Optical Resolution of *trans*-DL-1, 2-Epoxysuccinic Acid and Preparation of D-(−)-*erythro*-β-Hydroxyaspartic Acid[1]

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(Received July 27, 1967)

(−)-*trans* - 2, 3 - Epoxysuccinic acid ($[\alpha]_D$ = −100°), [(−)-epoxy acid], was prepared by Kuhn and Zell from D-tartaric acid by chlorination and subsequent alkaline treatment.[2] The configuration of the (−)-epoxy acid was determined as D- by converting the (−)-epoxy acid to D-(+)-malic acid.[2] However, the (−)-epoxy acid prepared from D-tartaric acid might not be a pure compound isomerically and optically, because two substitution reactions could be involved during the synthesis. On the other hand, it has been known that optically active (−)-epoxy acid was produced by *Penicillium viniferum* and *Monilia formosa*.[3] For preparative purpose, the fermentative method involving glucose and *Aspergillus fumigatus* was used which produced a relatively high yield of the (−)-epoxy acid. The specific rotation of the (−)-epoxy acid prepared by the fermentative method was reported to be −118° in ethanol.[4]

threo- and *erythro*-β-Hydroxyaspartic acids were first prepared by Dakin.[5] Kornguth and Sallach[6] prepared a mixture of *threo*- and *erythro*-isomers by condensation of glyoxylic acid with copper glycinate. They separated these two isomers by the use of ion-exchange column chromatography. Liwschitz *et al.*[7] reported the syntheses of *threo*- and *erythro*-DL-β-hydroxyaspartic acid by benzylamination and subsequent hydrogenolysis of *cis*- and *trans*-2, 3-epoxysuccinic acid. Recently, optically active *erythro*-(+)-β-hydroxyaspartic acid ($[\alpha]_D$ +51°) was prepared by the use of transaminase on dihydroxyfumaric acid and L-glutamic acid.[8] Optically active *erythro*-(+)-isomer was also synthesized ($[\alpha]_D$ +49°) by Miller[9] by ammonolysis of (−)-epoxy acid (95% pure) which was prepared by fermentation. β-Hydroxyaspartic acid was also found in a culture medium of some Azotobacter[10] and as a constituent of Phallicidine.[11] In the latter case, isolated β-hydroxyaspartic acid was identified as D-*erythro*-β-hydroxyaspartic acid ($[\alpha]_D$ −54°). Recently, Kaneko and Katsura[12] assigned the configurations of four isomers of β-hydroxyaspartic acid. In their study, optically active *erythro*-L-(+)-β-hydroxyaspartic acid ($[\alpha]_D$ +53.0°) was synthesized from (−)-epoxy acid which was derived from D-tartaric acid.

In this investigation, in order to examine the isomerical and optical purity of *trans*-(−)-2, 3-epoxysuccinic acid prepared by fermentation, optically pure *trans*-L-(+)-2, 3-epoxysuccinic acid (III) was prepared by resolution of *trans*-DL-epoxy acid (I) by the use of *l*-ephedrine. The *trans*-DL-epoxy acid was prepared by epoxidation of fumaric acid (Scheme 1).[13] The resolved (+)-epoxy acid (III) showed a specific rotation of +117.8°, which was the same specific rotation (−118°) in absolute value as that prepared by the fermentative method.[4] Therefore the (+)-epoxy acid is the antipode of that prepared by the fermentative method. According to these results, the epoxy acid obtained by fermentation could be the optically pure *trans*-isomer. Using the resolved (+)-*trans*-epoxy acid (III), optically active *erythro*-(−)-β-hydroxyaspartic acid (VII) (Scheme 1) was prepared by amination with benzylamine[7] to check the optical purity of the *erythro*-β-hydroxyaspartic acid obtained by the transamination reaction.[8] Reported specific rotations of optically active *erythro*-β-hydroxyaspartic

