On the Erlenmeyer Reaction. I. Mechanism of Threo-β-phenylserine Formation*

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The Erlenmeyer reaction[1] of glycine with benzaldehyde in the presence of excess of alkali results in the formation of N-benzal-β-phenylserine alkali salt and N-benzal-α, β-diphenyl-β-hydroxyethylamine. Recently many stereochemical studies of β-phenylserine have been published in connection with chloramphenicol. Vogler[2] has determined chemically that the structure of Erlenmeyer's phenylserine was only of the threo-type. The diastereoisomeric erythro-β-phenylserine was first isolated in pure state by Shaw and Fox[3] in 1950. They prepared pure erythro-β-phenylserine using a new method for separation of the threo and erythro isomers, and they also found a paper chromatographic solvent (S–F solvent) to separate these two diasteromers.

An interesting point in the Erlenmeyer synthesis of β-phenylserine is that the threo isomer is the predominant product. The erythro isomer, which is expected to form in the aldol condensation of glycine and benzaldehyde, has not been isolated from the final crystalline product. Shaw and Fox found by use of their paper chromatographic solvent that the threo isomer increased and the erythro isomer decreased during the reaction.

Quantitative analyses of the threo- and the erythro-phenylserine and glycine in the reaction mixture at different intervals of the reaction time have now been carried out to determine the ratio of these three amino acids. Table I shows the ratio of these three amino acids produced in the Erlenmeyer reaction.

From the results of the analyses, it may be inferred that the erythro isomer is converted into the threo isomer during the reaction.

In order to prove the hypothesis, either erythro or threo-phenylserine was mixed with ethanol, water, benzaldelyde and sodium hydroxide as in the Erlenmeyer reaction. It was found that if equimolar or less sodium hydroxide was used, threo- or erythro-phenylserine was not converted into its diastereomer. The resulting reaction mixture gave only the original threo or erythro isomer, which was identified by paper chromatography using the S–F solvent. However, by the use of an excess of alkali as in the Erlenmeyer reaction, both threo- and erythro-phenylserine were converted into a mixture of the isomers. When threo-, erythro- or a mixtuer of threo- and erythro-phenylserine were treated under Erlenmeyer's condition, one of the sodium salts of isomeric N-benzal-phenylserine crystallized out from the reaction mixture as the predominant product. After debenzalation of the crystalline

TABLE I. AMINO ACID RATIO IN THE ERLENMEYER REACTION

hr.	Gly	Threo-phe.ser.	Erytho-phe.ser.
1/4	48	28	24
1/2	39	39	22
1 1/2	23	52	25
4 1/2	18	62	20
24	11	73	16
50	9	77	14

1) E. Erlenmeyer Jr., *Ber.*, **25**, 3445 (1892); E. Erlenmeyer, Jr. and E. Frustuck, *Ann.*, **284**, 36 (1894); E. Erlenmeyer, Jr., *ibid.*, **307**, 70 (1899).
2) K. Vogler, *Helv. Chim. Acta*, **33**, 2111 (1950).
3) K. N. F. Shaw and S. W. Fox, *Abstr. of Papers, 18th Am. Chem. Soc. Meeting.*, p. 28N (1950); K. N. F. Shaw and S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3417, 3421 (1953).
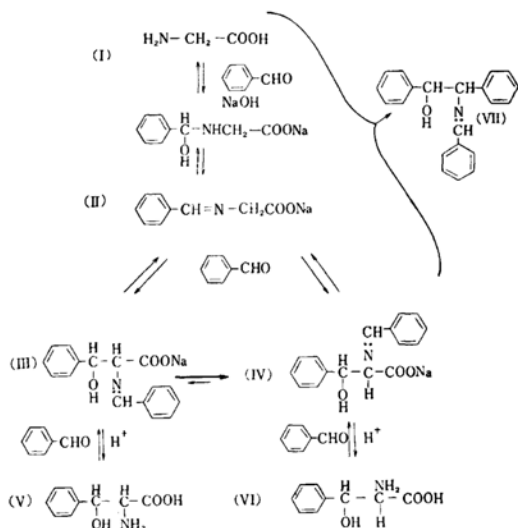
Fig. 1. Flow sheet of postulated mechanism of Erlenmeyer reaction.

product, paper chromatography showed that the product consisted of only the threo isomer. Each of the mother liquors arising from the above mentioned three different reactions, however, contained glycine and the threo- and erythro-phenylserine. These results suggest a possible-mechanism for the formation of Erlenmeyer's threo-phenylserine. The postulated pathways of the reaction are shown in Fig. 1.

