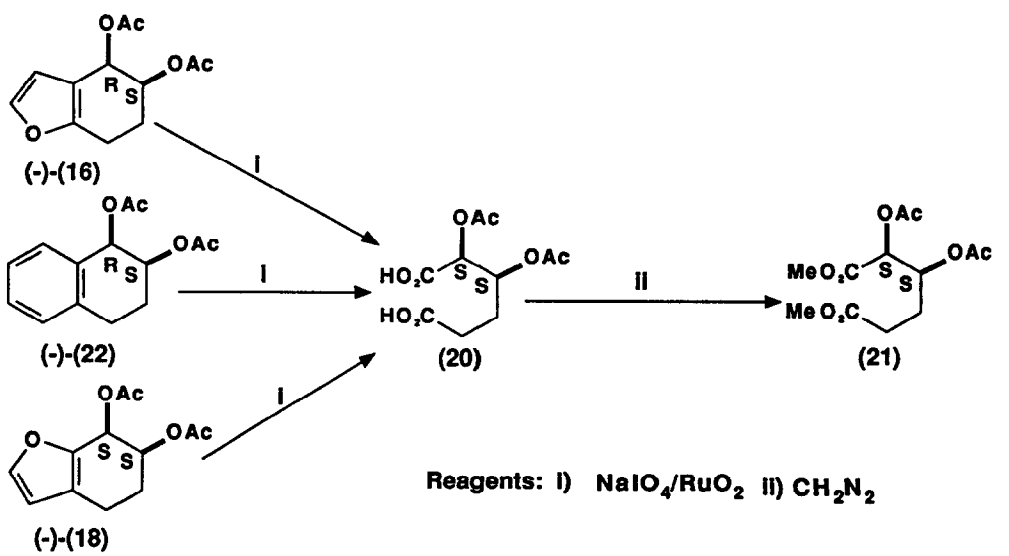
Scheme 3



Reagents: I) H_2 / Pd-C II) Ac_2O/ Pyridine

Treatment of the *cis*-tetrahydrodiol (-)-(11) with acetic anhydride (Scheme 3) to yield the *cis*-tetrahydrodiacetate (-)-(16), followed by oxidative cleavage of the aryl ring with $RuO_2/NaIO_4$ (Scheme 4), gave the ring-degradation product (20) which was converted directly (CH_2N_2) to dimethyl(2,3-diacetoxy)adipate (21), $[\alpha]_D$ -11.3 . When a homochiral sample of (-)-*cis*-1,2-diacetoxy-1,2,3,4-tetrahydronaphthalene (22) ($[\alpha]_D$ -170) of established (1R,2S)-absolute configuration[11,12], was similarly degraded and derivatised ($RuO_2/NaIO_4$; CH_2N_2) product (21) (now $[\alpha]_D$ -14) was obtained (Scheme 4). Thus a (2S,3S) configuration for (-)-dimethyl(2,3-diacetoxy)adipate (21) and a (4R,5S) configuration for the *cis*-dihydrodiol (+)-(8) were established.

Scheme 4



A comparison of the optical yields and absolute configurations of the chiral metabolites (6), (7) and (8), isolated from metabolism of substrates (4) and (6) by *P. putida* UV4, led us to speculate on the sequence

of reactions involved in their biosynthesis (Scheme 2). The initial bioconversion step involves benzylic oxidation of 2,3-dihydrobenzofuran (**4**) to yield an excess of the (+)-(3S) enantiomer (**6**). It is probable that, as in the benzylic hydroxylation of indan[6], a dioxygenase enzyme is involved in the biotransformation of 2,3-dihydrobenzofuran (**4**) into alcohol (+)-(**6**).

Formation of the *cis*-dihydrodiol (+)-(4R,5S)-(**8**) could also be accounted for by the subsequent dioxygenase-catalysed oxidation of alcohol (+)-(**6**) to form the unstable triol intermediate, (3S,4R,5S)-3,4,5-trihydroxy-2,3,4,5-tetrahydrobenzofuran (**23**) (Scheme 2). Spontaneous dehydration of the triol (**23**) in the heterocyclic ring will then give the *cis*-dihydrodiol metabolite. Biotransformation of the bicyclic arene substrate, 1,2-dihydronaphthalene by *P. putida* UV4 has been postulated[13] to occur *via* a similar oxidative metabolic sequence (monol --> triol --> diol).

The e.e. value (*ca.* 86%) of *cis*-dihydrodiol (+)-(**8**), isolated as a metabolite from racemic 3-hydroxy-2,3-dihydrobenzofuran (**6**), is consistent with a preferential rate of oxidation of the (+)-(3S) enantiomer of alcohol (**6**) to yield the (3S,4R,5S)-*cis,cis*-triol intermediate (**23**) and dehydration to give compound (+)-(**8**) [compared with the oxidation of (-)-(3R) enantiomer to the (3R,4S,5R) *cis,cis*-triol enantiomer (**23**) and dehydration to *cis*-dihydrodiol (-)-(**8**)].When substrate (-)-(**6**) was metabolised, the yield of compound (-)-(**7**) was very low(3%), the recovery of substrate was relatively high(38%), and none of the *cis*-diol (-)-(**8**) was isolated. Indirect evidence for its formation could however be taken from detection of phenol (**9**). A large scale biotransformation on substrate (-)-(**6**) should thus yield an enantiopure sample of *cis*-diol (-)-(**8**).

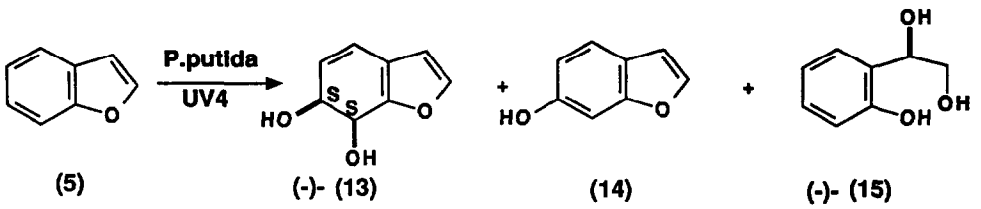
The faster rate of oxidation of the(-)-(3R) enantiomer of alcohol (**6**) to the corresponding ketone, and loss of the latter volatile metabolite during the biotransformation and subsequent workup , could account for the excess of the(+)-(3S) enantiomer recovered from the metabolism of the racemic substrate (**6**). The volatile ketone , 3(2H)-benzofuranone, was identified as a minor component by g.c.-m.s, analysis.

The e.e. value (*ca.* 70%) of phenolic alcohol (+)-(3S)-(**7**) from alcohol ± (**6**) and the exclusive formation of the (3S) and (3R) enantiomers of compound (**7**) respectively from the (+)-(3S) and (-)-(3R) enantiomer substrates (**6**), can again be explained by the monol --> triol oxidation *i.e.* (3S)-(**6**) --> (3S,4R,5S)-(**23**)[or (3R)-(**6**) --> (3R,4S,5R)-(**23**)] (Scheme 2). Spontaneous dehydration within the carbocyclic ring of triol enantiomers [(3S,4R,5S)-(**23**) and (3R,4S,5R) (**23**)] would thus account for the formation of phenolic alcohols (+)-(3S)-(**7**) and (-)-(3R)-(**7**). A faster rate of enzyme-catalysed oxidation of the (+)-(3S) enantiomer of alcohol (**6**) to triol (**23**) may thus explain both the large excess of the (+)-(3S) enantiomer of phenolic alcohol (**7**) (70% e.e.) and the (+)-(4R,5S) enantiomer of the *cis*-dihydrodiol (**8**) (86% e.e.).

Phenolic compounds (**7**) and (**9**) appear to be derived from the common intermediate triol (**23**). While phenol (**9**) could in principle be formed by dehydration of compound (**7**) it is more likely that it originated from the less stable *cis*-diol metabolite (**8**). Compounds (**6**) and (**7**) could be regarded as arene hydrates of the benzofurans (**5**) and (**9**), respectively. Preliminary studies on the dehydration of the 3-hydroxy-2,3-dihydrobenzofurans (**6**) and (**7**) to yield the corresponding benzofurans (**5** and **9**) indicate that this is a relatively slow process and is thus less likely to occur during the biotransformation and isolation procedures.

