Enzyme-catalysed synthesis and reactions of benzene oxide/oxepine derivatives of methyl benzoates†

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A series of twelve benzoate esters was metabolised, by species of the *Phellinus* genus of wood-rotting fungi, to yield the corresponding benzyl alcohol derivatives and eight salicylates. The isolation of a stable oxepine metabolite, from methyl benzoate, allied to evidence of the migration and retention of a carbomethoxy group (the NIH Shift), during enzyme-catalysed *ortho*-hydroxylation of alkyl benzoates to form salicylates, is consistent with a mechanism involving an initial arene epoxidation step. This mechanism was confirmed by the isolation of a remarkably stable, optically active, substituted benzene oxide metabolite of methyl 2-(trifluoromethyl)benzoate, which slowly converted into the racemic form. The arene oxide was found to undergo a cycloaddition reaction with 4-phenyl-1,2,4-triazoline-3,5-dione to yield a crystalline cycloadduct whose structure and racemic nature was established by X-ray crystallography. The metabolite was also found to undergo some novel benzene oxide reactions, including epoxidation to give an *anti*-diepoxide, base-catalysed hydrolysis to form a *trans*-dihydrodiol and acid-catalysed aromatisation to yield a salicylate derivative *via* the NIH Shift of a carbomethoxy group.

Introduction

Aromatic hydroxylation of monosubstituted benzene substrates, to yield the corresponding phenols, is a common biotransformation in eukaryotic (*e.g.* fungal) and prokaryotic (*e.g.* bacterial) cells.[1] Earlier studies, using more than twelve different fungal strains with several monosubstituted benzene substrates **1** (X = Y = H, R = Me, OMe, Cl, Br, CO_2Me, CO_2Bu^t), have shown that both *ortho*- and *para*-hydroxylation can occur, with *ortho*-phenols **5** generally found as the major metabolites (Scheme 1).[2–7] Furthermore, using deuterium-labelled substituted benzene substrates **1** (X or Y = H or D), *ortho*- and *para*-hydroxylation[2,5–7] was found to be accompanied by the migration and partial retention of deuterium label (the NIH Shift)[8–10] in the resulting phenols **5** and **5′**. This observation was taken as evidence for the initial formation of the corresponding equilibrating valence tautomeric arene oxide/oxepine intermediates **2/2′**–**4/4′**. Recent studies have, however, shown that the NIH Shift can also be accounted for without involvement of arene oxide/oxepine intermediates. Thus, the enzyme-catalysed formation of *cis*- or *trans*-dihydrodiol metabolites of arene substrates, followed by dehydration, or the direct insertion of an oxygen atom, can all involve similar cation (or radical) intermediates and result in the NIH Shift without the requirement for arene oxide intermediates.[11–17] In view of these alternative mechanisms, to account for the NIH Shift phenomenon occurring during aromatic hydroxylation,[11–17] unequivocal evidence for the formation of monocyclic arene oxide/oxepine intermediates **2/2′** in fungal cells was sought and reported in our preliminary communication.[7]

A more comprehensive study is now presented with particular focus on (i) provision of direct evidence for the fungal enzyme-catalysed formation of oxepine **2′a** from methyl benzoate **1a** (R = CO_2Me, X = Y = H,) and arene oxide **2d** from substrate **1** (R = CO_2Me, X = CF_3, Y = H) metabolites derived from the corresponding methyl benzoates, (ii) indirect evidence for the involvement of monocyclic 1,2-arene oxides/oxepines **2/2′** (derived from methyl benzoate **1a**, methyl 2-(trifluoromethyl)benzoate **1d**, methyl 3-fluorobenzoate **1e**, methyl 3-methylbenzoate **1f**, methyl 4-fluorobenzoate **1g**, methyl 4-chlorobenzoate **1h**, methyl 4-methylbenzoate **1i** and ethyl benzoate **1l**) based on the identification of the relevant methyl salicylate fungal metabolites and observation of the NIH Shift and (iii) reactivity studies of the most stable monocyclic 1,2-arene oxide metabolite **2d** isolated by enzyme-catalysed oxidation of methyl benzoate **1d** (R = CO_2Me, X = CF_3, Y = H). Novel benzene oxide reactions observed include cycloaddition, epoxidation, hydrolysis and aromatisation.

Results and discussion

Our previous studies of the fungal enzyme-catalysed aromatic hydroxylation of 2-D- and 4-D-labelled toluenes **1** (R = Me, X or Y = D) and anisoles **1** (R = OMe, X or Y = D) showed the characteristic migration and partial retention of the D label on a neighbouring carbon atom associated with the NIH Shift in the

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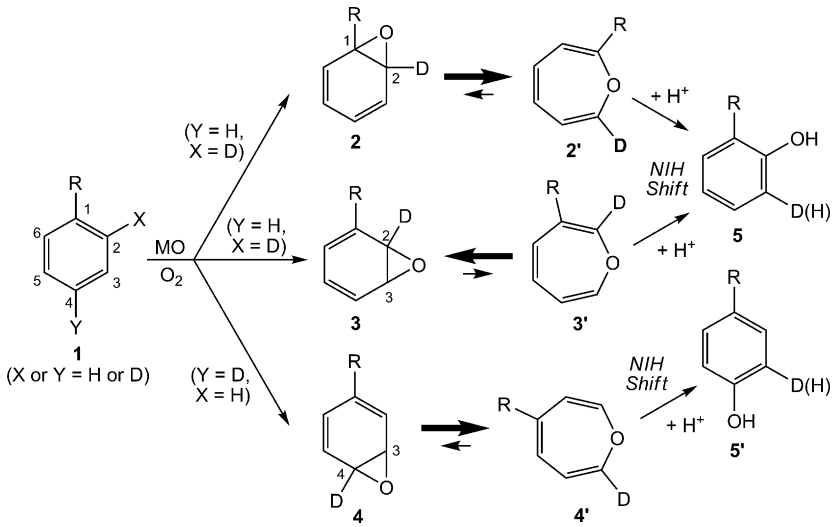


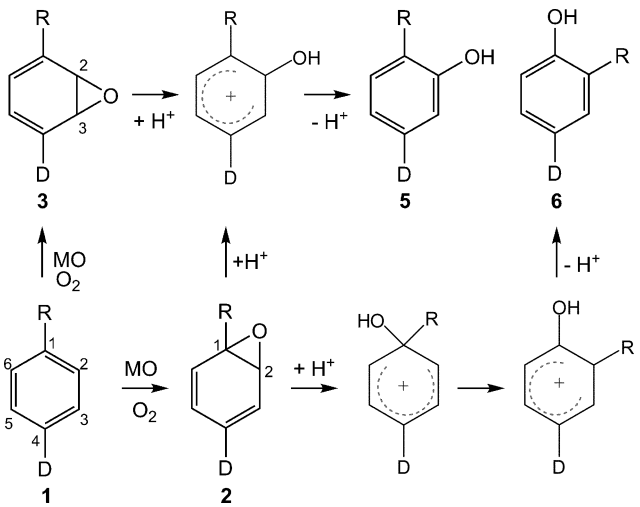
Scheme 1 Monooxygenase (MO)-catalysed epoxidation of substituted benzenes (**1**) to form 1,2- (**2**), 2,3- (**3**) and 3,4-benzene oxides (**4**) and the corresponding oxepines (**2′**, **3′**, **4′**), and acid-catalysed rearrangement to yield the corresponding *ortho*- (**5**) and *para*-phenols (**5′**).

ortho- (**5**, R = Me, 11, 65% D) and *para*-substituted phenols (**5′**, R = OMe, 49, 71% D, Scheme 1).[5]

