[CONTRIBUTION FROM THE NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH][1]

# SOME DERIVATIVES OF THE STEREOISOMERIC β-PHENYLSERINES. CONVERSION OF THE *THREO* TO THE *ERYTHRO* FORM

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β-Phenyl-DL-serine was first prepared by Erlenmeyer (1) through the sodium hydroxide-catalyzed condensation of benzaldehyde and glycine. The product thus obtained was shown to be the *threo* form by Carrara and Weitnauer (2) who related the compound to chloroamphenicol. Vogler (3) arrived at the same conclusion by the resolution of *threo*-β-phenyl-DL-serine (details not given) and the conversion of the D- and L-enantiomorphs to L-mandelic acid and L-phenylalanine respectively.

TABLE I

DERIVATIVES OF *threo* AND *erythro* β-PHENYL-DL-SERINES

| DERIVATIVE OF PHENYLSERINE | M.P.[a], °C. | FORMULA | ANALYSIS[b] | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Calculated | | | | Found | | | |
| | | | C | H | N | Cl | C | H | N | Cl |
| *threo* SERIES | | | | | | | | | | |
| N-Acetyl | 154–156 | $C_{11}H_{13}NO_4$ | 59.2 | 5.8 | 6.3 | | 59.3 | 6.1 | 6.3 | |
| N-Acetyl ethyl ester[c] | 180.5–182.5 | $C_{13}H_{17}NO_4$ | 62.2 | 6.8 | 5.6 | | 62.3 | 7.1 | 5.6 | |
| N-Acetyl methyl ester | 182–184 | $C_{12}H_{15}NO_4$ | 60.7 | 6.3 | 5.9 | | 60.5 | 6.7 | 5.8 | |
| N-Chloroacetyl ethyl ester | 140–141 | $C_{13}H_{16}NO_4Cl$ | 54.6 | 5.6 | 4.9 | 12.4 | 54.2 | 5.8 | 5.0 | 12.8 |
| N-Chloroacetyl methyl ester | 130–132 | $C_{12}H_{14}NO_4Cl$ | 53.0 | 5.2 | 5.2 | 13.1 | 52.6 | 5.1 | 5.2 | 12.9 |
| N-Dichloroacetyl | 162–164 | $C_{11}H_{11}NO_4Cl_2$ | 45.2 | 3.8 | 4.8 | 24.3 | 45.7 | 4.0 | 5.0 | 23.8 |
| *erythro* SERIES | | | | | | | | | | |
| N-Acetyl | 174.5–176.5 | $C_{11}H_{13}NO_4$ | 59.2 | 5.8 | 6.3 | | 58.7 | 5.9 | 6.3 | |
| N-Chloroacetyl | 115–117 | $C_{11}H_{12}NO_4Cl$ | 51.2 | 4.7 | 5.4 | 13.8 | 51.4 | 5.0 | 5.3 | 13–5 |
| N-Acetyl ethyl ester | 145.5–147.5 | $C_{13}H_{17}NO_4$ | 62.2 | 6.8 | 5.6 | | 61.9 | 6.9 | 5.6 | |
| N-Chloroacetyl ethyl ester | 97–98 | $C_{13}H_{16}NO_4Cl$ | 54.6 | 5.6 | 4.9 | 12.4 | 54.4 | 5.8 | 4.9 | 12.6 |

[a] Melting points are corrected. [b] Analyses by Mr. R. J. Koegel and Dr. W. C. Alford and their staffs of the National Institutes of Health. [c] After this work was complete Takagi and Ichikawa reported this cpd., m.p. 173–174°, *J. Pharm. Soc. Japan*, **71**, 1952 (1951).

Two independent reports of the preparation of the *erythro* isomer of β-phenyl-DL-serine have appeared. Shaw and Fox (4) reported in abstract form the isolation of the compound from the products of a modified Erlenmeyer condensation, and Elphinoff-Felkin and Felkin (5) synthesized the isomer by catalytic hydrogenation of α-oximinobenzoyl acetate.

The assignment of the *threo* configuration to the main product of the Erlenmeyer condensation and of the *erythro* configuration to the other isomer isolated by the method of Shaw and Fox (4) has recently been confirmed by enzymatic

[1] Public Health Service, Federal Security Agency.