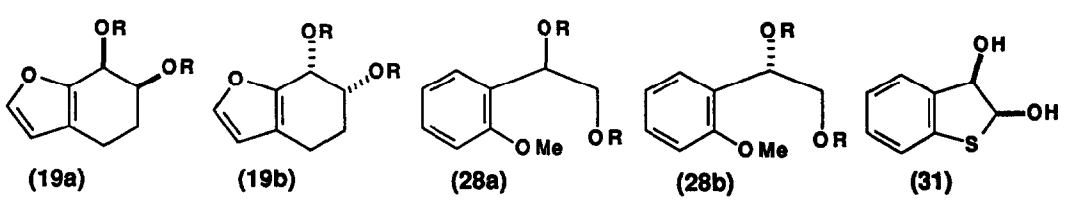
When benzofuran (**5**) was added as substrate to *P. putida* UV4 cultures and the bioproducts were extracted and purified by preparative TLC, three major metabolites were isolated (Scheme 5). None of these metabolites appeared to have been formed during biotransformation of 2,3-dihydrobenzofuran (**4**) and thus it is improbable that benzofuran (**5**) was formed as a metabolite of substrate (**4**)(Scheme 2).

Scheme 5



The least polar metabolite (R_f 0.83) was identified as 6-hydroxybenzofuran (**14**) by spectral analysis. A second more polar (R_f 0.39) metabolite (**13**) was found to decompose to 6-hydroxybenzofuran (**14**) when allowed to stand at ambient temperature. Metabolite (**13**), $[\alpha]_D$ -35 , showed ^{1}H-n.m.r. signals consistent with a *cis*-dihydrodiol structure (NOE at the alkene 4-H signal when the 3-H signal was irradiated) and it was identified as *cis*-6,7-dihydroxy-6,7-dihydrobenzofuran (**13**) which is isomeric with *cis*-dihydrodiol (**8**). Catalytic hydrogenation of *cis*-dihydrodiol (-)-(**13**) gave *cis*-tetrahydrodiol (**17**), $[\alpha]_D$ -91.5 , (Scheme 3). Esterification of the tetrahydrodiol (**17**) with (+)-(R)-MTPA acid gave a single di-MTPA diastereoisomer (**19a**) consistent with a homochiral sample of *cis*-tetrahydrodiol (-)-(**17**). In order to show that the other di-MTPA diastereoisomer (**19b**) [which would be formed if the enantiomer (+)-(**17**) were present] was distinguishable from (**19a**) by ^{1}H-n.m.r. spectral analysis, the tetrahydrodiol (-)-(**17**) was esterified with (-)-(S)-MTPA acid. The di-MTPA ester thus obtained ,which was the enantiomer of (**19b**), proved to be spectrally distinguishable. The absolute configuration of the *cis*-dihydrodiol metabolite (-)-(**13**) was determined by acetylation of the *cis*-tetrahydrodiol (-)-(**17**) to give the *cis*-tetrahydrodiacetate (-)-(**18**) (Scheme 3) followed by oxidative cleavage and subsequent methylation to yield (-)-dimethyl(2,3-diacetoxy)adipate (**21**), $[\alpha]_D$ -13.2 , (Scheme 4). This sequence established that the *cis*-dihydrodiol (-)-(**13**) was of the (6S,7S) configuration and was enantiomerically pure.

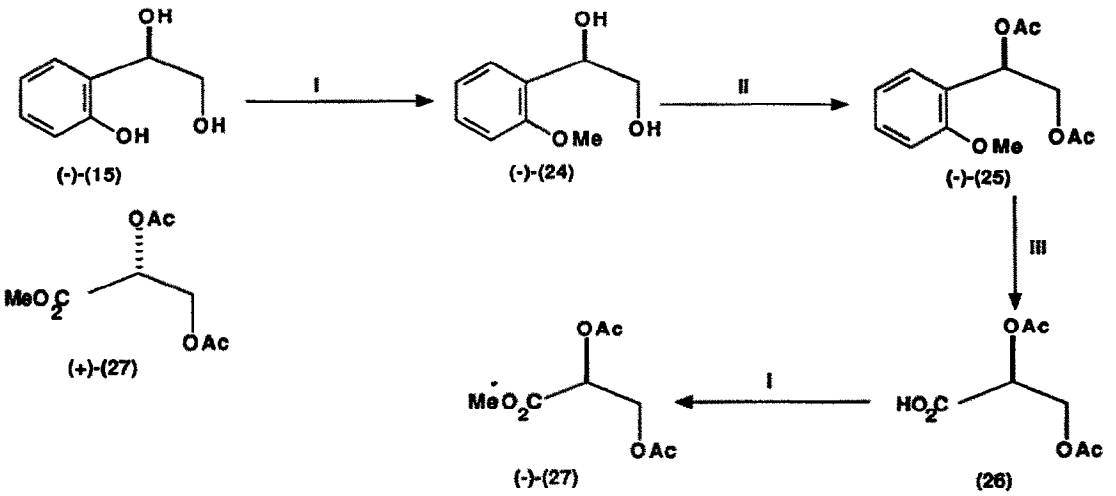
The most polar metabolite of benzofuran (**5**) (R_f 0.29) was identified as 1,2-dihydroxy-1-(2'-hydroxyphenyl)ethane (**15**), $[\alpha]$ -24 , by spectral analysis of the metabolite and its derivatives. This compound has also been found as a flavone metabolite in cultures of *Rhizopus nigricans*.[14] The trihydroxy metabolite (**15**), $[\alpha]_D$ -24°, was treated with diazomethane to yield the monomethyl ether (**24**), $[\alpha]_D$ -23 , which was reacted with (+)-(R)-MTPA acid to produce a mixture of diastereoisomers (**28a, 28b**) in the ratio 75:25. From the diastereoisomeric ratio the phenolic diol metabolite (**15**) was considered to have an e.e. value of *ca.* 50%.



R = MTPA

Conversion of metabolite (-)-(15) to the monomethyl ether (-)-(24), followed by acetylation to yield the diacetate (-)-(25), $[\alpha]_D$ -22 , oxidation with ruthenium tetroxide to give the carboxylic acid (26) and treatment with diazomethane,afforded methyl(2,3-diacetoxy)propanoate (-)-(27), $[\alpha]_D$ -8.0 (Scheme 6)

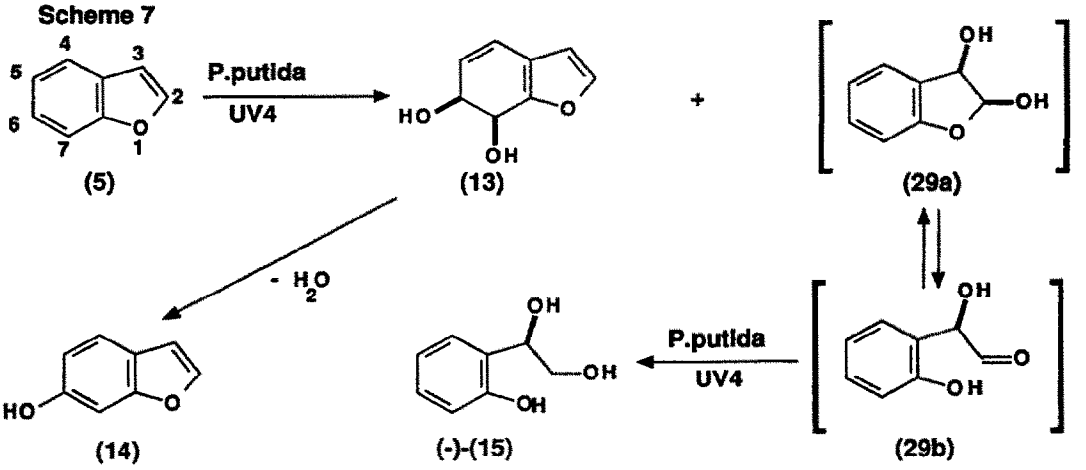
Scheme 6



Reagents: I) CH_2N_2 II) Ac_2O/pyridine III) $NaIO_4/RuO_2$

A sample of triester (27), $[\alpha]_D$ +16 , was synthesised from (R)-glyceric acid hemicalcium salt and was found to have the (2R) configuration. The bacterial metabolite (-)-(15) was thus formed with an excess (*ca.* 50%) of the (1R) enantiomer.

