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Side chain oxidation of aromatic compounds by fungi. 7. A rationale for sulfoxidation, benzylic hydroxylation, and olefin oxidation by *Mortierella isabellina*

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Abstract

The fungus *Mortierella isabellina* ATCC 42613 catalyses the biotransformation of substituted-aryl methyl sulfides to give sulfoxides of predominantly (*R*) configuration; of aryl-substituted alkenes to give chiral vicinal diols; and of various phenylalkanes and phenylcycloalkanes to give (*R*)-configuration benzylic alcohols. The nature of the products from all these processes may be accounted for by a single model based on restrictive space descriptors that can be used to rationalise these reactions, and which is proposed as a predictor of the outcome of the *M. isabellina*-catalyzed oxidations of similar substrates. © 1997 Elsevier Science B.V.

Keywords: Biocatalysis; Biotransformation; Epoxidation; Hydroxylation; *Mortierella isabellina* ATCC 42613; Sulfoxidation

1. Introduction

Biotransformations that involve the introduction of an oxygen atom into the substrate to give a chiral oxidised product are potentially of great value in synthetic organic chemistry. These reactions include hydroxylation of prochiral methylene groups, conversion of prochiral sulfides to chiral sulfoxides, and the enantioselective epoxidation of alkenes. This class of reactions, generally catalyzed by oxidase enzymes [1], are most often carried out using whole-cell biotransformation methodology. This requirement follows both from the intractable nature of the responsible membrane-bound oxygenase enzymes, and from their extensive cofactor requirements, which render impractical their use in isolated form. A further consequence of the nature of these enzymes is a limitation in the availability of structural information, frequently restricting their preparative utility as a result of difficulties in the prediction of substrate suitability and reaction products.

One approach to solving these problems has been the development of 'active site' models for individual substrate groups and microorganisms. Examples that have been developed include those describing the hydroxylation of amides by *Beauveria sulfurescens* [2–4], steroid hydroxyl-

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ations by *Calonectria decora* [5,6], oxidation of sulfides by *Helminthosporium* species [7], and benzylic hydroxylations by *Mortierella isabellina* [8,9]. These models have enjoyed some success in the rationalisation of the outcome of microbial oxidation reactions, but to date their application for the prediction of the products of oxidation of new substrates has been limited [10,11].

This paper reports a refinement of our original model [8] for *Mortierella isabellina*-catalyzed benzylic hydroxylations, and its application to biotransformations involving benzylic hydroxylation, olefin oxidation and sulfoxidation reactions.

2. Results and discussion

We have previously shown that both benzylic hydroxylation and sulfoxidation appear to be catalyzed by the same enzyme of *M. isabellina* [12], and that this enzyme may also be responsible for the oxidation of phenyl-substituted alkenes, via epoxides, to vicinal diols [9]. The enzyme has the characteristics of a cyt.P-450-dependent monooxygenase, and the model shown in Fig. 1 illustrates its application for the benzylic hydroxylation reaction. Fig. 1 represents a refinement of the original model [8]. A consideration of the biotransformation of *ortho*-substituted substrates, discussed below, has led to the inclusion of a space restriction in the neck of the aromatic binding pocket, A, and analysis of the biotransformations of alkyl substituted tetrahydronaphthalenes and indanes has resulted in the definition of the limits of the aliphatic binding pocket, B. In all of the studies discussed below, energy minimised conformations of substrate (MM^+, generated by HyperChem©) were used for dimensional analysis.

The biotransformations of aryl sulfides, hydrocarbons and olefin are summarised in Tables 1–3. The structures of biotransformation products were established by a combination of mass and NMR (both ^1H and ^{13}C) spectroscopy.

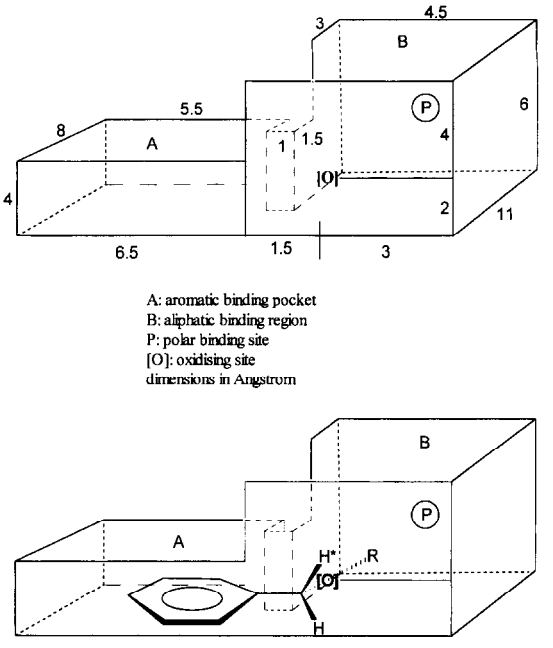
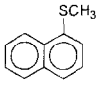


Fig. 1. (top) Model for the benzylic hydroxylase of *Mortierella isabellina*. (bottom) Benzylic hydroxylation of phenyl alkanes (R = alkyl) and benzyl cycloalkanes (R = cycloalkyl) by removal of H * to generate (R) alcohols. A: aromatic binding pocket, B: aliphatic binding region, P: polar binding site, [O]: oxidising site. Dimensions in Ångstrom.

Enantiomeric purities of sulfoxides were determined by analysis of their ^1H NMR spectra in the presence of the chiral shift reagents (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) [13] or (*R*)-(−)-N-(3,5-dinitrobenzoyl)-α-methylbenzylamine [14], and confirmed by correlation

Table 1
Oxidation of sulfides **1–11** to sulfoxides by *M. isabellina*

Substrate	Sulfoxide		
	yield (%)	configuration	e.e. (%)
1	10	*R*	30
2	20	*R*	53
3	23	*R*	30
4	12	*R*	44
5	12	*R*	50
6	35	*R*	60
7	15	*R*	84
8	21	*R*	70
9	4	*R*	6
10	10	*R*	69
11	13	*R*	64



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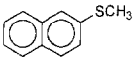

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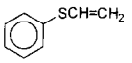

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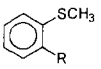
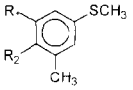
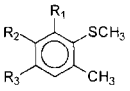


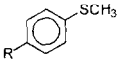
4 R = CH_3
5 R = C_2H_5



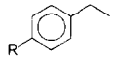
6 $R_1 = R_2 = H$
7 $R_1 = CH_3$, $R_2 = H$
8 $R_1 = H$, $R_2 = CH_3$



9 $R_1 = CH_3$, $R_2 = R_3 = H$
10 $R_1 = R_3 = H$, $R_2 = CH_3$
11 $R_1 = R_2 = H$, $R_3 = CH_3$



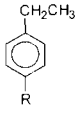
12a R = H
b R = CH_3
c R = C_2H_5
d R = $i.C_3H_7$
e R = $t.C_4H_9$



13a R = H
b R = CH_3
c R = C_2H_5
d R = halogen
e R = NO_2
f R = OCH_3
g R = CN

14 R = C_2H_5
15 R = $CH(OH)CH_3$

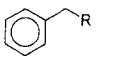
16a $R_1 = F$, $R_2 = H$
16b $R_1 = H$, $R_2 = F$



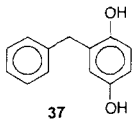
17 R = C_6H_5
18 R = $t.C_4H_9$

19

20



21 R = $CH(CH_3)_2$
22 R = $C(OH)(CH_3)_2$
23 R = $C(CH_3)_3$
24 R = $C(CH_3)_2CH_2OH$
25 R =
26 R =
27 R =
28 R =
29 R =
30 R =
31 R =
32 R =
33 R =
34 R =
35 R =
36 R = C_6H_5



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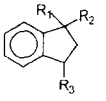
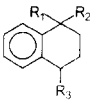
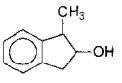


