594. *The β-Phenylserine Series. Part I.*

By Ernst D. Bergmann, H. Bendas, and W. Taub.

Esters of glycine condense with *p*-nitrobenzaldehyde in alcohol, to give esters of *N*-*p*-nitrobenzylidene-*threo*-β-*p*-nitrophenylserine (II). Hydrochloric acid hydrolyses the products to β-*p*-nitrophenylserine ester hydrochlorides. A number of aromatic aldehydes (but not all) undergo analogous condensations. Lithium aluminium hydride reduces the phenylserine esters to the corresponding alcohols.

Of the esters of other amino-acids investigated, only alanine esters behave analogously. From optically active alanine methyl ester, optically active condensation products are obtained.

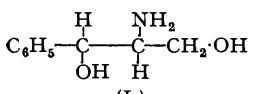
Some experiments expected to elucidate the configuration of β-phenylserine are recorded.

The most puzzling aspect of the recently established structure of chloramphenicol ("Chloromycetin") is the problem of the biogenesis of the compound: not only is the presence of a nitro-group and of the dichloroacetyl radical unusual, but also the biological synthesis of the corresponding alcohol (I) skeleton appears obscure. If one assumes that it is derived from the substituted β-phenylserine (the reverse reaction is known in the oxidation of chloramphenicol to *p*-nitrophenylserine; Smith and Worrel, *Arch. Biochem.*, 1950, **28**, 232), the problem is reduced to that of the biosynthesis of β-phenylserines. It will be recalled that the alcohols corresponding to alanine occur in Nature (Jacobs and Craig, *Science*, 1935, **82**, 16).

In analogy with the formation of serine from formaldehyde and glycine, one would expect a substituted β-phenylserine to be formed from a substituted benzaldehyde and glycine. Reactions of this type are not new in principle. Fifty years ago, Erlenmeyer (*Annalen*, 1899, **307**, 84; cf. *idem*, *ibid.*, 1904, **337**, 222; Erlenmeyer and Fruestueck, *ibid.*, 1895, **284**, 36) described the formation of β-phenylserine from benzaldehyde and glycine under the influence of strong alkali, its *N*-benzylidene derivative being the final product.* Erlenmeyer also stated that other aldehydes (except *o*-anisaldehyde) did not react analogously—this

* Erlenmeyer's discovery has been obscured by the uncertainty introduced into the field by the subsequent investigations of Forster and Rao (*J.*, 1926, 1943), Oesterlin (*Centralbl.*, 1929, II, 1398), and Fourneau and Billeter (*Bull. Soc. chim.*, 1940, **7**, 593). However, the link recently established between β-phenylserine and chloramphenicol (see below) proves not only the configuration, but also the constitution, of Erlenmeyer's amino-acid.

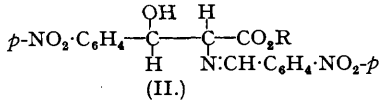
has now been confirmed in experiments with anisaldehyde. Rosenmund and Dornsaft (*Ber.*, 1919, **52**, 1734) and Dalgliesh (*J.*, 1949, 90; cf. Dalgliesh and Mann, *J.*, 1947, 658) extended the scope of the reaction somewhat, using sodium in ether in order to achieve the condensation of aromatic aldehydes and glycine esters; they applied the reaction to anisaldehyde, *p*-carbethoxyoxybenzaldehyde, *O*-carbethoxyvanillin, *OO*-dicarbethoxyprotocatechualdehyde, and *p*-nitrobenzaldehyde. The configuration of Erlenmeyer's β-phenylserine is identical with that of chloramphenicol (*threo*); this has been proved by Carrara and Weitnauer (*Gazzetta*, 1949, **79**, 856), by Vogler (*Helv. Chim. Acta*, 1950, **33**, 2111), by experiments already published from this laboratory (Bendas and E. Bergmann, *Bull. Res. Council Israel*, 1951, **1**, 131), and by others now reported through lithium aluminium hydride reduction of the ester to that alcohol (I) which on nitration gives chloramphenicol. This appears to strengthen the thesis that this condensation reaction represents the "natural" synthesis of the chloramphenicol skeleton, although, of course, the agent used for the condensation is not biogenic. Shaw and Fox (quoted by Vogler, *loc. cit.*) reported recently the isolation of the *erythro*-form of β-phenylserine by a modification of Erlenmeyer's method (cf. also Elphimoff-Felkin and Felkin, *Compt. rend.*, 1951, **232**, 241).



