56. *Some Derivatives of β-Phenylserine.*

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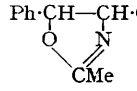
The possible inversion of the diastereoisomeric β-phenyl- and β-*p*-nitrophenyl-serines has been briefly studied and several *p*-substituted phenylserines have been prepared.

During our study of derivatives of β-phenylserine (I; R = R′ = H, X = CO_2H), considered as analogues of the antibiotic chloramphenicol (I; *threo*; R = $CO{\cdot}CHCl_2$, R′ = NO_2, X = $CH_2{\cdot}OH$), reports of similar investigations appeared from several sources. This paper therefore records only that part of our work which has not been adequately described elsewhere: our conclusions regarding the two *p*-nitrophenylserines have been previously but briefly published (Holland and Jenkins, *Chem. and Ind.*, 1951, 1092; Holland and Nayler, *ibid.*, 1952, 518).

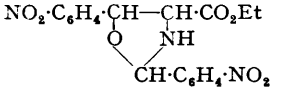
Although *threo*-phenylserine, the configuration of which has been determined by Billet (*Compt. rend.*, 1950, **230**, 1074) and by Vogler (*Helv. Chim. Acta*, 1950, **33**, 2111), has been known for some time the pure *erythro*-form had not been described when our work began and attempts were made to prepare it by inversion of the *threo*-isomer. Satisfactory methods for the preparation of *erythro*-phenylserine have since been reported by Shaw and Fox (Abstr., 118th A.C.S. meeting, 1950, p. 28N) and by Elphimoff-Felkin *et al.*, (*Compt. rend.*, 1951, **232**, 241; *Bull. Soc. chim.*, 1952, **19**, 252).

p-R′·C_6H_4·CH(OH)·CHX·NHR
(I)

Ph·CHCl·CH(NHAc)·CO_2Me
(III)



(II)



(IV)

Attempts to effect inversion at the β-carbon atom of *N*-acetyl-*threo*-phenylserine methyl ester *via* the *cis*-oxazoline (II) by reaction with thionyl chloride gave the β-chloro-derivative (III) as the only tractable product, even at −20° when some starting material remained unchanged. Treatment of the reaction product with pyridine, as described by Fry (*J. Org. Chem.*, 1950, **15**, 438) for the preparation of methyl 2-phenyl-L-oxazoline-4-carboxylate, also failed since the only transformation products isolated were the chloro-compound (III) and methyl α-acetamidocinnamate [no doubt formed from (III) by removal of hydrogen chloride]. Vogler (*loc. cit.*) similarly obtained a β-chloro-compound rather than an oxazoline from the corresponding ethyl ester, although Moersch, Rebstock, Moore, and Hylander (*J. Amer. Chem. Soc.*, 1952, **74**, 565) inverted *erythro*-*p*-nitrophenylserine by the oxazoline route.

The action of moist silver carbonate on (III) gave the original *N*-acetyl-*threo*-phenylserine methyl ester and since this reagent does not in general effect a Walden inversion it appears that the chloro-compound (III) also possesses the *threo*-configuration. Attempts to replace the chlorine of (III) by hydroxyl with inversion by the use of stronger bases were unsuccessful. Sodium hydroxide at ordinary temperature yielded mainly α-acetamidocinnamic acid together with an unsaturated neutral substance, $C_{10}H_{11}ON$, which

may have been acetamidostyrene or 2-methyl-5-phenyl-Δ^1-oxazoline. Sodium carbonate similarly removed hydrogen chloride to yield methyl α-acetamidocinnamate. This ester on hydrolysis with aqueous sodium hydroxide gave only the corresponding acid. The chlorine atom was also replaced by hydroxyl without inversion when (III) was converted by boiling dilute mineral acid into *threo*-phenylserine.

Two DL-forms of *p*-nitrophenylserine (I; R = H, R′ = NO_2, X = CO_2H) are known although their configurations have recently aroused controversy. Compounds of one series have been obtained by numerous workers by the nitration of *threo*-phenylserine or derivatives thereof; those of the other arise by a sequence of reactions from *p*-nitrobenzaldehyde and glycine ester. The latter reactants condense in ether in the presence of sodium (Dalgliesh, *J.*, 1949, 90) or in alcohol without a catalyst (Bergmann, Genas, and Bendas, *Compt. rend.*, 1950, **231**, 361; Bergmann, Bendas, and Taub, *J.*, 1951, 2673) to yield a compound $C_{18}H_{17}O_7N_3$ tentatively regarded by Dalgliesh as the oxazolidine (IV) but which from its infra-red spectrum appears to be the isomeric Schiff's base (V) (Bergmann, Zimkin, and Pinchas, *Rec. Trav. chim.*, 1952, **71**, 168). A compound, m. p. 148°, obtained earlier by Gerngross and Zühlke (*Ber.*, 1924, **57**, 1482) from *p*-nitrobenzaldehyde and glycine ester in alcohol and considered by them to be *p*-nitrobenzylideneglycine ester appears also to be this *p*-nitrophenylserine derivative, since authentic *p*-nitrobenzylideneglycine ester has m. p. 85—88° (see below). We have found that the compound (V) is more conveniently prepared in alcohol in the presence of triethylamine. Conversion into *p*-nitrophenylserine was then effected in good overall yield essentially as described by Dalgliesh (*loc. cit.*).

