Tetrahedron Letters,Vol.28,No.12,pp 1277-1280,1987 0040-4039/87 $3.00 + .00
Printed in Great Britain Pergamon Journals Ltd.

LARGE-SCALE ENZYMATIC SYNTHESIS OF DIASTEREOISOMERIC γ-HYDROXY L-GLUTAMIC ACIDS.

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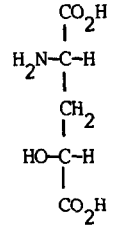
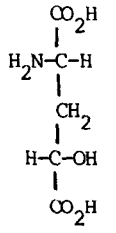
Summary : Transamination of cysteine sulfinic acid and γ-hydroxy α-cetoglutaric acid, catalyzed by immobilised glutamic oxaloacetic aminotransferase provides a convenient route to γ-hydroxy L-glutamic acids synthesis.

γ-hydroxy L-glutamic acids are natural products : erythro isomer , (2S,4R) 1a, is an intermediate in hydroxyproline metabolism in mammals (1), threo isomer (2S,4S) 1b, was isolated from Phlox decussata (2). They are obviously interesting chiral synthons, as for exemple in synthesis where the starting material, L-glutamic acid has to be modified on C4 position (3).

Ia   Ib

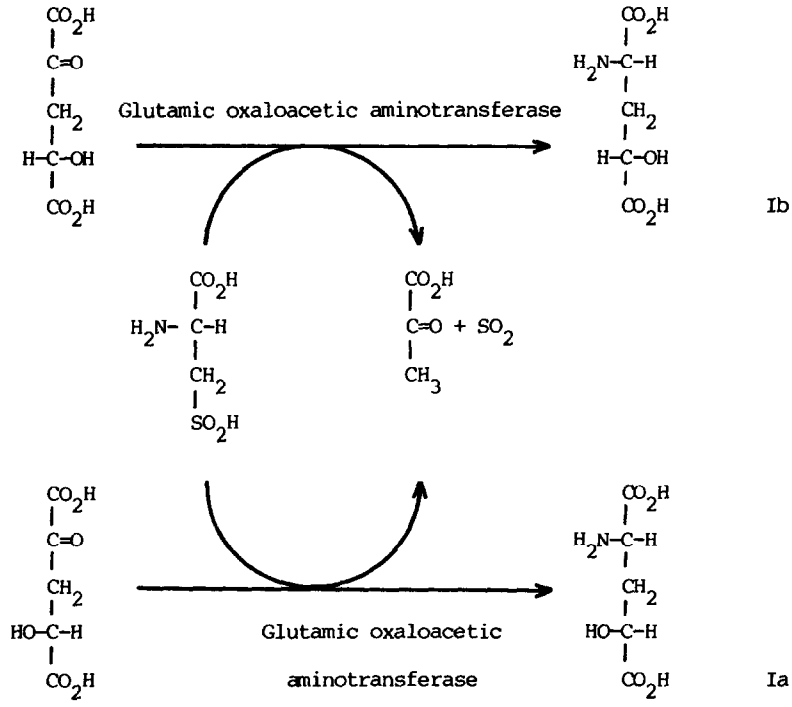
Chemical synthesis of these compounds has already been described, however, the procedure is long and requires a resolution of the racemates (4). Two commercial enzymes could be used for enzymatic synthesis : glutamic dehydrogenase (E.C. 1.4.1.3.) and glutamic oxaloacetic

aminotransferase (E.C. 2.6.1.1.). Only the former has already been used for a millimole scale synthesis of these acids (5,6), but a practical application is not possible without an efficient system for the regeneration of the expensive required cofactor (NADH). Although this problem was extensively studied lately and could be considered as resolved for at least a laboratory-scale synthesis (7), it still remains expensive. We had rather prefered to study the system, based on glutamic oxaloacetic aminotransferase utilisation (8).

In this paper we describe an aminotransferase-catalyzed synthesis of γ-hydroxy L-glutamic acids Ia and Ib, carried out on 0.1 mole scale, which could be easily scaled up.

Goldstone and Adams (9) showed that the D γ-hydroxy α-cetoglutaric acid is a good substrate for glutamic oxaloacetic aminotransferase from rat liver. With aspartic acid as an amino group donor, Vmax is 60 % of the observed value for natural substrate, Km is low (4 mM vs. 0.3 mM for α-cetoglutaric acid). However, the equilibrium position is unfavorable, and to get a good yield and to avoid problems in aminoacids separation, it is necessary to drive the reaction to complete consumption of the aminoacid donor.

We have realized this synthesis using an analog of aspartic acid, cysteine sulfinic acid (10). The transamination product, very unstable is decomposed into SO_2 and pyruvic acid (scheme).



To improve the stability of the enzyme in a long time running reactor, and to allow its recovery, it has been immobilised by covalent binding on activated polyacrylamide (PAN) following Whitesides' procedure (11). The immobilisation yield was 45 %.

Racemic γ-hydroxy α-cetoglutaric acid was prepared following (12) by condensation of oxaloacetic acid on glyoxylic acid in water at pH 7 and at room temperature. Oxaloacetic acid excess is destroyed by dropping the pH to 3. The resulting solution, once the pH adjusted again to 7 is used without further purification.

A representative experiment is described below : to 1 l of aqueous solution of D,L γ-hydroxy α-cetoglutaric acid (150 mM) and cysteine sulfinic acid (110 mM) were added 400 Units of immobilised glutamic oxaloacetic aminotransferase (150 ml). The reaction was monitored by HPLC and enzymatic assay (13). After 9 hours, cysteine sulfinic acid was completely consumed and the mixture contained both erythro and threo γ-hydroxy L-glutamic acids in equal amounts. The gel, containing 90 % of the original enzymatic activity, was separated by centrifugation. The solution was filtered on a DOWEX 50 X2 (H^+) column (300 ml) and hydroxyglutamic acids eluted with 0.5 N HCl. The pooled fractions were evaporated, taken up with water and chromatographied on a DOWEX 1X4 (AcO^-) column (200 ml) eluted with 0.5 N acetic acid. The pooled fractions were evaporated giving 15.4 g (yield : 86 %) of γ-hydroxy L-glutamic acids (98% pure by enzymatic assay). Separation of the isomers was performed on small scale (300 mg) by ion exchange chromatography on DOWEX 1X2 (15b). (NMR, IR and polarimetric data were in accordance with litterature (4, 6,14)). However ion exchange chromatography is not suitable for large scale separation. In this case, a chemical method, described by Benoiton and coll. (4) can be used : only the Ib isomer undergoes a lactonisation in strong acidic conditions.

The synthesis described here provides a practical route to γ-hydroxy L-glutamics acids. The final concentration of the product (100 mM) cannot be increased without a loss of enzymatic activity, but the procedure is simple enough to be carried out in batches of larger volume or in continuous reactor. The enzyme is cheap and stable. Starting hydroxycetoglutaric acid is prepared in water and no purification is necessary. Cysteine sulfinic acid is rather expensive but it can be prepared from cystin (15). Cheaper procedures based on utilisation of aspartic acid as an aminodonor are under investigation.

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(Received in France 22 December 1986)