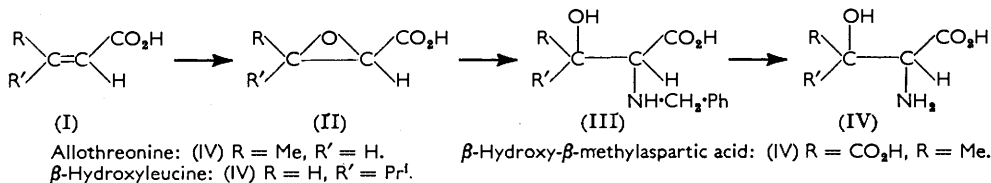


**207. Syntheses of  $\alpha$ -Amino- $\beta$ -hydroxy-acids. Part I. DL-Allothreonine, DL-erythro- $\beta$ -Hydroxyleucine, and DL-erythro- and threo- $\beta$ -Hydroxy- $\beta$ -methylaspartic Acid.**

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$\alpha$ -Amino- $\beta$ -hydroxy-acids have been prepared by the reaction of benzylamine with 2,3-epoxy-acids and subsequent hydrogenolysis of the *N*-benzyl derivatives. From *trans*-acids *erythro*-isomers are produced whereas *cis*-acids give rise to *threo*-compounds.

BESIDES serine and threonine, which have long been known to constitute building stones of proteins, other  $\alpha$ -amino- $\beta$ -hydroxy-acids, such as  $\beta$ -hydroxyleucine<sup>1</sup> and  $\beta$ -hydroxyaspartic acid<sup>2</sup> have recently been isolated from natural sources. Since these substances contain two asymmetric carbon atoms, two diastereoisomeric pairs exist, but most methods devised so far for their synthesis generally yield mixtures of the respective *erythro*- and *threo*-isomers.\* On investigating the opening of 2,3-epoxy-acids with benzylamine,<sup>4</sup> we found that this reaction represents a stereospecific method for the simultaneous introduction of amino- and hydroxyl-groups and should prove of value for the synthesis of  $\alpha$ -amino- $\beta$ -hydroxy-acids for which the required  $\alpha\beta$ -unsaturated fatty acids (I) are readily available. The latter can efficiently be epoxidised by hydrogen peroxide in the



presence of sodium tungstate,<sup>5</sup> to yield the 2,3-epoxy-acids (II). Reaction with benzylamine then results in the formation of  $\alpha$ -benzylamino- $\beta$ -hydroxy-acids (III) from which the free amino-acids (IV) are obtained by catalytic hydrogenolysis with 30% palladium-charcoal.

\* A stereospecific synthesis for allothreonine was described by Carter and Zirkle.<sup>3</sup>

<sup>1</sup> Kenner and Sheppard, *Nature*, 1958, **181**, 48.

<sup>2</sup> Saris and Virtanen, *Acta Chem. Scand.*, 1957, **11**, 1440.

<sup>3</sup> Carter and Zirkle, *J. Biol. Chem.*, 1949, **178**, 709.

<sup>4</sup> Liwschitz and Rabinsohn, *Bull. Res. Council Israel*, 1960, **9**, 55.

<sup>5</sup> Payne and Williams, *J. Org. Chem.*, 1959, **24**, 54.

The opening of the oxiran ring by ammonia or benzylamine could result in either  $\alpha$ -amino(or benzylamino)- $\beta$ -hydroxy-acids or  $\alpha$ -hydroxy- $\beta$ -amino(or benzylamino)-acids. According to the literature<sup>6</sup> oxirans to which a polar substituent (in the case at hand a carboxyl group) is attached are opened to produce the latter substances. It appeared, however, that the reaction in the cases investigated by us proceeded exclusively by the alternative course and in no instance were products other than the  $\alpha$ -benzylamino- $\beta$ -hydroxy-acids isolated, a fact which had already been observed by Carter and Zirkle in their synthesis of allothreonine.<sup>3</sup> Regarding the stereochemistry of the ring opening, it has been established that inversion of configuration occurs which is compatible with the  $S_N2$  mechanism postulated for this reaction.<sup>6</sup> By starting with *trans*-acids, one obtains exclusively *erythro*-isomers, and the corresponding compounds possessing the *threo*-configuration are formed from *cis*-acids.

