Biological Baeyer–Villiger Oxidation of Some Monocyclic and Bicyclic Ketones using Monooxygenases from *Acinetobacter calcoaceticus* NCIMB 9871 and *Pseudomonas putida* NCIMB 10007

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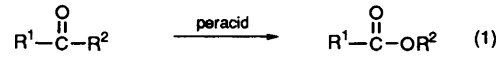
A. calcoaceticus NCIMB 9871 and *Ps. putida* NCIMB 10007 [grown on (+)-camphor] have been utilized as biocatalysts in Baeyer–Villiger oxidations. The former microorganism oxidized the racemic ketone **6** non-selectively but transformed the dihalogeno ketone (±)-**8** into optically active lactone **10** and recovered ketone. *Ps. putida* NCIMB 10007 oxidized the two enantiomers of the ketone **6** at different rates while both enantiomers of ketone (±)-**1** were converted into lactones, one enantiomer giving 3-oxabicyclooctenone preferentially, while the other enantiomer gave 2-oxabicyclooctenone. *Ps. putida* NCIMB 10007 contains two quite different types of monooxygenase enzyme, one using NADH as cofactor (labelled MO1) the other employing NADPH as cofactor (labelled MO2). Monooxygenase MO1 proved to be a selective efficient biocatalyst for the oxidation of bicyclic ketones such as **1** and **6** while monooxygenase MO2 is a useful catalyst for the oxidation of cyclopentanones **15–17**. Cofactor recycling was effected using dehydrogenase enzymes in preparative-scale experiments.

Baeyer–Villiger oxidation, an important transformation that is often used in synthetic organic chemistry,[1] can be accomplished using a peracid such as peracetic acid or *meta*-chloroperbenzoic acid [eqn. (1)]. In general, when different substituents



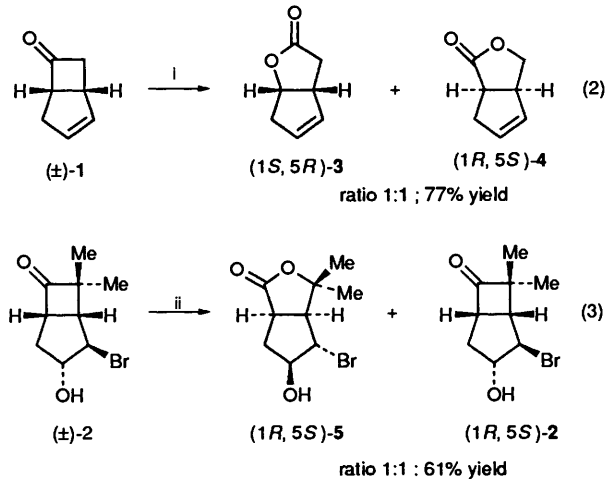
(1)

are attached to the carbonyl carbon atom, the one that can better accommodate a (partial) positive charge is the one that migrates to the incoming oxygen atom [R^2 in eqn. (1)].

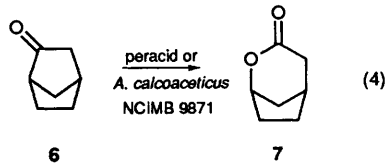
meta-Chloroperbenzoic acid is often the oxidant of choice for performing a Baeyer–Villiger oxidation on a small scale; however, caution must be exercised on operating such an oxidation on a multi-kilo scale owing to the intrinsic instability of the peracid. Other chemical oxidants have been explored as alternatives[2] but an equivalent oxidative bioconversion utilising dioxygen is an attractive proposition.

Enzyme-catalysed Baeyer–Villiger oxidations have been known for some time.[3] The bio-oxidation can be conducted using whole-cell systems or isolated partially purified protein (with the appropriate co-factor). The microorganism *Acinetobacter calcoaceticus* NCIMB 9871 and related bacterial species have been popular biocatalysts.[4] For example, workers in Exeter/Kent[5] and Marseille[6] have shown that bicyclo-[3.2.0]heptanones **1** and **2** are oxidized to the corresponding lactones **3–5** [Scheme 1, eqns. (2) and (3)]. These lactones are obtained in optically active, essentially optically pure, form through the operation of enantiodivergent or enantioselective pathways. The isolation of optically active products distinguishes the biological method from the alternative chemical strategies (*vide supra*). For comparison the ketone **1** is oxidized by *meta*-chloroperbenzoic acid or peracetic acid[7] to give racemic lactones **3** and **4** in the ratio > 10:1.

Oxidation of norbornanone **6** with *A. calcoaceticus* NCIMB 9871 (or peracid) gave the lactone **7** in racemic form [eqn. (4)], but the related dihalogeno ketone **8** better illustrates the significant differences that can occur between chemical and biological oxidants. *meta*-Chloroperbenzoic acid transforms the ketone **8** into the racemic lactones **9** and **10** in the ratio 3.5:1