Scheme 7



The mechanism of formation of 6-hydroxybenzofuran (**14**) may be analogous to that of 5-hydroxybenzofuran (**9**) and could be accounted for by dehydration of the *cis*-dihydrodiol metabolite (**13** or **8**) (Scheme 7)

The (S) configuration at the allylic position of the (-)-*cis*-dihydrodiol metabolite (**13**) fits the pattern of absolute stereochemistry observed for all homochiral *cis*-dihydrodiol metabolites resulting from bacterial oxidation of the polycyclic arenes *e.g.* naphthalene,[11] anthracene,[15] phenanthrene,[16] benz[a]anthracene,[17] quinoline[18] and benzo[b]thiophene.[19]

A possible biosynthetic pathway linking benzofuran to the trihydroxy metabolite (**15**) is shown in Scheme 7. This sequence has been postulated as a result of the recent observation[19] that benzo[b]thiophene is partially oxidized to (2R,3R)-2,3-dihydroxy-2,3-dihydrobenzothiophene (**31**) in cultures of *P. putida* UV4. It is reasonable to assume that benzofuran (**5**) will be similarly oxidized to yield 2,3-dihydroxy-2,3-dihydrobenzofuran (**29a**) as an unstable intermediate. The hemiacetal metabolite (**29a**) is expected to exist in equilibrium with the acyclic aldehyde tautomer (**29b**). Enzyme-catalysed reduction of aldehyde (**29b**) could account for the isolation of (-)-1,2-dihydroxy-1-(2'-hydroxyphenyl)ethane (**15**) as a metabolite.

The formation of metabolite (**15**) with a strong preference (75%) for the (1R) enantiomer could be consistent with the oxidation of benzofuran (**5**) to yield mainly the (2R,3R) enantiomer of *cis*-diol (**29a**). A similar (2R,3R) configuration was observed for the *cis*-diol obtained by oxidation of the heterocyclic ring in benzo[b]thiophene.[19] The possibility of enantioselective enzyme-catalysed reduction of the aldehyde enantiomers cannot however be excluded and this factor could affect the final e.e. value and configuration of metabolite (**15**).

Summary

This study has demonstrated the value of dioxygenase enzymes (from intact cultures of *P. putida* UV4) in the synthesis of potentially useful chiral metabolites of benzofuran and derivatives. *cis*-Dihydrodiols of benzofuran have been obtained by enzyme-catalysed oxidation either directly from benzofuran (metabolite **13**) or indirectly from 2,3-dihydrobenzofuran (metabolite **8**). Benzylic hydroxylation to yield 3-hydroxy-2,3-dihydrobenzofuran (**6**), followed by further oxidation to a transient *cis,cis*-triol intermediate (**23**) and subsequent dehydration, may account for the formation of the phenolic metabolites (**7**) and (**9**). An unstable *cis*-diol intermediate (**29a**) is postulated as the biosynthetic precursor of the trihydroxy metabolite (**15**). The chiral metabolites isolated (**6,7,8,13** and **15**) were generally found to be of high enantiomeric excess (70-98%) and their absolute configurations have been unequivocally established. The enantiomers of 3-hydroxybenzofuran (**6**) have been chemically resolved *via* their camphanate diastereoisomers.

Experimental

General experimental procedures. Biotransformations of substrates (**4**), (**5**), (±)-(**6**), (+)-(**6**) and (-)-(**6**) with growing cultures of *Pseudomonas putida* UV4 were carried out as previously reported.[8] Products were obtained by repeated manual extraction (EtOAc) of the NaCl-saturated culture medium from biotransformations, and careful concentration of the extract under reduced pressure. ^{1}H-N.m.r. were recorded using $CDCl_3$ as solvent (unless stated otherwise) at 300MHz, 500 MHz (General Electric QE300 and GNΩ-

500) and 400MHz (Bruker WP400) instruments. Flash chromatography and preparative t.l.c. were carried out with Merck Kieselgel 60 (250-400 mesh) and Merck Kieselgel $PF_{254 \text{ and } 366}$ respectively. Analytical t.l.c. was performed on Merck Kieselgel 60_{254} plates. Mass spectra were recorded at 70eV on a AEI-MS902 spectrometer updated by V.G. Instruments. Accurate molecular weights were determined by the peak-matching method, using perfluorokerosene as standard reference. Circular dichroism spectra were recorded on a JASCO J720 instrument. A Perkin-Elmer 214 polarimeter was used for all $[\alpha]_D$ measurements. Samples of 2,3-dihydrobenzofuran (**4**), benzofuran (**5**) ,(+)-(R) and (-)-(S)-MTPA acids and (-)-(1S)-camphanic chloride (Aldrich Chemical Co.) and the hemicalcium salt of (R)-glyceric acid (Fluka Chemical Co.) were purchased from the sources specified. (-)-(1R,2S)-*cis*-1,2-Diacetoxy-1,2,3,4-tetrahydronaphthalene (**22**) was available from previous work.[13,20]

Isolation and Identification of Bacterial Metabolite.

(a) 2,3-Dihydrobenzofuran Substrate (4). T.l.c. analysis (EtOAc:hexane, 75:25) of the crude extract, obtained from the biotransformation of 2,3-dihydrobenzofuran (0.16g, 1.3mmol), showed the presence of at least four components (R_f: 0.78, 0.66, 0.47, 0.36). Preparative t.l.c. (silica-gel) was used to separate the four components into pure metabolites (**9**), (**6**), (**7**) and (**8**) respectively.

5-Hydroxybenzofuran (9) The title compound was obtained as a low-melting solid (0.009g, 9%); R_f 0.78, m.p. 56-58°C (from diethyl ether/hexane); (Found: M^+ 134.0363 $C_8H_8O_2$ requires 134.0368); δ_H(500 MHz) 6.67 (1H, dd, $J_{3,2}$ 2.2 Hz, $J_{3,4}$ 0.7Hz, 3H), 6.82 (1H, dd, $J_{6,7}$ 8.8 Hz, $J_{6,4}$ 2.6Hz, 6-H), 7.02 (1H, d, $J_{4,6}$ 2.6 Hz, 4-H), 7.35 (1H, d, $J_{7,6}$ 8.8 Hz, 7-H), 7.59 (1H, d, $J_{2,3}$ 2.2 Hz, 2-H); m/z (%) 134 (M^+, 100). Phenol (**9**) was also obtained from spontaneous decomposition of metabolite (**8**).

(+)-(3S)-3-Hydroxy-2,3-dihydrobenzofuran (6). This alcohol was obtained as a yellow solid (0.019 g, 11%); R_f 0.66; m.p. 51-52°C (from diethyl ether/hexane) (lit.,[10] b.p. 122°C/15 mm Hg); $[\alpha]_D$ +47 (*c* 1.70, $CHCl_3$); δ_H(300 MHz) 2.92 (1H, br s, OH), 4.28(1H, dd, $J_{2,2'}$ 10.7 Hz, $J_{2,3}$ 2.5 Hz, 2-H), 4.40 (1H, dd, $J_{2,2'}$ 10.7 Hz, $J_{2',3}$ 6.5 Hz, 2'-H), 5.17 (1H, dd, $J_{3,2'}$ 6.5 Hz, $J_{3,2}$ 2.5 Hz, 3-H), 6.82 (1H, d, $J_{7,6}$ 8.1 Hz, 7-H), 6.90(1H, m, 5-H), 7.22(1H, m, 6-H), 7.34 (1H, d, $J_{4,5}$ 7.7 Hz, 4-H). The ^{1}H-n.m.r. spectral data of the isolated metabolite (+)-(**6**) was identical to that of an authentic sample prepared by synthesis.[10]

The e.e. value (73%) of metabolite (+)-(**6**) was determined by CSP-HPLC analysis [Chiralcel OB (250 x 4 mm) column, 10% propan-2-ol in hexane, 0.5 ml/min]. Small quantities of each enantiomer of alcohol (**6**) were also separated and isolated by CSP-HPLC (α 2.57). While the Chiralcel OB column gave an excellent separation of the enantiomers (+)-(**6**) and (-)-(**6**), the column was found to have a very short lifetime despite using recommended solvents only. Stereochemical correlation studies established the absolute configuration of bioproduct (+)-(**6**) as (3S) (see later).

