The tube was then weighed and the loss in weight of the sample recorded. The sample tube was then replaced in the bath and the process repeated until the desired amount of data had been obtained. The loss in weight for complete reaction corresponded to about 70 mg. for these compounds with the size sample used. The high boiling point of the compounds studied as well as of the olefins produced ensured that the loss in weight was due only to the gaseous reaction products and hence the loss in weight can be correlated to the extent of reaction.

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[CONTRIBUTION FROM THE DEPARTMENT OF ORGANIC CHEMISTRY, RESEARCH DIVISION, SHARP AND DOHME, INC.]

β-Phenylserine

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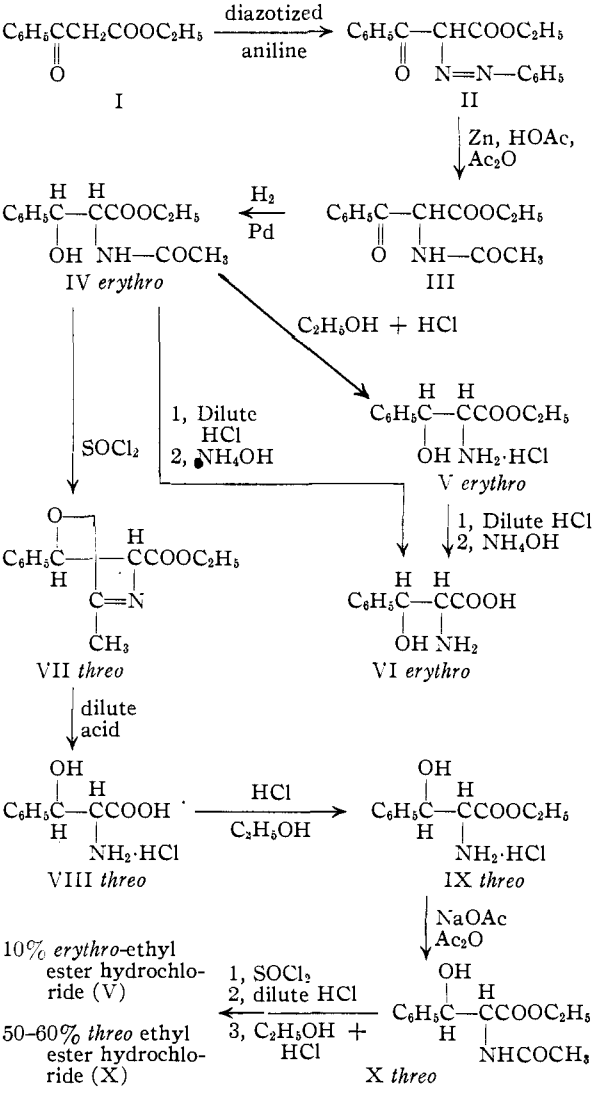
erythro-β-Phenylserine (VI) was prepared from ethyl benzoylacetate. The configuration of an intermediate, *erythro*-N-acetyl-β-phenylserine ethyl ester (IV), could be inverted by oxazoline formation with thionyl chloride. Hydrolysis of the oxazoline yielded *threo*-β-phenylserine hydrochloride (VIII). The configuration of *threo*-N-acetyl-β-phenylserine ethyl ester (X) was for the most part unchanged on treatment with thionyl chloride. No crystalline oxazoline was obtained from X.

The two diasteroisomers of β-phenylserine were required in connection with a biological investigation and a study of their preparation was undertaken. When the synthetic work was initiated, none of the published syntheses of β-phenylserine appeared promising as methods of preparing both diastereoisomeric forms. However, the synthesis of threonine and allothreonine from ethyl acetoacetate[1] provided an example of a successful preparation of both isomers of a β-substituted serine. Therefore, a new synthesis was devised in which *threo*- and *erythro*-β-phenylserine were synthesized from ethyl benzoylacetate. Hayes and Gever[2] attempted to use this method for the preparation of β-(2-furyl)-serine from ethyl 2-furoylacetate but were unable to separate one of the intermediates from the acetanilide that was formed as a by-product.

In this Laboratory, ethyl α-phenylazobenzoylacetate (II) was obtained by treating ethyl benzoylacetate (I) with benzenediazonium chloride by the method of Bulow and Neber.[3] Reductive acetylation of the phenylazo compound (II) yielded ethyl α-acetamidobenzoylacetate (III) with acetanilide as a by-product.

