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Side Chain Hydroxylation of Aromatic Compounds by Fungi. Part 5. Exploring the Benzylic Hydroxylase of *Mortierella isabellina*

Herbert L. Holland*, Maik Kindermann, Sudalaiyandi Kumaresan, and Tomislav Stefanac

Department of Chemistry, Brock University, St. Catharines, Ontario, L2S 3A1, Canada

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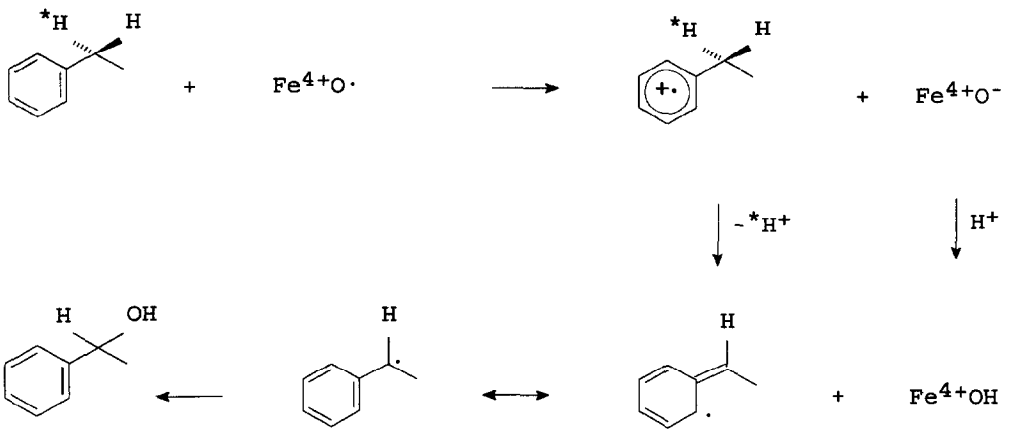
Abstract: The active site topography of the hydroxylase enzyme of *Mortierella isabellina* ATCC 42613, which carries out the benzylic hydroxylation of toluene, ethylbenzene, and related compounds, has been explored. Operating in a whole cell biotransformation mode, this enzyme shows selectivity in substrate processing based on the nature, position and size of substituent side chains close to the site of hydroxylation The results of determination of the yield and stereochemistry of hydroxylation of over twenty substrates and potential substrates, together with previously reported data, have been used to propose an active site model for the benzylic hydroxylase enzyme.

The fungus *Mortierella isabellina* ATCC 42613 carries out the benzylic hydroxylation of simple aromatic hydrocarbons such as toluene[1,2] and ethylbenzene,[3,4] in addition to performing similar biotransformations with more complex hydrocarbons[5] and other substituted aromatic compounds.[6-8] The enzyme which performs this reaction has the characteristics of a cyt.P-450 dependent mono-oxygenase.[3] We have studied the mechanistic details of this enzyme,[2,4] and have proposed a reaction route (Figure 1) involving the initial removal of an electron from the aromatic ring by an activated iron-oxygen species of the enzyme's cofactor. This oxidation is followed by loss of a proton from the resulting radical cation to generate a benzylic radical, which is then captured by a process involving homolytic cleavage of the cofactor's iron-oxygen bond to generate the product. The essential feature of this mechanism is its stepwise nature, with consequent separation of the events of oxidation (electron removal), hydrogen loss, and product formation. This is reflected in the observation that the stereochemistries of hydrogen removal (specifically pro-R)[4,9] and product formation (generally giving R alcohol with e.e. 25-35%)[1] are clearly independent of each other, a phenomenon which has also been noted by others for the enzymic cyt.P-450 catalyzed benzylic hydroxylation of ethyl benzene.[10]

As part of our continuing investigation of the biotransformations of organic compounds by *Mortierella isabellina*,[1-5,8,9,11] we have examined a large number of aromatic substrates. One consequence of this endeavour has been the emergence of data which can usefully define the substrate specificity of the benzylic hydroxylase enzyme. We have not attempted to isolate this enzyme: the isolation of such membrane-bound cyt. P-450 mono-oxygenases from fungal sources is fraught with difficulty, and in spite of preliminary results for steroid hydroxylating enzymes, no synthetically useful preparation has yet been

Scheme 1. Proposed route for benzylic hydroxylation by M. isabellina



obtained.[12,13] Useful models for fungal hydroxylating enzymes have nevertheless been developed, notably those for hydroxylation of amides by *Beauveria sulfurescens*,[14] and the hydroxylation of steroids by *Calonectria decora* and other fungi.[15] Active site models have also been proposed for the hydroxylation of monoterpenes by *Bacillus cereus*,[16] and O-demethylations carried out by *Streptomyces griseus*.[17]

The biotransformations performed as part of the present study are listed in Table 1. All products were identified by routine spectral analysis (see ***Experimental***): in the case of products derived from **2 - 4**, the regiochemistry of hydroxylation was confirmed by ^{13}C nmr analysis of the product of oxidation to the corresponding ketone. The structures of products derived from **6 - 8**, **11**, and **12** were derived by comparison of spectral data with published values. The inability of *M. isabellina* to hydroxylate **5**, **9**, and **10** is based on the recovery of the bulk of the substrate, together with a failure to isolate metabolic products at a level of $\geq 1\%$ of the added substrate.

