Toluene dioxygenase-catalysed oxidation route to angular *cis*-monohydrodiols and other bioproducts from bacterial metabolism of 1,2-dihydrobenzocyclobutene and derivatives

Derek R. Boyd,*[,a] Narain D. Sharma,[a] Timothy A. Evans,[a] Melanie Groocock,[a] John F. Malone,[a] Paul J. Stevenson[a] and Howard Dalton*[,b]

[a] *School of Chemistry, The Queen's University of Belfast, Belfast, UK BT9 5AG*
[b] *Department of Biological Sciences, The University of Warwick, Coventry, UK CV4 7AL*

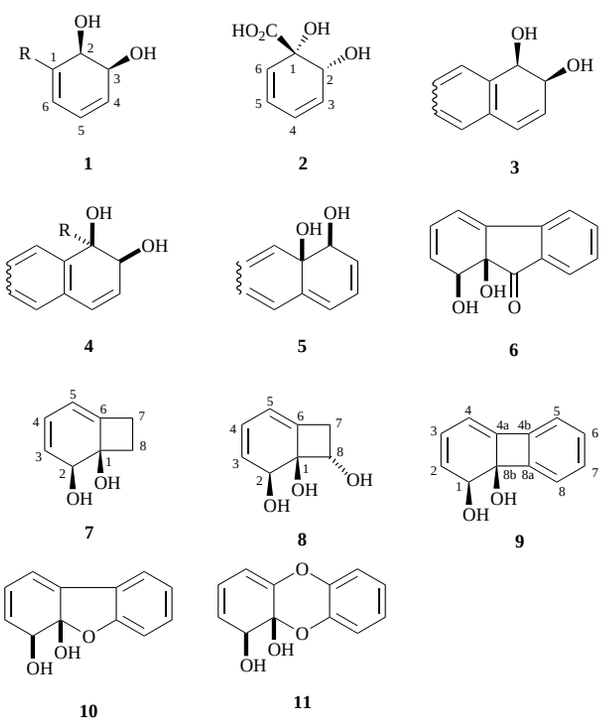
A mutant strain (UV4) of the soil bacterium *Pseudomonas putida*, containing toluene dioxygenase, has been used in the metabolic oxidation of 1,2-dihydrobenzocyclobutene 12† and the related substrates 1,2-dihydrobenzocyclobuten-1-ol 13 and biphenylene 33. Stable angular *cis*-monohydrodiol metabolites (1*R*,2*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2-diol 7, (1*S*,2*S*,8*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2,8-triol 8 and biphenylene-*cis*-1,8b-diol 9, isolated from each of these substrates, have been structurally and stereochemically assigned. The structure, enantiopurity and absolute configuration of the other *cis*-diol metabolites, (2*R*,3*S*)-bicyclo[4.2.0]octa-1(6),4-diene-2,3-diol 14 and *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene 16, and the benzylic oxidation bioproducts, 1,2-dihydrobenzocyclobuten-1-ol 13, 1,2-dihydrobenzocyclobuten-1-one 15 and 2-hydroxy-1,2-dihydrobenzocyclobuten-1-one 17, obtained from 1,2-dihydrobenzocyclobutene and 1,2-dihydrobenzocyclobuten-1-ol, have been determined with the aid of chiral stationary-phase HPLC, NMR and CD spectroscopy, and stereochemical correlation. X-Ray crystallographic methods have been used in the determination of absolute configuration of the di-camphanates 27 (from diol 7) and 32 (from diol 9), and the di-MTPA ester 29 (from diol 14) of the corresponding *cis*-diol metabolites. The metabolic sequence involved in the formation of bioproducts derived from 1,2-dihydrobenzocyclobutene 12 has been investigated.

Introduction

The initially formed *cis*-dihydrodiol bioproducts from the bacterial metabolism of mono- and poly-cyclic arenes generally result from dioxygenase-catalysed *cis*-dihydroxylation at unsubstituted arene bonds.[1,2] Thus biotransformation of monosubstituted benzene substrates normally occurs by oxidation at the 2,3-bond to yield *cis*-dihydrodiols (*e.g.* type **1**, R = Me, Et, F, Cl, CF_3, CH_2OAc).[2] *cis*-Diol formation at a substituted 1,2-bond (*ipso* bond) in monosubstituted benzene substrates has rarely been observed. An exception was found during the bacterial metabolism of benzoic acid where oxidation occurred at the 1,2-bond to yield a *cis*-diol of type **2**.[3] Polycyclic aromatic hydrocarbon metabolism by bacteria has similarly been found to proceed *via cis*-diol formation at unsubstituted carbon–carbon bonds (type **3**)[1,4,5] rather than at a substituted (type **4**) or an angular (type **5**) carbon–carbon bond. Mutant strains of either *Pseudomonas putida* (UV4),[2] or *Beijerinckia* (B8/36),[4] also described as *Sphingomonas yanoikuyae* (B8/36),[5] lack the *cis*-dihydrodiol dehydrogenase enzyme responsible for catechol formation, and therefore have made available *cis*-diols (type **1** and **3**) in sufficient quantities for use in chiral synthesis and kinetic studies.

Polycyclic arenes have been shown to undergo oxidative bacterial metabolism *via* angular *cis*-diols (*cis*-monohydrodiols) using strains of both *Brevibacterium*[6,7] and *Pseudomonas putida*.[8,9] The stable angular *cis*-diols **6**,[6,7,9] **7**,[8] **9**[8] and triol **8**[8] metabolites were isolated and subsequently identified by spectroscopic methods. Unstable angular *cis*-diols have also been proposed as initial metabolites of dibenzofuran (*e.g. cis*-monohydrodiol **10** from a *Pseudomonas* species),[6] and dibenzo-[1,4]dioxine (*e.g. cis*-monohydrodiol **11** from a *Staphylococcus auriculans* strain).[10]

While the absolute configurations of a large number of *cis*-dihydrodiols from monocyclic[1,2] and polycyclic[1,4,5] aromatic hydrocarbons are known, none of the angular *cis*-diol metabolite series, *e.g.* **6**–**9**, have yet been unequivocally assigned. The structure, absolute configuration and enantiopurity of the alcohol **13**, *cis*-diols **7**, **14**, keto alcohol **17** and triol **8** metabolites of 1,2-dihydrobenzocyclobutene **12**, the structure of *cis*-diol **16**, and the *cis*-monohydrodiol metabolite **9** of biphenylene **33** (several of which were reported in a preliminary communication[8]) are now discussed in detail. The results of the study on the metabolic sequence for biotransformation of 1,2-dihydrobenzocyclobutene **12**, in *P. putida* UV4, using both single enanti-

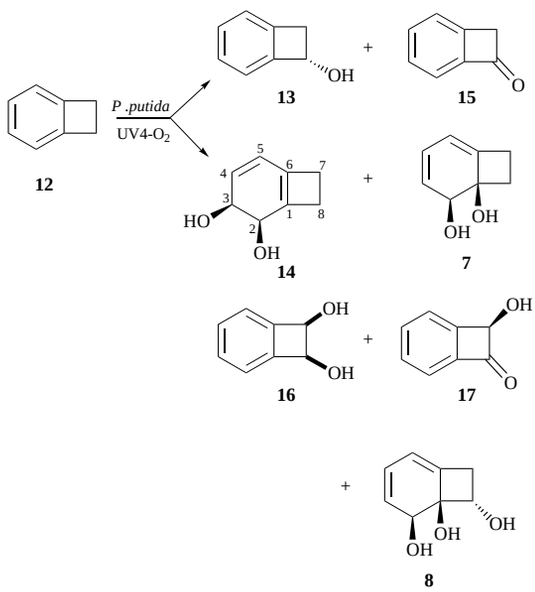


† Also described as 'benzocyclobutene' in the literature.

omers of the bacterial metabolite 1,2-dihydrobenzocyclobuten-1-ol **13** as substrate, are also included herein.