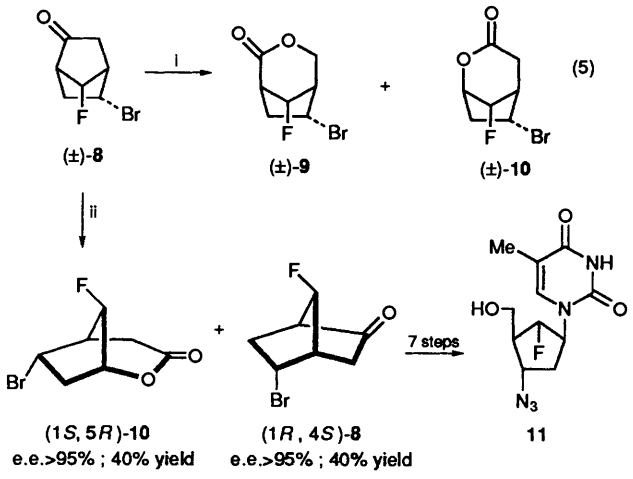


Scheme 1 *Conditions:* i, *Acinetobacter* TD63; ii, *A. calcoaceticus* NCIMB 9871, H_2O buffer, 30 °C



[Scheme 2, eqn. (5)]. The presence of the electron-withdrawing fluorine atom at C-7 dictates the preferential migration of the methylene group to the incoming oxygen atom. In contrast, *A. calcoaceticus* NCIMB 9871 produces the 1*S*,5*R*-lactone **10** and recovered 1*R*,4*S*-ketone **8** in an enantioselective reaction.[8] The latter compound was converted, in seven steps, into the AZT-analogue **11** which possessed significant anti-HIV activity.[9]

The problems with performing biological Baeyer–Villiger oxidations using whole-cell systems such as *A. calcoaceticus* NCIMB 9871 are the usual ones,[10] namely that yields of product(s) can be far from quantitative due to a variety of reasons including over-metabolism, the participation of side



Scheme 2 *Reagents and conditions:* i, $ClC_6H_4CO_3H$, CH_2Cl_2, $NaHCO_3$; ii, *A. calcoaceticus* NCIMB 9871, H_2O buffer, 30 °C

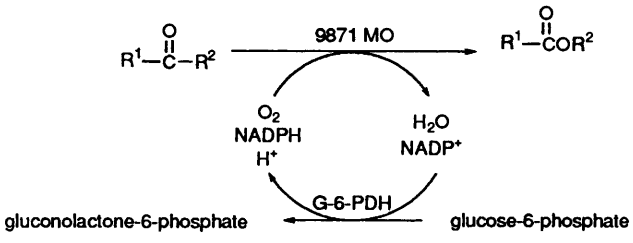
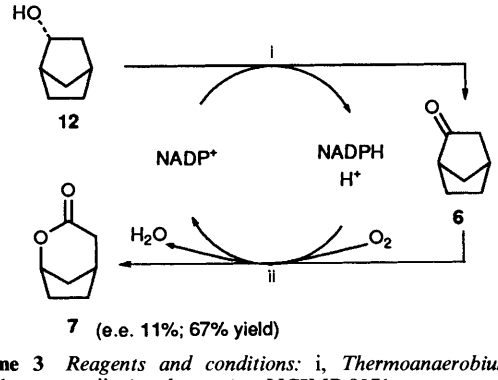


Fig. 1 9871 MO is the partially purified monooxygenase from *A. calcoaceticus* NCIMB 9871

reactions and entrainment of material in the cell debris. Most of these losses can be circumvented by isolating and using the partially purified enzyme and indeed the procedure for obtaining the monooxygenase from *A. calcoaceticus* NCIMB 9871 has been described.[11] Unfortunately, the enzyme utilizes NADPH as the co-factor; NADPH is expensive and co-factor recycling is difficult (Fig. 1).[12]

To some extent the problem can be overcome by employing another coupled enzyme system, *i.e.* an NADPH-dependent dehydrogenase and the monooxygenase working in tandem (Scheme 3). Thus, (±)-norbornenol **12** was oxidized with the

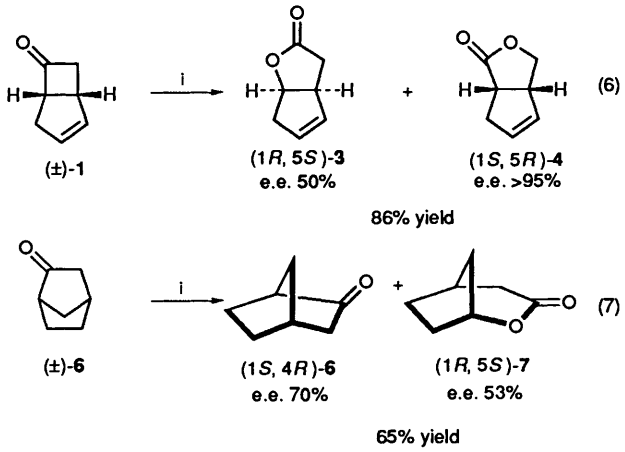


Scheme 3 *Reagents and conditions:* i, *Thermoanaerobium brockii* dehydrogenase; ii, *A. calcoaceticus* NCIMB 9871 monooxygenase

commercially available dehydrogenase from *Thermoanaerobium brockii* in a non-selective manner to give norbornanone **6** which was oxidized in the same pot to give the lactone **7**.[5b]

Only 3.5 mol% of $NADP^+$ is required for complete consumption of substrate, cofactor recycling being accommodated *in situ*.

The use of this coupled enzyme system might be considered when a cyclic secondary alcohol needs to be converted into the corresponding lactone, but its utility will be severely limited by



Scheme 4 *Conditions:* i, *Ps. putida* NCIMB 10007, H_2O, buffer, 1.5–4 h

the fact that only a very few characterised dehydrogenases employ $NADP^+$ as the cofactor. Thus, the range of substrates will be restricted to those oxidized by these few dehydrogenases.

