Model for the functional active site of Baeyer–Villigerases.[1] X-Ray crystal data for (1*S*,2*R*,5*R*,8*S*,1′*R*)-8-*endo*-benzoyloxy-*N*-(1′-phenylethyl)bicyclo[3.3.0]octane-2-*endo*-carboxamide

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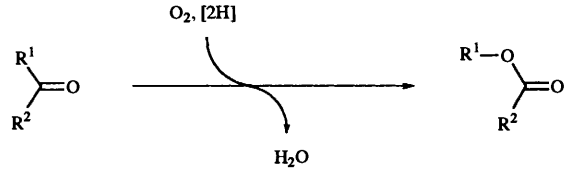
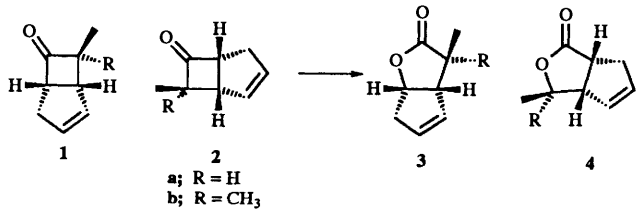
An active-site model for enzyme-catalysed Baeyer–Villiger reactions is proposed and tested by transformation of the tricyclic ketone **6** to the lactone **7** (>98% ee) using purified enzymes from *Acinetobacter* sp. NCIMB 9871 and *Pseudomonas putida* NCIMB 10 007 (MO1). The absolute stereochemistry of the lactone **7** was determined by a single-crystal X-ray diffraction structure determination of the (1*R*′)-α-methylbenzylamide benzoate derivative **11b**. Baeyer–Villiger reactions (and Baeyer–Villigerases) are classified by the stereochemistry of the active site and the hydroxy peroxide intermediates.

Introduction

Enzymes continue to attract attention as enantioselective catalysts for a wide range of reactions.[2] In particular, enzyme-catalysed Baeyer–Villiger reactions have tremendous potential for exploitation in the manufacture of fine chemicals and in organic synthesis. The only reagents consumed in the course of the reaction are dioxygen, a reductant and the ketone substrate which are transformed enantioselectively into the corresponding lactone plus water [eqn. (1)].[3] Until recently enzyme catalysis



was the sole way of achieving enantioselective Baeyer–Villiger oxidation and there has been only a single report of an abiotic equivalent.[4] The stereoselectivity of Baeyer–Villigerases is fascinating. Prochiral ketones are transformed as expected into chiral products; however, enantiomeric ketones are transformed into regioisomeric lactones. A good example is provided by the ring expansion of the enantiomeric bicyclic ketones **1** and **2** to give the regioisomeric lactones **3** and **4** (Scheme 1) mediated by



Scheme 1 *Reagent:* CHMO

† It is conceivable that the rearrangement of the hydroxy peroxide intermediate is initiated by the loss of proton which is shuttled to neutralise the departing flavin alkoxide.

the cyclohexanone mono-oxygenase (CHMO) from *Acinetobacter* sp. NCIMB 9871[5] and a range of other Baeyer–Villigerases.[6–8] A possible explanation for this result is that the enzyme has two catalytic sites, each of which binds a single enantiomer. However, the enzyme binds only one molecule of the prosthetic group flavine-adenine dinucleotide (FAD) and one of 1,4-dihydronicotinamide-adenine dinucleotide phosphate (NADPH). The amino acid sequence[9] indicates only single binding sites for each of the cofactors, and enantiomeric inhibitors cause identical inhibition.[10] Hence, it is highly likely that both enantiomers are processed at the same active site.

The mechanism of enzyme-catalysed Baeyer–Villiger ring expansions is believed to proceed by a similar mechanism to the abiotic reaction, except that a reduced flavin hydroperoxide is utilised instead of a peracid (or other hydroperoxide). Addition of a peroxide to a ketone yields an alkoxide. It is seems inconceivable that a group of this basicity could be generated unstabilised in an enzyme-active site, and since no metal ions are associated with this class of enzyme a proton or another electrophile must be donated to neutralise the charge. This of course could be donated prior to the addition of the peroxide to catalyse the addition, or in synchrony with addition. Throughout the rest of this discussion we will refer to the putative electrophile as a proton for the sake of simplicity. The origin of the proton, or the kinetics of its participation in the addition step, have no effect on the product-determining step, which is the rearrangement of the hydroxy peroxide. However, facile detachment of the proton in the course of the rearrangement dictates that the oxygen atom to which it is bound be placed in an optimum position for the exchange process.†[11] A central dogma of the currently accepted mechanism for the Baeyer–Villiger reaction[12] is that the bond undergoing migration in the hydroxy peroxide intermediate lies antiperiplanar to the oxygen–oxygen bond of the peroxide. Thus, if it is assumed that the three oxygen atoms participating in the rearrangement are fixed in space relative to each other, this is sufficient to define an ensemble capable of undergoing an enantioselective Baeyer–Villiger reaction. This can be readily visualised from the Newman project of the potential intermediates (Fig. 1), which are non-superimposable even if the substituents (R^1, R^2) are identical. This means that a

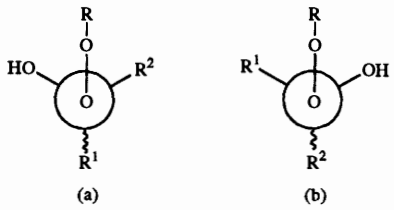


Fig. 1 The migrating bond is shown as a wavy line

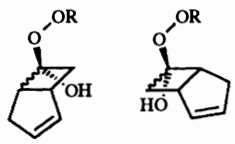


Fig. 2 The migrating bond is shown as a wavy line

Baeyer–Villiger reaction that occurs under stereoelectronic control must always proceed *via* a chiral transition state even with a pro-prochiral ketone (*e.g.*, acetone)!

It is well known that electronically unbiased bicyclo-[3.2.0]hept-2-en-6-ones react with nucleophiles predominantly on the *exo*-face.‡[13] Thus, the geometry of the transition state and the diastereofacial selectivity of nucleophilic attack suggest that hydroxy peroxides derived from the enantiomeric ketones **1a** and **2a** bind at the active site of CHMO such that the cyclobutanone rings are in overlapped positions and the cyclopentane rings lie at 90° to each other (Fig. 2). The three oxygen atoms (peroxide and hydroxy group) plus the migrating bond are in virtually identical positions in both intermediates (*cf.* Fig. 1, rotamer a), except for slight differences in bond lengths. It is a corollary of this mechanism that the enantiomers of the lactones **3** and **4** formed as minor components of the biotransformation result from attack of the ketones **1** and **2** by the hydroperoxide on the more hindered *endo* face.

We sought to demonstrate the fundamentals of this proposal by biotransformation of a composite construct of the structural elements of the ketones **1** and **2**. Conceptually, reduction of the alkene bonds, overlap of the cyclobutanone rings and fusion of the 1,2 C–C bonds gives the tricyclic ketone **6**. The alkene double bonds cannot be accommodated in such a structure because of valency constraints; however, the 2,3-dihydro analogue of the ketones **1a** and **2a** undergoes CHMO-mediated ring expansion with the same enantioselectivity as the unsaturated analogues.[14] The tricyclic ketone **6** has the additional advantage that attack on the *endo*-face should be disfavoured relative to the bicyclic ketones, which according to our model would increase enantioselectivity.

The [2 + 2] cycloaddition of alkyl ketenes to alkenes usually proceeds in rather poor yield. 2-Halogenoketenes give better yields;[15] however, in our case reductive removal of the extraneous halogen in the adduct would have given the 7-spirocyclopropane. Initially, we treated 4-bromobutyryl bromide and cyclopentadiene with triethylamine, but very low to zero yields of the adduct **5a** were obtained. However, poor but reproducible yields (44%) of the known bicyclic ketone **5b** were achieved with 4-chlorobutyryl chloride. As expected the *endo*-ethyl stereoisomer **5b** required for the 5-*exo-trig* free radical cyclisation was formed exclusively. However, it has been reported that this compound is reduced to the ethyl alkene **5c**[16] by tributyltin hydride. Moreover, Dowd has shown in a plethora of closely related systems that many other manifolds are accessible for the radical intermediate.[17] In the event, slow addition of TBTH to the chloroalkene **5b** in refluxing tetrahydrofuran (THF) gave the symmetrical tricyclic ketone **6** (75%). The ^{1}H NMR spectrum of this compound was complex, but the ^{13}C NMR spectrum had only six signals and integration of the signals clearly indicated a symmetrical structure. When the reaction was run in benzene or toluene, the *endo*-ethyl bicyclic ketone **5c** (10–15%) was also isolated. It constituted up to 40% of the product if the tin hydride were added quickly in one portion. Separation of the tricyclic ketone **6** and the ethyl bicyclic ketone **5c** by column chromatography was laborious. However, ozonolysis in methanol, followed by reductive work-up with dimethyl sulfide and Kugelrohr distillation or column chromatography, gave clean tricyclic ketone **6**. The expected keto dialdehyde resulting from cleavage of the alkene **5c** was apparently unstable, as shown by the presence of several slower spots on TLC. No attempt was made to isolate these by-products.