Results and discussion

Addition of 1,2-dihydrobenzocyclobutene **12** to growing cultures of *P. putida* UV4 yielded a mixture of seven metabolites consisting of 1,2-dihydrobenzocyclobuten-1-one **15**, 1-oxo-2-hydroxy-1,2-dihydrobenzocyclobutene **17**, 1,2-dihydrobenzocyclobuten-1-ol **13**, *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene **16**, (1*R*,2*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2-diol **7**, (2*R*,3*S*)-bicyclo[4.2.0]octa-1(6),4-diene-2,3-diol **14** and (1*S*,2*S*,8*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2,8-triol **8** (Scheme 1). Among

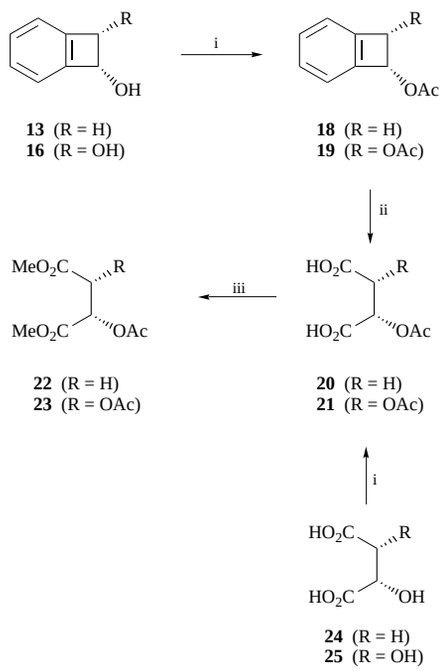


Scheme 1

the metabolites which could be isolated in pure form by an initial preparative layer chromatography (PLC) separation [silica gel, EtOAc–hexane (1:1)] were ketone **15** (R_f 0.90, 2%), keto alcohol **17** (R_f 0.80, 1%), alcohol **13** (R_f 0.75, 33%) and triol **8** (R_f 0.09, 9%). ^{1}H NMR Analysis of a further major fraction (R_f 0.34), indicated the presence of three *cis*-diols. PLC Separation by multiple elution of this mixture [silica gel, EtOAc–hexane (1:4)] yielded a high R_f component which was identified as *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene **16** (9%) and a low R_f band containing two diols. Semi-preparative reversed-phase HPLC separation of the latter diol mixture [Zorbax ODS column, H_2O–MeOH (70:30), $\alpha = 1.9$] yielded a pure diol from the early, more polar, fractions. This very stable metabolite was identified as (1*R*,2*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2-diol **7** (18%). The later, less polar, HPLC fractions afforded an unstable diol which was identified as (2*R*,3*S*)-bicyclo[4.2.0]octa-1(6),4-diene-2,3-diol **14** (0.14 g, 13%).

1,2-Dihydrobenzocyclobuten-1-one **15** and 1,2-dihydrobenzocyclobuten-1-ol **13** were readily identified as metabolites of 1,2-dihydrobenzocyclobutene **12** by comparison of their chromatographic and spectral characteristics with authentic samples and with the literature data.[11,12] The enantiomeric excess (ee) of the alcohol metabolite **13** ($[\alpha]_D$ −16) was estimated to be *ca.* 20% by chiral stationary phase (CSP) HPLC. The ee value was confirmed by ^{1}H NMR analysis of the derived methoxy(trifluoromethyl)(phenyl)acetate (MTPA) derivative. Using a semi-preparative Chiralcel OB column, small samples (*ca.* 0.10 g) of the early ($[\alpha]_D$ −88) and late ($[\alpha]_D$ +88) eluting enantiomers of 1,2-dihydrobenzocyclobuten-1-ol **13** were separated by HPLC for use as substrates.

The absolute configuration of the enzymatically formed (−)-1,2-dihydrobenzocyclobuten-1-ol **13** ($[\alpha]_D$ −16) was determined by converting it to the corresponding acetate **18** ($[\alpha]_D$ −8), followed by oxidative cleavage of the aryl ring with ruthenium tetroxide to furnish acetoxysuccinic acid **20** (Scheme 2). The crude diacid **20**, on treatment with diazomethane and subsequent PLC purification, gave dimethyl acetoxysuccinate **22** ($[\alpha]_D$ −2.2). The latter compound was assigned as (*S*) absolute configuration by comparison with an authentic sample of **22** ($[\alpha]_D$ −11), obtained by esterification of (*S*)-hydroxysuccinic acid [L-(−)-malic acid] **24** ($[\alpha]_D$ −28) *via* the dicarboxylic acid **20** and therefore the stereochemical correlation sequence shown in Scheme 2 unequivocally establishes the absolute



Scheme 2 *Reagents:* i, $(MeCO)_2O$–pyridine; ii, RuO_2–$NaIO_4$; iii, CH_2N_2

configuration of the (−)-1,2-dihydrobenzocyclobuten-1-ol metabolite **13** as (*S*).

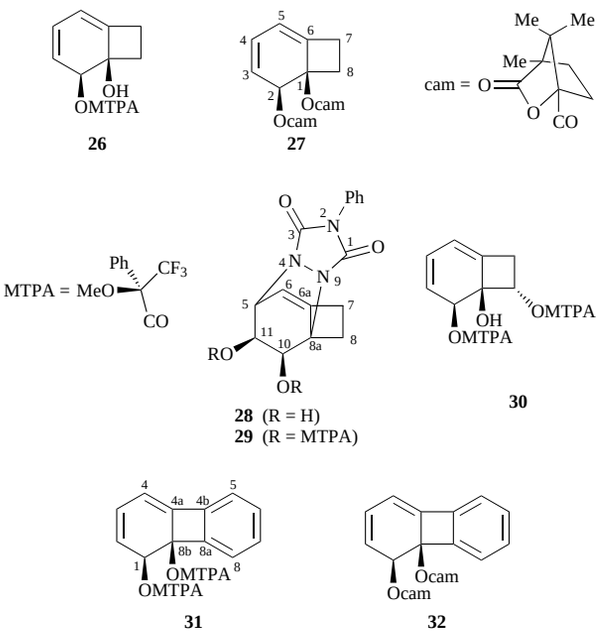
The toluene dioxygenase (TDO)-catalysed oxidation of benzocycloalkanes in *P. putida* UV4 was consistently found to yield enantiopure benzylic alcohol metabolites of (*R*) configuration where five-, six- and seven-membered non-aromatic rings were present.[8] An (*R*) configuration was assumed for metabolite **13** in the preliminary report[8] based upon the above trend and the expectation that a similar elution sequence, using the same CSPHPLC system, would be found for all (*R*) benzylic alcohol metabolites from benzocycloalkanes of differing ring size. The 1,2-dihydrobenzocyclobuten-1-ol metabolite **13** from *P. putida* UV4 was found to be exceptional in showing (a) a reverse elution sequence of enantiomers on CSPHPLC, (b) a relatively low ee (20%) and (c) a reverse absolute configuration (*S*), compared to benzocycloalkanes with larger non-aromatic rings.

1-Oxo-2-hydroxy-1,2-dihydrobenzocyclobutene **17** ($[\alpha]_D$ −36) was isolated as a very minor (1%) metabolite of 1,2-dihydrobenzocyclobutene **12** and was found to have an ee of 72% by CSPHPLC analysis. This ee value was confirmed when enantiopure samples of the keto alcohol **17** ($[\alpha]_D$ −50 and +51) were separated on a semi-preparative Chiralcel OB column. The absolute configuration of keto alcohol (−)-**17** was found to be (2*R*) by conversion to (*S*)-1,2-dihydrobenzocyclobuten-1-ol **13**.

The structure and relative stereochemistry of metabolite **16** was confirmed as *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene by a similar acylation and oxidation cleavage sequence to that used for 1,2-dihydrobenzocyclobuten-1-ol **13** (Scheme 2). Thus metabolite **16** was acetylated to yield *cis*-1,2-diacetoxy-1,2-dihydrobenzocyclobutene **19** which after oxidation (RuO_4) gave *meso*-2,3-diacetoxysuccinic acid **21**. Dimethylation of

diacid **21** (CH_2N_2) gave *meso*-dimethyldiacetoxysuccinate **23** whose structure was confirmed by its synthesis starting from *meso*-tartaric acid **25** (Scheme 2).