That the *p*-nitrophenylserine obtained in this way differs from that derived by the nitration of *threo*-phenylserine is clear from their different biological activity (Billet, *loc. cit.*; Molho and Molho-Lacroix, *Compt. rend.*, 1951, **233**, 1067) and from the distinctive melting points of several derivatives (Kopp, Larramona, and Welvart, *Compt. rend.*, 1951, **233**, 527; Moersch *et al.*, *loc. cit.*). In addition we have found that the two isomers are readily separable by paper partition chromatography. Both forms of *N*-acetyl-*p*-nitrophenylserine, with acetic anhydride, yielded 2-methyl-4-*p*-nitrobenzylidene-5-oxazolone, which is also obtained when *p*-nitrobenzaldehyde and aceturic acid are heated with acetic anhydride and sodium acetate.

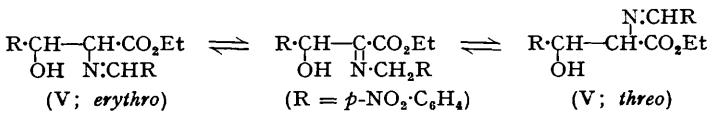
It has been generally assumed that the *p*-nitrophenylserine derived from *threo*-phenylserine itself has the *threo*-configuration and that the compounds derived from *p*-nitrobenzaldehyde are hence members of the *erythro*-series. In support of this, Kopp *et al.* (*loc. cit.*) described the preparation of an *ON*-diacetyl-*p*-nitrophenylserine ester from *erythro*-phenylserine, identical with the acetylation product of the *p*-nitrophenylserine ester obtained from *p*-nitrobenzaldehyde. It has, however, been claimed by Bergmann, Bendas, and Taub (*loc. cit.*) that condensation of *p*-nitrobenzaldehyde with glycine ester in alcohol gives compounds possessing the *threo*-configuration since lithium aluminium hydride reduction, presumably of the *p*-nitrophenylserine ester, apparently gave rise to *threo*-2-amino-1-*p*-nitrophenylpropane-1 : 3-diol (I; R = H, R′ = NO_2, X = CH_2·OH). Accordingly, we examined the lithium aluminium hydride reduction of compounds of both series and obtained *threo*-2-acetamido-1-*p*-nitrophenylpropane-1 : 3-diol (I; R = Ac, R′ = NO_2, X = CH_2·OH) from the ester of the nitration product of *N*-acetyl-*threo*-phenylserine and the *erythro*-diol from the acetyl derivative of the ester obtained from *p*-nitrobenzaldehyde. These results prove that the main product isolated from the condensation of *p*-nitrobenzaldehyde and glycine ester has the *erythro*-configuration. Attempts to reduce the unacetylated *erythro*-ester with lithium aluminium hydride were unsuccessful.

The claim by Billet and Marnay (*Compt. rend.*, 1951, **233**, 961) to have obtained the *threo*-form, m. p. 89°, of the Schiff's base (V) by the uncatalysed reaction of *p*-nitrobenzaldehyde and glycine ester in dry ether is apparently unfounded since it has been shown independently by Elphimoff-Felkin, Felkin, and Welvart (*Compt. rend.*, 1952, **234**, 1564) and by two of us (Holland and Nayler, *loc. cit.*) that the product is in fact *N*-*p*-nitrobenzylideneglycine ester. Authentic *N*-*p*-nitrobenzylidene-*threo*-*p*-nitrophenylserine ethyl ester prepared from *threo*-*p*-nitrophenylserine ester and *p*-nitrobenzaldehyde has m. p. 121—121·5°, evidence that this compound is not the isomeric oxazolidine (IV) being

afforded by the very close similarity of its ultra-violet absorption spectrum to that of the *erythro*-Schiff's base.

It is noteworthy that none of the various groups of workers who have condensed *p*-nitrobenzaldehyde with glycine ester has referred to the possibility of more than one form of *p*-nitrophenylserine derivative being present in a given reaction mixture. We have found, using paper chromatography, that the condensation in ethanol either with or without triethylamine gives rise to both *erythro*- and *threo*-compounds although the former are produced in greater quantity and, being the less soluble, constitute the form normally isolated. The erroneous conclusion by Bergmann, Bendas, and Taub (*loc. cit.*) as to the configuration of the major constituent may thus have been due to the reduction of a mixture of diastereoisomers and the fortuitous isolation of only the *threo*-form of the product.