It is apparent from the data presented in Table 1 that, whereas the *M. isabellina* benzylic hydroxylase will accept phenylcyclobutane (**1**) as substrate, it is unable to convert the higher phenylcycloalkanes **2 - 4**, these substrates being hydroxylated exclusively elsewhere in the molecule. The existence of other hydroxylase enzymes in *M. isabellina* is thought to be responsible for the formation of the non-benzylic alcohols from **1 - 4**: this view is strengthened by the fact that similar products have previously been found as a result of bioconversion of phenyl and substituted phenylcycloalkanes by a variety of micro-organisms,[18,19] suggesting the existence of enzymes capable of hydroxylating compounds such as **1 - 4** at a non-benzylic site. Other relevant experiments summarized in Table 1 are the failure of the *M. isabellina* hydroxylase to hydroxylate 2-phenylbutane (**5**) in appreciable yield, remarkable in view

Table 1. Products of biotransformation by M. isabellina

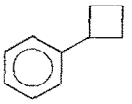
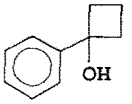
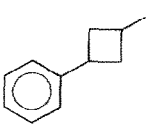
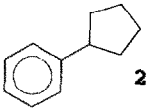
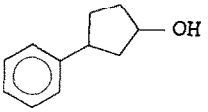
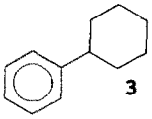
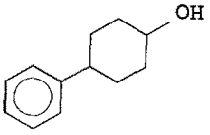
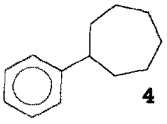
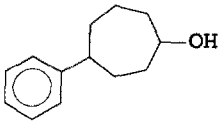
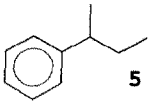
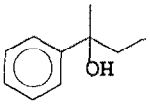
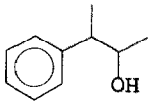
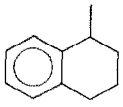
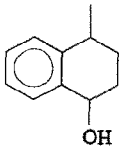
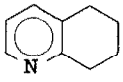
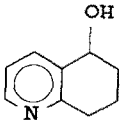
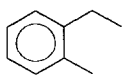
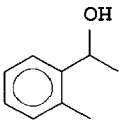
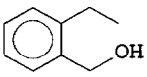
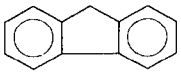
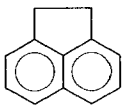
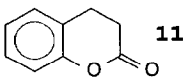
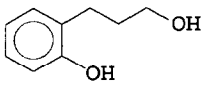
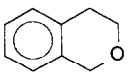
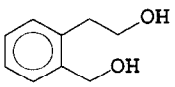
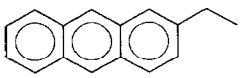
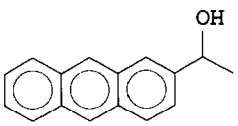
SUBSTRATE	PRODUCTS (YIELD, STEREOCHEMISTRY, ENANTIOMERIC EXCESS)
 **1**	 **1a** (18)  **1b** (4, cis) **1c** (4, trans)
 **2**	 **2a** (1, cis) **2b** (2, trans)
 **3**	 **3a** (13, trans)
 **4**	 **4a/4b** (2, cis/trans)
 **5**	 **5a** (trace)  **5b** (trace)
 **6**	 **6a** (8, trans) **6b** (7, cis)
 **7**	 **7a** (5, R, 30)

Table 1, continued

SUBSTRATE	PRODUCTS (YIELD, STEREOCHEMISTRY, ENANTIOMERIC EXCESS)
 **8**	 **8a** (7, S, 25)  **8b** (3)
 **9**	None
 **10**	None
 **11**	 **11a** (54)
 **12**	 **12a** (10)
 **13**	 **13a** (trace)

of the very efficient benzylic hydroxylation (85% conversion) of 2-phenylpropane to 2-phenyl-2-propanol by this organism,[3] the regioselective hydroxylation of **6** at the secondary benzylic site, and the formation of **8a** (with predominant R configuration), although *ortho*-diethylbenzene is not accepted by the enzyme.[3] The formation of hydroxyphenols from **11** and **12** may be rationalized with the knowledge that *M. isabellina* carries out the reduction of carbonyl groups in an irreversible manner.[3,11,20] Thus reduction of dihydrocoumarin (**12**) to the lactol, followed by hydrolysis and reduction of the resulting aldehyde could give rise to **11a**, while a similar sequence of events following benzylic hydroxylation of **12** at C-1 would produce **12a**.

