Stereoelectronic Control of the Base-catalysed Rearrangement of 2-Hydroxy-3-oxocarboxylates

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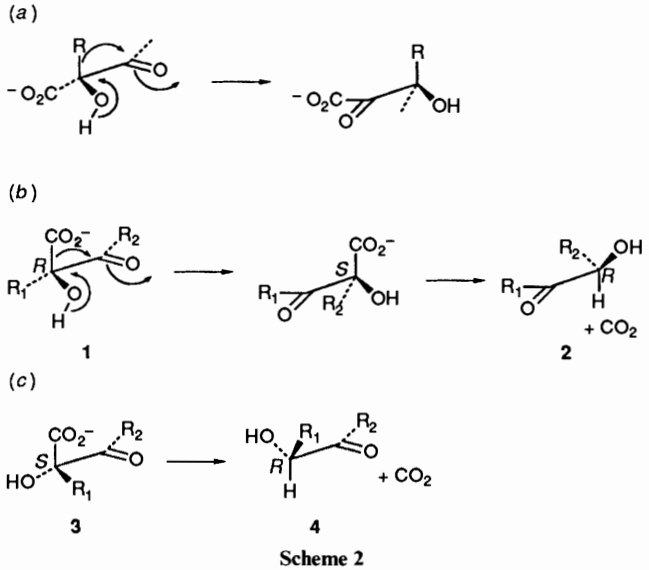
The stereochemistry of the alkali-catalysed rearrangement of α-acetohydroxybutyrate (2-ethyl-2-hydroxy-3-oxobutanoate) was studied. The rearrangement was found to proceed *via* a transition state in which a *syn* arrangement of the C–O bonds is preferred over the *anti* arrangement by a factor of ≥2:1.

During investigations into the stereochemistry of reactions of branched-chain amino acid metabolism, several tertiary ketol rearrangements have been studied which conform to the general transformation shown in Scheme 1. The relevant reactions are

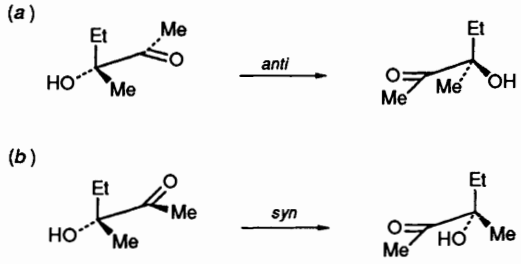
Scheme 1

shown in Scheme 2(*a*) and (*b*). In Scheme 2(*a*) is shown the rearrangement catalysed by the enzyme reductoisomerase (ketol-acid reductoisomerase, E.C. 1.1.1.86) of the pathway of valine–isoleucine biosynthesis (R = Me, valine pathway, R = Et, isoleucine pathway).[1] The overall stereochemistry of the transformation requires a *syn* arrangement of the carbon–oxygen bonds as shown.[2] In Scheme 2(*b*) is shown the rearrangement presumed to occur during conversion of (*R*)-2-hydroxy-3-oxocarboxylates **1** into (*R*)-ketols **2**, catalysed by acetolactate decarboxylase.[3,4] The rearrangement is postulated in order to explain the stereospecific conversion of (*R*)-substrates **1** into (*R*)-ketols **2** given that the preferred (*S*)-substrates **3** are converted into the isomeric (*R*)-ketols **4** [Scheme 2(*c*)].



Scheme 2

The rearrangement of Scheme 2(*b*) must also occur with the substrate in the conformation having a '*syn*' arrangement of the carbon–oxygen bonds. The rearrangement of Scheme 2(*b*) is also catalysed by alkali (pH > 13).[5] Since this rearrangement