The possibility that the two forms of *p*-nitrophenylserine might undergo inversion by way of the *N*-*p*-nitrobenzylidene derivatives of their esters was first suggested by Billet and Marnay (*loc. cit.*) to explain the spontaneous conversion in alcohol of a labile compound, m. p. 85°, obtained by Dalgliesh into the *erythro*-form of (V). Billet and Marnay considered the labile compound to be the *threo*-form of (V), but this has now been shown to have m. p. 121—121·5°. However, since inversion at the α-carbon atom of (V) could theoretically occur by prototropy as indicated below (cf. Harvill and Herbst, *J. Org. Chem.*, 1944, **9**, 21, and earlier papers) we briefly examined the behaviour of pure specimens of each diastereoisomer in ethanol.



After 1 hour's refluxing either pure Schiff's base was found to give rise on hydrolysis to a mixture of both forms of *p*-nitrophenylserine, but from the relative intensities of the spots on a paper chromatogram developed with ninhydrin the extent of inversion appeared to be fairly small. The prototropic mechanism advanced above suggested that a base might accelerate the attainment of equilibrium and it was found that in presence of 0·1 mol. of triethylamine each Schiff's base gave substantial amounts of each form of *p*-nitrophenylserine. There was, however, no clear evidence of preferential formation of one isomer. Similar results were obtained when the Schiff's bases were formed *in situ* by heating the *p*-nitrophenylserine esters with *p*-nitrobenzaldehyde and triethylamine in alcohol. It was established that the method of conversion into the amino-acids caused no appreciable inversion, although a little breakdown to glycine occurred during alkaline hydrolysis of the esters. Similarly the esters did not suffer significant inversion on brief heating with ethanolic triethylamine in the absence of aldehydes.

Elphimoff-Felkin, Felkin, and Welvart (*Compt. rend.*, 1952, **234**, 1627) recently recorded the conversion of the *threo*-ester into the *erythro*-Schiff's base and *vice versa*, and postulated a mechanism involving fission of the central carbon–carbon bond.

Several other substituted phenylserines have been prepared from the two *p*-nitrophenylserines. Catalytic hydrogenation afforded the two *p*-amino-compounds of which the *erythro*-isomer has been described by Dalgliesh, and these led by diazotisation to the hydroxy- and the chloro-compounds.

p-Hydroxyphenylserine was also prepared, in 15% overall yield, from *p*-(ethyl carbonato)benzaldehyde and glycine ester in the presence of sodium as described by Rosenmund and Dornsaft (*Ber.*, 1919, **52**, 1734). The configuration of the product could not be inferred from its melting point since those of the two *p*-hydroxyphenylserines differ by only a few degrees and, in common with the other diastereoisomeric pairs of amino-acids encountered in the present work, give no depression on admixture. Paper partition chromatography indicated, however, that it was probably *erythro*-*p*-hydroxyphenylserine.

In addition to the diazotisation method *threo*-*p*-chlorophenylserine was also obtained by direct condensation of *p*-chlorobenzaldehyde with glycine in aqueous-alcoholic sodium hydroxide.

None of these compounds possessed appreciable antibacterial activity.

Experimental

M. p.s are uncorrected.

N-*Acetyl*-threo-β-*phenylserine*.—To a solution of *threo*-phenylserine (36 g.) in N-sodium hydroxide (200 ml.), 2N-sodium hydroxide (520 ml.) and acetic anhydride (52 ml.) were run in concurrently with stirring during 1·5 hours while the temperature was kept at 0—5° and the mixture was kept alkaline. After storage at 0° overnight the filtered solution was acidified (pH 1) with concentrated hydrochloric acid and concentrated under reduced pressure to about 200 ml. On refrigeration the product separated almost quantitatively as colourless needles, m. p. 146—147° (decomp.), unchanged by recrystallisation from hot water (Weitnauer, *Gazzetta*, 1951, **81**, 156 gives m. p. 152°) (Found: C, 59·0; H, 5·9; N, 6·5. Calc. for $C_{11}H_{13}O_4N$: C, 59·2; H, 5·8; N, 6·3%).

Methyl ester. *N*-Acetyl-*threo*-phenylserine (20 g.) suspended in dry dioxan was treated with an excess of ethereal diazomethane with shaking and initial cooling. Next morning the excess of diazomethane was destroyed with acetic acid and the crude solid (19·3 g.) was collected and washed with ether. Recrystallisation from water (300 ml.)–alcohol (70 ml.) yielded the *ester* (15·4 g.), constant m. p. 174—180° (decomp.) (Found: C, 60·3; H, 6·6; N, 6·1. $C_{12}H_{15}O_4N$ requires C, 60·8; H, 6·3; N, 5·9%).