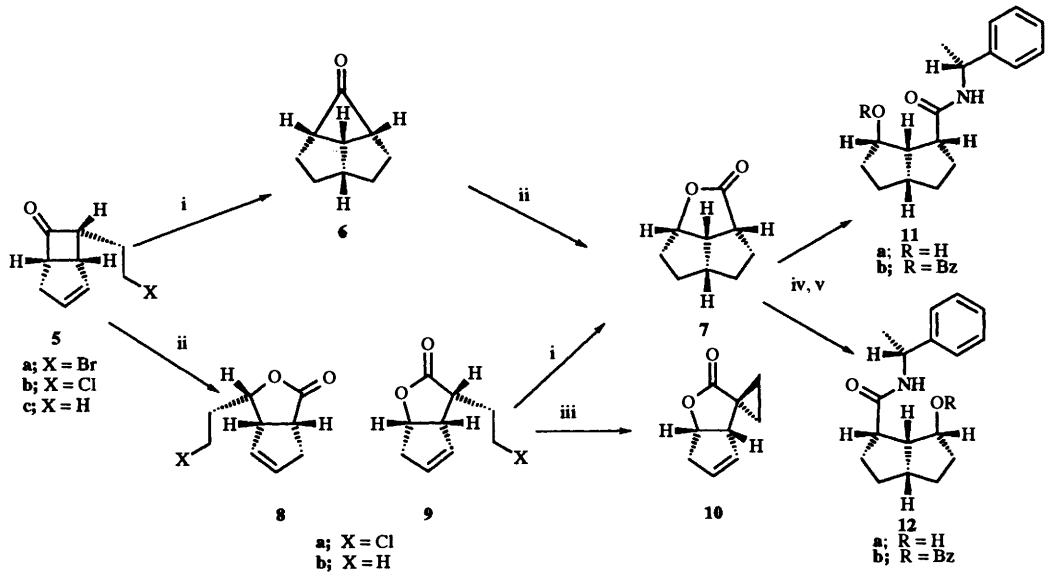
Racemic standards were prepared by conventional methodology. The tricyclic ketone **6** was readily oxidised to the known racemic lactone **7**[18] by hydrogen peroxide–acetic acid. The lactone **7** could also be prepared by inversion of the sequence of steps. Baeyer–Villiger oxidation of the racemic chloroethyl ketone **5b** with hydrogen peroxide–acetic acid gave a mixture (70:30) of the lactones **8a** and **9a**, both of which underwent free-radical cyclisation to the racemic lactone **7**. Apparently this cyclisation is faster than that of the bicyclo[3.2.0]heptanones **5a** and **5b**, because no *endo*-ethyl bicyclic lactones **8b**/**9b** were detected in any of the runs. The structure assignments for the chloroethyl lactones **8a** and **9a** were confirmed by treatment with DBU to give the cyclopropane **10** and untransformed lactone **8a** (Scheme 2).[19]

Biotransformation of the tricyclic ketone **6** with whole organisms broadly parallels that of the bicyclic ketones **1a** and **2a** (Table 1). *Acinetobacter* sp. and *Xanthobacter autotrophicus* gave tricyclic lactone **7** of high enantiomeric purity from the same enantiomeric series (entries 1, 3). These species also give the bicyclic lactones **3a** and **4a** from the same enantiomeric series.

The results obtained with the *Pseudomonas* putida strains are more complicated. The Exeter group has reported that *P. putida* grown on (+)-camphor oxidises the bicyclic ketones **1a** and **2a** to the lactones *ent*-**4a** and *ent*-**3a** respectively.[7] This selectivity was maintained when the purified NADH-dependent mono-oxygenase (MO1, which consists of two isoenzymes) from this species was employed, but the opposite enantioselectivity was achieved with the NADPH-dependent mono-oxygenase (MO2). In contrast, the Kent/Cardiff group found that *P. putida* grown on (+)-camphor gave the same lactones **3a** and **4a** as *Acinetobacter* sp. and the strain grown on (−)-camphor gives the opposite enantiomeric series albeit with only poor to fair enantiomeric excesses.[6] The transformation of the tricyclic ketone **6** by the *P. putida* strain grown on (+)-camphor gave essentially racemic lactone **7** whereas the strain grown on (−)-camphor gave the same enantiomer of the lactone **7** as *Acinetobacter* sp. These results underscore how difficult it is to interpret stereoselectivity, when multiple forms of the same enzyme activity are present. The ring expansion of the ketone **6** with the purified enzymes (entries 2, 6) was essentially 100% enantioselective and the stereoselectivity was congruent with that of ring expansion of the bicyclic ketones **1** and **2**.

At this stage of the work we were not able to assign an absolute configuration to the tricyclic lactone **7** although the two enantiomers were distinguishable by chiral GC. Previous work with *Acinetobacter* sp. NCIMB 9871 mediated Baeyer–Villiger ring expansions of bicyclo[3.2.0]heptan-6-ones suggested that modestly sized 7-*endo*-substituents (*e.g.*, methyl

‡ Bicyclo[3.2.0]heptan-6-one **1a**/**2a** and its 7,7-dimethyl- and 7,7-dichloro- derivatives are all reduced by sodium boranuide in ethanol at room temperature to give an ~80:20 mixture of *endo*:*exo* alcohols.



Scheme 2 *Reagents and conditions:* i, Bu_3SnH, THF, AIBN, reflux; ii, H_2O_2, acetic acid, water or Baeyer–Villigerase; iii, DBU, CH_2Cl_2; iv, BuLi, (*R*)-(+)-α-methylbenzylamine; v, BzCl, py

Table 1 Biotransformation of the tricyclic ketone **6** to the lactone **7**

Entry	Organism	ee (%)[a]
1	*Acinetobacter* sp. NCIMB 9871	87.5[b]
2	*Acinetobacter* sp. NCIMB 9871, purified enzyme	>98
3	*Xanthobacter autotrophicus* DSM 431	87.5
4	*Pseudomonas putida* NCIMB 10007, (+)-camphor[c]	−5.5[d]
5	*P. putida* NCIMB 10007, (−)-camphor[c]	56.6
6	*P. putida* NCIMB 10007, (+)-camphor[c] purified enzyme (MO1), NADH	>−98[d]

[a] Enantiomeric excess of the slowest eluting analyte by GC. [b] 75.4% Conversion, all others 100% conversion. [c] Substrate on which the organism was grown. [d] Enantiomeric excess relative to the other enantiomer.

1b/**2b**) slightly improve stereoselectivity relative to the unsubstituted bicycles (*e.g.*, **1a**/**2a**). If this observation were also true for the 2-chloroethyl bicyclic ketone **5b** then ring expansion to the lactones **8a** and **9a** followed by free radical cyclisation to the lactone **7** would enable the absolute stereochemistry to be provisionally assigned.

Whole-cell biotransformation by *Acinetobacter* sp. NCIMB 9871 of the *endo*-chloroethyl ketone **5b** gave a mixture of the lactones **8a** and **9a** (27:73; 82%, 97% ee respectively). *Xanthobacter* DSM 431 whole-cell conversion of the same ketone **5a** gave a similar mixture of the lactones **8a** and **9a** (27:73), but both were enantiomerically pure (>99% ee). In both cases all the ketone **5a** was consumed and hence some material must have been lost to catabolic processes. The mixture **8a**/**9a** from the *Acinetobacter* sp. NCIMB 9871 whole-cell biotransformation underwent tin hydride-mediated cyclisation to give the same enantiomer of the lactone **7** (92.7% ee) as that produced by the same organism from the tricyclic ketone **6**. The calculated ee of the tricyclic lactone **7** based on the ratio of the chloroethyl lactones **8a** and **9a** and their ee is 92.9% assuming the predominant regioisomers are from opposite enantiomeric series or 48.7% if they are from the same enantiomeric series.

Although this sequence provides good circumstantial evidence for the stereochemistry of the tricyclic lactone **7** it cannot be regarded as definitive. We therefore sought a derivative suitable for circular dichroism (cd) spectroscopy or X-ray crystallographic structure determination, by opening the lactone ring. The methodology was initially developed using racemic lactone **7** and was then applied to material derived from biotransformation with *Acinetobacter* sp. NCIMB 9871.

