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A Proposal for The Origin of Stereoselectivity in Enzyme Catalysed Baeyer-Villiger Reactions

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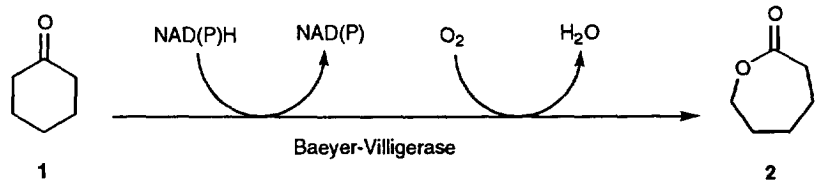
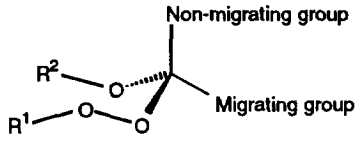
Abstract: The mechanism for flavoenzyme catalysed Baeyer-Villiger ring expansions is proposed to proceed via a 4-hydroxy-1,2,5-trioxane adduct formed from the ketone and a flavin hydroperoxide. In consequence the enantioselectivity of the transformation is dictated by which side of the flavin cofactor reacts with the ketone. Copyright © 1996 Elsevier Science Ltd

The Baeyer-Villiger reaction is the conversion of a ketone (usually cyclic) into the corresponding ester or lactone mediated by a hydroperoxide[1]. Although the abiotic reaction has been known for almost a hundred years[2], it is only some 20 years ago that an organism capable of achieving this transformation on a preparative scale was first developed. An *Acinetobacter sp.* (NCIMB 9871) grown on cyclohexanone **1** as a sole carbon source, was able to oxidise it to ε-caprolactone **2**[3]. The yellow flavin enzyme (cyclohexanone mono-oxygenase; CHMO) responsible was isolated and sequenced. Its versatility and enantioselectivity has been demonstrated in a series of studies by Walsh[4], Roberts[5], Furstoss[6], Taschner[7], and Knowles[8]. Baeyer-Villigerase activity has now been demonstrated in over a hundred organisms; five enzymes have been purified to homogeneity, but no crystal structure has been reported.

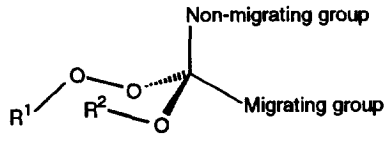


The mechanism of the Baeyer-Villiger reaction precedes *via* nucleophilic addition of hydroperoxide to the ketone to give a hydroxy-peroxide, which undergoes rearrangement to the lactone. In the abiotic reaction with weak peracids (such as peracetic acid) the rearrangement is the rate limiting step. The enantioselectivity demonstrated by all Baeyer-Villigerases can be rationalised by the formation of peroxides with the configurations shown in Figure 1. The most important feature of these structures is that the migrating group must be anti-periplanar to the peroxide bond in order to overlap with the anti-bonding orbital of this bond[9]. Given that these proposals are correct, the enantioselectivity of any Baeyer-Villigerase mediated ring expansion is solely a function of the diastereofacial selectivity of the enzyme for the ketone substrate. The tricyclic ketone **6** (Figure 2) can only be attacked by nucleophiles from the *exo*-face, because *endo*-attack is disfavoured by interactions with the cyclopentane rings. Hence individually the intermediates (a) and (b)

shown in Figure 1 can only give enantiomerically pure lactones (**8** and **ent-8** respectively) with this substrate. This has been tested experimentally; all five purified Baeyer-Villigerases give the lactones (**8** or **ent-8**) with >98% ee[10].