These observations were consistent with the involvement of fungal monooxygenase (MO) enzymes and the intermediacy of arene oxides/oxepines **2**/**2′**–**4**/**4′**, (Scheme 1). The benzene oxides (**2–4**) were assumed to be the initial metabolites existing in a state of rapid equilibration with the corresponding oxepine valence tautomers (**2′**–**4′**) at ambient temperature. The balance between monosubstituted benzene oxides (**2–4**) and oxepine tautomers (**2′**–**4′**) was dependent on the substituent position.[8–10] Thus 1,2- (**2**) and 3,4- (**4**) arene oxide tautomers are not detectable by NMR spectroscopy; these compounds exist almost exclusively in the oxepine forms **2′** and **4′** (Scheme 1). Conversely, the 2,3-arene oxide **3** is strongly preferred over the corresponding oxepine tautomer **3′** at equilibrium. Due to their instability and propensity to undergo further metabolism, no unequivocal evidence (*e.g.* NMR spectroscopy or X-ray crystallography) of benzene oxide/oxepine metabolite formation, from the corresponding substituted benzene substrates, is currently available. Thus, the 3,4-arene oxide/oxepine derivatives **4**/**4′** (R = Cl and Br), from chloro- and bromo-benzenes **1** (R = Cl and Br), were synthesised chemically but were only detected using ^{14}C-labelled halobenzene substrates, with monooxygenase enzymes (CYP-450 isoenzymes) present in liver microsomal fractions employing radiochemical detection methods.[18,19]

While fungal *para*-hydroxylation of the monohalogenated benzene substrates **1** (R = Cl, Br, X = Y = H), to give the corresponding phenols **5′** (R = Cl, Br, X = Y = H), was observed in some cases, *ortho*-hydroxylation was found to be the major metabolic pathway using seven of the ten fungi studied.[5,6] Employing the most efficient fungal strains (*Rhizopus stolonifer* and *Rhizopus arrhizus*), *ortho*-hydroxylation of chlorobenzenes **1** (R = Cl, X = D, Y = H) and bromobenzenes **1** (R = Br, X = D, Y = H) was found to be the dominant metabolic pathway (Scheme 1).[5] With these two fungal strains, the NIH Shift of a deuterium atom was observed during formation of the phenols (**5**, R = Cl; 67–73% D; R = Br; 64–69% D). On the assumption that arene oxide intermediates are generally involved during *ortho*-hydroxylation of toluene **1** (R = Me, X = D, Y = H), anisole **1** (R = OMe, X = D, Y = H), chlorobenzene **1** (R = Cl, X = D, Y = H), and bromobenzene **1** (R = Br, X = D, Y = H) in fungi,[5,6] the NIH Shift could be accounted for by the formation of either 1,2- (**2**), or 2,3-arene oxides (**3**) followed by acid catalysed aromatisation to give the same *ortho*-phenol **5**, (Scheme 1, R = Me, OMe, Cl, Br).

To distinguish between the formation of arene oxide types **2** or **3** during *ortho*-hydroxylation, 4D-chlorobenzene **1** (R = Cl) and 4D-bromobenzene **1** (R = Br) were used as substrates with whole cells of *R. stolonifer* and *R. arrhizus* (Scheme 2). These substrates were selected since it was anticipated that any 1,2-arene oxide **2** (R = Cl or Br) formed would isomerise largely to the corresponding phenol **6** (R = Cl or Br), with concomitant migration of the halogen atom, and that the other phenol **5** (R = Cl or Br) would only be found as a minor product (Scheme 2). This type of NIH Shift of a halogen atom was well precedented during earlier



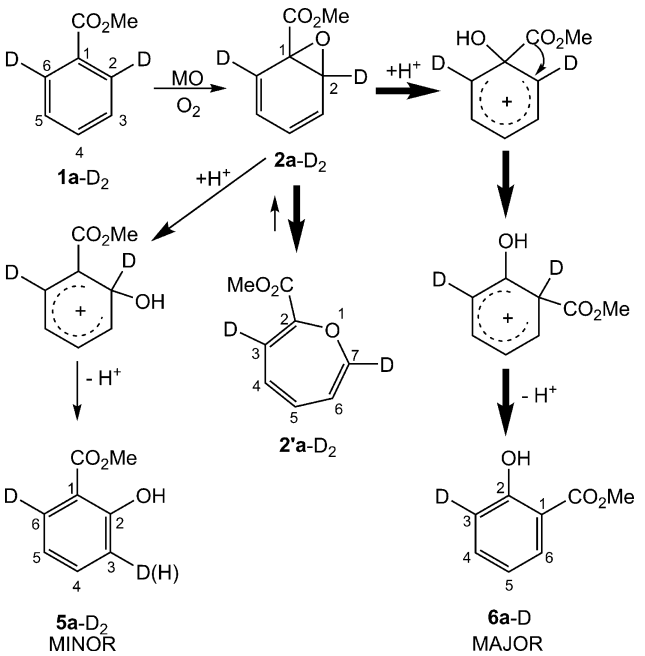
Scheme 2 Monooxygenase (MO)-catalysed epoxidation of monosubstituted benzenes (**1**) to form 1,2- (**2**) and 2,3-benzene oxides (**3**), and acid-catalysed rearrangement to yield the corresponding *ortho*-phenols (**5** and **6**).

enzyme-catalysed aromatic hydroxylations and isomerisations of α-halogeno epoxides to α-halogeno ketones.[6] In practice, however, ^{1}H-NMR and MS analyses of the resulting products showed that only *ortho*-phenol **5** was formed *i.e.* no migration of halogen atom R to yield phenol **6** had occurred.[6] Whilst this negative result was consistent with the formation of the 2,3-arene oxide of chlorobenzenes (**3**, R = Cl or Br), rather than the 1,2-arene oxide (**2**, R = Cl or Br), more convincing evidence was sought for the involvement of arene oxides **2** or **3** during aromatic hydroxylations by fungi.

Our earlier studies, on the synthesis of the parent oxepine **2′a** (Scheme 3), and oxepine derivatives of methyl benzoate **1a** substituted by deuterium atoms or Me groups, showed that this type of compound was relatively stable and that aromatisation involved almost exclusive migration of the carbomethoxy group from the minor arene oxide tautomer **2a** to give the corresponding *ortho*-phenol **5a** (minor) and **6a** (major, Scheme 3).[20,21] Investigations, undertaken in these laboratories,[24–27] into the biological role of chloromethane (CH_3Cl), a volatile metabolite released by many species of *Phellinus*, a widespread genus of white rot fungi, have demonstrated that the genus is unusual in utilising CH_3Cl rather than *S*-adenosylmethionine as the biological methyl donor in the biosynthesis of the secondary metabolites methyl benzoate, methyl salicylate and methyl 2-furoate. However, studies of the time course of incorporation of label from deuteriated methionine (the metabolic precursor of CH_3Cl) into methyl salicylate by *P. pomaceus* was significantly different from that into methyl benzoate. The latter observation suggests that methyl salicylate is formed in the fungus not by methylation of salicylic acid but by *o*-hydroxylation of methyl benzoate.[24] This conclusion is consistent with the finding that isolated mycelia of the fungus could not use CH_3Cl in methylation of salicylic acid.[26] Later studies[22] on *P. tremulae* detected, as secondary metabolites, not only methyl benzoate **1a** and methyl salicylate **5a/6a**, but also 2-carbomethoxyoxepine **2′a** (Scheme 3). This observation led to speculation that, given the propensity of the arene oxide **2a** to rearrange with migration of the carbomethoxy group, oxepine tautomer **2′a** may be an intermediate in methyl salicylate **5a/6a** biosynthesis from methyl benzoate **1a**. Further investigations[23] using labelled benzoic acid, in experiments with the fungus, provided strong support for this hypothesis.