We were unable to effect hydrolysis of the lactone **7** with aq. lithium hydroxide, even under forcing conditions, and indeed it was resistant to a number of common nucleophiles. This is undoubtedly due to the rigid bicyclic framework, which retards ejection of the incipient alkoxide or alcohol. Full details will be reported elsewhere. Similarly, treatment of racemic tricyclic lactone **7** with (*R*)-α-methylbenzylamine even under reflux at 220 °C failed to open the lactone ring; however, this *was* achieved with the lithium salt of the amine. The reaction was highly capricious; TLC frequently indicated partial or full conversion into a product, but starting material was isolated upon work-up. This presumably indicates that the intermediate tetrahedral aminol is capable of reversion to starting materials. Perseverance and close attention to the work-up conditions gave the desired amides **11a** and **12a** in a 50:50 ratio as an oil. Benzoylation with a large excess of benzoyl chloride (to avoid diastereoisomeric discrimination) gave the diastereoisomeric benzoates **11b** and **12b**. In a similar manner the enantiomerically enriched lactone **7** from the *Acinetobacter* sp. NCIMB 9871 whole-cell biotransformation of the ketone **6** (Table 1, entry 1) was transformed into the hydroxy amide **11a** and the benzoate **11b**. The ^1H NMR spectra of the diastereoisomeric hydroxy amides **11a** and **12a** showed distinct signals for the α-hydroxy and hydroxy protons but the benzylic methyl doublets were virtually superimposed. The benzylic methyl groups of the benzoate diastereoisomers **11b** and **12b** appeared at δ 0.87 and 1.29, respectively, indicating that the methyl group in the material from the *Acinetobacter* sp. NCIMB 9871-derived lactone **7** lies above the plane of the benzoate ring in the deshielding cone. The requisite orientation of the methyl group was demonstrated by molecular modelling.[20] However there are seven bonds which are capable of rotation and three accessible conformation states for the ring system (*exo,exo*; *exo,endo*; *endo,exo* envelopes) each of which can exist in two twist forms. Hence complete exploration of the conformational space could not be guaranteed.

The absolute configuration of the benzoate **11b** was determined to be (1*S*,2*R*,5*R*,8*S*,1′*R*) by single-crystal X-ray diffraction, by reference to the known stereochemistry of the

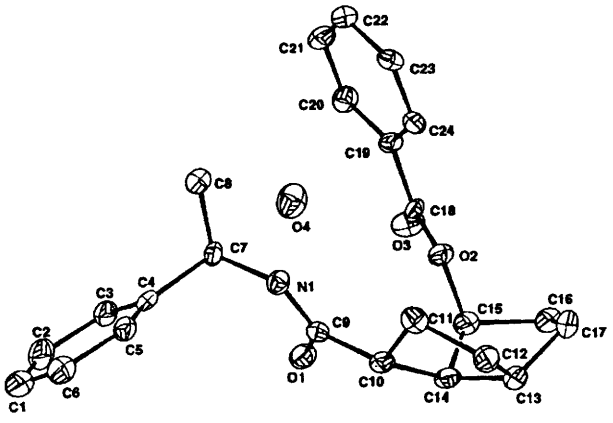
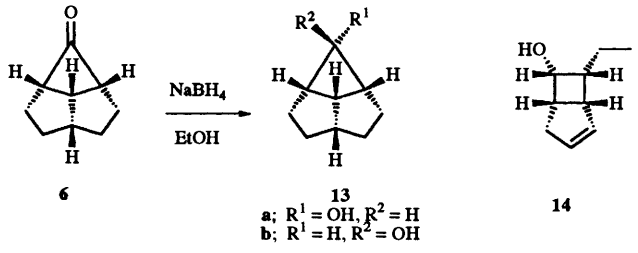


Fig. 3 X-ray crystal structure of (1*S*,2*R*,5*R*,8*S*,1′*R*)-8-*endo*-benzoyloxy-*N*-(1′-phenylethyl)bicyclo[3.3.0]octane-2-*endo*-carboxamide **11b**

(*R*)-α-methylbenzylamine moiety (Fig. 3). The asymmetric unit also contained one molecule of water.

As noted above, bicyclo[3.2.0]hept-2-en-6-ones **1a** and **2a** are reduced by boranuide with a facial selectivity of attack of about 80:20 at room temperature and are typically oxidised by *Acinetobacter* sp. NCIMB 9871 with ees in the range 90–95%. It appeared possible that the facial selectivity of reduction of the tricyclic ketone **6** could be correlated with the enantioselectivity of Baeyer–Villiger oxidation. Reduction of the tricyclic ketone **6** with sodium boranuide in ethanol gave exclusively the *endo*-alcohol **13a** [eqn. (2)]. High-field ^{1}H NMR spectroscopy



gave no evidence for the epimeric *exo*-alcohol **13b** in the crude reaction mixture; however, we would not have been able to detect small amounts (< 3%) by this technique and so we turned to capillary GC-MS which has a wider dynamic range. Full-scan runs (30–600 *m*/*z*) were analysed as single-ion chromatograms. The only additional peak detected in the single-ion chromatograms was for the *endo*-bicyclic alcohol **14** (0.63%). Based on the mass spectrum of this peak and assuming that the ionisation efficiency of the *exo*-tricyclic alcohol **13b** is comparable to that of the *endo*-tricyclic alcohol **13a**. The isomeric ratio should be at least 99.95:0.05. We attempted to prepare the *exo*-alcohol **13b** by Mitsunobo inversion using *p*-nitrobenzoic acid or chloroacetic acid;[21] however, the *endo*-alcohol **13a** was recovered as the sole product.

Several groups have proposed models for the active site of Baeyer–Villigerases. In Furstoss' model the stereoselectivity is determined by the ability of the active site to distinguish groups of differing sizes.[22] Roberts suggested that suppression of bridgehead migration in 7-*exo*-methylbicyclo[3.2.0]heptan-6-ones is due to steric interactions between the peroxide and the 7-*exo*-methyl substituent as a consequence of the antiperiplanar arrangement of the peroxide and the migrating bond.[5]

Finally, Taschner in the proceedings of a conference,[23] has proposed hydroxy peroxide structures virtually identical with ours; however, the corresponding molecular models in this

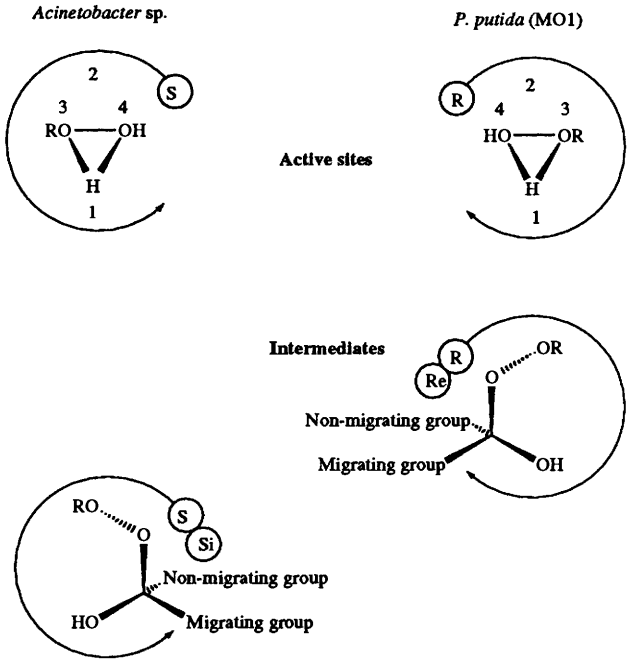


Fig. 4

report have different configurations at the centre to which the flavin peroxide is attached. The results we present here and analysis of prior work indicates that at least for the small and medium ring mono-, bi- and tri-cyclic ketones investigated thus far, the configuration of the hydroxy peroxide intermediate, modulated by the diastereoselectivity of addition of the peroxide, controls the stereoselectivity of the reaction (Fig. 2). There have been several prior attempts to correlate the regioselectivity of abiotic Baeyer–Villiger ring expansions with the facial selectivity of addition, but these could not come to a clear conclusion because the facial selectivity was ambiguous[24] and, moreover, the configuration of the hydroxy peroxide could not be controlled. The tricyclic ketone **6** has a very high if not total selectivity for attack from the *exo*-face and as suggested above the configuration of the hydroxy peroxide of Baeyer–Villigerases is fixed. Hence, ketone **6** can only be transformed by a pure chiral catalyst with less than 100% ee if one of these criteria breaks down. Thus, it can be used as a tool for assessing the configuration of the active site of Baeyer–Villigerases and abiotic asymmetric Baeyer–Villiger reactions.