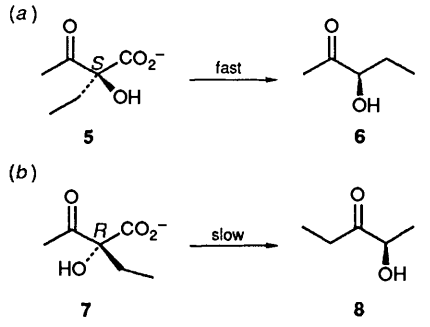
Scheme 3

is accompanied by racemisation, it too must proceed, at least in part, from a conformation with a *syn* arrangement of the carbon–oxygen substituents. The stereochemical course of these transformations suggested that there might be an inherent stereoelectronic preference for a *syn* arrangement of the carbon–oxygen substituents over the *anti* arrangement. However, an investigation of stereoelectronic control in a simple tertiary ketol rearrangement indicated a 1.8:1 preference for an *anti* mode [Scheme 3(*a*), (*b*)].[6] Therefore it was pertinent to enquire whether this preference would be maintained, or reversed by the introduction of the charged carboxylate group as the migrating entity. In order to investigate this question, it was necessary to bring about rearrangement of an optically-active substrate and to carry out a chiral analysis of the product. Because of the extreme lability of the 3-oxocarboxylate substrates, normal methods of chiral analysis were difficult to apply. However, a method has been devised based on the stereoselectivity and stereospecificity of the enzyme acetolactate decarboxylase (ADC) [(*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase, E.C. 4.1.1.5].

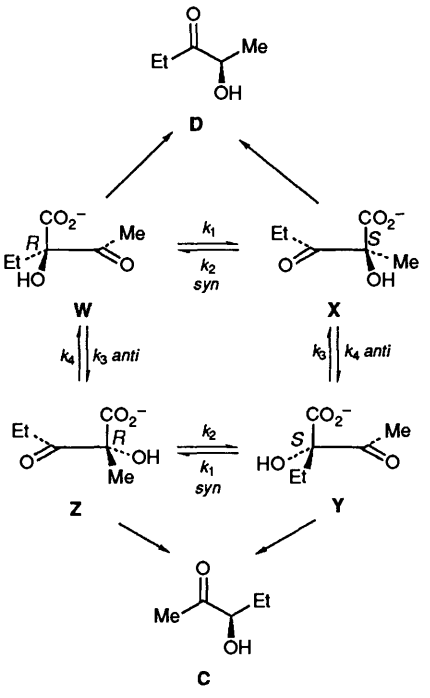
The substrate chosen for this investigation was 2-ethyl-2-hydroxy-3-oxobutanoate (α-acetohydroxybutyrate) **5**, the biological precursor of isoleucine. It had been shown that the action of acetolactate decarboxylase on this substrate in racemic form led to a rapid decarboxylation of the (*S*)-substrate **5** to (*R*)-3-hydroxy-2-pentanone **6** followed by a slower decarboxylation of the (*R*)-substrate **7** to the isomeric (*R*)-2-hydroxy-3-oxopentanone **8** (Scheme 4).[4]

It was clear from this work that if ADC-catalysed decarboxylation of racemic α-acetohydroxybutyrate were allowed to proceed to a conversion of 50%, all of the (*S*)-substrate **5** would be removed leaving optically pure (*R*)-substrate **7**. Alkali-catalysed rearrangement of this optically pure (*R*)-substrate **7** could proceed *via* either an *anti* or *syn* mode, and the respective products could further rearrange to generate the cyclic equilibrating system of the four species, **W**, **X**, **Y** and **Z** shown in Scheme 5.

Species **W** and **Y** are constitutionally identical and consequently would be present in equal concentrations at

