Site selective oxidation of tricyclo[3.3.1.1^{3,7}]decane (adamantane) and some of its derivatives using fungi of the genus *Absidia*

PERKIN 2

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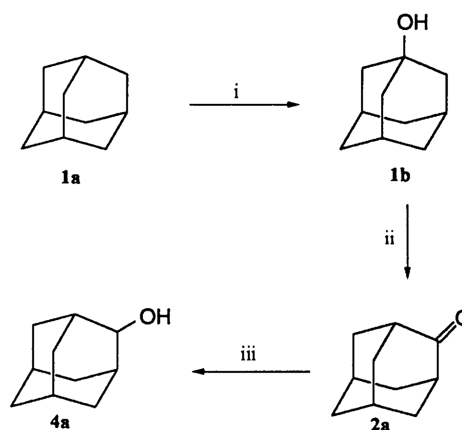
Tricyclo[3.3.1.1^{3,7}]decane 1a has been converted into 1-hydroxytricyclo[3.3.1.1^{3,7}]decane 1b, tricyclo[3.3.1.1^{3,7}]decane-1,4ax-diol 4b, tricyclo[3.3.1.1^{3,7}]decane-1,3-diol 3b and to a lesser extent, 2-hydroxytricyclo[3.3.1.1^{3,7}]decane 4a (20 to 40% overall yield) using the microorganisms *Absidia glauca* (IMI 239693), *A. cylindrospora* (IMI 342950), *A. spinosa* (IMI 193887), *A. spinosa* var. *biappendiculata* (IMI 238610) and *A. cylindrospora* var. *nigra* (IMI 240053) as biocatalysts. In addition, *A. cylindrospora* (IMI 342950) converted tricyclo[3.3.1.1^{3,7}]decane-1-carboxylic acid 1c into 4ax-hydroxytricyclo[3.3.1.1^{3,7}]decane-1-carboxylic acid 4c with almost complete regioselectivity. Tricyclo[3.3.1.1^{3,7}]decane derivatives 1d–g were used as biohydroxylation substrates with *A. cylindrospora* (IMI 342950), giving selective biohydroxylation at the 4ax- and/or the 3-position. The 4ax-selectivity was confirmed by X-ray crystal structure determinations of 4b, 4c and 4j.

Since Peterson and Murray first used *Rhizopus nigricans* in the hydroxylation of progesterone,[1] microbial oxidations of organic substrates, especially steroids, have been extensively studied and reviewed.[2] Although numerous chemical[3] and biomimetic[4] methods of oxidation have been utilised, biotransformations involving monooxygenase enzymes in whole-cell systems have continued to be the method of choice for chemists seeking remote hydroxy-functionalisation at unactivated carbon centres. Whole-cell systems are preferred over the use of isolated enzymes for the following reasons. (*i*) The enzymes involved are usually membrane bound and are relatively inactive outside the cell environment in which they normally operate. The required enzymes may be deactivated during procedures designed to isolate these proteins from the cells. (*ii*) The enzymes are cofactor-dependent and isolated enzymes require the purchase and employment of expensive cofactors.

A major impediment in the field of fungal biohydroxylations has been the difficulty in predicting the regio- and stereochemistry of the oxidation reaction, although active site models for the fungi *Calonectia decora*[5] and *Beauveria sulfurescens*[6] and more recently, *Absidia blakesleeana*[7] have been proposed. In this paper, we report the regiospecific biohydroxylation of tricyclo[3.3.1.1^{3,7}]decane, **1a**, and derivatives such as tricyclo[3.3.1.1^{3,7}]decane-1-carboxylic acid, **1c**.

The biooxidation of hydrocarbon **1a** was of interest because the large scale production of the important pharmaceutical intermediate 2-hydroxytricyclo[3.3.1.1^{3,7}]decane, **4a**, is not straightforward (Scheme 1), being based on the Geluk–Schlattmann adamantanone synthesis.[8] The problem arises in step (ii). This step produces excessive tarring, making the isolation and refining stages of the ketone **2a** difficult, costly and low yielding.

The dual requirement of an improved industrial process for the synthesis of alcohol **4a** and improved predictability of oxidation sites on hydrocarbon substrates led us to investigate the biohydroxylation of hydrocarbon **1a** with fungi of the genus *Absidia*.† This particular genus was chosen because in an initial screening using 24, 48 and 72 old flask cultures of the fungi *Cunninghamella blakesleeana* (IMI 223658), *Beauveria sulfurescens* (IMI 341084) and *Absidia cylindrospora* (IMI 342950), only the latter could effect substrate hydroxylation on hydrocarbon **1a** at substrate concentrations of 200 mg dm^{-3}.



Scheme 1 *Reagents and conditions:* i. O_2, Cu catalyst; ii. conc H_2SO_4, heat; iii. $NaBH_4$, EtOH

Results and discussion

The hydrocarbon **1a** was incubated with 14 fungi from the

† X-Ray crystal structures of 4-substituted tricyclo[3.3.1.1^{3,7}]decane compounds derived from *Absidia*-catalysed biotransformations were presented in our preliminary communication (Colin H. Ridyard, Roger A. Whittaker, Stanley D. Higgins, Stanley M. Roberts, Andrew J. Willetts, Patrick D. Bailey and Georgina M. Rosair, *Chem. Commun.*, 1996, 1833) and the details were deposited at the Cambridge Crystallographic Data Centre.

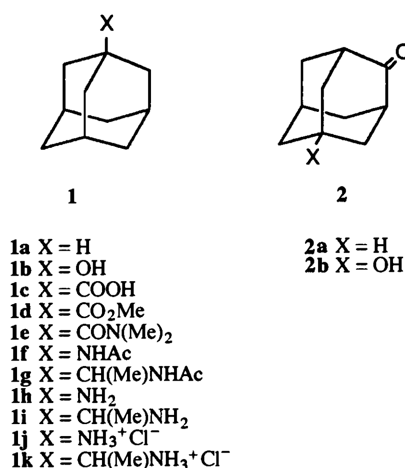
Table 1 Optimised conversions of hydrocarbon **1a** with fungi of the genus *Absidia*

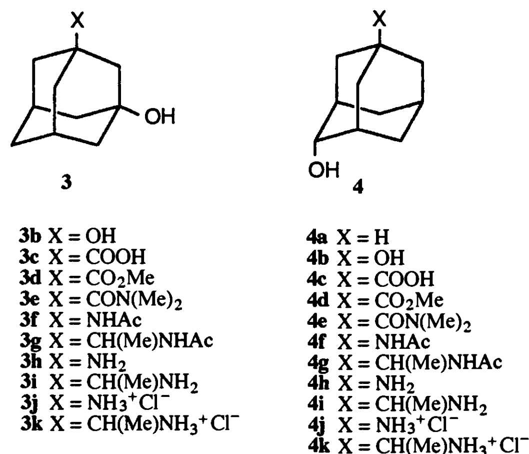
Fungus	Product (%) **1b**	**4b**	**3b**	**4a**	Conversion time/h[a]	Age of flask culture/h[b]	Type
A. glauca	24	8	4	3	260	48	2
A. cylindrospora	3	14	22	—	120	48	1
A. spinosa	3	13	20	—	120	73	1
A. cylind. var. *nigra*	17	4	6	2	150	48	2
A. spin. var. *biapp*	20	2	4	1	144	72	2

[a] From addition of **1a** to flask culture. [b] Age on addition of **1a**.

genus *Absidia*. These fungi were *Absidia blakesleeana* (IMI 343040), *A. repens* (IMI 238605), *A. anomala* (IMI 238606), *A. californica* (IMI 238609), *A. psychrophila* (IMI 197671), *A. cuneospora* (IMI 078403), *A. coerulea* (IMI 202719), *A. griseola* (IMI 239487), *A. pseudocylindrospora* (IMI 240050), *A. glauca* (IMI 239693), *A. cylindrospora* (IMI 342950), *A. spinosa* (IMI 193887), *A. spinosa* var. *biappendiculata* (IMI 238610) and *A. cylindrospora* var *nigra* (240053). Nearly all of the fungi gave some conversion, however the better conversions in solution (20 to 40%) were obtained with just five of the species (Table 1). *A. cylindrospora* and *A. spinosa* initially gave the alcohol **1b** which was subsequently converted into diols **3b** and **4b**. *A. glauca*, *A. spinosa* var. *biappendiculata* and *A. cylindrospora* var. *nigra* gave mainly the alcohol **1b** with smaller quantities of **3b**, **4b** and **4a**. Two types of product profiles were observed in the overall screening. Type 1 refers to those fermentations where the target alcohol **4a** was not present in the fermentation beer on GC analysis.