38 $R_1 = R_2 = R_3 = H$
39 $R_1 = CH_3$, $R_2 = R_3 = H$
40 $R_1 = C_2H_5$, $R_2 = R_3 = H$
41 $R_1 = i.C_3H_7$, $R_2 = R_3 = H$
42 $R_1 = R_2 = CH_3$, $R_3 = H$
43 $R_1 = CH_3$, $R_2 = OH$, $R_3 = H$
44 $R_1 = CH_3$, $R_2 = H$, $R_3 = OH$
45 $R_1 = CH_2OH$, $R_2 = R_3 = H$
46 $R_1 = C_2H_5$, $R_2 = OH$, $R_3 = H$
47 $R_1 = i.C_3H_7$, $R_2 = H$, $R_3 = OH$
48 $R_1 = C(OH)(CH_3)_2$, $R_2 = R_3 = H$



50 $R_1 = R_2 = R_3 = H$
51 $R_1 = CH_3$, $R_2 = R_3 = H$
52 $R_1 = R_2 = CH_3$, $R_3 = H$
53 $R_1 = C_2H_5$, $R_2 = R_3 = H$
54 $R_1 = C_2H_5$, $R_2 = H$, $R_3 = OH$
55 $R_1 = i.C_3H_7$, $R_2 = R_3 = H$
56 $R_1 + R_2 = O$, $R_3 = H$
57 $R_1 = i.C_3H_7$, $R_2 = H$, $R_3 = OH$
58 $R_1 = C(OH)(CH_3)_2$, $R_2 = H$, $R_3 = OH$



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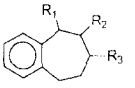
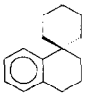


59 $R_1 = R_2 = R_3 = H$
60 $R_1 = OH$, $R_2 = R_3 = H$
61 $R_1 = R_3 = H$, $R_2 = OH$
62 $R_1 = R_2 = H$, $R_3 = OH$



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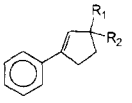
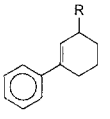
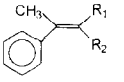


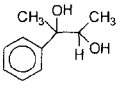
65 $R_1 = R_2 = H$
66 $R_1 = OH$, $R_2 = H$
67 $R_1 + R_2 = O$



68 R = H
69 R = OH



70 $R_1 = R_2 = CH_3$
71 $R_1 = CH_3$, $R_2 = H$
72 $R_1 = H$, $R_2 = CH_3$
73 $R_1 = CH_2OH$, $R_2 = CH_3$
74 $R_1 = CH_2OH$, $R_2 = H$



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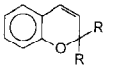
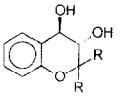
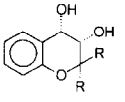


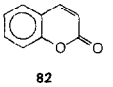
76 R = H
77 R = CH_3



78 R = H
79 R = CH_3



80 R = H
81 R = CH_3



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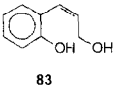

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Scheme 1.

Table 2
Hydroxylation of hydrocarbons by *M. isabellina*

Substrate	Benzylic alcohol			Other alcohols
	yield (%)	confign.	e.e. (%)	product (%)
14	2	—	0	
16a	6	*R*	52	
16b	3	*R*	18	
17	1	*R*	52	
18	2	*R*	36	
19	0			
20	0			
21	1	*R*	35	**22** (8)
23	2	*R*	55	**24** (3)
25	6	*R*	> 95	**26** (2)
				27 (8)
				28 (4)
29				**30** (2)
				31 (1)
				32 (5)
33				**34** (1)
				35 (2)
36	1			**37** (0.5)
39	**43** + **44** (4)			**45** + **49** (3)
40	**46** (2)	unknown		
41	**47** (1)	unknown		**48** (1)
42	14	*R*	15	
52	11	*R*	6	
53	**54** (0.3)	unknown		
55	**57** (2)	unknown		**56** (4)
				58 (4)
59	**60** (3)	*R*	48	**61** (3)
				62 (4)
63	0			1
64	0			0.2

Table 3
Oxidation of alkenes by *M. isabellina*

Substrate	Products	Yield (%)	Configuration	E.E. (%)
65	**66** + **67**	12		
68	**69**	2		0
70	**73**	7		
71	**74**	10		
	75	1	not determined	
72	**75**	8	not determined	
76	**78**	4	3*R*,4*R*	78
	80	2	3*R*,4*S*	78
	82	7		
	83	11		
77	**79**	12	3*S*,4*R*	70
	81	1	3*S*,4*S*	70

with optical rotation data where available. Use of the NMR shift reagents give consistent, configurationally dependent chemical shift patterns that have been correlated with data obtained from both (*R*) and (*S*) sulfoxides of established configuration [13,14]. The configurational assignments of Table 1 are in full agreement with the complexation model proposed for MPAA interaction with sulfoxides [13]. Enantiomeric ratios of alcohols were determined by analysis of the appropriate C*H*OH resonance in the presence of tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium (III), the method being calibrated where necessary (e.g. analysis of the benzylic hydroxylation product of **25**) by use of the racemic mixture generated by sodium borohydride reduction of the corresponding ketone.

2.1. Oxidation of sulfides

The model has not previously been applied to the oxidation of phenyl sulfides by *M. isabellina*, but the data presented in Table 1 for the oxidation of the series of aryl alkyl sulfides **1–11** (for structures see Scheme 1), together with our earlier work in this area [15,16], can all be rationalised by the use of Fig. 1 (lower) in which the benzylic methylene group is replaced by a sulfur atom. Oxidation would then be expected to result in the formation of sulfoxides with predominantly (*R*) configuration, as observed (Refs. [15,16] and Table 1), with the highest enantiomeric excesses being found for those substrates whose substituents result in an optimal fit into the binding pockets A and/or B. Substrates in this category include Ph–S-*n*-propyl ((*R*) sulfoxide, e.e. 100%, [16]) and Ph–S-*i*-propyl ((*R*) sulfoxide, e.e. 83%, [16]), both of which contain alkyl groups that are an optimal fit into pocket B; and *p*-Br–Ph–S–methyl ((*R*) sulfoxide, e.e. 100%, [16]) and the *p*-alkyl–Ph–S–methyl series (**12**), in which high e.e.'s of (*R*) sulfoxide are formed when the para-alkyl group is sufficiently large (as in **12c**,

d, and **e**) for the aryl substituents to be an optimal fit in pocket A.

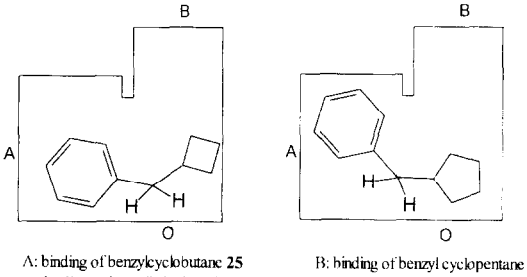
This analysis is strengthened by the results presented in Table 1. Methyl 1-naphthyl sulfide (**1**) is a poor substrate, as its oxidation would require the naphthyl ring to occupy pocket A in a 'sideways' orientation; methyl 2-naphthyl sulfide (**2**) is marginally better, as its aryl substituent can occupy pocket A in the preferable 'lengthways' orientation, and as a result the yield and e.e. of sulfoxide from **2** are very similar to those obtained from thioanisole [16]. The results obtained from oxidation of the series of methyl-substituted substrates **4–11** also serve to define the spatial limits of the aryl binding pocket A. It is clear that *ortho*-substituted examples (**4**, **5**, and **9–11**), particularly the di-*ortho*-methyl compound **9**, are poorly tolerated by the enzyme, but that di-*meta*- and *meta,para*-substituted compounds (**6–8**) can be reasonable substrates.