Methyl α-*Acetamido*-β-*chloro*-β-*phenylpropionate*.—Thionyl chloride (40 ml.) was added dropwise during 1 hour to a stirred suspension of *N*-acetyl-*threo*-phenylserine methyl ester (20 g.) in dry chloroform (20 ml.) at about 0°. After being kept overnight at 0° the solution was concentrated under reduced pressure at room temperature and the remaining red syrup was diluted with chloroform and extracted with 5 small portions of water. Evaporation of the dried chloroform layer yielded a dark brown syrup, which solidified when rubbed with ethyl acetate. The yellow solid was washed with ethyl acetate–ether (1 : 1) and finally ether, to yield 9·05 g. of *methyl* α-*acetamido*-β-*chloro*-β-*phenylpropionate*. A further 0·82 g. was obtained by concentration of the filtrates and cautious dilution with ether. Recrystallisation from 50% aqueous alcohol gave a white powder, m. p. 128—128·5° (Found: C, 56·6; H, 5·7; N, 5·1; Cl, 13·7. $C_{12}H_{14}O_3NCl$ requires C, 56·4; H, 5·5; N, 5·5; Cl, 13·9%).

Action of Bases on Methyl α-*Acetamido*-β-*chloro*-β-*phenylpropionate* (III).—(a) *Silver carbonate*. To a solution of silver nitrate (0·74 g.) in water (2·5 ml.) there was added aqueous potassium carbonate (0·3 g. in 2·5 ml.), followed by alcohol (5 ml.) and the β-chloro-ester (1 g.). The mixture was heated under reflux for 4 hours, filtered, and concentrated under reduced pressure; *N*-acetyl-*threo*-phenylserine methyl ester (0·25 g.) separated, having m. p. 174—180° not depressed by an authentic specimen.

(b) *Sodium hydroxide*. A mixture of the chloro-ester (2·55 g.) with 1·01N-sodium hydroxide (24·7 ml.) and dioxan (10 ml.) was shaken for 1 hour, and the resulting clear solution kept at ordinary temperature for 2 days, then concentrated under reduced pressure to a small volume; some white solid separated. This was removed, washed with water, and dried *in vacuo* ($CaCl_2$), to yield 0·44 g., m. p. 113—114° with slight gas evolution. After several crystallisations from carbon tetrachloride and from aqueous alcohol, followed by sublimation *in vacuo*, the *substance* had m. p. 113—117°, after shrinking from 106° (Found: C, 74·4; H, 6·5; N, 9·1. $C_{10}H_{11}ON$ requires C, 74·5; H, 6·8; N, 8·7%). It rapidly decolorised bromine water.

The alkaline filtrate on acidification evolved carbon dioxide and deposited α-acetamidocinnamic acid (1·32 g., 64%), m. p. 186—188° (decomp.) after crystallisation from water and drying at 80°/0·5 mm. (P_2O_5) (Found: C, 64·1; H, 5·6; N, 7·1. Calc. for $C_{11}H_{11}O_3N$: C, 64·4; H, 5·4; N, 6·8%). Erlenmeyer and Früstück (*Annalen*, 1895, **284**, 47) gave m. p. 190—191° (decomp.) for the anhydrous acid. This, when heated with acetic anhydride, yielded its azlactone, m. p. 153—154°, identical with the compound obtained on heating phenylserine with the same reagent (Bergmann and Delis, *ibid.*, 1927, **458**, 76).

(c) *Sodium carbonate*. The chloro-ester (1·5 g.) and sodium carbonate (0·34 g.) in 50% aqueous dioxan (40 ml.) were refluxed for 3 hours and concentrated *in vacuo* to a small volume. A gum separated which solidified. This crude *methyl* α-*acetamidocinnamate* (1·05 g.) was crystallised several times from benzene and then had m. p. 124—127° (slight decomp.) (Found: C, 65·3; H, 6·1. $C_{12}H_{13}O_3N$ requires C, 65·8; H, 5·9%). It rapidly decolorised bromine water, and when shaken with N-sodium hydroxide yielded α-acetamidocinnamic acid almost quantitatively.

Action of Dilute Hydrochloric Acid on (III).—The chloro-ester (2 g.) was refluxed in 10% hydrochloric acid (30 ml.) for 4 hours, developing an odour of phenylacetaldehyde, and after extraction with ethyl acetate was evaporated *in vacuo*, treated with water and concentrated

again. The residual gum was dissolved in a little water, an excess of triethylamine added, the mixture evaporated *in vacuo*, and the residue washed with chloroform to remove triethylamine hydrochloride and leave crude *threo*-phenylserine (1·07 g.), characterised as the *N*-acetyl derivative.