The structure of the angular *cis*-diol metabolite **7** was based mainly upon ^{1}H NMR and mass spectroscopy, and chiroptical (CD) data. The absence of vicinal coupling between the protons 2-H and 3-H is consistent with a dihedral angle of *ca.* 90°, and an axial orientation for 2-H. Thus the equatorial secondary OH and axial tertiary OH groups must adopt a *cis*-configuration in common with dihydrodiols formed from TDO-catalysed oxidation of arenes using *P. putida* UV4. Further evidence for the angular *cis*-diol structure of metabolite **7** was provided by esterification of one hydroxy group. Treatment with both (*R*)- and (*S*)-forms of MTPA-chloride yielded only the corresponding mono-MTPA esters **26** due to esterification of the less hindered secondary OH group. ^{1}H NMR Analysis of the MTPA esters **26** confirmed that the angular *cis*-diol **7** was enantiopure (>98% ee). Treatment of the *cis* diol **7** with the less



26 **27** **28** (R = H) **29** (R = MTPA) **30** **31** **32**

bulky reagent, (1*S*)-camphanic acid chloride, resulted in diesterification of both secondary and tertiary OH groups to yield a crystalline dicamphanate derivative **27** ($[\alpha]_D$ −229). X-Ray crystallographic analysis of dicamphanate **27** (Fig. 1) confirmed the *cis*-relationship between the equatorial OH group at C-2 and the axial OH at C-1 in the parent diol **7** and also confirmed the orthogonal relationship between protons 2-H and 3-H which had been suggested by ^{1}H NMR spectroscopy. The absolute configuration is established as (1*R*,2*S*) for compounds **27** and **7**. The diene C^3=C^4–C^5=C^6 in ester **27** has helicity *M* with torsion angle $\tau = -15°$.

The relative stability of the angular *cis*-diol **7** may account for the successful synthesis and isolation of the monoester **26** and diester **27** derivatives. The formation of these derivatives was noteworthy since earlier attempts to obtain either mono- or di-MTPA derivatives directly from *cis*-dihydrodiol metabolites of monosubstituted benzenes or polycyclic aromatic hydrocarbons were unsuccessful due to their aromatization (dehydration).[2]

The third *cis*-diol metabolite of 1,2-dihydrobenzocyclobutene **12** proved to be very unstable and particular care was required during the HPLC purification step. The structure of diol **14** was established by ^{1}H NMR analysis and in particular from the *vicinal* coupling constant ($J_{2,3}$ 6.5 Hz) which was totally consistent with the *cis*-geometry. Due to the possibility of traces of achiral phenolic decomposition products being present in diol **14**, the optical rotation observed ($[\alpha]_D$ −16) should be regarded

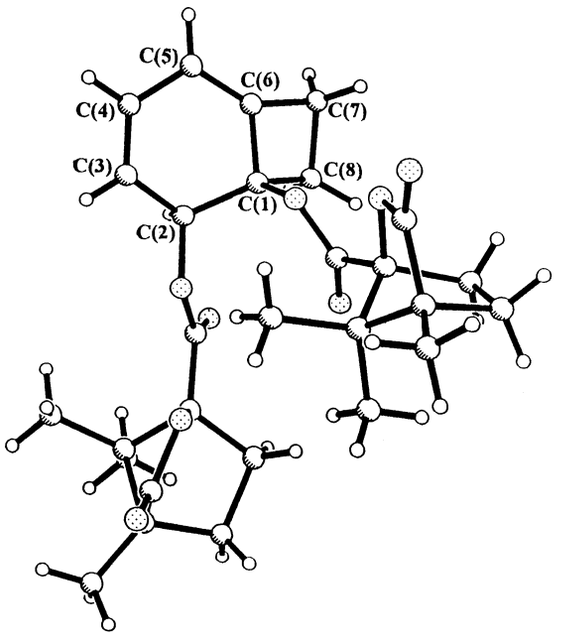


Fig. 1 A projection of molecule **27**

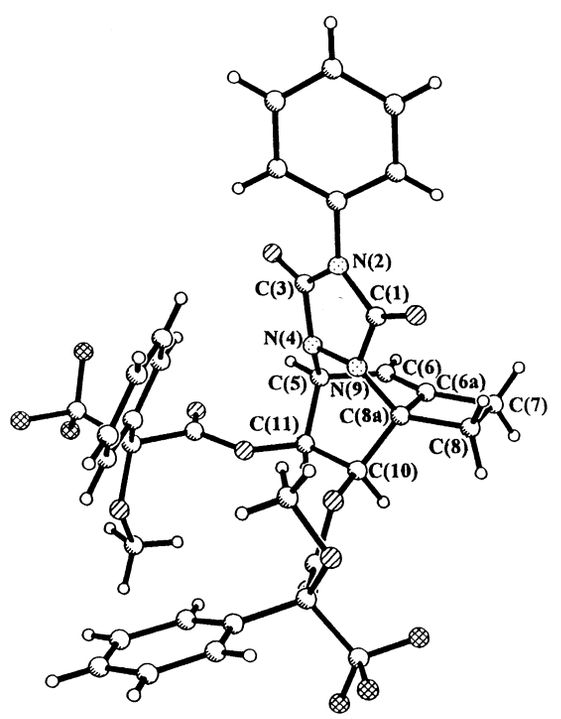


Fig. 2 A projection of molecule **29**

as a minimal value. The structure and absolute stereochemistry of *cis*-dihydrodiol **14** was unequivocally established by formation of a stable cycloadduct with 4-phenyl-1,2,4-triazoline-3,5-dione **28** ($[\alpha]_D$ +2). The di-MTPA derivatives **29** formed using (*R*)-MTPA **29a** ($[\alpha]_D$ +16) and (*S*)-MTPA **29b** ($[\alpha]_D$ +32), respectively, gave ^{1}H NMR spectral data which indicated that the *cis*-diol metabolite **14** was enantiomerically homogeneous (>98% ee). The di-MTPA cycloadduct **29b** ($[\alpha]_D$ +32) gave suitable crystals for an X-ray crystal structure analysis (Fig. 2) which established the absolute configuration of the parent *cis*-diol **14** ($[\alpha]_D$ −16) as (2*R*,3*S*). This configuration is identical to that found for the *cis*-dihydrodiol metabolites formed at unsubstituted bonds in both monosubstituted[2] and *ortho*-disubstituted[13] benzene substrates. The relative instability of *cis*-dihydrodiol **14** compared with *cis*-monohydrodiol **7** could be accounted for by the weak electron donating character of the

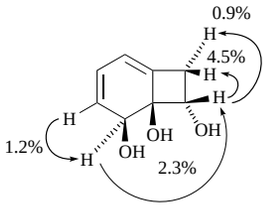


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Fig. 3 NOE interactions in the ^{1}H NMR spectrum of triol **8**

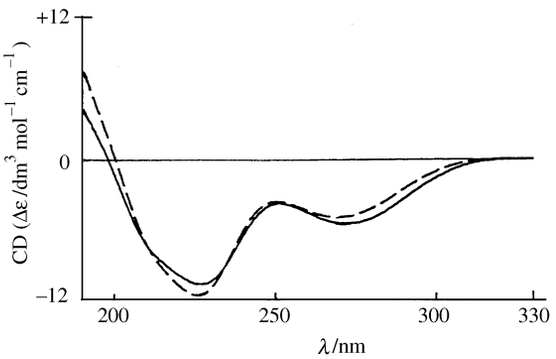
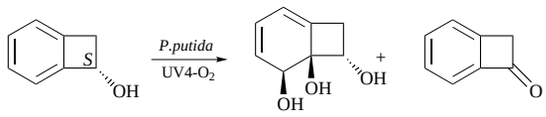


Fig. 4 CD Spectra of diol **7** (– – –) and triol **8** (——)

alkyl substituents, leading to a faster rate of aromatization,[14] and the ring strain associated with the cyclobutene ring.