It became increasingly clear to us that if a useful bio-catalyst was to emerge for use in Baeyer–Villiger oxidations it was essential to find and investigate monooxygenases that have NADH as a cofactor. The microorganism *Pseudomonas putida* NCIMB 10007 [grown on (+)-camphor] contains such enzymes.[13]

Oxidation of the bicyclic ketone **1** with *Ps. putida* NCIMB 10007 gave the lactones (1*R*,5*S*)-**3** and (1*S*,5*R*)-**4** in a reaction [eqn. (6), Scheme 4] which is enantio-complementary to the one described in eqn. (2), Scheme 1.

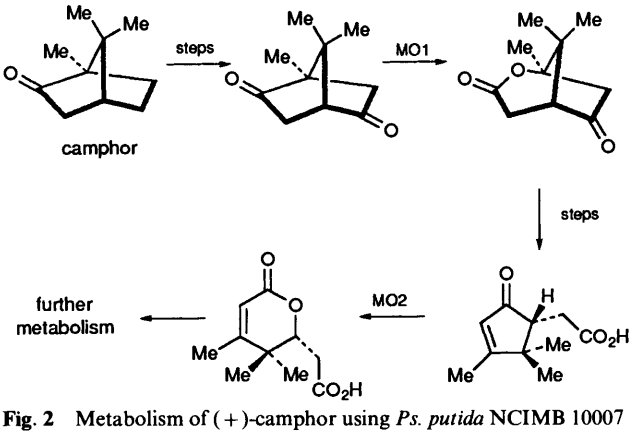


Fig. 2 Metabolism of (+)-camphor using *Ps. putida* NCIMB 10007

Furthermore, *Ps. putida* 10007 catalysed oxidation of norbornanone **6** in a modestly enantioselective fashion [eqn. (7), Scheme 4] giving the lactone (1*R*,5*R*)-**7** in 53% e.e. after *ca.* 45% conversion [*cf.* eqn. (4)].[14]

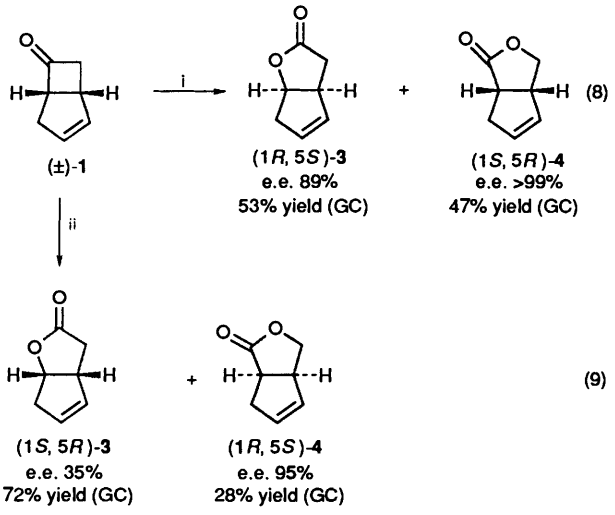
Ps. putida when grown on (+)-camphor gave rise to two sets of monooxygenases, one (labelled MO1 in Fig. 2) whose natural function is to metabolize the ketocamphane and the second (labelled MO2 in Fig. 2) raised to oxidize an α,β-unsaturated cyclopentanone that appears further down the metabolic pathway.[15] The two proteins MO1 and MO2 are simple to obtain by ammonium sulfate precipitation and can be separated by ion-exchange chromatography using DEAE-cellulose.[16]

Further purification of protein MO1 confirmed the previous observation[16] that MO1 from (+)-camphor grown cells comprises two different isozymes [2,5-diketocamphane monooxygenase (25 DKCMO) and 3,6-diketocamphane monooxygenase (36 DKCMO)] that have complementary specificities for the two enantiomers of camphor acting as surrogate substrates.

Table 1 Oxidation of bicycloheptenones using 2,5-diketocamphane monooxygenase (25DKCMO) and 3,6-diketocamphane monooxygenase (36DKCMO) obtained from MO1 over 1 h

Substrate	Enzyme	Products (e.e. %)		Product ratio (conversion)
(±)-**1**	25DKCMO	(+)-**4** (100)	(+)-**3** (82 ± 7)	1:1.3 (100)
	36DKCMO	(+)-**4** (72)	(+)-**3** (10)	1:1.3 (30)
(±)-**6**	25DKCMO	(−)-**7** (60)	(+)-**6** (*ca.* 20)	(20)
	36DKCMO	(−)-**7** (>90)	(+)-**6** (>90)	(48)

Thus, the protein MO1 contains at least two NADH-dependent monooxygenases. However, it was found to oxidize the unnatural ketone **1** in an enantiodivergent and highly selective manner. After complete consumption of the substrate the 1*S*,5*R*-lactone **4** was obtained (99% e.e.) together with 1*R*,5*S*-lactone **3** (89% e.e.) [eqn. (8), Scheme 5]. The NADPH-