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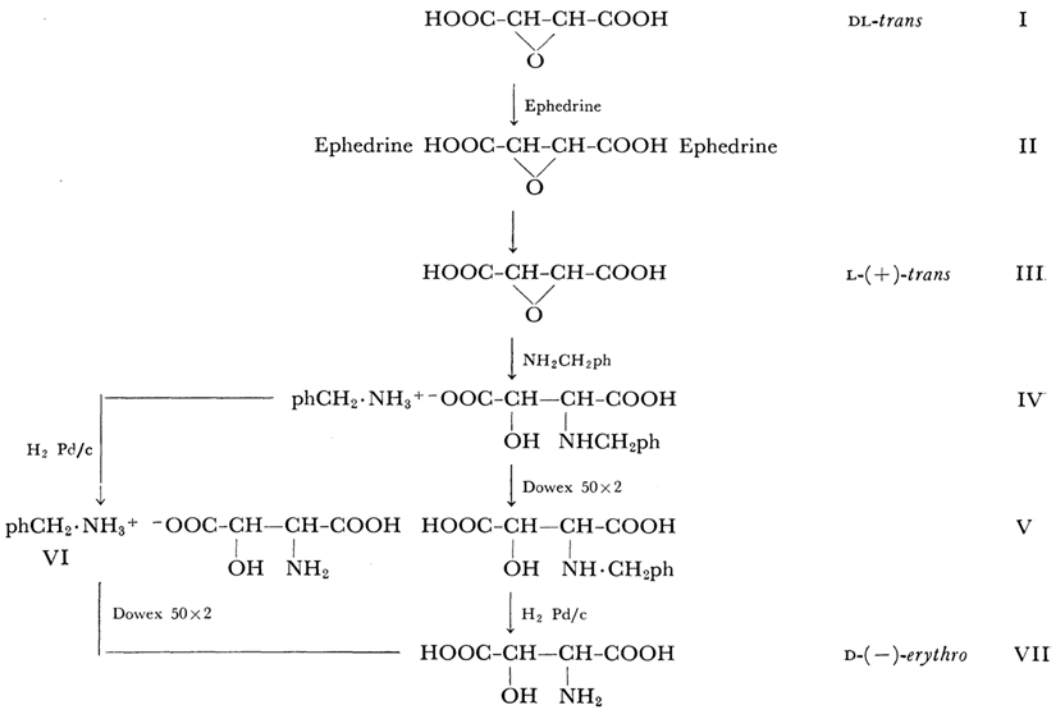

Scheme 1.

acid were not consistent ($[\alpha]_D^{25}=+51°$,[8] $-54°$,[11] $+49°$,[9] $+53°$[12] in 1 N HCl). Optically active *erythro*-β-hydroxyaspartic acid has not yet been prepared from optically pure *trans*-2, 3-epoxysuccinic acid.

The reaction of epoxy acid with aqueous ammonia is rather slow so that stronger reaction conditions were required (120°C, 30 hr).[2,12] In the ammonolysis reaction, formation of a small amount of *threo*-β-hydroxyaspartic acid was observed. On the other hand, however, (+)-epoxy acid (III) reacted easily with benzylamine in aqueous solution by refluxing for 4 hr. The resulting *N*-benzylamino acid (V) was hydrogenolyzed catalytically by the use of palladium on charcoal. In this process, only *erythro*-β-hydroxyaspartic acid (VII) was formed. This would suggest that the amination reaction accompanied a complete Walden inversion. The specific rotation of isolated D-(−)-*erythro*-β-hydroxyaspartic acid (VII) was found to be $-59°$ which was a greater absolute value than that prepared enzymatically ($+51°$).[8]

Experimental[14]

DL-*trans*-2, 3-Epoxysuccinic Acid (I). The I was prepared by Payne and Williams,[13] mp 207—210°C. Found: C, 36.38; H, 3.05%. Calcd for $C_4H_4O_5$: C, 36.48; H, 3.17%.

Optical Resolution of *trans*-DL-2, 3-Epoxysuccinic Acid. The epoxy acid, 6.6 g (0.05 mol), and *l*-ephedrine, 8.25 g (0.05 mol), were dissolved in 10 m*l* of hot methanol and 90 m*l* of acetone was added. After cooling, crystallization of the ephedrine salt began by seeding with an authentic specimen which was obtained in another crystallization experiment. After standing 30 min at room temperature, the crystals were filtered and washed with acetone. The crystals, 5.8 g (50%), were recrystallized from methanol and acetone, yield 5.5 g (47.6%), mp 198°C, $[\alpha]_D^{25}$ +2.1° (*c* 5.15, MeOH). The melting point and the specific rotation did not change after further purification.

Found: C, 62.46; H, 7.33; N, 6.18%. Calcd for $C_{24}H_{34}N_2O_7$: C, 62.32; H, 7.41; N, 6.06%.

(+)-*trans*-2, 3-Epoxysuccinic Acid (III). Ephedrine salt, 11.0 g (0.024 mol), was dissolved in 10 m*l* of water. The solution was applied to a column of Dowex 50×2 (H-form, 100—200 mesh, 2 cm×40 cm) and washed with water until the effluent became neutral. The effluent was evaporated to dryness under reduced pressure. Crude (+)-epoxy acid, 3.0 g (97.5%) was obtained. This was recrystallized from dioxane and *n*-hexane. (+)-Epoxy acid-dioxane adduct was obtained. This adduct lost dioxane easily at room temperature and thus free (+)-epoxy acid (III) was obtained, mp 188°C, $[\alpha]_D^{25}$ +117.8° (*c* 2.6, EtOH).