The reaction profiles for Type 1 biohydroxylations follow the pattern described in Fig. 1. The same profiles were also





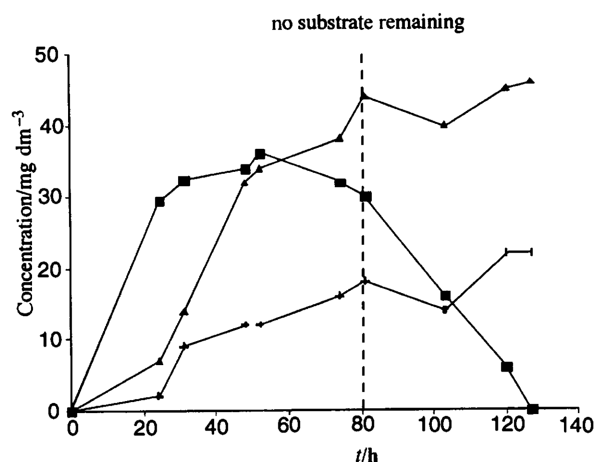


Fig. 1 Reaction profile for the biohydroxylation of hydrocarbon **1a** using the Type 1 fungus *Absidia cyclindrospora* (IMI 342950). Key: ■ (**1b**), + (**4b**), ▲ (**3b**).

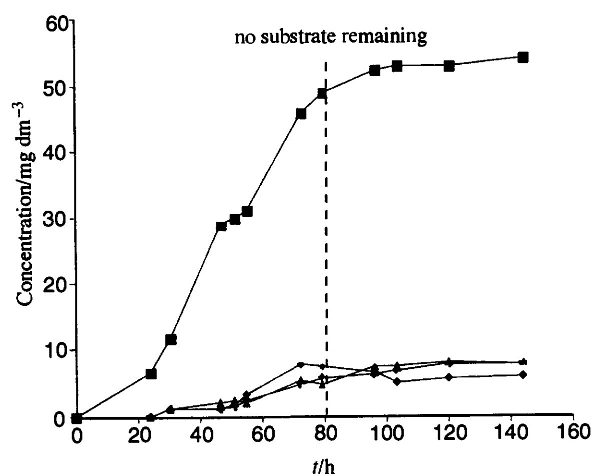


Fig. 2 Reaction profile for the biohydroxylation of hydrocarbon **1a** using the Type 2 fungus *Absidia glauca* (IMI 239693). Key: ■ (**1b**), ▲ (**4b**), + (**3b**), ◆ (**4a**).

observed with the biohydroxylations catalysed by *A. repens* (IMI 238605) and *A. anomala* (IMI 238606), although their overall product yields were much lower. By way of contrast, a second product distribution profile (Fig. 2) was observed and was highly conserved among the fungi that produced quantities of the target alcohol **4a** (Table 1). These biohydroxylations were defined as Type 2 biohydroxylations. The product profiles of Type 2 oxidations further differed from those generated by Type 1 systems in that the diol concentrations **3b** and **4b** were greatly reduced and the alcohol **1b** remained in solution without being metabolised.

The Type 1 biohydroxylation pattern appeared analogous (with respect to monohydroxylation) to results obtained with cytochrome $P450_{CAM}$ while Type 2 systems gave results similar to those obtained with the mammalian cytochrome $P450_{LM2}$.[9]

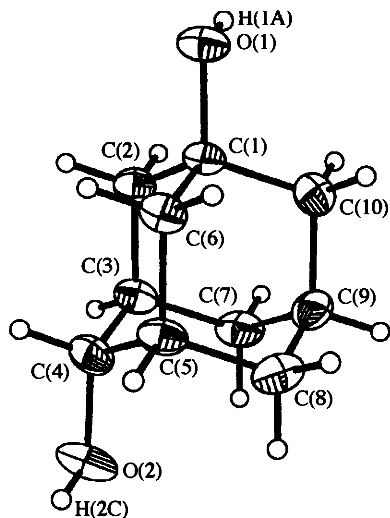


Fig. 3 An ORTEP drawing of the diol **4b** highlighting the axial geometry of the hydroxy group at C-4

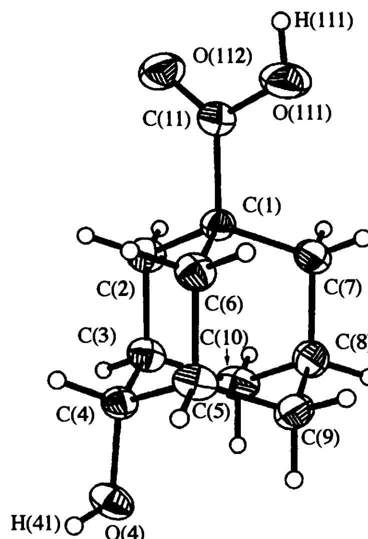


Fig. 5 An ORTEP drawing of the hydroxy acid **4c** highlighting the axial geometry of the hydroxy group at C-4

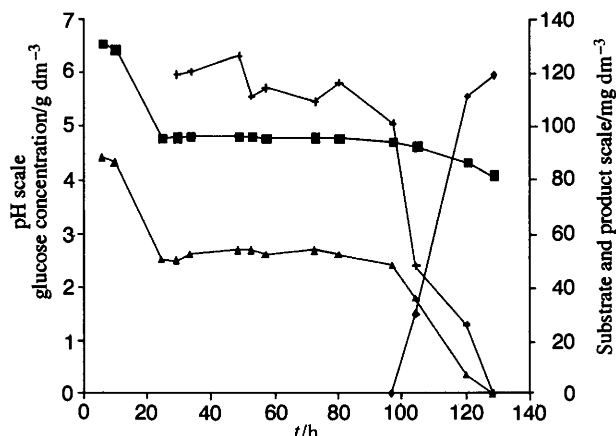


Fig. 4 Biohydroxylation of carboxylic acid **1c** (+) showing the relationship between pH (■), glucose concentration (▲) and product **4c** (◆) formation

The diols **3b** and **4b** were identified by comparison with authentic samples prepared by literature methods[10,11] and the stereochemistry of the diol **4b** was confirmed by X-ray crystallography (Fig. 3).

The reaction profile illustrated in Fig. 1 suggests the following. (*i*) Up to approximately 30 h, the substrate **1a** is converted into the monohydroxylated derivative **1b**, traces of which are converted to the diols **3b** and **4b**. (*ii*) Between 30 and 80 h, the substrate **1a** is converted into the monohydroxylated derivative **1b**, which is itself being converted at a similar rate to the diols **3b** and **4b**. Steady-state concentrations of **1b** were not constant in the different biotransformations of **1a**. (*iii*) After 80 h, hydroxylation of **1a** is complete and the amount of **1b** declines due to continued conversion into **3b** and **4b**.