The removal of this by-product by ordinary recrystallization procedures was extremely difficult but repeated water extraction of a benzene solution of the mixture readily eliminated all the acetanilide. Reduction of the ketone (III) was carried out by a catalytic hydrogenation. Palladium catalyst (5% on charcoal) was used and the reduction ceased completely when one mole of hydrogen had been absorbed. The product (IV) had a sharp melting point and appeared to consist of a single diastereoisomer.

Treatment of the N-acetyl-β-phenylserine ethyl ester (IV) with hydrogen chloride in absolute ethanol yielded *erythro*-β-phenylserine ethyl ester hydrochloride (V). *erythro*-β-Phenylserine (VI) could be prepared indirectly by acid hydrolysis of the ester hydrochloride (V) and directly by acid hydrolysis of the *erythro*-N-acetyl-β-phenylserine ethyl ester



(IV). The yields were higher by the latter route, but intermediate isolation of the ester hydrochloride, possessing a characteristic melting point, gave an added check on the stereochemical purity of the final product.

(1) K. Pfister, C. A. Robinson, A. C. Shabica and M. Tishler, THIS JOURNAL, **71**, 1101 (1949).

(2) K. Hayes and G. Gever, *J. Org. Chem.*, **16**, 269 (1951).

(3) C. Bulow and P. Neber, *Ber.*, **45**, 3732 (1912).

The configuration of the groups about the β-carbon atom as they occur in *erythro*-N-acetyl-β-phenylserine ethyl ester (IV) was inverted by oxazoline formation with thionyl chloride. *threo*-2-Methyl-4-carbethoxy-5-phenyl-2-oxazoline hydrochloride (VII) was obtained as a sticky, hygroscopic, white product. Hydrolysis of the oxazoline (VII) with dilute acid yielded *threo*-β-phenylserine hydrochloride (VIII).

Although the configurational transformation brought about by thionyl chloride occurs with N-acylated esters of both threonine and allothreonine[1,4] evidence already has appeared that only the transformation from the *erythro* to the *threo* configuration occurs in compounds having the β-phenyl-β-hydroxyethylamine nucleus. This has been demonstrated with α,β-diphenyl-β-hydroxyethylamine by Weijlard, *et al.*,[5] and with 1-phenyl-2-acetamidopropane-1,3-diol by Moersch and Moore.[6]

To test the effect of thionyl chloride on *threo*-N-acetyl-β-phenylserine ethyl ester (X), this compound was prepared by acetylating *threo*-β-phenylserine ethyl ester hydrochloride (IX). On treating the *threo*-N-acetyl-β-phenylserine ethyl ester (X) with thionyl chloride in the same way the *erythro* compound was treated, no crystalline oxazoline hydrochloride was obtained. However, hydrolysis of the reaction mixture with acid and subsequent esterification (HCl and alcohol) of the hydrolysis product yielded small (10%) amounts of *erythro*-β-phenylserine ethyl ester hydrochloride. Large quantities (50–60%) of the more soluble *threo*-β-phenylserine ethyl ester hydrochloride were isolated and the only effect of making the conditions of the thionyl chloride reaction more drastic was increased tar formation. Vogeler[7] has already reported that an oxazoline was not found when *threo*-N-acetyl-β-phenylserine ethyl ester was treated with thionyl chloride. It is not known whether the small amount of *erythro* compound actually resulted from an oxazoline-type of inversion or whether it resulted from some other series of reactions. Nevertheless, the marked difference in behavior of *erythro*- and *threo*-N-acetyl-β-phenylserine ethyl ester toward thionyl chloride is striking.

Interest in the β-phenylserines has been stimulated by their similarity and ease of conversion to chloramphenicol type compounds and many publications have recently appeared dealing with their synthesis and stereochemistry. Bergmann, *et al.*,[8,9] report that certain aromatic aldehydes condense with glycine ester in absolute alcohol to give β-phenylserines of which β-phenylserine itself and the *p*-nitro analog were claimed to have the *threo* configuration. However, various investigators[10] have demonstrated by chemical and biological means that the β-*p*-nitrophenylserine prepared by Bergmann's method actually possesses the *erythro* configuration. Likewise, in this Laboratory, the condensation of benzaldehyde with glycine ethyl ester under the conditions reported by Bergmann has been found to yield *erythro*-β-phenylserine ethyl ester hydrochloride after acid-catalyzed alcoholysis of the Schiff base. Although the yield was only of the order of 20% this reaction coupled with the aqueous acid hydrolysis of the ester hydrochloride is probably the best method for the preparation of *erythro*-β-phenylserine.