In a wider context, the authors of the latter study[23] pointed out that salicylic acid plays a key role in the induction of plant resistance to pathogens acting as a signal compound for inducing systemic acquired resistance. In plants, salicylic acid is normally biosynthesised from phenylalanine *via trans*-cinnamic acid and benzoic acid. The latter compound is presumably converted to salicylic acid by monooxygenase-catalysed attack *via* the arene oxide/oxepine. Interestingly, methyl salicylate does not induce systemic acquired resistance. Thus methylation of benzoic acid by *P. tremulae* effectively shuts down induction of resistance by blocking synthesis of salicylic acid.

In the first part of the current investigation, time course studies, employing HPLC analysis and using authentic reference compounds, showed that the naturally occurring metabolites methyl benzoate **1a**, methyl salicylate **5a/6a** and oxepine **2′a**, previously identified in *P. tremulae*, were also present in aqueous cultures of *P. ribis* (Fig. 1). After growth for 10 days, the concentration of methyl benzoate **1a** and oxepine **2′a** increased up to a peak after



Scheme 3 MO-catalysed epoxidation of 2,6-D_2-methyl benzoate (**1a**-D_2) to form 1,2-arene oxide/oxepine (**2a**-D_2/**2′a**-D_2) and acid-catalysed rearrangement to yield the corresponding *ortho*-phenols (**5a**-D_2 and **6a**-D).

18 days. The concentration of methyl salicylate **5a/6a** increased more slowly and appeared to level off after 20 days. Extraction of metabolites from the culture medium after 20 days followed by preparative layer chromatography (PLC) purification gave a sufficient quantity of oxepine **2′a** (14 mg in 2 L) for detailed NMR analysis. The results of this time course experiment are consistent with oxepine **2′a** and methyl salicylate **5a/6a** both being formed from methyl benzoate **1a** in *P. ribis* by a biosynthetic pathway similar to that established for *P. tremulae* and *P. pomaceus.* HPLC analysis (Fig. 1) did not however provide an answer to the question of carbomethoxy group migration occurring during the biosynthesis of methyl salicylate **5a/6a**.

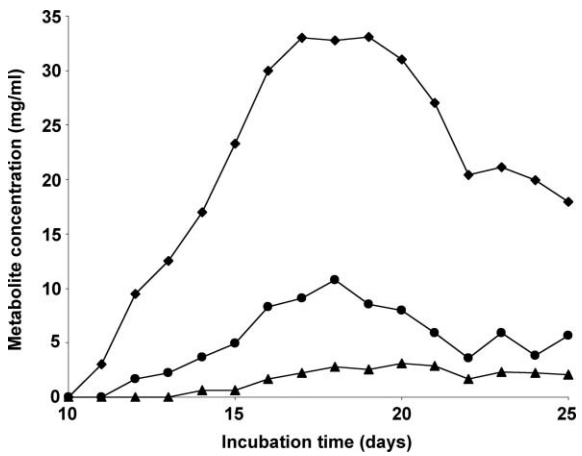


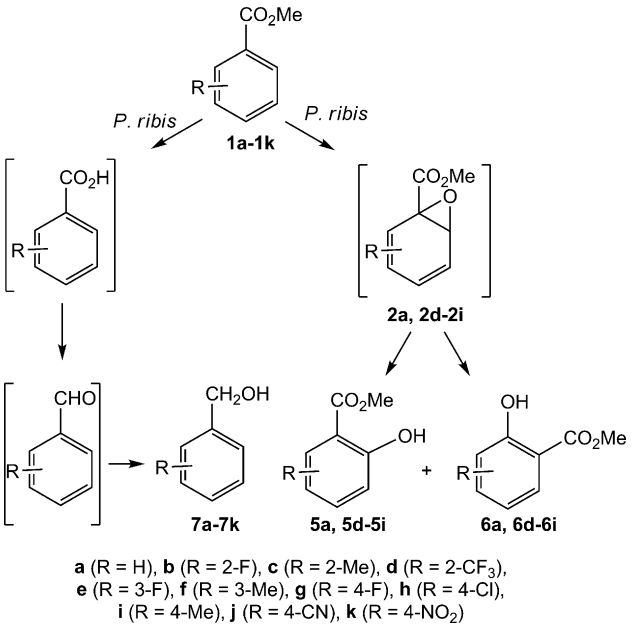
Fig. 1 Formation of the natural metabolites **1a** (●) **2′a** (◆) and **5a/6a** (▲) in *P. ribis*.

The second part of the study was thus designed to determine whether or not carbomethoxy group migration occurred during the biosynthesis of methyl salicylate **5a/6a** (as found during our

earlier *in vitro* studies);[20,21] it involved the addition of several selectively deuteriated methyl benzoates **1a**, as supplementary substrates to *P. ribis* followed by GC–MS analysis of the metabolites. The interpretation of results obtained from these experiments was more complex, as the natural secondary metabolites **1a**, **2′a** and **5a/6a**, identified during the time course study (Fig. 1), were always present together with the corresponding deuterium-labelled metabolites. Addition of methyl pentadeuteriobenzoate **1a**-D_5 ($C_6D_5CO_2CH_3$) to the growing cultures (after 11 days' growth) of *P. ribis* resulted in the maximum incorporation of label into metabolites **2′a**-D_5 and **5a**-D_4/**6a**-D_4 after 4 days. Furthermore, the resulting oxepine metabolite **2′a** (2.2 mg L^{-1}) showed a higher incorporation (21% D_5) compared with the methyl salicylate bioproduct **5a** (or **6a**, 7% D_4, 0.8 mg L^{-1}). Similarly, when trideuteriomethyl benzoate **1a**-D_3 ($C_6H_5CO_2CD_3$) was added as substrate to growing *P. ribis* cultures, the incorporation into the oxepine **2′a**-D_3 (5 mg L^{-1}) after 3 days was also higher (37% D_3) compared with methyl salicylate **5a**-D_3/**6a**-D_3 (1 mg L^{-1}, 16% D_3). These observations were consistent with the metabolic pathway proposed earlier (**1a** → **2a** ⇌ **2′a** → **5a/6a**, Scheme 3), based on the use of other types of labelled precursors ($C_6H_5{}^{13}CO_2H$ and CD_3Cl).[23,25]

The synthesis and metabolism of the dideuteriated substrate, methyl 2,6-dideuteriobenzoate **1a**-D_2 ($C_6D_2H_3CO_2CH_3$), was then studied using ^{1}H-, ^{2}H-, and ^{13}C-NMR spectroscopy and GC–MS analyses (Scheme 3). Addition of substrate **1a**-D_2 to 11-day-old *P. ribis* cultures, followed by extraction of the bioproducts after a further 10-day incubation period, enabled ^{1}H-NMR and GC–MS analysis to be carried out. The results revealed that, in the methyl salicylate metabolite **5a/6a**, incorporation of deuterium at C-6 was relatively low (10% D) compared with that at C-3 (45% D), indicating that it had, mainly, structure **6a** and that the NIH Shift of the carbomethoxy group had occurred (**1a** → **2a**→ **6a**, Scheme 3). A higher incorporation of deuterium atoms into oxepine **2′a**-D_2 (67% D_2 at C-3 and C-7) was again observed. Furthermore, the ^{1}H-NMR spectrum of methyl salicylate **5a/6a** showed a significant reduction (45%) in the signal of the proton attached to C-3, consistent with an NIH Shift of the carbomethoxy group being the major pathway (as found during the earlier *in vitro* studies).[20,21] The observation was further supported by ^{2}H- and ^{13}C-NMR analyses which showed a strong peak for the deuterium atom at C-3, much weaker peak at C-6, and a correspondingly strong β-shift peak at C-4 and weak β-shift peak at C-6 in the ^{13}C-spectrum. These weaker signals in the ^{2}H- and ^{13}C spectra, combined with a small reduction in the proton signal at C-6 (11%), were also consistent with a minor aromatisation pathway involving an NIH Shift of a deuterium atom rather than the carbomethoxy group (**1a** → **2a**→ **5a**, Scheme 3).