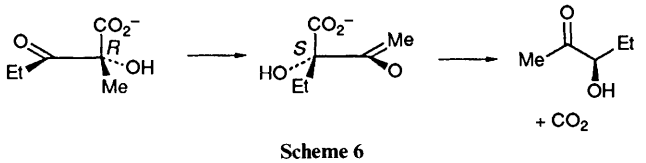


Scheme 4



Scheme 5

equilibrium. The same is true of species **X** and **Z**. The kinetics of the system can therefore be described by only four rate constants. Also, because of the cyclic nature of the equilibrating system, only three of these rate constants are independent. Thus if k_4 is chosen as the dependent rate constant, $k_4 = k_2k_3/k_1$. The conversions of Scheme 4 also indicated that ADC could be used for chiral analysis of the products, since the enantiomers of the substrate, **W** and **Y** would give constitutionally different ketols **D** and **C**, respectively. This method of chiral analysis had already been used to determine the absolute configuration of the α-acetohydroxybutyrate produced by the enzyme acetolactate synthase [acetolactate pyruvate-lyase (carboxylating), E.C. 4.1.3.18] of the pathway of isoleucine biosynthesis.[7] The (*S*)-enantiomer of the rearrangement product **X** would give ketol **D** on ADC-catalysed decarboxylation, by analogy with the transformation of Scheme 4(*a*). However, the corresponding (*R*)-enantiomer **Z** would only undergo decarboxylation after prior rearrangement with carboxylate migration to (*S*)-2-ethyl-2-hydroxy-3-oxobutanoate as shown in Scheme 6. The product of decarboxylation of the rearranged compound would be ketol **C**. The ketols expected to be produced from the equilibrating species are shown in Scheme 5.



Scheme 6

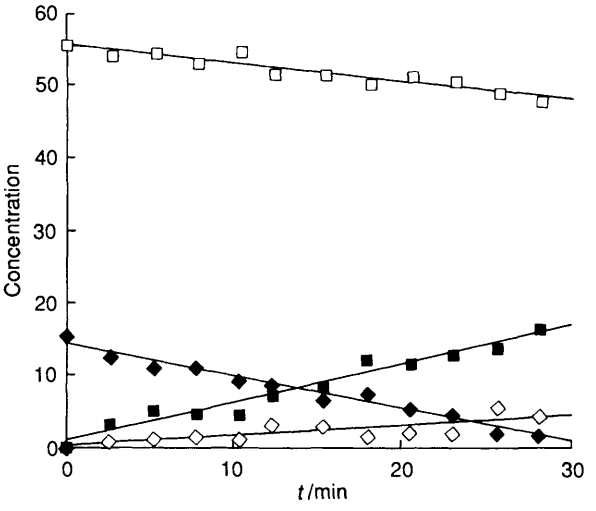
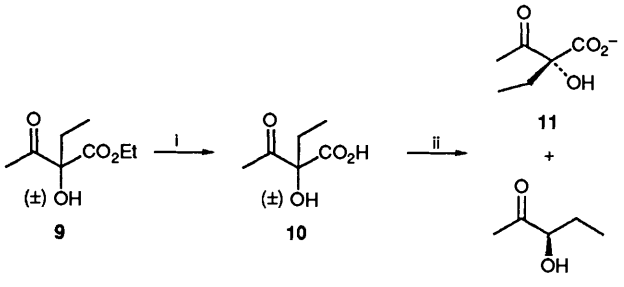


Fig. 1 Decarboxylation of the products of alkali-catalysed rearrangement of 2-ethyl-2-hydroxy-3-oxobutanoate, **W** (Scheme 5) catalysed by ADC. Products formed: □ = **A**; ◆ = **B**; ■ = **C**; ◇ = **D**. The changes in concentrations during the interval in which [**B**] changed from 14.37 (t = 0) to zero (t = 31.4 min) were: △[**A**] = 7.69 ± 0.60; △[**C**] = 4.31 ± 0.69; △[**D**] = 16.71 ± 0.88 (arbitrary concentration units).

The required α-acetohydroxybutyrate was prepared by enzyme-catalysed hydrolysis of the corresponding ethyl ester **9** (Scheme 7) using pig liver esterase (PLE). This procedure was known to be non-enantioselective.[4] Also, because it was carried out at pH 7.2 there was no danger of rearrangement occurring during hydrolysis.



Scheme 7 *Enzymes:* i, PLE; ii, ADC

The (*RS*)-α-acetohydroxybutyrate **10** (Scheme 7) was treated with ADC. The reaction was monitored by ^{1}H NMR and was stopped after >50% decarboxylation of the substrate, *i.e.* at a point where all of the (*S*)-enantiomer of the substrate would have undergone decarboxylation. The reaction mixture was filtered through a micropore filter and lyophilised to remove the ketol produced in the decarboxylation. The residual (*R*)-α-acetohydroxybutyrate **11** was treated with 1.5 mol dm^{-3} sodium hydroxide and the tertiary ketol rearrangement was followed by ^{1}H NMR. Rearrangement was allowed to proceed until the mixture contained 21% of rearranged product and 79% starting material. The reaction mixture was brought to pH 8.4 and lyophilised. The residue was dissolved in water and treated with ADC. The formation of ketol products was followed by ^{1}H NMR until all of the rearranged product **X** + **Z** (Scheme 5) had disappeared. The rate of disappearance of the carboxylates and the evolution of ketol products, as determined by integration of the ^{1}H NMR signals attributable to these various species, is shown in Fig. 1. Species **A** corresponds to the sum of (*R*)-α-acetohydroxybutyrate **W** (Scheme 5) and its enantiomer **Y**. Similarly **B** = **X** + **Z**. From the slopes of the concentration *vs.* time plots, linear regression analysis gave the best estimates of the changes in concentration between t = 0 and t when [**B**] = 0. In the following discussion, the concentration changes of Fig.