The triol metabolite **8** proved to be the most polar of the bioproducts isolated after 1,2-dihydrobenzocyclobutene **12** was added to growing cultures of *P. putida* UV4. The ^{1}H NMR and mass spectroscopy data of metabolite **8** indicated a structure similar to the angular *cis*-monohydrodiol **7**. As protons 2-H and 3-H were uncoupled, an orthogonal relationship between them was present, as in *cis*-diol **7**. The proximity of protons 7-H↔ 8-H, 2-H↔8-H (3.5 Å) and 2-H↔3-H was evident from their NOE interactions and from consideration of the X-ray structure of compound **27**. The absence of any NOE interactions between protons 2-H and 7-H is entirely consistent with the triol **8** having OH groups on adjacent carbon atoms (C-2, C-1 and C-8, Fig. 3). Further NMR studies allied to stereochemical correlation however, allowed the position and the relative/absolute configuration at C-8 in triol **8** to be unequivocally established as (8*S*) by (i) comparison of the chemical shift value of the allylic proton 2-H (δ 4.53) relative to the non-allylic proton 8-H (δ 4.17), (ii) analysis of the 2D-COSY ^{1}H NMR spectrum indicating allylic coupling between 5-H and 7-H, (iii) selective ^{1}H-decoupling on 7-H while observing the non-decoupled ^{13}C NMR spectrum where multiple collapse was observed on C-5, C-6 and C-8, and (iv) addition of (−)-(*S*)-1,2-dihydrobenzocyclobuten-1-ol **13** as substrate (see later). Metabolite **8** ($[a]_D$ −197) yielded di-MTPA esters **30** on treatment with (*R*)- and (*S*)-MTPA-Cl due to selective esterification of the secondary OH groups on C-2 and C-8. ^{1}H NMR Analysis of the di-MTPA esters **30** indicated that the triol **8** was optically pure (>98% ee). The (1*S*,2*S*) absolute configuration of triol **8** ($[a]_D$ −197) was deduced from its CD spectrum. The similarity of CD spectra of the (1*R*,2*S*)-diol **7** and triol **8** which both contain the diagnostic skew-diene chromophore is consistent with each having an identical (2*S*) configuration (Fig. 4).

The sequence of enzyme-catalysed reactions involved in the conversion of 1,2-dihydrobenzocyclobutene **12** to triol **8** was studied using (*S*)- and (*R*)-1,2-dihydrobenzocyclobuten-1-ol **13** and racemic 1,2-dihydrobenzocyclobuten-1-ol **13**, containing a deuterium atom at C-1, as substrates with *P. putida* UV4. Addition of (*S*)-1,2-dihydrobenzocyclobuten-1-ol **13** resulted in its total biotransformation to yield triol **8** (8%) and 1,2-dihydrobenzocyclobuten-1-one **15** (92%) as shown in Scheme 3. Since the stereochemistry of the angular *cis*-diol moiety in triol **8**, derived from (*S*)-1,2-dihydrobenzocyclobuten-1-ol, has been

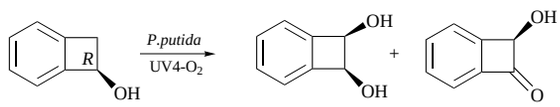


13 **8** **15**

Scheme 3

identified as (1*S*,2*S*) from the CD spectrum, the configuration at all three chiral centres in triol **8** ($[a]_D$ −197) will thus be (1*S*,2*S*,8*S*) confirming the *trans* relationship between the OH group on C-8 and the OH groups on C-1 and C-2. The sample of triol **8**, isolated from the metabolism of racemic C-1 deuteriated alcohol **13**, was found to have totally retained the original deuterium atom at the C-8 position as shown by the ^{1}H NMR and mass spectroscopy data. This observation proves that the integrity of the chiral centre in (*S*)-1,2-dihydrobenzocyclobuten-1-ol during its conversion to triol **8** is maintained.

(*R*)-1,2-Dihydrobenzocyclobuten-1-ol **13** proved to be a rather poor substrate compared with the (*S*)-enantiomer; it was only partially metabolized (Scheme 4) yielding a mixture of *cis*-



13 **16** **17**

Scheme 4

diol **16** (36%), keto alcohol **17** (27%) and recovered substrate (27%). This sample of metabolite **17** was found to have identical absolute stereochemistry to that isolated from 1,2-dihydrobenzocyclobutene **12**.

The formation of different metabolites from enantiomeric benzylic alcohols, *i.e.* alcohol (*S*)-**13**→triol **8** (Scheme 3), and alcohol (*R*)-**13**→diol **16** and keto alcohol **17** (Scheme 4), has also been observed during earlier metabolism studies using *P. putida* UV4, where a much stronger preference for triol formation was noted when (*S*)-3-hydroxy-2,3-dihydrobenzofuran was used as the substrate compared with the (*R*)-enantiomer.[15]

When the angular *cis*-monohydrodiol (1*R*,2*S*)-**7** was used as a substrate, no evidence of triol **8** (or other metabolites) was found. This observation supports the view that triol **8** was being formed *via* the metabolic sequence **12**→**13**→**8**. The hydroxy ketone **17** was isolated as a metabolite when either *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene **16** or 1,2-dihydrobenzocyclobuten-1-one **15** were used as substrates. The metabolic pathway involved in the formation of compound **17** from 1,2-dihydrobenzocyclobutene **12** remains unclear. Either sequence **12**→**13**→**16**→**17** or **12**→**13**→**15**→**17** (or a combination of both) may be applicable.

From these studies with the bacterium *P. putida* UV4, employing a range of substrates, it is evident that three metabolites of 1,2-dihydrobenzocyclobutene **12**, *i.e.* (*S*)-1,2-dihydrobenzocyclobuten-1-ol **13**, (1*R*,2*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2-diol **7** and (2*R*,3*S*)-bicyclo[4.2.0]octa-1(6),4-diene-2,3-diol **14**, may be considered as primary products. However, 1,2-dihydrobenzocyclobuten-1-one **15**, *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene **16** and (1*S*,2*S*,8*S*)-bicyclo[4.2.0]-octa-3,5-diene-1,2,8-triol **8** appear to be secondary metabolites derived from 1,2-dihydrobenzocyclobuten-1-ol **13**. The only identified tertiary metabolite was 2-hydroxybenzocyclobuten-1-one **17** which could be derived from either of the secondary metabolites cyclobutanone **15** or *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene **16**.

The TDO-catalysed metabolism of 1,2-dihydrobenzocyclobutene **12**, in *P. putida* UV4, was much more complex than previously observed with other members of the benzocycloalkene series containing five-, six- and seven-membered rings,

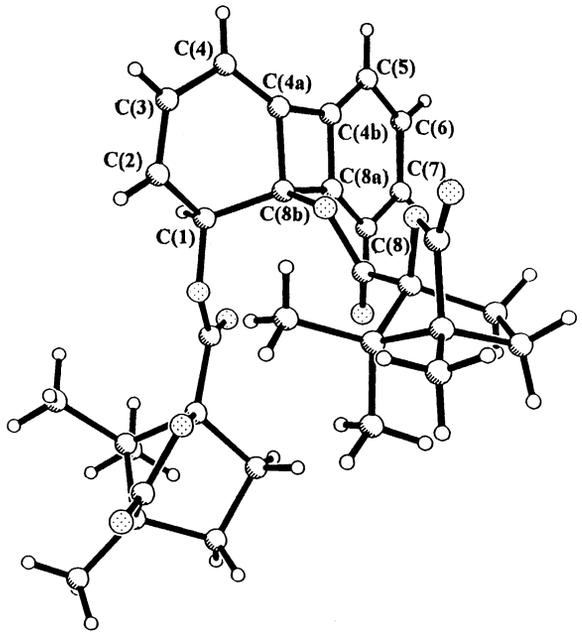
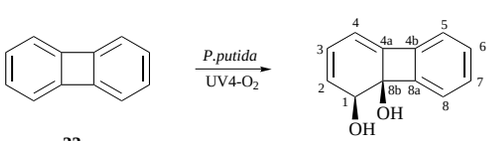


Fig. 5 A projection of molecule **32**

where mainly benzylic oxidation products (benzylic alcohols and ketones) were found.[8] It would appear that when the ring size is sufficiently small the dioxygenase enzyme can accept and metabolize the substrate in a manner similar to *ortho*-disubstituted arenes *i.e.* yielding *cis*-diol metabolites. It has recently been reported[16] that naphthalene dioxygenase (NDO)-catalysed oxidation of 1,2-dihydrobenzocyclobutene **12**, in growing cultures of *Pseudomonas fluorescens*, yielded racemic 1,2-dihydrobenzocyclobuten-1-ol **13** and 1,2-dihydrocyclobuten-1-one **15** as the only metabolites.

A single metabolite was isolated when biphenylene **33** was added to *P. putida* UV4 cultures. This product was identified as *cis*-monodihydrodiol **9** ($[\alpha]_D$ +429) on the basis of its spectral and chromatographic characteristics (Scheme 5). The absence



33 **9**

Scheme 5

of coupling between the orthogonal protons 1-H and 2-H in *cis*-diol **9**, as was found for protons 2-H and 3-H in diol **7** and triol **8**, was characteristic of the angular *cis*-monohydrodiol moiety. Synthesis of the di-MTPA derivatives **31** allowed the enantiomeric excess (>98% ee) to be determined.

