Selective biohydroxylation of 1-substituted adamantanes using *Absidia cylindrospora* (I.M.I. 342950)

Patrick D. Bailey,[a] Stanley D. Higgins,[b] Colin H. Ridyard,[b] Stanley M. Roberts,[c] Georgina M. Rosair,[a] Roger A. Whittaker[b] and Andrew J. Willetts[d]

[a] *Department of Chemistry, Heriot-Watt University, Riccarton, Edinburgh, UK EH14 4AS*
[b] *Peboc Division of Eastman Chemical (UK) Ltd, Llangefni, Anglesey, Gwynedd, UK LL77 7YQ*
[c] *Robert Robinson Laboratory, Department of Chemistry, University of Liverpool, PO Box 147, Liverpool, UK L69 3BX*
[d] *Department of Biological Sciences, University of Exeter, Exeter, Devon, UK EX4 4QD*

The biohydroxylation of 1-substituted adamantanes using *Absidia cylindrospora* in a whole-cell oxidation system exclusively generated 3-hydroxy and 4_{ax}-hydroxy derivatives; the assignments were confirmed by three X-ray crystal structure determinations.

Derivatives of adamantane **1** display a wide range of valuable properties,[1] and efficient synthetic routes to such compounds are consequently very important. Of particular interest are 1,3- and 1,4-disubstituted analogues, which may possess anti-viral activity (*c.f.* rimantidine **2g**),[2] but standard synthetic routes to such compounds are extremely long, cumbersome and inefficient. We therefore decided to explore whether enzymic oxidation[3,4] of 1-substituted adamantanes might generate the 3- or 4-hydroxy derivatives cleanly, quickly and efficiently. Fungal whole-cell systems were selected for study, because these biocatalysts are known to possess membrane-bound cytochrome P450 monooxygenase enzymes, which might achieve the desired hydroxy-functionalisation at one or more unactivated carbon atoms. From an initial screening of 14 fungi, *Absidia cylindrospora* (I.M.I. 342950) was selected as the most promising (based on its ability to oxidize adamantane **1** initially to 1-hydroxyadamantane **2a**, and then further to the diols **3a** and **4a**).

Once appropriate conditions for the hydroxylation of adamantane had been established, a range of 1-substituted adamantanes were used as substrates, and the results are summarised in Table 1.†

The products **3a–f** and **4a–f** were readily separated by column chromatography [R_f (**4**) > R_f (**3**)], and the positions of hydroxylation were established by analysis of their NMR spectra. The literature contains some ambiguity concerning the assignment of stereochemistry of 1,4-disubstituted adamantanes (for example, see refs. 5–7), and we therefore sought to provide unequivocal identification of structures **4a–f**. For compounds **4a** and **4b**, single crystal X-ray structure determinations were carried out, and the axial geometry of the 4-substituents are shown in Figs. 1 and 2.‡ Compound **4e** was hydrolysed with aqueous sodium hydroxide, and acidification with HCl generated the crystalline hydrochloride salt **4h**, whose structure was also determined by X-ray diffraction (Fig. 3).‡ Compounds **4c** and **4f** were hydrolysed to **4b**, enabling us to confirm their axial geometry, and it was inferred that **4d** also possessed the 4-axial stereochemistry; this would be in agreement with one other reported biohydroxylation of this type,[5] although it is interesting that microbial oxidation of diamantanol by *Rhizopus nigricans* occurs exclusively at the apical C–H positions.[8]

The striking feature of the oxidations was the formation of only 3-hydroxy and 4_{ax}-hydroxy derivatives. Thus, remote functionalisation of 1-substituted adamantanes by *Absidia cylindrospora* gives direct access to these important compounds, for which standard chemical methods would be impracticable.