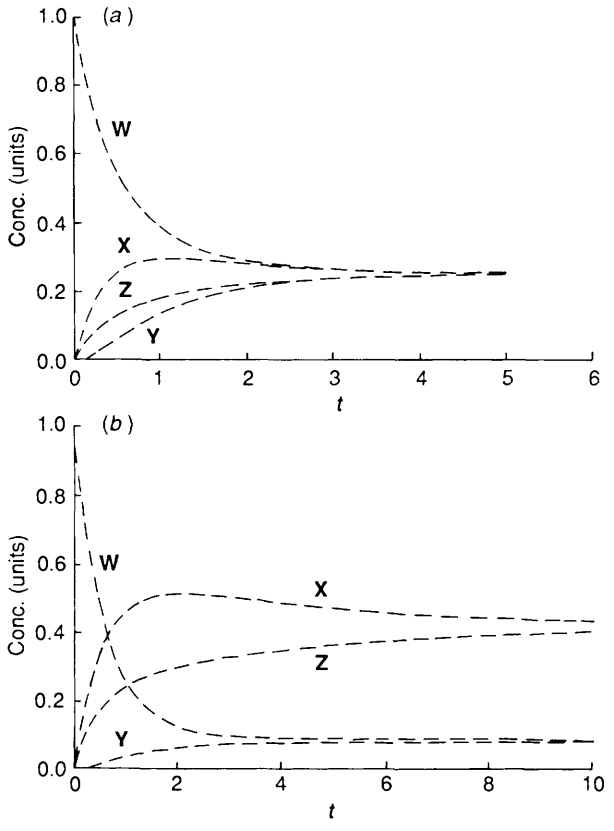


Fig. 2 Simulation of the evolution of the equilibria of Scheme 5 starting with [W] = 1, [X] = [Y] = [Z] = 0: (*a*) with $k_1 = k_2 = 1, k_3 = k_4 = 0.5$; (*b*) with $k_1 = 1, k_2 = 0.2, k_3 = 0.5, k_4 = 0.1$

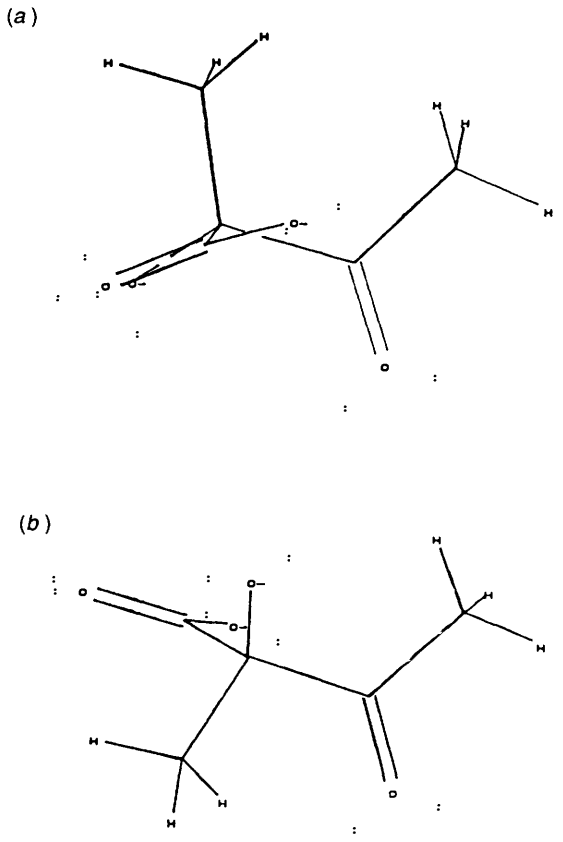


Fig. 3 Energy-minimised conformations of α-acetolactate (2-hydroxy-2-methyl-3-oxobutanoate) with the O=C–C–CO_2^- dihedral angle fixed at 90°, (*a*) with a '*syn*' and (*b*) with an '*anti*' conformation with respect to the C–O bonds