Using the terms of abiotic asymmetric synthesis, Baeyer–Villigerases act by reagent control rather than by reactant control. It is therefore appropriate to classify enantioselective Baeyer–Villiger reactions according to the arrangement of groups in the catalytic site rather than the intermediates themselves. This can easily be done by application of the Cahn–Ingold–Prelog rules for chiral structures which have no asymmetric centres as shown in Fig. 4. The two oblique links to the hydrogens indicate relative position rather than chemical bonds. Thus, for the *Acinetobacter* sp. site, the hydrogen (or other electrophile) lies above the plane and takes highest priority ⟨1⟩, followed by the phantom position also above the plane ⟨2⟩, then the flavin proximal oxygen ⟨3⟩, followed by the flavin distal oxygen ⟨4⟩ which overall gives an *S*-configuration. The use of a phantom site may appear to be unprecedented at first sight; however, the same analysis would be applied to a hypothetical chiral tetrahedral carbonium ion.

The absolute configuration of the intermediates can be assigned as *R* or *S* by increasing the priority of the migrating

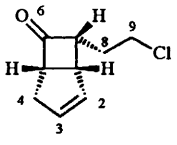
group over that of the non-migrating group. Thus, the *Acinetobacter* sp. intermediate has the *S*-configuration. A better system (which does not require assignment of relative priorities to the migrating and non-migrating groups) is to define the face over which the migrating group moves. The centre at which migration occurs is orientated towards the viewer with the migrating group upper most. The face is defined by the centre from which migration occurs, the two oxygens to which it is attached and the non-migrating group. Using the usual rules for trigonal centres in which the peroxide takes priority over the hydroxy, this defines a *si* migration for the *Acinetobacter* sp. intermediate. This nomenclature is readily applied and will facilitate discussion of both biotic and abiotic Bayer–Villiger reactions.

Experimental

NMR spectra were run on a Bruker WM-360 or AMX-360 spectrometer running DISNMR or UXNMR, respectively, at 360 MHz for protons and 90 MHz for carbon-13 in $CDCl_3$ unless noted otherwise. ^1H NMR coupling constants (*J*/Hz) reported to one decimal place of accuracy were calculated using the computer program Multiplet (release NMRUC46.3 and 50.1) and have a digital resolution of 0.3 Hz unless indicated otherwise. Spin simulations were performed using RACCOON.

GC-MS was run on an HP5890 gas chromatograph with a capillary column, linked to a Trio-1 mass spectrometer running Lab-Base 3.0, with an electron-impact ionisation (EI) source at 70 eV. Other low-resolution EI mass spectra were run on Varian CH-5 or VG-Platform II spectrometers. Ion isotope patterns were confirmed by comparison with those calculated using HiMass. High-resolution, chemical ionisation (CI) and fast-atom bombardment (FAB) mass spectra were run at the SERC mass spectrometry centre at Swansea.

TLC was run on aluminium plates precoated with silica gel (Merck $60F_{254}$) and the spots were visualised using UV and/or dodecaphosphomolybdic acid (3% in ethanol) followed by heating. Optical rotations ($[\alpha]_D/10^{-1}$ deg cm^2 g^{-1}) were measured on an Optical Activity AA-1000 polarimeter at ambient temperature. FT-IR spectra were run on a Perkin-Elmer 1600 spectrometer, and UV spectra on a Perkin-Elmer Lambda 2 spectrophotometer.



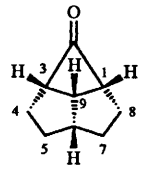
5b

Racemic 7-*endo*-(2′-chloroethyl)bicyclo[3.2.0]hept-2-en-6-one 5b[19]

Triethylamine (50.5 g, 69.6 cm^3, 0.5 mol) as a solution in dichloromethane (150 cm^3) was added to a mixture of cyclopentadiene (131 g, 1.99 mol) and 4-chlorobutyryl chloride (70 g, 0.5 mol) in dichloromethane (250 cm^3) dropwise at 0 °C, with overhead mechanical stirring. The reaction mixture was left overnight; triethylamine hydrochloride salt was filtered off and washed with diethyl ether (200 cm^3). The combined organic phases were washed successively with water (2 × 200 cm^3), saturated aq. sodium hydrogen carbonate (3 × 100 cm^3) and water (2 × 100 cm^3). Drying over magnesium sulfate, evaporation, and purification by column chromatography [elution with light petroleum (bp 40–60 °C) and diethyl ether (5% increment)] gave a yellowish oil (34.2 g, 40%); $\nu_{max}(CHCl_3)$/cm^{-1} 3054, 2959, 2852 and 1769 (str, C=O); δ_H(360 MHz) 5.90 (1 H, dd, *J* 5.7 and 2.6, 2-H), 5.75 (1 H, dq, *J* 5.8 and 2, 3-H), 3.86 (1 H, m), 3.68 (2 H, m) 3.61 (2 H, t, *J* 6.6 9-H_2), 2.65 (1 H, dm, *J* 17.3, fine coupling not resolved, 4-H_{endo}), 2.41 (1 H, ddq, *J* 17.2, 9.2 and 2.0, 4-H_{exo}), 2.05 (1 H, m, 8-H) and 1.85 (1 H, m, 8-H); δ_C(90 MHz) 213.9 (s, C-6); 135.2 (d, C-2); 129.2 (d, C-3); 61.9 (d, C-1); 59.8 (d, C-5); 43.1 (t, C-9); 42.0 (d, C-7); 34.1 (t, C-4) and 28.2 (t, C-8); *m*/*z* 170 (M$^+$, 1.2), 142 (13, M − CO), 93 (33), 91 (47), 80 (22), 79 (100), 78 (30), 77 (66), 66 (67), 65 (76) and 55 (72).

Tricyclo[4.2.1.0^{3,9}]nonan-2-one 6

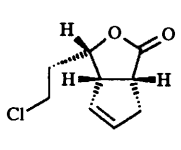
Tributyltin hydride (20.54 g, 0.071 mol) as a solution in THF (20 cm^3) was added dropwise to a refluxing mixture of 7-(2-chloroethyl)bicyclo[3.2.0]hept-2-en-6-one **5b** (12.0 g, 0.071 mol) and 2,2′-azo(2-methylpropionitrile) (AIBN) (0.023 g, 0.5 mol%) in THF (50 cm^3) under argon. The reaction mixture was refluxed overnight. Purification of the crude product by column chromatography (twice), over silica eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, gave the tricyclic ketone as an amber oil (7.21 g, 75%); ν_{max}(neat)/cm^{-1} 2949, 2862 and 1769 (str, C=O), 1470 and 1052; δ_H(360 MHz) 3.45 (2 H, m, 1- and 3-H), 3.16 (1 H, q, *J* 7.6, 9-H) 2.84 (1 H, m, 6-H) and 1.99, 1.82 and 1.53 (8 H, 3 m, 4-, 5-, 7- and 8-H_2); δ_C(90 MHz) 220.8 (s, C-2), 62.95* (d, C-1 and -3), 45.6 (d, C-9), 42.8 (d, C-6), 33.1* (t, C-4 and -8) and 29.2* (t, C-5 and -7) peaks marked with* have double intensity; *m*/*z* 136 (21% Found: M$^+$, 136.088 8. $C_9H_{12}O$ requires M, 136.088 8), 108 (33, M − CO), 80 (100), 79 (57), 67 (73) and 55 (36).



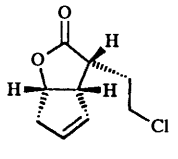
6

Separation of a mixture of tricyclo[4.2.1.0^{3,9}]nonan-2-one 6 and racemic 7-*endo*-ethylbicyclo[3.2.0]hept-2-en-6-one 5c by ozonolysis

The mixture (3 g, 60:40; **6**:**5c** was dissolved in methanol (25 cm^3) and was ozonolysed initially at −78 °C. The reaction mixture was allowed to warm to room temp. and monitored by TLC (benzene eluent; **6** R_f 0.32 and **5c** R_f 0.4). After *ca.* 3 h, the ozone flow was terminated and dimethyl sulfide (15 cm^3, 15.2 g, 25 mmol) added. The mixture was left for 1 h and evaporated to approx. 5 cm^3. The residue was added to water (30 cm^3), extracted with hexane (3 × 10 cm^3), and the extract was dried over sodium sulfate and evaporated to give a clear oil consisting predominantly (by NMR and TLC) of the tricyclic ketone **6**. Filtration column chromatography with light petroleum–diethyl ether (60:40) as eluent gave pure tricyclic ketone **6** (950 mg, 53% recovery). The aqueous phase was further extracted with dichloromethane (3 × 10 cm^3) and the extract was dried over magnesium sulfate. No tricyclic ketone **6** was detected by TLC.