3,5-Dihydroxybenzofuran (7) and cis-4,5-Dihydroxy-4,5-dihydrobenzofuran (8). The most polar metabolites were tentatively identified as 3,5-dihydroxybenzofuran (**7**) (R_f 0.47) and *cis*-4,5-dihydroxy-4,5-dihydrobenzofuran (**8**) (R_f 0.36) from their ^{1}H-n.m.r. spectra. Since the compounds were present as minor metabolites in the crude mixture (<1%), an insufficient quantity of material was available for full characterization.

However, larger samples of the dihydroxylation products (7) and (8), obtained by metabolism of 3-hydroxy-2,3-dihydrobenzofuran (6) as substrate, allowed their complete structural analysis [see *(b)*.]. Thus the presence of dihydroxy compounds (7) and (8) as minor metabolites of 2,3-dihydrobenzofuran (4) was confirmed.

(b) (±)-3-Hydroxy-2,3-dihydrobenzofuran Substrate (6). A large scale biotransformation of racemic 3-hydroxy-2,3-dihydrobenzofuran (6) (7.0g, 0.05mol,) over 20h followed by the normal isolation procedure gave mainly the recovered substrate (2.96g, 42%) which was found to be < 5% e.e by CSP-HPLC analysis. The metabolites (9) (R_f 0.78, 0.31g, 4%), (7) (R_f 0.47, 0.55g, 7%) and (8) (R_f 0.36, 0.43g, 5%) were separated from the substrate (6) (R_f 0.66) by flash chromatography and by preparative t.l.c. A subsequent small scale biotransformation (0.08g, 24 h) of racemic alcohol (6) gave recovered alcohol (+)-(6) (0.0018g, 2%, 60% e.e.), metabolites (7) (8) (9) (0.020g, 25%) and traces of the ketone, 3(2H)-benzofuranone. The two samples of metabolites (9) (5-hydroxybenzofuran), isolated from the metabolism of both substrates (4) and (6) were spectrally indistinguishable.

(+)-(3S)-3,5-Dihydroxy-2,3-dihydrobenzofuran (7). This metabolite was isolated as an oil (0.55 g, 7%), $[\alpha]_D$ +14.3 (*c* 1.41, MeOH); (Found: M^+ 152.0483 $C_8H_8O_3$ requires 152.0473); δ_H (500MHz, $(CD_3)_2CO$), 4.25 (1H, dd, $J_{2,2'}$ 10.2 Hz, $J_{2,3}$ 3.1 Hz, 2-H), 4.46 (1H, dd, $J_{2',2}$ 10.2 Hz, $J_{2',3}$ 6.9 Hz, 2'-H), 4.52(1H, br s, 3-OH), 5.30 (1H, dd, $J_{3,2'}$ 6.9 Hz, $J_{3,2}$ 3.1 Hz, 3-H), 6.60 (1H, d, $J_{7,6}$ 8.4 Hz, 7-H), 6.70 (1H, dd, $J_{6,7}$ 8.4 Hz, $J_{6,4}$ 2.6 Hz, 6-H), 6.88(1H, d, $J_{4,6}$ 2.6 Hz, 4-H), 7.88 (1H, br s, 5-OH); m/z(%) 152 (M^+, 31), 134 (M^+-H_2O, 5), 86(12), 31(100). The e.e. value was estimated to be *ca.* 70% by CSP-HPLC (Chiralcel OB, 15% propan-2-ol in hexane, 0.5 ml/min, α1.29) and ^{1}H-n.m.r. spectral analysis of its di-MTPA esters. Metabolite (+)-(7) was assigned the (3S) configuration by c.d. spectral comparison with (+)-(3S)-3-hydroxy-2,3-dihydrobenzofuran (6). The configuration for metabolite (+)-(7) was unequivocally confirmed by later experiments. A comparison of $[\alpha]_D$ values of metabolite (7), derived from the racemate ($[\alpha]_D$ + 14.3) and from the (3S)enantiomer ($[\alpha]_D$ + 22.5) of substrate (6), gave a somewhat similar (*ca.* 64% e.e.), but less reliable e.e. value compared with the CSP-HPLC/^{1}H-n.m.r. method.

(+)-(4R,5S)-cis-4,5-Dihydroxy-4,5-dihydrobenzofuran (8). The *cis*-dihydrodiol (8) (0.43g, 5%) was obtained as a crystalline white solid, m.p. 101-103°C (from chloroform/hexane) $[\alpha]_D$ +16° (*c* 0.92, MeOH); (Found: C, 62.9; H, 5.3. $C_5H_8O_3$ requires C, 63.2; H, 5.2%); δ_H (300 MHz) 2.40(2H,br s,OH), 4.54 (1H, m, 5-H), 4.63(1H, d, $J_{4,5}$ 5.9 Hz, 4-H), 5.80 (1H, dd, $J_{6,7}$ 10.0 Hz, $J_{6,5}$ 2.6 Hz, 6-H), 6.39 (1H, dd, $J_{7,6}$ 10.0Hz, $J_{7,5}$ 2.3Hz, 7-H), 6.50 (1H, d, $J_{3,2}$ 1.8 Hz, 3-H), 7.29 (1H, d, $J_{2,3}$ 1.8Hz, 2-H); m/z(%) 152(M^+, 92), 134(M^+-H_2O, 73), 123(100).Confirmation of the structural assignment for *cis*-dihydrodiol (8) was provided by nuclear Overhauser enhancement (NOE) of selected ^{1}H signals (400 MHz). Thus, irradiation of the ^{1}H-n.m.r. signal at 6.50δ (3-H) showed enhancement at 7.29δ (2-H, 1.2%) and 4.63δ (4-H, 0.6%).Catalytic hydrogenation (10% Pd/C in EtOAc, 1-atmosphere, r.t.) of *cis*-dihydrodiol (8) to yield the *cis*-tetrahydrodiol (11), $[\alpha]_D$ -92 (see later) followed by treatment with the acid chloride derived from (+)-(R)-MTPA acid yielded a diastereoisomeric mixture of di-MTPA esters (12a/12b). ^{1}H-N.m.r. analysis gave a diastereoisomeric ratio of 93:7 which corresponded to an e.e. value of 86% for metabolite (8).

Fractional crystallization of the diastereoisomeric mixture (12a/12b) from diethyl ether/hexane yielded a pure sample of the major diastereoisomer (12a), m.p. 95-96°, (Found: C, 57.3; H, 3.9. $C_{28}H_{29}F_6O_7$ requires

C, 57.3; H, 4.1%); δ_H (500MHz) 2.17(1H, m, 6-H), 2.29 (1H, m, 6'-H), 2.80(2H, m, 7-H and 7'-H), 3.30(3H, s, OMe), 3.50 (3H, s, OMe), 5.45 (1H, ddd, $J_{5,6'}$ 12.1 Hz, $J_{5,6} = J_{5,4}$ 3.3 Hz, 5-H), 6.03 (1H, d, $J_{4,5}$ 3.3 Hz, 4-H), 6.39 (1H, d, $J_{3,2}$ 1.7 Hz, 3-H), 7.21-7.42 (9H, m, Ar-H), 7.53(2H, m, Ar-H).The ^{1}H-n.m.r. spectrum of the minor diastereoisomer (**12b**) showed different chemical shifts at 3.34 (3H, s, OMe), 3.46 (3H, s, OMe), 6.23 (1H, d, $J_{4,5}$ 3.3 Hz, 4-H). The absolute configuration of the *cis*-dihydrodiol (+)-(**8**)was initially given as (4R,5S) from the ^{1}H-n.m.r. spectral characteristics[7] of the di-MTPA esters **12a/12b** and was later unequivocally established by the stereochemical correlation sequence outlined in Scheme 4.