1 are taken as the basis of calculations of the amounts of the various species converted and produced in the product analysis using ADC. Because alkali-catalysed rearrangement (Scheme 7) had been allowed to proceed only to 21% of rearranged product, the unreacted carboxylate **A** (= **W** + **Y**, Scheme 5) would consist largely of the (*R*)-enantiomer (**W**, Scheme 5). This conclusion is supported by the slow decarboxylation of species **A** compared with that of the rearranged species **B** (= **X** + **Z**). The relatively rapid decarboxylation of the latter also suggested that it consisted predominantly of the (*S*)-enantiomer (**X**, Scheme 5). Decarboxylation of **A** was not followed to completion, because over an extended period, non-enzymatic decarboxylation would have been significant. However, the data obtained allowed calculation of limiting values of the *syn*/*anti* ratio in the carboxylate migration step. As the first case, let it be assumed that none of ketol **C** is derived from **A** (*i.e.* [**Y**] = 0, Scheme 5). In other words, all of ketol **C** is derived from species **Z**. It follows that the decrease in [**A**] is attributable solely to the decarboxylation of **W** giving ketol **D**. Thus the rest of **D** (16.71–7.69 = 9.02) would be derived from **X**, *i.e.* [**X**] = 9.02. Since, in this limiting case, none of ketol **C** would be derived from **A**, all of the ketol **C** produced would be derived from **Z**, *i.e.* [**Z**] = 4.31. The ratio [**X**]/[**Z**] = 9.02 (±1.06)/4.31 (±0.69) = 2.09 (±0.20) thus gives a lower limit for the ratio k_1/k_3 (Scheme 5) indicating a two-fold preference for a *syn* mode of rearrangement over an *anti* mode. An upper limit is less easy to arrive at. The second limiting case could be taken as that in which *all* of species **C** would be derived from **A** (*i.e.* [**Y**] = [**C**], Scheme 5), but this is clearly unrealistic. As a guide, the evolution of the species in Scheme 5 was simulated using a numerical integration computer program (FACSIMILE), with $k_1 = k_2 = 1, k_3 = k_4 = 0.5$. The results are shown in Fig. 2(*a*), from which it can be seen that the increase in concentration of species **Y** lags behind that of **Z** until almost complete equilibration has been reached. Accordingly, on this basis a reasonable upper limit for the ratio **Z**:**Y** would be 1:1. Thus half of ketol **C** would be derived from **Y** (=2.15) and thus the remainder of NMR species **A** [7.69 (±0.60) − 2.15 (±0.35) = 5.54 (±0.69)] would be converted into ketol **D**. The remainder of ketol **D** [16.71 (±0.88) − 5.54 (±0.69) = 11.17 (±1.12)] would therefore be derived from species **X**. The ratio **X**:**Z** would thus be 11.17 (±1.12):2.15 (±0.35) indicating an upper limit for k_1/k_3 of 5.2 (±0.19). In fact, from the simulation of Fig. 2(*a*), the ratio [**X**]/[**Z**] can be plotted against time. When this is done it is seen that the value of [**X**]/[**Z**] for 20% conversion falls from 2 only to 1.98. Indeed, even after 60% conversion, the value falls only to 1.7. It is therefore probable that the true value of k_1/k_3 (Scheme 5) lies closer to 2.09 than to 5.2.

