Stereochemical congruence of Baeyer–Villigerases

David R. Kelly,*[a] Christopher J. Knowles,[b] Jassem G. Mahdi,[a] Michael A. Wright,[b] Ian N. Taylor,[b] Stanley M. Roberts,[c] Peter W. H. Wan,[c] Gideon Grogan,[d] Sandrine Pedragosa-Moreau[d] and Andrew J. Willetts[d]

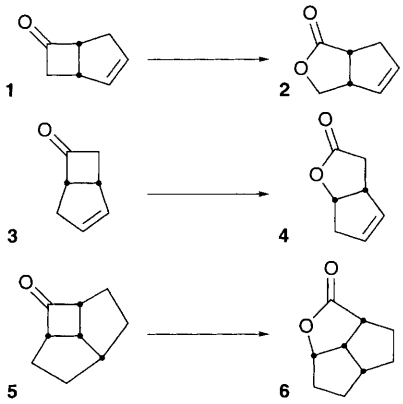
[a] *Department of Chemistry, University of Wales, College of Cardiff, PO Box 912, Cardiff, UK CF1 3TB*
[b] *Biological Laboratory, The University of Kent, Canterbury, Kent, UK CT2 7NJ*
[c] *Department of Chemistry, University of Exeter, Stocker Road, Exeter, UK EX4 4QD*
[d] *Department of Biological Sciences, University of Exeter, Stocker Road, Exeter, UK EX4 4QG*

The enantiomeric bicyclic ketones 1, 3 and the tricyclic ketone 5 undergo stereochemically congruent Baeyer–Villiger oxidations with CHMO from *Acinetobacter* sp., CPMO from *Pseudomonas* sp. as well as 2,5-DKCMO, 3,6-DKCMO and MO2 from *P. putida*; in every case the tricyclic ketone 5 is transformed with >96% ee. *N*-terminal sequences for the FAD/NADPH linked enzymes from *Acinetobacter* sp., *Pseudomonas* sp. and a novel CHMO from *R. coprophilus* have high homology with each other but no homology with the FMN/NADH linked enzymes; 2,5-DKCMO and 3,6-DKCMO.

We have recently drawn attention to the enzyme catalysed Baeyer–Villiger oxidation of the ketones **1**, **3**.[1,2] A mechanism was proposed in which these ketones bind at the active site of the Baeyer–Villigerase in the orientations shown in Scheme 1 and are oxidised predominantly to the lactones **2**, **4**. In support of this proposal the prochiral tricyclic ketone **5** was prepared and underwent stereoselective ring expansion with the predicted enantioselectivity (>98% ee) to give the lactone **6**.[3] This outcome has been rationalised by postulating a unique configuration for the peroxide intermediate formed from the flavin hydroperoxide and the ketone.[4]

Here we report new stereochemically congruent Baeyer–Villiger oxidations of the ketones **1**, **3** and **5**. In the five cases (Table 1, entries 1,[3] 3, 12, 13, 15) in which highly purified enzyme has been employed the tricyclic ketone **5** is transformed with almost 100% stereoselectivity and with a stereoselectivity congruent with the bicyclic ketones **1**, **3**. The whole cell transformations are more variable reflecting the activity of multiple monooxygenases.

The ketones **1**, **3** are transformed with low enantiomeric excesses (entry 3) by CPMO from *Pseudomonas* sp., whereas the tricyclic ketone **5** gives enantiomerically pure **6**. The yields of the lactones **2**, **4** (2.5, 74% respectively) indicate that this enzyme has very low activity for ring expansion with migration of the methylene group and consequently the majority of the ketone **1** is transformed through the methine migration manifold to the lactone *ent*-**4**. *Nocardia* sp. and NV-2[2] give bicyclic lactones **2**, **4** and tricyclic lactone **6** from related enantiomeric series (entries 4, 5).



Scheme 1

Pseudomonas putida has a repertoire of at least three monooxygenases for the camphor degradation pathway. There are two NADH/FMN linked monooxygenases; 2,5-DKCMO and 3,6-DKCMO which are specific for the oxidised catabolite of (+)- and (−)-camphor respectively. A mixture of these enzymes is designated the MO1 fraction. In addition there is NADPH linked monooxygenase activity (MO2) which degrades the subsequent cyclopentenones in the two pathways.[5] This may also consist of individual enzymes for each enantiomer.