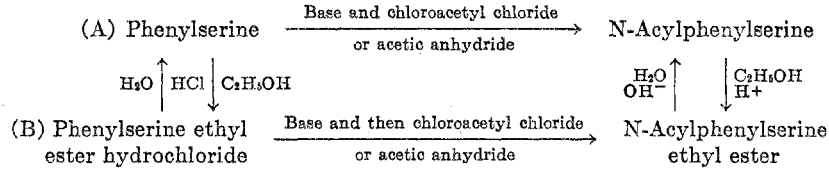
oxidation (6) with rattlesnake venom, of the L-forms of the DL pairs to D- and L-mandelic acid respectively.

In this paper there is reported the experimental details for the preparation of the stereoisomers employing the procedure outlined by Shaw and Fox (4) together with some reactions of the isomers. The yield of pure *threo* and *erythro* forms obtained in this way was 45 and 3% respectively.

The isomerides were converted into a number of derivatives (Table I) not previously reported. In the *threo* series the N-acetyl amino acid and its ethyl ester were prepared by the reactions shown in the accompanying figure. Identical products were obtained in each case by either route thus indicating that no conversion of one form to the other occurred during any of the transformations. The chloroacetyl ester was obtained by route B and the methyl esters listed were obtained by the action of excess diazomethane on the appropriate N-acyl-amino acid. The N-dichloroacetyl *threo* derivative was obtained by a Schotten-Baumann reaction between dichloroacetyl chloride and *threo* β-phenyl-DL-serine.

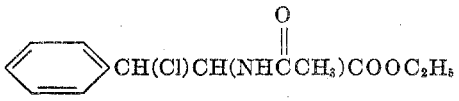
The N-chloroacetyl *erythro* isomer was prepared by both routes leading to identical compounds. The N-acetyl derivative was prepared by reaction A and both *erythro* ethyl esters were obtained by reaction B.

FIGURE




Because the preparative methods described in the experimental section for the isomeric β-phenyl-DL-serines led to such a large proportion of the *threo* isomer the possibility of inverting the configuration at the β-carbon was investigated. Reaction of N-acetyl *threo*-β-phenyl-DL-serine ethyl ester with thionyl chloride in chloroform followed by acid hydrolysis did lead to the *erythro* isomer (isolated as its ethyl ester hydrochloride) in poor yield (7%). Also isolated was about 40% of material with a wide melting range which was probably a mixture of *threo* and *erythro* ester hydrochlorides.

When the acid hydrolysate from the inversion was extracted with ether and the ether then extracted with aqueous sodium bicarbonate there was isolated by concentration of the organic solvent a neutral compound that analyzed correctly for ethyl α-acetamido-β-chlorohydrocinnamate (I) of unknown con-




I

figuration. This was shown to be the correct structure by its conversion to ethyl α-acetamidocinnamate with one mole of alkali, which in turn was hy-

drolyzed to α-acetamidocinnamic acid by addition of a second mole of alkali. The latter compound was identified by comparison with an authentic sample.[2]

From the bicarbonate extract mentioned above there was isolated by acidification and subsequent ether extraction followed by removal of the ether a small amount of phenylpyruvic acid, identified by comparison of its 2,4-dinitrophenylhydrazone with an authentic sample.[2] Incidental to this identification a hitherto unreported form of this derivative, m.p. 160–162° was isolated by crystallization from ethyl acetate and petroleum ether. It was readily converted to the form usually obtained from water or alcohol (7), m.p. 192–193°, by crystallization from these solvents; a mixture of the two forms melted at 192–193°.

## EXPERIMENTAL[3]

*Preparation of the isomeric β-phenyl-*DL*-serines* (4, 8). To a solution of 254 g. of glycine and 204 g. of sodium hydroxide in 850 ml. of water at room temperature there was added 720 g. of benzaldehyde. The whole was stirred until the solution first became clear and then until the solid benzylidene derivative of the sodium salt of the β-phenyl-DL-serines began to crystallize. The reaction mixture was allowed to stand overnight in the ice-box and the next morning the solid cake was broken up and treated with 650 ml. of concentrated hydrochloric acid. The liberated benzaldehyde was extracted with ether and to the aqueous phase there was added 200 ml. of concentrated ammonium hydroxide followed by sufficient acetic acid to bring the solution to *p*H 6.0 ± 0.5. After standing overnight in the ice-box the precipitated β-phenyl-DL-serine (x) was collected by filtration. This, material after two recrystallizations from water, with the aid of charcoal, gave 270 g. (45%) of *threo*-β-phenyl-DL-serine. There was also isolated about 30 g. of organic material, m.p. 125–127°, insoluble in water which was not further investigated. Upon esterification with ethanol and anhydrous hydrogen chloride the *threo* acid yielded the corresponding ester, m.p. 139–141°, [lit. (2) m.p. 138°].

