A Simple Strategy for obtaining both Enantiomers from an Aldolase Reaction: Preparation of L- and D-4-Hydroxy-2-ketoglutarate

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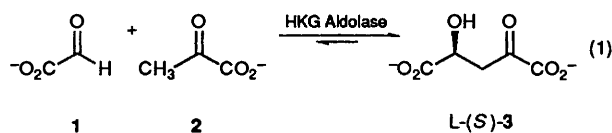
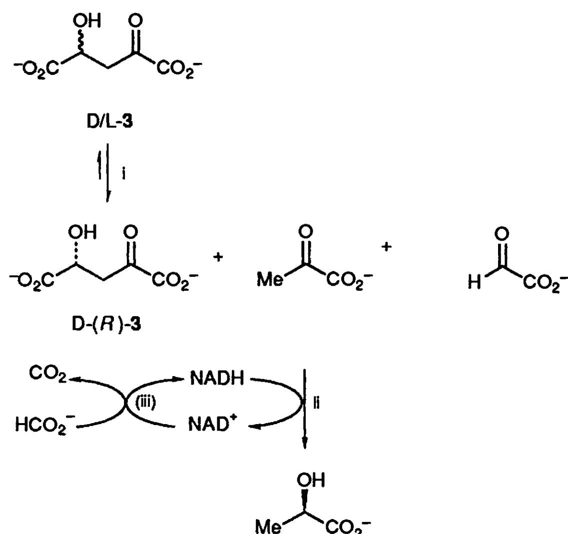
Both L- (>95% e.e.) and D- (60% e.e.) 4-hydroxy-2-ketoglutarate 3 have been prepared on a mmol scale using 4-hydroxy-2-ketoglutarate (HKG) aldolase. Substrate specificity studies indicate that HKG aldolase isolated from different sources (*Escherichia coli*, bovine liver and rat liver) is highly specific for both pyruvate and glyoxylate.

Amongst those enzymes capable of catalysing stereospecific carbon–carbon bond formation, the aldolases have been shown to be particularly useful.[1] For example, fructose-1,6-diphosphate aldolase from rabbit muscle[2] and *Escherichia coli*[3] will utilise a wide range of unnatural aldehydes as the electrophilic component of the aldol reaction and has been used to prepare a number of natural[4] and unnatural[5] sugar derivatives. In order to extend the range of aldolases available for synthesis we have studied the aldolase that condenses glyoxylate **1** with pyruvate **2** to give 4-hydroxy-2-ketoglutarate



3 [eqn. (1)]. The enzyme from *E. coli* is known to produce the L-(*S*)-isomer of **3**[6] whereas both the rat liver[7] and bovine liver[8] enzymes are reported to give racemic material. Herein we describe a procedure for using the *E. coli* HKG aldolase to prepare both L-(*S*)-**3** and D-(*R*)-**3**. 4-HKG aldolase was isolated from *E. coli* K-12 cells according to the literature procedure.[6] The reported equilibrium constant for this reaction is 0.76 dm^3 $mmol^{-1}$ in phosphate buffer (pH 7.3).[6] In order to overcome the problem of the unfavourable equilibrium, the condensation of pyruvate and glyoxylate was carried out at a relatively high (50 mmol dm^{-3}) concentration. This lead to a quantitative (>95%) conversion of pyruvate and glyoxylate to L-(*S*)-**3** as evidenced by ^1H NMR. After termination of the reaction, isolation of the product on ion-exchange resin gave L-(*S*)-**3** in 70% yield. The optical purity of the product was determined by conversion into the dimethyl ester ($AgNO_3$ followed by MeI, reflux[9]) followed by ^1H NMR spectroscopy (250 MHz) in the presence of 2,2,2-trifluoro-1-(3-anthryl)ethanol (e.e. > 95%) [NB. the addition of 2,2,2-trifluoro-1-(3-anthryl)ethanol to a solution of the racemic dimethyl ester resulted in the expected separation of the two methyl ester signals which integrated 1:1]. The absolute configuration was assigned as *S* by oxidative decarboxylation (H_2O_2)[10] of L-(*S*)-**3** to L-(*S*)-malic acid.

To obtain the D-isomer of 4-HKG we envisaged the system shown in Scheme 1. Preferential HKG aldolase catalysed cleavage of the L-isomer of racemic 4-HKG should occur leaving the D-isomer in solution. To ensure that the reaction went to completion lactate dehydrogenase was added which reduced the pyruvate to lactate. Catalytic NADH was added and recycled by the formate/formate dehydrogenase system. When the reaction was monitored by ^1H NMR spectroscopy the appearance of lactate could be detected and the reaction was allowed to proceed to *ca.* 50% conversion when the incubation was stopped. Isolation of the remaining 4-HKG by ion-exchange chromatography and analysis by chiral shift NMR



Scheme 1 Enzymes: i, L-4-hydroxy-2-ketoglutarate aldolase; ii, L-lactate dehydrogenase; iii, formate dehydrogenase

spectroscopy as above indicated an enantiomeric excess of 60%. Treatment with H_2O_2 converted D-(*R*)-**3** into D-(*R*)-malic acid.