The data from Table 1, together with previously reported biotransformations performed by *M. isabellina*, ATCC 42613,[1-5,11], have been summarized in Tables 2 and 3 under the headings of acceptable (Table 2) and non-acceptable (Table 3) substrates, with the predominant site of hydroxylation indicated by arrows where appropriate. Phenyl cyclopropane is included in Table 2: even though no hydroxylation products were isolated from this substrate,[5] it appears to enter the active site of the enzyme and act as a metabolically activated suicide substrate, binding to the enzyme following a cyclopropyl-allyl radical rearrangement and preventing further substrate turnover.[5,21,22] Evidence for this view was obtained by a sequential incubation involving first phenylcyclopropane, followed by ethylbenzene: no 1-phenylethanol could be detected in the biotransformation extract. A similar sequence of experiments involving phenylcyclobutane followed by ethylbenzene gave rise to the formation of 1-phenylethanol in the usual manner, albeit in reduced yield, a factor which may be attributed to cell death during the lengthy biotransformation period (144 hours) necessitated by such experiments.

The salient features of Tables 2 and 3 which led to the development of the model proposed for the active site of the *M. isabellina* hydroxylase are these: the inability of the enzyme to process 1-ethylnaphthalene or 2-ethylanthracene, although 2-ethylnaphthalene is a good substrate; the limit of C_4 in acceptable ring size of phenylcycloalkane substrates; the inability of the enzyme to hydroxylate 2-phenylbutane or *ortho*- substituted ethylbenzenes other than 2-ethyltoluene, and only then with perturbation of stereochemistry; and the efficient hydroxylation of benzo-fused cycloalkanes such as indane or tetralin, but lack of hydroxylation of larger molecules such as fluorene and acenaphthene. The active site model proposed in Figure 1 accounts for all these observations, and involves a specific phenyl binding pocket close to a sterically constrained site **X** for electron removal and processing of the radical cation intermediate (c.f.. Scheme 1). Among potential substrates with tertiary benzylic centres, the site is able to accept only 2-phenylpropane, phenylcyclopropane, and (marginally) phenylcyclobutane: the effective spatial requirements of the conformationally flexible 2-phenylbutane and *ortho*-diethylbenzene preclude their acceptance into the active site. 1-Methyltetralin is hydroxylated only at C-4 for the same reason, and although 2-ethyltoluene is a substrate, its binding into the active site is obviously abnormal in view of the formation of product (**8a**) with predominant S stereochemistry, in contrast to the otherwise ubiquitous