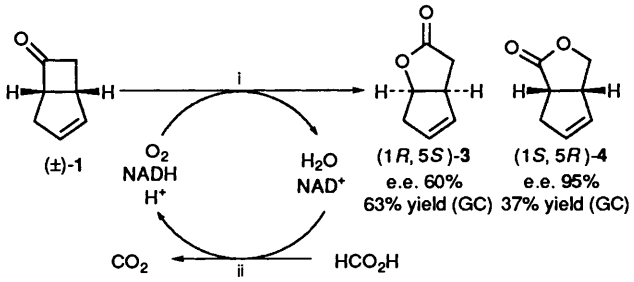
Scheme 5 *Conditions:* i, MO1, $NADH^+$, H_2O buffer, O_2, 1 h; ii, MO2, $NADPH^+$, H_2O buffer, O_2, 2 h

dependent system MO2 was much less selective giving (1*R*,5*S*)-lactone **4** (95% e.e.) as the minor product and the (1*S*,5*R*)-lactone **3** (35% e.e.) as the major product [eqn. (9), Scheme 5].[17]

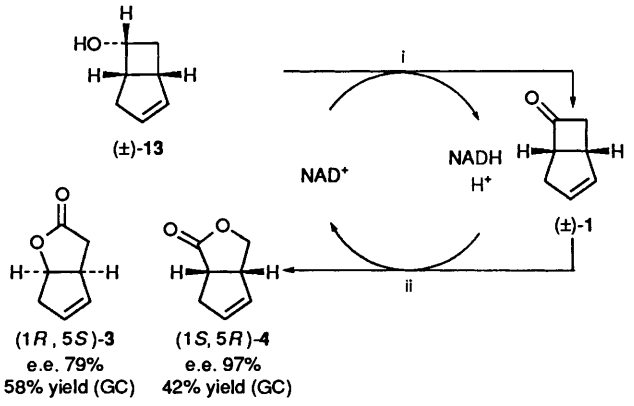
Further studies of MO1's NADH-dependent isoenzymes (*i.e.* 2,5-diketocamphane monooxygenase and 3,6-diketocamphane monooxygenase) with unnatural ketones (±)-**1** and (±)-**6** confirmed that the results obtained with MO1 represent a combination of two different and very distinct patterns of biotransformation (Table 1). The 2,5-diketocamphane monooxygenase isoenzyme was the more stereoselective isoenzyme with the bicyclo[3.2.0] ketone, whereas for the ketone **6** the greater stereoselectivity was exhibited by the 3,6-diketocamphane monooxygenase isoenzyme.

In preparative runs involving MO1 and the ketone (±)-**1** the cofactor was recycled using formate and formate dehydrogenase (Scheme 6). (Note that separation of MO1 from MO2 is unnecessary in such circumstances since MO2 does not function in the absence of NADPH.) The difference in the optical purity of compound (1*R*,5*S*)-**3** as recorded in Schemes 5 and 6 probably reflects a different ratio of the two diketocamphane monooxygenase isozymes remaining in the preparation.

The *endo*-alcohol **13**[18] is readily oxidized to the optically active lactone **3** and **4** by coupling two enzyme-catalysed oxidative processes; thus, conversion of alcohol into lactone is



Scheme 6 *Conditions:* i, MO1 (not separated from MO2), H_2O buffer; ii, formate dehydrogenase from *Candida boidinii*, H_2O



Scheme 7 *Conditions:* i, Horse liver alcohol dehydrogenase, H_2O buffer; ii, MO1, O_2

accomplished using only a small amount of cofactor (Scheme 7). Obviously, horse liver alcohol dehydrogenase oxidizes the alcohol **13** to the ketone **1** with little or no enantioselectivity.

Further control can be implemented by incorporating a third type of enzyme-catalysed transformation. By using the ester **14**[19] as the substrate and engaging *Pseudomonas cepacia* lipase as the hydrolysis catalyst optically active alcohol **13** is fed into the system and the products **3** and **4** are shown (by GC) to be formed in vastly different amounts (at 47% conversion residual acetate is almost optically pure) (Scheme 8). These results are simply explained by the facts that although the hydrolase-catalysed reaction is highly enantioselective, the HLAD oxidation is not selective and the monooxygenase forms the 2-oxabicyclooct-6-en-3-one and the 3-oxabicyclooct-6-en-2-one from the (1*R*,5*S*)-ketone **1** in the ratio 1:3.5.

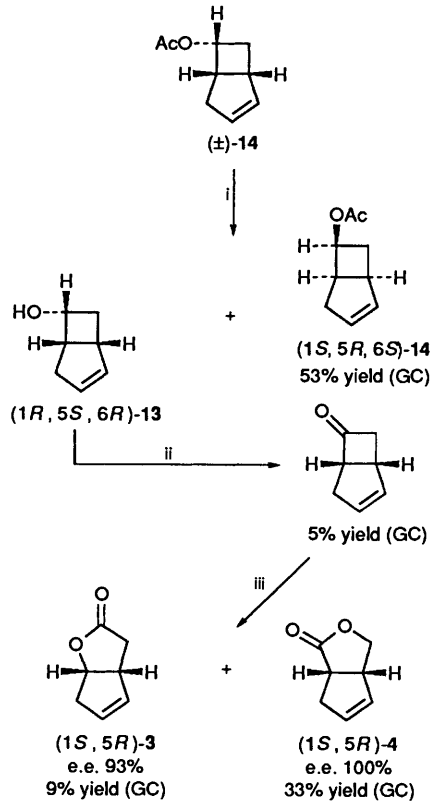
The processing of the (1*R*,5*S*,6*R*)-acetate **14** through three enzyme-catalysed reactions is reminiscent of similar cascades in the carbohydrate field[20] and in the synthesis of some amino acids.[21]

The availability of a variety of methods of recycling NAD^+ is a noteworthy advantage of using NAD^+-linked Baeyer–Villiger monooxygenases.