The di-MTPA esters **31** proved difficult to crystallize. However, the dicamphanate derivative **32** ($[\alpha]_D$ −70) provided a suitable crystal for X-ray structure analysis (Fig. 5) and the absolute configuration of metabolite **9** ($[\alpha]_D$ +429) was established as (1*S*,8b*R*). In diester **32** the equatorial group on C-1 and the axial group on C-8b are *cis* and the diene C^2=C^3–C^4=C^{4a} has helicity *M* with torsion angle $\tau = -19°$. Individual molecules of ester **32** adopt the same conformation, including that of the camphanate groups, as ester **27** but the molecular packing differs, leading to a different lattice. Recent studies[17] of the bacterial oxidation of biphenylene **33**, using a carbazole dioxygenase (CDO) as biocatalyst, have shown that the isolated *cis*-monohydrodiol metabolite **9** was of opposite absolute configuration, (1*R*,8b*S*), to that found in this study using TDO. Formation of *cis*-monohydrodiol **9**, using either the TDO or CDO enzymes, provides the first example of enantiocomplementarity during dioxygenase-catalysed *cis*-dihydroxylation of arenes. Similar examples, supporting this concept, have recently been reported during TDO- and NDO-catalysed benzylic hydroxylation,[18] alkene *cis*-dihydroxylation[19,20] and sulfoxidation.[21,22] The absolute configuration of each of the three stable angular *cis*-monohydrodiols **7**, **8** and **9**, obtained by TDO-catalysed oxidation, was identical and is consistent with a similar orientation within the active site of the enzyme. Since either enantiomer of the angular *cis*-diol **9** may be formed using TDO or CDO systems, in the absence of information about the dioxygenase type, it is difficult to predict the absolute configuration of other angular *cis*-diol metabolites.

Experimental

1,2-Dihydrobenzocyclobutene **12**,[11] 1,2-dihydrobenzocyclobuten-1-ol **13**[11] and *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene **16**[12] were synthesised by the literature procedures. Biphenylene **33** was obtained from the Aldrich Chemical Company. Substrates **12**, (+)-**13**, (−)-**13**, (±)-**13** and **33** were each metabolized using growing cultures of *Pseudomonas putida* UV4 according to the reported method.[23] The bioproducts were harvested by solvent extraction (EtOAc) of the sodium chloride-saturated aqueous solution containing the biotransformed material, and concentration of the combined extracts under reduced pressure. A ^{1}H NMR spectrum of the crude mixture of bioproducts, obtained from each run, was routinely examined prior to any purification procedure.

^{1}H NMR Spectra of compounds were recorded using General Electric QE 300, GNΩ-500, and Bruker WP400 instruments using $CDCl_3$ as solvent. Coupling constants *J* are quoted in Hz. Mass spectra were recorded at 70 eV on a AEI-MS902 spectrometer updated by VG Autospec Instruments. Elemental microanalyses were performed on a Perkin-Elmer 2400 CHN microanalyser. Accurate molecular weights were determined by the peak matching method, using perfluorokerosene as standard reference. Flash chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and $PF_{254/366}$ respectively. Merck Kieselgel $60F_{254}$ analytical plates were used for normal TLC. Optical rotation ($[\alpha]_D$) measurements were carried out with a Perkin-Elmer 214 polarimeter at ambient temperature (*ca.* 20 °C) at a concentration of 0.005 g cm^{-3} and are given in units of 10^{-1} deg cm^2 g^{-1}. Circular dichroism (CD) spectra were recorded on a JASCO J720 instrument in acetonitrile solvent.

Metabolism of 1,2-dihydrobenzocyclobutene 12

The crude mixture of products, obtained from the biotransformation of 1,2-dihydrobenzocyclobutene **12** (0.6 g, 5.7 mmol) over 24 h, was separated into five bands by PLC [EtOAc–hexane (1:1)]. 1,2-*Dihydrobenzocyclobuten*-1-*one* **15** was isolated as an oil (0.014 g, 2%), R_f 0.90; δ_H(300 MHz) 4.00 (2H, s, 1-H, 2-H) and 7.36–7.55 (4H, m, Ar-H). It was spectrally indistinguishable from an authentic sample.

(−)-(2R)-1-*Oxo*-2-*hydroxy*-1,2-*dihydrobenzocyclobutene* **17** (0.008 g, 1%), R_f 0.80, mp 64–66 °C (from hexane–isopropyl alcohol); $[\alpha]_D$ −36 ($CHCl_3$) (Found: M^+, 134.0367. $C_8H_6O_2$ requires *M*, 134.0368); δ_H(300 MHz) 5.82 (1H, s, 2-H) and 7.30–7.70 (4H, m, Ar-H); *m*/*z* 134 (M^+, 24%) and 105 (100); ν_{max}(neat)/cm^{-1} 1758 (C=O) and 3608 (OH); CD λ/nm ($\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}) 340.6 (3.39), 289.4 (−6.98) and 282.2 (−3.46). The enantiopurity of metabolite **17** ($[\alpha]_D$ −36) was estimated to be 72% by CSPHPLC analysis [Chiralcel OB column; hexane–isopropyl alcohol (9:1), $\alpha = 1.9$].

(−)-(1S)-1,2-*Dihydrobenzocyclobuten*-1-*ol* **13** (0.228 g, 33%), R_f 0.75, mp 56–58 °C (from hexane) (lit.,[11] mp 58 °C); $[\alpha]_D$ −16 ($CHCl_3$); δ_H(300 MHz) 2.23 (1H, s, OH), 3.03 (1H, dd, $J_{2,2'}$ 14.5, $J_{2',1}$ 1.6, 2′-H), 3.62 (1H, dd, $J_{2,2'}$ 14.4, $J_{2,1}$ 5.0, 2-H), 5.31 (1H, dd, $J_{1,2}$ 5.0, $J_{1,2'}$ 1.6, 1-H) and 7.13–7.33 (4H, m, Ar-H). The enantiopurity of metabolite **13** ($[\alpha]_D$ −16) was estimated to be

ca. 20% by CSPHPLC analysis [Chiralcel OB column; hexane–isopropyl alcohol (9:1), $\alpha = 2.1$].

A fourth band, R_f 0.34, contained a mixture of three compounds and the least polar component, cis-1,2-*dihydroxy*-1,2-*dihydrobenzocyclobutene* **16**, was isolated from the mixture by multiple elution PLC [EtOAc–hexane (1:4)], (0.07 g, 9%), mp 126–128 °C (from $CHCl_3$) (lit.,[24] mp 127.5–128 °C; δ_H(300 MHz) 5.38 (2H, s, 1-H and 2-H) and 7.35 (4H, m, Ar-H).

The remaining low R_f mixture was separated into (1R,2S)-*bicyclo*[4.2.0]*octa*-3,5-*diene*-1,2-*diol* **7**, and (2*R*,3*S*)-*bicyclo*-[4.2.0]*octa*-1(6),4-*diene*-2,3-*diol* **14** by semi-preparative reversed-phase HPLC [Zorbax ODS column, 9.4 × 250 mm; H_2O–MeOH (7:3), $\alpha = 1.9$].

(−)-(1R,2S)-*Bicyclo*[4.2.0]*octa*-3,5-*diene*-1,2-*diol* **7** was isolated from the first fractions (0.143 g, 18%), mp 235 °C (from $CHCl_3$–hexane); $[\alpha]_D$ −166 ($CHCl_3$) (Found: M^+, 138.0684. $C_8H_{10}O_2$ requires *M*, 138.0681); δ_H(500 MHz) 2.15 (2H, m, 8-H), 2.70 (1H, m, 7-H), 3.16 (1H, m, 7-H), 4.63 (1H, s, 2-H), 5.65 (1H, d, $J_{3,4}$ 9.6, 3-H), 5.74 (1H, d, $J_{5,4}$ 4.6, 5-H) and 5.96 (1H, dd, $J_{4,3}$ 9.6, $J_{4,5}$ 4.3, 4-H); *m*/*z* 138 (M^+, 4%) and 120 ($M^+ - H_2O$, 73); CD λ/nm ($\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}) 269 (−4.85) and 226 (−1.15). The enantiomeric excess of diol **7** was determined as >98% by ^{1}H NMR analysis of the mono-MTPA ester derivatives **26** which showed diagnostic MeO singlets at δ 3.60 [(*R*)-MTPA] and 3.53 [(*S*)-MTPA].