Table 2. Acceptable substrates for M. isabellina hydroxylase

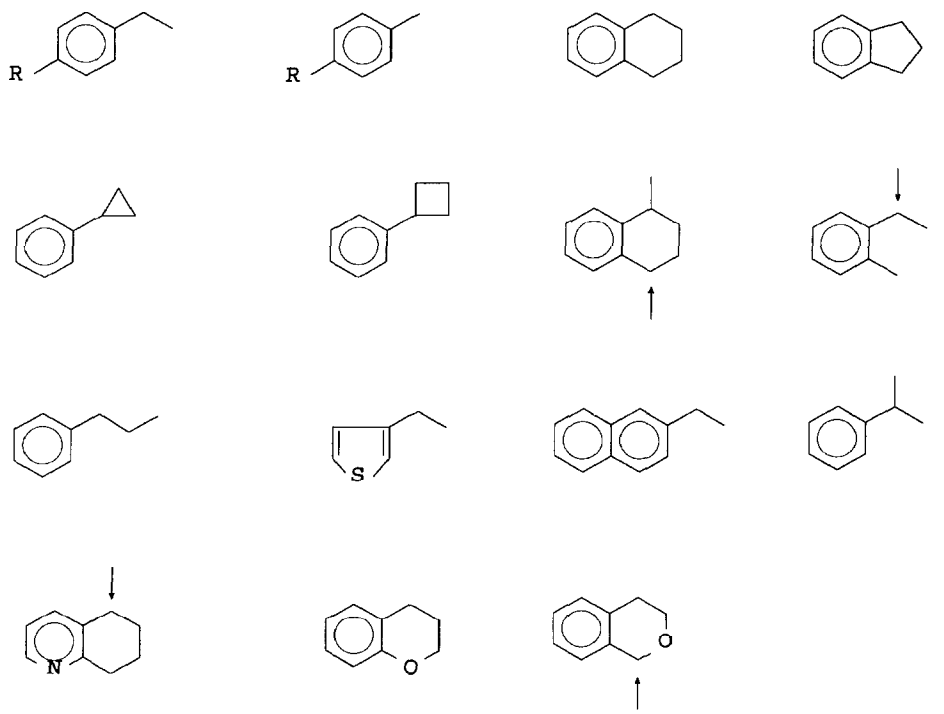


Table 3. Non-acceptable substrates for M. isabellina hydroxylase

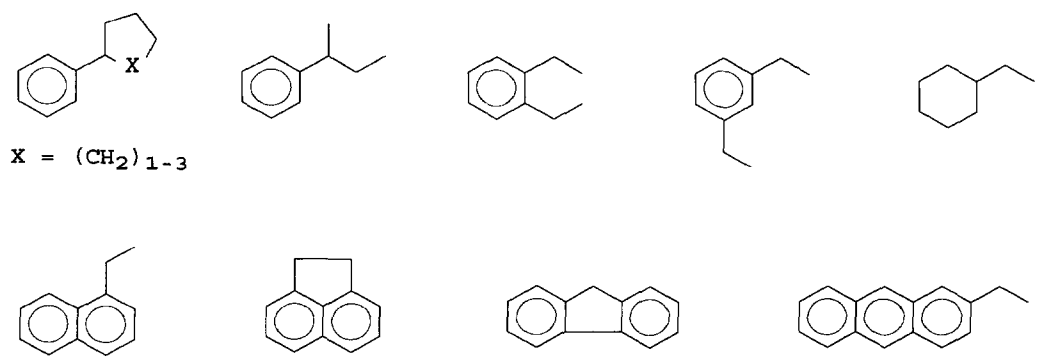
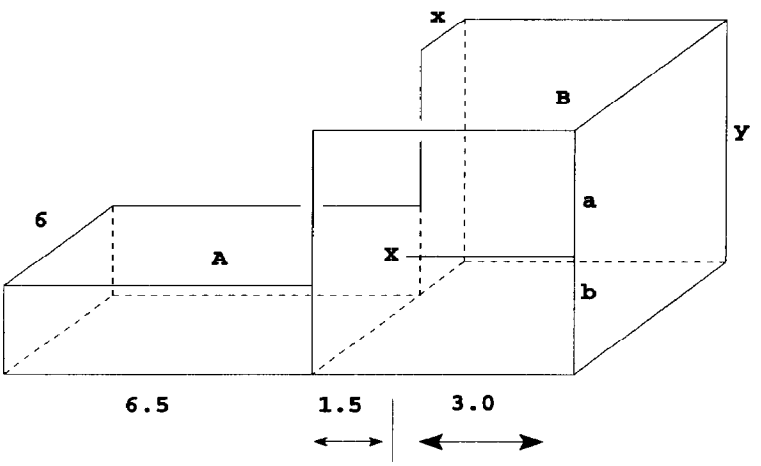


Figure 1. Active site model for M. isabellina hydroxylase

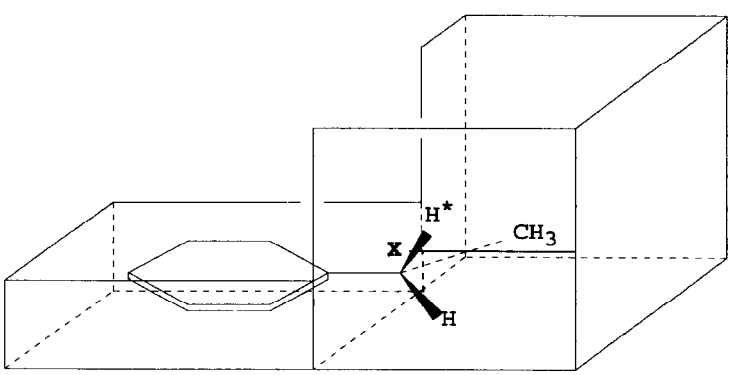


A: *aromatic binding pocket*

B: *aliphatic binding region*

X: *oxidation centre*

Substrate binding to M. isabellina hydroxylase



production of R benzylic alcohols by *M. isabellina* catalyzed biotransformations of aromatic hydrocarbons.