Experimental[11]

Ethyl α-Phenylazobenzoylacetate (II).—Aniline (46.6 g., 0.50 mole) in a mixture of 200 ml. of concentrated hydrochloric acid and 500 g. of ice, was diazotized by the slow addition of an ice-cold solution of 35 g. (0.50 mole) of sodium nitrite in 160 ml. of water. The diazonium salt solution was added over the course of one hour to a well-stirred mixture of 450 g. (3.2 moles) of sodium acetate (hydrate), 300 ml. of water, 96.0 g. (0.50 mole) of ethyl benzoylacetate and 1800 ml. of ethyl alcohol held at 0° by external cooling. After the addition of the diazonium salt was complete, the volume of the reaction mixture was made up to four liters with ice-water and stirring was continued for another hour.

The product was collected by filtration and thoroughly washed with water. After drying (dry wt. 133.5 g.) it was washed with 300 ml. of hexane to yield 128 g. (86.6%) of a bright yellow product melting at 63–65°. Bulow and Hailer[13] reported that this compound melted at 65°.

Ethyl α-Acetamidobenzoylacetate (III).—A solution of 133 g. (0.45 mole) of ethyl α-phenylazobenzoylacetate in 355 ml. of glacial acetic acid was added to a well-stirred mixture of 203 g. (3.1 moles) of zinc dust, 675 ml. of acetic acid and 141 ml. (1.5 moles) of acetic anhydride as rapidly as the maintenance of a temperature of 15–20° by external ice cooling (*ca.* 1 hour) would permit. The mixture was stirred for 45 minutes at 25–30° and the zinc acetate and excess zinc dust were removed by filtration and thoroughly washed with glacial acetic acid. The combined filtrates were concentrated under reduced pressure to remove most of the acetic acid and anhydride.

The residual oil was dissolved in two liters of benzene and the solution was extracted with twelve 1,400-ml. portions of water. Each water wash, before being discarded, was passed successively through two separatory funnels containing 500 ml. of benzene. The oily residue (105 g.) obtained by combining and concentrating the benzene solutions was dissolved in 150 ml. of anhydrous ether. Crystallization was rapid and, after storage overnight at 0°, the product was collected and washed with cold ether (wt. 75.8 g.). Recrystallization from 100 ml. of ether yielded 72.0 g. (64.3%) of product melting at 75–80°. A sample, prepared for analysis by recrystallization first from ether and then from xylene, melted at 80–82°.

Anal. Calcd. for $C_{13}H_{15}O_4N$: C, 62.65; H, 6.07; N, 5.62. Found: C, 62.78; H, 6.15; N, 5.65.

***erythro*-N-Acetyl-β-phenylserine Ethyl Ester (IV).**—Ethyl α-acetamidobenzoylacetate (30.0 g., 0.12 mole) dissolved in glacial acetic acid (250 ml.) was hydrogenated at atmospheric pressure and temperature in the presence of 2.5 g. of 5% palladium-on-charcoal catalyst. A total of 2,980 ml. of hydrogen (theory 2,920 ml.) was absorbed in 2.5 hours. The catalyst was removed by filtration and the filtrate was concentrated under reduced pressure until heavy crystal deposition prevented any further evaporation. Fifty ml. of isopropyl alcohol was added and the product was allowed to stand at 0° for 18 hours. The first crop weighed 27.5 g.

(4) J. Attenburrow, D. F. Elliott and G. F. Penny, *J. Chem. Soc.*, 310 (1948); D. F. Elliott, *Nature*, **162**, 857 (1948); K. Pfister, C. A. Robinson, A. C. Shabica and M. Tishler, THIS JOURNAL, **70**, 2297 (1948).

(5) J. Weijlard, K. Pfister, E. Swanezy, C. A. Robinson and M. Tishler, *ibid.*, **73**, 1216 (1951).

(6) G. W. Moersch and A. C. Moore, U. S. Patent 2,513,346 (1950).

(7) K. Vogeler, *Helv. Chim. Acta*, **33**, 2111 (1950).

(8) E. D. Bergmann, M. Genas and H. Bendas, *Compt. rend.*, **231**, 361 (1950).

(9) E. D. Bergmann, H. Bendas and W. Taub, *J. Chem. Soc.*, 2673 (1951).