8a



9a

Racemic 4-*endo*-(2-chloroethyl)-3-oxabicyclo[3.3.0]oct-6-en-2-one 8a and racemic 4-*endo*-(2-chloroethyl)-2-oxabicyclo[3.3.0]-oct-6-en-3-one 9a [19]

Hydrogen peroxide (2.5 g, 73.53 mmol, 8.3 cm^3 of a 30% aq. solution) was added to a solution of 7-*endo*-(2-chloroethyl)bicyclo[3.2.0]hept-2-en-6-one **5b** (5 g, 29.4 mmol) in acetic acid–water (78 cm^3; 7:1) at 0 °C. The reaction mixture was left overnight, quenched with water and extracted with dichloromethane. The organic layer was washed with aq. sodium hydrogen carbonate until the pH was neutral to universal indicator paper and the solution tested negative for peroxide (starch–iodide paper). The organic layer was dried over sodium sulfate, filtered, and the solvent was evaporated off under reduced pressure to leave a mixture of two regioisomeric lactones **8a** and **9a** as a mobile oil; ratio 70:30 determined by 360 MHz spectroscopy ^1H NMR ($CDCl_3$); ν_{max}(neat)/cm^{-1} 3062w, 2791, 2928, 2861, 1766str, 1182, 1147, 1116 and 994; *m/z* (CI, NH_3) 206 (32%), 204 (100, M + NH_4^+), 189 (1.5) and 187 [5, M + H Found: 187.052 60. $C_9H_{12}ClO_2$ requires (M + H) 187.052 60]. The mixture was separated by analytical capillary GLC (30 m, DB17) programmed 40 °C(1) X7 to 250 °C (20) and analysed by EI-MS. The retention times were 23.27 and 24.17 min and the ratio of the uncorrected total ion currents 4:96. The higher ion current from the 3-oxa isomer **8a** is due to ease of acylium ion cleavage, loss of carbon monoxide, and allylic cleavage which results in the extremely stable $C_5H_6^+$ ion.

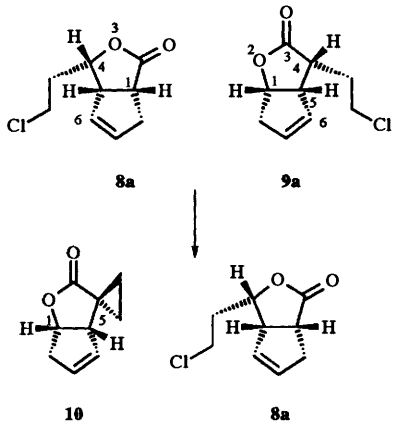
Compound **8a** 24.17 min; *m/z* 160 (2%), 158 (7, M − CO), 123 (3, M − CO − Cl), 91 (8), 79 (10), 77 (12), 67 (21), 66 (100) and 65 (15).

Compound **9a** 23.27 min; *m/z* 170 (3%), 168 (9, M − H_2O), 124 (45), 105 (52), 93 (48), 91 (48), 79 (100), 78 (55), 77 (64), 55 (80) and 39 (67).

The two components were isolated by silica gel column chromatography eluting with light petroleum (bp 40–60 °C) and diethyl ether in 5% increments to give the 3-oxa lactone **8a** (3.780 g, 69%) and the 2-oxa lactone **9a** (1.628 g, 29.5%).

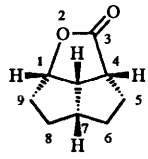
4-*endo*-(2-Chloroethyl)-3-oxabicyclo[3.3.0]oct-6-en-2-one **8a**; δ_H(360 MHz) 5.94 (1 H, m, 6-H), 5.63 (1 H, m, 7-H), 4.84 (1 H, dt, *J* 7 and 6, 4-H) 3.7 (2 H, m, 10-H_2), 3.69 (1 H, m, 1-H), 3.27 (1 H, td, *J* 8.1 and 1.4, 5-H), 2.85 (1 H, ddt, *J* 17.1, 3.8 and 2.1, 8-H_{endo}) 2.7 (1 H, ddq, *J* 17.2, 8.4 and 2.6, 8-H_{exo}) and 2.12 (2 H, m, 9-H_2); δ_C(90 MHz; DEPT) 179.7 (4°, C-2, 134.0 (CH, C-6 126.7 (CH, C-7), 78.5 (CH, C-4, 50.0 (CH, C-1), 43.5 (CH, C-5), 41.4 (CH_2, C-10), 36.4 (CH_2, C-8) and 35.0 (CH_2, C-9).

4-*endo*-(2-Chloroethyl)-2-oxabicyclo[3.3.0]oct-6-en-3-one **9a**; δ_H(360 MHz) 5.88 (1 H, m, 6-H), 5.63 (1 H, m, 7-H), 5.06 (1 H, m, 1-H), 3.87 (1 H, ddd, *J* 11.1, 7.3 and 5.5, 10-H), 3.75 (1 H, ddd, *J* 11.1, 7.2 and 5.4, 10-H), 3.62 (1 H, m, 4-H), 3.05 (1 H, dt, *J* 8.5 and 6.4, 5-H), 2.73 (2 H, m, 8-H_2), 2.32 (1 H, m, 9-H) and 2.01 (1 H, m, 9-H); δ_C(90 MHz) 177.26 (s, C-3), 131.56 (d, C-6), 126.79 (d, C-7), 80.99 (d, C-1), 49.56 (d, C-5), 42.91 (t, C-10), 42.24 (d, C-4), 39.65 (t, C-8) and 29.64 (t, C-9).



Spiro{cyclopropane-1,4'-2'-oxabicyclo[3.3.0]oct-6'-en-3'-one} 10 [19]

A mixture of racemic 4-*endo*-(2-chloroethyl)-3-oxabicyclo[3.3.0]oct-6-en-2-one **8a** and racemic 4-*endo*-(2-chloroethyl)-2-oxabicyclo[3.3.0]oct-6-en-3-one **9a** (0.5 g, 2.69 mmol; ratio 70:30) and 1,8-diazabicyclo[5.4.0]undec-7-ene (0.409 g, 2.69 mmol) in acetonitrile (5 cm^3) was refluxed overnight. The reaction mixture was neutralised with dil. hydrochloric acid, and acetonitrile was evaporated off under reduced pressure. The crude product was then extracted with dichloromethane (2 × 20 cm^3) and washed with water (10 cm^3) to give a mixture of racemic 4-*endo*-(2-chloroethyl)-3-oxabicyclo[3.3.0]oct-6-en-2-one **8a** and (spiro{cyclopropane-1,4'-2'-oxabicyclo[3.3.0]oct-6'-en-3'one} **10** (0.411 g; ratio 71:29) as determined by 360 MHz ^1H NMR spectroscopy.



7

Racemic 2-oxatricyclo[5.2.1.0^{4,10}]decan-3-one 7 [18] **from Baeyer–Villiger oxidation**

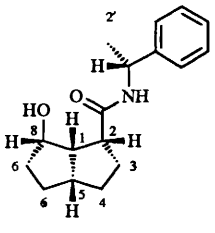
Hydrogen peroxide (0.313 g, 9.2 mmol, 1.04 cm^3 of a 30% aq. solution) was added to tricyclo[4.2.1.0^{3,9}]nonan-2-one **6** (0.5 g, 3.7 mmol) in acetic acid–water (8 cm^3; 7:1) at 0 °C and the mixture was stirred for 4 h (0 °C; room temp.). The reaction mixture was quenched with water and extracted with dichloromethane. The organic layer was washed with sodium hydrogen carbonate until the pH was neutral to universal indicator paper and the solution tested negative for peroxide when using starch–iodide paper. The organic layer was dried over sodium sulfate, filtered, and the solvent evaporated off under reduced pressure to give the racemic tricyclic lactone **7** (0.51 g, 91%) as a mobile oil ν_{max}(neat)/cm^{-1} 2956, 2868, 1763 (str, C=O), 1178 and 1000; δ_H(360 MHz) 4.94 (1 H, ddd, *J* 7.0, 5.2 and 1.7, 1-H), 3.19 (1 H, td, *J* 9.3 and 7.2, 10-H), 3.05 (1 H, ddd, *J* 9.3, 8.4 and 5.1, 4-H), 2.64 (1 H, m, 7-H), 2.14 (3 H, m), 1.87 (3 H, m) and 1.48 (2 H, m); δ_C(90 MHz) 181.0 (s, C-3), 84.6 (d, C-1), 50.6 (d, C-4), 46.0 (d, C-10), 45.2 (d, C-7), 34.4 (t, C-5), 31.7 (overlapping t, C-8 and -9) and 28.8 (t, C-6); ^{13}C–^1H correlation: 84.6–4.94, 50.06–3.19, 46.00–2.64 and 45.2–3.05; *m/z* (EI-MS) 153 (5%, M + H^+), 152 (1. Found: 152.0837. Calc. for $C_9H_{12}O_2$: M, 152.0837), 108 (10), 93 (15), 80 (100), 79 (40) and 67 (82); *m/z* (CI, NH_3) 170 (100%, M + NH_4^+) and 153 (15, M + H^+).