2.2. Benzylic hydroxylation of hydrocarbons

In order to extend our original studies of the biotransformation of substituted ethylbenzenes (**13**) [12], we have examined the biotransformation by *M. isabellina* of a series of compounds designed to test the spatial limits of both the aryl binding pocket A (compounds **14** and **16–20**), and the aliphatic binding region B (**21**, **23**, **25**, **29**, **33**, **36**, **39–42**, **52**, **53**, **55**, **59**, **63**, and **64**), the results of which are presented in Table 2. Yields of benzylic hydroxylation products were generally low, as observed for **13**, consistent with the observation that enzymic sulfoxidation is generally more facile than hydroxylation [9].

m-Ethyltoluene (**14**) presents the enzyme with a regiochemical choice of benzylic hydroxylation sites, but the sole product (**15**) appears to result from the expected preferred binding of the methyl group in pocket A and the ethyl group in pocket B. Similar specificity is observed in the hydroxylation of *p*-ethyltoluene to give *p*-tolylethanol [12]. Pocket A can clearly accommodate the small substituent fluorine in the *ortho* or *meta* position (**16**), but the enzyme is unable to hydroxylate *ortho*- or *meta*-dialkyl-substituted examples such as **19** and **20**. The bulky *para*-substituted examples **17** and **18** are hydroxylated in very low yield, albeit with the usual (*R*) stereoselectivity.

The series of benzylalkyl and -cycloalkyl compounds **21**, **23**, **25**, **29**, and **33** was examined as part of a programme aimed at defining the limits of the alkyl binding region B. Benzylic hydroxylation was observed for both *i*-propyl (**21**) and *t*-butyl (**23**) substituted phenylmethanes, but in both cases the major products (**22** and **24**, respectively) resulted from hydroxylation of the alkyl residue. Hydroxylation of benzylcyclopropane by *M. isabellina* has previously been reported to give the (*R*)-benzylic alcohol (e.e. 62%) as the sole product [17], but biotransformation of the higher benzyl cycloalkanes **25**, **29**, and **33** resulted in the formation of products hydroxylated in the alkyl ring. In only one instance (benzylcyclobutane, **25**), was benzylic hydroxylation observed (e.e. 100%). These data serve to confirm the limits of the alkyl binding pocket, B, as shown in Fig. 2: binding of benzylcyclobutane to allow benzylic hydroxylation is possible but crowds pocket B, as shown in Fig. 2A: the higher benzylcycloalkanes cannot bind in this manner, but can bind so as to permit hydroxylation in the alkyl ring, shown in Fig. 2B. Hydroxylations of **21**



A: binding of benzylcyclobutane **25** leading to benzylic hydroxylation

B: binding of benzyl cyclopentane **29** leading to cycloalkyl hydroxylation

Fig. 2. Binding of benzyl cycloalkanes from the top perspective of the model. (A) Binding of benzylcyclobutane **25** leading to benzylic hydroxylation. (B) Binding of benzyl cyclopentane **29** leading to cycloalkyl hydroxylation.

and **23** in the alkyl substituent may also occur as a result of their binding in a manner analogous to that illustrated in Fig. 2B. Diphenylmethane (**36**) undergoes benzylic hydroxylation in very low yield, and is also transformed to phenol **37**.

The depth and height of pocket B was explored by the use of alkyl-substituted indanes (**39–42**) and tetrahydronaphthalenes (**52, 53, 55, 63,** and **64**) as substrates. Hydroxylation at the remote benzylic carbon was observed as the major product of biotransformation for 1-methylindane (**39**), 1-ethylindane (**40**), 1-methyltetrahydronaphthalene (**51**) [8], 1-ethyltetrahydronaphthalene and the corresponding 1,1-dimethyl substituted substrates **42** and **52**, but not for the 1-*i*-propyl substituted examples **41** and **55**, or for the spiroalkanes **63** and **64**. These observations serve to establish the overall dimensions of pocket B shown in Fig. 1. This analysis is illustrated in Fig. 3 for an acceptable (**52**) and non-acceptable (**63**) substrate.

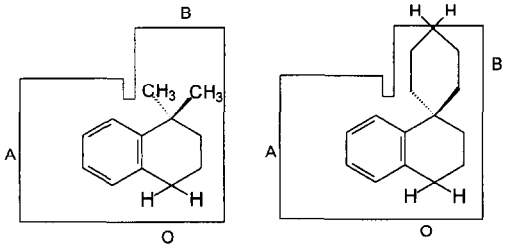
Although indane (**38**) and tetrahydronaphthalene (**51**) are hydroxylated by *M. isabellina* exclusively at the benzylic position [12,18], benzocycloheptene (**59**) is also hydroxylated to give the non-benzylic alcohols **61** and **62**. This may again be a reflection of the limitations of site B for acceptance of a larger cycloalkyl residue, resulting in some binding analogous to that illustrated in Fig. 2B, leading to the formation of **61** and **62**.

2.3. Biotransformation of phenylalkenes

Biooxidation of olefins to epoxides is a common feature of hydroxylase enzyme activity [1]. The resulting epoxides may be hydrolysed enzymatically (catalyzed by an epoxide hydrolase enzyme) or non-enzymatically (at pH < 7). As *M. isabellina* does not appear to possess any substantial epoxide hydrolase activity [19] and carries out its biotransformations at pH 5.0–5.5, epoxidation of olefins by this microorganism is manifested by their conversion to vicinal diols with an absolute stereochemistry determined by the enantioselectivity of the epoxidation [9,20]. In general, olefinic substrates are also susceptible to hydroxylation at an activated allylic position [1].

Table 3 presents the results obtained from biotransformation of the phenylcycloalkenes **65** and **68**, phenylbutenes **70–72**, and chromenes **76** and **77**. *M. isabellina* does not hydroxylate phenylcyclopentane or phenylcyclohexane at the benzylic position [8], and analogously no products of olefin oxidation were obtained from the biotransformations of **65** or **68**. In both cases, products resulted from regiospecific hydroxylation at C-3, a reaction that can be explained by substrate binding as illustrated in Fig. 4 for phenylcyclohexene, **68**.

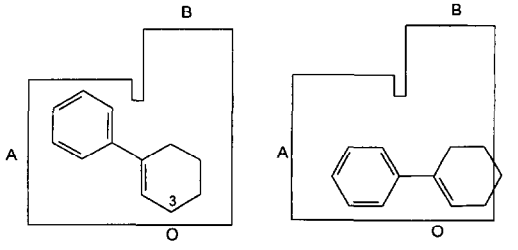
Biotransformation of the phenylbutenes **70–72** extended our study of phenylpropene



A: binding of **52** leading to benzylic hydroxylation

B: spiro compound **63** exceeding the binding limits in the aliphatic region B

Fig. 3. Binding of substituted tetrahydronaphthalenes. (A) Binding of **52** leading to benzylic hydroxylation. (B) Spiro compound **63** exceeding the binding limits in the aliphatic region B.



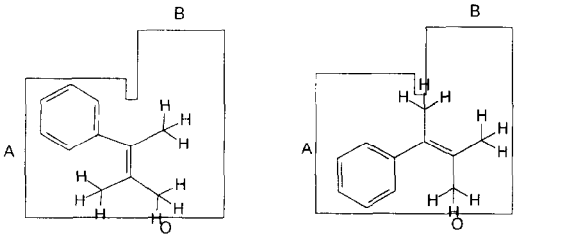
A: binding of **68** leading to hydroxylation at C-3

B: oxidation at C-1(2) of **68** exceeding the binding limits in the aliphatic region B

Fig. 4. Binding of phenylcyclohexene **68**. (A) Binding of **68** leading to hydroxylation at C-3. (B) Oxidation at C-1(2) of **68** exceeding the binding limits in the aliphatic region B.

metabolism by *M. isabellina* [9], and again presents a consistent picture that can be analysed by the application of our model. As shown in Fig. 5, preferred binding (Fig. 5A) of 3-methyl-2-phenyl-2-butene (**70**) would occur so as to expose the (*E*)-methyl group to the oxidising centre, resulting in regiospecific formation of **73**. The alternative binding shown in Fig. 5B results in severe interaction of the C-1-methyl group with the restricted region of the aromatic binding pocket. (*E*)-2-phenylbutene (**71**) is similarly transformed predominantly to give **74**, but the (*Z*)-isomer **72** is oxidised only at the olefinic bond, resulting in a *threo/erythro* mixture of diols **75**, a mode of reaction that now becomes preferred as the absence of the (*E*)-methyl group (R_1 in structures **70–74**) results in a substrate that no longer exceeds the sideways limits of pocket B for binding leading to olefin oxidation.