Indirect evidence for the intermediacy of arene oxides/oxepines **2/2′**, from biotransformation of methyl benzoate **1a** (R = H) and many of the corresponding substituted methyl benzoate substrates **1d** (R= 2-CF_3), **1e** (3-F), **1f** (3-Me), **1g** (4-F), **1h** (4-Cl), **1i** (4-Me), and ethyl benzoate **1l**, was obtained from GC–MS analysis of extracts from cultures of *P. ribis* and *P. pomaceus* (Scheme 4 and Table 1).



Scheme 4 Enzyme-catalysed reduction and hydroxylation of methylbenzoates (**1**) to yield benzylic alcohols (**7**) and substituted methyl salicylates (**5/6**).

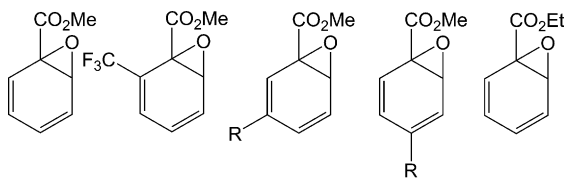
In all cases, the normally produced methyl salicylate **5a/6a** was obtained but, in addition, from the corresponding substrates

Table 1 Relative ratios of bioproducts observed from biotransformations of alkyl benzoates **1a–l** in *P. ribis* and *P. pomaceus*

Alkyl benzoate	Alkyl salicylate products[a]		Arene oxide/oxepine products [a]	
1a	**5a/6a**	++	**2′a** [b]	++
1b	ND		ND	
1c	ND		ND	
1d	**5d/6d**	+	**2d** [b]	++
1e	**5e/6e**	++	ND	
1f	**5f/6f**	++	ND	
1g	**5g/6g**	++	ND	
1h	**5h/6h**	++	ND	
1i	**5i/6i**	++	ND	
1j	ND		ND	
1k	ND		ND	
1l	**5l**	++	**2′l**	+

[a] Average relative proportions estimated by GC–MS analysis. [b] Structure determined by NMR analysis. ND = not detected; ++ indicates presence at approximately similar concentration to natural bioproducts (**2′a**, **5a/6a**); + indicates presence at a lower concentration than natural bioproducts (**2′a**, **5a/6a**).

(**1d–i**), six other methyl salicylates **5d–i**/**6d–i** were also found as concomitant biotransformation products. It is probable that, in common with the parent methyl benzoate **1a**, which yielded oxepine **2′a** and methyl salicylate **5a**/**6a**, aromatisation of the corresponding arene oxides **2d–i** was responsible for the formation of methyl salicylates **5d–i**/**6d–i**. This conclusion was further supported by ^{1}H-NMR evidence of the NIH Shift of the carbomethoxy group in the formation of methyl salicylates **6a**, **6d**, and **6g** during the biotransformations of compounds **1a**, **1d** and **1g** and by acid-catalysed rearrangements of the corresponding oxepine **2′a** and arene oxide **2d** (see Scheme 5). The observation of migration of the carbomethoxy group, during these enzyme-catalysed *ortho*-hydroxylations of methyl benzoates in *P. ribis* and *P. pomaceus*, is of considerable relevance in the context of the *ortho*-hydroxylation of 4-hydroxybenzoic acid to yield gentisic acid (2,5-dihydroxybenzoic acid) in other microbial species.[28,29] It was evident, from earlier aromatisation studies of the 1,2-arene oxide of benzoic acid, that migration of the carboxyl group did not occur.[21] Now, in light of our findings, it would seem much more likely that a carboxylic acid derivative, *e.g.* an ester or thioester, is the migrating group, which is then hydrolysed back to the carboxyl group found in gentisic acid.



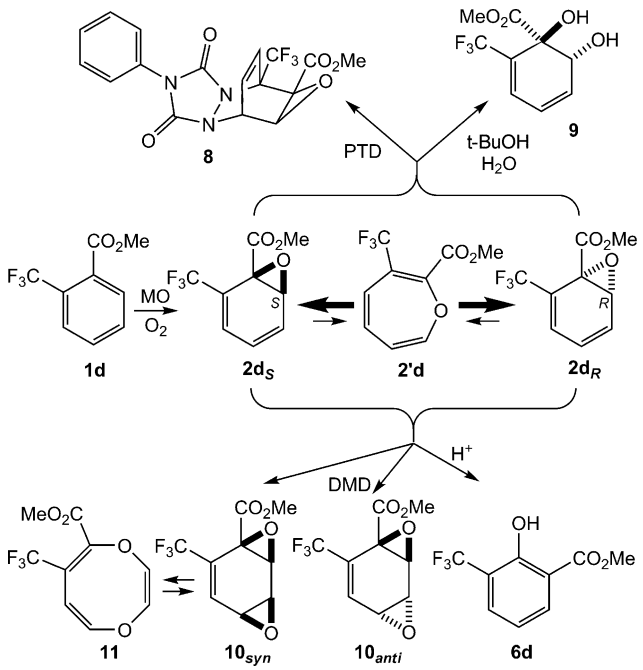
2a **2d** **2e** (R = 3-F) **2f** (R = 3-Me) **2g** (R = F) **2h** (R = Cl), **2i** (R = Me) **2l**

Enzyme-catalysed reduction of the ester group in the methyl benzoate substrates **1a–k**, consistently, yielded the corresponding benzylic alcohols **7a–k** as metabolites (Scheme 4), although only traces of benzyl alcohol **7d** were detected. The benzylic alcohol bioproducts, being of less importance to this study, were not isolated and were only identified by comparison of mass spectral data with those of authentic standards or with the MS data of the library Wiley 275-pc (Aligent Technologies). It is probable that the biotransformation of methyl benzoates **1a–k**, to the corresponding benzylic alcohols **7a–k**, was initiated *via* enzyme-catalysed hydrolysis to give the corresponding carboxylic acids, as previously reported for methyl benzoate substrate **1a** in growing cultures of the fungi *R. stolonifer* and *R. arrhizus*.[5,6] In microbial systems, the reduction of carboxylic acids to the corresponding aldehydes and benzylic alcohols is well precedented (Scheme 4).

Direct GC–MS evidence was obtained for the formation of oxepine/arene oxide metabolite **2′l**/**2l** from ethyl benzoate **1l**. Although insufficient material was available for NMR analysis and complete characterisation, identification of a molecular ion at *m*/*z* 166, and the similarity of its fragmentation pattern relative to oxepine **2′a** coupled with its retention time relative to the ethyl salicylate metabolite, provided strong evidence in favour of the initial formation of arene oxide **2l** and its preferred existence as the corresponding oxepine tautomer (**2′l**).

