Biotransformations with Acetolactate Decarboxylase: Unusual Conversions of Both Substrate Enantiomers into Products of High Optical Purity

David H. G. Crout* and Daniel L. Rathbone

Department of Chemistry, University of Warwick, Coventry CV4 7AL, U.K.

Acetolactate decarboxylase catalyses the decarboxylation of both enantiomers of 2-ethyl-2-hydroxy-3-oxobutanoate to isomeric ketols of high optical purity and both enantiomers of α-acetolactate (2-hydroxy-2-methyl-3-oxobutanoate) to acetoin (3-hydroxybutan-2-one) of high optical purity.

Acetolactate decarboxylase [(*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxylase, EC 4.1.1.5][1] catalyses the decarboxylation of α-acetolactate (2-hydroxy-2-methyl-3-oxobutanoate) (**1**) and α-acetohydroxybutyrate (2-ethyl-2-hydroxy-3-oxobutanoate) (**2**), which are, respectively, biological precursors of valine and isoleucine. The *S*-isomers of the substrates (**1**) and (**2**) are the preferred substrates for the enzyme from *Aerobacter aerogenenes*.[2] The *R*-isomers are decarboxylated also but at a lower rate. It was shown that the product of decarboxylation of (*S*)-α-acetolactate (**1**) was (*R*)-acetoin [(*R*)-3-hydroxybutan-2-one] (**3**),[2,3] that the intermediate was protonated at the carbon atom that originally bore the carboxylate group,[4] and that the decarboxylation therefore proceeded with overall inversion of configuration (Scheme 1).

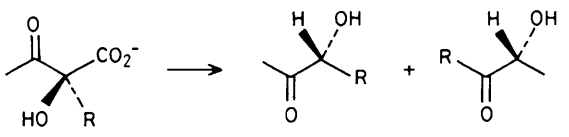
A solution of racemic α-acetohydroxybutyrate (**2**) was treated with an experimental sample of acetolactate decarboxylase from a *Bacillus* species.† The decarboxylation was followed by ^{1}H n.m.r., which indicated an initial rapid decarboxylation; this was accompanied by the appearance of signals attributable to the expected product ketol [as (**4**)] (Scheme 1). When 50% of the substrate had undergone decarboxylation, a second, slower decarboxylation began, accompanied by the appearance of signals attributable to the isomeric ketol (**5**). The product ketols were extracted into carbon tetrachloride and examined by ^{1}H n.m.r. (400 MHz) in the presence of an approximately two-fold molar excess of (*S*)-2,2,2-trifluoro-1-(9-anthryl)ethanol.[5] The ketols (**4**) and (**5**) were shown to have enantiomeric excesses (e.e.s) of 93 and 95%, respectively.

Hill and his co-workers[2] had suggested that the slow decarboxylations of the *R*-isomers of the substrates (**1**) and (**2**) were attributable to the known base-catalysed tertiary ketol rearrangement of α-acetohydroxy acids, a process that proceeds with migration of the carboxylate group.[6] This process causes racemisation of optically active α-acetolactate (**1**). It must therefore be accompanied, at least in part, by overall inversion of configuration in this degenerate rearrangement (Scheme 2). It was later pointed out[3] that although base-catalysed rearrangement was unlikely, as this only occurred *in vitro* at pH 12.9, an alternative possibility was that the enzyme itself catalysed the rearrangement of the *R*-isomer. Such a process would be consistent with the foregoing results, since enzyme-catalysed rearrangement of (*R*)-2-ethyl-2-hydroxy-3-oxobutanoate (**6**) into (*S*)-2-hydroxy-2-methyl-3-oxopentanoate (**7**), followed by stereospecific decarboxylation with protonation of the carbon atom to which the carboxylate group had been transferred, would lead to the observed 2-hydroxypentan-3-one (**5**) of high optical purity (Scheme 2).