(c) (+)-(3S)-3-Hydroxy-2,3-dihydrobenzofuran Substate (6). A small-scale biotransformation of the (3S)-3-hydroxy-2,3-dihydrobenzofuran (**6**) (0.049g, 0.36mmol, $[\alpha]_D$ +67) was carried out over a 22h period. The normal extraction and preparative t.l.c. separation of the crude mixture of bioproducts gave:

5-Hydroxybenzofuran (9), (R_f 0.78, 0.006 g, 13%) .

(+)-*(3S)-3-Hydroxy-2,3-dihydrobenzofuran* (*6*), (R_f 0.66, 0.009 g,18%, >98% e.e. by CSP-HPLC analysis)

(+)-*(3S)-3,5-Dihydroxy-2,3-dihydrobenzofuran (7),* (R_f 0.47, 0.012 g, 22%, $[\alpha]_D$ +22.5 (*c* 0.67, MeOH), >98% e.e. by CSP-HPLC and ^{1}H-n.m.r. analysis of the di-MTPA ester.

(+)-(4R,5S)-cis-4,5-Dihydroxy-4,5-dihydrobenzofuran (8), (R_f 0.36, 0.002 g, 4%), was assigned the (4R,5S) configuration by analysis of the ^{1}H-n.m.r. spectral data of the di-MTPA ester (**12a**) derived from the *cis*-tetrahydrodiol (**11**). The analysis also showed that the *cis*-dihydrodiol was >98% e.e.

(d) (-)-(3R)-3-Hydroxy-2,3-dihydrobenzofuran Substrate (6). A small-scale biotransformation of (-)-(3R)-3-hydroxy-2,3-dihydrobenzofuran (**6**) (0.048g, 0.35mmol, $[\alpha]_D$ -67 , 22h) followed by the normal isolation procedure afforded :

5-Hydroxybenzofuran (9),(R_f 0.78, <0.001 g, <2%).

(-)-*(3R)-3-Hydroxy-2,3-dihydroxybenzofuran (6),* (R_f 0.66, 0.018 g, 38 , >98% e.e. by CSP-HPLC analysis).

(-)-(3R)-3,5-dihydroxy-2,3-Dihydrobenzofuran (7), (R_f 0.47, 0.002g, 3%). An insufficient quantity of metabolite (**7**) was available for accurate $[\alpha]_D$ measurement. However it was shown, by ^{1}H-n.m.r. spectral analysis of its di-MTPA ester, to be of > 98% e.e. and of the (3R) configuration.

(e) Benzofuran Substrate (5). A medium scale biotransformation of benzofuran (**5**) (2.25 g, 19mmol, 24h) and separation of the crude bioproducts by ethyl acetate extraction followed by flash chromatography on silica-gel [dichloromethane (100%) --> dichloromethane:methanol (93:7)] gave three metabolites (eluted in the

sequence **14**, **13**, and **15**) which were further purified by multiple elution preparative t.l.c. [silica-gel, ethyl acetate: hexane, 55:45].

6-Hydroxybenzofuran (14). This least polar metabolite (R_f 0.83) was obtained as a colourless oil (0.30 g, 12%) (lit.[21] oil); δ_H (500 MHz) 6.69 (1H, dd, $J_{3,2}$ 2.2 Hz, $J_{3,4}$ 1.1 Hz, 3-H), 6.80 (1H, dd, $J_{5,4}$ 8.4 Hz, $J_{5,7}$ 2.2 Hz, 5-H), 7.00 (1H, br d, $J_{7,5}$ 2.2 Hz, 7-H), 7.42 (1H, d, $J_{4,5}$ 8.4 Hz, 4-H), 7.52(1H, d, $J_{2,3}$ 2.2 Hz, 2-H); m/z(%) 134(M^+, 100). Spontaneous decomposition of metabolite (**13**) at ambient temperature also resulted in the formation of 6-hydroxybenzofuran (**14**).

(-)-(6S,7S)-cis-6,7-Dihydroxy-6,7-dihydrobenzofuran (13). The *cis*-diol metabolite (**13**) (R_f 0.39) was isolated as a white solid (0.98g, 34%), m.p. 61-63°C (decomp.) (from chloroform/hexane), $[\alpha]_D$ -35 (*c* 0.96, MeOH); (Found: M^+ 152.0483 $C_8H_8O_3$ requires 152.0473); δ_H (300MHz) 3.99(2H, br s, OH), 4.57-4.65 (2H, m, 6-H and 7-H), 5.67(1H, dd, $J_{5,4}$ 9.6 Hz, $J_{5,6}$ 2.1 Hz, 5-H), 6.24 (1H, dd, $J_{4,5}$ 9.6 Hz, $J_{4,6}$ 2.5 Hz, 4-H), 6.29(1H, d, $J_{3,2}$ 1.8 Hz, 3-H), 7.35 (1H, d, $J_{2,3}$ 1.8 Hz, 2-H); m/z (%) 152 (M^+, 100), 134 (M^+-H_2O, 29), 123(98). Confirmation of structural assignment to the *cis*-diol (**13**) was provided by an NOE experiment (400MHz, C_5D_5N). Thus, irradiation at 6.43δ (3-H) gave enhancement of signals at 7.60δ (2-H, 1.7%) and 6.37δ (4-H, 1.0%). Catalytic hydrogenation [10% Pd/C in EtOAc, 1-atmosphere, r.t.] of *cis*-dihydrodiol (**13**) to yield the corresponding *cis*-tetrahydrodiol (**17**) $[\alpha]_D$ -91.5°, (Scheme 3) followed by treatment with the acid chloride derivative of (+)-(R)-MTPA acid gave the diastereoisomer (**19a**) as an oil which decomposed on silica-gel during chromatography. (Found: M^+ 586.1458. $C_{28}H_{24}F_6O_7$ requires 586.1426); δ_H (300MHz) 2.08-2.18 (2H, m, 5-H and 5'-H), 2.63 (2H, m, 4-H and 4'-H), 3.34 (3H, s, OMe), 3.50 (3H, s, OMe), 5.51 (1H, m, 6-H), 6.20 (1H, d, $J_{3,2}$ 2.0 Hz, 3-H), 6.36 (1H, d, $J_{7,6}$ 3.7 Hz, 7-H), 7.25-7.65 (11H, m, Ar-H); m/z(%) 586(M^+,<1), 352(18), 189(100). The ^{1}H-n.m.r. spectrum of ester (**19a**) was consistent with the presence of only one enantiomer of *cis* -dihydrodiol (**13**) *i.e.* >98% e.e. This conclusion was confirmed by esterification of (+)-(**17**) with (-)-(S)-MTPA acid to yield the di-MTPA ester (**19b**); δ_H (300 MHz), 1.94 (2H, m, 5-H and 5'-H), 2.60 (2H, m, 4-H and 4'-H), 3.38(3H, s, OMe), 3.53(3H, s, OMe), 5.51 (1H, m, 6-H), 6.24 (1H, d, $J_{3,2}$ 2.0 Hz, 3-H), 6.47 (1H, d, $J_{7,6}$ 3.6 Hz, 7-H), 7.12-7.64 (11H, m, Ar-H). The absolute configuration of the *cis*-dihydrodiol metabolite (-)-(**13**) was tentatively assigned as (6S,7S) by ^{1}H-n.m.r. spectral analysis of (**19a**) in comparison with (**19b**) and was later confirmed by following the stereochemical correlation sequence shown in Scheme 4.