Addition of methyl 2-(trifluoromethyl)benzoate **1d** (Scheme 4), as a substrate with *P. ribis*, was found to result in the formation of a remarkably stable arene oxide **2d**, as a major bioproduct, based on GC–MS analysis. When this biotransformation was conducted on a larger scale, it became possible to separate, using PLC, a pure sample of the unnatural arene oxide **2d** from the other bioproducts, including naturally occurring metabolites (**1a**, **2′a**, **6a** and **7a**), the corresponding salicylate (**5d** or **6d**) and benzyl alcohol (**7d**). The isolated sample of arene oxide **2d** (73 mg) was sufficient for characterisation and a limited number of reactivity studies. ^{1}H-NMR and ^{13}C-NMR spectroscopy suggested that the derived metabolite existed, in solution, exclusively as the arene oxide valence tautomer **2d**. Interestingly, in addition to the other metabolites, GC–MS analysis also revealed the presence of a minor arene oxide/oxepine metabolite (<1% relative to arene oxide **2d**) from substrate **1d**; it could not be detected by NMR spectroscopy. On the basis of the MS data, and the earlier precedent for a thermal oxygen walk rearrangement of a monocyclic arene oxide,[30] it was presumed to be an isomeric arene oxide or oxepine artefact of arene oxide **2d** formed during GC–MS analysis.

Surprisingly, arene oxide **2d** was found to be optically active ($[\alpha]_D$ −14, $CHCl_3$), and its circular dichroism spectrum in MeOH showed a strong negative Cotton effect at 242 nm. However, on storage at *ca.* 4 °C the sample racemised to give $\mathbf{2d}_S/\mathbf{2d}_R$ (Scheme 5). A repeat biotransformation of substrate **1d** gave a sample of arene oxide **2d** but with no optical rotation. This observation can be rationalised in terms of a MO-catalysed epoxidation to yield an excess of one arene oxide enantiomer $\mathbf{2d}_S$ (or $\mathbf{2d}_R$) with only a trace of the oxepine tautomer **2′d** being present at equilibrium, leading to a relatively slow rate of racemisation which had gone to completion in the second experiment. Earlier synthetic studies of arene oxide derivatives of polycyclic aromatic hydrocarbons (PAHs), from enantiopure precursors, showed that while some were optically active and configurationally stable, others were found to racemise rapidly according to structure *via* an



8 **9** **1d** $\mathbf{2d}_S$ **2′d** $\mathbf{2d}_R$ **11** $\mathbf{10}_{syn}$ $\mathbf{10}_{anti}$ **6d**

Scheme 5 MO-catalysed epoxidation of methyl 2-(trifluoromethyl)-benzoate **1d** to form 1,2-arene oxide $\mathbf{2d}_S/\mathbf{2d}_R$ and aromatisation to yield the methylsalicylate **6d**, cycloaddition to yield cycloadduct **8**, hydrolysis to yield *trans*-dihydrodiol **9** and epoxidation to yield *syn*- and *anti*-benzene dioxides $\mathbf{10}_{syn}$ and $\mathbf{10}_{anti}$.

undetected oxepine tautomer.[10] It was assumed that, in common with other benzene oxides **3** (R = Cl, Br)[31] and PAH oxides,[10] spontaneous arene oxide racemisation (**2d**$_S$ ⇌ **2d**$_R$) would occur rapidly *via* the corresponding undetected oxepine **2′d**.

A sample of arene oxide **2d**$_S$/**2d**$_R$, stored at 4 °C for several weeks, was used for reactivity studies (Scheme 5). Thus, it was first reacted with 4-phenyl-1,2,4-triazoline-3,5-dione to give the arene oxide-derived cycloadduct **8**. From X-ray crystallographic analysis of compound **8** (Fig. 2),† it was confirmed that the dienophile had added exclusively *anti* to the epoxide ring (possibly due to its steric effect) and that the cycloadduct **8** was indeed racemic.

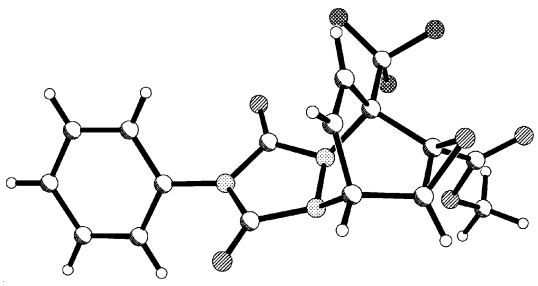


Fig. 2 X-Ray crystal structure of cycloadduct **8**.†

Reaction of arene oxide **2d**$_S$/**2d**$_R$ under weakly basic conditions (reflux phosphate buffer, pH 8.1) resulted in hydrolysis of the epoxide ring to yield the corresponding *trans*-dihydrodiol **9** (Scheme 5). Chiral stationary phase (CSP) GC–MS analysis indicated that the *trans*-dihydrodiol **9** was also racemic. Earlier attempts to hydrolyse other substituted benzene oxides (including **2a**) were unsuccessful,[10] and it had been assumed that epoxide hydrolase enzymes were required to catalyse the reaction. This first example of the chemical hydrolysis of a benzene oxide appears to be due to its unusual chemical stability and susceptibility to attack by OH anion.

The third reaction involved treatment of arene oxide **2d**$_S$/**2d**$_R$ with dimethyldioxirane (DMD) as oxidant (Scheme 5). Whilst epoxidation of the deactivated vinyl group, bearing an electron-withdrawing CF_3 group, was not observed, epoxidation of the unsubstituted alkene bond yielded mainly *anti*-dioxide **10**$_{anti}$ (*ca.* 90%) and a small proportion of *syn*-dioxide **10**$_{syn}$ (*ca.* 10%). The major *anti* isomer **10**$_{anti}$ was purified by column chromatography. Unfortunately a pure sample of the minor *syn* isomer **10**$_{syn}$ could not be obtained. However, the ^{1}H-NMR spectrum of an enriched fraction containing traces of *anti* isomer **10**$_{anti}$, and GC–MS data allowed the *syn*-dioxide **10**$_{syn}$ to be identified. The relative stereochemistry of the dioxides (**10**$_{anti}$ and **10**$_{syn}$) was determined by NMR spectral analysis. Both benzene dioxides **10**$_{anti}$ and **10**$_{syn}$ were found to be racemic by CSP GC–MS analysis, thus providing further evidence of spontaneous racemisation of the arene oxide precursor **2d**$_S$/**2d**$_R$. Similar types of *syn*- and *anti*-benzene dioxides have recently been used as valuable synthetic intermediates to form the corresponding *cis*- and *trans*-dihydrodiol derivatives.[32,33] Whilst the *anti*-benzene dioxides, in the latter study, were found to be stable at elevated temperatures, by contrast the corresponding *syn*-benzene dioxides were found to undergo an electrocyclic rearrangement above 80 °C to yield the corresponding achiral eight-membered 1,4-dioxocin rings. GC-IR-MS analysis of the benzene dioxide fraction **10**$_{syn}$ also showed evidence of a minor (*ca.* 5%) isomeric component derived from the *syn*-dioxide **10**$_{syn}$ which was tentatively identified as the corresponding achiral 1,4-dioxocin **11**, most likely formed by thermal isomerisation.

In the final reaction, treatment of arene oxide **2d**$_S$/**2d**$_R$ with trifluoroacetic acid resulted in aromatisation and migration of the carbomethoxy group (the NIH Shift) in a similar manner to that found with the transient arene oxide tautomers (**2a**) of the oxepine metabolite **2′a**. The question of whether the isomerisation of the arene oxide intermediates **2** to yield the corresponding phenols **5** and **6** in *P. ribis* and *P. pomaceus* is spontaneous or under enzyme-control has not been addressed. It has however been reported that enzymes present in bacteria can catalyse this epoxide–carbonyl isomerisation process, *e.g.* styrene oxide and propylene oxide to the corresponding aldehyde and ketone isomers.[34] Thus, while acid-catalysed isomerisation of arene oxides to phenols has been demonstrated in the current study, the possibility of an enzyme-catalysed process being involved in the biosynthesis of the methyl salicylates (**5a/6a**, **5d–i/6d–i**) from the corresponding arene oxides cannot be excluded.

