701. *Syntheses of α-Amino-β-hydroxy-acids. Part II.** erythro- *and* threo-β-*Hydroxy*-DL-*aspartic Acid.*

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The *erythro*- and *threo*-isomers of β-hydroxy-DL-aspartic acid have been synthesized stereospecifically by reaction of *trans*- and *cis*-epoxysuccinic acid, respectively, with benzylamine, and catalytic hydrogenolysis of the *N*-benzyl derivatives.

β-Hydroxyaspartic acid which had been found in pancreatic digests of casein,[1] and whose enzymic formation by a transamination between glutamate and oxaloglycollate had been established by two independent groups,[2,3] was first synthesized by Dakin by reaction between chloromalic acid and ammonia.[4] He obtained a mixture of the racemic *erythro*- and the *threo*-isomer which was separated by fractional crystallization from water. Kornguth and Sallach recently prepared it by condensation of sodium glyoxylate with copper glycinate.[5] These authors also developed a method for the separation of the mixture of the diastereoisomers obtained, with the aid of Dowex 1 ion-exchange columns. Franklin [6] described a synthesis by catalytic hydrogenation of ethyl α-oxo-β-phenylhydrazonosuccinate and hydrolysis of the resulting amino-ester, which yielded a mixture of the *threo*- and *erythro*-isomers in a ratio of **3 : 4**.

In all these instances, mixtures of the two stereoisomeric racemates were obtained, but no synthesis for the stereospecific preparation of either of them has been published up to now.† Having found that α-amino-β-hydroxy-acids can be prepared stereospecifically by reaction of either *cis*- or *trans*-epoxy-acids with benzylamine and hydrogenolysis of the resulting *N*-benzyl derivatives,[7] we undertook to extend this method to the synthesis of the *threo*- and *erythro*-β-hydroxyaspartic acids, by starting with maleic and fumaric

* Part I, Liwschitz, Rabinsohn, and Perera, *J.*, 1962, 1116.

† In ref. 5 mention is made of a modification of Dakin's method by W. Shive (personal communication) in which chloromalate is treated with ammonia at room temperature for 8 days instead of being heated in an autoclave for 10 hr. This procedure yielded 95% of the *erythro*- and only 5% of the *threo*-form, irrespective of whether fumarate or maleate was the starting compound. This should be due to the preponderant formation of *trans*-epoxysuccinic acid which is the more stable isomer, in both cases at hand. We were, however, unable to reproduce these results on the basis of this information.

[1] Sallach and Kornguth, *Biochim. Biophys. Acta*, 1959, **34**, 582.
[2] Sallach and Peterson, *J. Biol. Chem.*, 1956, **223**, 629.
[3] Garcia-Hernandez and Kun, *Biochim. Biophys. Acta*, 1957, **24**, 78.
[4] Dakin, *J. Biol. Chem.*, 1921, **48**, 273.
[5] Kornguth and Sallach, *Arch. Biochem. Biophys.*, 1960, **91**, 39.
[6] Franklin, *J.*, 1960, 4709.
[7] Liwschitz, Rabinsohn, and Perera, *J.*, 1962, 1116.

acid, respectively. Epoxidation of these acids by hydrogen peroxide with sodium tungstate as catalyst has been described by Payne *et al.*[8] On reaction of either of the epoxy-acids with benzylamine in aqueous solution, the benzylamine salt of the *threo-* or *erythro-N*-benzyl-β-hydroxyaspartic acid was obtained in high yield. These compounds, as well as the free acids, obtained by acidification of aqueous solutions of the salts to pH 4 with hydrochloric acid, had distinctly different infrared spectra, although their physical properties (m. p.s, solubilities, etc.) were similar. Hydrogenolysis had to be carried out in glacial acetic acid as ethanol and other solvents were unsuitable. It was best to use directly the benzylamine salt which initially dissolved in glacial acetic acid; the free *N*-benzyl-β-hydroxyaspartic acid was then precipitated in a finely divided state which was much more susceptible to the catalytic hydrogenolysis which was performed, as usual, at 70—80° with a 30% palladium chloride–Norite catalyst. The reaction time in the case of the *erythro*-isomer was much longer, and complete hydrogenolysis was effected only after about 20 hours. Both of the hydroxyaspartic acids, being insoluble in glacial acetic acid, were deposited together with the catalyst, from which they were freed by recrystallization from hot water. To the more soluble *erythro*-isomer ethanol was added to achieve more complete precipitation. Neither compound was mixed with its epimer. The *threo*-isomer was analyzed by means of an automatic amino-acid analyzer and the elutogram proved it to be entirely pure. The vanadate test,[9] carried out on the tartaric acids derived by deamination with nitrous acid, according to Greenstein's method,[10] was positive in the case of the *threo*-isomer (giving rise to DL-tartaric acid) but negative with the *erythro*-isomer (from which *meso*-tartaric acid resulted). Both acids gave a positive biuret reaction which is characteristic of α-amino-β-hydroxy-acids.[7] We have been unable to find a solvent system for separation of the diastereoisomers by paper chromatography.