(10) G. W. Moersch, *et al.*, THIS JOURNAL, **74**, 565 (1952); M. Kopp, *et al.*, *Compt. rend.*, **233**, 527 (1951); and D. Billet and C. Marnay, *ibid.*, **233**, 961 (1951).

(11) For identification purposes in this work all β-phenylserine products were converted to the ethyl ester hydrochlorides which possessed distinctive melting points as reported by Shaw and Fox.[12] The microanalyses were carried out by Mr. Kermit Streeter and his staff. All melting points are uncorrected.

(12) K. N. F. Shaw and S. W. Fox, p. 28N, Abstracts of Papers, 118th Meeting of the American Chemical Society, Sept., 1950.

(13) C. Bulow and E. Hailer, *Ber.*, **35**, 923 (1902).

(m.p. 139–141°) and by concentrating the mother liquor a second crop of 1.4 g. (m.p. 135–137°) was obtained. The combined yield was 95.6%. Recrystallization of a sample from isopropyl alcohol gave a product melting at 142.5–143.5°.

Anal. Calcd. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.58. Found: C, 62.13; H, 6.83; N, 5.59.

***erythro*-β-Phenylserine Ethyl Ester Hydrochloride (V).**—*erythro*-N-Acetyl-β-phenylserine ethyl ester (56.5 g., 0.225 mole) was dissolved in 225 ml. of an approximately half saturated solution of hydrogen chloride in absolute alcohol and the clear solution was allowed to stand at room temperature. After 15 hours, heavy crystal deposition had occurred and 225 ml. of absolute ether was added to the mixture. It was cooled at 0° for 3 hours and filtered. The ethanol washed product weighed 35.0 g. (63.4%) and melted at 180–181° with decomposition.

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.56; N, 5.70; Cl, 14.43. Found: C, 53.91; H, 6.56; N, 5.71; Cl, 14.42.

***erythro*-β-Phenylserine (VI). A. By Hydrolysis of *erythro*-β-Phenylserine Ethyl Ester Hydrochloride.**—A solution of 34.5 g. of *erythro*-β-phenylserine ethyl ester hydrochloride in 175 ml. of 10% hydrochloric acid was heated under reflux for two hours. After vacuum concentration to dryness the total weight of solid product was 30.4 g. (theory for β-phenylserine hydrochloride is 30.6 g.). The crystalline product was dissolved in 25 ml. of water, treated with Darco, and neutralized with concentrated ammonia. After standing at 0° overnight, a first crop of 15.0 g. was obtained. Concentration of the filtrate yielded an additional 8.2 g., resulting in a total yield of 92.0%.

The combined products were recrystallized from a mixture of water and dimethylformamide and 16.1 g. of pure material was obtained. A sample dried at 100° *in vacuo* melted with decomposition at 199–200°.

Anal. Calcd. for $C_9H_{11}O_3N$: N, 7.72. Found: N, 7.75.

B. By Acid Hydrolysis of *erythro*-N-Acetyl-β-phenylserine Ethyl Ester.—The procedure used was the same as for the hydrolysis of the ester hydrochloride (above). An over-all yield of 92% was obtained.

***threo*-β-Phenylserine Hydrochloride (VIII).**—Two grams of *erythro*-N-acetyl-β-phenylserine ethyl ester was added to 6 ml. of thionyl chloride at 0°. After five minutes, 40 ml. of absolute ether was added and the product separated in the form of fine needles. Filtration and washing with absolute ether gave 1.70 g. (79.5%) of the oxazoline hydrochloride (VII) as sticky white crystals.

This product was added to 30 ml. of 10% hydrochloric acid and heated under reflux for two hours. The solution was concentrated until crystallization occurred and the residue was placed in a vacuum desiccator over sodium hydroxide pellets. Dry, powdery *threo*-β-phenylserine hydrochloride (1.30 g., 94.9% based on the oxazoline hydrochloride) was obtained which melted with decomposition at 164–166°.

Esterification with absolute alcohol and hydrogen chloride yielded *threo*-β-phenylserine ethyl ester hydrochloride (92.8%) melting at 134–137°. Recrystallization from alcohol yielded material having the characteristic melting point of 137.5–139°.

Anal. Calcd. for $C_{11}H_{16}O_3NCl$: C, 53.77; H, 6.56; N, 5.70; Cl, 14.43. Found: C, 53.86; H, 6.69; N, 5.69; Cl, 14.42.