Biotransformations using *P. putida* (entries 6–14) are dominated by the two enzymes of the MO1 fraction which both yield *ent*-**2**, *ent*-**4**, *ent*-**6** (entries 11, 12 and 13). Crude preparations of MO2 gave stereochemically divergent results with the bicyclic and tricyclic ketones (entries 7, 9 and 14). However highly purified MO2 (entry 15) gave the expected tricyclic lactone **6** (96% ee) and the bicyclic lactones **2**, **4** with congruent stereochemistry. MO2 is a more efficient catalyst

Table 1 Biotransformation of the ketones **1**, **3**, **5** to the lactones **2**, **4**, **6**

Entry	Enzyme, organism[a]	Ee (%)[b] (% yield by GC[c])		
		2	**4**	**6**
1	*Acinetobacter* sp. CHMO, PE	>98 (50)	>98 (50)	>98[3]
2	*X. autotrophicus*, WC[12]	85.6 (7.5)	48.6 (70)[10]	87.5[3]
3	*Pseudomonas* sp., CPMO, PE	78 (2.5)	33 (74)	>98
4	*Nocardia* sp., WC	71 (54)	81 (46)	>98
5	NV-2, WC	76 (54)	85 (46)	34
	P. putida; *grown on* (+/−)-*camphor*			
6	WC	—	—	−21 (36)
7	MO2, CE	—	—	−60 (75)
	P. putida; *grown on* (−)-*camphor*			
8a	WC (Kent)	−70 (22)	−22.5 (78)[10]	−56.5[3d]
8b	WC (Exeter)	—	—	−30 (39)
9	MO2, CE	—	—	−89 (78)
	P. putida; *grown on* (+)-*camphor*			
10a	WC (Kent)	64 (22)	17 (78)[10]	−5.5[3]
10b	WC (Exeter)	>−95 (26)	−50 (60)[11]	−13 (39)
11	MO1 fraction			>−98 (72)[3]
12	2,5-DKCMO[d], PE	−100 (44)	−82+/−7 (56)[11]	>−98 (80)
13	3,6-DKCMO[d], PE	−72 (17)	−10 (13)[11]	>−98 (86)
14	MO2, CE	95 (28)	35 (72)[12]	−77 (82)
15	MO2, PE	98 (34)	50 (66)	>96 (100)

[a] WC whole cell; CE crude enzyme; PE highly purified enzyme. [b] Negative ee's indicate an excess of the other enantiomer. [c] Entries without yields were quantitative conversions. [d] This result was erroneously reported as the enantiomeric product in our prior reports.[1,3]

Table 2 Baeyer–Villigerase *N*-terminal residues[a]

Residue number	*C. radicola*, SMO[7]	*R. coprophilus*,‡ CHMO	*Acinetobacter* sp., CHMO	*Pseudomonas* sp., CPMO	*R. rhodocrous*, SMO[7]	*P. putida*, 2,5-DKCMO[12]	*P. putida*, 3,6-DKCMO[12]
−13 to 1	—	Thr	—	Thr, Thr, Met, Thr Thr, Met, Thr, Thr Glu, Gln, Leu, Gly Met, Asn	—	—	Ala
2	**Ala**	**Ala**	[Met]	Asn	—	**Met**	**Met**
3	Glu	Gln (*Thr*)	*Ser*	*Ser*	Met	*Gln*	*Glu*
4	Trp	Thr	Gln	Val	Asn	Ala	Thr
5	Ala	*Ile*	*Leu* [Lys]	Asn	Gly	**Gly**	**Gly**
6	Glu	His	Met	Asp	Gln	Phe	Leu
7	Glu	Gly (Thr)	Asp	Lys	His	Phe	Ile
8	**Phe**	*Val*	**Phe**	*Leu*	Pro	Gly	Phe
9	**Asp**	**Asp**	**Asp**	**Asp**	Arg	Thr	His
10	**Val**	*Ala*	*Ala*	**Val**	**Val**	**Pro**	**Pro**
11	*Leu*	**Val**	*Ile*	*Leu*	**Val**	**Tyr**	**Tyr**
12	**Val**	**Val**	**Val**	*Leu*	**Val**	Asp	Met
13	*Val*	**Ile**	**Ile**	**Ile**	Ala	Leu	Tyr
14	**Gly**	**Gly**	**Gly**	**Gly**	Ala	**Pro**	**Pro**
15	**Ala**	**Ala**	*Gly*	**Ala**	Pro	Thr	Gly
16	**Gly**	**Gly**	**Gly**	**Gly**	Asp	*Arg*	*Lys*
17	**Ala**	**Phe**	**Phe**	**Phe**	Ala	*Thr*	*Ser*
18	**Gly**	**Gly**	**Gly**			**Ala**	**Ala**
19	**Gly**	**Gly**	**Gly**			Arg	Ala
20 to 28		*Ile*, **Tyr**, **Ala**, **Val**, His, **Lys**	*Leu*, **Tyr**, **Ala**, **Val**, Lys, **Lys**, Leu, Tyr [Arg], Asp			**Gln**	**Gln**