Isolation of the arene oxide **2d**$_S$/**2d**$_R$, oxepines **2′a** and **2′l**, and observation of the NIH Shift of the carbomethoxy group, during aromatisation of several metabolites (**2′a**, **2d**, **2′g** and **2′i**), and the formation of methyl salicylates as bioproducts (**5a/6a**, **5d–i/6d–i**), are consistent with the enzyme-catalysed formation of eight arene oxides (**2a**, **2d–i** and **2l**) as initial metabolites from the corresponding benzoate ester substrates (**1a**, **1d–i** and **1l**). It has been found that the solutions of arene oxides **2a**, **2i** and **2l** exist preferentially as the corresponding oxepines (**2′a**, **2′i** and **2′l**)[19] and, with the exception of compound **2d**, it is probable that most of the other arene oxides (**2e–h**) prefer to exist as the corresponding oxepine tautomers (**2′e–2′h**).

Conclusion

The following results were obtained, when whole cell fungal biotransformations of methyl benzoate **1a** and derivatives **1b–k** were examined using two members of the *Phellinus* genus (*P. ribis* and *P. pomaceus*).

(i) Unequivocal NMR evidence for the formation of two arene oxide–oxepine metabolites, one existing preferentially as an oxepine valence tautomer (**2′a**) and the other as an arene oxide (**2d**$_S$/**2d**$_R$), was found.

(ii) Arene oxide **2d**$_S$/**2d**$_R$ was initially formed with an enantiomeric excess due to monooxygenase-catalysed epoxidation but spontaneously racemised on storage at 4 °C.

(iii) Strong evidence for the formation of a further oxepine (**2′l**), and indirect evidence for the involvement of arene oxide-oxepine intermediates was obtained from the formation of methyl salicylates **5a/6a**, **5d–i/6d–i** and from GC–MS analysis.

(iv) The NIH Shift of a carbomethoxy group during the biotransformation process has been established using D-labelled methyl benzoate **1a** and substituted methyl benzoates (*e.g.* **1d** and **1g**) and during acid-catalysed rearrangement of oxepines **2′a** and arene oxide **2d**$_S$/**2d**$_R$.

(v) The enzyme-catalysed reduction of the methyl benzoates (**1a–1k**), to the corresponding benzylic alcohols (**7a–k**), has been observed and was found to be the sole identified metabolic pathway in some cases (**1b**, **1c**, **1j**, **1k**).

(vi) Due to the unusual stability of arene oxide $\mathbf{2d}_S/\mathbf{2d}_R$, it has been possible to form a Diels–Alder cycloadduct (whose structure was confirmed by X-ray crystallography), to provide the first examples of a base-catalysed hydrolysis of a benzene oxide ($\mathbf{2d}_S/\mathbf{2d}_R$) to yield a *trans*-dihydrodiol **9** and of a monoepoxidation of a benzene oxide ($\mathbf{2d}_S/\mathbf{2d}_R$) to yield *syn*- ($\mathbf{10}_{syn}$) and *anti*- ($\mathbf{10}_{anti}$) benzene dioxides.

Experimental

NMR (^{1}H and ^{13}C) spectra were recorded on Bruker Avance DPX-300 and DPX-500 instruments and mass spectra were run at 70 eV, on a VG Autospec Mass Spectrometer, using a heated inlet system. Accurate molecular weights were determined by the peak matching method, with perfluorokerosene as the standard.

GC–MS analyses were performed on an Agilent 6890 gas chromatograph 5973 Mass Selective Detector system whilst GC-IR-MS studies utilised a Hewlett-Packard 5890GC/5965B Infrared Detector/5790 Mass Selective Detector system. An Agilent Technologies Ultra 1 (12 m × 0.25 mm × 0.17 μm) capillary column was used for GC–MS analyses, and chiral GC separations were performed with a Supelco Gamma-Dex 120 (30 m × 0.25 mm × 0.25 μm) capillary column. Elemental microanalyses were carried out on a PerkinElmer 2400 CHN microanalyser. For optical rotation ($[a]_D$) measurements (*ca.* 20 °C, 10^{-1} deg cm^2 g^{-1}), a PerkinElmer 341 polarimeter was used. Circular dichroism spectroscopy was carried out using a Jasco J-720 instrument using a sample concentration of *ca.* 0.5 g mL^{-1} in spectroscopic grade methanol. Flash column chromatography and PLC were performed on Merck Kieselgel type 60 (250–400 mesh) and $PF_{254/366}$ respectively. Merck Kieselgel type $60F_{254}$ analytical plates were used for TLC.

Authentic samples of the methyl benzoates **1a–k** and methyl salicylates **5a**, **5c**, **5f** and **5i** were obtained commercially while oxepine **2′a** was synthesised by using the reported method.[20,21] A commercial sample of 2,3,4,5,6-pentadeuteriobenzic acid (benzoic acid-D_5) was methylated with diazomethane to give methyl 2,3,4,5,6-pentadeuteriobenzoate (methyl benzoate-D_5) **1a** in quantitative yield, while a sample of CD_3-labelled methyl benzoate (methyl benzoate-D_3) was prepared by the standard method using hexadeuteriodimethyl sulfate and sodium hydroxide.

Synthesis of methyl 2,6-dideuteriobenzoate 1a-D_2

A mixture of sodium benzoate (5 g, 34.7 mmol) and rhodium(III) chloride (1 g, 4.8 mmol) dissolved in anhydrous DMF (100 cm^3) and D_2O (50 cm^3) was refluxed for 18 h. The reaction mixture was extracted with Et_2O (3 × 100 cm^3) and the combined extracts were washed successively with saturated NaCl solution and water. The organic phase was dried ($MgSO_4$), and the solvent removed under reduced pressure to yield the crude product, which was sublimed (100 °C / 0.07 mmHg) using Kugelrohr apparatus. 2,6-Dideuteriobenzoic acid (2.74 g, 64%, >95% d_2) was obtained as a white crystalline solid, mp 121.5–123 °C, ν_{max}/cm^{-1} 1687 (C=O); δ_H (500 MHz, $CDCl_3$) 7.41(2 H, d, *J* 7.5, 3-H and 5-H), 7.54 (1 H, t, *J* 7.5, 4-H); *m*/*z* (EI) 124 (M$^+$, 67%), 107 (43), 106 (100), 79 (95).

To a solution of 2,6-dideuteriobenzoic acid (2 g, 16 mmol) in Et_2O (60 cm^3) was added, drop-wise at 0 °C, a solution of diazomethane in Et_2O until the yellow colour of diazomethane persisted. After leaving the reaction mixture in an ice bath for 1 h, excess diazomethane was destroyed by the addition of AcOH. The crude product, obtained on removal of solvent, was purified by distillation (Kugelrohr) to yield methyl 2,6-dideuteriobenzoate **1a**-D_2 as a clear liquid (1.73 g, 78%, >95% d_2), bp 90 °C / 0.05 mm (oven temp.), ν_{max}/cm^{-1} 1724 (C=O); δ_H(500 MHz, $CDCl_3$) 3.85(3 H, s, CO_2Me), 7.36 (2 H, dd, *J* 7.0, 3-H and 5-H), 7.97 (1 H, t, *J* 7.0, 4-H); *m*/*z* (EI) 138 (M$^+$, 24%), 122 (45), 121 (100).