(-)-(1R)-1,2-Dihydroxy-1-(2'-hydroxyphenyl)ethane (15). Metabolite (**15**) was isolated as a low-melting solid (R_f 0.29, 0.93 g, 32%), m.p. 69-71°C, (lit.[13] oil) which resisted attempts at recrystallization), $[\alpha]_D$ -24 (*c* 0.76, MeOH); δ_H(500 MHz, $(CD_3)_2CO$), 3.58 (1H, dd, $J_{2a,2b}$ 10.7 Hz, $J_{2a,1}$ 7.8 Hz, 2a-H), 3.73(1H, dd, $J_{2b,2a}$ 10.7 Hz, $J_{2b,1}$ 3.9 Hz, 2b-H), 5.00(1H, dd, $J_{1,2a}$ 7.8 Hz, $J_{1,2b}$ 3.9 Hz, 1-H), 6.80(2H, m, 3'-H and 5'-H), 7.09 (1H, ddd, $J_{4',3'} = J_{4',5'}$ 7.9 Hz, $J_{4',6'}$ 1.8 Hz, 4'-H), 7.26 (1H, dd, $J_{6',5'}$ 7.5 Hz, $J_{6',4'}$ 1.8 Hz, 6'-H); m/z(%) 154 (M^+, 19), 123(100), 107(17), 77(28).The absolute configuration of metabolite (-)-(**15**) was shown to be (1R) by following the stereochemical correlation sequence drawn in Scheme 6 . Conversion of the phenolic OH group in metabolite (-)-(**15**) to a methyl ether (**24**) using an excess of CH_2N_2 (0°C, 12h) followed by diesterification with the acid chloride of (+)-(R)-MTPA acid gave the diastereoisomeric mixture (**28a/28b**) as a viscous oil (Found: M^+ 600.1559. $C_{29}H_{26}F_6O_7$ requires 600.1583). The major diastereoisomer (**28a**) showed the following ^{1}H-n.m.r. spectral characteristics: δ_H(500MHz), 3.40 (3H, s, OMe), 3.45 (3H, s, OMe),

3.84 (3H, s, 2'-OMe), 4.54 (1H, dd, $J_{2a,2b}$ 12.2 Hz, $J_{2a,1}$ 7.8 Hz, 2a-H), 4.67 (1H, dd, $J_{2b,2a}$ 12.2 Hz, $J_{2b,1}$ 2.5 Hz, 2b-H), 6.55 (1H, dd, $J_{1,2a}$ 7.8 Hz, $J_{1,2b}$ 2.5 Hz, 1-H), 6.79 (1H, dd, $J_{5',4} = J_{5',6}$ 7.5 Hz, 5'-H), 6.88 (1H, d, $J_{3',4'}$ 8.4 Hz, 3'-H), 6.93 (1H, dd, $J_{6',5'}$ 7.5 Hz, $J_{6',4'}$ 1.5 Hz, 6'-H), 7.29-7.44 (11H, m, Ar-H). The minor diastereoisomer (**28b**) showed different characteristic ^{1}H n.m.r. signals at δ3.38 (3H, s, OMe), 3.42(3H, s, OMe), 3.86(3H, s, 2'-OMe), 6.70 (1H, dd, $J_{1,2a}$ 7.0 Hz, $J_{1,2b}$ 3.1 Hz, 1-H), m/z(%) 600(M^+, 4), 367(25), 189(100).From the di-MTPA ester ratio (**28a:28b**) of 75:25 an e.e. value of *ca.* 50% was estimated for the metabolite (-)-(**15**) while the (1R) absolute configuration was assigned by using the stereochemical correlation sequence shown in Scheme 6 .

Absolute Configuration Determination of Metabolites

(i) (+)-(3S)-3-Hydroxy-2,3-dihydrobenzofuran (6)

Racemic 3-hydroxy-2,3-dihydrobenzofuran[10] (**6**) (0.32g, 2.4mmol) was treated at room temperature with (-)-(1S)-camphanic chloride (0.58g, 2.7mmol) in dry pyridine (5ml) to yield a crystalline mixture of camphanate diastereoisomers (**10a/10b**) (0.66 g, 90%); (Found: C, 68.7, H, 6.4 $C_{18}H_{20}O_5$ requires C, 68.4, H, 6.3%); m/z(%) 316(M^+, 30), 119(110). Fractional recrystallization from diethyl ether/hexane gave pure diastereoisomer (**10b**), m.p. 107-108°C; $[\alpha]_D$ -109 (*c* 1.25, $CHCl_3$); δ_H(300 MHz) 0.90 (3H, s, Me), 0.93 (3H, s, Me), 1.09(3H, s, Me), 1.62-1.71 (1H, m, CH_2), 1.84-2.05 (2H, m, CH_2), 2.35-2.43 (1H, m, CH_2), 4.56(1H, dd, $J_{2,2'}$ 11.7 Hz, $J_{2,3}$ 2.2 Hz, 2-H), 4.65 (1H, dd, $J_{2',2}$ 11.7 Hz, $J_{2',3}$ 6.4 Hz, 2'-H), 6.41 (1H, dd, $J_{3,2'}$ 6.4 Hz, $J_{3,2}$ 2.2 Hz, 3-H), 6.94 (2H, m, 5-H and 7-H), 7.32 (1H, m, 6-H), 7.46 (1H, d, $J_{4,5}$ 7.7 Hz, 4-H). Diastereoisomer (**10a**) was obtained as the more soluble isomer from the enriched mother liquor by further fractional crystallizations, m.p. 90-92°C; $[\alpha]_D$ +55 (*c* 1.23, $CHCl_3$); δ_H(300MHz), 0.83 (3H, s, Me), 1.01(3H, s, Me), 1.09(3H, s, Me), 1.62-1.71 (1H, m, CH_2), 1.85-2.04 (2H, m, CH_2), 2.36-2.44 (1H, m, CH_2), 4.62 (2H, m, 2-H and 2'-H), 6.41 (1H, dd, $J_{3,2}$ 6.1 Hz, $J_{3,2'}$ 2.6 Hz, 3-H), 6.93 (2H, m, 5-H and 7-H), 7.31 (1H, m, 6-H), 7.45 (1H, d, $J_{4,5}$ 7.4 Hz, 4-H).The absolute configuration of diastereoisomer (**10b**), $[\alpha]_D$ -109 , was determined by X-ray crystal structure analysis as (3R) (Figure 1) relative to the camphanate ester group [derived from (1S)-camphanic chloride].

Crystal Data for the Camphanate Ester (10b):

$C_{18}H_{20}O_5$, M=316.4, orthorhombic, $\underline{a}$ = 24.020(11), $\underline{b}$ = 9.262(8), $\underline{c}$ = 7.295(4)Å, U = 1622.9(1.8)Å^3, Z = 4, space group $P2_12_12$ (No.18), F(000) = 672, D_c = 1.29g cm^{-3}, $\mu(MoK_\alpha)$ = 0.9 cm^{-1}, $\lambda(MoK_\alpha)$ = 0.71073Å. Cell dimensions and the orientation matrix for data collection were obtained from least squares refinement of the setting angles of 27 reflections, in the range $8<2\theta<23°$, using a Siemens P3/V2000 diffractometer. 2170 unique data were measured using $\theta/2\theta$ scans, θ scan range 1.2° + α_1/α_2 splitting, $3\leq2\theta\leq55°$, scan speed 5-15° min^{-1}. The intensity of a standard reflection was measured after every 100 data and showed no significant change. The structure was determined by the direct methods procedures of SHELXS86 and refined by full-matrix least squares (SHELX76) with allowance for anisotropic thermal parameters for non-hydrogen atoms. The configuration of the molecule was chosen to conform to the *known* (1S-) absolute configuration of the camphanate moiety. This established the absolute configuration at C-3 as R. All hydrogens were located in a difference Fourier synthesis but were included in the final stages of refinement at positions calculated from the geometry of the molecule (C-H = 1.08Å) with common isotropic temperature factors for methyl, methylene,

tertiary CH and aromatic hydrogens which refined to final values of U = 0.11(1), 0.09(1), 0.08(3) and 0.09(2)Å^2, respectively. In the final cycles the 953 reflections with I>2σ(I) gave R = 0.070, R_w = 0.064 with weighting scheme w = 2.54/[σ^2(Fo) + 0.000543 Fo^2]. The maximum residual electron density was 0.09 eÅ^{-3}. An ORTEP projection of the molecule is shown in Figure 1 . Tables of atomic coordinates, thermal parameters and all derived results have been deposited with The Director, Cambridge Crystallographic Data Centre.