The filtrate from (x) was concentrated to dryness and then suspended in 1500 ml. of absolute ethanol which in turn was saturated with anhydrous hydrogen chloride. The hot mixture was filtered to remove inorganic material and the filtrate was allowed to stand overnight. After concentration to 500 ml. and cooling there was obtained 20 g. (3%) of *erythro*-β-phenyl-DL-serine ethyl ester hydrochloride, m.p. 175–178°, which on recrystallization from ethanol was raised to 180–182° [lit. (5) m.p. 186° (block)]. The ester on hydrolysis by refluxing one hour in 3 *N* hydrochloric acid yielded the free amino acid.

*N-Acetyl-threo-β-phenyl-*DL*-serine.* (*A*). *threo*-β-Phenyl-DL-serine (50 g.) was acetylated with 30 g. of acetic anhydride and 2 *N* sodium hydroxide in a conventional Schotten-Baumann reaction. The precipitate that formed upon acidification of the reaction was collected and the filtrate was extracted with ethyl acetate to obtain more product. The ethyl acetate solution was concentrated, cooled, and the crystals thus obtained were combined with those first obtained upon acidification of the reaction mixture. Recrystallization of the combined crystals gave 35.5 g. (63%) of N-acetyl-*threo*-β-phenyl-DL-serine, m.p. 154–156°.

(*B*). To 5 g. of N-acetyl *threo*-β-phenyl-DL-serine ethyl ester, dissolved in 50 ml. of ethanol there was added 0.8 g. of sodium hydroxide in 20 ml. of water. The mixture was allowed to stand overnight at room temperature; the alcohol was removed in a stream of air following which the residue was acidified and extracted with ethyl acetate. Concentration of the solvent yielded 2.5 g. (56%) of material, m.p. 145°, which upon recrystallization from water yielded 1.0 g. of the N-acylamino acid, m.p. 154–156°.

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[2] The author is indebted to Dr. J. P. Greenstein for a sample of α-acetamidocinnamic acid and to Dr. Alton Meister for one of β-phenylpyruvic acid, 2,4-dinitrophenylhydrazone.

[3] All m.p.'s are corrected.

*N-Acetyl-erythro-β-phenyl*-DL-*serine*. In a manner similar to that described in (*A*) above 1.2 g. of *erythro*-β-phenyl-DL-serine was reacted with 1 g. of acetic anhydride to give 0.9 g. (67%) of N-acetyl compound, m.p. 164–167°. The melting point was raised to 174.5–176.5° by recrystallization from ethyl acetate.

*N-Chloroacetyl-erythro-β-phenyl*-DL-*serine*. (*A*). *erythro*-β-Phenyl-DL-serine (1.8 g.) was chloroacetylated by the Schotten-Baumann procedure using 1.6 g. of chloroacetyl chloride. The product was isolated by extraction followed by concentration of the solvent, and addition of petroleum ether, b.p. 35–65°, to induce crystallization. There was thus obtained 1.2 g. (52%) of crystals, m.p. 113–116°, which on recrystallization from ethyl acetate and petroleum ether gave 0.8 g. of the desired acid, m.p. 115–117°.

(*B*). N-Chloroacetyl-*erythro*-β-phenyl-DL-serine ethyl ester (0.55 g.) was hydrolyzed with 0.08 g. of sodium hydroxide in a manner analogous to that described above for the *threo*-N-acetyl ester. There was obtained 0.130 g. (26%) of the acid, m.p. 107–109° which on recrystallization gave 0.060 g., m.p. 115–117°.