[a] Identical residues in the FAD/NADPH or FMN/NADH linked enzymes are marked in bold, very closely related residues are marked with italics. Residues in square brackets are those determined by analysis of the gene[7] which differ from those determined in the current study. Residues in curved brackets are alternative assignments, due to ambiguity in the determination.

with cyclopentanones than bicyclic ketones (rate ratio *ca.* 1 : 0.3). It is likely therefore that it has even greater discrimination against transformation of the tricyclic ketone **5**. Consequently the products observed with the crude preparations (entries 7, 9, 14) probably result from the presence of traces of the MO1 enzymes, although no contaminating bands could be detected by gel electrophoresis.

It is striking that the only Baeyer–Villigerases with a preference for the formation of *ent*-**2**, *ent*-**4** and *ent*-**6** are the DKCMO enzymes which utilise FMN and NADH cofactors whereas all the other enzymes utilise FAD and NADPH. This suggests that these enzymes may represent two evolutionarily distinct classes of Baeyer–Villigerases. To shed light on this hypothesis, *N*-terminal analysis by Edman degradation was performed on CHMOs from *R. coprophilus*‡ and *Acinetobacter* sp., and CPMO from *Pseudomonas* sp. The sequence of the CHMO from *Actinobacter* sp. has been determined previously from the gene,[6] and that of two steroid monooxygenases[7] and the two MO1 enzymes by Edman degradation[8] (Table 2).

Excellent homology is shown between the four of the five FAD/NADPH linked enzymes. The GlyXGlyXGlyGly sequence which is a common binding site motif for the adenosine moiety[9] was located in the *C. radicicola* SMO, the two CHMOs and the CPMO (residue numbers 14–19). The *R. rhodocrous* SMO has little homology with the other FAD/NADPH linked enzymes, except for an apolar region (residues 10–13).[7] There is no apparent homology between the FAD/NADPH linked enzymes and the FMN/NADH linked enzymes. Whereas the latter have seven identical and three very closely related residues out of nineteen in the alignment.[8]

Footnotes

† Abbreviations: *Acinetobacter* sp., *Acinetobacter* sp. NCIMB 9871; *C. radicicola*, *Cylindrocarpon radicicola*; *Nocardia* sp., *Nocardia* sp. NCIMB 11399; NV-2, NV-2 (a black yeast); *P. putida*, *Pseudomonas putida* NCIMB 10007; *Pseudomonas* sp., *Pseudomonas* sp., NCIMB 9872; *R. coprophilus*, *Rhodococcus coprophilus* WT-1; *R. rhodocrous*, *Rhodococcus rhodocrous*; *X. autotrophicus*, *Xanthobacter autotrophicus* DSM431, NCIMB 10811; MO, monooxygenase; CH, cyclohexanone; CP, cyclopentanone; DKC, diketocamphane; S, steroid.

‡ The Baeyer–Villigerase activity of this species has not been reported previously. The CHMO was induced by growth on cyclohexanol. It is a 58 KDa protein with a FAD prosthetic group and has a specific cofactor requirement for NADPH. It transforms ketones **1**, **3** to lactones **2**, **4** (100% ee in each case) but has not been tested with the tricyclic ketone **5**.

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