The dimensional limits of Figure 1 (Å) are derived from molecular modelling studies of the relevent substrates and potential substrates using Tripos Associates Alchemy© software. The dimensions labelled **x**, **y**, **a**,and **b** are currently undefined, but the absolute stereochemistry of the enzyme's action dictates that the oxidation centre **X** must lie above the mean plane of the aromatic ring in order to abstract the pro-R hydrogen ($H^{\bullet}$) and generate product of R chirality. Further refinement of the spatial limitations of the active site using larger benzo-fused cycloalkanes and other substituted tetralins is necessary, and the role of heteroatoms in determining the regiospecificity of hydroxylation remains to be clarified, but Figure 1 nevertheless serves as the first working model for the prediction of the efficiency and regiochemistry of benzylic hydroxylation by *M. isabellina.*

Experimental

Apparatus, materials, and methods: instrumental methods and routine analytical procedures were those previously described.[5] Gas chromatographic analysis was performed on an HP5890 instrument fitted with a J&W Scientific DB1 capillary column. *Mortierella isabellina* ATCC 42613 (identical to NRRL 1757) was maintained on 4% malt agar slopes, grown at 27°C and stored at 4°C. Biotransformations were performed and products isolated and purified using the standardized procedures described elsewhere.[3,23]

Preparation of substrates: the substrates used were commercial samples, with the exception of the phenylcycloalkanes **1, 2,** and **4**; these were prepared by a common route described below for the preparation of phenylcyclopentane. A solution of cyclopentanone (12.62 g, 0.15 mole) in ether (50 mL)was added slowly to an ice-cooled solution of phenyl magnesium bromide in ether (0.15 mole). The resulting mixture was stirred at room temperature for 1.5 h: conventional work-up afforded 1-phenylcyclopentanol (87%), which was dissolved in ethyl acetate (250 mL) containing conc. hydrochloric acid (4 drops), and the resulting solution placed in a 500 mL Parr hydrogenation vessel together with 1g of 10% palladium on charcoal. Hydrogenation was carried out at 25-40 psig for 2h, after which the catalyst was removed by filtration, the filtrate washed (satd. $NaHCO_3$), dried and evaporated. Distillation (bp 73°C, 1mm; lit.[24] bp 118°C, 25mm) gave the final product **2** in an overall yield of 71% from the starting ketone: PMR: δ 1.3-2.1 (8H, m, CH_2), 2.9-3.1 (1H, q, H-1), 7.0-7.6 (5H, m, aromatic H's) ppm; CMR δ 25.6 (2C), 34.4 (2C), 46.0, 125.7, 127.1 (2C), 128.2 (2C), and 146.6 ppm. Also prepared by this route were phenylcyclobutane (**1**, overall 82%), bp 88-90°C, 25mm (lit.[25] bp 89-91°C, 25mm): PMR δ 1.8-2.5 (6H, m, CH_2's), 3.56 (1H, quintet, H-1), and 7.1-7.45 (5H, m, aromatic H's) ppm; CMR δ 18.3 (C-3), 29.8(C-2,-4), 40.4(C-1), 125.9(C-4'), 126.3, 128.2 (aromatic C's) and 146.3 (C-1') ppm, and phenylcycloheptane (**4**, overall 77%), bp 77°C, 1mm (lit.[26] bp 114.5°C, 10mm): PMR δ 1.5-2.0 (12H, m, CH_2's), 2.65 (1H, m, H-1), and 7.1-7.4 (5H, m, aromatic H's) ppm; CMR δ 27.3 (2C, C-4,-5), 28.0 (2C, C-3,-6), 36.8 (2C, C-2,-7), 47.1 (C-1), 125.9, 126.8 (2C), 128.3 (2C), and 146.8 (C-1') ppm.

Incubations with *M. isabellina*: these were performed by the standard procedure referenced above. Yields and (where appropriate) enantiomeric excesses of products are listed in Table 1. All yields refer to isolated, purified material. Characteristic spectral data of the products are listed below under the approriate substrate headings.

Phenylcyclobutane (1)

1-Phenylcyclobutanol (**1a**); oil; PMR δ 1.5-2.7 (6H, m, CH_2's), 7.2-7.5 (5H, m, aromatic H's) ppm; CMR δ 13.1 (C-3), 36.6 (2C, C-2,-4), 115.1 (C-1), 125.1, 127.2, 128.4 (aromatic C's), and 146.0 (C-1') ppm; MS m/z(%) 148(M^+,8), 131(8), 120(55), 104(100), 91(5), 78(12). *cis/trans*-3-Phenylcyclobutanol (**1b** + **1c**); oil; PMR δ 2.0-3.0 (4H, CH_2's), 3.6-3.8 (1H, m, H-3), 4.3 (0.5H, quintent, H-1), 4.6 (0.5H, quintet, H-1), and 7.2-7.4 (5H, m, aromatic H's) ppm; CMR δ 29.0/32.9 (C-3), 39.2/40.9 (C-2), 63.4/66.2 (C-1), 125.8/ 126.0 (C-4'), 126.6, 128.4 (C-2',-3'), and 145.2 (C-1') ppm; MS m/z(%) 148(M^+,2), 130(5), 104(100), 91(25), 78(10).