(a) *Acinetobacter* sp. NCIMB 9871, *P. putida* NCIMB 9872, *P. putida* NCIMB 10007 (MO2)



(b) 2,5-diketocamphane 1,2-monoxygenase and 3,6-diketocamphane 1,2-monoxygenase from *P. putida* NCIMB 10007 (MO1)

Figure 1. R^1 = flavin or acyl residue, R^2 = flavin or proton. Schematic representations of enantiomeric intermediates for Baeyer-Villiger reactions. The species indicated produce Baeyer-Villigerases which give lactones that can be rationalised by these intermediates. MO1 is monooxygenase one from this species, which is NADH dependant. MO2 is NADPH dependant.

The currently accepted mechanism for Baeyer-Villigerases is shown in the upper circuit in Figure 2. Baeyer-Villigerases are flavoproteins (usually containing non-covalently bound FAD **3**), in which the reduced flavin **4** is oxidised by dioxygen to give a flavin hydroperoxide **5**. Nucleophilic addition to the ketone **6** gives the hydroxy-peroxide intermediate **7** which rearranges to give lactone **8** and a hydroxy-flavin **9**. Elimination of water completes the cycle to give FAD **3**.

The origin of the stereoselectivity in the rearrangement of the hydroxy-peroxide **7** is still a matter of conjecture. If the O-O bond of this intermediate is fixed in space and defined as a reference point, then it is rotation about the C-O(-O-) bond of the peroxyhemiacetal which dictates whether intermediates (a) or (b) are accessed. If one rotomer can be formed preferentially, a stereoselective transformation will occur. In principle this could be achieved by immobilisation (binding) of the carbon framework of the former ketone moiety. However Baeyer-Villigerases accept a diverse range of ketones, which undergo ring expansion with congruent stereochemistry, hence this is unlikely. The alternative is to bind the hydroxyl **7** or alkoxyl group **11**. One possibility is intramolecular addition of the hydroxyl group in the intermediate **7** to the C-4 carbonyl group but this would require substantial activation of the latter by pyrimidisation and/or protonation. The proton might be donated in concert with addition of the alcohol (or alkoxide), but it is not clear how this could be achieved without loss of the adjacent proton on N-3. A more persuasive alternative is to postulate removal of the proton at N3 in the hydroperoxide **5**. The peroxidic hydrogen can then be shared with O4 *via* a hydrogen bond bridge[11]. This not only increases the nucleophilicity of the peroxide but also produces a potent electrophile adjacent to it. Self immolation to form a dioxetane is prevented by the rigidity of the ring system.

Addition of the peroxidic anion **10** to the ketone **6**, yields the alkoxide **11** which undergoes ring closure on C4 of the isoalloxazine to give the 4-hydroxy-1,2,5-trioxane **12** (Figure 2, 3). This has all the requisite bonding and non-bonding orbitals optimally aligned for Baeyer-Villiger rearrangement under stereoelectronic control and abstraction of the C-4 hydroxyl proton initiates rearrangement (Figure 3) to give the lactone **8** and

hydroxy flavin **9**. A trioxane has previously been suggested as an intermediate in an abiotic intramolecular Baeyer-Villiger reaction[12].