Fungal biotransformation procedures

Phellinus ribis Karst var. *ribis* (NCWRF-FPRL 42) and *Phellinus pomaceus* (Pers) Maire (NCWRF FPRL 33A) were acquired from the National Collection of Wood-Rotting Fungi, Princes Risborough Laboratory, Building Research Establishment, Aylesbury, Bucks, England.

Culture media and cultural conditions

P. pomaceus and *P. ribis* were grown on a medium containing glucose (30 g L^{-1}), mycological peptone (5 g L^{-1}), chloramphenicol (0.025 g L^{-1}) and NaCl (0.5 g L^{-1}). Cultures were incubated at 25 °C, with shaking (120 rpm), in 2000 mL conical flasks containing 300 mL of medium up to 25 days. Samples were taken daily for HPLC analysis. The inoculum was a mycelial suspension (3 mL) prepared by homogenising mycelia (60 mg wet weight) in sterile distilled water (40 mL) for 30 s with an Ultra Turrax homogeniser. For supplementation and labelling experiments, after 11 days' growth, mycelia and medium were asceptically sampled and the samples (10 mL) transferred to 100 mL sterile conical flasks. These cultures were then supplemented at the 0.5 mmol level with a series of methyl esters of monosubstituted benzoic acids (**1b–k**) and ethyl benzoate (**1l**) and incubated for a further 2–5 days. For labelling experiments, methyl 1,2,3,4,5-pentadeuteriobenzoate **1a**-D_5, methyl 2,6-dideuteriobenzoate **1a**-D_2 and trideuteriomethyl benzoate **1a**-D_3 were added to the cultures.

GC–MS identification of products of biotransformation from supplementation experiments

At the end of the incubation period, the medium and mycelia were extracted with Et_2O (2 × 20 mL) and the extract dried (Na_2SO_4) and concentrated (1 mL). A portion of this extract (1 μL) was analysed by GC–MS with the mass spectrometer operating in the electron impact full scanning mode measuring ion currents between *m*/*z* 30 and 500. The GC oven was programmed to hold at an initial temperature of 30 °C for 1 min, ramp at 10 °C to 300 °C and hold this temperature for 5 min. The injector port and transfer line temperatures were set at 250 °C and the sample was injected in the splitless mode. Retention times and mass spectra of salicylates and oxepines, identified from the biotransformations, were compared directly with those of authentic standards except for the oxepine of ethyl benzoate. The presence of this oxepine was inferred from comparison of its mass spectra and retention time with that of the oxepine of methyl benzoate **2′a**, for which an authentic standard was available.

Determination of incorporation of deuterium into bioproducts during labelling experiments

Percentage incorporation from methyl 1,2,3,4,5-pentadeuteriobenzoate **1a**-D_5, methyl 2,6-dideuteriobenzoate **1a**-D_2 and trideuteriomethyl benzoate **1a**-D_3 into oxepine **2′a** and methyl salicylate **6a** was determined by both GC–MS, ^{1}H-NMR and ^{13}C-NMR analyses. For GC–MS, the GC operating conditions were as described above but the mass spectrometer was employed in the selected ion monitoring mode measuring ion currents at *m/e* 152, 153, 154, 155, 156 and 157. Incorporation of label into oxepine **2′a** and methyl salicylate **6a** was determined as the ratio of the various ion currents, after correction for natural abundances, measured at the expected retention time of the authentic standard compounds.

Methyl 2-oxepinecarboxylate 2′a

Yellow oil (0.335 g, 65%); R_f 0.5, ν_{max}/cm^{-1} 1726 (C=O); δ_H (500 MHz, $CDCl_3$) 3.82 (3 H, s, CO_2Me), 5.78 (1 H, dd, $J_{6,5}$ 6.2, $J_{6,7}$ 5.2, 6-H), 5.94 (1 H, d, $J_{7,6}$ 5.2, 7-H), 6.35 (1 H, dd, $J_{4,5}$ 10.5, $J_{4,3}$ 6.1, 4-H), 6.47 (1 H, dd, $J_{5,4}$ 10.5, $J_{5,6}$ 6.2, $J_{5,3}$ 0.5, 5-H), 6.85 (1 H, dd, $J_{3,4}$ 5.6, $J_{3,5}$ 0.5, 3-H); δ_C (125 MHz, $CDCl_3$) 52.45, 118.08, 123.91, 128.56, 132.90, 133.59, 137.02, 163.25; *m/z* (EI) 154 (M^+, 85%), 121 (12), 93 (26), 65 (100). The isolated bioproduct **2′a** was spectroscopically indistinguishable from a chemically synthesised sample.[20,21]

Biotransformation of methyl 2-(trifluoromethyl)benzoate 1d with *P. ribis*

Methyl 2-(trifluoromethyl)benzoate **1d** (0.5 mmol) was added to each of six flasks each containing a growing culture of *P. ribis* (10 mL, day 14); the culture media were left for 8 days at ambient temperature. The combined culture broth was then centrifuged and the aqueous phase extracted with Et_2O (3 × 50 cm^3). The Et_2O extract was dried (Na_2SO_4) and carefully concentrated to yield the crude product as a yellow oil. ^{1}H-NMR analysis of the crude product showed evidence of the presence of natural metabolites **2′a**, **5a**/**6a** and **7a**, derived from methyl benzoate **1a**, and also metabolites **2d$_S$**/**2d$_R$**, **5d**/**6d** and **7d**, derived from methyl 2-(trifluoromethyl)benzoate **1d**. The arene oxide metabolite **2d$_S$**/**2d$_R$** was found to be one of the major metabolites; it was purified by PLC (20% Et_2O in pentane).

2-(Trifluoromethyl)-7-oxabicyclo[4.1.0]hepta-2,4-diene-1-carboxylic acid methyl ester 2d$_S$/2d$_R$

Colourless oil (73 mg, 11%); R_f 0.20 (20% Et_2O in pentane); $[\alpha]_D$ −14 (*c* 0.35, $CHCl_3$); (Found: M^+, 220.0351. $C_9H_7O_3F_3$ requires 220.0347); δ_H (500 MHz, $CDCl_3$) 3.86 (3 H, s, CO_2Me), 4.23 (1 H, m, $J_{6,5}$ 3.0, $J_{6,4}$ 1.8, 6-H), 6.59 (1 H, m, $J_{4,6}$ 1.8, 4-H), 6.70 (1 H, m, $J_{5,6}$ 3.0, $J_{5,4}$ 6.4, 5-H), 6.97 (1 H, m, $J_{3,4}$ 6.4, $J_{3,5}$ 1.3, 3-H); δ_C (125 MHz, $CDCl_3$) 51.67, 59.59, 126.59, 128.82, 128.90, 129.42, 166.58; *m/z* (EI) 220 (M^+, 100%), 189 (18), 173 (16), 161 (19), 145 (18), 133 (49), 113 (39), 83 (23), 59 (40).