Racemic 2-oxatricyclo[5.2.1.0^{4,10}]decan-3-one 7 [18] ***via* free radical cyclisation**

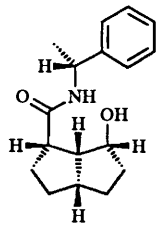
TBTH (1.17 g, 4.03 mmol) as a solution in THF (0.5 cm^3) was added dropwise to a refluxing mixture of racemic 4-*endo*-(2-chloroethyl)-3-oxabicyclo[3.3.0]oct-6-en-2-one **8a** and racemic 4-*endo*-(2-chloroethyl)-2-oxabicyclo[3.3.0]oct-6-en-3-one **9a** (0.5 g, 2.69 mmol; ratio 70:30) and AIBN (28 mg, 0.17 mmol) in THF (4 cm^3) under argon. The reaction mixture was refluxed overnight. Purification of the crude product by column chromatography (twice) over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, gave the racemic tricyclic lactone **7** as an amber oil (0.319 g, 78%).

(1*S*,4*R*,7*R*,10*S*)-2-Oxatricyclo[5.2.1.0^{4,10}]decan-3-one 7 *via* free radical cyclisation

The cyclisation described above was repeated using a mixture of 4-*endo*-(2-chloroethyl)-3-oxabicyclo[3.3.0]oct-6-en-2-one **8a** and 4-*endo*-(2-chloroethyl)-2-oxabicyclo[3.3.0]oct-6-en-3-one **9a** (0.2 g, 1.08 mmol; ratio 27:73; 82 and 97% ee from an *Acinetobacter* sp. NCIMB 9871 biotransformation), and AIBN (11 mg, 0.067 mmol) in THF (1.2 cm^3) with TBTH (0.375 g, 1.29 mmol) in THF (0.1 cm^3). Purification of the crude product by column chromatography (twice) over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, gave the tricyclic lactone **7** as an amber oil (0.135 g, 81%; 92.7% ee).



11a

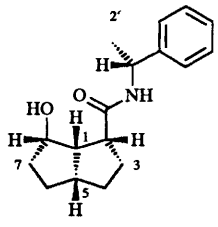


12a

(1*RS*,2*SR*,5*SR*,8*RS*,1′*R*)-8-Hydroxy-*N*-(1′-phenylethyl)-bicyclo[3.3.0]octane-2-carboxamide 11a/12a. Attempted preparation using (*R*)-(+)-α-methylbenzylamine

The optical rotation of (*R*)-(+)-α-methylbenzylamine used in this experiment and all subsequent experiments was $[\alpha]_D^{21}$ = +35.6 (neat) {lit.[25] $[\alpha]_D^{25}$ +39.2–39.7 (neat)}. The racemic lactone **7** (0.6 g, 4 mmol) and (*R*)-(+)-α-methylbenzylamine (0.72 g, 6 mmol) were dissolved in THF (2 cm^3) and the solution was stirred under argon at room temp. for 2 days and at 70 °C for 1 day. Further (*R*)-(+)-α-methylbenzylamine (0.48 g, 4 mmol) was added. The THF was evaporated off and the reaction mixture was heated at 160 °C overnight. Further (*R*)-(+)-α-methylbenzylamine (0.24 g, 2 mmol) was added and the reaction mixture was refluxed at 220 °C overnight. Hexamethylphosphoric triamide (HMPA) (3 cm^3) was added and the reaction was left to proceed for a further night at room temp. The mixture was dissolved in dichloromethane (20 cm^3), which was extracted successively with dil. hydrochloric acid (3 × 5 cm^3) and water (3 cm^3), dried over sodium sulfate, and evaporated to give the lactone **6** (0.532 g, 89%).

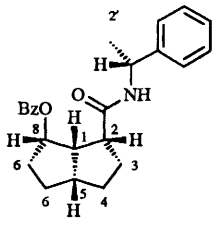
Using lithium (*R*)-(+)-α-methylbenzylamide. Butyllithium (0.58 g, 9.02 mmol) as a solution in hexanes (5.7 cm^3; 1.6 mol dm^{-3}) was added slowly to a solution of (*R*)-(+)-α-methylbenzylamine (1.04 g, 8.6 mmol) in THF (5 cm^3) at −78 °C. The reaction mixture was warmed slowly until a red-brown colour appeared. A solution of tricyclic lactone **7** (0.6 g, 3.95 mmol) in THF (1 cm^3) was added dropwise to the (*R*)-(+)-α-methylbenzylamine anion at 0 °C. After *ca.* 2 h, the reaction mixture changed from red-brown to yellowish in colour. The reaction mixture was left for a further hour and was then quenched with aq. ammonium chloride (10%; 30 cm^3). The THF was removed under reduced pressure and the crude product was extracted with dichloromethane (3 × 10 cm^3), and the extract was washed successively with dil. hydrochloric acid (10 cm^3), aq. sodium hydrogen carbonate (10 cm^3) and water (5 cm^3). The solvent was removed and the crude product was purified by column chromatography over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, to give the hydroxy amides **11a** and **12a** (0.62 g, 58%) ν_{max}(neat)/cm^{-1} 3295br (OH, NH), 2956, 2868, 1760, 1643, 1548 and 1451; δ_H(360 MHz) 7.31 (5 H, m, ArH), 6.08 (1 H, br s, exchangeable with D_2O > 15 min., NH), 5.16 (1 H, q, *J* 6.5, 1′-H), 4.94* and 4.82* (1 H, 2 br s, exchangeable with D_2O < 15 min., OH), 4.32* and 4.25* (1 H, 2 br s, 8-H), 2.50 (3 H, m), 2.20 (1 H, m), 2.04 (1 H, m), 1.88–1.52 (6 H, m) and 1.53 (3 H, 2 overlapped d*, *J* 6.5, 2′-H$_3$). Peaks marked with an asterisk are assigned to individual diastereoisomers; δ_C(90 MHz) 175.7 (s, C=O), 143.1* and 143.0* (2 s, Ar-C), 128.5, 127.1 and 125.9** (3, Ar CH), 74.6* and 74.5* (2, C-8), 53.21 (d), 49.5 (d), 48.9 (d), 48.8 (d), 42.5 (d) 36.1, 32.1 and 31.0 (4 t, CH$_2$) and 21.8* and 21.6* (2 q, C-2′). Adjacent peaks marked with an asterisk are due to similar carbons from different diastereoisomers. Peaks marked with a double asterisk integrate for two carbons, indicating the overlap of signals from the two diastereoisomers; *m/z* 273 (1%. Found: M$^+$, 273.1729. $C_{17}H_{23}NO_2$ requires *M*, 273.1729), 223 (3), 182 (3), 156 (14), 155 (10), 154 (16), 153 (10), 152 (18), 142 (12), 141 (15), 108 (17), 106 (59), 105 (19), 86 (42), 84 (68) and 80 (100).



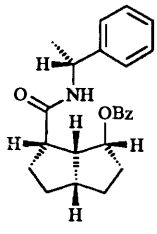
11a

(1*S*,2*R*,5*R*,8*S*,1′*R*)-8-Hydroxy *N*-(1′-phenylethyl)bicyclo-[3.3.0]octane-2-carboxamide 11a

The procedure used was similar to that described above for racemic lactone **7**. Enantiomerically enriched tricyclic lactone **7** (87.5% ee) was obtained from an *Acinetobacter* sp. NCIMB 9871 whole-cell biotransformation of the ketone **6**. (*R*)-(+)-α-methylbenzylamine anion was generated from butyllithium (0.068 g, 1.09 mmol; 0.7 cm^3; 1.6 mol dm^{-3} in hexanes) and (*R*)-(+)-α-methylbenzylamine (0.116 g, 0.96 mmol) in THF (3 cm^3) to which was added a solution of the lactone **7** (0.073 g, 0.48 mmol) in THF (3 cm^3). Purification of the crude product by column chromatography over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, gave the hydroxy amide **11a** (0.118 g, 90%), δ_H(360 MHz) 7.31 (5 H, m, ArH), 6.05 (1 H, br s, NH), 5.16 (1 H, quin., *J* 6.5, 1′-H), 4.25 (1 H, br s, 8-H), 2.50–1.2 (11 H, m) and 1.50 (3 H, d, *J* 6.5, 2′-H$_3$).