(−)-(2*R*,3*S*)-*Bicyclo*[4.2.0]*octa*-1(6),4-*diene*-2,3-*diol* **14** was isolated from the second fraction as an unstable solid (0.10 g, 13%); $[\alpha]_D$ −16 ($CHCl_3$) (Found: M^+, 138.0685. $C_8H_{10}O_2$ requires *M*, 138.0681); δ_H(500 MHz) 2.63 (2H, m, 7-H), 2.70 (1H, m, 8-H), 2.80 (1H, m, 8-H), 4.06 (1H, d, $J_{2,3}$ 6.5, 2-H), 4.44 (1H, m, $J_{3,5}$ 2.2, 3-H), 5.75 (1H, dd, $J_{5,4}$ 9.6, $J_{5,3}$ 2.2, 5-H) and 5.86 (1H, dd, $J_{4,5}$ 9.6, $J_{4,3}$ 2.2, 4-H); *m*/*z* 138 (M^+, 90%) and 120 ($M^+ - H_2O$, 39); CD λ/nm ($\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}) 268 (−2.46), 215 (−1.41) and 199.8 (−1.48). The enantiopurity of diol **14** was determined as >98% by ^{1}H NMR analysis of the di-MTPA ester derivatives **29** of the triazolinedione adduct **28** which showed characteristic MeO singlets at δ 3.08 and 3.56 [(*R*)-MTPA, **29a**], or δ 3.16 and 3.46 [(*S*)-MTPA, **29b**].

The most polar band (R_f 0.09) contained a single compound which was identified as (−)-(1*S*,2*S*,8*S*)-*bicyclo*[4.2.0]*octa*-3,5-*diene*-1,2,8-*triol* **8** (0.08 g, 9%), mp 118–119 °C (from CH_2Cl_2); $[\alpha]_D$ −197 ($CHCl_3$) (Found: C, 61.8; H, 6.2. $C_8H_{10}O_3$ requires C, 62.3; H, 6.5%); δ_H(500 MHz) 2.92 (1H, m, 7-H), 3.17 (1H, m, 7′-H), 4.17 (1H, dd, $J_{8,7'} = J_{8,7}$ 7.1, 8-H), 4.53 (1H, s, 2-H), 5.58 (1H, d, $J_{3,4}$ 9.6, 3-H), 5.79 (1H, d, $J_{5,4}$ 3.2, 5-H) and 5.86 (1H, dd, $J_{4,3}$ 9.6, $J_{4,5}$ 3.2, 4-H); *m*/*z* 136 ($M^+ - H_2O$, 24%); CD λ/nm ($\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}) 273 (−5.43) and 226 (−1.05). The enantiopurity of triol **8** was determined as >98% by ^{1}H NMR analysis of the di-MTPA ester derivatives **30** which showed characteristic MeO singlets at δ 3.50 and 3.54 [(*R*)-MTPA], or δ 3.33 and 3.36 [(*S*)-MTPA].

Separation of (+)-(*R*)- and (−)-(*S*)-1,2-dihydrobenzocyclobuten-1-ol 13 by CSPHPLC

Racemic 1,2-dihydrobenzocyclobuten-1-ol **13**[11] (0.2 g) was separated into pure enantiomers using a Chiralcel OB semi-preparative HPLC column [9.4 × 250 mm; isopropyl alcohol–hexane (10:90), 0.01 g injections, $\alpha = 2.1$]. The first isomer to elute was (−)-(*S*)-**13**, mp 57–59 °C; $[\alpha]_D$ −88 ($CHCl_3$). The later eluting isomer was (+)-(*R*)-**13**, mp 58–60 °C; $[\alpha]_D$ +88 ($CHCl_3$).

Metabolism of (+)-(*R*)-1,2-dihydrobenzocyclobuten-1-ol 13

An enantiopure sample of (+)-(*R*)-**13** (0.1 g) was metabolised over a 24 h period, using *P. putida* UV4 under standard conditions, and the aqueous extracts were worked up by the normal procedure. PLC Separation of the crude mixture of bioproducts gave *cis*-diol **16** (0.04 g) and 1-oxo-2-hydroxy-1,2-dihydrobenzocyclobutene **17** (0.01 g), which were identical to the samples obtained from biotransformation of 1,2-dihydrobenzocyclobutene **12**. (*R*)-1,2-Dihydrobenzocyclobuten-1-ol **13** (0.03 g) was also recovered.

Metabolism of (−)-(*S*)-1,2-dihydrobenzocyclobuten-1-ol 13

An enantiopure sample of (−)-(*S*)-**13** (0.1 g) was metabolized and the products isolated in a similar manner to the metabolites of the (+)-(*R*)-enantiomer **13**. 1,2-Dihydrobenzocyclobuten-1-one **15** (0.06 g) and triol **8** (0.02 g) were isolated and found to be identical in all respects to the samples isolated from metabolism of 1,2-dihydrobenzocyclobutene **12**.

Metabolism of biphenylene 33

The crude product, extracted from the biotransformed substrate **33** (0.1 g, 6.5 mmol) appeared to contain only one metabolite which was identified as 1,8b-dihydrobiphenylene-*cis*-1,8b-diol **9** after purification by PLC [EtOAc:hexane (1:1)], (0.08 g, 66%), mp 119–121 °C (from $CHCl_3$); $[\alpha]_D$ +429 (MeOH) (Found: M^+, 186.1686. $C_{12}H_{10}O_2$ requires *M*, 186.1681); δ_H(500 MHz) 4.62 (1H, s, 1-H), 5.81 (1H, dd, $J_{2,3}$ 9.3, $J_{2,4}$ 1.6, 2-H), 6.07 (1H, dd, $J_{4,2}$ 1.6, $J_{4,3}$ 4.4, 4-H), 6.16 (1H, dd, $J_{3,4}$ 4.4, $J_{3,2}$ 9.3, 3-H) and 7.30–7.46 (4H, m, Ar-H); *m*/*z* 186 (M^+, 42%) and 168 ($M^+ - H_2O$, 100); CD λ/nm ($\Delta\varepsilon$/dm^3 mol^{-1} cm^{-1}) 325 (+1.86), 227 (−2.9) and 200 (−3.1). Conversion of the *cis*-diol **9** ($[\alpha]_D$ +429) to the di-MTPA derivatives **31** and subsequent ^{1}H NMR analysis allowed the ee value to be determined (>98%) from the characteristic MeO singlets at δ 3.45 and 3.65 [(*R*)-MTPA], or δ 3.30 and 3.66 [(*S*)-MTPA].

Assignment of relative and absolute configuration of metabolites and derivatives

(i) Conversion of (−)-(*S*)-1,2-dihydrobenzocyclobuten-1-ol 13 to (−)-(2*S*)-dimethyl acetoxysuccinate 22

The metabolite 1,2-dihydrobenzocyclobuten-1-ol **13** ($[\alpha]_D$ −16; 0.4 g, 3.3 mmol), on treatment with Ac_2O–pyridine, gave the acetoxy derivative **18** (0.320 g, 60%), bp 130–133 °C/3 mmHg (lit.,[11] bp 80–84 °C/1.5 mmHg), $[\alpha]_D$ −8.2 ($CHCl_3$). To a solution of the acetate **18** ($[\alpha]_D$ −8.2; 0.2 g, 1.2 mmol) in CCl_4 (5 cm^3), was added CH_3CN (5 cm^3), water (7 cm^3), $NaIO_4$ (7 g) and a catalytic amount (*ca.* 0.001 g) of ruthenium(IV) oxide hydrate. The reaction mixture was stirred at room temperature (72 h), treated with hydrochloric acid (20 cm^3; 1.5 M) saturated with NaCl, and then extracted with EtOAc (3 × 50 cm^3). The ethyl acetate extract was washed with saturated aqueous NaCl (30 cm^3), dried (Na_2SO_4) and the solvent distilled off under reduced pressure to yield an oily residue of acetoxysuccinic acid **20** (0.12 g, 57%). The ^{1}H NMR spectrum of this sample was consistent with structure **20** and the sample was used without further purification. A solution of the crude acetoxysuccinic acid **20** (0.1 g) in methanol (1 cm^3) was reacted (4 h, 0 °C) with an excess of an ethereal solution of CH_2N_2. The solvents and excess CH_2N_2 were removed with a stream of nitrogen and the residue purified by column chromatography (hexane–EtOAc) to give (−)-(2*S*)-dimethyl acetoxysuccinate **22** as a colourless oil (0.08 g, 73%), bp 90–94 °C/3 mm Hg; $[\alpha]_D$ −2.2 ($CHCl_3$) (Found: C, 47.0; H, 6.1. $C_8H_{12}O_6$ requires C, 47.1; H, 5.9%); δ_H(500 MHz) 2.14 (3H, s, O_2CMe), 2.91 (2H, m, 3-H and 3′-H), 3.73 (3H, s, CO_2Me), 3.78 (3H, s, CO_2Me) and 5.48 (1H, dd, $J_{2,3} = J_{2,3'}$ 6.0, 2-H). Comparison of the $[\alpha]_D$ value (−2.2) of the latter sample of dimethyl acetoxysuccinate **22** with that of a sample synthesised from (*S*)-hydroxysuccinic acid **24** ($[\alpha]_D$ −18.2), established an (*S*)-configuration for (−)-1,2-dihydrobenzocyclobuten-1-ol **13**.