Biotransformation of the chromenes **76** and **77** was examined to investigate the putative existence of a polar binding site, P, in region B of the model. The existence of P was suggested by the contrast in our earlier data from the hydroxylations by *M. isabellina* of tetrahydronaphthalene (**50**) (resulting in (1*R*)-alcohol, e.e. 33% [12]) and chroman (resulting in (1*R*)-alcohol, e.e. 98% [21]), which indicated that placement of an electronegative atom in a region of the substrate that would occupy the rear of the aliphatic binding region B had a profound influence on the steric course of hydroxylation. This effect was again observed for the biotransformations of **76** and **77**. The *trans*-diols **78** ((*3R,4R*), e.e 78%) and **79** ((*3S,4R*), e.e. 70%) were obtained in much higher optical purity than the analogous (1*R*,2*R*)-diol formed from dihydronaphthalene (e.e. 33%, [12]), suggesting the existence of site P capable of interacting with an electronegative atom of the substrate in region B of the model. A similar polar binding site has been proposed in a model for the sulfoxidation reactions performed by *Helminthosporium* and may operate by interacting with the substrate so as to restrict its motion in the active site [7].



A: favoured binding of **70** leading to hydroxylation at the (*E*)-methyl group

B: disfavoured binding of **70** leading to hydroxylation at the (*Z*)-methyl group

Fig. 5. Binding of phenylbutene **70**. (A) Favoured binding of **70** leading to hydroxylation at the (*E*)-methyl group. (B) Disfavoured binding of **70** leading to hydroxylation at the (*Z*)-methyl group.

The biotransformations of **76** and **77** were complicated by formation of *cis*-diols **80** and **81**, which can result from non-stereoselective acid-catalysed opening of an intermediate 3,4-epoxide [22]. Similar side reactions are seen during the biotransformations by *M. isabellina* of indene and indeneoxide [12], but their formation can be avoided by control of the pH of the biotransformation medium [23]. The additional products **82** and **83** formed from **76** presumably arise via competing oxidation at C-2: analogous products are formed during the biotransformation of **76** by *P. putida* [24].

The model presented in Fig. 1 can thus be applied to biotransformations by *M. isabellina* which involve sulfoxidation, benzylic hydroxylation, and olefin oxidation reactions. These processes are all disparate functions of monooxygenase enzymes: if indeed, as suggested by the model, they are catalyzed by the same enzyme of *M. isabellina*, then the model of Fig. 1 may serve as an active site model for this enzyme. However, in the absence of any definitive data on the number and function of monooxygenase enzymes expressed by *M. isabellina*, this conclusion is tentative, and Fig. 1 must be viewed as an empirical tool for the prediction of the outcome of the oxidative biotransformations carried out by this fungus.

3. Experimental

3.1. Apparatus, materials and methods

Melting points were determined on a Kofler heating stage. Infrared spectra were recorded with an Analect 6260FX spectrometer. NMR spectra were recorded at 200 MHz (routine ^{1}H) or 50 MHz (^{13}C) with a Bruker AC200 spectrometer using $CDCl_3$ as solvent and $CHCl_3$ as internal standard. Enantiomeric ratios of sulfoxides were determined at 500 MHz (Bruker AC500) by ^{1}H NMR analysis of the S–CH_3 resonance in the presence of 3 equivalents of (*S*)-(+)-α-methoxyphenylacetic acid (MPAA) or two equivalents of (*R*)-(−)-N-(3,5-dinitrobenzoyl)-α-methylbenzylamine. Enantiomeric ratios of alcohols were determined at 200 MHz by analysis of the appropriate C*H*OH resonance in the presence of tris[3-(heptafluoropropylhydroxymethylene)-*d*-camphorato] europium (III). Optical rotations were obtained in the stated solvent at ambient temperature with a Rudolph Autopol III polarimeter. Mass spectra (EI mode) were obtained with a Kratos 1S instrument. Thin layer chromatography was performed on Merck silica gel 60F-254 and flash column chromatography used silica gel, 230–400 mesh.

3.2. Maintenance of microorganisms

Mortierella isabellina ATCC 42613, obtained from the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, USA, was maintained on 4% malt agar slopes, grown at 27°C and stored at 4°C.

3.3. Preparation of substrates

Sulfides **1–11** were either commercial samples or were prepared by reaction of the corresponding thiophenol or thionaphthol with iodomethane in the presence of potassium hydroxide [13]. *Ortho-* and *meta*-fluoroethylbenzenes **16a** and **16b** were prepared from the corresponding anilines via the diazonium fluoroborate salts [25]. *p-t*-Butylethylbenzene was prepared from *p-t*-butylacetophenone, and the cycloalkyl phenyl methanes **25**, **29**, and **33** were obtained from the corresponding cycloalkyl phenyl ketones, by Huang–Minlon reduction [26]. The 1-alkylindans **39**, **40**, and **41** were prepared via reaction of 1-indanone with the appropriate Grignard reagent as described below for **39**.

1-Methylindane (**39**). 1-Indanone (5.0 g) was added at room temperature to a stirred solution of methyl magnesium iodide (prepared from 1.36 g of magnesium and 3.54 ml of iodomethane in 40 ml ether), and the resulting mixture stirred for 16 h. The mixture was then cooled in an ice bath, and hydrochloric acid (50 ml of 15% HCl) added slowly. The resulting mixture was stirred at room temperature for 1 h, then extracted with ether. The extract was washed with satd. aq. $NaHCO_3$, water, satd. NaCl, dried and evaporated to yield 1-methylindene (4.89 g, 99%). This was dissolved in ethyl acetate (100 ml), 10% Pd/C (0.5 g) added, and the mixture hydrogenated at 35 psi in a Parr apparatus for 2 h. The catalyst was removed by filtration and the filtrate evaporated to yield 94% 1-methylindane (**39**) as a clear oil ^{1}H NMR δ 1.27 (3H, d), 1.57 (2H, m), 2.85 (2H, m), 3.16 (1H, m), and 7.15 (4H, m) ppm; ^{13}C NMR δ 19.9, 31.4, 34.7, 39.4, 123.2, 124.3, 126.1, 143.8, and 148.7 ppm; MS *m/z*(%) 132(36), 117(100). Similarly obtained were:

1-Ethylindane (**40**) from 1-indanone and ethyl magnesium bromide; clear oil, ^{1}H NMR δ 0.99 (3H, t), 1.35–1.70 (1H, m), 2.25 (1H, m), 2.88 (2H, d of q), 2.70–3.10 (3H, m), and 7.12 (4H, m) ppm; ^{13}C NMR δ 12.0, 27.7, 31.4, 32.9, 46.5, 123.6, 124.4, 125.9, 126.2, 144.1 and 147.6 ppm; MS *m/z*(%) 144(30), 131(12), 117(99), 103(35), 91(100);

1-*i*-Propylindane (**41**) from 1-indanone and *i*-propyl magnesium bromide; clear oil; ^{1}H NMR δ 0.76 (3H, d), 0.98 (3H, d), 1.86 (1H, m), 2.01 (2H, m), 2.79 (2H, m), 3.06 (1H, m), and 7.14

(4H, m) ppm; ^{13}C NMR δ 21.0, 26.6, 30.8, 31.6, 51.2, 124.2, 125.7, 126.0, 144.4 and 146.2 ppm; MS m/z(%) 160(20), 117(100);

1-Ethyl-1,2,3,4-tetrahydronaphthalene (**53**) from 1-tetralone and ethyl magnesium iodide; clear oil; 0.96 (3H, t), 1.50–1.95 (6H, m), 2.64–2.76 (3H, m), 7.04–7.23 (4H, m); ^{13}C NMR δ 11.9, 19.9, 26.9, 29.4, 29.8, 39.2, 125.3, 125.4, 128.6, 129.0, 137.1, and 141.3 ppm; MS m/z(%) 160(18), 131(100), 115(9), 91(15);

1-*i*-Propyl-1,2,3,4-tetrahydronaphthalene (**55**) from 1-tetralone and *i*-propyl magnesium iodide; clear oil; ^{1}H NMR δ 0.72 (3H, t), 0.98 (3H, t), 1.50–2.10 (4H, m), 2.22 (1H, d of q), 2.70 (3H, m), and 7.02–7.21 (4H, m); ^{13}C NMR δ 17.4, 21.2, 21.3, 23.3, 29.8, 31.4, 43.5, 125.0, 125.2, 128.1, 128.8, 137.9, and 140.2 ppm; MS m/z(%) 174(12), 146(20), 131(100), 118(28), 91(25).

