Oxidation of some prochiral 3-substituted cyclobutanones using monooxygenase enzymes: a single-step method for the synthesis of optically enriched 3-substituted γ-lactones

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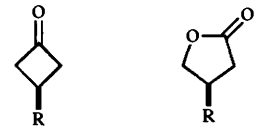
Oxidation of the prochiral cyclobutanones **1–5** using *Acinetobacter calcoaceticus* NCIMB 9871 and *Pseudomonas putida* NCIMB 10007 monooxygenases (MO1 and MO2) furnishes the corresponding lactones **6–10** in moderate to excellent yield and enantiomeric purity. These enzymes show enantiocomplementarity in all but one case.

Introduction and Background Information

Following the seminal work of Trudgill,[1] Walsh,[2] Furstoss[3] and Taschner,[4] further work on the Baeyer–Villiger oxidation of cyclic ketones has proven it to be a very efficient way of producing enantiomerically enriched lactones. This approach is particularly interesting as far as overall yields are concerned when using a prochiral substrate. Herein we describe our results concerning the oxidation of different 3-substituted cyclobutanones **1–5** to the corresponding lactones **6–10** using three different types of biocatalysts, *i.e.* whole cells of *Acinetobacter calcoaceticus* NCIMB 9871 and two partially purified monooxygenase activities (MO1 and MO2) from *Pseudomonas putida* NCIMB 10007.[5]



1 R = Bu **6**
2 R = Bu^i **7**
3 R = CH_2Ph **8**
4 R = CH_2 **9**
5 R = CH_2OCH_2Ph **10**

Results and discussion

The various starting cyclobutanones were prepared as previously described[6] *via* dichloro ketene cycloaddition on the corresponding olefin, followed by hydrodehalogenation to the corresponding cyclobutanone. The cyclobutanones **1–5** were subsequently oxidized using whole-cell cultures of *A. calcoaceticus* NCIMB 9871 or monooxygenases isolated from *P. putida*. This last bacterial strain has been shown to contain two sets of monoxygenases, one NADH-linked (labelled MO1) and one NADPH-linked (labelled MO2).[5] These purified enzymes were used together with the appropriate co-factor recycling processes.[5] The results we have obtained in the course of this study are summarized in Table 1.

When oxidized by whole cells of *A. calcoaceticus*, 3-butylcyclobutanone **1** led preferentially to the corresponding (*S*)-lactone **6**, whereas MO1 and MO2 afforded the (*R*)-antipode of this lactone. The ee of the lactone product proved to be much higher when the purified enzymes were used. The enantiomeric purity of **6** was assessed by gc using a chiral column (Lipodex E) and the absolute configuration was elucidated by comparison of the optical rotation with the literature value.[7] (Note that, for the transformations using MO1 and MO2, all the data in Table 1 were generated by GC measurements on aliquots of the reaction mixture.)

3-Isobutylcyclobutanone **2** was processed by *A. calcoaceticus* NCIMB 9871 and the two enzymes from *P. putida* NCIMB 10007 in a highly selective fashion. Both *P. putida* MO1 and *P. putida* MO2 gave the (*R*)-lactone **7** with good enantiomeric excess (ee) (> 85%). The whole cells of *A. calcoaceticus* gave the (*S*)-lactone **7** in 84% enantiomeric excess. The estimations of ee were made by GC using a chiral stationary phase (Lipodex E). The absolute configuration of the lactone was determined by comparison with an authentic sample.†

Table 1 Oxidation of 3-substituted cyclobutanones with monooxygenases

Substrate	Organism or enzyme[a]	Product	Conversion[b] (%)	Yield[c] (%)	Ee (%)
1	NCIMB 9871	(*S*)-**6**	95	68	17
	MO1	(*R*)-**6**	100	ND[e]	69
	MO2	(*R*)-**6**	93	ND	54
2	NCIMB 9871	(*S*)-**7**	98	56	84
	MO1	(*R*)-**7**	78	ND	91
	MO2	(*R*)-**7**	97	ND	85
3	NCIMB 9871	(*S*)-**8**	100	57	82
	MO1	(*S*)-**8**	58	40	15
	MO2	(*S*)-**8**	37	26	20
4	NCIMB 9871	(*S*)-**9**	100	83	95 (96)
	MO1	(*R*)-**9**	48	38	7
	MO2	(*S*)-**9**	71	69	14
5	NCIMB 9871	(*S*)-**10**	100	89	55 (53)
	MO1	(*S*)-**10**	98	74	74
	MO2[d]	(*R*)-**10**	95	ND	90

[a] NCIMB 9871: *Acinetobacter calcoaceticus* NCIMB 9871; MO1: NADH dependent monooxygenase from *Pseudomonas putida* NCIMB 10007; MO2: NADPH-dependent monooxygenase from *Pseudomonas putida* NCIMB 10007. [b] Conversion measured by GC; reaction mixtures showed only two components, starting material and product. [c] Isolated yield. [d] MO2 partially purified by FPLC (Q-Sepharose column). [e] ND not determined.

† This analysis was performed by R. McCague (Chiroscience).