Glycine (I) reacted with benzaldehyde to yield benzal glycine (II). The resulting II reacted again with benzaldehyde forming erythro-N-benzal-phenylserine (III) and threo-N-benzalphenylserine (IV). In solution both III and IV exist as a mixture. However,

(A) if the solubility of IV is small and it crystallizes easily, and

(B) if there is an equilibrium between III and IV under the reaction conditions,

the erythro isomer III is converted in to threo isomer IV by crystallization. Erlenmeyer's threo-phenylserine formation may be considered as a typical asymmetric transformation reaction.

In the conversion of V→III→IV, about 65~75% of the threo isomer was isolated in crystal form IV. It is necessary to use more than twice the equivalent of alkali to crystallize IV in the conversion of erythro to threo isomer. The filtrate of the crystalline reaction product contains III, IV and glycine, the last being an alkali degradation product of phenylserine. The reaction yielded N-benzal-α, β-diphenyl-β-hydroxyethylamine (VII) as a by-product. Paper chromatography showed that a longer reaction time resulted in an increased formation of VII.

In the conversion reaction, the α- or β-carbon atom or both carbon atoms must be racemized or inverted. Optically active erythro-phenylserine was used to determine which carbon

atom racemized or inverted during the reaction. When D-erythro-phenylserine was treated with benzaldehyde and alkali as in Erlenmeyer's reaction, condition, the mixture had a very high rotatory power which decreased rapidly as is shown in Fig. 2. After about 4.5 hr., the solution lost almost all optical activity and crystals were precipitated out. The expected optically active threo isomer was not obtained, but the crystal was identified as sodium salt of racemic N-benzal-threo-phenylserine (IV).
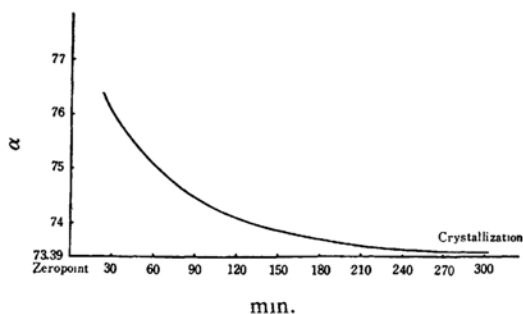

Fig. 2. Mutarotation of D-erythro-β-phenylserine.

This study showed that both the α- and β-carbon atoms were racemized during the reaction. In the absence of benzaldehyde, D-erythro-phenylserine did not racemize so rapidly and it retained more than 75% of its optical activity after 4.5 hr. The racemization at the α- and β-carbon atoms is not only due to the alkali but the N-benzal residue, which is important in the conversion process. Such knowledge suggests the equilibrium between III and IV as is shown in Fig. 3.

Optically active erythro-phenylserine has been isolated by Fone[4] using an enzymic method and he determined its configuration
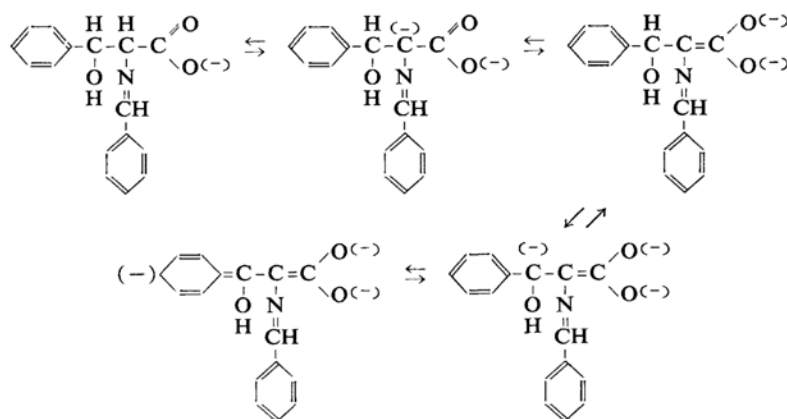

Fig. 3. Equilibrium between III and IV.