Experimental

Melting points were determined in a Fisher–Johns apparatus.

Benzylamine Salt of N-*Benzyl*-threo-β-*hydroxy*-DL-*aspartic Acid*.—To *cis*-epoxysuccinic acid [8] (4 g.) in water (10 ml.) was added benzylamine (11 g.), and the mixture was heated under reflux for 3 hr. After cooling, the salt which crystallized was filtered off and washed with acetone. The *product* (9·4 g., 90%) had m. p. 187—188° (from water or methanol) [Found: C, 62·4; H, 6·8; N(total), 8·1; N(Van Slyke), 4·1. $C_{18}H_{22}N_2O_5$ requires C, 62·4; H, 6·4; N(total), 8·1; N(Van Slyke), 4·1%].

N-*Benzyl*-threo-β-*hydroxy*-DL-*aspartic Acid*.—The benzylamine salt (6·9 g.) was dissolved in 15% sodium hydroxide solution (8 ml.), and the liberated benzylamine was extracted with ether. The solution was then acidified to pH 4 with hydrochloric acid, whereupon the *product* was precipitated quantitatively. It was recrystallized from a large volume of water and had m. p. 225—226° (Found: C, 55·2; H, 5·6; N, 6·0. $C_{11}H_{13}NO_5$ requires C, 55·2; H, 5·4; N, 5·9%).

threo-β-*Hydroxy*-DL-*aspartic Acid*.—The benzylamine salt of *N*-benzyl-*threo*-β-hydroxy-DL-aspartic acid (5·3 g.) was dissolved in glacial acetic acid (200 ml.), and the catalyst (0·3 g. of 3 : 10 palladium chloride–Norite) was added quickly before the free acid was precipitated. Hydrogenolysis was carried out in a Parr low-pressure apparatus for 5 hr. at 70—80°. The *threo*-β-hydroxyaspartic acid was precipitated and was separated from the catalyst by recrystallization from water (yield 2 g., 90%). It gave positive ninhydrin and biuret reactions (Found: C, 32·6; H, 4·9; N, 9·4. Calc. for $C_4H_7NO_5$: C, 32·2; H, 4·7; N, 9·4%).

Benzylamine Salt of N-*Benzyl*-erythro-β-*hydroxy*-DL-*aspartic Acid*.—To *trans*-epoxysuccinic acid [8] (2·4 g.) in water (5 ml.) was added benzylamine (6 g.), and the mixture was heated under reflux for 4 hr. The excess of benzylamine was extracted with ether, and to the aqueous layer was added acetone which precipitated the *benzylamine salt* (5·5 g., 87%), m. p. 184—186° (from aqueous ethanol) (Found: C, 62·3; H, 6·5; N, 8·1. $C_{18}H_{22}N_2O_5$ requires C, 62·4; H, 6·4; N, 8·1%).

[8] Payne and Williams, *J. Org. Chem.*, 1959, **24**, 54.
[9] Matchett, Legault, Nimmo, and Notter, *Ind. Eng. Chem.*, 1944, **36**, 851.
[10] Benoiten, Winitz, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1957, **79**, 6192.

N-*Benzyl*-erythro-β-*hydroxy*-DL-*aspartic Acid.*—The benzylamine salt (5 g.) was dissolved in water (5 ml.) and hydrochloric acid was added to pH 4. The *product* was precipitated quantitatively, m. p. 222° (from a large volume of water) (Found: C, 55·1; H, 5·5; N, 6·0. $C_{11}H_{13}NO_5$ requires C, 55·2; H, 5·4; N, 5·9%).

erythro-β-*Hydroxy*-DL-*aspartic Acid.*—The benzylamine salt of *N*-benzyl-*erythro*-β-hydroxy-DL-aspartic acid (2·7 g.) was dissolved in glacial acetic acid (120 ml.), and the catalyst (0·5 g.) was added. Hydrogenolysis was carried out under the conditions used for the *threo*-isomer for 20 hr. The product was extracted from the catalyst by hot water. After filtration and addition of ethanol it was precipitated (0·8 g., 70%). It gave positive ninhydrin and biuret reactions (Found: C, 32·4; H, 4·8; N, 9·4. Calc. for $C_4H_7NO_5$: C, 32·2; H, 4·7; N, 9·4%).

Nitrous Acid Deamination of threo- *and* erythro-β-*Hydroxy*-DL-*aspartic Acid to* DL-*Tartaric and* meso-*Tartaric Acid.*—Each of the two diastereoisomers (0·025 g.) was treated with nitrous acid.[10] After completion of the deamination, as indicated by the disappearance of the ninhydrin reaction,[11] the solution was neutralized and a sample (3 ml.) of each was submitted to the vanadate test [9] by adding a 2% sodium metavanadate solution (0·4 ml.) while shaking, followed by 50% acetic acid (0·1 ml.). The sample derived from the *threo*-isomer (DL-tartaric acid) developed a red colour, in contradistinction to that prepared from the *erythro*-isomer (*meso*-tartaric acid) which did not change colour and remained yellow like a control.

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[11] Sallach, *J. Biol. Chem.*, 1957, **229**, 437.