threo-β-p-*Nitrophenylserine*.—*N*-Acetyl-*threo*-phenylserine was nitrated essentially as described by Woolley (*J. Biol. Chem.*, 1950, **185**, 293) except that 0·3 mol. of sodium nitrite was included in the cold fuming nitric acid. The resulting *N*-acetyl-*threo-p*-nitrophenylserine (67·5%) separated from aqueous alcohol as cream-coloured crystals, m. p. 191—192° (decomp.) (Found: C, 49·1; H, 4·9; N, 10·0. Calc. for $C_{11}H_{12}O_6N_2$: C, 49·3; H, 4·5; N, 10·4%). Hydrolysis was accomplished in 72% yield essentially as described by Woolley, the amino-acid crystallising from water (charcoal) as a cream-coloured powder, m. p. 180—181° (decomp.) (dependent considerably on the rate of heating). Analysis indicated 1·5 mol. of water of crystallisation (Billet, *loc. cit.*, reported only 1 mol.) (Found: C, 42·8; H, 5·15; N, 10·7. Calc. for $C_9H_{10}O_5N_2,1{\cdot}5H_2O$: C, 42·7; H, 5·14; N, 11·1%).

erythro-β-p-*Nitrophenylserine*.—A solution of glycine ethyl ester hydrochloride (43·5 g.) in hot absolute alcohol (250 ml.) was added to a solution of sodium (7·15 g.) in absolute alcohol (175 ml.) and after 1 hour sodium chloride was removed. To the filtrate was added *p*-nitrobenzaldehyde (93·9 g.) followed by triethylamine (4·5 ml.), and the mixture shaken occasionally until the reaction appeared to be virtually complete. Next morning the intermediate *p*-nitrobenzylidene derivative (V), m. p. 142—143°, was filtered off, washed with alcohol and then ether (yield 99·6 g., 82%). A sample recrystallised from methanol or benzene formed colourless needles, m. p. 146—147° (Found: C, 55·8; H, 4·3; N, 11·0. Calc. for $C_{18}H_{17}O_7N_3$: C, 55·8; H, 4·4; N, 10·8%).

The intermediate (133·2 g.) was heated under reflux with absolute alcohol (1330 ml.), and 2·58N-alcoholic hydrochloric acid (201 ml., 1·5 equiv.) was added to the boiling suspension. Dissolution was rapid and after about 2 minutes the *p*-nitrophenylserine ester hydrochloride began to separate. After 10 minutes' refluxing the mixture was cooled and stored in the ice-chest overnight, to give 72·5 g. of product, m. p. 176—179° (decomp.), a further 5 g. being obtained from the mother-liquors on concentration. The crude hydrochloride (133·5 g.) was stirred for 3 hours at room temperature in N-sodium hydroxide (913 ml.)–alcohol (270 ml.). Neutralisation of the resulting yellow solution with N-hydrochloric acid gave *erythro*-β-*p*-nitrophenylserine as a cream-coloured powder (*ca.* 100%), m. p. 187—188° (decomp.) after recrystallisation from water and drying (P_2O_5) at 80°/0·5 mm. (Found: C, 47·9; H, 5·1; N, 12·2. Calc. for $C_9H_{10}O_5N_2$: C, 47·8; H, 4·4; N, 12·4%).

In another experiment the filtrates at each stage were subjected to the same reactions as the corresponding isolated solids, the *p*-nitrobenzaldehyde liberated on acid treatment of the Schiff's base being removed before the final alkaline hydrolysis by evaporation and extraction of the residue with benzene and ether. Aqueous solutions (0·3%) from each crop of solid amino-acid, together with the corresponding mother-liquors, were compared with pure specimens of the two *p*-nitrophenylserines by partition chromatography on Whatman No. 1 paper with butanol–formic acid–water (10 : 1 : 10). Results indicated a 50% overall yield of pure *erythro-p*-nitrophenylserine (R_F 0·48) and a further 8% apparently consisting mainly of the *erythro*-compound but also containing a little of the *threo*-form (R_F 0·41); the mother-liquors appeared to contain substantial amounts of both forms and also some glycine (R_F 0·12). In an experiment without triethylamine, *i.e.*, essentially as described by Bergmann *et al.* (*loc. cit.*), the chromatographic results were qualitatively similar but the total overall yield of solid amino-acid was only 9%.

N-*Acetyl*-erythro-p-*nitrophenylserine*.—*erythro-p*-Nitrophenylserine was acetylated as described for *threo*-phenylserine, the *derivative* crystallising from water as pale yellow platelets, m. p. 170° (decomp.) (Found: C, 47·5; H, 4·7; N, 10·3. $C_{11}H_{12}O_6N_2,0{\cdot}5H_2O$ requires C, 47·6; H, 4·7; N, 10·1%). The m. p. and analysis were unchanged when the compound was dried (P_2O_5) at 80°/0·2 mm. for 3 hours. This derivative depressed the m. p. of the *threo*-isomer.