Phenylcyclopentane (2)

cis/trans-3-Phenylcyclopentanol (**2a/2b**): oil; PMR δ 1.5-2.5 (6H, m, CH_2's), 3.2-3.4 (1H, m, H-3), 4.3-4.6 (1H, m, H-1), and 7.1-7.4 (5H, m, aromatic H's) ppm; CMR δ 32.6 (C-4), 35.7/36.0 (C-5), 42.9 (C-3), 44.0/44.3 (C-2), 73.7 (C-1), 125.9 (C-4'), 127.0, 128.3 (C-2',-3'), 127.1 (C-1'), 145.5 (C-1') ppm; MS m/z(%) 162(M^+,18), 144(73), 129(85), 120(100), 105(35), 91(30), 77(20). Jones' oxidation of **2a/2b** (50 mg) afforded 3-phenylcyclopentanone in 85% yield: oil; CMR δ 31.1 (C-4), 39.8 (C-5), 42.2 (C-3), 45.7 (C-2), 126.7, 128.4, 143.0 (aromatic C's), and 218.3 (C-1) ppm; MS m/z(%) 160(M^+,69), 131(12), 117(32), 104(100).

Phenylcyclohexane(3)

trans-4-Phenylcyclohexanol (**3a**): mp 115-117°C (lit.[27] mp 118°C); PMR δ 1.4-2.6 (8H, m, CH_2's), 3.6-3.8 (1H, m, H-4), and 7.1-7.4 (5H, m, aromatic H's) ppm; CMR δ 32.4, (C-3), 36.0 (C-2), 43.4 (C-4), 70.6 (C-1), 126.0 (C-4'), 126.7, 128.3 (C-2',-3'), and 145.2 (C-1') ppm (lit.[28] δ_C 32.7, 35.5, 44.5, 70.1 ppm); MS m/z(%) 176(M^+,20), 158(100), 143(69), 130(54), 117(50), 104(79), 91(65), 77(21). Jones' oxidation of **3a** (50 mg) afforded afforded 4-phenylcyclohexanone (44 mg): oil; CMR δ 33.9 (C-3,-5), 41.3 (C-2,-6), 42.7 (C-4), 126.5, 126.6, 128.6, 144.7 (aromatic C's), and 211.0 (C-1) ppm; MS, M^+ 174.

Phenylcycloheptane (4)

cis/trans-4-Phenylcycloheptanol (**4a/4b**): oil; PMR δ 1.3-2.2 (10H, m, CH_2's), 2.5-2.8 (1H, m, H-4), 3.9-4.1 (1H, m, H-1), and 7.1-7.4 (5H, m, aromatic H's) ppm; CMR δ 21.4/23.3 (C-6), 29.4 /31.4 (C-3), 35.7/36.5 (C-7), 37.0/37.6, 37.1/37.8 (C-2,-5), 46.7/47.0 (C-4), 71.5/72.7 (C-1), 125.9, 126.6, 128.3 (aromatic C's), and 149.2 (C-1') ppm; MS m/z(%) 190(M^+,45), 172(18), 144(76), 129(38), 118(100), 104(76), 91(88). Jones' oxidation of **4a/4b** (50 mg) gave 4-phenylcycloheptanone (42 mg): oil; CMR δ 23.7 (C-6), 31.8 (C-3), 38.3 (C-5), 42.8, 43.7 (C-2,-7), 48.6 (C-4), 126.1, 126.4, 128.4 (aromatic C's), 147.5 (C-1'), and 214.5 (C-1) ppm; MS M^+ 188.

2-Phenylbutane (**5**)

2-Phenyl-2-butanol (**5a**) and 3-phenyl-2-butanol (**5b**) were identified in trace amounts (≤ 1%) by comparison with authentic standards in gc analysis of the biotransformation extract, but could not be obtained in sufficient quantity and purity for spectral and configurational analysis.

1-Methyltetralin (**6**)

cis/trans-4-Methyl-1,2,3,4-tetrahydro-1-naphthol (**6a** + **6b**): oil; PMR δ 1.19 and 1.24 (total 3H, each d, CH_3), 1.4-2.0 (4H, m, CH_2's), 2.6-2.9 (1H, m, H-4), 4.70 and 5.30 (total 1H, each t, H-1), and 7.0-7.6 (4H, m, aromatic H's) ppm; CMR δ 23.2/23.5 (CH_3), 26.8/27.2 (C-3), 29.4/29.7 (C-2), 32.3/32.5(C-4), 68.4/68.6 (C-1), 127.1 (2C), 128.0, 128.2 (C-5,-8), 138.5 (C-8a), 142.0 (C-4a) ppm (lit.[29] C-1 69.0(*c*)/ 69.2(*t*), C-2 29.8(*c*)/30.3(*t*), C-3 27.2(*c*)/27.4(*t*), C-4 32.5(*c*)/32.6(*t*)). MS m/z(%) 162(M^+,32), 144(100), 129(51), 117(43), 104(55), 91(47).