*N-Dichloroacetyl-threo-β-phenyl*-DL-*serine*. By the Schotten-Baumann procedure 8 g. of *threo*-β-phenyl-DL-serine was reacted with 10 g. of dichloroacetyl chloride to give 7.5 g. of crystals, m.p. 153–157°. These upon recrystallization from water yielded 5.0 g. (43%) of the desired compound, m.p. 162–164°.

*N-Acetyl-threo-β-phenyl*-DL-*serine ethyl ester*. (*A*). *threo*-β-Phenyl-DL-serine ethyl ester hydrochloride (25.7 g.) was neutralized with a slight excess of alkali and extracted into ethyl acetate. To this dried extract (sodium sulfate) 10 g. of acetic anhydride was added and after one-half hour the resulting precipitate was collected to give 24 g. (91%) of the desired acyl ester, m.p. 180–182°. Recrystallization of a small sample for analysis from ether raised the m.p. to 180.5–182.5°.

(*B*). N-Acetyl-*threo*-β-phenyl-DL-serine (14.5 g.) was esterified by refluxing 24 hours in 250 ml. of absolute ethanol containing 1 ml. of sulfuric acid. After removal of the excess alcohol and addition of ether the unreacted acid was extracted with sodium bicarbonate. After removal of the solvent the product was recrystallized from alcohol to give 1.6 g. (10%) of ester, m.p. 177–180°. A mixture melting point with a sample from method *A* was found to be 177–180°.

*N-Acetyl-erythro-β-phenyl*-DL-*serine ethyl ester*. In a manner similar to that used in (*A*) for the corresponding *threo* compound the amino ester from 3.1 g. of the ester hydrochloride was reacted with 1.25 g. of acetic anhydride in ethyl acetate. The solution was washed with dilute hydrochloric acid and bicarbonate solution, following which the solvent was concentrated and upon cooling 2.4 g. (75%) of the acetyl ester was obtained, m.p. 145.5–147.5°.

*N-Chloroacetyl-threo-β-phenyl*-DL-*serine ethyl ester*. The amino ester in ether obtained from 4.9 g. of the corresponding ester hydrochloride was treated with 0.9 ml. of chloroacetyl chloride. After one-half hour the ether solution was washed with water, dried, and concentrated. Addition of petroleum ether, b.p. 35–65°, caused the precipitation of 1.6 (56%) of N-chloroacetyl *threo* ester, m.p. 140–141°.

*N-Chloroacetyl-erythro-β-phenyl*-DL-*serine ethyl ester*. In a like manner the amino ester in ether from 2.6 g. of the *erythro* ester hydrochloride was reacted with 0.7 ml. of chloroacetyl chloride. The resulting precipitate was dissolved with bicarbonate and more chloroacetyl chloride was added. Isolation of the product as in the previous case gave 1.1 g. (37%) of the N-chloroacetyl *erythro* ester, m.p. 97–99°.

*N-Acetyl-threo-β-phenyl*-DL-*serine methyl ester*. N-Acetyl-*threo*-β-phenyl-DL-serine (4.4 g.) was reacted with excess diazomethane (9) in ether. The excess diazomethane was destroyed with hydrochloric acid and the precipitated ester was collected. There was thus obtained 5 g. of material of m.p. 140–170° which upon two recrystallizations from methanol gave 1.1 g. (24%) of methyl ester, m.p. 182–184°.

*N-Chloroacetyl-threo-β-phenyl*-DL-*serine-methyl-ester*. In like manner 20 g. of N-chloroacetyl-*threo*-β-phenyl-DL-serine was reacted with excess diazomethane to give 16.5 g. (78%) of the methyl ester, m.p. 129.5–132° after crystallization from ethyl acetate. Recrystallization from the same solvent gave 14.5 g., m.p. 130–132°.