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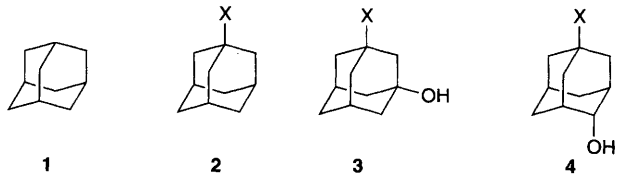

1 **2** **3** **4**

a X = OH
b X = CO_2H
c X = CO_2Me
d X = CHMeNHAc
e X = NHAc
f X = $CONMe_2$
g X = $CHMeNH_2$
h X = NH_2.HCl

Table 1 Oxidation of 1-substituted adamantanes **1** by *Absidia cylindrospora* (I.M.I. 342950)

Starting material **2**	**3** formed (%)	**4** formed (%)
X = OH	20	14[a]
X = CO_2H	0	40[a]
X = CO_2Me	21	29[b]
X = CHMeNHAc	36	12[c]
X = NHAc	16	37[a]
X = $CONMe_2$	trace	18[b]

[a] Structure determined by X-ray crystallography. [b] Structure assigned by conversion to **4b**. [c] Axial geometry inferred by analogy.

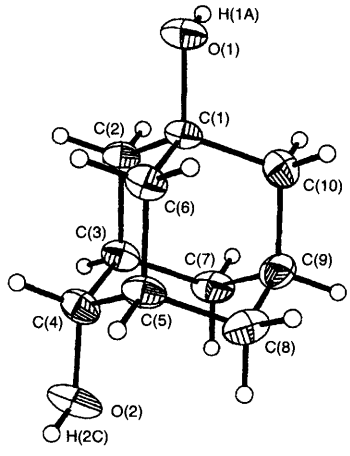

Fig. 1 ORTEP view of the crystal structure of **4a**

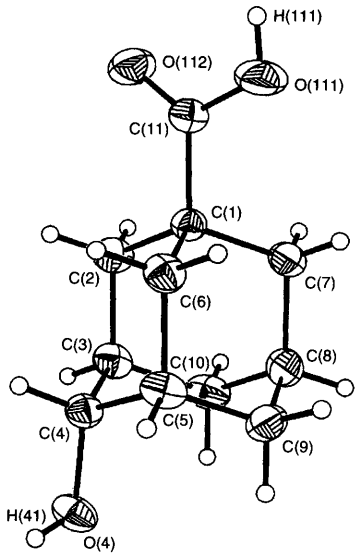

Fig. 2 ORTEP view of the crystal structure of **4b**

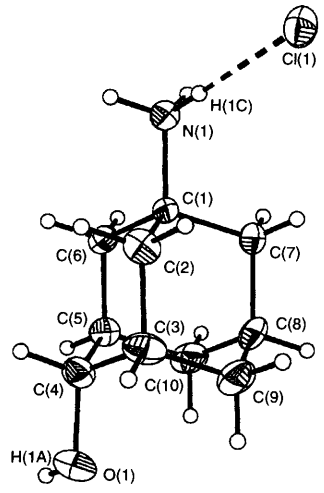

Fig. 3 ORTEP view of the crystal structure of **4h**

C. H. R.). We also thank Dr H. Geluk of Solvay-Duphar for authentic samples of adamantane diols. We are grateful for the assistance of Drs P. Richardson and P. Sutton (Department of Chemistry, University of Exeter).

Footnotes

† *General procedure* for biotransformations: *Absidia cylindrospora* (I.M.I. 342950) was grown on 5% malt extract agar under ambient conditions; a 0.25 cm^2 section of actively growing fungus was transferred into fermentation medium [50 cm^3 of a solution of corn steep liquor (20 g) and glucose (10 g) in deionized water (1 dm^{-3}), adjusted to pH 8 with NaOH(aq.)], pre-inoculated for 72 h (23 °C with orbital shaking), and then transferred to 500 cm^3 of fermentation medium (orbital shaking for 24–48 h at 23 °C, until required). The substrate (**2a–f**, 0.1–1 g) was dissolved in DMF or methanol (5 cm^3), added to the culture, and incubated at 23 °C with orbital shaking for 120–150 h. After filtration, the filtrate was continuously extracted with dichloromethane (800 cm^3) for 24 h, the organic phase was dried ($MgSO_4$), the extracts were absorbed onto C60 Sorbsil silica (1 g) by solvent evaporation, and the products were isolated by column chromatography.