5,6,7,8-Tetrahydroquinoline (**7**)

5-Hydroxy-5,6,7,8-tetrahydroquinoline (**7a**): oil; PMR δ 1.7-2.3 (4H, m, H-6,-7), 2.75-3.15 (2H, m, H-8), 3.60 (1H, br.s, exchanges D_20, OH) 4.70 (1H, m, H-5), and 7.15, 7.70, and 8.45 (each 1H, m, aromatic H's) ppm; CMR δ 18.4 (C-7), 30.8, 31.7 (C-6,-8), 67.3 (C-5), 122.3 (C-3), 136.5 (C-4a), 139.2 (C-4), 145.5 (C-2) and 155.7 (C-8a) ppm; MS m/z(%) 149(M^+,22), 131(100); $[\alpha]_D$ -12.3° (c = 0.12, ethanol), ee 28% , R configuration, based on $[\alpha]_D$ +44 for the S enantiomer,[30] ee 33% by PMR determination.

2-Ethyltoluene (**8**)

1-(2'-Methylphenyl)ethanol (**8a**): oil; PMR δ 1.40 (3H, d, H-2), 2.3 (3H, s, aryl-CH_3), 5.10 (1H, q, H-1), 7.0-7.4 (4H, m, aromatic H's) ppm; $[\alpha]_D$ -14 (c = 0.4, ethanol), ee 25%, S configuration based on $[\alpha]_D$ + 55.4 (R configuration).[31] 2-Ethylbenzyl alcohol (**8b**): oil; PMR δ 1.45 (3H, t, CH_3), 2.70 (2H, q, CH_2), 4.70 (2H, s, CH_2OH), 7.0-7.4 (4H, m, aromatic H's) ppm.

Dihydrocoumarin (**11**)

3-(2'-Hydroxyphenyl)propanol (**11a**): oil; PMR δ 2.5 (2H, m, H-2), 3.1 (2H, t, H-3), 3.9 (2H, t, H-1), 6.8-7.5 (4H, m, aromatic H's); MS m/z(%) 152(M^+,83), 134(77), 107(100), 91(70), 77(62).

Isochroman (**12**)

2-(2'-Hydroxymethylphenyl)ethanol (**12a**): oil; PMR δ 3.1 (2H, t, H-2), 4.05 (2H, t, H-1), 4.85 (2H, s, CH_2OH), and 7.3-7.6 (4H, m, aromatic H's) ppm; CMR δ 35.0 (C-2), 62.7, 63.0 (C-1 and CH_2OH), 126.7, 128.5, 129.7, 130.0, 138.1 and 139.3 (aromatic C's) ppm; MS m/z(%) 152(M^+,3), 134(20), 104(100), 91(37), 77(38).

2-Ethylanthracene (**13**)

1-(2'-Anthracenyl)ethanol (**13a**) was identified in incubation extracts from **13** by comparison of GC traces with those of authentic samples (estimated yield ca. 0.1%), but could not be obtained in sufficient quantity and purity for spectral and configurational analysis.

Sequential incubations of phenylcyclopropane followed by ethylbenzene, and phenylcyclobutane followed by ethylbenzene were carried out as follows: the fungal mycelia recovered from the incubation of *M. isabellina* with the initial substrate was re-distributed over 15 1L Erlenmeyer flasks, each containing 250 mL of distilled water, and then a solution of ethylbenzene (1 g) in 95% ethanol (30 mL) added at the rate of 2 mL per flask. The flasks were stoppered in the usual way and returned to the rotary shaker for a further 72h, after which time product isolation and charaterization were carried out in the usual manner. From *M. isabellina* recovered from phenylcylopropane biotransformation, no detectable conversion of ethyl benzene to 1-phenylethanol was observed. *M. isabellina* recovered from biotransformation of phenylcyclobutane gave 1-phenylethanol (0.15 g, 14%), $[\alpha]_D$ +11.0 (e.e. 26%).[3]