The diastereoisomer (**10b**) (0.059g, 0.19mmol, $[\alpha]_D$ -109°) was hydrolysed by refluxing (2.5h) in aqueous THF (0.2ml water in 4ml THF) containing potassium hydroxide (0.047g, 0.82mmol). The crude product was purified by preparative t.l.c. [silica-gel, diethyl ether:hexane, 50:50], followed by recrystallization from diethylether-hexane to yield (-)-(3R)-3-hydroxy-2,3-dihydrobenzofuran (**6**), (0.018g, 72%); m.p. 57-58°C; $[\alpha]_D$ -67 (*c* 0.51, $CHCl_3$).

Similar treatment of the camphanate diastereoisomer of (3S) configuration (**10a**) (0.089g, 0.28mmol, $[\alpha]_D$ +55) gave (+)-(3S)-3-hydroxy-2,3-dihydrobenzofuran (**6**), (0.026g, 68%); m.p. 57-58°C, $[\alpha]_D$ +67 (*c* 0.63, $CHCl_3$). Both (+) and (-) enantiomers of alcohol (**6**) were spectrally indistinguishable from the racemic sample and the metabolite ($[\alpha]_D$ +47) isolated from the biotransformation of 2,3-dihydrobenzofuran (**4**).

(ii) (+)-(4R,5S)-cis-4,5-Dihydroxy-4,5-dihydrobenzofuran (8) and (-)-(6S,7S)-cis-6,7-Dihydroxy-6,7-dihydrobenzofuran (13)

The *cis*-dihydrodiol metabolite (+)-(**8**) (0.151g, 0.99mmol, $[\alpha]_D$ +16°) in ethyl acetate solution (8ml, containing one drop of triethylamine) was hydrogenated at ambient temperature and pressure (24h) in the presence of a 10% Pd/C catalyst (0.020g). The reaction mixture was filtered and concentrated under reduced pressure to yield the crude product (**11**)which was purified by preparative t.l.c. [silica-gel, ethyl acetate:hexane, 65:35].*(-)-(4R,5S)-cis-4,5-Dihydroxy-4,5,6,7-tetrahydrobenzofuran (11)*, was obtained as a white solid which crystallised from chloroform/hexane (0.058g, 38%); m.p. 125-126°C; $[\alpha]_D$ -92.5 (*c* 0.36, MeOH); Found: M^+, 154.0630. $C_8H_{10}O_3$ requires 154.0630); δ_H(300 MHz) 1.91-2.10 (2H, m, 6-H and 6'-H), 2.35 (2H, br s, OH), 2.56-2.81 (2H, m, 7-H and 7'-H), 3.95 (1H, m, 5-H), 4.64 (1H, d, $J_{4,5}$ 3.3 Hz, 4-H), 6.41 (1H, d, $J_{3,2}$ 1.6 Hz, 3-H), 7.29 (1H, d, $J_{2,3}$ 1.6 Hz, 2-H); m/z(%), 154(M^+, 19), 110(100), 109(33).To a solution of compound (**11**) (0.046g, 0.30mmol) in dry pyridine (0.3ml) was added an excess of acetic anhydride (0.2 ml, 2.2 mmol) and the reaction mixture was allowed to stand overnight at room temperature. The pyridine was removed azeotropically (using toluene) under reduced pressure. The residue was treated with water (8 ml) and extracted with diethyl ether (2 x 25 ml). The combined extracts were washed with water (10 ml), dried (Na_2SO_4) and concentrated to give *(-)-(4R,5S)-cis-4,5-diacetoxy-4,5,6,7-tetrahydrobenzofuran (16)* (0.062 g, 87%). Preparative t.l.c. purification [silica-gel, diethyl ether:hexane, 40:60] furnished the pure diacetate (**16**) as a colourless oil; $[\alpha]_D$ -133 (*c* 0.43, $CHCl_3$); (Found: M^+ 238.0844. $C_{12}H_{14}O_5$ requires 238.0841); δ_H(300 MHz) 2.01 (1H, m, 6-H), 2.07 (3H, s, OAc), 2.09(3H, s, OAc), 2.28 (1H, m, 6'-H), 2.68-2.93 (2H, m, 7-H and 7'-H), 5.18 (1H, ddd, $J_{5,6'}$ 14.9 Hz, $J_{5,6}$=$J_{5,4}$ 3.5 Hz, 5-H), 5.97 (1H, d, $J_{4,5}$ 3.5 Hz, 4-H), 6.33 (1H, d, $J_{3,2}$ 1.5 Hz, 3-H), 7.28 (1H, d, $J_{2,3}$ 1.5 Hz, 2-H); m/z(%) 238 (M^+, 8), 178(14), 153(19), 136(55), 118(100).

The *cis*-dihydrodiol metabolite (-)-(**13**) (0.212g, 1.39mmol, $[\alpha]_D$ -35°) was hydrogenated, under similar conditions to those used for the metabolite (+)-(**8**), and the product purified by preparative t.l.c. to yield *(-)- (6S,7S)-cis-6,7-Dihydroxy-4,5,6,7-tetrahydrobenzofuran(17)* as a white solid (0.187 g, 87%); m.p. 116-

118°C (from chloroform/hexane); $[\alpha]_D$ -91.5° (*c* 0.98, MeOH); (Found: C, 62.0; H, 6.3. $C_8H_{10}O_3$ requires C, 62.3; H, 6.5%); δ_H (300 MHz), 1.89 (2H, m, 5-H and 5'-H), 2.38-2.63 (2H, m, 4-H and 4'-H), 2.74 (2H, br s, OH), 3.96 (1H, m, 6-H), 4.73 (1H, d, $J_{7,6}$ 3.9 Hz, 7-H), 6.22 (1H, d, $J_{3,2}$ 1.7 Hz, 3-H), 7.36(1H, d, $J_{2,3}$ 1.7 Hz, 2-H); m/z(%) 154(M^+, 16), 110(100), 109(27).Treatment of the tetrahydrodiol (**17**) (0.124g, 0.81mmol, $[\alpha]_D$ -91.5°) with acetic anhydride/pyridine followed by a similar workup and purification procedure (t.l.c. solvent; ether:hexane, 40:60) to that used for compound (**16**) yielded *(-)-(6S,7S)-cis-6,7-diacetoxy-4,5,6,7-tetrahydrobenzofuran(18)* as a colourless oil (0.171 g, 89%); $[\alpha]_D$ +37° (*c* 1.75, $CHCl_3$); (Found: M^+, 238.0832. $C_{12}H_{14}O_5$ required 238.0841); δ_H (300 MHz), 1.90 (1H, m, 5-H), 2.04 (3H, s, OAc), 2.09 (3H, s, OAc), 2.12 (1H, m, 5'-H), 2.49-2.69 (2H, m, 4-H and 4'-H), 5.18 (1H, ddd, $J_{6,5'}$ 12.0 Hz, $J_{6,5} = J_{6,7}$ 3.7 Hz, 6-H), 6.18 (1H, d, $J_{7,6}$ 3.7 Hz, 7-H), 6.24 (1H, d, $J_{3,2}$ 1.6 Hz, 3-H), 7.36 (1H, d, $J_{2,3}$ 1.6 Hz, 2-H); m/z(%), 238(M^+, 2), 178(19), 153(22), 136(100).

Catalytic hydrogenation of (+)-(1R,2S)-*cis*-1,2-dihydroxy-1,2-dihydronaphthalene, $[\alpha]_D$ +246° ($CHCl_3$)[11,13] to yield (-)-(1R,2S)-*cis*-1,2-dihydroxy-1,2,3,4-tetrahydronaphthalene, $[\alpha]_D$ -39° ($CHCl_3$)[13,20] and subsequent treatment with acetic anhydride/pyridine afforded (-)-(1R,2S)-*cis*-1,2-diacetoxy-1,2,3,4-tetrahydronaphthalene (**22**) $[\alpha]_D$ -170° ($CHCl_3$)[11,20].