The involvement of cytochrome P450 monooxygenase(s) was implied, because the Type 1 biohydroxylation was completely inhibited in the presence of the cytochrome P450-specific inhibitor, 1-aminobenzotriazole.[12] The ability of the fungi undertaking Type 1 biohydroxylation to effect methylene biooxidation on the alcohol **1b** to give the diol **4b**, coupled with their inability to effect methylene biooxidation of the hydrocarbon **1a**, suggested that two cytochrome P450 monooxygenases were involved in the overall biotransformation.

The failure to obtain reasonable quantities of alcohol **4a** *via* direct biohydroxylation of hydrocarbon **1a** prompted an investigation into other biological methods for its preparation. Because **1b** could be hydroxylated to the diols **3b** and **4b** by *A. cylindrospora* it was reasoned that the carboxylic acid **1c** could similarly be hydroxylated at the C-3 and C-4 positions. The latter product could then be isolated and decarboxylated to give **4a**. Biohydroxylation of the carboxylic acid **1c** (Fig. 4) provided the 4ax-derivative **4c**‡ the structure of which was determined by X-ray crystallography (Fig. 5).

A change in morphology of the microorganism accompanied the formation of **4c**. The submerged culture changed from a dissociated pellatised mycelium to a higher buoyancy, aggregated pellatised mycelium. Furthermore, there were obvious differences between the filtered mycelium recovered from the successful (cream coloured) and the unsuccessful (pigmented) biohydroxylations (marked with an asterisk in Table 2).

It is noteworthy that addition of the substrate **1c** to a 24 h old flask culture of *A. cylindrospora* (IMI 342950) caused a levelling-off in the pH and glucose concentrations (Fig. 4). The formation of **4c** and the change in morphology were accompanied by a renewed decrease in the pH and recommencement of glucose utilisation. The time between substrate introduction and product formation was not constant and varied between two and four days.

The observed patterns of change to the pH, residual glucose and colony morphology suggests that the hydroxylation of substrate **1c** may be a serendipitous consequence of the change in enzyme profile needed to trigger a different developmental phase of the life cycle of the fungus.

Biotransformations utilising *A. cylindrospora* (IMI 342950) as the biocatalyst were conducted on the tricyclo-[3.3.1.1^{3,7}]decane derivatives **1d**, **1e**, **1f** and racemic **1g**. With the exception of racemic substrate **1g**, the preferred site of hydroxylation was the 4-axial (4ax) position but the regioselectivity was not as well defined as for the acid **1c** since hydroxylation at the 3-position was also observed (Table 3).

The axial geometry in the product **4g** was unconfirmed because it could not be isolated in a suitably crystalline form for X-ray characterisation. The axial geometry in **4d** and **4e** was characterised by comparison of their hydrolysis products with the authentic hydroxy acid **4c**. Alkaline hydrolysis of **4f** generated the amine free-base **4h** which was crystallised from ethereal hydrogen chloride as the hydrochloride salt **4j**. X-Ray crystallographic analysis of **4j** revealed once again an axial

‡ There are several chemical methods described in the literature that give the C-4 hydroxylated acid (**4c**) as a mixture of equatorial and axial isomers,[13,14] however, these pathways consist of a number of steps going through the intermediacy of the 1,4-hydroxy ketone (**2b**).

Table 2 Biohydroxylation of carboxylic acid **1c** with the fungus *A. cylindrospora* (IMI. 342950)

Reaction no.	Initial [**1c**]/mg dm^{-3}	Final [**4c**]/mg dm^{-3}	Initial [glucose]/g dm^{-3}	Time to reach max [**4c**]/h	Biomass[a]/g dm^{-3}
1	150	124	4	70	2.88
2	150	124	8	70	2.74
3	150	123	6	70	3.9
4	500	—	6	—	1.36*
5	1000	—	6	—	0.84*
6 (blank)	—	—	6	—	3.8
7	150	119	6	105	3.9
8	150	123	12	105	3.8
9	250	—	6	—	1.76*

[a] An asterisk represents pigmented mycelium on filtration.

Table 3 The biohydroxylation of substrates **1d–g** catalysed by the fungus *Absidia cylindrospora* (IMI 342950)

Substrate	Conc./mg dm^{-3} [a]	Products	% Yield (isolated)
1d	200	**3d**	21
		4d	29
1e	200	**4e** (+2 trace products)	18
1f	2000	**3f**	16
		4f	37
1g	800	**3g**	36
		4g	12

[a] Initial substrate concentration.

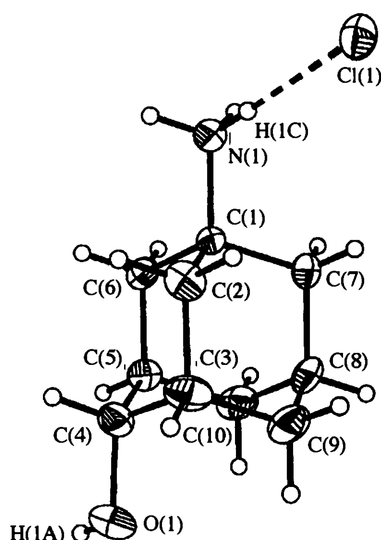


Fig. 6 An ORTEP drawing of the amine salt **4j** highlighting the axial geometry of the hydroxy group at C-4

hydroxy group at C-4 (Fig. 6). The axial-hydroxylation at C-4 was evidently a highly-conserved characteristic within these biotransformations. The biohydroxylation of the racemic substrate **1g** was not highly enantioselective. The major component **3g** was found to be dextrorotatory with an enantiomeric excess of 6%. The minor component **4g** was also found to be dextrorotatory with an enantiomeric excess of 36.5%.

The amine free-bases (**3h**, **3i** and **4i**) and salts (**3j**, **3k** and **4k**) were prepared in a similar manner to **4h** and **4j**, and the salts **3j**, **4j**, **3k** and **4k** were tested for anti-HIV activity using C8166 cells infected with HIV-1 MN at 37 °C. Three of the compounds, **3j**, **4j** and **4k** were found to be pro-active at higher concentrations giving over 100% antigen gp120 and virus yield relative to infected control cells. Compound **3k** was found to inhibit viral proliferation at high concentrations (> 1000 μM) as seen by the reduction in the antigen and virus yield.

Oxidation at the C-3 and C-4 positions of the hydrocarbon nucleus (especially with substrate **1f** which was used in the original site-modelling studies) correlate well with the 5.5 Å[6] rule and its latter modifications[15] for the fungus *Beauveria sulfurescens* (ATCC 7159). It is possible that the monooxygenase effecting hydroxylation at C-3 and C-4 in the substrates **1c–g** was identical to the second enzyme postulated in the Type 1 biohydroxylation that effected C-3 and C-4 hydroxylation of the alcohol **1b**. There is no obvious rationale as to why oxidation occurred almost exclusively at C-4 on the acid **1c** and the amide **1e** but at C-3 *and* C-4 on the other substrates used.

It is notable that like *A. cylindrospora*, inhibitor studies on *B. sulfurescens* (IMI 12939) have indicated the intermediacy of cytochrome P450 monooxygenase(s) in the biohydroxylation of xenobiotics.[16] The similarity in the products resulting from the biotransformation of substrate **1f** and the organisms' susceptibilities to cytochrome P450-specific inhibitors would suggest that their oxygenating species are related. However, the inability of *B. sulfurescens* to transform the hydrocarbon **1a** is a significant difference between it and *A. cylindrospora* and is further evidence of the latter organism possessing more than one cytochrome P450 monooxygenase.