DL-Allothreonine, not contaminated by either its epimer or the two  $\alpha$ -hydroxy- $\beta$ -benzylaminobutyric acids, was obtained by reaction of benzylamine with  $\alpha\beta$ -epoxybutyric acid (from crotonic acid). This was unequivocally proved by paper chromatography.<sup>7</sup> Among the methods employed to detect the eventual formation of  $\alpha$ -hydroxy- $\beta$ -amino-acids in all cases under discussion, one was that of Larsen and Kjaer<sup>8</sup> by which  $\alpha$ -mono-amino-acids can be distinguished from other ninhydrin-positive substances by treatment with methanolic cupric nitrate before development of the ninhydrin colour which then either is absent or appears at most as a very faint violet spot, in contrast to other amino-acids in which the carboxyl group is further from the amine function so that complex formation with copper does not then occur. DL-Allothreonine was also obtained directly by treating the epoxy-acid with ammonia, thus confirming that the glycidic acid was an intermediate in Carter and Zirkle's synthesis of allothreonine from  $\alpha$ -bromo- $\beta$ -hydroxybutyric acid.<sup>3</sup>

DL-*erythro*- $\beta$ -Hydroxyleucine which had previously been prepared by Abderhalden,<sup>9</sup> by Buston and Bishop,<sup>10</sup> and by a stereospecific synthesis by Dalby *et al.*,<sup>11</sup> was obtained similarly from *trans*- $\alpha\beta$ -epoxy- $\gamma$ -methylvaleric acid (which was not isolated) and it conformed to the *erythro*-isomer described by Kenner and Sheppard.<sup>1</sup>

The two  $\beta$ -hydroxy- $\beta$ -methylaspartic acids recently obtained as a mixture of the racemic epimers by Greenstein and his co-workers<sup>12</sup> were formed as separate entities when starting with citraconic and mesaconic acid, respectively. The  $R_F$  values for these two acids were in accordance with those given by Greenstein *et al.* for their solvents. The *threo*-isomer was expected to result from citraconic and the *erythro*-isomer from mesaconic acid. This was confirmed by the infrared spectra and by applying the vanadate test<sup>13</sup> to the two methyltartaric acids obtained by treating the  $\beta$ -hydroxy- $\beta$ -methylaspartic acids with nitrous acid according to the procedure of Benoiton *et al.*<sup>14</sup> The *threo*-isomer gave a red colour but the *erythro*-isomer failed to react. This represents not only an extension of the vanadate test to substituted tartaric acids, but also shows that the *erythro*-compound was entirely free from its epimer, since the colour test is very sensitive.

All  $\alpha$ -amino- $\beta$ -hydroxy-acids prepared gave a positive biuret reaction. We have observed that any two of the following three groups cause a compound to give a positive biuret reaction: amine, hydroxyl, and amide. Thus, not only asparagine, serine, threonine, or  $\alpha$ -amino-acid amides (*e.g.*, leucine-amide) give a positive biuret reaction, but also lactamide, ethanolamine, and diethanolamine.

<sup>6</sup> Parker and Isaacs, *Chem. Rev.*, 1959, **59**, 737.

<sup>7</sup> Shaw and Fox, *J. Amer. Chem. Soc.*, 1953, **75**, 3421.

<sup>8</sup> Larsen and Kjaer, *Biochim. Biophys. Acta*, 1960, **38**, 148.

<sup>9</sup> Abderhalden, *Z. physiol. Chem.*, 1938, **251**, 164.

<sup>10</sup> Buston and Bishop, *J. Biol. Chem.*, 1955, **215**, 217.

<sup>11</sup> Dalby, Kenner, and Sheppard, *J.*, 1960, 968.

<sup>12</sup> Benoiton, Winitz, Colman, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1959, **81**, 1726.

<sup>13</sup> Matchett, Legault, Nimmo, and Notter, *Ind. Eng. Chem.*, 1944, **36**, 851.

<sup>14</sup> Benoiton, Winitz, Birnbaum, and Greenstein, *J. Amer. Chem. Soc.*, 1957, **79**, 6192.

## EXPERIMENTAL

M. p.s were determined in a Fisher-Johns apparatus.

*N-Benzyl-DL-allothreonine*.—A mixture of *trans*- $\alpha\beta$ -epoxybutyric acid<sup>8</sup> (5.1 g.), water (7 ml.), and benzylamine (8 g.) was heated under reflux for 2 hr. After cooling, the product which was precipitated in almost quantitative yield was filtered off and recrystallised from aqueous ethanol. It had m. p. 248° (Found: C, 63.1; H, 7.1; N, 6.5. Calc. for  $C_{11}H_{15}NO_3$ : C, 63.1; H, 7.2; N, 6.7%). Attenburrow *et al.*<sup>15</sup> prepared this compound by a different route, but it seems that their product contained a small amount of *N*-benzyl-DL-threonine which lowered the m. p. to 238° and caused hydrogenation to yield "slightly impure allothreonine."