Cycloaddition of 4-phenyl-1,2,4-triazoline-3,5-dione to arene oxide 2d$_S$/2d$_R$ to yield (±)-*anti*-10-carbomethoxy-1-(trifluoromethyl)-4-phenyl-9-oxa-2,4,6-triazatetracyclo[5.3.2.0^{2,6}.0^{8,10}]dodec-11-ene-3,5-dione 8

4-Phenyl-1,2,4-triazoline-3,5-dione (18 mg, 0.1 mmol) was added to a solution of arene oxide **2d$_S$**/**2d$_R$** (15 mg, 0.068 mmol) in CH_2Cl_2 (4 mL) and the reaction mixture stirred at ambient temperature for 3 days. The crude product obtained after removal of the solvent was purified by PLC (7% MeOH in $CHCl_3$) to give the cycloadduct **8** as colourless plates (19 mg, 70%); mp 164–65 °C (EtOAc–hexane); R_f 0.62 (7% MeOH in $CHCl_3$); $[\alpha]_D$ 0.0 (*c* 1.0, $CHCl_3$); (Found: M^+, 395.0749. $C_{17}H_{12}F_3N_3O_5$ requires 395.0730); δ_H (500 MHz, $CDCl_3$) 3.91 (3 H, s, CO_2Me), 4.02 (1 H, d, $J_{8,7}$ 4.5, 8-H), 5.51 (1 H, ddd, $J_{7,11}$ 1.7, $J_{7,8}$ 4.5, $J_{7,12}$ 6.0, 7-H), 6.32 (1 H, dd, $J_{12,11}$ 8.4, $J_{12,7}$ 6.0, 12-H), 6.49 (1 H, dd, $J_{11,12}$ 8.4, $J_{11,7}$ 1.7, 11-H), 7.5 (5 H, m, C_6H_5); *m/z* (EI) 395 (M^+, 33%), 308 (25), 220 (39), 189 (20), 161 (14), 133 (19), 119 (100), 91 (28), 59 (31).

Crystal data for compound 8. $C_{17}H_{12}F_3N_3O_5$, $M = 395.3$, monoclinic, $a = 10.584(3)$, $b = 10.448(3)$, $c = 14.677(4)$ Å, $\beta = 90.31(1)$, $U = 1623.1(8)$ Å^3, $T = 150(2)$ K, Mo-Kα radiation, $\lambda = 0.71073$ Å, space group $P2_1/c$ (no. 14), $Z = 4$, $F(000) = 808$, $D_x = 1.618$ g cm^{-3}, $\mu = 0.143$ mm^{-1}, Bruker SMART CCD-detector diffractometer, ϕ/ω scans, $4.8° < 2\theta < 56°$, measured/independent reflections: 4219/2371, $R_{int} = 0.046$, direct methods solution, full-matrix least squares refinement on F_0^2, anisotropic displacement parameters for non-hydrogen atoms; all hydrogen atoms located in a difference Fourier synthesis but included at positions calculated from the geometry of the molecules using the riding model, with isotropic vibration parameters. $R_1 = 0.072$ for 1828 data with $F_0 > 4\sigma(F_0)$, 254 parameters, $\omega R_2 = 0.233$ (all data), GoF = 1.14, $\Delta\rho_{min,max} = -0.67/0.09$ e Å^{-3}. CCDC reference number 668670.†

Base-catalysed hydrolysis of arene oxide 2d$_S$/2d$_R$ to yield (±)-*trans*-6-(trifluoromethyl)-3,5-cyclohexadien-1,2-diol-1-carboxylic acid methyl ester 9

A mixture of arene oxide **2d$_S$**/**2d$_R$** (16 mg, 0.073 mmol), ^{t}BuOH (2 mL) and pH 8.1 buffer solution (4 mL) was heated under reflux until the starting material had been consumed (6 h). The solvents were removed under reduced pressure, the concentrate diluted with water (5 mL), the mixture extracted (3 × 5 mL) with EtOAc and the extract dried (Na_2SO_4). The residue, left after the removal of solvent, was purified by PLC (50% EtOAc in hexane) to give *trans*-diol **9** as colourless oil (6 mg, 35%); R_f 0.48 (50% EtOAc in hexane); $[\alpha]_D$ 0.0 (*c* 0.5, $CHCl_3$); δ_H (500 MHz, $CDCl_3$) 3.89 (3 H, s, CO_2Me), 5.09 (1 H, br s, 2-H), 6.05 (1 H, m, 4-H), 6.18 (1 H, d, $J_{3,4}$ 10.4, 3-H), 6.67 (1 H, d, $J_{5,4}$ 5.5, 5-H). *m/z* (EI) 238 (M^+, 48%), 179 (53), 161 (100), 150 (28), 142 (48), 133 (75), 102 (47).

Epoxidation of arene oxide 2d$_S$/2d$_R$ to yield (±)-*anti*-5-(trifluoromethyl)-3,8-dioxatricyclo[5.1.0.0^{2,4}]oct-5-ene-4-carboxylic acid methyl ester 10$_{anti}$/10$_{syn}$

A solution of arene oxide **2d$_S$**/**2d$_R$** (17 mg, 0.077 mmol) in acetone (1 mL) maintained at 0 °C was treated with an excess of dimethyldioxirane solution in acetone (10 mL, 0.03 M). The reaction mixture was kept at 0 °C until almost all the starting compound had reacted (~ 7 days). The solvents were carefully

removed under reduced pressure and the residue purified by silica gel column chromatography (hexane → 30% Et_2O in hexane). The earlier fractions gave a mixture of dioxides $\mathbf{10}_{syn}/\mathbf{10}_{anti}$. The later fractions on concentration yielded a pure sample of the major dioxide $\mathbf{10}_{anti}$ as a colourless oil (6.5 mg, 36%); R_f 0.14 (30% Et_2O in hexane); (Found: M^+, 236.0293. $C_9H_7O_4F_3$ requires 236.0296); δ_H (500 MHz, $CDCl_3$) 3.32 (1 H, m, $J_{4,3}$ 3.9, $J_{4,5}$ 4.4, 4-H), 3.81 (1 H, dd, $J_{3,2}$ 1.7, $J_{3,4}$ 3.9, 3-H); 3.82 (3 H, s, CO_2Me), 3.93 (1 H, dd, $J_{2,3}$ 1.7, $J_{2,4}$ 0.7, 2-H), 6.75 (1 H, m, $J_{5,4}$ 4.4, 5-H); *m/z* (EI) 236 (M^+, 11%), 220 (23), 207 (22), 163 (12), 149 (50), 101 (21), 84 (32), 59 (49), 43 (100). Dioxide $\mathbf{10}_{syn}$: δ_H (500 MHz, $CDCl_3$) 3.66 (1 H, d, $J_{2,3}$ 1.3, 2-H), 3.84 (3 H, s, CO_2Me), 4.57 (1 H, m, $J_{4,3}$ 7.0, $J_{4,5}$ 3.7, 4-H), 4.72 (1 H, dd, $J_{3,2}$ 1.3, $J_{3,4}$ 7, 3-H), 6.59 (1 H, m, $J_{5,4}$ 3.7, 5-H).

Acid-catalysed aromatisation of arene oxide $2d_S/2d_R$ to yield 2-hydroxy-3-(trifluoromethyl)benzoic acid methyl ester 6′d

A solution of arene oxide $\mathbf{2d}_S/\mathbf{2d}_R$ (20 mg, 0.091 mmol) in $CHCl_3$ (2 mL) was treated with TFA at room temperature. The mixture was left at room temperature overnight. The solvent was evaporated and the crude product purified by PLC (10% EtOAc in hexane) to give phenol **6′d** as a light yellow oil (12 mg, 60%); R_f 0.54 (10% EtOAc in hexane); (Found: M^+, 220.0338. $C_9H_7O_3F_3$ requires 220.0347); δ_H (500 MHz, $CDCl_3$) 4.00 (3 H, s, CO_2Me), 6.95 (1 H, m, $J_{5,6}$ 7.8, 5-H), 7.77 (1 H, d, $J_{4,5}$ 7.5, 4-H), 8.04 (1 H, d, $J_{6,5}$ 7.8); δ_C (125 MHz, $CDCl_3$) 52.79, 113.61, 118.34, 118.87, (120.0, 122.10, 124.27, 126.44, quartet), 132.98, 133.76, 159.74, 170.06; *m/z* (EI) 220 (M^+, 87%), 188 (100), 169 (61), 160 (58), 141 (19), 132 (45), 113 (35), 63 (37).

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