There was no correlation between the Type 1 and Type 2 biohydroxylations of hydrocarbon **1a** and the active-site model previously proposed for *A. blakesleeana*.[7] There are four reasons for this. (*i*) A totally different three-dimensional active site for the planar aromatic nucleus of flavanones would be anticipated when compared to the tetrahedral cage associated with hydrocarbon **1a** and its related substrates. (*ii*) *A. blakesleeana* (IMI 343040) gave a very poor hydroxylation of hydrocarbon **1a** (3 to 8%). (*iii*) The *A. blakesleeana* active-site model relies on two anchor points and the oxygen atoms of the central flavanone ring are 7.53 and 4.98 Å away from the hydroxylated carbon atom (*cf.* O–C4 is 4.25 Å and O–C3 is 3.77 Å on the alcohol **1b**). (*iv*) There was no evidence to suggest the flavanone hydroxylation was mediated by a cytochrome P450 monooxygenase (*e.g.* laccases are capable of catalysing aromatic hydroxylations[17]).

The active site model for the fungus *Calonectia decora*[5] was also found to be inapplicable to this particular study.

The 3- and 4ax-hydroxylation of the substrates used is conveniently characterised by the model described in Fig. 7. The model is similar to that proposed by Fonken *et al.*[6] since there is an interaction between a nucleophilic atom or functional group at C-1 and an electrophilic locus within the enzyme active-site. However, to allow for the hydroxylation at the C-3 and C-4ax positions, the interaction would have to be either non-bonding or very weakly bonding. The orientation of both C-1 and C-2 are important since the β-hydrogen at C-2 is equidistant (5.005 Å) from the C-3* and C-4ax* hydrogen atoms. It is these two hydrogen atoms that meet the required criteria for hydrogen abstraction because the C–H bonds are aligned parallel to the plane of the haem oxyform.[18]

Topographical models of the tertiary structure of eukaryotic cytochrome P450 monooxygenases have been constructed by aligning their sequences with that of cytochrome $P450_{CAM}$ and then fitting the eukaryotic sequences to the X-ray crystallographic model template of the prokaryotic enzyme.[19] This was necessary because the eukaryotic membrane-bound cytochrome P450 monooxygenases are very labile enzyme systems and are generally unstable under conditions where cellular integrity is disrupted. However, active-site models based on this approach are considered unreliable due to the relatively rigid substrate specificity and electron transport system characterised by cytochrome $P450_{CAM}$.[20] Indirect studies based on *in situ* rearrangement of phenyl–iron complexes[20] and site-directed mutagenesis[21] have indicated that the apolar active-site around

the haem and the substrate access channel of eukaryotic membrane-bound cytochrome P450 monooxygenases are larger or more flexible than the prokaryotic cytosolic cytochrome $P450_{CAM}$. This in turn accounts for the broader substrate specificities observed with the eukaryotic cytochrome P450 monooxygenases.[22] These studies have also concluded that in the eukaryotic model, the hydrophobic pocket enclosing the haem active-site has a high area to depth ratio (shallow active-site model). It is difficult to make any fine correlation between the shallow active-site model and the axial hydroxylation model because the latter is based on a tetrahedral cage and the former is based on studies with 'shallow' conjugated aromatic systems. However, it is noteworthy that in order for the axial hydroxylation model to apply, it is essential that the substrate approach the haem lengthways as opposed to side-on (Fig. 8) and this alone could be indicative of a high area to depth ratio prevailing in the system.

From the information available in the literature and the reported observations in this paper, it is postulated that the C-1 substituent and the C-2 carbon are orientated within an interfacial area between the hydrophobic pocket and the substrate access channel. This means that the tricyclo[3.3.1.1^{3,7}]decane nucleus has some freedom of motion

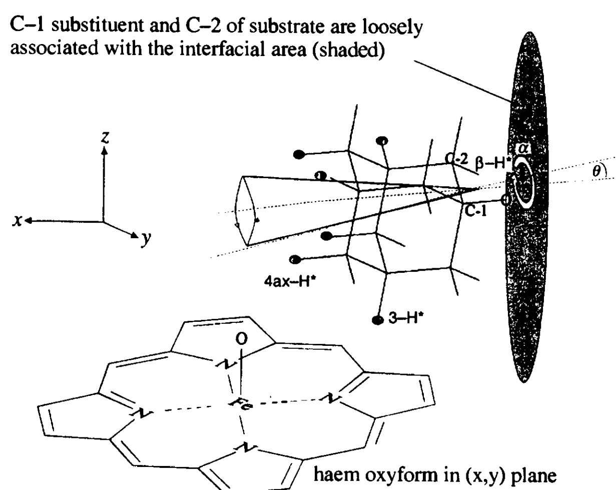


Fig. 7 Axial hydroxylation model postulated for 1-substituted tricyclo[3.3.1.1^{3,7}]decane derivatives

within the hydrophobic pocket to account for hydroxylation at the C-3 and C-4ax positions, and the relatively hydrophilic substituent at C-1 is orientated away from the hydrophobic pocket in the direction of the substrate access channel. This interfacial area forms what could be described as a 'hydrophilic groove' within the hydrophobic pocket and lies in a plane (y,z) approximately perpendicular to the plane of the haem oxyform (x,y).

There was a substantial amount of evidence in favour of the axial hydroxylation model in Fig. 7. (*i*) Inhibitor studies confirmed the intermediacy of one or more cytochrome P450 monooxygenases. The activity of the haem oxyform and the required parallel orientation of the C–H bond are consistent with current theory on cytochrome P450 mediated biohydroxylations. (*ii*) Axial hydroxylation at C-4 had been confirmed in a number of substrates by X-ray crystallography regardless of the substituent at C-1. (*iii*) Hydroxylation at C-3 was confirmed by mass spectroscopic, NMR, IR and melting point data in a number of substrates. (*iv*) In all cases where C-3 and C-4 hydroxylation occurred together, they always appeared at the same time and in similar ratios for a single substrate. There was no evidence to suggest that C-3 and C-4 hydroxylations were catalysed by separate enzymes (*e.g.* by the hydroxylation at C-3 preceding that at C-4). (*v*) The substrate orientation and motion in the axial hydroxylation model is the *only* way to account for the hydroxylation observed. The motion is essential to account for the spatial differences between the substituent at C-1 and the C-3 and C-4 carbon atoms. The orientation is necessary to allow hydrogen abstraction as a prelude to oxygen rebound. A second limiting model where the interfacial area lies parallel to the plane of the haem (Fig. 9) would not apply as this would lead to hydroxylation at the C-3 and C-4eq positions. (*vi*) Because different C-1 substituents give different ratios of C-3 : C-4ax products and considering the substrate orientation and motion, it would be impossible for bond formation between a C-1 substituent and a single amino acid residue to occur *and* to see hydroxylation at both C-3 and C-4ax positions. However, there had to be some association between substituent and active-site because some regioselectivity was observed (*e.g.* no C-2 and C-4eq hydroxylation was observed in any of the substrates).