11b

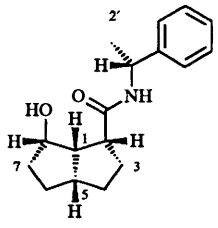


12b

(1*RS*,2*SR*,5*SR*,8*RS*,1′*R*)-8-*endo*-Benzoyloxy-*N*-(1′-phenylethyl)-bicyclo[3.3.0]octan-2-*endo*-carboxamide 11b/12b

Benzoyl chloride (0.7 cm^3, 0.85 g, 6 mmol) as a solution in dichloromethane (0.5 cm^3) was added dropwise during 1 h to a stirred mixture of the hydroxy amide **11a/12a** (0.16 g, 0.586 mmol) and pyridine (1 cm^3, 0.98 g, 12.3 mmol) in dichloromethane (2 cm^3). The reaction mixture was left overnight and the crude product was then extracted with dichloromethane (10 cm^3), and the extract was washed successively with dil. hydrochloric acid (2 × 5 cm^3), aq. sodium hydrogen carbonate (2 × 5 cm^3) and water (5 cm^3). The solvent was removed and the crude product was purified by column chromatography over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, to give the benzoates **11b** and **12b** (0.152g, 68%), ν_{max}(neat)/cm^{-1} 2963, 2869, 1720 and

1645; δ_H(300 MHz) 8.02–7.08 (10 H, m, ArH), 5.69 (1 H, apparent br q, *J* 4.6, 8-H), 5.54 (1 H, br d, *J* 6.8, NH), 4.75 (1 H, m, 1'-H), 3.00 (1 H, m), 2.80 (1 H, m), 2.68 (1 H, m), 2.22–1.90 (4 H, m), 1.80–1.55 (3 H, m), 1.30–1.5 (1 H, m), 1.29* (3 H/2, d, *J* 6.8, 2'-H_3) and 0.87* (3 H/2, d, *J* 6.9, 2'-H_3). Peaks marked with an asterisk are from single diastereoisomers.



11b

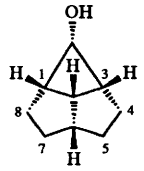
(1*S*,2*R*,5*R*,8*S*,1'*R*)-2-*endo*-Benzoyloxy-*N*-(1'-phenylethyl)-bicyclo[3.3.0]octane-2-*endo*-carboxamide 11b

Benzoyl chloride (0.602 g, 0.5 cm^3 4.28 mmol as a solution in dichloromethane (0.7 cm^3) was added dropwise (30 min) to a stirred mixture of the hydroxy bicycle **11a** (0.118 g, 0.43 mmol), 4-(dimethylamino)pyridine (0.53 g, 4.34 mmol) and triethylamine (0.1 cm^3) in dichloromethane (5 cm^3). The reaction mixture was left overnight, extracted with dichloromethane (10 cm^3), and washed successively with dil. hydrochloric acid (2 × 5 cm^3), aq. sodium hydrogen carbonate (2 × 5 cm^3) and water (5 cm^3). The solvent was removed and the crude product was purified by column chromatography over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, to give the bicyclic benzoate **11b** (0.052 g, 33%). Further column chromatography (3 times) and slow evaporation of the column fractions gave crystals suitable for X-ray crystallography, ν_{max}(neat)/cm^{-1} 2962, 2862, 1717 and 1645; δ_H(360 MHz) 8.02–7.08 (10 H, m, ArH), 5.64 (1 H, m, 8-H), 5.50 (1 H, br d, *J* 6.8, NH), 4.75 (1 H, m, 1'-H), 3.00 (1 H, m, 2.80 (1 H, m), 2.69 (1 H, m), 2.20–2.00 (2 H, m), 1.94 (2 H, m), 1.88–1.60 (3 H, m), 1.45 (1 H, m) and 0.88 (3 H, d, *J* 6.9, 2'-H_3); δ_C(90 MHz; DEPT) 172.3 (C, C=O amide), 165.6 (C, C=O benzoate), 143.4 (C, Ar amide), 132.8 (CH, Ar), 131.1 (C, Ar benzoate), 129.7, 128.6, 128.4, 127.2 and 126.18 (5 CH, ArCH), 78.8 (CH, C-1) 48.8, 48.7, 48.6 and 42.5 (4 CH, C-2, -5, -8, -1'), 35.1, 32.1, 31.4 and 29.8 (4 CH_2) and 28.4 (CH_3, C-2'); *m/z* 378 (0.8%, M + H^+), 377 (3, M^+. Found: M^+; 377.1991. $C_{24}H_{27}NO_3$ requires M, 377.1991), 349 (2.5), 255 (3), 212 (3.4), 120 (100), 105 (53), 77 (18) and 58 (11).

X-Ray structure determination

Cell dimensions and intensity data were obtained using a FAST TV area detector diffractometer with graphite monochromated Mo-Kα radiation (λ = 0.710 69 Å), following previously described procedures.[26] The structure was solved by direct methods[27] and refined (on F^2) by full-matrix least-squares.[28]

Crystal data. $C_{24}H_{29}NO_4$, M_r = 395.48, monoclinic, space group $P2_1$, a = 12.172(5), b = 6.815(3), c = 13.361(4) Å and β = 107.33(2)°, V = 1058.0(5) $Å^3$, Z = 2, D_x = 1.241 g cm^{-3}, T = 120 K, crystal size 0.12 × 0.10 × 0.10 mm^3. Data were recorded within $1.75 \leqslant \theta \leqslant 24.99°$, $-2 \leqslant h \leqslant 13$, $-5 \leqslant k \leqslant 5$, $-14 \leqslant l \leqslant 14$, giving 2851 measurements and 2651 unique (R_{int} = 0.0435). Refinement with 263 parameters gave R_1 = 0.0442, wR_2 = 0.0932 for 1507 data with $I > 2\sigma(I)$ (0.0745, 0.1008 respectively for all data) GOF on F^2 = 0.726. Non-hydrogen atoms were refined anisotropically. Hydrogens were inserted in idealised positions with U_{iso}-values set at 1.5 × U_{eq} for the parent atoms, except for the water hydrogens, which were freely refined. The atomic coordinates, anisotropic displacement parameters of the non-hydrogen atoms, and tables of bond lengths and angles have been deposited as supplementary material with the Cambridge Crystallographic Data Centre.§



13a

Tricyclo[4.2.1.0^{3,9}]nonan-2-*endo*-ol 13a

Tricyclo[4.2.1.0^{3,9}]nonan-9-one **6** (0.356 g, 2.62 mmol) as a solution in ethanol (5 cm^3) was added slowly to a solution of sodium boranuide (0.2 g, 5.41 mmol) in ethanol (10 cm^3) at 0 °C. The reaction mixture was quenched with ice after 2 h. Ethanol was removed under reduced pressure and the crude product was extracted with dichloromethane (20 cm^3), and the extract was washed successively with dil. hydrochloric acid (4 cm^3), aq. sodium hydrogen carbonate (2 × 2 cm^3) and then with water (2 × 2 cm^3). Purification of the crude product by column chromatography over silica, eluted with light petroleum (bp 40–60 °C)–diethyl ether in 5% increments, gave the tricyclic alcohol **13a** as an oil (0.246 g, 68%), ν_{max}(neat)/cm^{-1} 3365br (OH), 2950, 1469 and 1338; δ_H(360 MHz) 4.41 (1 H, t, *J* 7.0, 2-H); 2.85 (2 H, m, 1-H and 3-H), 2.62 (2 H, m, 6- and 9-H) and 1.85, 1.72, 1.70 and 1.53 (9 H, m, 4-, 5-, 7- and 8-H, OH); δ_C(90 MHz) 64.32 (d, C-2), 45.78 (d, C-6 or C-9), 45.53 (d, C-6 or C-9), 43.63* (d, C-1 and -3) and 33.83* and 24.84* (t, C-4, -5, -7 and -8). Peaks marked with an asterisk have double intensity; *m/z* 138 (1%, M^+. Found: M^+, 138.1044. $C_9H_{14}O$ requires M, 138.1044), 120 (35, M − H_2O), 110 (12), 105 (28), 94 (39), 93 (38), 92 (48), 91 (40), 80 (49), 79 (70) and 67 (100). Purity and isomeric ratio were determined by capillary GC-MS [30M DB5 capillary column, temperature programme 30 °C(10), X4 °C, 200 °C(10)]. Several full-scan runs were analysed as single-ion chromatograms (SIC) at *m/z* 138 (M^+), 120 (M^+ − H_2O) and 67 (base peak of the *endo*-alcohol **13**). The traces were truncated on either side of the peak for the *endo*-alcohol **13** (t_R 27.83 min) to maximise the displayed peak heights. No peaks whatsoever were found in the *m/z* 120, 137 and 138 chromatograms. A low-level background continuum was found for *m/z* 67. The total-ion chromatogram and an SIC of *m/z* 66 showed a single peak and a good spectrum for the *endo*-bicyclic alcohol **14** (0.63%, t_R 23.30 min). We could reasonably expect to find a peak with 10% of the ion current of this peak and hence this indicates that the isomeric purity of the *endo*-alcohol **13** was at least 99.95%. No corrections were made for ionisation efficiency.