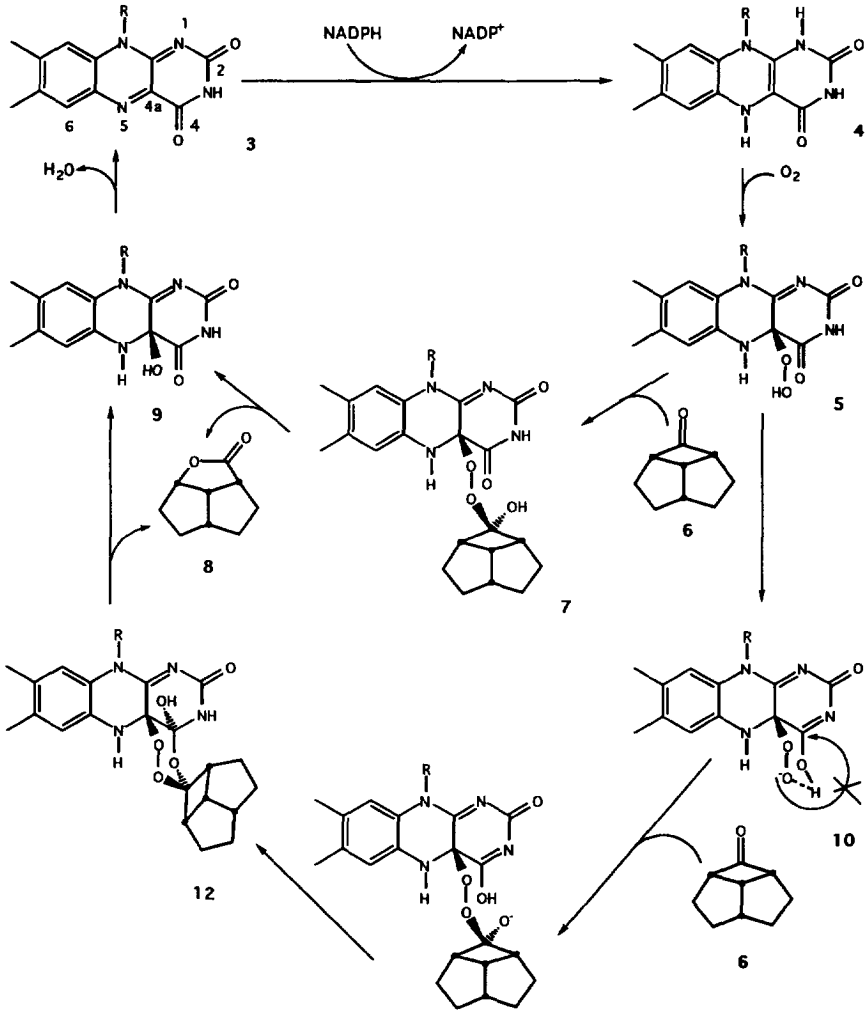


Figure 2. R= Adenosine diphosphate ribosyl

The stereochemistry shown in Figures 2 and 3 is that expected for reactions catalysed by the CHMO from *Acinetobacter* sp. NCIMB 9871. The mechanism requires that the hydroperoxide is attached to the *si* face of FAD. However previous workers have suggested that reaction occurs on the *re* face of FAD, because this is the face utilised by *p*-hydroxybenzoate hydroxylase (HBH)[13] and several other aromatic hydroxylases. HBH has some amino acid sequence homology with CHMO and glutathione reductase, but this is not surprising because all three utilise NADPH to donate hydrogen to the *re* face of FAD[14,15] and the nucleotide binding domains are probably derived from a common ancestral gene[16]. However, there is no evidence for substantial homology outside these domains.

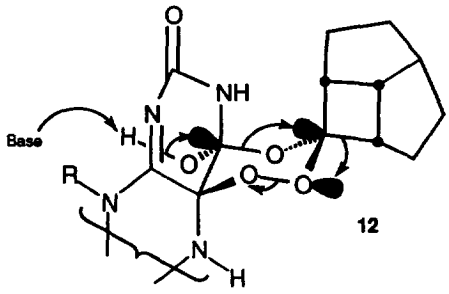


Figure 3. Transition state for the Baeyer-Villiger rearrangement. Anti-bonding orbitals are shown as black lobes.

The mechanism suggested above further requires that the enantiocomplementary transformations by 2,5-diketocamphane 1,2-monoxygenase and 3,6-diketocamphane 1,2-monoxygenase from *P. putida* NCIMB 10007 occur on the *re* face of FAD. If the proposals described here can be substantiated this would be the first example of a reaction in which the enantioselectivity is a consequence of diastereofacial selection on a nucleotide cofactor. Similar considerations apply to the oxidation of sulfides to sulfoxides and the decomposition of the aldehydic peroxide adduct of luciferase by flavin monoxygenases.

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