4) W. S. Fone, *J. Biol. Chem.*, **204**, 323 (1953).

biologically. In this study D- and L-erythro-phenylserine were prepared by resolution of N-benzoyl-DL-erythro-phenylserine using quinine. The D-isomer was isolated in an optically pure state but the L-isomer had only 75% of its optical activity. The configuration of these isomers was also determined chemically. The (−)-erythro-phenylserine was reduced with hydrogen iodide and red phosphorus giving D(+)-phenylalanine. The (−)-erythro isomer has a sweet taste, and (+)-erythro-phenylserine has a bitter taste. These results show that the (−)-erythro isomer has the D_s-configuration and (+)-erythro isomer has the L_s-configuration.

Discussion

The fact that the mechanism of threo-phenylserine formation is based on the racemization of $α$- and $β$-carbon atoms has been mentioned above. However, another explanation is possible: the $α−β$ carbon linkage could be cleft during the reaction and the resulting benzaldehyde and N-benzal-glycine could react again to reform the threo isomer IV. Such a mechanism is conceivable since the linkage of the $α−β$ carbon is not stable in the alkaline condition; phenylserine is completely decomposed to glycine and benzaldehyde after boiling for 1 hr. with 1 N sodium hydroxide. Elphimoff-Felkin[5] studied the transformation of ethyl threo-p-nitro-$β$-phenylserinate to the corresponding erythro isomer in alcoholic solution. The conversion reaction was explained by the cleavage of the $α−β$ carbon bond of the threo isomer and consequent reforming of the erythro isomer from the resulting p-nitro-benzaldehyde and glycine ethylester. However, the explanation can not be applied successfully in the Erlenmeyer reaction. In the reaction of threo-p-nitro-phenylserine[6] with benzaldehyde and alkali, the sodium salt of N-benzal-threo-p-nitro-penylserine was crystallized out easily from the reaction mixture. The mother liquor contained threo- and erytho-p-nitro-phenylserine and only a trace amount of glycine. After two days' standing at room temperature, however, the mother liquor of the reaction mixture contained threo- and erythro-p-nitrophenylserine, glycin and also a small amount of phenylserine, the last being a reaction product of benzaldehyde and glycine produced by the cleavage reaction. These results showed that the cleavage and resynthesis of phenylserine are not so important in the cases of short reaction time, although it may be impor-

ant in the cases of prolonged reaction time. The fact that the phenylserine was not formed rapidly in the reaction of p-nitro-phenylserine and benzaldehyde, suggests that the mechanism shown in Figs. 1 and 3, which contains racemization of the $α$- and $β$-carbon atoms and asymmetric transformation is a more reasonable pathway in the Erlenmeyer reaction.

The N-benzal residue in the reaction is important in the racemization and also in the formation and stabilization of many equilibrium systems. The $β$-carbon atom connected with the hydroxyl group is also interesting in regard to the formation of the conjugated double bond system from the $β$-phenyl residue to the N-benzal residue or to the carboxylate group. In the same reaction condition, L-tyrosine was not racemized at all with benzaldehyde and alkali.

The racemization mechanism of the Erlenmeyer reaction is complex. Two asymmetric carbons are racemized simultaneously. The observed data by rotatory power showed that the racemization proceeded as a pseudo 1st order reaction; the racemization constant, K, is 0.156 min^{-1}. The specific rotation of N-benzal D-erythro-phenylserine at zero time has a tremendously high value $[α]_D^{27} = 13000°$ which is calculated from the racemization constant.