1,1-Dimethylindane (**42**). This was prepared in 66% yield from 1-indanone and dimethylzinc/titanium tetrachloride by the procedure described [27] to give the product as a clear oil; ^{1}H NMR δ 1.1 (6H, s), 2.2 (2H, t), 3.2 (2H, t) and 7.5 (4H, m) ppm; ^{13}C NMR δ 28.8 (2C), 30.6, 41.4, 43.8, 121.9, 124.8, 126.1, 126.3, 142.6 and 152.4 ppm; MS m/z(%) 146(20), 131(100), 91(14). Similarly prepared was:

1,1-Dimethyl-1,2,3,4-tetrahydronaphthalene (**52**) from 1-tetralone; clear oil, 60%; ^{1}H NMR δ 1.5 (6H, s), 1.8 (2H, t), 2.0 (2H, m), 3.0 (2H, t), and 7.5 (4H, m) ppm; ^{13}C NMR δ 19.7, 30.7, 31.9 (2C), 33.8, 39.3, 125.8, 126.3, 126.6, 129.9, 136.0 and 145.7 ppm; MS m/z(%) 160(21), 145(100), 129(15), 119(17).

10,11-Benzospiro[5,5]undecane (**63**) and 9,10-benzospiro[4,5]decane (**64**) were prepared by cyclization of the corresponding phenylcycloalkanols using fluorosulfuric acid as described [28] and gave spectral data consistent with those previously reported [28].

1-Phenylcyclopentene (**65**) and 1-phenylcyclohexene (**68**) were obtained in overall yields of 60–70% by reaction of phenyl magnesium bromide with the corresponding cycloalkanone, followed by treatment of the resulting alkanol with 15% HCl as described above for the preparation of **39**, and exhibited the following spectral data: **65**; oil; ^{1}H NMR δ 2.7 (2H, m), 3.2 (2H, m), 3.4 (2H, m), 6.8 (1H, t) and 7.8–8.3 (5H, m) ppm; ^{13}C NMR δ 23.3, 33.1, 33.3, 125.5, 126.7, 127.1, 128.2, 128.6, 129.4, 136.7, and 142.4 ppm; MS m/z(%) 144(100), 129(65), 115(36). **68**; oil; ^{1}H NMR δ 1.6–1.9 (4H, m), 2.1–2.5 (4H, m), 6.1 (1H, t), and 7.0–7.5 (5H, m) ppm; ^{13}C NMR δ 22.0, 23.1, 25.9, 27.4, 124.6, 126.4, 127.1, 128.1, 129.9, 136.6 and 142.7 ppm; MS m/z(%) 158(100), 143(36), 130(62), 115(42).

(*E*)- and (*Z*)-2-phenyl-2-butenes (**71** and **72**) were isolated from the mixture obtained by dehydration of 2-phenyl-2-butanol with HCl using the method described above for the preparation of **39**. The resulting mixture of (*E*)-2-phenyl-2-butene (70%), (*Z*)-2-phenyl-2-butene (25%) and 2-phenyl-1-butene (5%) was distilled at 185°C (Vigreux column) and the residue collected to give (*E*)-2-phenyl-2-butene (**71**) in 12% isolated yield from the mixture, of $\geq$ 95% purity as determined by ^{1}H NMR, δ 1.7 (3H, d), 2.0 (3H, s), 5.8 (1H, q) and 7.1–7.4 (5H, m) ppm. The distillate from the above distillation (ca. 1:1 *E*:*Z* isomers) was then separated by chromatography on silver nitrate-impregnated silica gel (500 g silica gel containing 15 g $AgNO_3$), eluting with hexane. Thirty fractions of 100 ml were collected and (*Z*)-2-phenyl-2-butene (**72**) of $>$ 98% purity obtained in 5% yield from the mixture as a clear oil by evaporation of fractions 19–23; ^{1}H NMR δ 1.6 (3H, d), 2.1 (3H, s), 5.6 (1H, q), and 7.2–7.4 (5H, m) ppm.

2,2-Dimethylchromene (**77**) was obtained by dehydration of 2,2-dimethylchroman-4-ol [29] (2.5 g) in dry toluene (50 ml) using *p*-toluenesulfonic acid (0.1 g) as catalyst. The mixture was refluxed under argon for 15 min, cooled, washed (5% NaOH, water), dried and evaporated to give **77** in 81% yield; oil, ^{1}H NMR δ 1.49 (6H, s), 5.5 (1H, d), 6.2 (1H, d), and 6.7–7.1 (4H, m) ppm; ^{13}C NMR δ 27.7 (2C),

75.9, 116.2, 120.6, 121.1, 126.2, 128.9, 130.5, and 152.9 ppm.

3.4. Incubations with M. isabellina

Two slopes of *M. isabellina* ATCC 42613 were used to inoculate 15 1 l Erlenmeyer flasks each containing 200 ml of an autoclaved medium composed of glucose (40 g), yeast extract (5 g), sodium chloride (5 g), dibasic potassium phosphate (5 g) and soya flour (5 g) per l of distilled water. The flasks were allowed to stand overnight at 27°C, then placed on a rotary shaker at 180 rpm, and growth continued for a further 72 h at 27°C. The fungus was then harvested by centrifugation and resuspended in 15 1 l Erlenmeyer flasks each containing 200 ml of distilled water. Substrate (1 g in 30 ml of 95% ethanol) was then distributed among the flasks, which were replaced on the rotary shaker at 180 rpm, 27°C for a further 48 h (for sulfoxidation) or 72 h (for hydroxylation or olefin oxidation). The fungus and aqueous medium were then separated by filtration as before, the aqueous medium extracted with dichloromethane (continuous extraction, 72 h), and the fungus discarded. Concentration of the medium extract gave the crude product, which was treated as described below.

3.5. Isolation and characterization of products

The crude biotransformation extracts obtained as described above were examined by TLC, using ether or 10% methanol/ether as solvent, and then submitted to flash chromatography using a hexane–ethyl acetate or benzene–ether 10% stepwise gradient, followed by an ethyl acetate–methanol or ether–methanol 5% stepwise gradient if necessary. The yields and e.e. values quoted in the tables refer to purified, homogeneous material and, unless otherwise stated, arise from the combination of (only) homogeneous column fractions without further purification (e.g. crystallization) that could lead to changes in stereochemical enrichment values [30]. Products were identified by a combination of NMR and mass spectral analysis. Spectral and optical rotation data for products obtained in this study are listed below under the appropriate substrate heading. Yields and enantiomeric excesses are reported in the tables.

Methyl 1-naphthyl sulfide (**1**): methyl 1-naphthyl sulfoxide; ^{1}H NMR δ 2.86 (3H, s) and 7.5–8.2 (7H, m); MS m/z(%) 190(52), 175(100); $[\alpha]_D$ +158 (c 2.4, $CHCl_3$).

Methyl 2-naphthyl sulfide (**2**): methyl 2-naphthyl sulfoxide; ^{1}H NMR δ 2.80 (3H, s) and 7.5–8.2 (7H, m); MS m/z(%) 190(58), 175(100); $[\alpha]_D$ +90 (c 1.0, $CHCl_3$).

Methyl vinyl sulfide (**3**): methyl vinyl sulfoxide; ^{1}H NMR δ 5.85 (1H, m), 6.15 (1H, m), 6.55 (1H, m) and 7.4–7.7 (5H, m); MS m/z(%) 152(20), 109(38), 104(100); $[\alpha]_D$ +143 (c 0.7, acetone).

o-Tolyl methyl sulfide (**4**): *o*-tolyl methyl sulfoxide; ^{1}H NMR δ 2.36 (3H, s, Ar–CH_3), 2.67 (3H, s, S–CH_3), 7.1–7.4 (3H, m), and 7.96 (1H, d) ppm; MS m/z(%) 154(100), 137(60), 77(82); $[\alpha]_D$ +91 (c 0.4, acetone).