Biotransformations

The following methods were used for all the biotransformations except for *Pseudomonas putida* NCIMB 10 007 purified enzyme. The progress of the reactions was monitored by packed column GC (Porapak PS, mesh 80–100; 2 mm × 6 ft), isothermal at 225 °C, with the injector and field ionisation decorption (FID) detector at 250 °C and helium carrier gas at a flow rate of 50 cm^3 min^{-1}. A 100 mm^3 aliquot of the reaction mixture was centrifuged at 13 000 g for 2 min and 5 mm^3 of this was injected into the GC. 3-Cyanopyridine was used as an internal standard. When the substrate was no longer detectable the cells were removed by centrifugation and the supernatant was extracted with ethyl acetate (3 × 25 cm^3, analytical grade), and the extract was dried over sodium sulfate, filtered, and

§ For details of the system, see Instructions for Authors in the January Issue.

evaporated to constant volume on a rotary evaporator. GC yields were estimated by comparison of the area of the lactone peak(s) against that of the ketone peak at time zero with reference to the internal standard. With one exception the yields were quantitative. The *Acinetobacter* sp. NCIMB 9871 whole-cell biotransformation gave a 75% conversion into lactone plus a trace of residual ketone.

The crude product was analysed by chiral GC on Lipodex D (0.25 mm × 50 m, Macherey-Nagel, Duren, Germany). The injector and FID detector temperatures were set at 250 °C and helium was used as carrier gas at 1.4 bar‖ with a split ratio of 1:50. The isothermal temperature programmes and retention times (min) were 170 °C **7** 18.1, 18.9; 160 °C **9a** 54.3, 56.4; **8a** 67.1, 69.1. The crude product was purified by flash column chromatography over silica gel and elution with light petroleum (bp 40–60 °C) containing increasing increments of diethyl ether. The purified products gave satisfactory ^{1}H NMR spectra. Isolated yields were typically 40–60%.

Acinetobacter sp. NCIMB 9871,[29] *Xanthobacter autotrophicus* DSM 431 and *Pseudomonas putida* NCIMB 10 007[6] were grown and maintained as described previously.

***Acinetobacter* sp. NCIMB 9871.** (*i*) *Whole-cell biotransformations.*—The medium (10 dm^3) was inoculated (2.5–5% v/v) with a late-exponential-phase culture. Cells were harvested in the mid-to-late log phase of growth by centrifugation at 4 °C and 10 000 g for 15 min. The cells were washed and resuspended in phosphate buffer (25 cm^3; 50 m mol dm^{-3}, pH 7.1). The substrate (25 mg) was added to an Erlenmeyer flask (250 cm^3) containing the cell suspension, sealed with a serum cap and incubated at 30 °C on a gyratory stirrer at 200 rpm.

(*ii*) *Isolated enzyme biotransformations.*—(1*S*,4*R*,7*R*,10*S*)-2-*Oxatricyclo*[5.2.1.0^{4,10}]*decan*-3-*one* **7**. The tricyclic ketone **6** (25 mg, 0.18 mmol) was added to an Erlenmeyer flask containing glycine–NaOH (25 mmol, pH 8), NADP (0.1 mmol), glucose 6-phosphate (20 mmol), purified cyclohexanone monooxygenase (20 U; 0.6 mg of protein) and glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (10 U) in a total volume of 25 cm^3 with water. The product was analysed and purified as described above.

Purification of cyclohexanone monooxygenase from *Acinetobacter* sp. NCIMB 9871. Cells were harvested in the mid-to-late log phase (40 dm^3 culture volume) by centrifugation at 4 °C and 10 000 g for 15 min. The cells were washed, resuspended in KH_2PO_4–Na_2HPO_4 buffer (25 cm^3, 50 mmol dm^{-3}; pH 7.1) and disrupted by sonication at 200 W, 0.25 s^{-1} cycles for 20 min in an ice-bath. The cell debris was removed by sonication (40 000 g for 20 min). The soluble fraction was obtained by ultracentrifugation at 200 000 *g* for 180 min. The supernatant was fractionated using a 50–80% aq. ammonium sulfate cut. The precipitate was dialysed and aliquots (500 mg) of protein were loaded onto a HiLoad Q-Sepharose HP (Pharmacia Ltd) anion-exchange column (HR 16/10; 22 cm^3 bed volume) and eluted at 2.5 cm^3 min^{-1} with a linear gradient of potassium chloride (0–500 mmol dm^{-3}) in phosphate buffer (25 mmol dm^{-3}; pH 7.1). Fractions containing cyclohexanone monooxygenase activity but not lactonase or cyclohexanol dehydrogenase activity were pooled, and stored precipitated at −20 °C. The dialysed Q-Sepharose fraction was purified to homogeneity on a Reactive red 120 column (Sigma Chemicals Ltd, 10 cm^3 bed volume). The extract (maximum 25 mg of protein) was applied at 4 °C at a flow rate of 0.5 cm^3 min^{-1}. The column was washed with phosphate buffer (40 cm^3; 25 mmol dm^{-3}; pH 7.1) and the cyclohexanone mono-oxygenase fraction was eluted with phosphate buffer (25 mmol dm^{-3}; pH 7.1) containing nicotinamide adenine dinucleotide phosphate (NADP) (2 mmol dm^{-3}). These fractions were pooled, precipitated using aq. ammonium sulfate, and stored at −20 °C. A single band was present on sodium dodecyl sulfate/polyacydamide gel electrophoreris (SDS/PAGE).

***Xanthobacter autotrophicus* DSM 431.** These whole-cell biotransformations were run in a manner identical with those of *Acinetobacter* sp. NCIMB 9871 except that a smaller volume of medium (1 dm^3) was inoculated more heavily (5–10% v/v).

***Pseudomonas putida* NCIMB 10 007.** (*i*) *Whole-cell biotransformations at Kent.*—These cells were cultivated using techniques described previously[6] using either (+)-camphor or (−)-camphor as sole carbon source. Cells were harvested in the mid-to-log phase of growth by centrifugation at 4 °C and 20 000 g for 10 min. The cells were washed and resuspended in phosphate buffer (25 cm^3; 50 mmol dm^{-3}; pH 7.1). The substrate (25 mg) was added to an Erlenmeyer flask (250 cm^3) containing the cell suspension, sealed with a serum cap, and incubated at 30 °C on a gyratory stirrer at 200 rpm.

(*ii*) *Isolated enzyme biotransformation at Exeter.* (1R,4S,7R,10R)-2-*Oxatricyclo*[5.2.1.0^{4,10}]*decan*-3-*one ent*-**7**.—The tricyclic ketone **6** (7.5 mg, 0.055 mmol), the partially purified monooxygenase (MO1) from *Pseudomonas putida* NCIMB 10 007 grown on (+)-camphor[7] (38.5 mg), and NADH (55 mg, 0.070 mmol) were added to TRISMA buffer (50 mmol dm^{-3}; pH 7.1; 5.5 cm^3). The mixture was stirred at 30 °C and monitored by GC, using a BP1 column (injector 280 °C, FID detector 280 °C), isothermal 150 °C. The ketone **6** eluted at 2.73 min and the lactone **7** after 4.98 min. At completion, the mixture was extracted with ethyl acetate (3 × 5 cm^3), dried over magnesium sulfate, filtered, and evaporated to give the tricyclic lactone *ent*-**7** as an oil (6 mg, 72%); $[\alpha]_D^{25} = +62$ ($c = 1$, $CHCl_3$). The product was shown to be 100% pure, > 98% ee by GC, over Lipodex D, injector 220 °C, FID detector 260 °C, with the temperature program 120 °C (15 min) X 35 °C min^{-1}, 155 °C (10 min). A racemic sample showed two peaks, at t_R 18.82 and 19.58 min; the area of the peak eluting at 18.82 min was increased when the racemic sample was spiked with the biotransformation product.

‖ 1 bar = 10^5 Pa.

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