In summary, the enzyme MO1 (comprising two diketocamphane monooxygenases) is a readily available NAD^+/NADH-linked Baeyer–Villiger monooxygenase capable of performing enantioselective transformations on two bicyclic substrates. The monooxygenase MO2 appears to be a less efficient catalyst for the production of optically active bicyclic lactones.

Reference to Fig. 2 can lead to a simple rationale for these results: the protein MO1 is induced to metabolize a chiral bicycloalkanone while the protein MO2 is raised to oxidize a monocyclic ketone. Thus, in a further development of this programme it was of interest to determine if protein MO1 or protein MO2 was the better catalyst for the oxidation of the simple cyclopentanones **15**–**17**.

Oxidation of the ketones **15**–**17** with whole cells of *Ps. putida*



Scheme 8 *Conditions:* i, *Ps. cepacia* lipase, H_2O buffer; ii, HLAD, NAD^+; iii, MO1, NADH, O_2

Table 2 Whole-cell biotransformations of some 2-substituted cyclopentanones by (+)-camphor-grown *Ps. putida* NCIMB 10007

	Substrate		
	15	**16**	**17**
Concentration (g dm^{-3})	1	1	1
Time (*T*/h)	7	5	10
Conversion (%)[a]	16	35	42
% Recovered (−)-ketone	38	53	49
Optical rotation $[\alpha]_D$[b]	−23	−39	−78
Enantiomeric excess (%)	18	34	62
% Isolated (−)-lactone	15	33	39
Optical rotation $[\alpha]_D$[b]	−35	−29	−29
Enantiomeric excess (%)	77	88	84
E_p[c]	8.9 ± 0.5	23 ± 2	21 ± 4

[a] Conversion: % conversion of ketone to lactone as measured by GC (error ± 5%) on an aliquot taken just before the isolation of the products. [b] Optical rotations $[\alpha]_D$ were measured using chloroform as solvent (c = 1 g 100 cm^{-3}). Estimated error in the readings ± 15%.

[c] E_p = Enantiomeric ratio = $\frac{\ln[1 - c(1 + \text{e.e.}_p)]}{\ln[1 - c(1 - \text{e.e.}_p)]}$

c = conversion

NCIMB 10007 gave the corresponding *S* δ-lactones **18–20** with modest enantioselectivity (*E*) (Table 2).[22] The optical purities of the lactones were assessed by formation of the corresponding *ortho*-esters[23] as recommended by Furstoss.[24] The residual ketones were oxidized using *meta*-chloroperoxybenzoic acid and the resultant lactones were analysed in the manner described above. The NADH-dependent monooxygenase converted these ketones into the corresponding lactones with low selectivity (Table 3) but, as we suspected, the NADPH-dependent monooxygenase was much more selective, transforming the *S*-ketones of the substrates **15–17** almost exclusively (Table 4).

Table 3 NADH-dependent biotransformations of some 2-substituted cyclopentanones by partially purified MO1 from (+)-camphor grown *Ps. putida* NCIMB 10007

	Substrate		
	15	**16**	**17**
Concentration (g dm^{-3})	0.75	1	0.5
Time (*T*/h)	4	1	5
Conversion (%)[a]	29	38	18
% Recovered (−)-ketone	14[b]	48	35
Optical rotation $[\alpha]_D$	—	−47	—
Enantiomeric excess (%)	9	48	22
% Isolated (−)-lactone	16	34	11
Optical rotation $[\alpha]_D$	—	−26	—
Enantiomeric excess (%)	58	74	90
E_p	4.7 ± 0.1	10.4 ± 0.4	23.0 ± 0.6

[a] Conversion: % conversion of ketone to lactone based on GC analysis of the isolated crude product (error ± 2%). [b] The ketone is volatile and losses were incurred during work-up and purification.

Table 4 NADPH-dependent biotransformations of some 2-substituted cyclopentanones by partially purified MO2 from (+)-camphor grown *Ps. putida* NCIMB 10007

	Substrate		
	15	**16**	**17**
Concentration (g dm^{-3})	0.75	1	1
Time (*T*/h)	1.5	1.5	1.5
Conversion (%)	47	44	34
% Recovered (−)-ketone	26	51	44
Optical rotation $[\alpha]_D$	−57	−55	−46
Enantiomeric excess (%)	—	75	59
% Isolated (−)-lactone	40	35	29
Optical rotation $[\alpha]_D$	−33	−35	−32
Enantiomeric excess (%)	95	92	95
E_p	104 ± 21	52 ± 5	63 ± 4

The whole set of results described above using *Pseudomonas putida* NCIMB 10007 as the biocatalyst were obtained after growing the organism on (+)-camphor to induce the relevant Baeyer–Villiger monooxygenases. Therefore, it was of further interest to ascertain if a different substrate selectivity was observed using organisms grown on (−)-camphor. Interestingly, there was no difference in selectivity (*e.g.* Table 5). Data obtained in this study confirmed that whichever enantiomer of camphor is used in the growth medium the same portfolio of monooxygenases (including the same ratio of 2,5- and 3,6-diketocamphane monoxygenases) is produced.[16]

On-going work in these laboratories involves a study of the ability of the monooxygenases MO1 and MO2 to oxidize 2-substituted cyclohexanones and 5-substituted cyclopent-2-enones and the results of these investigations will be the subject of a later report.