A hydrophilic nature at the interface between the substrate access channel and the hydrophobic pocket could limit the accessibility of highly lipophilic substrates such as **1a** to the

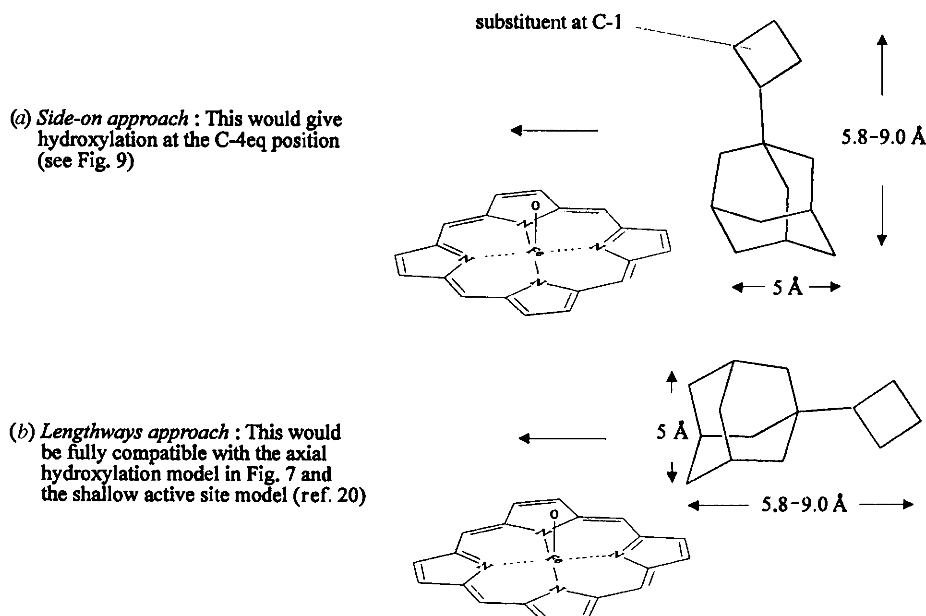


Fig. 8 A diagram representing the approach of the substrate to the haem oxyform. The variation in the length of the substrate is dependent on the type of substituent at C-1.

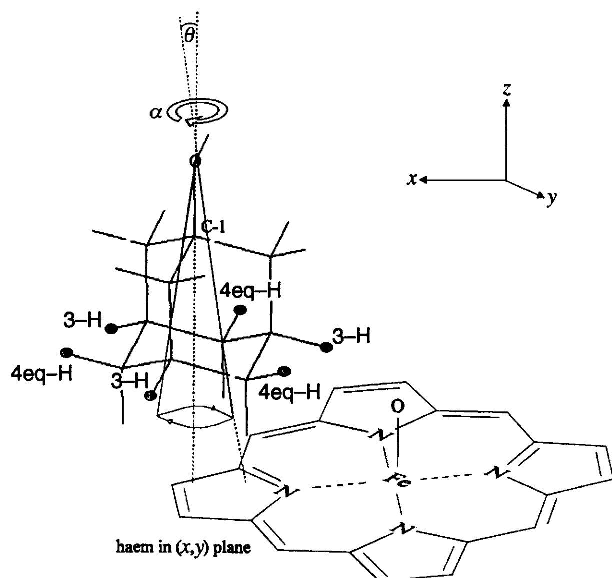


Fig. 9 Equatorial hydroxylation model for 1-substituted tricyclo[3.3.1.1^{3,7}]decane derivatives. This would be a consequence of the side on approach seen in Fig. 8. Alternatively, this model could arise as a consequence of the interfacial plane lying parallel to the plane of the heme.

active site of the monooxygenase and explain the apparent prerequisite of substitution to the nucleus of **1a** as a prelude to methylene oxyfunctionalisation. This hypothesis assumes that the differences in the ratios of 4-substituted to 3-substituted products seen with substrates **1b–g** was a consequence of the way in which the functional group at C-1 of the adamantane nucleus interacted with the interfacial area lying in the plane perpendicular to the haem moiety. For example, the excellent regioselectivity seen in the biohydroxylation of the acid **1c** may have been due to a very strong interaction of the carboxylic acid function at C-1 with the hydrophilic groove of the monooxygenase, restricting the molecular motion described by the angles α and θ. This restriction of motion could have effectively locked the axial C–H bond in the required parallel orientation. The strong interaction could be due to the non-covalent bonding of the carboxy group to an amino acid residue situated in the apoprotein.

Experimental

Preparation of fermentation medium

This was prepared by dissolving glucose (10 g) and corn steep liquor (20 g) in deionised water (1 dm^3), adjusting to pH 8 with dilute caustic soda and autoclaving at 15 psi and 121 °C for 20 min.

Maintenance of the fungi

Each of the fungi was grown on 5% malt extract agar at ambient temperature in an incubator protected from UV radiation.

Preparation of the culture

A 0.25 cm^2 section of actively growing fungus (*i.e.* from the expanding peripheral edges of the mycelial mat) was cut from the Petri dish, aseptically inoculated into 50 cm^3 corn steep liquor medium in a 100 cm^3 conical flask and then incubated for 72 h at 23 °C and 160 rpm in an orbital incubator. It was then transferred aseptically to a 1 dm^3 conical flask containing the same medium (500 cm^3) and incubation continued at 23 °C and 160 rpm until required for substrate addition.

Biohydroxylation of tricyclo[3.3.1.1^{3,7}]decane-1-carboxylic acid 1c

The substrate acid **1c** (75 mg, 0.42 mmol) was dissolved in absolute ethanol (1 cm^3) and added to a 24 h old culture of *A. cylindrospora* (IMI 342950) in corn steep liquor medium (500 cm^3). The seeded medium was then incubated at 23 °C and 160 rpm for a further 76–120 h to enable the required biotransformation to occur. The spent medium was acidified to below pH 3 with 36% hydrochloric acid. The acidified filtrate was continuously extracted with methylene chloride (800 cm^3) for 24 h using an 800 cm^3 extractor column fitted with a condenser and a sintered disc distributor. The organic extracts were dried with anhydrous magnesium sulfate and adsorbed onto C60 Sorbsil silica (1 g) by solvent evaporation. The crude product **4c** (55 mg) was isolated by column chromatography using a 0 to 50% acetone in light petroleum (40–60 °C) elution gradient over a C60 Sorbsil silica column (30 g silica, 2.5 cm internal diameter).

Recrystallisation from 1 : 1 acetone–light petroleum afforded 32 mg (40%) of **4c**; mp 194–5 °C (lit.,[13] 196–198 °C); ν_{max}(KBr)/cm^{-1} 3300–2400, 3460, 2940, 2860, 2640, 1710, 1460, 1330, 1280, 1260, 1230, 1195, 1160, 1100, 1040; δ_H(250 MHz; CD_3OD) 1.43–2.17 (13 H, m, CH and CH_2s), 3.82 (1 H, m, C*H*-OH); δ_C(62.9 MHz; CD_3OD) 28.64 (CH), 31.05 (2 × CH_2), 35.32 (2 × CH), 39.21 (2 × CH_2), 40.17 (CH_2), 40.92 (quat. C), 74.37 (CH–OH) and 181.22 (C=O) (Found: M$^+$, 196.110 47. $C_{11}H_{16}O_3$ requires 196.109 95).

Analysis of glucose content in biohydroxylation of 1c

This was measured using a glucose oxidase–peroxidase bioassay. The chromogenic substance used was 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). The enzymes used were glucose oxidase (EC 1.1.3.4), (Sigma) and Grade II peroxidase (Boehringer Mannheim).