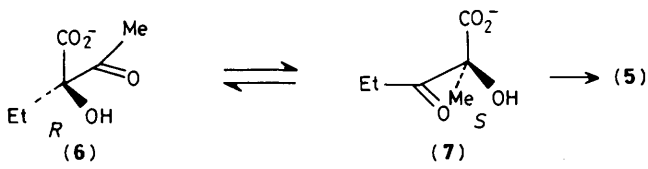
If this interpretation were correct, it could be predicted that decarboxylation of racemic α-acetolactate (both enantiomers) should lead exclusively to (−)-(*R*)-acetoin (**3**), the *R*-isomer (**8**) of the substrate being converted into (*R*)-acetoin *via* prior rearrangement into (*S*)-α-acetolactate (**1**) (Scheme 3). This prediction was tested by complete decarboxylation of racemic α-acetolactate (**1**) to (−)-(*R*)-acetoin (**3**). N.m.r. analysis as before indicated an e.e. of 92%. This conversion therefore appears to be an unusual example of the enzymic conversion of a racemic substrate essentially into a single enantiomer of the product.

The substrates for these decarboxylations were prepared by prior enzymic hydrolysis of the corresponding methyl or ethyl esters, using pig liver esterase (PLE), which catalysed hydrolysis of both enantiomers of the substrates. During hydrolysis, some non-enzymic decarboxylation occurred, accounting for the small amount of racemic ketol produced. When racemic methyl α-acetolactate was hydrolysed by PLE in the presence of the decarboxylase, the acetoin produced, after complete hydrolysis and decarboxylation of the substrate, had an e.e. of >98%.

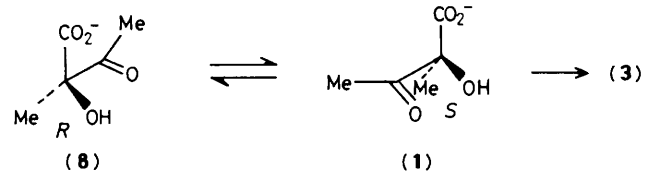
The absolute configurations of the ketols (**4**) and (**5**) have not been assigned. However, since in the presence of the chiral solvating agent the methyl singlet attributable to the ketol (**4**) appears in the upfield position, and the methyl



Scheme 1



Scheme 2



Scheme 3

† Kindly provided by Novo Industri S/R.

doublet attributable to the ketol (**5**) appears in the downfield position, as with the corresponding signals for (−)-(*R*)-acetoin (**3**), both ketols provisionally may be assigned the *R*-configuration. Additional circumstantial evidence for these assignments is the production of (−)-(*R*)-acetoin (**3**) from racemic α-acetolactate already described.

The results described here lend further weight to the suggestion[3] that acetolactate decarboxylase catalyses the decarboxylation of the *R*-enantiomers of the substrates *via* tertiary ketol rearrangement with carboxylate group migration, to the isomeric ketol (or to the identical ketol in the case of the degenerate rearrangement of α-acetolactate) with the *S*-configuration. If this interpretation is correct, the enzyme shows a capability for catalysing a tertiary ketol rearrangement with carboxylate migration that contrasts with the tertiary ketol rearrangement of (*S*)-α-acetolactate (**1**) and (*S*)-α-acetohydroxybutyrate (**7**) in the pathways respectively of valine and isoleucine biosynthesis.[2,7] These rearrangements, catalysed by the enzyme reductoisomerase, occur with migration of the methyl and ethyl groups, respectively.

We thank the S.E.R.C. for financial support.

Received, 7th May 1987; Com. 621

References

1 F. C. Stormer, *J. Biol. Chem.*, 1967, **242**, 1756.
2 R. K. Hill, S. Sawada, and S. M. Arfin, *Bioorg. Chem.*, 1979, **8**, 175.
3 D. H. G. Crout, J. Littlechild, and S. M. Morrey, *J. Chem. Soc., Perkin Trans. 1*, 1986, 105.
4 D. H. G. Crout, J. Littlechild, M. B. Mitchell, and S. M. Morrey, *J. Chem. Soc., Perkin Trans. 1*, 1984, 2271.
5 W. H. Pirkle and D. J. Hoover, *Top. Stereochem.*, 1982, **13**, 263.
6 D. H. G. Crout and C. J. R. Hedgecock, *J. Chem. Soc., Perkin Trans. 1*, 1979, 1982.
7 F. B. Armstrong, E. L. Lipscomb, D. H. G. Crout, M. B. Mitchell, and S. R. Prakash, *J. Chem. Soc. Perkin Trans. 1*, 1983, 1197.