Experimental

General Information.—Diethyl ether, dimethoxyethane and tetrahydrofuran were dried and distilled from sodium/benzophenone. Ethyl acetate was distilled from phosphorus

Table 5 Oxidation of ketone (±)-**16** using NADPH-dependent monooxygenase(s) (MO2) isolated from *Ps. putida* NCIMB 10007 grown on (+)- and (−)-camphor

Growth substrate	(*R*)-(+)-Camphor	(*S*)-(−)-Camphor
Concentration (g dm^{-3})	1	1
Time (*T*/h)	1.5	2.5
Conversion (%)	44	32
% Recovered (*R*)-ketone **16**	51	25
Optical rotation $[\alpha]_D$	−55	−39
Enantiomeric excess (%)	75	44
% Isolated (*S*)-lactone **19**	35	15
Optical rotation $[\alpha]_D$	−35	−36
Enantiomeric excess (%)	92	95
E_p	52 ± 5	61 ± 3

pentoxide. Light petroleum (40–60 °C) and dichloromethane were distilled from calcium hydride. Chloroform was washed with water, dried over potassium carbonate and distilled from calcium chloride. Other reagents and solvents were used as commercially supplied. Thin layer chromatography (TLC) was performed on pre-coated glass plates (Merck silica gel 60 F254). The plate was visualized using a *para*-anisaldehyde dip or a mixture of ceric sulfate and ammonium molybdate followed by heating. Flash column chromatography was performed over silica gel (Merck silica gel 60, 40–63 mm). Gas chromatography was performed with a Shimadzu GC-14A gas chromatograph equipped with a capillary column: BP1 25 μm, Lipodex D, 25m. ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Brüker AM 250 spectrometer using the deuterium lock, for $CDCl_3$ solutions. Chemical shifts (δ) were quoted in ppm and coupling constants (*J*) in Hz. IR spectra were recorded on a Nicolet Magna-IR 550 spectrometer on a liquid film between sodium chloride plates. Mass spectra were recorded on a VG ZAB-F spectrometer at the SERC Mass Spectrometry Centre, Swansea, or a Kratos Profile HV 3000 spectrometer at the University of Exeter. Optical rotations were measured on an Optical Activity AA-1000 polarimeter and are recorded in units of 10^{-1} deg cm^2 g^{-1}. Optical density was measured on a Spectronic 20 Spectrophotometer. Solutions of compounds in organic solvents were dried using anhydrous magnesium sulfate.

Origin of Substrates.—Norbornanone **6** was obtained from the Aldrich Chemical Company. Bicyclo[3.2.0]hept-2-en-6-one **1** was a gift from Glaxo Group Research. 5-Bromo-7-fluoronorbornan-2-one[8] **8**, bicyclo[3.2.0]hept-2-en-6-*endo*-ol[17] **13**, bicyclo[2.2.1]heptan-2-ol[27] **12**, and 6-*endo*-acetoxybicyclo[3.2.0]hept-2-ene **14**[18] were prepared as described elsewhere. 2-Substituted cyclopentanones were prepared from cyclopentanone *via* radical alkylation with the appropriate alkene in the presence of silver(I) oxide[25] or from methyl cyclopentanone-2-carboxylate *via* alkylation and decarboxylation.[26]

Maintenance and Growth of Microorganisms.—*Acinetobacter calcoaceticus* NCIMB 9871 was maintained and used as prescribed. The NADPH-dependent monooxygenase from this bacterium was obtained as described previously.[28] *Pseudomonas putida* (strain no. NCIMB 10007) was obtained from NCIMB (Aberdeen, UK) and maintained on nutrient agar slopes at 28 °C. The organism was routinely grown on a basal salts medium: NH_4Cl (2 g), K_2HPO_4 (8.2 g), KH_2PO_4 (3.1 g), yeast extract (0.1 g), tryptone (0.1 g), $MgSO_4 \cdot 7H_2O$ (0.4 g), $MnSO_4 \cdot H_2O$ (0.05 g), $CaCl_2 \cdot H_2O$ (0.01 g), $NaMoO_4$ (0.01 g), $FeSO_4 \cdot 7H_2O$ (0.05 g dm^{-3}), (+)-Camphor (2.5 g dm^{-3}) was added prior to autoclaving the media at 15 psi for 15 min. A 100 cm^3 culture of bacteria was inoculated from a slope and after 24 h growth at 28 °C on an orbital shaker (150 rpm) was transferred to 1 dm^3 of media. After a further 24 h of growth this culture was used to inoculate 12 dm^3 of sterile media in a 20 dm^3 glass fermenter which was sparged with sterile air at 4 dm^3 min^{-1}. Growth of the organism was monitored by transferring 1 cm^3 aliquots of growing cell suspension to a plastic cuvette and reading the absorbance at 500 nm on a Spectronic 20 spectrophotometer. Cells were harvested after approximately 18 h of growth. *Pseudomonas cepacia* (also known as *Pseudomonas fluorescens*) lipase was purchased from Biocatalysts (Sketty, Swansea, UK).