(ii) Conversion of *cis*-1,2-dihydroxy-1,2-dihydrobenzocyclobutene 16 to *meso*-dimethyl 2,3-diacetoxysuccinate 23

cis-1,2-Dihydroxy-1,2-dihydrobenzocyclobutene **16** (0.1 g, 0.7 mmol) was treated with an excess of acetic anhydride in dry pyridine using a method similar to that described for 1,2-dihydrobenzocyclobuten-1-ol **13**, to afford *cis*-1,2-diacetoxy-

1,2-dihydrobenzocyclobutene **19**. PLC Purification [diethyl ether–hexane (1:4)] gave compound **19** (0.1 g, 83%), bp 140 °C/2 mmHg (lit.,[24] 105–110 °C/0.4 mmHg); δ_H(300 MHz) 2.04 (6H, s, OCOMe), 6.12 (2H, s, 1-H and 2-H) and 7.22–7.32 (4H, m, ArH).

Diacetate **19** (0.16 g, 0.73 mmol) was subjected to oxidative cleavage (RuO_2–$NaIO_4$) using a similar procedure to that described for compound **18**. Purification of the product by PLC [diethyl ether–hexane (2:3)] gave *meso*-dimethyl 2,3-diacetoxysuccinate **23** (0.15 g, 79%), mp 97–99 °C (from MeOH) (Found: C, 45.6; H, 5.4. $C_{10}H_{14}O_8$ requires C, 45.8; H, 5.4%); δ_H(500 MHz) 2.19 (6H, s, O_2CMe), 3.84 (6H, s, CO_2Me) and 5.67 (2H, s, 2-H, 3-H). Compound **23** was found to be spectrally and chromatographically indistinguishable from an authentic sample of *meso*-dimethyl 2,3-diacetoxysuccinate.

(iii) Conversion of (−)-(1*R*,2*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2-diol 7 to (−)-(1*R*,2*S*)-*cis*-1,2-bis{(3,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptan-1-yl)carbonyloxy}bicyclo[4.2.0]octa-3,5-diene 27

The angular *cis*-monohydrodiol metabolite **7** (0.05 g, 0.36 mmol), $[\alpha]_D$ −166 ($CHCl_3$), on reaction with (−)-(1*S*)-3-oxo-4,7,7-trimethyl-2-oxabicyclo[2.2.1]heptane-1-carbonyl chloride (camphanic chloride; 0.173 g, 0.8 mmol) in dry pyridine and subsequent PLC purification [Et_2O–hexane (70:30)] of the crude product gave colourless crystals of the dicamphanate **27** (0.15 g, 83%), mp 163–164 °C (from MeOH); $[\alpha]_D$ −229 ($CHCl_3$) (Found: C, 67.1; H, 6.8. $C_{28}H_{34}O_8$ requires C, 67.5; H, 6.9%); δ_H(500 MHz) 0.95 (3H, s, Me), 0.99 (3H, s, Me), 1.07 (6H, s, Me), 1.09 (3H, s, Me), 1.10 (3H, s, Me), 1.66 (1H, m, H_{cam}), 1.91 (1H, m, H_{cam}), 2.07 (1H, m, H_{cam}), 2.15 (1H, m, H_{cam}), 2.45 (2H, m, H_{cam}), 2.72 (2H, m, H_{cam}), 5.56 (1H, d, $J_{2,3}$ 9.8, 3-H), 5.83 (1H, m, 3-H), 5.88 (1H, s, 2-H) and 6.02 (1H, m, 4-H); *m*/*z* 498 (M^+, 2.5%), 120 (100).

X-Ray crystal structure analysis of compound 27

Crystal data. $C_{28}H_{34}O_8$, M = 498.6. Orthorhombic, a = 31.593(7), b = 11.267(7), c = 7.334(1) Å, V = 2610.6(8) Å^3, λ = 0.710 73 Å, space group $P2_12_12$ (No. 18), Z = 4, D_x = 1.268 g cm^{-3}. Colourless prisms, dimensions 0.96 × 0.60 × 0.57 mm, μ(Mo-Kα) = 0.92 cm^{-1}, F(000) = 1064.

Data collection and processing. Siemens P3 diffractometer, ω scan, scan width 1.0°, 4 < 2θ < 60°, h: 0→44, k: 0→15, l: 0→10; graphite-monochromated Mo-Kα radiation; 4340 unique reflections measured giving 2258 with $I > 2\sigma(I)$.

Structure analysis and refinement. Direct methods (SHELXS86).[25] Full-matrix least-squares refinement on F^2 (SHELXL-93)[26] with all non-hydrogen atoms anisotropic and hydrogens in calculated positions using the riding model with U_{iso}(H) = 1.2U(eq) for the attached atom. Final R_1 = 0.065 (for 2258 data), wR_2 = 0.269 (all data), GOF = 0.84, maximum residual electron density 0.17 e Å^{-3}. A projection of the molecule is shown in Fig. 1.‡

(iv) Conversion of (−)-(2*R*,3*S*)-bicyclo[4.2.0]octa-1(6),4-diene-2,3-diol 14 to (10*R*,11*S*)-10,11-dihydroxy-2-phenyl-2,3,5,7,8,8a-hexahydro-1*H*-5,8a-ethanocyclobuta[*c*][1,2,4]triazolo[1,2-*a*]-pyridazine-1,3-dione 28

To a stirring solution of *cis*-diol metabolite **14** (0.02 g, 0.14 mmol; $[\alpha]_D$ −16) in CH_2Cl_2 (1 cm^3) was added, dropwise, a solution of freshly sublimed 4-phenyl-1,2,4-triazoline-3,5-dione (0.03 g, 0.17 mmol) in CH_2Cl_2 (1 cm^3) at room temperature. Upon completion of the reaction (*ca.* 3 h), CH_2Cl_2 was evaporated off and the resulting crude product was purified by PLC (EtOAc) to give (10*R*,11*S*)-10,11-dihydroxy-2-phenyl-2,3,5,7,8,8a-hexahydro-1*H*-5,8a-ethanocyclobuta[*c*][1,2,4]triazolo[1,2-*a*]pyridazine-1,3-dione **28** (0.03 g, 66%), mp 171–172 °C ($CHCl_3$–hexane); $[\alpha]_D$ +2 (pyridine) (Found: C, 60.9; H, 4.5; N, 13.3. $C_{16}H_{15}N_3O_4$ requires C, 61.3; H, 4.8; N, 13.4%); δ_H(300 MHz) 2.70 (1H, m, 8-H), 2.87 (1H, m, 8-H), 3.10 (1H, m, 7-H), 3.25 (1H, m, 7-H), 3.92 (1H, dd, $J_{11,10}$ 8.2, $J_{11,5}$ 2.2, 11-H), 4.03 (1H, d, $J_{10,11}$ 8.2, 10-H), 4.88 (1H, dd, $J_{5,11}$ 2.2, $J_{5,6}$ 5.6, 5-H), 5.99 (1H, d, $J_{6,5}$ 5.6, 6-H) and 7.26–7.47 (5H, m, Ar-H); *m*/*z* 313 (M^+, 20%) and 253 (100).