To a biphasic mixture of the diacetate (**22**) (0.124g, 0.50mmol), and sodium metaperiodate (3.26 g, 15mmol) in carbon tetrachloride (2ml), acetonitrile (2ml) and water (3ml) was added ruthenium(IV) oxide hydrate (0.001g). After stirring the reaction mixture at room temperature (75h), a solution of hydrochloric acid (20ml, 1.5M) saturated with NaCl was added and the product extracted with ethyl acetate (3 x 50 ml). The combined ethyl acetate extracts were washed with a saturated solution of NaCl, dried (Na_2SO_4) and concentrated under reduced pressure to give a brown oil. ^{1}H-n.m.r. spectral analysis of the crude reaction product confirmed that the aromatic ring of diacetate (**22**) had cleaved and the diacid (**20**) had been formed. A solution of the oily residue containing the diacid in methanol (1ml) was treated with an excess of freshly prepared solution of CH_2N_2 in diethyl ether (4h, 0°C). The solvents and excess of diazomethane were removed under a steam of nitrogen and the residue purified by flash chromatography (silica-gel, hexane:diethyl ether, 90:10 --> 50:50) to give *(2S,3S)-Dimethyl(2,3-diacetoxy)adipate (21)*, as a colourless oil (0.035 g, 24%), $[\alpha]_D$ -14° (*c* 2.92, $CHCl_3$); (Found: M^+-OMe 259.0829. $C_{11}H_{15}O_7$ requires 259.0818); δ_H(500 MHz), 1.97(1H, m, CH_2), 2.07(3H, s, OAc), 2.06-2.15(1H, m, CH_2), 2.18(3H, s, OAc), 2.37(2H, m, CH_2), 3.68(3H, s, CO_2Me), 3.79(3H, s, CO_2Me), 5.30(2H, m, 2-H and 3-H); m/z(%) 259(M^+-OMe, 5), 217(M^+-COMe, 16).

Treatment of (-)-(4R,5S)-*cis*-4,5-diacetoxy-4,5,6,7-tetrahydrobenzofuran (**16**) (0.034 g, 0.14 mmol, $[\alpha]_D$ -133°) with RuO_2/$NaIO_4$ followed by the isolation of product , as described for diacetate (-)-(**22**), furnished ester (**21**) (0.007 g, $[\alpha]_D$ -11.3 , 18%) as an oil which was spectrally indistinguishable from the methyl ester of the degradation product obtained from diacetate (-)-(**22**).

Similar oxidative cleavage (RuO_2/$NaIO_4$) of the furan ring of (-)-(6S,7S)-*cis*-6,7-diacetoxy-4,5,6,7-tetrahydrobenzofuran (**18**) (0.138 g, 0.58 mmol, $[\alpha]_D$ -137°) once again yielded (2S,3S)-dimethyl(2,3-diacetoxy)adipate (**21**) (0.039 g, $[\alpha]_D$ -13.2 , 23%) as an oil.

Since the absolute configuration, (1R,2S), of (+)-*cis*-1,2-dihydroxy-1,2-dihydronaphthalene ($[\alpha]_D$ +246°) is known from past studies [11,12], the derived dimethyl(2,3-diacetoxy)adipate (**21**, $[\alpha]_D$ -14°) will have a (2S,3S) configuration. Stereochemical correlation of the product (-)-(**21**) with the tetrahydrodiacetate

derivatives (-)-(**16**) and (-)-(**18**) (and through to the *cis*-dihydrodiol precursors) allows the (4R,5S) and (6S,7S) configurations to be assigned to the cis-dihydrodiols (+)-(**8**) and (-)-(**13**) respectively.

(-)-(1R)-1,2-Dihydroxy-1-(2'-hydroxyphenyl)ethane (15).

A solution of metabolite (**15**) (0.239 g, 0.16 mmol, $[\alpha]_D$ -24) in methanol (2 ml) was treated with an excess of ethereal solution of CH_2N_2 as reported earlier. The methylated product was purified by preparative t.l.c. [silica-gel, ethyl acetate:hexane, 60:40) to furnish *1,2-dihydroxy-1-(2'-methoxyphenyl)ethane (24)* (0.185 g, 71%) as a semi solid, $[\alpha]_D$ -23 (*c* 0.61, MeOH); (Found: M^+ 168.0792. $C_9H_{12}O_3$ requires 168.0786); δ_H(500 MHz), 2.55(2H, br s, OH), 3.68(1H, dd, $J_{2a,2b}$ 11.1 Hz, $J_{2a,1}$ 8.0 Hz, 2a-H), 3.80(1H, dd, $J_{2b,2a}$ 11.1 Hz, $J_{2b,1}$ 3.5 Hz, 2b-H), 3.84(3H, s, OMe), 5.05(1H, dd, $J_{1,2a}$ 8.0 Hz, $J_{1,2b}$ 3.5 Hz, 1-H), 6.88(1H, d,$J_{3',4'}$ 8.4 Hz, 3'-H), 6.98(1H, ddd, $J_{5',4'}$=$J_{5',6'}$ 7.5 Hz, $J_{5',3'}$ 1.0 Hz, 5'-H), 7.28(1H, m, 4'-H), 7.38(1H, dd, $J_{6',5'}$ 7.5 Hz, $J_{6',4'}$ 1.6 Hz, 6'-H); m/z(%) 168(M^+, 12), 137(100), 107(36).

Acetylation of diol (**24**) (0.169 g, 1.0 mmol, $[\alpha]_D$ -23) with acetic anhydride (0.7 ml, 7.4 mmol) in pyridine yielded crude *1,2-diacetoxy-1-(2'-methoxyphenyl)ethane (25)* as an oil (0.238g, 94%). Purification by preparative t.l.c. [silica gel, diethyl ether:hexane, 35:65] gave the diacetate (**25**) as a colourless oil which crystallized from diethyl ether/hexane as white needles, m.p. 59-60°C; $[\alpha]_D$ -22 (*c* 1.62, $CHCl_3$); (Found: C, 61.8, H, 6.5. $C_{13}H_{16}O_5$ requires C, 61.9; H, 6.3); δ_H(300 MHz), 2.01(3H, s, OAc), 2.13(3H, s, OAc), 3.84(3H, s, OMe), 4.30(2H, m, 2a-H and 2b-H), 6.40(1H, dd, $J_{1,2a}$ 6.5 Hz, $J_{1,2b}$ 3.3 Hz, 1-H), 6.86-6.97(2H, m, 3'-H and 5'-H), 7.25-7.34(2H, m, 4'-H and 6'-H); m/z(%) 252(M^+,1), 192(12), 150(24), 137(100).

Employing the oxidative cleavage ($RuO_2/NaIO_4$) procedure as described for the diacetate (**22**), 1,2-diacetoxy-1-(2'-methoxyphenyl)ethane (**25**, 0.204 g, 0.81 mmol, $[\alpha]_D$ -22) was converted to 2,3-diacetoxypropanoic acid (**26**) which was treated directly with ethereal CH_2N_2 solution. Purification of the product by flash chromatography [silica-gel, hexane:diethyl ether, 90:10 ---> 50:50] gave *methyl(2,3-diacetoxy)propanoate (27)* (0.079 g, 48%) as a viscous colourless oil $[\alpha]_D$ -8.0°(*c* 2.7, $CHCl_3$) (lit. $[\alpha]_D$ -12.5 [22] and $[\alpha]_D$ +11.3 [23]); δ_H(300 MHz), 2.07(3H, s, OAc), 2.17(3H, s, OAc), 3.77(3H, s, OMe), 4.40(1H, dd, $J_{3,3'}$ 12.1 Hz, $J_{3,2}$ 5.4 Hz, 3-H), 4.48(1H, dd, $J_{3',3}$ 12.1 Hz, $J_{3',2}$ 3.3 Hz, 3'-H), 5.31(1H, dd, $J_{2,3}$ 5.4 Hz, $J_{2,3'}$ 3.3 Hz, 2-H); m/z(%) 145(14), 103(17), 43(100).

A comparison of the $[\alpha]_D$ value (-8) of the triester (**27**) with that of a synthetic sample of (2R)-methyl (2,3-diacetoxy)propanoate (**27**), $[\alpha]_D$ +15.8 , synthesised from (R)-glyceric acid (hemicalcium salt), showed that the metabolite (**15**) has a (1R) configuration and an e.e. value of *ca.* 51%. Spectral analysis of the di-MTPA esters of the diol derivative (**24**) confirmed the e.e. value.

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