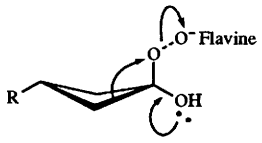
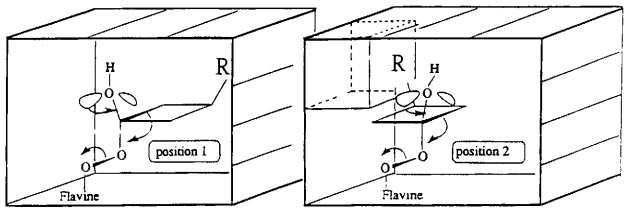


Fig. 1 Preferred mode of ring expansion catalysed by MO1



Preferential formation of (*S*)-(**7**); (*S*)-(**8**) and (*S*)-(**9**)

Preferential formation of (*S*)-(**10**)

Fig. 2 Disposition of 3-substituted cyclobutanones in the active site of monooxygenase in *A. calcoaceticus* NCIMB 9871

3-Benzylcyclobutanone **3** behaves in a very different manner. Thus, whole cells of *A. calcoaceticus* gave a reasonable yield of the (*S*)-enantiomer of the lactone **8**. The ee was estimated by ^{19}F NMR spectroscopy following lithium aluminium hydride reduction of **8** to afford the corresponding diol and then formation of the bis-Mosher's ester. The absolute configuration was confirmed by comparison of $[\alpha]_D$ values with those pre-recorded in the literature.[8] In contrast to compounds **1** and **2**, the same enantiomer [(*S*)-lactone] is formed with MO1 and MO2, however with poor enantiomeric purities and low yields.

Whole cells of *A. calcoaceticus* completely transformed ketone **4** into the lactone (*S*)-**9** which showed an excellent ee. This biotransformation, conducted on 0.5 g of **4** in a 1 dm^3 culture thus affords 450 mg (83% yield) of lactone **9** showing an optical purity of 96%. In contrast, the crude MO1 and MO2 enzymes afforded the lactone **9** with moderate yields and poor ee's. Moreover, in this case MO1 led to (*R*)-**9** whereas MO2 afforded (*S*)-**9** antipode. The ee of **9** was judged by formation of the acetal[9] followed by chiral GC analysis. The absolute configuration was established by comparison of its specific rotation, $[\alpha]_D^{20}$ −5.0 (*c* 1, $CHCl_3$), with that observed by Honda *et al.*[10] for authentic (*R*)-lactone.

Surprisingly, oxidation of the ketone **5** with MO1 and *A. calcoaceticus* NCIMB 9871 furnished the same [(*S*)-(+)]-enantiomer of the lactone **10**. On the other hand, using FPLC-purified MO2 a high ee (90%) of the (*R*)-lactone **10** was obtained. The enantiomeric excesses were measured using GC (Lipodex E) and the absolute configuration was established from literature precedent.[11]

For the monooxygenase MO1 from *Pseudomonas putida* NCIMB 10007 the preferred mode of ring expansion described in Fig. 1[12] is observed for the ketones **1**, **2** and **5**. For the ketones **3** and **4** MO1 shows little selectivity. Contrariwise, *A. calcoaceticus* NCIMB 9871 shows good selectivity with substrates **2**, **3** and **4** and the mode of ring expansion is in accord with the previously proposed 'cubic model'.[13] In these three cases the side chain is relatively compact and position 1 (Fig. 2) is preferred by the substrate in the active site. For ketone **1** with the elongated butyl side chain there is little stereoselectivity in the ring expansion reaction while for the ketone **5**, with the still more sterically demanding benzyloxymethyl side chain, the unusual mode of ring expansion is observed, leading to the conclusion that position 2 (Fig. 2) is preferred by the substrate during the ring-expansion process.

The crude mono-oxygenase MO2 gives a consistent mode of ring expansion for some 2-substituted cyclopentanones[5] and some 2-substituted cyclohexanones but is not the biocatalyst of choice for the oxidation of the 3-substituted cyclobutanones described herein, except perhaps for the formation of the lactone (*R*)-**10** using FPLC-purified enzyme.

Overall the present study shows that biocatalysts can be used to prepare important 3-substituted γ-lactones in optically active form. It is interesting to note, in this context, that the lactone (*R*)-**9** is a key intermediate in the synthesis of several lignan derivatives which possess interesting anti-leukemic activities.[10] Also the lactone **10** has been shown to be a valuable chiron for Factor I, an autoregulator for the production of antibiotics in *Streptomycetes*.[14] In many ways the monooxygenases from *A. calcoaceticus* NCIMB 9871 and *P. putida* NCIMB 10007 show complementary stereoselectivities and, with increased knowledge of the ring-expansion patterns, the correct enzyme can be chosen, with greater confidence, to perform a desirable, synthetically useful bio-Baeyer Villiger reaction.

Experimental

Oxidation of 3-benzylcyclobutanone 3 using *Acinetobacter calcoaceticus* NCIMB 9871

The ketone **3** (48 mg) dissolved in ethanol (1.9 cm^3) was added to an Erlenmeyer flask containing cells of *Acinetobacter calcoaceticus* NCIMB 9871 suspended in pH 7.1 phosphate buffer (192 cm^3). The mixture was agitated at 30 °C for 6 h (100% conversion by GC) and extracted with ethyl acetate (3 × 30 cm^3). The combined extracts were dried ($MgSO_4$) and evaporated and the residue was chromatographed over silica (eluent: diethyl ether–light petroleum, 4:6) to give the (*S*)-lactone **8** (30 mg), $[\alpha]_D^{23}$ −6.4 (*c* 2.0 $CHCl_3$) (lit.,[9] $[\alpha]_D$ −8.6). Other physical data were in accord with the literature values.

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