ABTS (125 mg) was dissolved in 0.1 mol dm^{-3} pH 7 phosphate buffer (125 cm^3). To this solution was added a solution of glucose oxidase (2.5 mg) and peroxidase (10 mg) dissolved in the same buffer (50 cm^3). The reagent was made up to volume (250 cm^3) with more buffer. To 1 cm^3 samples containing glucose concentrations over the range 0 to 2.5 × 10^{-4} mol dm^{-3} was added 4 cm^3 of the ABTS reagent and after mixing, the samples were left for 30 min at room temperature. The reactions were quenched with 4 mol dm^{-3} hydrochloric acid (0.5 cm^3), the absorbance of the solutions measured at 410 nm, and the data used to plot a standard curve. This was then used in conjunction with A_{410} values of appropriate dilutions of fermentation beer treated similarly with ABTS reagent in order to establish the glucose concentrations.

Analysis of tricyclo[3.3.1.1^{3,7}]decane-1-carboxylic acid 1c and 4ax-hydroxytricyclo[3.3.1.1^{3,7}]decane-1-carboxylic acid 4c[23]

Aliquots of fermentation beer (2.5 cm^3) were acidified with 36% hydrochloric acid (2 drops) and extracted with ethyl acetate (3 × 4 cm^3). The extracts were dried with anhydrous magnesium sulfate and solvent exchanged to 25% methanol in diethyl ether (*ca.* 7 cm^3). The solutions were treated with diazomethane until pale yellow and left to stand for 30 min. Excess diazomethane was removed by gently purging with nitrogen. The solutions were made up to volume in 10 cm^3 volumetric flasks and analysed by GC using 1-hydroxyadamantane as the internal standard. The GC column was a glass 5″ × $\frac{1}{4}$′ packed with 10% Apiezon L on chromosorb W/AL 100–120. The carrier gas was N_2, the column temperature 175 °C, and the injector and detector ovens both 300 °C.

Synthesis of tricyclo[3.3.1.1^{3,7}]decane-1-4ax-diol 4b

The hydroxy ketone **2b**[24] (5 g, 30 mmol) was dissolved in ethanol (200 cm^3) and the solution was heated to reflux. A

solution of sodium borohydride (1.3 g, 34 mmol) in water (28.6 cm^3) was added to the solution. The reaction mixture was heated under reflux for 3.5 h or until no starting material was observed (GC). Hydrochloric acid (36% w/v, a few drops) was added to the cooled reaction mixture pH < 5.0. The aqueous ethanolic solution was decanted and evaporated to provide a white residue which was treated with hot ethyl acetate (200 cm^3 at 60 °C) and filtered to remove inorganic material. The ethyl acetate solution was evaporated to dryness to yield a white crystalline solid consisting of axial and equatorial isomers of **4b** (4.6 g; GC assay 96.2% by area). A small sample (*ca.* 500 mg) of the isomeric mixture was dissolved in acetone (15 cm^3) and adsorbed onto Sorbsil C60 silica (2 g). The silica/product residue was put on the top of a Sorbsil C60 silica column (30 g to 2.5 cm ID) and eluted with 32% acetone in light petroleum (40–60 °C). The first fractions contained the less polar axial isomer **4b** (80 mg) whereas later fractions contained the more polar equatorial isomer (67 mg). Mps > 320 °C (lit.,[11] axial 336–337 °C; equatorial 352–353 °C).

For the axial isomer **4b**: δ_H(250 MHz; CD_3OD) 1.34–1.47 (m, 2 H), 1.55–1.79 (m, 6 H), 1.95–2.17 (m, 5 H), 3.63–3.70 (m, C*H*–OH); δ_C(62.9 MHz; CD_3OD) 30.68 (2 × CH_2), 31.41 (CH), 37.42 (2 × CH), 44.18 (2 × CH_2), 46.06 (CH_2), 67.92 (quat. C), 74.13 (CH–OH) (Found M^+, 68.1153. $C_{10}H_{16}O_2$ requires 168.1150).

For the equatorial isomer: δ_H(250 MHz; CD_3OD) 1.34–1.47 (m, 2 H), 1.55–1.79 (m, 6 H), 1.95–2.17 (m, 5 H), 3.63–3.70 (m, C*H*–OH); δ_C(62.9 MHz; CD_3OD) 30.96 (CH), 36.13 (2 × CH_2), 38.36 (2 × CH), 39.60 (2 × CH_2), 46.01 (CH_2), 68.16 (quat. C), 73.44 (CH–OH) (Found: M^+, 168.1149. $C_{10}H_{16}O_2$ requires 168.1150).

Biohydroxylation of tricyclo[3.3.1.1^{3,7}]decane 1a

The substrate hydrocarbon (100 mg, 0.74 mmol) was dissolved in DMF (5 cm^3, 90 °C) and added to a culture (48–72 h old) of the appropriate fungus in corn steep liquor medium (500 cm^3). The seeded medium was incubated at 23 °C and 160 rpm for 120–150 h (subject to GC analysis). The fermentation medium was filtered with gentle suction and the filtrate was continuously extracted with methylene chloride (800 cm^3) for 24 h using an 800 cm^3 extractor column fitted with a condenser and sintered disc distributor. The organic extracts were dried with anhydrous magnesium sulfate and adsorbed onto C60 Sorbsil silica (1 g) by solvent evaporation. The crude products, usually **3b** and **4b** were isolated by column chromatography (Type 1 extracts) using a 0 to 50% acetone in light petroleum (40–60 °C) elution gradient over a C60 Sorbsil silica column (30 g silica, 2.5 cm internal diameter) wet-packed with light petroleum. After recrystallisation from acetone–light petroleum (*ca.* 1 : 1), the products were obtained as white crystals (10–18 mg of each, typical overall yields *ca.* 20%).

Analysis of **3b** revealed ν_{max}(KBr)/cm^{-1} 3600 to 3200, 3220, 2930, 2850, 1300, 1135, 1020; δ_H(250 MHz; solvent $CDCl_3$–acetone; standard Me_4Si) 1.25 (s, 2 H) 1.63 (m, 10 H), 2.32 (d, 2 H); δ_C(62.9 MHz; $CDCl_3$–acetone) 32.54 (2 × CH), 35.81 (1 = CH_2), 44.64 (4 × CH_2), 53.21 (1 × CH_2), 70.67 (2 × quat. C) (Found: M^+, 168.1144. $C_{10}H_{16}O_2$ requires 168.1150).

Analysis of **4b** as before. Identification of **1b** and **4a** was by GC and TLC (multiple systems) comparison with authentic samples.

Analysis of tricyclo[3.3.1.1^{3,7}]decane 1a biohydroxylation. Aliquots of fermentation (2.5 cm^3) were spiked with an aqueous internal standard solution containing **4a**, in the case of Type 2 analyses, the aliquot had to be run on the GC system before and after internal standard addition to compensate for its initial presence. The beers could not be directly extracted with organic solvents because **3b** and **4b** were insufficiently lipophilic. Compound **1a** could not be directly analysed as it formed a microdispersion with limited solvation.

Screening. The substrate hydrocarbon **1a** (100 mg, 0.74 mmol) was dissolved in DMF (5 cm^3, 90 °C) and added to 24, 48 and 72 h old cultures of the appropriate fungus. Samples of fermentation beer (2.5 cm^3) were taken 2–4 times daily over a 5–7 day period and analysed by the GC method outlined above.

Chemical synthesis of *N*,*N*-dimethyltricyclo[3.3.1.1^{3,7}]decane-1-carboxamide 1e

Tricyclo[3.3.1.1^{3,7}]decane-1-carbonyl chloride (5.0 g, 25 mmol) was dissolved in THF (30 cm^3) and cooled to below 5 °C. A 40% w/v aqueous solution of dimethylamine (10 cm^3, *ca.* 80 mmol) was added to the cooled solution with stirring whilst maintaining the temperature below 10 °C. The solution was maintained this way for a further 30 min then poured into water (350 cm^3) whereupon crystallisation occurred. The crystals were collected by suction filtration, air dried and recrystallised from light petroleum (40–60 °C) at −7 °C to give **1e** (2.74 g, 53%).