Conversion of compound 28 to (10*R*,11*S*)-10,11-di-[(*R*)-methoxytrifluoromethylphenylacetoxy]-2-phenyl-2,3,5,7,8,8a-hexahydro-1*H*-5,8a-ethanocyclobuta[*c*][1,2,4]triazolo[1,2-*a*]-pyridazine-1,3-dione 29a

A solution of cycloadduct **28** (0.01 g, 0.03 mmol) in dry pyridine (0.5 cm^3) containing 4-dimethylaminopyridine (0.005 g) was treated with (+)-MTPA chloride (0.018 g, 0.07 mmol derived from R-MTPA). The mixture was heated at 55 °C for 36 h. Pyridine was removed under reduced pressure from the reaction mixture, by forming an azeotrope with toluene. The residue was purified by PLC [MeOH:$CHCl_3$ (2:98)] to afford the di-MTPA ester **29a** (0.014 g, 60%), mp 150–152 °C ($CHCl_3$–hexane); $[\alpha]_D$ +16 ($CHCl_3$) (Found: C, 58.0; H, 3.7; N, 5.9. $C_{36}H_{29}F_6N_3O_8$ requires C, 58.0; H, 3.9; N, 5.6%); δ_H(500 MHz) 2.60 (1H, m, 8-H), 3.02 (1H, m, 8-H), 3.08 (3H, s, OMe), 3.56 (3H, s, OMe), 3.67 (2H, m, 7-H), 5.21 (1H, d, $J_{10,11}$ 8.6, 10-H), 5.25 (1H, dd, $J_{5,11}$ 2.2, $J_{5,6}$ 5.6, 5-H), 5.32 (1H, dd, $J_{11,5}$ 2.2, $J_{11,10}$ 8.6, 11-H), 6.12 (1H, m, $J_{6,5}$ 5.6, 6-H) and 7.30–7.47 (15H, m, Ar-H).

Conversion of compound 28 to (10*R*,11*S*)-10,11-di-[(*S*)-methoxytrifluoromethylphenylacetoxy]-2-phenyl-2,3,5,7,8,8a-hexahydro-1*H*-5,8a-ethanocyclobuta[*c*][1,2,4]triazolo[1,2-*a*]-pyridazine-1,3-dione 29b

Using (−)-MTPA-chloride (derived from S-MTPA) the cycloadduct **28** was esterified to yield the di-MTPA ester **29b**, mp 139–140 °C (from $CHCl_3$–hexane); $[\alpha]_D$ +32 ($CHCl_3$) (Found: C, 57.8; H, 3.9; N, 5.9. $C_{36}H_{29}F_6N_3O_8$ requires C, 57.8; H, 3.9; N, 5.6%); δ_H(500 MHz) 2.84 (1H, m, 8-H), 3.05 (2H, m, 7-H, 8-H), 3.16 (3H, s, OMe), 3.46 (3H, s, OMe), 3.76 (1H, m, 7-H), 5.13 (1H, dd, $J_{5,11}$ 2.3, $J_{5,6}$ 5.3, 5-H), 5.16 (1H, dd, $J_{11,10}$ 8.6, $J_{11,5}$ 2.3, 11-H), 5.51 (1H, d, $J_{10,11}$ 8.6, 10-H), 6.11 (1H, m, $J_{6,5}$ 5.3, 6-H) and 7.31–7.56 (15H, m, Ar-H).

X-Ray crystal structure analysis of compound 29b

Crystal data. $C_{36}H_{29}F_6N_3O_8$, M = 745.6. Orthorhombic, a = 11.970(3), b = 16.096(6), c = 17.972(6) Å, V = 3463(2) A^3, λ = 0.710 73 Å, space group $P2_12_12_1$ (No. 19), Z = 4, D_x = 1.43 g cm^{-3}. Colourless blocks, dimensions 0.93 × 0.72 × 0.65 mm, μ(Mo-Kα) = 1.22 cm^{-1}.

Data collection and processing. Siemens P3 diffractometer, ω scan, scan width 1.2°, 3.5 < 2θ < 56°, h: 0→15, k: 0→21, l: 0→23; graphite-monochromated Mo-Kα radiation; 4664 unique reflections measured giving 2468 with $I > 2\sigma(I)$.

Structure analysis and refinement. Direct methods (SHELXS86).[25] Full-matrix least-squares refinement on F^2 (SHELXL-93)[26] with all non-hydrogen atoms anisotropic and hydrogens in calculated positions using the riding model with U_{iso}(H) = 1.2U(eq) for the attached atom. Final R_1 = 0.057 (for 2468 data), wR_2 = 0.138 (all data), GOF = 1.03, maximum residual electron density 0.18 e Å^{-3}. A projection of the molecule is shown in Fig. 2.

(v) (−)-(1*S*,2*S*,8*S*)-Bicyclo[4.2.0]octa-3,5-diene-1,2,8-triol 8

Triol **8** ($[\alpha]_D$ −197, $CHCl_3$) derived from the metabolism of (1*S*)-1,2-dihydrobenzocyclobuten-1-ol **13** was assigned a (1*S*,2*S*,8*S*) configuration based on CD spectral comparison with (−)-(1*R*,2*S*)-bicyclo[4.2.0]octa-3,5-diene-1,2-diol **7** (Fig. 4).

‡ Atomic coordinates, thermal parameters and bond length and angles have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Instructions for Authors, *J. Chem. Soc., Perkin Trans. 1*, 1997, Issue 1. Any request to the CCDC for this material should quote the full literature citation and the reference number 207/110.

(vi) Conversion of (+)-(1*S*,8b*R*)-1,8b-dihydrobiphenylene-*cis*-1,8b-diol 9 to (−)-(1*S*,8b*R*)-*cis*-1,2-bis{(4,7,7-trimethyl-3-oxo-2-oxabicyclo[2.2.1]heptan-1-yl)carbonyloxy}biphenylene 32

Biphenylene-*cis*-diol **9** (0.1 g, 0.54 mmol; $[\alpha]_D$ +429) was reacted with (−)-(1*S*)-camphanyl chloride (0.25 g, 1.16 mmol) in dry pyridine (1 cm^3) at ambient temperature. PLC Purification [Et_2O–hexane (70:30)] yielded the dicamphanate **32** as a white crystalline solid (0.19 g, 65%), mp 178–180 °C (MeOH–Me_2CO), $[\alpha]_D$ −72 ($CHCl_3$) (Found: C, 70.2; H, 6.1. $C_{32}H_{34}O_8$ requires C, 70.3; H, 6.3%); δ_H(300 MHz) 0.86 (3H, s, Me), 0.98 (3H, s, Me), 1.00 (3H, s, Me), 1.06 (3H, s, Me), 1.10 (3H, s, Me), 1.13 (3H, s, Me), 1.75 (2H, m, H_{cam}), 1.96 (3H, m, H_{cam}), 2.35 (2H, m, H_{cam}), 2.58 (1H, m, H_{cam}), 5.75 (1H, d, $J_{2,3}$ 9.8, 2-H), 5.78 (1H, s, 1-H), 6.19 (1H, d, $J_{4.3}$ 4.6, 4-H), 6.29 (1H, dd, $J_{3,4}$ 4.6, $J_{3,2}$ 2.1, 3-H) and 7.31–7.59 (4H, m, Ar-H).

X-Ray crystal structure analysis of compound 32

Crystal data. $C_{32}H_{34}O_8$, M = 546.6. Monoclinic, a = 7.224(3), b = 11.333(8), c = 17.854(12) Å, β = 98.65(4)°, V = 1445(2) $Å^3$, λ = 0.710 73 Å, space group $P2_1$ (No. 4), Z = 2, D_x = 1.256 g cm^{-3}. Colourless blocks, dimensions 0.98 × 0.92 × 0.80 mm, μ(Mo-Kα) = 0.90 cm^{-1}.

Data collection and processing. Siemens P3 diffractometer, ω scan, scan width 2.0°, $4 < 2\theta < 60°$, h: −10→10, k: 0→15, l: 0→25; graphite-monochromated Mo-Kα radiation; 3902 unique reflections measured giving 2809 with $I > 2\sigma(I)$.

Structure analysis and refinement. Direct methods (SHELXS86).[25] Full-matrix least-squares refinement on F^2 (SHELXL-93)[26] with all non-hydrogen atoms anisotropic and hydrogens in calculated positions using the riding model with U_{iso}(H) = 1.2U(eq) for the attached atom. Final R_1 = 0.090 (for 2809 data), wR_2 = 0.251 (all data), GOF = 1.08, maximum residual electron density 0.51 e $Å^{-3}$. A projection of the molecule is shown in Fig. 5.

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