2-Ethylphenyl methyl sulfide (**5**): 2-ethylphenyl methyl sulfoxide; ^{1}H NMR δ 1.26 (3H, t), 2.62–2.76 (2H, d of q), 2.67 (3H, s), 7.2–7.4 (3H, m) and 7.97 (1H, d) ppm; MS m/z(%) 168(20), 152(100), 137(85); $[\alpha]_D$ +111 (c 0.5, acetone).

3-Methylphenyl methyl sulfide (**6**): 3-methylphenyl methyl sulfoxide; ^{1}H NMR δ 2.41 (3H, s, Ar–CH_3), 2.69 (3H, s, S–CH_3), 7.2–7.4 (3H, m), and 7.96 (1H, d) ppm; MS m/z(%) 154(22), 138(100); $[\alpha]_D$ +84 (c 0.4, acetone).

3,5-Dimethylphenyl methyl sulfide (**7**); 3,5-dimethylphenyl methyl sulfoxide; ^{1}H NMR δ 2.36 (6H, s, Ar–CH_3), 2.69 (3H, s, S–CH_3), 7.08 (1H, d), and 7.22 (2H, d) ppm; MS m/z(%) 168(92), 153(100); $[\alpha]_D$ +124 (c 0.5, EtOH).

3,4-Dimethylphenyl methyl sulfide (**8**): 3,4-dimethylphenyl methyl sulfoxide; ^{1}H NMR δ 2.29 and 2.31 (each 3H, s, Ar–CH_3), 2.67 (3H, s, S–CH_3), and 7.2–7.4 (3H) ppm; ^{13}C NMR δ

19.13, 19.20, 43.3, 120.5, 123.8, 129.9, 137.5, 139.5 and 142.2 ppm; MS m/z(%) 168(60), 153(100); $[\alpha]_D$ +87 (c 2.2, EtOH).

2,6-Dimethylphenyl methyl sulfide (**9**): 2,6-dimethylphenyl methyl sulfoxide; ^{1}H NMR δ 2.60 (6H, s, Ar–CH_3), 2.85 (3H, s, S–CH_3), 7.0–7.2 (3H, m) ppm; MS m/z(%) 168(100), 151(55); $[\alpha]_D$ −4 (c 0.6, EtOH).

2,5-Dimethylphenyl methyl sulfide (**10**): 2,5-dimethylphenyl methyl sulfoxide; ^{1}H NMR δ 2.30 and 2.38 (each 3H, s, Ar–CH_3), 2.65 (3H, s, S–CH_3), 7.0/7.1 (2H, ABq) and 7.75 (1H, d) ppm; ^{13}C NMR δ 17.6, 21.0, 42.1, 123.2, 130.6, 131.6, 137.5 and 143.5 ppm; MS m/z(%) 168(100), 151(88), 91(55); $[\alpha]_D$ +121 (c 1.3, EtOH).

2,4-Dimethylphenyl methyl sulfide (**11**): 2,4-dimethylphenyl methyl sulfoxide; ^{1}H NMR δ 2.32 and 2.34 (each 3H, s, Ar–CH_3), 2.64 (3H, s, S–CH_3), and 7.2–7.4 (3H) ppm; ^{13}C NMR 17.6, 20.9, 41.9, 122.8, 127.8, 131.1, 133.6, 140.6, 140.8 ppm; MS m/z(%) 168(100), 153(40), 151(35), 91(90); $[\alpha]_D$ +142 (c 1.05, EtOH).

3-Ethyltoluene (**14**): 1-(3-methylphenyl)ethanol (**15**); ^{1}H NMR δ 1.48 (3H, d), 2.35 (3H, s), 4.85 (1H, q), and 7.06–7.27 (4H, m) ppm; ^{13}C NMR δ 21.4, 25.1, 70.4, 122.4, 126.1, 128.2, 128.4, 138.1, and 145.8 ppm; MS m/z(%) 136(47), 121(58), 119(95), 91(100); $[\alpha]_D$ 0 (c 0.6, EtOH).

2-Fluoroethylbenzene (**16a**); 1-(2-fluorophenyl)ethanol; ^{1}H NMR δ 1.47 (3H, d), 5.15 (1H, q), 6.94–7.25 (3H, m) and 7.45 (1H, m) ppm; ^{13}C NMR δ 23.9, 84.5, 115.2, 124.2, 126.6, 128.7, 132.7 and 159.8 ppm; MS m/z(%) 140(35), 125(100), 105(25), 97(99); $[\alpha]_D$ +24.3 (c 3, EtOH).

3-Fluoroethylbenzene (**16b**); 1-(3-fluorophenyl)ethanol; ^{1}H NMR δ 1.47 (3H, d), 4.88 (1H, q), and 6.89–7.35 (4H, m) ppm; ^{13}C NMR δ 25.2, 69.8, 112.3, 114.2, 120.9, 130.0, 148.6 and 163.0 ppm; MS m/z(%) 140(43), 125(100), 97(88); $[\alpha]_D$ +2.2 (c 2, EtOH).

4-Ethylbiphenyl (**17**); 1-(4-biphenyl)ethanol; mp 73–76°C; ^{1}H NMR δ 1.54 (3H, d), 4.96 (1H, q), and 7.25–7.61 (9H, m) ppm; ^{13}C NMR δ 25.1, 70.2, 125.9, 127.1, 127.3, 128.8, 140.6, 140.9, and 144.9 ppm; MS m/z(%) 198(73), 183(100); $[\alpha]_D$ +15 (c 0.3, EtOH).

4-*t*-butylethylbenzene (**18**); 1-(4-*t*-butylphenyl)ethanol; ^{1}H NMR δ 1.32 (9H, s), 1.49 (3H, d); 4.87 (1H, q), and 7.2–7.4 (4H, m) ppm; ^{13}C NMR δ 24.9, 31.4, 34.5, 70.2, 125.2, 125.4, 142.8, and 150.5 ppm; MS m/z(%) 178(28), 163(72), 161(31), 160(33), 145(100); $[\alpha]_D$ +12.6 (c 0.8, EtOH).

2-Methyl-1-phenylpropane (**21**); 2-methyl-1-phenyl-1-propanol; ^{1}H NMR δ 0.8 (3H, d), 1.01 (3H, d), 1.96 (1H, q), 4.85 (1H, d) and 7.32 (5H, m) ppm; ^{13}C NMR δ 18.2, 19.1, 35.3, 80.1, 126.6, 127.4, 128.2 and 143.7 ppm; MS m/z(%) 150(18), 133(28), 105(25), 91(100); $[\alpha]_D$ +7 (c 0.3, EtOH), and 2-methyl-2-phenyl-2-propanol (**22**); ^{1}H NMR δ 1.21 (6H, s), 2.75 (2H, s), and 7.18 (5H, m) ppm; ^{13}C NMR δ 29.1, 49.7, 70.6, 126.4, 128.1, 130.4, and 137.8 ppm; MS m/z(%) 150(7), 135(15), 117(15), 92(100).

2,2-Dimethylphenylpropane (**23**); 2,2-dimethyl-1-phenyl-1-propanol; ^{1}H NMR δ 0.92 (9H, s), 4.39 (1H, s) and 7.28–7.32 (5H, m) ppm; ^{13}C NMR δ 25.9, 35.7, 82.5, 127.3, 127.5, 127.6 and 142.3 ppm; MS m/z(%) 149(100), 131(19), 117(36), 91(22); $[\alpha]_D$ +19.2 (c 0.4, EtOH), and 2,2-dimethyl-3-phenyl-1-propanol (**24**); ^{1}H NMR δ 0.87 (6H, s), 2.58 (2H, s), 3.32 (2H, s), and 7.14–7.28 (5H, m) ppm; ^{13}C NMR δ 23.9, 36.4, 71.2, 125.9, 127.6, 130.5 and 138.7 ppm; MS m/z(%) 164(18), 133(10), 92(100).