Experimental

Quantitative Analysis of the Amino Acid in the Erlenmeyer Reaction.—A mixture of glycine (37.0 g.), water (400 ml.) and sodium hydroxide (70.0 g.) was shaken vigorously with a mixture of ethyl alcohol (100 ml.) and benzaldehyde (1.06 g.) under cooling. The mixture became clear and then a crystalline paste was formed gradually which solidified after several hours. About 0.5 g. of aliquot was taken from the reaction mixture after 15 min., 30 min., 1.5 hr., 4.5 hr., 24 hr. and 50 hr. These samples were acidified immediately with acetic acid, and a small amount of water was added. The resulting benzaldehyde was extracted with ether. The aqueous layer was adjusted to pH 10 with sodium carbonate solution, then extracted with ether to eliminate diphenylethanolamine. The aqueous solution was neutralized and evaporated in vacuo, and the residue was subjected to paper chromatography and also to dinitrophenylation of glycine and phenylserine. The ratio of glycine to phenylserine was determined by paper chromatography (solvent: n-BuOH : AcOH : H$_2$O/4 : 1 : 1). The corresponding spots were cut off and these were eluted with hot water. The ratio of glycine to phenylserine was determined colorimetrically, using the standard ninhydrin method[7]. The threo- and erythro-phenylserines were isolated by the Seki's method[8]. The DNP-amino acid mixture of each

5) I. Elphimoff-Felkin et al., *Compt. rend.*, 234, 154, 1627 (1952).
6) G. Ehrhart, *Chem. Ber.*, 86, 483 (1953).

7) S. Akabori and S. Mizushima, " Protein Chemistry (Tampakushitsu Kagaku)'', Vol. 1, Kyoritsushuppan, (1954), p. 209.
8) T. Seki, *J. Biochem.* (Tokyo), 47, 253 (1960).

sample was columnchromatographed, using Amberlite IR-112 resin (Na+) and an elution solvent composed of pH 3.1 citric acid buffer (1/10 M): ethyl methyl ketone : tetrahydrofurane/10 : 1 : 1.

Eluted solutions were collected with a fraction collector. The DNP-derivatives were separated into 3 fractions; the first band was composed of DNP-erythro-phenylserine and DNP-glycine, the second was dinitrophenol and the third was DNP-threo-phenylserine. The second band at 430 mμ disappeared on acidification to pH 1. The ratio of glycine and erythro-phenylserine to threo-phenylserine was determined colorimetrically at 430 mμ. The ratios of glycine, threo- and erythro-phenylserine were calculated from the data measured above. The results are shown in Table I.

Reaction of Phenylserine with Benzaldehyde and Alkali.

—(A) *When a Small Proportion of Alkali was Used.*—In either case threo-phenylserine monohydrate (1.0 g.) or erythro-phenylserine (0.91 g.) was mixed with water (15 ml.), sodium hydroxide (0.2 g.), benzaldehyde (1.0 ml.) and ethanol (6 ml.). The mixture was shaken until it became clear and was allowed to stand overnight. The reaction mixture was diluted with hydrochloric acid; the resulting benzaldehyde was extracted with ether. The solution was neutralized and then examined by paper chromatography with the S-F solvent. Neither the threo nor the erythro isomer was converted.

(B) *When a Large Proportion of Alkali was Used.*—(1) Threo-phenylserine monohydrate (1.0 g.) was mixed with water (5 ml.), sodium hydroxide (0.6 g.), benzaldehyde (1 ml.) and ethanol (2 ml.). The clear solution began to deposit crystals after 1 hr. It was allowed to stand overnight at room temperature. After filtration and washing with ethanol, 1.30 g. of the crystalline *N*-benzal derivative IV was obtained. The IV was treated with 1 N hydrochloric acid and the resulting benzaldehyde was extracted with ether and the aqueous layer was evaporated to dryness. The phenylserine hydrochloride in the residue was extracted with absolute alcohol. After evaporation of the alcohol, the residual syrup was treated with silver carbonate and hydrogen sulfide to eliminate hydrochloric acid. The aqueous solution was evaporated. The resulting crude phenylserine was recrystallized from water, 0.75 g. of the threo-phenylserine monohydrate was obtained. The isolated phenylserine was shown to consist of pure threo isomer by paper chromatography with the S-F solvent (Found: N, 7.07%).

(2) A mixture of threo-phenylserine monohydrate (0.50 g.) and erythro-phenylserine (0.46 g.) was treated with sodium hydroxide (0.7 g.), water (5 ml.), benzaldehyde (1 ml.) and ethanol (2 ml.) as in the previous experiment. The clear solution began to deposit crystals after 2 hr. or earlier on seeding with IV. After standing overnight at room temperature, 0.95 g. of IV was isolated. The crystals were treated in the same way as above, 0.62 g. of pure threo-phenylserine monohydrate was isolated (Found: N, 7.18%).