Biotransformation of 5-endo-*Bromo*-7-anti-*fluorobicyclo*-[2.2.1]*heptan-2-one with* Acinetobacter calcoaceticus NCIMB 9871.—Cells grown up in 6 dm^3 of medium were collected by centrifugation and resuspended in buffer (350 cm^3, pH 7.1, KH_2PO_4 2 g dm^{-3}, Na_2HPO_4 4 g dm^{-3}). The ketone **8** (190 mg) was added to the mixture which was then shaken at 30 °C for 2.5 h. The cells were removed by centrifugation and the aqueous phase was continuously extracted with dichloromethane (2 × 500 cm^3, 2 × 2 days). The combined organic extracts were dried and evaporated and the residue was chromatographed over silica gel using dichloromethane as eluent. The lactone **10** was obtained as a white solid (40%), m.p. 79–80 °C; $[\alpha]_D^{20.5}$ −12 (*c* 0.78 $CHCl_3$) e.e. >95% as judged by ^1H NMR spectroscopy using a chiral shift reagent [Eu(hfc)$_3$]; $\nu_{max}(CHCl_3)/cm^{-1}$ 3014 and 1750; $\delta_H(CDCl_3)$ 5.22 (1 H, d, *J* 50.5, 8-H), 4.74–4.36 (2 H, m, 1-H, 6-H), 3.32 (1 H, ddd, *J* 17, 7, 4 4-*endo*-H), 3.01 (1 H, m, 4-*exo*-H), 2.82 (2 H, m, 5-H and 7-*exo*-H), 2.42 (1 H, dd, *J* 16, 5, 7-*endo*-H) (Found: C, 38.0; H, 3.8. $C_7H_8BrFO_2$ requires C, 37.7; H, 3.6%). Optically active starting material was also obtained (96 mg), $[\alpha]_D^{20.5}$ −27 (*c* 0.95 $CHCl_3$) (e.e. >95% as estimated by ^1H NMR using the chiral shift reagent): other physical constants were identical with those reported previously.[8]

Conversion of Norbornanol **12** *into the Lactone* **7** *using the* Thermoanaerobium brockii *Alcohol Dehydrogenase/Monooxygenase* 9871 *Coupled Enzyme System.*—To a stirred solution of the alcohol **12** (60 mg, 14 mmol dm^{-3}) in tris/HCl buffer (pH 9.0, 40 cm^3) at 30 °C was added *T. brockii* alcohol dehydrogenase (100 units) and monooxygenase from *A. calcoaceticus* 9871 (40 units) and $NADP^+$ (5 mmol dm^{-3}). After 5.5 h, the mixture was extracted with dichloromethane (3 × 50 cm^3), dried and evaporated. The residue was chromatographed over silica gel using dichloromethane as eluent to give the title lactone (50 mg) as a white solid.

General Procedures for the Whole-cell Biotransformation using Ps. putida NCIMB 10007.—The medium (10 dm^3) was inoculated and grown to an optical density of 0.9 at 500 nm. The cells were then harvested by centifugation (3900 rpm) at 30 °C for 20 min. The cells were resuspended in a phosphate buffer solution (21 mmol dm^{-3}, pH 7.1; 1 dm^3) and the suspension was used immediately for biotransformations. The substrate which was predissolved in ethanol was added to the cell suspension, with the concentration being varied in the range 0.25–1 g dm^{-3}, depending on the substrate. The scale in this system was varied in the range 0.5–1.0 g. The whole mixture was put into a gyratory shaker at 30 °C (200 rpm). The biotransformation was monitored by periodic sampling of aliquots (0.5 cm^3) which were extracted with ethyl acetate (0.5 cm^3) and analysed by GC. After completion of the biotransformation the cells were removed by centrifugration (3900 rpm) at 30 °C for 20 min and the supernatant was extracted with ethyl acetate (2 × volume of the aqueous phase). The combined extracts were dried and solvent was removed under reduced pressure to give a crude residue. The lactone and the recovered ketone were separated and purified by flash column chromatography.

Partial Purification of NADH- and NADPH-dependent Activities from Ps. putida 10007.—Cells were harvested by centrifugation at 4000 rpm for 30 min and the cell paste from 12 dm^3 of growth medium was resuspended in 300 cm^3 of phosphate buffer (pH 7.1, 21 mmol dm^{-3}). The cells were disrupted by sonication at an amplitude of 22 μm for four 30 s periods with 1 min intervals in an ice-bath. The cell debris was removed by centrifugation (12 000 rpm for 20 min) and the supernatant taken to 50% ammonium sulfate saturation and left for 1 h at 0 °C. The precipitate was centrifuged (12 000 rpm, 20 min) and the pellet discarded. The supernatant was taken to 75% ammonium sulfate saturation and left for 1 h at 0 °C. The precipitate was centrifuged as above and the supernatant discarded. The pellet was dissolved in a minimum of phosphate buffer and dialysed against sequential 5 dm^3 portions of the same until all the native cofactor had been removed. The protein solution was then lyophilised and stored at 4 °C until required. This ammonium sulfate cut contained both the NADH- and NADPH-dependent monooxygenase activities used in the following biotransformations.