Analysis of **1e** revealed ν_{max}/cm^{-1}(Nujol) 2900, 1620, 1450, 1370, 1160, 1050; mp 79–80 °C; δ_H(250 MHz; $CDCl_3$) 1.69–1.80 (m, 6 H), 2.00–2.11 (m, 9 H), 3.10 (s, 2 × CH_3); δ_C(62.9 MHz; $CDCl_3$) 29.57 (3 × CH), 36.72 (3 × CH_2), 37.41 (quat. C), 38.53 (2 × CH_3), 38.83 (3 × CH_2), 177.24 (*C*=O) (Found: M^+, 207.163 09. $C_{13}H_{21}NO$ requires 207.162 31).

Biohydroxylation of *N*,*N*-dimethyltricyclo[3.3.1.1^{3,7}]decane-1-carboxamide 1e to 4ax-hydroxy-*N*,*N*-dimethyl-tricyclo[3.3.1.1^{3,7}]decane-1-carboxamide 4e

The amide (**1e**, 100 mg, 0.48 mmol) was dissolved in absolute ethanol (1.0 cm^3) and added to a 48 h old culture of *Absidia cylindrospora* (IMI 342950) in corn steep liquor medium (500 cm^3). The seeded medium was incubated with reciprocal shaking at 23 °C and 160 rpm for three days. The fermentation medium from two such reactions was filtered with gentle suction through a GF/B filter (9 cm) and the filtrate was continuously extracted with methylene chloride (800 cm^3) for 24 h using an 800 cm^3 extractor column fitted with a condenser and sintered disc distributor. The organic extracts were dried with anhydrous magnesium sulfate and adsorbed onto C60 Sorbsil silica (1 g). The crude product (**4e**, 46 mg, 22%) was isolated by column chromatography using a 0 to 100% ethyl acetate in light petroleum (40–60 °C) elution gradient over a C60 Sorbsil silica column (38 g silica, 2.5 cm internal column diameter) wet-packed with light petroleum (40–60 °C). Recrystallisation from methylene chloride–light petroleum gave **4e** as white crystalline needles (39 mg, 18%).

Analysis of **4e** revealed, mp 108–9 °C; δ_H(250 MHz; $CDCl_3$) 1.89–2.24 (m, 13 H), 3.05 (s, 6 H, 2 × C*H*$_3$), 3.82–3.89 (m, C*H*–OH); δ_C(62.9 MHz; $CDCl_3$) 27.78 (CH), 30.07 (2 × CH_2), 34.64 (2 × CH), 38.02 (2 × CH_2), 38.43 (CH_2), 38.60 (2 × CH_3), 41.02 (quat. C), 73.50 (CH–OH), 176.31 (C=O) (Found: M^+, 223.157 13. $C_{13}H_{21}NO_2$ requires 223.157 23).

Refluxing **4e** in 6 mol dm^{-3} HCl gave **4c**, mp 194–195 °C; ν_{max}(KBr)/cm^{-1} 3600–3300, 3300–2400, 2940, 2860, 2640, 1710, 1460, 1330, 1260, 1195, 1100, 1040.

Chemical synthesis of methyl tricyclo[3.3.1.1^{3,7}]decane-1-carboxylate 1d

Tricyclo[3.3.1.1^{3,7}]decane-1-carbonyl chloride (3.0 g, 15 mmol) was dissolved in methylene chloride (20 cm^3) and added dropwise to a solution of methanol (760 mm^3, 600 mg, 18.8 mmol) and triethylamine (2100 mm^3, 15 mmol) in methylene chloride (30 cm^3) whilst maintaining the temperature below 5 °C. The solvent was removed by evaporation and the ester was obtained as a low melting solid (2.3 g, 79%) by flash column

chromatography using light petroleum (40–60 °C) (300 cm^3 over 15 g C60 Sorbsil silica).

Analysis of **1d** revealed, mp 38–41 °C; ν_{max}(KBr)/cm^{-1} 2930, 2850, 1730, 1455, 1435, 1340, 1235, 1080; δ_H(250 MHz; $CDCl_3$) 1.60–2.05 (m, 15 H), 3.65 (S, 3 H, CH_3–O); δ_C(62.9 MHz; $CDCl_3$) 27.94 (3 × CH), 36.47 (3 × CH_2), 38.79 (3 × CH_2), 40.63 (quat. C), 51.52 (CH_3–O), 178.23 (C=O).

Biohydroxylation of methyl tricyclo[3.3.1.1^{3,7}]decane-1-carboxylate 1d

The ester (**1d**, 100 mg, 0.52 mmol) was dissolved in absolute ethanol (1.0 cm^3) and added to a 48 h old culture of *Absidia cylindrospora* (IMI 342950) in corn steep liquor medium (500 cm^3). The seeded medium was incubated with reciprocal shaking at 23 °C and 160 rpm for three days. The fermentation medium from two such reactions was filtered with gentle suction through a GF/B filter (9 cm) and the filtrate was continuously extracted with methylene chloride (800 cm^3) for 24 h using an 800 cm^3 extractor column fitted with a condenser and sintered disc distributor. The organic extracts were dried with anhydrous magnesium sulfate and adsorbed onto C60 Sorbsil silica (1 g). The products were isolated by column chromatography using a 20 to 50% ethyl acetate in light petroleum (40–60 °C) elution gradient over a C60 Sorbsil silica column (34 g silica, 2.5 cm internal column diameter). The product **4d** eluted initially, closely followed by **3d**. Product **4d** (64 mg, 29%) and product **3d** (46 mg, 21%) were isolated as pale yellow oils.

Analysis of **3d** revealed, δ_H(250 MHz; $CDCl_3$) 1.54–1.92 (m, 12 H), 2.24–2.33 (m, 2 H), 3.65 (s, 3 H, O–CH_3); δ_C(62.9 MHz; $CDCl_3$) 30.21 (2 × CH), 34.97 (CH_2), 37.67 (2 × CH_2), 44.03 (quat. C), 44.26 (2 × CH_2), 46.33 (CH_2), 51.73 (O–CH_3), 68.27 (quat. C), 176.88 (C=O) (Found: M^+, 210.126 36. $C_{12}H_{18}O_3$ requires 210.125 60).

Analysis of **4d** revealed, δ_H(250 MHz; $CDCl_3$) 1.38–2.28 (m, 13 H), 3.65 (s, 3 H, O–CH_3), 3.87–3.95 (m, CH–OH); δ_C(62.9 MHz; $CDCl_3$) 27.18 (CH), 29.88 (2 × CH_2), 34.03 (2 × CH), 37.91 (2 × CH_2), 39.93 (CH_2), 40.14 (quat. C), 51.64 (O–CH_3), 73.41 (CH–OH), 177.71 (C=O) (Found: M^+, 210.125 60. $C_{12}H_{18}O_3$ requires 210.125 60).

Saponification with 1 mol dm^{-3} NaOH gave **4c**, mp 194–195 °C.