(3) Erythro-phenylserine (0.91 g.) was treated with sodium hydroxide (1.0 g.), water (5 ml.), benzaldehyde (1 ml.) and ethanol (2 ml.). After 2 hr. crystallization began. The crystallization proceeded more rapidly after seeding of IV, 1.0 g. of crystals was isolated. It was treated as above, 0.65 g. of pure threo-phenylserine monohydrate was isolated. The threo isomer was converted into its copper complex.

Found: N, 6.48. Calcd. for $(C_9H_{10}O_3N)_2Cu$: N, 6.43%.

(4) D(-)-Erythro-phenylserine dioxane adduct (89.8 mg.) and benzaldehyde (80 mg.) were dissolved in 5 ml. of a mixture of water (10 ml.), ethanol (2 ml.) and sodium hydroxide (0.8 g.). The rotatory power of the solution was observed. In the first 30 min. of the reaction, the value of $[\alpha]_D$ was too great to observe. The mutarotation curve is shown in Fig 2. After 4.5 hr. the $[\alpha]_D$ reached almost zero and crystallization began in the solution. The crystals were filtered off. The analytical data of the crystals, paper chromatography and the rotatory power (in 2 N hydrochloric acid) of the isolated phenylserine showed that the crystal was the pure DL-threo-*N*-benzal-phenylserine sodium salt.

Found: N, 4.74. Calcd. for $C_{16}H_{14}O_3NNa$: N, 4.81%.

In the above reaction without benzaldehyde, D(-)-erythro-phenylserine maintained about 76% of its optical activity after 4.5 hr. of treatment.

Reaction of Threo-*p*-nitro-phenylserine with Benzaldehyde and Alkali.

— The threo-*p*-nitrophenylserine was prepared by the method of Ehrhart[6]. Threo-*p*-nitro-phenylserine (1.13 g.) was treated with sodium hydroxide (0.2 g.), water (15 ml.), benzaldehyde (1 ml.) and ethanol (4 ml.). The clear solution began to deposit crystals after 2 hr. The crystals were filtered off after 6 hr., and were converted into free *p*-nitro-phenylserine. The product was found to consist of the pure threo isomer by chromatography. The mother liquor of the reaction mixture contained both the threo- and the erythro-*p*-nitro-phenylserine and a trace amount of glycine. However, after 50 hr. of standing at room temperature, the mother liquor was found to contain phenylserine and an increased amount of glycine in addition to the threo- and erythro-*p*-nitro-phenylserine.

Benzoyl Erythro- and Threo-phenylserine.

— Benzoyl-DL-erythro-phenylserine was prepared by the method of Shaw and Fox. Erythro-phenylserine was treated with benzoylchloride in the usual manner. M. p. 183~183.5°C (decomp.).

Found: C, 67.53; H, 5.48; N, 4.81%.

Benzoyl-DL-threo-phenylserine was prepared as above. M. p. 158°C (decomp.).

Found: C, 67.40; H, 5.43%.

Resolution of Benzoyl-DL-erythro-phenylserine.

—*N*-Benzoyl-DL-erythro-phenylserine (28.5 g.) and quinine (27.2 g.) were dissolved in hot mixture of methanol (60 ml.) and acetone (40 ml.). When the solution had cooled down, crystallization of quinine salt began after seeding with an authentic specimen which was obtained during the crystallization experiment. After standing overnight in a refrigerator, the crystals were collected by filtration. The product after washing with cold acetone weighed 17.0 g. The quinine salt was recrystallized from 300 ml. of

99% ethanol, 14.0 g. of the quinine salt was obtained; m. p. 214~217°C (decomp.). The mother liquor from the recrystallization was combined with original mother liquor. From this, an additional 7.7 g. of quinine salt was isolated.

Found: C, 70.63; H, 6.31; N, 6.88. Calcd. for $C_{36}H_{39}O_6N_3$: C, 70.91; H, 6.45; N, 6.89%.

$[\alpha]_D^{22} = -105.0°$ (c 0.401, 99% ethanol).