*DL-Allothreonine*.—(a) *From N-benzyl-DL-threonine*. *N*-Benzyl-DL-allothreonine (5 g.) was suspended in glacial acetic acid (120 ml.) and the catalyst (0.3 g. of 3:10 palladium chloride-Norite) added. Hydrogenolysis was carried out in a Parr low-pressure apparatus for 5 hr. at 60–70°. After cooling, the catalyst was filtered off and part of the substance which adhered to it was extracted by cold formic acid. After removal of both solvents *in vacuo*, the product recrystallised from water in almost quantitative yield. It had m. p. 260° (decomp.) (Found: C, 40.2; H, 7.7; N, 11.7. Calc. for  $C_4H_9NO_3$ : C, 40.2; H, 7.6; N, 11.8%).

(b) *From trans- $\alpha\beta$ -epoxybutyric acid*. To *trans*- $\alpha\beta$ -epoxybutyric acid (8 g.) was added 25% ammonia solution (145 ml.), and the stoppered flask was set aside at room temperature for 4 days. After evaporation to dryness *in vacuo*, the residue was dissolved in a small quantity of water, and DL-allothreonine (5 g., 54%) was precipitated by addition of ethanol. Chromatography<sup>7</sup> showed both products to be pure allothreonine.

*N-Benzyl-DL-erythro- $\beta$ -hydroxyleucine*.—(a) *Epoxidation of trans-4-methylpent-2-enoic acid*. An inhomogeneous mixture of this acid<sup>10</sup> (13.7 g.) and water (60 ml.) was stirred and heated to 50°, and sodium hydroxide (2.4 g.) in water (20 ml.) was added dropwise. Heating was stopped, sodium tungstate (4 g.) was added at once, and 30% hydrogen peroxide (18 ml.) during 5 min. The temperature remained at about 50° and the pH at about 5 throughout the reaction (if necessary more sodium hydroxide solution, as above, was added). After 1 hr. the temperature started to drop. The mixture was then cooled to 40° and sodium hydroxide solution added (total 1 equiv.). Part of the water was then evaporated *in vacuo* at 40°, up to a volume of 40 ml.

(b) *Reaction with benzylamine*. The above solution containing the sodium salt of the epoxy-acid was heated with benzylamine (19 g.), under reflux for 2 hr. After cooling, the excess of benzylamine was extracted with ether and the pH of the aqueous solution was made about 5–6 with hydrochloric acid. The precipitate was washed with acetone and then with ether. The product (15.7 g., 55%) had m. p. 224–228° (from dimethylformamide) (Found: C, 64.7; H, 7.9; N, 6.0.  $C_{13}H_{19}NO_3$  requires C, 65.7; H, 8.0; N, 5.9%).

*DL-erythro- $\beta$ -Hydroxyleucine*.—*N-Benzyl-DL-erythro- $\beta$ -hydroxyleucine* (5 g.) was suspended in glacial acetic acid (200 ml.) and catalyst (0.3 g.) was added. The hydrogenolysis was carried out for 12 hr. at about 70°. The catalyst and the substance which adhered to it were filtered off and the solvent was removed *in vacuo*. The residue was taken up in a small quantity of water and the amino-acid was precipitated with acetone. The remainder of the substance was then freed from the catalyst by extraction with boiling water and precipitation with acetone (total, 2 g., 65%); it had m. p. 219–220° (Found: C, 48.5; H, 8.7; N, 9.5. Calc. for  $C_6H_{13}O_3N$ : C, 49.0; H, 8.9; N, 9.5%). The infrared spectrum conformed to that of the *erythro*-isomer described by Dalby *et al.*<sup>11</sup>

*trans-Epoxymethylsuccinic Acid*.—(a) *As the disodium salt*. To mesaconic acid (22 g.) and water (60 ml.), sodium hydroxide (10 g.) in water (15 ml.) was added with stirring. This was followed by sodium tungstate (9.3 g.) and 30% hydrogen peroxide (20 ml.). The temperature was kept at 63–64°, at first by cooling, then by heating for 1½ hr. To maintain a pH of ~4 0.5N-sodium hydroxide was added. Most of the water was then removed *in vacuo* and the residue poured into a large volume of acetone, whereby the disodium salt was obtained as a viscous oil. The acetone was decanted and fresh acetone added; after this operation had been repeated twice, the substance crystallised in quantitative yield when scratched.

(b) *As the barium salt*. The disodium salt (24 g.) was dissolved in the minimum quantity of

<sup>15</sup> Attenburrow, Elliott, and Penny, *J.*, 1948, 310.

water and poured with stirring into a hot solution of barium chloride (40 g.) in water (120 ml.). The barium salt was precipitated at once in quantitative yield.