2-*Methyl*-4-p-*nitrobenzylidene*-5-*oxazolone*.—*p*-Nitrobenzaldehyde (2 g.), aceturic acid (1·83 g.), and fused sodium acetate (0·87 g.) in acetic anhydride (5 ml.) were heated on the water-bath for 1 hour. After cooling, the resulting *oxazolone* (2·2 g.) was washed first with 50% aqueous alcohol and then with ether and crystallised from alcohol–acetone (charcoal) as bright yellow needles, m. p. 186—187° (Found: C, 56·9; H, 3·9; N, 12·2. $C_{11}H_8O_4N_2$ requires C, 56·9; H, 3·5; N, 12·1%). The same compound was formed when either form of *N*-acetyl-*p*-nitrophenylserine was heated for a few minutes with acetic anhydride.

threo-p-*Nitrophenylserine Ethyl Ester Hydrochloride*.—A suspension of *threo-p*-nitrophenylserine (7 g.) in ethanol (100 ml.) was refluxed in a stream of dry hydrogen chloride for 4 hours. Evaporation of the clear yellow solution *in vacuo* left a syrup which on treatment with ethyl acetate and ether yielded a crystalline deliquescent solid (5·80 g.), crystallising from acetonitrile as non-deliquescent needles of the *hydrochloride*, m. p. 157—159° (softening at 154°) (Found: C, 45·3; H, 5·0; N, 9·65; Cl, 12·2. $C_{11}H_{15}O_5N_2Cl$ requires C, 45·4; H, 5·2; N, 9·6; Cl, 12·2%). The free ethyl ester was liberated by treating an ice-cold suspension of the hydrochloride with the calculated quantity of potassium carbonate solution, and crystallised from benzene as needles, m. p. 129—130° (Found: C, 52·0; H, 5·6; N, 11·3. Calc. for $C_{11}H_{14}O_5N_2$: C, 52·0; H, 5·55; N, 11·0%). The *erythro*-ester, similarly prepared and purified, formed needles, m. p. 114—115° (Found: C, 52·1; H, 5·4; N, 11·2%). The m. p.s of these esters depend markedly on the rate of heating, which may account for discrepancies in the literature.

N-*Acetyl*-threo-p-*nitrophenylserine Ethyl Ester*.—A solution of the *threo*-ester hydrochloride (2·91 g.) in ice-water (30 c.c.) was treated with acetic anhydride (2·5 ml.), followed by a solution of sodium acetate trihydrate (5 g.) in water (10 ml.), shaken at room temperature overnight, acidified with hydrochloric acid, and cooled to 0°, and the crude product collected (2·56 g.). N-*Acetyl*-threo-p-*nitrophenylserine ethyl ester* crystallised from 50% aqueous alcohol in needles, m. p. 182—184° (softening at 177°) (Found: C, 52·9; H, 5·4; N, 9·7. $C_{13}H_{16}O_6N_2$ requires C, 52·7; H, 5·4; N, 9·5%). The *erythro-N*-acetyl-ester, prepared similarly and crystallised from water, formed needles, m. p. 148—149° (softening at 144°) (Found: C, 52·7; H, 5·7; N, 9·2%). Moersch *et al.* (*loc. cit.*) give m. p. 158—159°.

threo-2-*Acetamido*-1-p-*nitrophenylpropane*-1 : 3-*diol*.—Lithium aluminium hydride (0·48 g.) in dry ether (50 ml.) was added during 2 hours to a stirred solution of *N*-acetyl-*threo-p*-nitrophenylserine ethyl ester (1·48 g.) in dry dioxan (75 ml.), then stirred for a further 2·5 hours, treated successively with water (5 ml.) and 5N-hydrochloric acid (5 ml.), and evaporated. An aqueous solution (20 ml.) of the residue was extracted with ethyl acetate (4 × 50 ml.). The combined extracts were dried (Na_2SO_4) and evaporated *in vacuo*, leaving a gum which on trituration with ethyl acetate yielded a pale brownish-yellow solid (0·29 g.). Recrystallisation from acetone gave a practically colourless powder, m. p. 165—167° not depressed by admixture with an authentic specimen of *threo*-2-acetamido-1-*p*-nitrophenylpropane-1 : 3-diol prepared by Long and Troutman's method (*J. Amer. Chem. Soc.*, 1949, **71**, 2473) (Found: C, 52·0; H, 5·6; N, 11·0. Calc. for $C_{11}H_{14}O_5N_2$: C, 52·0; H, 5·55; N, 11·0%).

erythro-2-Acetamido-1-*p*-nitrophenylpropane-1 : 3-diol, similarly prepared from the *erythro*-ester in 11% yield and crystallised from acetone, had m. p. 191—193° (Long and Troutman give m. p. 195°) (Found: C, 52·5; H, 5·8; N, 10·9%).

N-p-*Nitrobenzylidene*-threo-p-*nitrophenylserine Ethyl Ester*.—To a hot solution of *threo-p*-nitrophenylserine ethyl ester (10 g.) in chloroform (500 ml.) was added *p*-nitrobenzaldehyde (6 g.) and anhydrous sodium sulphate (5 g.). After 3 days at room temperature the filtered mixture on concentration *in vacuo* and trituration with ether at low temperature yielded N-p-*nitrobenzylidene*-threo-p-*nitrophenylserine ethyl ester* as a white powder (12·25 g.). This was best purified by repeated crystallisation from dry ether, forming needles, m.p. 121—121·5°, absorption max. at 2690 Å ($E^{1\%}_{1\,cm.}$ 621 in $CHCl_3$) (Found: C, 56·0; H, 4·8; N, 11·2. $C_{18}H_{17}O_7N_3$ requires C, 55·8; H, 4·4; N, 10·85%).