*Inversion of the threo-stereoisomer to the erythro form.* The N-acetyl-*threo*-β-phenyl-DL-serine ethyl ester (60 g.) was suspended in 350 ml. of chloroform and 19 ml. of thionyl chloride was added. After refluxing for two hours the chloroform was removed in a stream of air and the residue was heated 2 hours in 475 ml. of 2 *N* hydrochloric acid on the steam-bath. The cooled solution was extracted with ether and the ether solution in turn was extracted with aqueous bicarbonate. The original aqueous layer from the ether extraction was taken to dryness *in vacuo*. The residue was esterified with ethanol by saturation with anhydrous hydrogen chloride and allowing the reaction to stand overnight. Concentration of the alcohol solution, followed by chilling yielded 9 g. of *erythro*-β-phenyl-DL-serine ethyl ester hydrochloride, m.p. 167–175°. Several recrystallizations from alcohol yielded 4 g. (7%) of the ester hydrochloride, m.p. 178–180°. Addition of ether to the mother liquors caused the precipitation of 23 g. (40%) of material of m.p. 115–150° which presumably was a mixture of ester hydrochlorides.

The ethereal extract obtained above was taken to dryness to yield 6 g. of ethyl-α-acetamido-β-chlorohydrocinnamate, m.p. 105–107°. Recrystallization from ethyl acetate and petroleum ether gave m.p. 106–108°.

*Anal.*[4] Calc'd for $C_{13}H_{16}ClNO_3$: C, 57.8; H, 5.9; N, 5.2; Cl, 13.2.
Found: C, 57.8; H, 6.2; N, 5.2; Cl, 13.0.

From the bicarbonate extract obtained above there was isolated by acidification, ether extraction, removal of the solvent, and recrystallization from water 0.3 g. of phenylpyruvic acid, m.p. 153–155° [lit. (10) m.p. 154–155°].

*Ethyl α-acetamidocinnamate.* To 2.7 g. of ethyl α-acetamido-β-chlorohydrocinnamate in 20 ml. of alcohol there was added 5 ml. of 2 *N* sodium hydroxide. After standing overnight at room temperature the solvent was removed in a stream of air; the residue was taken up in ethyl acetate, washed with sodium bicarbonate solution, and dried with sodium sulfate. Concentration of the solvent followed by the addition of petroleum ether yielded 1.6 g. (69%) of ethyl α-acetamidocinnamate, m.p. 96–98°.

*Anal.*[4] Calc'd for $C_{13}H_{15}NO_3$: C, 66.9; H, 6.4; N, 6.0.
Found: C, 66.7; H, 6.7; N, 6.1.

*α-Acetamidocinnamic acid.* To 1.2 g. of ethyl α-acetamidocinnamate in 20 ml. of ethanol there was added 0.2 g. of sodium hydroxide in 20 ml. of water. After standing for four hours the alcohol was removed by a stream of air following which the residue was acidified and extracted with ether. Removal of the ether yielded 1.0 g. (94%) of crystals m.p. 183–188°. Several recrystallizations from water gave α-acetamidocinnamic acid, m.p. 192–194°, not depressed upon admixture with an authenic sample.[2]

*Phenylpyruvic acid 2,4-dinitrophenylhydrazone.* Phenylpyruvic acid (isolated above) (70 mg.) was reacted at room temperature with an excess of a saturated solution of 2,4-dinitrophenylhydrazine in hydrochloric acid. The collected precipitate weighed 90 mg. (60%) and upon recrystallization from ethyl acetate and petroleum ether had m.p. 162–164°.

*Anal.*[5] Calc'd for $C_{15}H_{12}N_4O_6$: C, 52.3; H, 3.5; N, 16.3.
Found: C, 52.2; H, 3.5; N, 15.9.

Recrystallization of the crystals from water led to material of m.p. 192–194°. A mixture of either form with an authentic sample gave m.p. 192–194°.

## SUMMARY

1. Ten new derivatives of the stereoisomeric β-phenyl-DL-serines have been prepared and characterized.

2. *Threo*-β-phenyl-DL-serine has been converted in low yield (7%) into the

[4] Analysis by Mr. R. J. Kogel of the National Cancer Institute.

[5] Analysis by Dr. W. C. Alford and associates of the microanalytical laboratory, National Institutes of Health.

*erythro* isomer by inversion at the β-carbon atom through the action of thionyl chloride on the ethyl ester of the N-acetyl *threo* compound. Also isolated from this inversion were ethyl α-acetamido-β-chlorohydrocinnamate and β-phenylpyruvic acid.

3. A new form of the 2,4-dinitrophenylhydrazone of β-phenylpyruvic acid is described.

4. Experimental details for the preparation of the isomeric β-phenyl-DL-serines are given.

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