Found: C, 36.33; H, 3.14%. Calcd for $C_4H_4O_5$: C, 36.38; H, 3.05%.

Benzylamine Salt of D-(−)-*N*-Benzyl-*erythro*-β-hydroxyaspartic Acid (IV). A mixture of (+)-epoxy acid (III) (1.75 g), water (6 m*l*), and benzylamine (4.0 g) was refluxed for 4 hr. After cooling,

14) All temperature measurements were uncorrected. All optical rotation measurements were carried out by the use of a Rudolph model 80 polarimeter with PEC-101 photometer.

the excess of benzylamine was extracted with ether, and to the aqueous layer was added 100 m*l* of acetone which precipitated the benzylamine salt of (−)-*N*-benzyl amino acid (IV), (4.15 g, 86%). This was recrystallized from water and ethanol. Yield, 3.80 g (78.8%), mp 196°C, $[\alpha]_D^{25}$ −4.7° (*c* 5.6, H_2O).

Found: C, 59.59; H, 6.26; N, 7.76%. Calcd for $C_{18}H_{24}N_2O_6$: C, 59.33; H, 6.64; N, 7.69%.

D-(−)-*N*-Benzyl-*erythro*-β-hydroxyaspartic Acid (V). Benzylamine salt (IV), 3.0 g (0.0083 mol), was dissolved in 10 m*l* of water. The solution was applied to a column of Dowex 50×2 (H-form, 100—200 mesh, 2 cm×15 cm) and eluted with a mixture of water and ethanol (50 : 50 v/v). The effluents were combined and evaporated to dryness *in vacuo*. Crude D-(−)-*N*-benzyl-*erythro*-β-hydroxyaspartic acid (V), 1.8 g (91%), was obtained. This was recrystallized from water, yield, 1.5 g (76.2%), mp 219°C dec., $[\alpha]_D^{25}$ −21.0° (*c* 3.2, N HCl).

Found: C, 55.41; H, 5.42; N, 5.95%. Calcd for $C_{11}H_{13}NO_5$: C, 55.23; H, 5.48; N, 5.85%.

D-(−)-*erythro*-β-Hydroxyaspartic Acid (VII). D-(−)-*N*-Benzyl-*erythro*-β-hydroxyaspartic acid (V), 2.39 g (0.01 mol), was dissolved in 50 m*l* of a mixture of water and ethanol (50 : 50 v/v). To this solution, 1.0 g of 5% palladium on charcoal was added and the hydrogenolysis was carried out at room temperature. When the hydrogen uptake ceased, the catalyst was removed by filtration. The filtrate was evaporated to dryness under reduced pressure. The crude VII was recrystallized from water, yield, 1.05 g (70.5%), no clear mp. It colored brown and gradually decomposed above 200°C, $[\alpha]_D^{25}$ −59.5° (*c* 1.28, N HCl).

Found: C, 32.16; H, 4.72; N, 9.44%. Calcd for $C_4H_7O_5$: C, 32.22; H, 4.73; N, 9.39%.

The benzylamine salt of D-(−)-*N*-benzyl-*erythro*-β-hydroxyaspartic acid (IV), (1.8 g), was dissolved in 50 m*l* of water. To this solution, 1.0 g of 5% palladium on charcoal was added and the hydrogenolysis was carried out at room temperature. When the hydrogen uptake ceased, the catalyst was removed by filtration. This filtrate was placed on a column of Dowex 50×2 (H-form, 100—200 mesh), 2 cm×15 cm) in order to remove benzylamine, and β-hydroxyaspartic acid was eluted with 5% pyridine solution. The effluents were combined and concentrated *in vacuo*. The remaining crude VII was recrystallized from water. Yield, 0.46 g (62%). No clear mp. It colored brown and gradually decomposed above 200°C, $[\alpha]_D^{25}$ −59.7° (*c* 1.12, N HCl). The specific rotation dod not change after further recrystallization. Elution volumes of the synthesized D-(−)-*erythro*-β-hydroxyaspartic acid in the phoenix K-5000 automatic amino acid analyzer are in a range of 60.9 m*l*—63.0 m*l* in several analyses. Elution volumes of standard DL-*threo*- and DL-*erythro*-β-hydroxyasparitc acid are 52.5 m*l* and 62 m*l* respectively.

This work was supported by Grant no. NsG-689 of the National Aeronautics and Space Administration, U. S. A.