The *erythro*-Schiff's base, similarly prepared, had m. p. 146—147°, undepressed by admixture with the product from *p*-nitrobenzaldehyde and glycine ester; it had an absorption max. at 2700 Å ($E^{1\%}_{1\,cm.}$ 635 in $CHCl_3$).

erythro-p-*Aminophenylserine*.—*erythro-p*-Nitrophenylserine (30 g.) in 1·37N-hydrochloric acid (200 ml.) and alcohol (200 ml.) was hydrogenated at ordinary temperature and pressure on Adams's catalyst, absorption being complete in 5 hours. The resulting pale yellow solution was filtered into a little water containing a trace of sodium dithionite and concentrated under reduced pressure under hydrogen. The syrupy residue was treated with a little water and the concentration twice repeated. The final residue was taken up into water (70 ml.) containing a little sodium dithionite and neutralised to pH 7 with ammonia, to precipitate *p*-aminophenylserine (22·7 g.). Recrystallisation from aqueous alcohol gave a cream-coloured powder, m. p. 205—207° (decomp.) (Found: C, 50·6; H, 6·9; N, 13·0. Calc. for $C_9H_{12}O_3N_2,H_2O$: C, 50·5; H, 6·6; N, 13·1%).

threo-p-*Aminophenylserine*.—*threo-p*-Nitrophenylserine (30 g.) was reduced and worked up as described above, to give 73% of threo-p-*aminophenylserine*, m. p. 205° (decomp.) (rapid heating from 180°), from aqueous alcohol (Found: C, 50·0; H, 6·5; N, 13·0%).

N^1-*Acetyl*-threo-p-*aminophenylserine*.—A solution of *N*-acetyl-*threo-p*-nitrophenylserine

(3·23 g.) in alcohol (100 ml.) was hydrogenated at ordinary temperature and pressure on Adams's catalyst, some solid separating towards the end of the reduction which was complete in 1 hour. The mixture was heated with further alcohol (50 ml.) and the resulting solution filtered. On cooling of the filtrate N^1-*acetyl*-threo-p-*aminophenylserine* separated and a further crop was obtained from the concentrated mother-liquors (charcoal). The product (2·08 g.) had m. p. 124—125°, not increased by recrystallisation from alcohol (Found: C, 53·5; H, 6·4; N, 11·8. $C_{11}H_{14}O_4N_2,0·5H_2O$ requires C, 53·4; H, 6·1; N, 11·3%).

erythro-p-*Hydroxyphenylserine*.—A solution of *erythro-p*-aminophenylserine (4·5 g.) in a mixture of sulphuric acid (4·9 ml.) and water (35 ml.) was kept at −5° to 0° whilst a solution of sodium nitrite (1·44 g.) in water (5 ml.) was added dropwise with stirring during 20 minutes. After a further 10 minutes' stirring below 0° the solution was heated on the steam-bath for 10 minutes (negative diazo-test), kept for 1 hour at room temperature, clarified with charcoal, and neutralised to pH 7 with ammonia. erythro-p-*Hydroxyphenylserine* separated immediately as a cream-coloured powder (2·67 g.) and a further 0·48 g. was obtained from the mother-liquor. The product, crystallised from 30% acetic acid, had m. p. 212° (decomp.) after becoming brown at 200° (Found, after drying over P_2O_5 at 100°/0·5 mm.: C, 54·7; H, 5·9; N, 7·0. $C_9H_{11}O_4N$ requires C, 54·8; H, 5·6; N, 7·1%).

threo-p-*Hydroxyphenylserine*.—*threo-p*-Aminophenylserine (9·89 g.) was diazotised, etc., essentially as described for the *erythro*-compound. The resulting brown solution on neutralisation with ammonia and refrigeration gave 1·33 g. of brown intractable material which was discarded. The residual liquor was concentrated *in vacuo* to yield a pale brown solid which was dried, washed with hot alcohol, and then treated with successive small quantities of water (37 ml. in all) till free from sulphate. The residual pale yellow powder threo-p-*hydroxyphenylserine* was purified by adding an equal volume of alcohol to its solution in hot water. The product separated slowly on refrigeration as a pale yellow powder, m. p. 188° (decomp.). It was dried (P_2O_5) at 100°/0·5 mm. (Found: C, 54·8; H, 5·9; N, 7·0%).

An attempt to convert *N*^{1}-acetyl-*threo-p*-aminophenylserine into the *N*-acetyl-*p*-hydroxy-compound by a similar method gave intractable material.