***threo*-N-Acetyl-β-phenylserine Ethyl Ester (X).**—To a mixture of 40 ml. of acetic acid, 15 g. (0.18 mole) of anhydrous sodium acetate and 40 g. (0.16 mole) of *threo*-β-phenylserine ethyl ester hydrochloride (prepared by method of Carrara and Weitnauer[14]), acetic anhydride (16.5 ml., 0.17 mole) was added slowly with agitation. The temperature rose to about 80° and the solution became thick with crystals. After standing at room temperature for two hours, the mixture was poured into 500 ml. of water. The white product, washed until chloride free, weighed 39.0 g. (95.0%) and melted at 173–175°. A sample recrystallized from dioxane melted at 175–176.5°.

Anal. Calcd. for $C_{13}H_{17}O_4N$: C, 62.14; H, 6.82; N, 5.58. Found: C, 62.36; H, 6.77; N, 5.56.

Treatment of *threo*-N-Acetyl-β-phenylserine Ethyl Ester with Thionyl Chloride.—This experiment, and the identification of products by formation of the ester hydrochlorides was carried out in the same manner as described for the preparation of *threo*-β-phenylserine hydrochloride. The *erythro*-ethyl ester hydrochloride is much less soluble in alcohol than its *threo* isomer.

(14) G. Carrara and G. Weitnauer, *Gazz. chim. ital.*, **79**, 856 (1949).

WEST POINT, PA.

[CONTRIBUTION FROM THE SQUIBB INSTITUTE FOR MEDICAL RESEARCH]

Streptomycin. X.[1] The Structure of Mannosidostreptomycin[2]

BY JOSEF FRIED AND HOMER E. STAVELY

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Evidence is presented to show that the glycosidic linkage between the D-mannose and N-methyl-L-glucosamine moieties in mannosidostreptomycin extends to position 4 of the latter and that the former most probably exists in the pyranoid form. Mannosidostreptomycin is therefore represented by formula IV. Proof for the site of attachment of the D-mannose to the N-methyl-L-glucosamine moiety rests on the degradation of fully methylated N-acetyldihydromannosidostreptomycin by acid hydrolysis followed by acetylation and chromatography to 2,4-diacetyl-(V) and 1,2,4-triacetyl-3,6-dimethyl-N-methyl-α-L-glucosamine (VI). Additional evidence is adduced by periodate oxidation of mannosido- and N-pentaacetyl-mannosidostreptomycin. The pyranose structure of the N-methyl-L-glucosamine moiety in streptomycin and mannosidostreptomycin is confirmed by degradation of fully methylated N-acetyldihydrostreptomycin to a trimethyl-N-methyl-L-glucosamine, which is shown to possess the pyranoid structure XI.

Mannosidostreptomycin has been shown by stepwise degradation[1] to be composed of D-mannose (I), streptobiosamine (II) and streptidine (III),[3] joined glycosidically in the order: D-mannose → N-methyl-L-glucosamine → L-streptose → streptidine. Moreover, it has been shown that the glycosidic linkage between the D-mannose and the N-methyl-L-glucosamine moieties, is of the α-type and involves one of the three hydroxyl groups of the

(1) Paper IX of this series: H. E. Stavely and J. Fried, THIS JOURNAL, **71**, 135 (1949).

(2) Presented in part before the Division of Biological Chemistry of the American Chemical Society, Chicago, Ill., April, 1948.

(3) For a discussion of the structure of streptobiosamine and streptidine see the chapter "The Chemistry of Streptomycin" by R. V. Lemieux and M. L. Wolfrom in "Advances in Carbohydrate Chemistry," Vol. III, 1948, Academic Press Inc., New York, N. Y. Pertinent findings since the appearance of that review include the demonstration that streptose possesses the L-*lyxo*-configuration (F. A. Kuehl, Jr., M. N. Bishop, E. H. Flynn and K. Folkers, THIS JOURNAL, **70**, 2613 (1948); M. L. Wolfrom and C. W. DeWalt, *ibid.*, **70**, 3148 (1948)), that streptidine probably has the all-*trans*-configuration (M. L. Wolfrom, S. M. Olin and W. J. Polglase, *ibid.*, **72**, 1724 (1950); O. Wintersteiner and A. Klingsberg, *ibid.*, **70**, 885 (1948)) and that the anomeric structure of the L-streptose–streptidine linkage is of the β-type (M. L. Wolfrom, M. J. Cron and R. M. Husband, Abstr. of Papers 118th Meeting, Am. Chem. Soc., 7R, (1950)).