Fifteen grams of the quinine salt was dissolved in ethanol, and to this solution, concentrated aqueous ammonia was added. The solution was then evaporated in vacuo to remove alcohol. The aqueous ammonia was added again and the evaporation was repeated until the ethanol was completely removed. The separated quinine base began to crystallize. The mixture was allowed to stand for a few hours for complete precipitation. The quinine was filtered off, then the filtrate was extracted three times with ether in order to remove residual quinine. The aqueous solution was concentrated in vacuo to 80 ml. and then acidified with 6 N hydrdrochloric acid to pH 2. The precipitated optically active benzoyl-erythro-phenylserine was filtered off. It was recrystallized from 35% ethanol, 5.7 g. of active benzoyl-erythro-phenylserine was obtained. M. p. 188~189°C (decomp.).

$[\alpha]_D^{23} = -29.3°$ (c 0.647, 99% ethanol).

Found: C, 67.27; H, 5.38; N, 4.90. Calcd. for $C_{16}H_{15}O_4N$: C, 67.36; H, 5.30; N, 4.91%.

D-Erythro-phenylserine.—(−)-N-Benzoyl-erythrophenylserine (8.0 g.) was heated under reflux with 90 ml. of 15% hydrobromic acid for 3 hr. After hydrolysis, the solution was extracted three times with ether. After evaporation of the aqueous fraction under reduced pressure, water was added and the process repeated to remove the excess of hydrobromic acid. The residue was treated with silver carbonate and hydrogen sulfide. The dehydrobrominated solution was evaporated in vacuo, and the residue was dissolved in 15 ml. of hot water, then 15 ml. of dioxane was added to the solution. Two and a half grams of (−)-erythro-phenylserine dioxane adduct was crystallized out.

$[\alpha]_D^{26} = -53.7°$ (c 1.091, 2 dm, 2 N hydrochloric acid).

Found: C, 58.65; H, 6.70; N, 6.19. Calcd. for

$(C_9H_{11}O_3N)_2C_4H_8O_2$: C, 58.65; H, 6.71; N, 6.22%.

Free (−)-erythro-phenylserine was prepared from the aqueous solution of the free amino acid by addition of much ethanol. The (−)-erythro-phenylserine has a sweet taste.

M. p. 193°C (decomp.). $[\alpha]_D^{25} = -68.0°$ (c 0.426, 2 N hydrochloric acid).

Found: C, 59.61; H, 6.12; N, 7.74. Calcd. for $C_9H_{11}O_3N$: C, 59.66; H, 6.12; N, 7.73%.

Reduction of (−)-Erythro-phenylserine.—A half gram of (−)-erythro-phenylserine dioxane adduct was heated at 150~160°C for 2 hr. with 15 ml. of hydrogen iodide and 0.6 g. of red phosphorus. The reaction mixture was filtered. It was then evaporated under reduced pressure, water was added and the process was repeated to remove the excess of hydrogen iodide. The residue was treated with silver carbonate and hydrogen sulfide, 160 mg. of D-phenylalanine was obtained.

$[\alpha]_D^{25} = +31.4°$ (c 0.780, 2 dm., water)

Found: C, 65.53; H, 6.65; N, 8.46. Calcd. for $C_9H_{11}O_2N$: C, 65.44; H, 6.71; N, 8.48%.

L-Erythro-phenylserine. — N - Benzoyl - L - erythrophenylserine was isolated from the mother liquor of the quinine salt as shown above for the separation of D-erythro isomer, (+)-N-benzoyl-erythrophenylserine, m. p. 182~183°C (decomp.).

$[\alpha]_D^{25} = +22.6°$ (c 0.973, 99% ethanol).

Found: C, 67.52; H, 5.44; N, 4.97. Calcd. for $C_{16}H_{15}O_4N$: C, 67.36; H, 5.30; N, 4.91%.

L-Erythro-phenylserine, m.p. 179~183°C (decomp.).

The L-erythro isomer has a bitter taste.

$[\alpha]_D^{25} = +51.2°$ (c 0.757, 2 N hydrochloric acid).

Found: N, 7.59. Calcd. for $C_9H_{11}O_3N$: N, 7.73%.

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