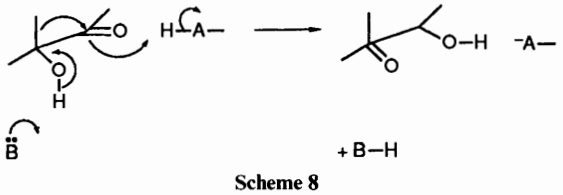
The assumption that $k_1 = k_2$, $k_3 = k_4$ might also be questioned, because there is a greater relief of steric strain at the migration origin in the conversion **W** to **X** than in the conversion **X** to **W** (and correspondingly for the other interconversions).[9] Thus if values for the rate constants are set at $k_1 = 1, k_2 = 0.2, k_3 = 0.5$ ($k_4 = 0.1$), the evolution of **W**, **X**, **Y** and **Z** as computed by the FACSIMILE program is as shown in Fig. 2(*b*). From this it can be seen that 20% conversion (of **W**), [**Y**] is still nearly zero. On this basis, therefore, the true value for k_1/k_3 would again be expected to lie much closer to the value 2.09 than to 5.2.

It can therefore be concluded that the substitution of a carboxylate group for an alkyl group causes the '*anti*' preference in the simple case (Scheme 3)[6] to switch to a '*syn*' preference in the rearrangement of α-acetohydroxybutyrate.

Possible reasons for this reversal were explored using the molecular mechanics program PCMODEL. As in the case of simple tertiary ketol rearrangement, it was assumed that the

minimum energy pathway would be one in which the C–CO_2^- bond would be parallel to the π system of the carbonyl group at the migration terminus. With this restriction, the conformation with *syn* and *anti* arrangements of the C–O bonds in α-acetolactate were minimised. The minimum energy conformations are shown in Figs. 3(*a*) and (*b*), respectively. The *syn*-conformer was found to have an energy 23 kJ mol^{-1} above that of the *anti*-conformer. As in the simple case, these energies (to which dipole–dipole repulsions make the major contribution) would be significantly reduced by solvation, no account of which is taken in these calculations. However, it is significant that this energy difference is considerably smaller than the corresponding difference for *syn* and *anti* conformers in the simple case (Scheme 3) which was found to be 35 kJ mol^{-1}. The difference is largely attributable to the conformation of the carboxylate group [Fig. 3(*a*)], which adopts the best average conformation to oppose simultaneously the C–O^- and C=O dipoles. (The molecular mechanics calculations assumed a perturbed charge distribution for the carboxylate group with fractional point charges of −0.28 and −0.81 of the electronic charge at the carboxyl and anionic oxygen atoms respectively). Corresponding minimisations were carried out for α-acetohydroxybutyrate in the '*syn*' and '*anti*' conformations. The energy difference between the two conformers is now only 11 kJ mol^{-1}. The minimum energy '*anti*' conformers for α-acetolactate and α-acetohydroxybutyrate are nearly superimposable. With the '*syn*' conformers the carboxylate oxygen atoms are displaced in the two conformers by 0.21 and 0.16 Å from each other, and there is lower bonding strain in the '*anti*' conformer of α-acetohydroxybutyrate relative to the '*syn*' isomer than for the corresponding α-acetolactate conformers. It would therefore require only a small differential effect of solvation of the '*syn*' and '*anti*' conformers of α-acetohydroxybutyrate for the scales to be tipped in favour of the '*syn*' conformer. It can be concluded that the '*syn*' preference revealed in the present investigations is attributable to the net result of finely balanced combinations of electronic, steric and solvation effects.

It remains to consider whether in the enzymatic reactions catalysed by ADC and reductoisomerase other factors may intervene to increase the preference for a '*syn*' mode of rearrangement. The most obvious suggestion is for there to be simultaneous general base–general acid catalysis (Scheme 8).



Scheme 8

Protonation of the carbonyl group as an isolated event would be slow, even if the process were to resemble a gas-phase reaction as proposed as a general description of enzymatic reactions by Dewar.[10] However, during carboxylate migration, the basicity of the carbonyl oxygen atom steadily increases to the point where proton transfer would become very fast. It is a question therefore as to the timing of the deprotonation, migration and protonation steps. If protonation were to occur as an early point during carboxylate migration, there would be a significant positive dipole–dipole interaction in the '*syn*'-conformer that would be negative in the '*anti*'-isomer. Full expression of this effect could be seen when the full zwitterionic

12 **13**

Scheme 9

species were modelled (Scheme 9). The '*syn*' conformer **12** (Scheme 9) was now more stable than the '*anti*' conformer **13** by 84 kJ mol^{-1}. These calculations ignore the effect of the charged conjugate acid and conjugate base of the presumed general base and general acid catalytic groups respectively. However, given that these are disposed so as not to prevent at least partial expression of the dipolar interaction, a considerable advantage might accrue to the '*syn*' transition state relative to the '*anti*' transition state.