No clear separation of *threo-* and *erythro-p*-hydroxyphenylserine could be obtained by paper partition chromatography. However, with water-saturated *n*-butanol containing 10% of diethylamine as the mobile phase *erythro-p*-hydroxyphenylserine gave a compact spot after development with ninhydrin, whereas the *threo*-isomer gave a long diffuse spot. The *p*-hydroxyphenylserine prepared by Rosenmund and Dornsaft's method (*loc. cit.*) gave a compact spot, indicating it to be the *erythro*-form.

erythro-β-p-*Chlorophenylserine*.—*erythro-p*-Aminophenylserine (4·5 g.) in hydrochloric acid (12 ml.) and water (5 ml.) was diazotised with sodium nitrite (1·44 g.) in water (4·5 ml.), and the resulting mixture poured into a solution of cuprous chloride in concentrated hydrochloric acid (9 ml.) at 0° (from copper sulphate, 6·55 g.). After 1 hour's stirring and storage for a further 1·5 hours, the mixture was heated to 70° for 5 minutes and then diluted with hot water (50 ml.). Copper was removed by hydrogen sulphide, and the filtered solution concentrated several times, with the addition of water, under reduced pressure. The residue was dissolved in hot water (20 ml.) (charcoal) and neutralised to pH 7 with triethylamine while still hot. After cooling to 0°, the precipitated erythro-p-*chlorophenylserine* (3·47 g.) was collected and crystallised from aqueous alcohol, to give a colourless powder, m. p. 178° (decomp.) after becoming brown at 170° (Found, after drying over P_2O_5 at 100°/0·5 mm.: C, 47·9; H, 5·3; N, 6·5; Cl, 15·8. $C_9H_{10}O_3NCl,0·5H_2O$ requires C, 48·1; H, 4·9; N, 6·3; Cl, 15·8%).

erythro-p-Chlorophenylserine was acetylated as described for *threo*-phenylserine, the *acetyl* derivative separating from water as a colourless powder, m. p. 165° (decomp.) (Found: N, 5·4; Cl, 13·8. $C_{11}H_{12}O_4NCl$ requires N, 5·4; Cl, 13·8%).

N-*Acetyl*-threo-β-p-*chlorophenylserine*.—*N*^{1}-Acetyl-*threo-p*-aminophenylserine (5 g.) was diazotised, etc., essentially as described for the preparation of *erythro-p*-chlorophenylserine. The reaction mixture was cooled at room temperature and the precipitated N-*acetyl* derivative (2·0 g.) washed with 5N-hydrochloric acid and then with water. A further 0·5 g. was obtained by extracting the mother-liquors with ethyl acetate. Recrystallisation from water gave a powder, m. p. 176° (decomp.), depressed on admixture with *N*-acetyl-*erythro*-β-*p*-chlorophenylserine (Found: C, 51·0; H, 4·4; N, 5·9; Cl, 13·8. $C_{11}H_{12}O_4NCl$ requires C, 51·3; H, 4·7; N, 5·4; Cl, 13·8%).

threo-β-p-*Chlorophenylserine*.—(a) *From the above* N-*acetyl derivative*. This derivative (1·03 g.) was heated under reflux with 50% (v/v) hydrochloric acid (15 ml.) for 5·5 hours, and the mixture cooled and filtered. The filtrate was concentrated under reduced pressure several times with

water; the final residue was dissolved in water (2 ml.), alcohol (2 ml.) added, and the mixture heated and then adjusted to pH 7 with triethylamine. After refrigeration overnight the precipitated threo-p-*chlorophenylserine* (0·47 g.) was collected and crystallised from aqueous alcohol, then having m. p. 175—176° (decomp.) (Found: N, 6·7. $C_9H_{10}O_3NCl$ requires N, 6·5%).

(b) *From* p-*chlorobenzaldehyde and glycine.* To a mixture of *p*-chlorobenzaldehyde (7·03 g.) and glycine (1·88 g.) in 50% aqueous alcohol (20 ml.) a solution of sodium hydroxide (3·5 g.) in water (10 ml.) was added during 5 minutes, with rapid shaking which was continued for 1 hour, and the resulting suspension was then kept overnight at room temperature. The product was filtered off, washed with alcohol, then suspended in water (50 ml.), acidified to pH 4 to 5 with acetic acid, and shaken with ether. *threo*-β-*p*-Chlorophenylserine (1·73 g.) was collected and washed with water and ether, the aqueous layer of the filtrate affording a further 1·41 g. on concentration and addition of alcohol. Recrystallisation from water (charcoal) gave a powder, m. p. 179° (decomp.) (Found: C, 49·9; H, 4·6; N, 6·2. Calc. for $C_9H_{10}O_3NCl$: C, 50·1; H, 4·6; N, 6·5%).

The acetyl derivative of this product had m. p. 174—175° (decomp.), undepressed on admixture with *N*-acetyl-*threo*-β-*p*-chlorophenylserine.

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