‡ *X-Ray crystallographic data.* Data were collected on a Siemens P4 diffractometer (Mo-Kα radiation, λ = 0.71073 Å, ω mode, θ range 2.8–25.0°(2.4–30.0° for **4b**) at 293 K. Data collection and reduction were performed using the program XSCANS.[9] Direct methods solution and refinements (full-matrix least-squares on F^2) were performed using *SHELXTL/PC* Ver. 5.03 γ test.[10] Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre (CCDC). See Information for Authors, Issue No. 1. Any request for this material should quote the full literature citation and the reference number 182/147.

Crystal data for **4a** (tricyclo[3.3.1.1]decane-1,4_{ax}-diol); colourless crystal (0.2×0.3×0.4 mm) from acetone, $C_{10}H_{16}O_2$, M = 168.23, monoclinic, space group Cc, a = 10.1600(10), b = 10.4080(10), c = 8.3820(10) Å, β = 92.070(10)°, V = 885.8(2) Å^3, Z = 4, D_c = 1.261 g cm^{-3}, μ = 0.086 mm^{-1}, absorption corrections (ψ scans) were applied; R_1 = 0.0503, wR_2 = 0.1080 and goodness-of-fit 1.057 for 687 unique observed data and 110 parameters. All non-hydrogen atoms were refined anisotropically, hydroxy H atom positions were calculated on the assumption that hydrogen bonding occurred (O–H bond staggered, and the O–H vector extrapolated to the shortest intermolecular oxygen contact), and all other hydrogen atom positions were calculated and assigned isotropic temperature factors; 1.5 times the attached oxygen U_{eq} for hydroxy hydrogens, and 1.2 times the attached carbon U_{eq} for C–H hydrogens. Residual electron density: +0.22 and −0.20 eÅ^3. For **4b** (4_{ax}-hydroxytricyclo[3.3.1.$1^{3,7}$]decane-1-carboxylic acid); colourless crystal (0.38×0.17×0.2 mm) from acetone, $C_{11}H_{16}O_3$, M = 196.24, monoclinic, space group $P2_1/n$, a = 6.7164(13), b = 11.240(2), c = 13.527(3) Å, β = 102.66(3)°, V = 996.4(3) Å^3, Z = 4, D_c = 1.308 g cm^{-3}, μ = 0.094 mm^{-1}, R_1 = 0.0570, wR_2 = 0.1180 and goodness-of-fit 1.111 for 1241 unique observed data and 135 parameters. All non-hydrogen atoms were refined anisotropically, carboxylate and hydroxy H atoms were refined isotropically, and all other hydrogen atom positions were calculated and assigned isotropic temperature factors; 1.2 times the attached carbon U_{eq}. Residual electron density: +0.22 and −0.23 eÅ^3. For **4h** (4_{ax}-hydroxytricyclo[3.3.1.$1^{3,7}$]decane-1-ylamine hydrochloride); colourless crystal (1.0×0.5×0.5 mm) from acetone, $C_{10}H_{18}OClN$, M = 203.69, orthorhombic, space group $Pna2_1$, a = 14.3910(10), b = 10.2370(10). c = 7.0560(10) Å, V = 1039.5(2) Å^3, Z = 4, D_c = 1.308 g cm^{-3}, μ = 0.33 mm^{-1}; R_1 = 0.0297, wR_2 = 0.0719 and goodness-of-fit 1.026 for 1016 unique observed data and 135 parameters. All non-hydrogen atoms were refined anisotropically, amine and hydroxy H atoms were refined isotropically, and all other hydrogen atom positions were calculated and assigned isotropic temperature factors; 1.2 times the attached carbon U_{eq}. Residual electron density: +0.18 and −0.18 eÅ^3.

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