Biohydroxylation of 1-ethanamidotricyclo[3.3.1.1^{3,7}]decane 1f

The ethanamide (**1f**, 1000 mg, 5.18 mmol) was dissolved in absolute ethanol (1.0 cm^3) and added to a 48 h old culture of *Absidia cylindrospora* (IMI 342950) in corn steep liquor medium (500 cm^3). The seeded medium was incubated with reciprocal shaking at 23 °C and 160 rpm for six days. The fermentation medium from two such reactions was filtered with gentle suction through a GF/B filter (9 cm) and the filtrate was continuously extracted with methylene chloride (800 cm^3) for 24 h using an 800 cm^3 extractor column fitted with a condenser and sintered disc distributor. The organic extracts were dried over anhydrous magnesium sulfate and adsorbed onto C60 Sorbsil silica (2 g). The products were isolated by column chromatography using a 10 to 60% acetone in light petroleum (40–60 °C) elution gradient over a C60 Sorbsil silica column (34 g silica, 2.5 cm internal column diameter). Compound **4f** was obtained from early fractions as long needle-like crystals (850 mg, 37%) after recrystallisation from acetone. The product **3f** (304 mg, 16%) eluted at a slower rate and was obtained as small orthorhombic crystals after recrystallisation from acetone. The isolated yield of **3f** was significantly reduced since initial fractions were contaminated with **4f**.

Analysis of **4f** revealed, mp 175–176 °C; δ_H(250 MHz; CD_3OD) 1.39–1.50 (m, 2 H), 1.85 (s, 3 H, CH_3–CO), 1.96–2.17 (m, 12 H), 3.87–3.94 (m, CH–OH), 7.40 (bs, Ac–NH–); δ_C(62.9 MHz; CD_3OD) 23.77 (CH), 30.13 (CH_3–CO), 30.89 (2 × CH_2), 36.36 (2 × CH), 40.79 (2 × CH_2), 42.38 (CH_2), 52.12 (quat. C), 74.13 (CH–OH), 172.59 (C=O) (Found: M^+, 209.141 82. $C_{12}H_{19}NO_2$ requires 209.141 58).

Analysis of **3f** revealed mp 224–226 °C; δ_H(250 MHz; CD_3OD) 1.50–1.74 (m, 6 H), 1.82 (s, 3 H, CH_3–CO), 1.87–1.99 (m, 6 H), 2.16–2.25 (m, 2 H); δ_C(62.9 MHz; CD_3OD) 23.69 (CH_2), 31.95 (2 × CH), 36.04 (CH_2), 40.96 (2 × CH_2), 44.84 (2 × CH_2), 48.91 (CH_3–CO), 55.11 (quat. C), 69.47 (quat. C), 172.53 (C=O) (Found: M^+, 209.141 60. $C_{12}H_{19}NO_2$ requires 209.141 58).

Biohydroxylation of *N*-(1-tricyclo[3.3.1.1^{3,7}]decylethyl)-ethanamide 1g

The ethanamide (**1g**, 400 mg, 1.81 mmol) was dissolved in absolute ethanol (1 cm^3) and added to a 48 h old culture of *Absidia cylindrospora* (IMI 342950) in corn steep liquor medium (500 cm^3). The seeded medium was incubated with reciprocal shaking at 23 °C and 160 rpm for two days. The fermentation medium from two such reactions was filtered with gentle suction through a GF/B filter (9 cm) and the filtrate was continuously extracted with methylene chloride (800 cm^3) for 24 h using an 800 cm^3 extractor column fitted with a condenser and sintered disc distributor. The organic extracts were dried with anhydrous magnesium sulfate and adsorbed onto C60 Sorbsil silica (3 g). The products were isolated by column chromatography using a 0 to 65% acetone in light petroleum (40–60 °C) elution gradient over a C60 Sorbsil silica column (35 g silica, 2.5 cm internal column diameter). From a total of four fermentations, 1600 mg of **1g** yielded (after recrystallisation) **3g** (620 mg, 36%, from methylene chloride) and **4g** (211 mg, 12%, from methylene chloride–light petroleum).

Analysis of **3g** revealed, mp 171–173 °C; ee = 6% (+-isomer); ν_{max}(KBr)/cm^{-1} 3400, 3280, 2940, 2900, 1635, 1530, 1160, 1110, 1060; δ_H(250 MHz; CD_3OD) 1.04 (d, *J* 8,§ 3 H, CH–CH_3), 1.39–1.78 (m, 12 H), 1.95 (s, 3 H, CH_3–CO), 2.14–2.22 (m, 2 H), 3.65–3.75 (m, 1 H, Ac–CH–Me), 7.6 (d, *J* 8, NH); δ_C(62.9 MHz; CD_3OD) 14.59 (CH_3–CH), 22.59 (CH_2), 31.80 (2 × CH), 36.66 (CH_2), 38.12 (2 × CH_2), 40.94 (CH_3–CO), 45.36 (2 × CH_2), 46.85 (Ac–CH–Me), 53.56 (quat. C), 69.22 (quat. C), 172.44 (C=O) (Found: M^+, 237.174 08. $C_{14}H_{23}NO_2$ requires 237.172 88).

Analysis of **4g** revealed, ee = 36.5% (+-isomer); δ_H(250 MHz; CD_3OD) 1.05 (d, *J* 8, 3 H, CH–CH_3), 1.35–1.45 (m, 2 H), 1.45–1.68 (m, 6 H), 1.82–1.99 (m, 6 H, including CH_3–CO), 2.11–2.20 (m, 2 H), 3.61–3.69 (m, 1 H, Ac–CH–Me), 3.71–3.78 (m, CH–OH), 7.6 (d, *J* 8, NH); δ_C(62.9 MHz; CD_3OD) 14.59 (CH_3–CH), 22.62 (CH_2), 29.13 (2 × CH_2), 31.45 (2 × CH), 35.49 (2 × CH_2), 36.55 (CH), 38.22 (CH_3–CO), 39.33 (Ac–CH–Me), 53.79 (quat. C), 74.89 (CH–OH), 172.55 (C=O) (Found: M^+, 237.171 96. $C_{14}H_{23}NO_2$ requires 237.172 88).

Enantiomeric excesses

The free base (**4i** or **3i**, 3 mg) was dissolved in aqueous acetone (1 : 1 v/v, 100 mm^3). The solution was treated with commercial Marfey's reagent (1 mg in 50 mm^3 acetone) and sodium hydrogen carbonate (40 mm^3, 1 mol dm^{-3}). The mixture was heated at 40 °C for 1 h cooled and acidified with dilute hydrochloric acid (20 mm^3, 2 mol dm^{-3}). The solution was run on a 25 cm ODS 2 HPLC column using a mobile phase of 1% aqueous acetic acid (55%) and acetonitrile (45%) running at 1 cm^3 min^{-1}. Detection was by UV-VIS absorbance at 340 nm.

General method for preparing the amine hydrochloride salts

The hydroxyethanamides **3f**, **4f**, **3g** and **4g** (*ca.* 1–2 mmol) were suspended in aqueous sodium hydroxide (10% w/v, 30 cm^3) and refluxed for 22 h. The corresponding free base was extracted from the cooled solution with diethyl ether (5 × 15 cm^3) and

§ *J* Values are given in Hz.

the dried (anhydrous magnesium sulfate) organic phase was treated with ethereal hydrogen chloride (*ca.* 7% w/v) to precipitate the amine hydrochloride salt. The salts (**3j**, **4j**, **3k** and **4k**) were recrystallised from methanol–ethyl methyl ketone.

Acknowledgements

We thank the SERC and Peboc Division of Eastman Chemical (UK) Ltd for financial support (CASE award to C. H. R.). We also thank Dr H. Geluk of Solvay-Duphar for the samples of the tricyclo[3.3.1.1^{3,7}]decanediols. We are also grateful for the assistance of Drs P. Richardson and P. Sutton (Dept. of Chemistry, University of Exeter). We thank Dr N. Mahmoud (MRC) for carrying out the anti-HIV assays.

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Paper 6/02721K
Received 18*th April* 1996
Accepted 13*th May* 1996