Cyclobutylphenylmethane (**25**); cyclobutyl phenyl methanol; ^{1}H NMR δ 1.78–2.06 (6H, m), 2.62 (1H, m), 4.57 (1H, d), and 7.25–7.34 (5H, m) ppm; ^{13}C NMR δ 17.8, 24.8, 42.5, 78.4, 126.2, 127.5, 129.3 and 143.2 ppm; MS m/z(%) 162(13), 144(10), 129(12), 107(100); $[\alpha]_D$ +8.6 (c 0.8, EtOH), 1-benzylcyclobutanol (**26**); ^{1}H NMR δ 1.8–2.2 (6H, m), 2.9 (2H, s), and 7.24–7.33 (5H, m) ppm; ^{13}C NMR δ 12.2, 35.5, 45.6, 75.1, 126.6, 128.3, 130.0, and 137.5 ppm; MS m/z(%) 162(15), 147(5), 134(27),

116(36), 91(100), and a 2:1 mixture of 2-benzylcyclobutanol (**27**) and 3-benzylcyclobutanol (**28**), identified by ^{13}C NMR signals at δ 26.7/28.7, 37.4/39.6, 41.2/43.0, and 64.0/66.2 ppm.

Cyclopentylphenylmethane (**29**); 1-benzylcyclopentanol (**30**); ^{1}H NMR δ 1.49–1.77 (6H, m), 2.81 (2H, s) and 7.12–7.26 (5H, m) ppm; ^{13}C NMR δ 23.5, 39.5, 47.5, 74.1, 126.5, 128.3, 130.2, and 138.3 ppm; MS m/z(%) 176(9), 158(36), 92(100), and 2-benzylcyclopentanol (**31**), (2:1 c/t mixture) ^{1}H NMR δ 1.17–1.92 (7H, m), 2.60/2.67 (total 2H, d), 3.78 (1H, t), and 7.06–7.16 (5H, m) ppm; MS m/z(%) 176(16), 158(11), 117 (22), 92(100), 3-benzylcyclopentanol (**32**), 1:1 c/t mixture; ^{1}H NMR δ 1.7–2.1 (7H, m), 2.60/2.68 (total 2H, d), and 7.12–7.3 (5H, m) ppm; MS m/z(%) 176(22), 158(25), 117(100), 92(59).

Cyclohexylphenylmethane (**33**); *cis*-4-benzylcyclohexanol (**34**); ^{1}H NMR δ 0.9–2.0 (9H, m), 2.84 (2H, d), 3.55 (1H, m) and 7.12–7.32 ppm; ^{13}C NMR δ 31.1, 35.6, 38.8, 43.3, 71.1, 125.8, 128.2, 129.1, and 141.0 ppm; MS m/z(%) 190(23), 172(21), 92(76), 81(100), and *trans*-4-benzylcyclohexanol (**35**); ^{1}H NMR δ 1.4–1.8 (9H, m), 2.54 (2H, d), 3.96 (1H, m) and 7.12–7.32 ppm; ^{13}C NMR 26.8, 32.3, 38.4, 42.8, 67.0, 125.7, 128.1, 129.1 and 141.1 ppm; MS m/z(%) 190(23), 172(14), 92(75), 81(100).

Diphenylmethane (**36**); diphenylmethanol, identified by comparison with an authentic standard, and 2,5-dihydroxyphenylphenylmethane (**37**); ^{1}H NMR δ 3.93 (2H, s), 6.57–6.64 (3H, m), and 7.20–7.32 (5H, m) ppm; ^{13}C NMR δ 36.3, 114.1, 116.7, 117.6, 126.4, 128.6, 128.7, 130.4, 139.7, 147.7, and 149.6 ppm; MS m.z(%) 200(74), 183(19), 181(34), 165(16), 152(23), 122(100).

1-Methylindane (**39**); a mixture of 1-hydroxymethylindane (**45**) and 1-methyl-2-indanol (**49**) (c/t, 70:30) identified by characteristic ^{1}H NMR signals for **45** at δ 3.73 (2H, d) and for **46** at δ 5.09 (1H, m), and by ^{13}C NMR signals for **45** at δ 72.0 and for **46** at δ 61.5/62.1 ppm, and a mixture of 1-methyl-1-indanol (**43**) and 3-methyl-1-indanol (**44**) identified by characteristic ^{1}H NMR signals for **43** at δ 1.55 (3H, s) and for **44** at δ 1.27 (3H, d) and 5.21 (1H, dd) ppm and by ^{13}C NMR signals at δ 81.2 (C-1 of **43**) and 75.1 (C-1 of **44**) ppm.

1-Ethylindane (**40**); 1-ethyl-1-indanol (**46**); ^{1}H NMR δ 0.95 (3H, t), 1.73–2.36 (4H, m), 2.65–3.0 (2H, m) and 7.23–7.37 (4H, m) ppm; ^{13}C NMR δ 8.6, 29.5, 33.0, 39.4, 84.1, 122.8, 124.9, 126.6, 128.2, 143.2 and 147.4 ppm; MS m/z(%) 162(10), 144(15), 133(100); $[\alpha]_D$ insufficient material.

1-*i*-Propylindane (**41**); a mixture of 3-*i*-propyl-1-indanol (**47**); ^{1}H NMR included δ 0.73 (3H, d), 0.97 (3H, d), 5.24 (1H, t) and 7.14–7.50 (4H, m) ppm; ^{13}C NMR included δ 17.9, 21.0, 31.3, 31.9, 48.9 and 75.6 ppm, and 1-(2-hydroxy-2-propyl)indane (**48**); ^{1}H NMR included δ 1.14 (3H, s), 1.26 (3H, s), and 7.14–7.50 (4H, m) ppm; ^{13}C NMR included δ 25.9, 28.6, 29.7, 38.2, 56.5 and 74.5 ppm.

1,1-Dimethylindane (**42**); 3,3-dimethyl-1-indanol; ^{1}H NMR δ 1.2 (2H, d), 1.5 (6H, s), 1.9 (1H, m), 2.4 (1H, m), 5.3 (1H, t) and 7.2–7.5 (4H, m) ppm; ^{13}C NMR δ 30.4, 39.5, 52.3, 74.9, 122.7, 124.5, 127.4, 129.0, 149.7 and 152.3 ppm; MS m/z(%) 162 (31), 147(100), 129(73); $[\alpha]_D$ −2.5 (c 2, $CHCl_3$).

1,1-Dimethyl-1,2,3,4-tetrahydronaphthalene (**52**); 4,4-dimethyl-1,2,3,4-tetrahydro-1-naphthol; ^{1}H NMR δ 1.2 (3H, s), 1.35 (3H, s), 1.6–1.9 (4H, m), 4.8 (1H, t), and 7.2–7.5 (4H, m) ppm; ^{13}C NMR δ 29.2, 31.7, 31.9, 34.3, 34.7, 69.3, 126.4, 127.0, 128.4, 128.7, 138.1 and 146.1 ppm; MS m/z(%) 176(28), 143(100), 120(34); $[\alpha]_D$ −2.9 (c 2, MeOH).

1-Ethyl-1,2,3,4-tetrahydronaphthalene (**53**); 4-ethyl-1,2,3,4-tetrahydro-1-naphthol (**54**); ^{1}H NMR δ 1.0 (3H, t), 2.1–2.95 (6H, m), 2.65 (1H, m), 4.80 (1H, m), and 7.3–7.7 (4H, m) ppm; ^{13}C NMR δ 11.9, 26.9, 27.2, 29.8, 39.2, 68.4, 125.3, 125.4, 129.0, 135.7 and 140.3 ppm; MS m/z(%) 161(100), 147(6), 129(10); $[\alpha]_D$ insufficient material.

1-*i*-Propyl-1,2,3,4-tetrahydronaphthalene (**55**); 1-tetralone (**56**), identified by comparison

with authentic material, 4-*i*-propyl-1,2,3,4-tetrahydro-1-naphthol (**57**) (*c/t* mixture), ^{1}H NMR δ 0.7 (3H, d), 1.13 (3H, d), 2.1–2.6 (4H, m), 4.74 (1H, t), and 7.1–7.9 (4H, m) ppm; ^{13}C NMR included signals at δ 73.2/74.8 ppm; MS *m/z*(%) 146(100), 129(40), and 4-(2-hydroxy-2-propyl)-1,2,3,4-tetrahydro-1-naphthol (**58**) obtained in admixture with **57** and identified by NMR signals at δ 1.30 (6H, s) (^{1}H) and δ 68.0 ppm (^{13}C).