Biotransformations of Ketones by NADH- and NADPH-dependent Monooxygenase Activities: Typical Procedure.—For NADH-dependent biotransformations, the ketone (0.6 mmol) was magnetically stirred in phosphate buffer (pH 7.1, 21 mmol dm^{-3}; 100 cm^3), containing 60 μmol of NADH, 5 units of NADH-dependent monooxygenase 20 mmol of sodium formate and 10 units of formate dehydrogenase. When the reaction had proceeded to either the required stage (≈40% bioconversion as monitored by GLC) or showed no signs of continuing below this level, the mixture was extracted with ethyl acetate (3 × 50 cm^3). The organic extract was dried ($MgSO_4$) and evaporated under reduced pressure. The lactone and residual ketone were separated by chromatography over silica. For NADPH-dependent biotransformations, the ketone (0.6 mmol) was magnetically stirred in phosphate buffer (pH 7.1, 21 mmol dm^{-3}; 100 cm^3), containing 30 μmol of NADPH, 2 mmol of glucose-6-phosphate, 10 units of NADPH-dependent monooxygenase and 100 units of glucose-6-phosphate dehydrogenase. The reactions were monitored and worked up as above.

Identification of Products from Ps. putida NCIMB 10007 *Biotransformations.*—2-Oxabicyclo[3.2.1]octan-3-one **7**, 2-oxabicyclo[3.3.0]oct-6-en-3-one **3** and 3-oxabicyclo[3.3.0]oct-6-en-2-one **4** were identical with samples obtained previously. For biotransformations involving the cyclopentanones **15–17** the isolated lactone and recovered ketone were identified on the basis of their ^1H and ^{13}C NMR and by comparison of their retention times on GC and R_f on TLC with racemic samples, prepared by chemical Baeyer–Villiger oxidation of the corresponding cyclopentanone using *meta*-chloroperbenzoic acid.

Measurements of the Enantiomeric Excesses of the Lactones **18–20** *and Optically Active Ketones* **15–17**.—The enantiomeric excess of the lactone was obtained by forming the acetal derivatives with (2*R*,3*R*)-(−)-butane-2,3-diol as described by Saucy *et al.*[22] and Furstoss *et al.*[23] The recovered ketone was oxidized by *m*-CPBA to give the lactone and analysed as described above. The lactones **18–20** were fully characterized from the following physical data.

Nonan-5-olide **18**. R_f 0.25 (hexane–ethyl acetate, 7:3) (Found: $[M]^+$, 156.115 46. $C_9H_{16}O_2$ requires $[M]^+$, 156.115 03); ν_{max}(neat)/cm^{-1} 2957, 2873, 1735 and 1241; δ_H 4.26 (1 H, m, CH), 2.50 (2 H, m, $COCH_2$), 2.00–1.20 (10 H, m, 5 × CH_2) and 0.88 (3 H, t, *J* 7.0, CH_3); δ_C 171.72 (CO), 80.36 (CH), 35.39 (CH_2), 29.31 (CH_2), 27.66 (CH_2), 26.92 (CH_2), 22.34 (CH_2), 18.33 (CH_2) and 13.74 (CH_3).

Undecan-5-olide **19**. R_f 0.25 (hexane–ethyl acetate, 7:3) (Found: $[M + H]^+$, 185.1542. $C_{11}H_{20}O_2$ requires $[M + H]^+$, 185.1542); ν_{max}(neat)/cm^{-1} 2932, 2859, 1736 and 1242; δ_H 4.26 (1 H, m, CH), 2.50 (2 H, m, CH_2CO), 2.00–1.20 (14 H, m, 7 × CH_2) and 0.88 (3 H, t, *J* 7.0, CH_3); δ_C 171.80 (CO), 80.48 (CH), 35.79 (CH_2), 31.61 (CH_2), 29.40 (CH_2), 29.00 (CH_2), 27.75 (CH_2), 24.82 (CH_2), 22.47 (CH_2), 18.43 (CH_2) and 13.93 (CH_3).

Tridecan-5-olide **20**. R_f 0.25 (hexane–ethyl acetate, 7:3) (Found: $[M]^+$, 212.177 88. $C_{13}H_{24}O_2$ requires $[M]^+$, 212.177 63); ν_{max}(neat)/cm^{-1} 2930, 2859, 1739 and 1242; δ_H 4.26 (1 H, m, CH), 2.50 (2 H, m, CH_2CO), 2.00–1.20 (18 H, m, 9 × CH_2) and 0.88 (3 H, t, *J* 7.0, CH_3); δ_C 171.68 (CO), 80.38 (CH), 35.76 (CH_2), 31.73 (CH_2), 29.33 (CH_2), 29.33 (CH_2), 29.33 (CH_2), 29.08 (CH_2), 27.70 (CH_2), 24.82 (CH_2), 22.51 (CH_2), 18.37 (CH_2) and 13.92 (CH_3).

Conversion of the Acetate **14** *into the Lactones* **3** *and* **4**.—The racemic ester **14** (0.12 mmol), NAD^+ (0.012 mmol), *P. cepacia* lipase (20 mg) horse liver alcohol dehydrogenase (40 mg, 1.7 μ mg^{-1}) and *Pseudomonas putida* monooxygenase (20 mg) were added to phosphate buffer (21 mmol dm^{-3}, pH 7.1; 12 cm^3). The reaction mixture was stirred at 30 °C and monitored by GC (Lipodex D).

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