We have also investigated the substrate specificity of 4-HKG aldolase. Previous work on the rat liver enzyme had indicated a tolerance for a wide range of glyoxylate and pyruvate analogues[7] whereas the *E. coli* and bovine liver enzymes have been less well studied. Our results with all three enzymes have revealed a high degree of specificity for both glyoxylate and pyruvate. Small variations in the structure of the substrates (*e.g.* replacement of pyruvate by α-ketobutyrate) resulted in either very low or no conversion into product.† Our results with the rat liver enzyme are at considerable variance with previous work,[7] a discrepancy that we have not yet been able to resolve.

In summary, we have described a practical method for the preparation of both L-(>95% e.e.) and D-(60% e.e.) 4-hydroxy-2-ketoglutarate on a mmol scale.

Experimental

Preparation of L-(S)-**3**.—To a mixture of sodium glyoxylate (114 mg, 1.0 mmol) and sodium pyruvate (110 mg, 1.0 mmol) was added sodium phosphate buffer (10 cm^3, 50 mmol dm^{-3}; pH 6.8) and 4-HKG aldolase (10 cm^3; 3.8 U in pH 6.8, 50 mmol dm^{-3} phosphate buffer). The solution was left at 27 °C for 24 h after which time the reaction was shown by TLC to have gone to completion (BuOH–AcOH–H_2O 5:2.5:2.5). The solution was lyophilised and the crude product (511 mg) redissolved in water (15 cm^3) and applied to a column of Dowex, 1-X8–200

† Full details of these experiments will be reported elsewhere.

resin (HCO_3^- form, 80 × 17 mm). The column was eluted stepwise with an increasing concentration of aqueous triethylammonium hydrogen carbonate (50 cm^3 of 50 mmol dm^{-3}, 100 cm^3 of 100 mmol dm^{-3}, 100 cm^3 of 150 mmol dm^{-3}, 150 cm^3 of 200 mmol dm^{-3}, 50 cm^3 of 250 mmol dm^{-3}) and fractions (4 cm^3) collected. Tubes 32–67 containing L-**3** were combined and lyophilised to yield the bistriethylammonium salt of L-**3** as a white powder (273 mg). This was redissolved in water (15 cm^3), treated with Dowex 50-X8 (Na^+ form, 5 cm^3) for 30 min, filtered and lyophilised to give the disodium salt of L-(*S*)-**3** as a hygroscopic solid (152 mg, 70%); $[\alpha]_D^{30}$ −7.4.* Compound **3** possessed full spectroscopic data in accord with its structure.

Preparation of D-(*R*)-**3**.—To a solution of D/L-**3** disodium salt (500 mg, 2.43 mmol) in sodium phosphate buffer (pH 7.3; 50 mmol dm^{-3}, 10 cm^3) was added sodium formate (170 mg, 2.50 mmol), formate dehydrogenase (10 U, 16.5 mg), nicotinamide adenine dinucleotide (reduced) (8 mg, 0.0012 mmol) and L-lactate dehydrogenase (100 U, 200 mm^3, pH 7.3 phosphate buffer). The solution was mixed and HKG aldolase (3.8 U, 10 cm^3, pH 6.8 phosphate buffer) added. The reaction was shaken at 37 °C and after 64 h ^1H NMR analysis showed the reaction to have gone to 51% conversion. The solution was lyophilised and D-(*R*)-**3** isolated as described above (191 mg, 78%); $[\alpha]_D^{30}$ = +2.4.†

* The specific rotation for this compound has not previously been reported.

† The slightly low value obtained is due to the hygroscopic nature of the sample.

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References

1 H. G. Davies, R. H. Green, D. R. Kelly and S. M. Roberts, *Biotransformations in Preparative Organic Chemistry: The Use of Isolated Enzymes and Whole Cell Systems in Synthesis*, Academic Press, London, 1989, pp. 221–231.
2 M. D. Bednarski, E. S. Simon, N. Bischofberger, W.-D. Fessner, M.-J. Kim, W. Lees, T. Saito, H. Waldmann and G. M. Whitesides, *J. Am. Chem. Soc.*, 1989, **111**, 627.
3 C. H. von der Osten, A. J. Sinskey, C. F. Barbas III, R. L. Pederson, Y.-F. Wang and C.-H. Wong, *J. Am. Chem. Soc.*, 1989, **111**, 3924.
4 N. J. Turner and G. M. Whitesides, *J. Am. Chem. Soc.*, 1989, **111**, 624; M. Schultz, H. Waldmann, W. Vogt and H. Kunz, *Tetrahedron Lett.*, 1990, **31**, 867.
5 W. Schmid and G. M. Whitesides, *J. Am. Chem. Soc.*, 1990, **112**, 9670.
6 H. Nishihara and E. E. Dekker, *J. Biol. Chem.*, 1972, **247**, 5079; E. E. Dekker, H. Nishihara and S. R. Grady, *Methods Enzymol.*, 1975, **42**, 285.
7 J. M. Scholtz and S. M. Schuster, *Bioorg. Chem.*, 1984, **12**, 229.
8 R. D. Kubes and E. E. Decker, *J. Biol. Chem.*, 1969, **244**, 1919; R. S. Lane, A. Shapley and E. E. Decker, *Biochemistry*, 1971, **10**, 1353.
9 L. Garanti, U. M. Pagnoni and F. Bellesia, *Ann. di Chimica*, 1973, **63**, 759.
10 S. C. Gupta and E. E. Decker, *J. Biol. Chem.*, 1984, 259, 10012.

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