Benzocycloheptene (**59**); 1,2-benzocyclohepten-3-ol (**60**); identified by comparison with an authentic sample, $[\alpha]_D$ +14 (*c* = 1.0, $CHCl_3$), 1,2-benzocyclohepten-4-ol (**61**); ^{1}H NMR δ 1.62 (2H, m), 1.87 (2H, m), 2.73 (2H, t), 3.02 (2H, t) 3.81 (1H, m) and 7.07–7.26 (4H, m) ppm; ^{13}C NMR δ 24.3, 35.7, 40.7, 44.7, 69.3, 126.3, 126.6, 129.0, 130.7, 136.5 and 143.5 ppm; MS *m/z*(%) 162(31), 144(35), 129(81), 118(100); $[\alpha]_D$ −10.7 (c 0.7, MeOH), ee 56%, and 1,2-benzocyclohepten-5-ol (**62**); ^{1}H NMR δ 1.57 (4H, m), 2.65 (2H, t), 2.88 (2H, t), 3.94 (1H, m), and 7.1 (4H, m) ppm; ^{1}H NMR δ 30.3, 36.5, 73.5, 126.3, 129.0, and 142.2 ppm; MS *m/z*(%) 162(13), 144(28), 129(100).

10,11-Benzospiro[5,5]undecane (**63**) and 9,10-benzospiro[4,5]decane (**64**); both gave mixtures of unidentified alcohols with ^{1}H NMR including signals at δ 2.80 (2H, t, benzylic CH_2), 4.50/4.62/4.77 (total 1H, m) ppm.

1-Phenylcyclopentene (**65**); 3-phenylcyclopent-2-en-1-one (**67**), ^{1}H NMR δ 2.6 (2H, t), 3.1 (2H, t), 6.6 (1H, s) and 7-4-7.7 (5H, m) ppm; ^{13}C NMR included signals at δ 28.6, 35.2 and 209.1 ppm; ir ν_{max} 1704 cm^{-1}; MS *m/z*(%) 158(100), 129(88), 115(60), and 3-phenylcyclopent-2-en-1-ol (**66**), ^{1}H NMR δ 1.8–2.0 (2H, m), 2.6 (2H, t), 5.0 (1H, m), 6.2 (1H, d) and 7.2–7.6 (5H, m) ppm; ^{13}C NMR included signals at δ 28.7, 35.8 and 79.9 ppm; ir ν_{max} 3400 cm^{-1}; MS *m/z*(%) 160(60), 142(30), 129(38), 115(39).

1-Phenylcyclohexene (**68**); 3-phenylcyclohex-2-en-1-ol (**69**); ^{1}H NMR δ 1.7 (2H, m), 2.0 (2H, m), 2.4 (2H, m), 4.4 (1H, m), 6.1 (1H, d) and 7.2–7.4 (5H, m) ppm; ^{13}C NMR δ 19.6, 27.5, 31.7, 66.3, 125.4, 126.6, 127.4, 128.3, 140.3 and 142.0 ppm; MS *m/z*(%) 174(98), 156(62), 145(100), 131(84), 115(23).

3-Methyl-2-phenyl-2-butene (**70**); (*E*)-1-hydroxymethyl-2-phenyl-2-butene (**73**); ^{1}H NMR δ 1.7 (3H, s), 2.1 (3H, s), 4.3 (2H, s), and 7.1-7.4 (5H, m) ppm; ^{13}C NMR δ 17.9, 20.3, 63.8, 125.7, 126.3, 128.0, 130.4, 134.4 and 144.5 ppm.

(*E*)-2-Phenyl-2-butene (**71**); (*E*)-2-phenyl-2-buten-1-ol (**74**); ^{1}H NMR δ 2.1 (3H, s), 4.3 (2H, d), 5.9 (1H, t) and 7.1–7.4 (5H, m) ppm; MS *m/z*(%) 148(11), 130(6), 115(12), 105(100), and 2-phenyl-2,3-butanediol (**75**) (*threo/erythro* mixture, 3:2), ^{1}H NMR δ 09./1.1 (total 3H, d), 1.5/1.6 (total 3H, s), 3.8–4.1 (1H, m), and 7.2–7.4 (5H, m) ppm; MS *m/z*(%) 166(1), 148(3), 135(9), 121(100).

(*Z*)-2-Phenyl-2-butene (**72**); 2-phenyl-2,3-butanediol (**75**) (*threo/erythro* mixture, 3:2), ^{1}H NMR δ 09./1.1 (total 3H, d), 1.5/1.6 (total 3H, s), 3.8–4.1 (1H, m), and 7.2–7.4 (5H, m) ppm; MS *m/z*(%) 166(1), 148(3), 135(9), 121(100).

Chromene (**76**); *trans*-chromane-3,4-diol (**78**); mp 97–99°C (Ref. [24] mp 97–98°C (3*R*,4*R*)); ^{1}H NMR δ 4.0–4.3 (3H, m), 4.6 (1H, d), 6.9 (2H, m) and 7.2–7.4 (2H, m) ppm; ^{13}C NMR δ 66.4, 68.3, 69.0, 116.9, 121.5, 126.7, 127.2, 130.0 ppm; MS *m/z*(%) 166(37) 148(6), 131(6), 122(100); $[\alpha]_D$ −18 (c 1.08, THF) (Ref. [24] $[\alpha]_D$ −22 (c 0.49, THF) for >98% ee (3*R*,4*R*) material), *cis*-chromane-3,4-diol; mp 158–160°C (Ref. [24] mp 159–161°C (3*R*,4*S*)); ^{1}H NMR δ 4.1–4.25 (3H, m), 4.8 (1H, d), 6.8–7.0 (2H, m) and 7.2–7.4 (2H, m) ppm; MS *m/z*(%) 166(30), 148(10), 122(100); $[\alpha]_D$ +50.5 (c 1, THF) (Ref. [24] $[\alpha]_D$ +63 (c 0.77, THF) for >98% ee (3*R*,4*S*) material), chromenone (**82**), identified by comparison with authentic material, and (*Z*)-3-(2-hydroxyphenyl)-2-propen-1-ol (**83**); mp 105–107°C (Ref. [31] mp 110°C); ^{1}H NMR δ 4.3 (2H, d), 6.1 (1H, m), 6.6 (1H, d) and 6.9–7.2 (4H, m) ppm; MS *m/z*(%) 150(14), 131(100), 121(19), 107(16).

2,2-Dimethylchromene (**77**); *trans*-2,2-dimethylchromane-3,4-diol (**79**); mp 71–73°C (Ref. [32] mp 57–58°C (racemate); Ref. [24] mp 93–94°C (3*S*,4*R*)); ^{1}H NMR δ 1.1 (3H, s), 1.5 (3H, s), 3.6 (1H, d), 4.5 (1H, d), and 6.7–7.3 (4H, m) ppm; ^{13}C NMR δ 18.6, 26.7, 69.6, 76.2, 78.4, 116.8, 120.7, 123.2, 127.4, 129.4, and 152.2 ppm; MS *m/z*(%) 194(41), 176(1), 136(10), 123(100); $[\alpha]_D$ +25.5 (c 1.0, $CHCl_3$) (Ref. [24] $[\alpha]_D$ +38 (c 1.01, $CHCl_3$) for > 98% ee (3*S*,4*R*) material), and *cis*-2,2-dimethylchromane-3,4-diol (**81**); mp 96–98°C (Ref. [24] mp 113–114°C (3*S*,4*S*)); ^{1}H NMR δ 1.3 (3H, s), 1.5 (3H, s), 3.8 (1H, d), 4.8 (1H, d), and 6.8–7.5 (4H, m) ppm; ^{13}C NMR δ 23.4, 24.8, 65.3, 71.7, 77.5, 116.9, 121.3, 122.5, 128.9, 129.4, and 152.5 ppm; $[\alpha]_D$ −10.3 (c 0.7, $CHCl_3$) (Ref. [24] $[\alpha]_D$ −15 (c 0.63, $CHCl_3$) for > 98% ee (3*S*,4*S*) material).

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