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References

1. Holland, H.L. and Munoz, B. *Bio-org. Chem.* **1988**, *16*, 388.
2. Holland, H.L.; Brown, F.M.; Conn, M. *J. Chem. Soc. Perkin Trans. 2* **1990**, 1651.
3. Holland, H.L.; Bergen, E.J.; Chenchaiah, P.C.; Khan, S.H.; Munoz, B.; Ninniss, R.W.; Richards, D. *Can. J. Chem.* **1987**, *65*, 502.
4. Holland, H.L.; Brown, F.M.; Munoz, B; Ninniss, R.W. *J. Chem. Soc. Perkin Trans. 2* **1988**, 1557.
5. Holland, H.L.; Chernishenko, M.J.; Conn, M.; Munoz, A.; Manoharan, T.S.; Zawadski, M.A. *Can. J. Chem.* **1990**, *68*, 696.
6. Kutney, J.P.; Singh, M.; Hewitt, G.; Salisbury, P.J.; Worth, B.R.; Servizi, J.A.; Martens, D.W.; Girdon, R.W. *Can. J. Chem.* **1981**, *59*, 2334.
7. Kutney, J.P.; Berset, J.-D.; Hewitt, G.M., Singh, M. *Appl. Environ. Microbiol.* **1988**, *54*, 1015.
8. Holland, H.L.; Conn, M.; Chenchaiah, P.C.; Brown, F.M. *Tetrahedron Lett.* **1988**, *29*, 6393.
9. Holland, H.L.; Carter, I.M.; Chenchaiah, P.C.; Khan, S.H.; Munoz, B.; Ninniss, R.W.; Richards,D. *Tetrahedron Lett.* **1985**, *26*, 6409.
10. White, R.E.; Miller, J.P.; Favreau, L.V.; Battacharyya, A. *J. Am. Chem. Soc.* **1986**, *108*, 6024.
11. Holland, H.L.; Manoharan, T.S.; Schweizer, F. *Tetrahedron Asymmetry* **1991**, *2*, 335.
12. Cresnar, B; Breskvar, K.; Hudnick-Plevnik, T. *Biochem. Biophys. Res. Commun.* **1985**, *133*, 1057.
13. Samata, T.B.; Ghosh, D.K. *J. Steroid Biochem.* **1987**, *28*, 327.
14. Archelas, A.R.; Furstoss, R.; Waegell, B.; Le Petit, J.; Devize, L. *Tetrahedron* **1984**, *40*, 355.
15. Holland, H.L. *Organic Synthesis with Oxidative Enzymes*, VCH Publishers: New York. 1992; pp. 76-82.

16. Liu, W.-G.; Goswami, A.; Steffek, R.P.; Chapman, R.L.; Sariaslani, F.S.; Steffens, J.J.; Rosazza, J.P.N. *J. Org. Chem.* **1988**, *53*, 5700.
17. Sariaslani, F.S.; Rosazza, J.P.N. *Enzyme Microbiol. Technol.* **1984**, *6*, 242.
18. Fonken, G.S.; Herr, M.E.; Murray, H.C. US Patent 3,281,330 (1964): *Chem. Abstr.* **1967**, *66*, 9974r.
19. Fonken, G.S.; Herr, M.E.; Murray, H.C. US Patent 3,392,171 (1968): *Chem. Abstr.* **1968**, *69*, 58586w.
20. Holland, H.L., Rand, C.G.; Viski, P.; Brown, F.M. *Can J. Chem.* **1991**, *69*, 1989..
21. Suckling, C.J.; Nonhebel; D.C.; Brown, L.; Suckling, K.E.; Seilman, S.; Wolf, C.R. *Biochem.J.* **1985**, *232*, 199.
22. Bowry, V.W.; Ingold, K.U. *J. Am. Chem. Soc.* **1991**, *113*, 5699.
23. Holland, H.L.; Carter, I.M. *Can. J. Chem.* **1982**, *60*, 2420.
24. Kooijman, E.C.; Strang, A. *Rec. Trav. Chim.* **1953**, *72*, 342.
25. Shabarov, Y.S.; Donskaya, N.A.; Levina, R.Y. *Zh. Obshch. Khim.* **1963**, *33*, 3434.
26. Chromow, S.I.; Balenkowa, E.S. *Dokl Akad. Nauk S.S.S.R.*. **1953**, *89*, 1025. *Chem. Abstr.* **1954**, *48*, 6383i.
27. Ungnade, H.E. *J. Org. Chem.* **1948**, *13*, 361.
28. Kalinowski, H.O.; Berger, S.; Braun, S. *Carbon-13 NMR Specroscopy*, J. Wiley: London. 1988; pp. 259-263.
29. Schneider, H.-J.; Agrawal, P.K. *Org. Mag. Res.* **1984**, *22*, 180.
30. Boyd, D.R.; Bushman, D.R.; Davies, R.J.H.; Dorrity, M.R.J.; Hamilton L.; Jerina, D.M.; Levin,W.; McCullough, J.J.; McMordie, R.A.S.; Malone, J.F.; Porter, H.P. *Tetrahedron Lett.* **1991**, *32*, 1991.
31. Brown, H.C.; Park, W.S.; Cho, B.T. *J. Org. Chem.* **1986**, *51*, 1934.