(c) *The free epoxy-acid.* To the barium salt, suspended in ether (300 ml.) and water (5 ml.), was added, with stirring, concentrated sulphuric acid (23 g.) in ether (70 ml.). After 24 hours' stirring at room temperature, the barium sulphate was filtered off and extracted with ether, and most of the ether was removed from the combined ethereal solutions. Pouring the residue into light petroleum (200 ml.) precipitated the free *epoxy-acid*. This was redissolved in ether and reprecipitated by light petroleum in almost quantitative yield. It had m. p. 155—156° (Found: C, 41.2; H, 4.5.  $C_5H_8O_5$  requires C, 41.1; H, 4.1%).

*N-Benzyl-DL-erythro- $\beta$ -hydroxy- $\beta$ -methylaspartic Acid.*—To *trans*-epoxymethylsuccinic acid (7.5 g.) water (12 ml.) and benzylamine (18 g.) were added and the mixture was heated under reflux for 4.5 hr. After cooling, the excess of benzylamine was extracted with ether, and the aqueous layer was acidified with hydrochloric acid to pH 4—5. The *product* (10 g., 82%) had m. p. 168—169° (from methanol) (Found: C, 56.9; H, 5.6; N, 5.3.  $C_{12}H_{15}NO_4$  requires C, 56.9; H, 5.9; N, 5.5%).

*DL-erythro- $\beta$ -Hydroxy- $\beta$ -methylaspartic Acid.*—*N-Benzyl-DL-erythro- $\beta$ -hydroxy- $\beta$ -methylaspartic acid* (3 g.) was suspended in glacial acetic acid (100 ml.), and the catalyst (0.5 g.) was added. Hydrogenolysis was carried out as usual for 5 hr. Since the *product* adhered to the catalyst, it was extracted with boiling water, from which it crystallised on cooling (1.1 g., 60%); it had m. p. 243—244° (Found: C, 36.6; H, 5.8; N, 8.5.  $C_5H_9NO_5$  requires C, 36.8; H, 5.5; N, 8.6%).

*cis-Epoxymethylsuccinic Acid.*—Citraconic acid was oxidised in the same manner as mesaconic acid, but the amount of the sodium tungstate catalyst was 3 moles %. The disodium salt was obtained in 90% yield. The barium salt could not be prepared by the procedure used for the *trans*-acid, because of its solubility in water. Therefore, isolation of the free epoxy-acid was not undertaken, and benzylamine was added to the disodium salt.

*N-Benzyl-DL-threo- $\beta$ -hydroxy- $\beta$ -methylaspartic Acid.*—To disodium *cis*-epoxymethylsuccinic acid (9.5 g.) in water (20 ml.) was added benzylamine (18 g.), and the mixture was heated under reflux for 2.5 hr. After cooling, the excess of benzylamine was extracted with ether, and the aqueous solution acidified with hydrochloric acid to pH 4—5. The *product* (10.6 g., 90%) had m. p. 218° (from water) (Found: C, 57.2; H, 6.2; N, 5.3.  $C_{12}H_{15}NO_5$  requires C, 56.9; H, 5.9; N, 5.5%).

*DL-threo- $\beta$ -Hydroxy- $\beta$ -methylaspartic Acid.*—*N-Benzyl-DL-threo- $\beta$ -hydroxy- $\beta$ -methylaspartic acid* (5 g.) was suspended in glacial acetic acid (180 ml.) and the catalyst (0.3 g.) added. Hydrogenolysis was carried out for 5 hr. The catalyst was then filtered off and the solvent evaporated *in vacuo*. The residue, recrystallised from water, had m. p. 234—236° (2.4 g., 78%) (Found: C, 37.0; H, 5.6; N, 8.6.  $C_5H_9NO_5$  requires C, 36.8; H, 5.6; N, 8.6%). The *N*-benzoyl derivative melted at 166° (Benoiton *et al.*<sup>12</sup> reported m. p. 163—164° for the benzoyl derivative of the diastereoisomeric mixture).

*Nitrous Acid Deamination of DL-erythro- and -threo- $\beta$ -Hydroxy- $\beta$ -methylaspartic Acid to the Corresponding Methyltartaric Acids.*—0.25 g. of each of the two  $\beta$ -hydroxy- $\beta$ -methylaspartic acids was deaminated with nitrous acid according to the procedure of Benoiton *et al.*<sup>14</sup> After neutralisation a sample (3 ml.) of each was treated as follows.<sup>13</sup> A 2% sodium metavanadate solution (0.4 ml.) was added while shaking, followed by 50% acetic acid (0.1 ml.). The sample derived from the *threo*-isomer developed a red colour but that of the *erythro*-isomer was unaffected. Chromatography of both diastereoisomers by the procedure of Benoiton *et al.*<sup>12</sup> gave identical  $R_F$  values which conformed to those given by these authors for their  $\beta$ -hydroxy- $\beta$ -methylaspartic acid.