Experimental

^{1}H NMR spectra were determined at 220 MHz using a Perkin-Elmer R34 spectrometer or at 400 MHz using a Bruker WH400 spectrometer. Coupling constants *J* are given in Hz. Software used was PCMODEL (Serena Software, Bloomington, Indiana, USA), TECHNICURVE (Ashton Scientific Ltd, Ashton Clinton, Bucks., UK) and FACSIMILE (Computer Science and Systems Division, Harwell, UK). Acetolactate decarboxylase was a gift from Dr. S. E. Godtfredsen, Novo Industri, Copenhagen, Denmark. Pig liver esterase was purchased from Boehringer.

Alkali-catalysed Rearrangement of (R)-α-*Acetohydroxybutyrate and Chiral Analysis of the Product.*—Ethyl α-acetohydroxybutyrate[8] (60 mg, 0.34 mmol), was dissolved in phosphate buffer (0.5 mol dm^{-3}, pH 7.2, 3 cm^3). To the solution at 37 °C was added pig liver esterase (100 mm^3, 130 U). Ester hydrolysis was followed by ^{1}H NMR (220 MHz). ^{1}H NMR spectra were recorded at intervals of 20 min, and after 2 h, hydrolysis was complete. The ^{1}H NMR spectrum consisted of signals attributable to ethanol and to (±)-α-acetohydroxybutyrate; δ_H 0.85 (3 H, t, *J* 7.2, *Me*CH_2), 1.9 (2 H, m, MeCH_2) and 2.29 (3 H, s, *Me*CO) (signals attributable to α-acetohydroxybutyrate) and δ_H 1.2 (3 H, t, *J* = 7.0, *Me*CH_2) and 3.68 (2 H, q, *J* 7.0 Hz, MeCH_2) (signals attributable to ethanol). To the solution was added acetolactate decarboxylase (1 mg, 15 U). The progress of decarboxylation was monitored by ^{1}H NMR. Initially, signals attributable to 3-hydroxypentan-2-one appeared; δ_H 0.9 (3 H, t, *J* 7.3, *Me*CH_2), 1.8 (2 H, m, MeCH_2) and 2.1 (3 H, s, MeCO). After 45 min, the ^{1}H NMR spectrum indicated that the decarboxylation of (*S*)-α-acetohydroxybutyrate was complete and signals attributable to 2-hydroxypentan-3-one, the decarboxylation product of the (*R*)-enantiomer, began to appear (of which the most characteristic was the doublet; δ_H 1.3 (*J* 7.2) attributable to the *Me*CH(OH) group). The reaction mixture was filtered by centrifugation (4,500 g) at −10 °C through a 10,000 MW cut-off micro-pore filter (Centricon-10). The filtered solution was lyophilized. (If protein was not removed in this way, the product tended to decompose during lyophilization). The lyophilized solid was dissolved in aqueous sodium hydroxide (1.5 mol dm^{-3}, 3 cm^3) and filtered through glass wool to give a clear solution. ^{1}H NMR spectra of the solution were recorded at intervals of 15 min. After 1 h, signals attributable to both α-acetohydroxybutyrate and 2-hydroxy-2-methyl-3-oxopentanoate were observed; δ_H 0.95 (3 H, t, *J* 7.2, *Me*CH_2CO) and 1.4 [3 H, s, *Me*C(OH)]. The rearrangement was allowed to continue until 20% of the starting material had been converted. The solution was acidified to pH 8.4 (HCl, 1.2 mol dm^{-3}), lyophilized, and the residual solid was dissolved in water (0.9 cm^3). The solution was filtered and acetolactate decarboxylase (0.5 mg, 7 U) was added. The subsequent decarboxylation at 25 °C was monitored by 400 MHz NMR with water suppression and data acquisition at intervals of 3 min. The rates of disappearance of 3-oxocarboxylates and the rates of production of ketols were monitored as described above. The results are illustrated in Fig. 1.

Acknowledgements

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