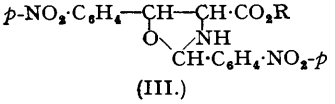
(I.)

The rather unexpected observation (E. Bergmann, Genas, and Bendas, *Compt. rend.*, 1950, **231**, 361) is, therefore, of interest, that *p*-nitrobenzaldehyde and glycine methyl (or ethyl) ester condense easily, at room temperature and without a catalyst, in methanolic solution to give the *N*-*p*-nitrobenzylidene derivative (II) (m. p. 144°) of the corresponding *p*-nitrophenylserine ester, which crystallises partly from the reaction mixture. If excess of alcoholic hydrochloric acid and ether is added to the reaction mixture or to the mother-liquor from (II), the hydrochloride of the *p*-nitrophenylserine ester is precipitated. The condensation is fairly fast; no difference in yield of the hydrochloride was observed for the mixture of 2 mols. of aldehyde (*m*-nitrobenzaldehyde and glycine ethyl ester were used in these experiments) and 1 mol. of ester after 24, 64, or 110 hours. The yield was 52%; it was increased to 74% if the molar ratio aldehyde : ester was changed to 1 : 1; this points to an equilibrium being reached in the condensation under the conditions employed.

Dalgliesh (*loc. cit.*) observed in the sodium condensation of glycine methyl ester and *p*-nitrobenzaldehyde two products of the composition of (II), melting at 85° and 139°, respectively. He ascribed formula (II) to the lower-melting, less stable product, and the oxazolidine formula (III) to the higher-melting stable isomer. This assignment has to be reversed; the higher-melting substance (m. p. 144°) is (II). This has been proved by infra-red analysis, which revealed the presence of a C=N bond (absorption at 1656 cm.$^{-1}$), whilst the oxazolidines have no absorption band at this wave-length and are characterized by a band triplet in the region of 1100—1200 cm.$^{-1}$ (E. Bergmann, Zimkin, and Pinchas, *Rec. Trav. chim.*, in the press; Daasch and Hanninen, *J. Amer. Chem. Soc.*, 1950, **72**, 3673). Indeed, experiments to be reported elsewhere (E. Bergmann, Zimkin, and Pinchas, *loc. cit.*) have shown that the stable products formed from amino-alcohols and aromatic aldehydes are usually Schiff's bases and not oxazolidines, the conjugation between the C=N double bond and the aromatic nucleus stabilizing the open form.



(II.)



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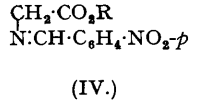


(IV.)

The configuration of (II) and of the corresponding *p*-nitrophenylserine is the same (*threo*) as in Erlenmeyer's β-phenylserine and in chloramphenicol, as reduction with lithium aluminium hydride led to the racemic form of *threo*-2-amino-1-*p*-nitrophenylpropane-1 : 3-diol (Rebstock, Crookes, Controulis, and Bartz, *J. Amer. Chem. Soc.*, 1949, **71**, 2458). The simple condensation between *p*-nitrobenzaldehyde and glycine ester gives, therefore, not only the correct structure, but also the correct configuration of the chloramphenicol molecule. This reduction was made possible by the observation (Felkin, *Compt. rend.*, 1950, **230**, 304) that lithium aluminium hydride reduces ester groups faster than the nitro-group. However, the yields leave much to be desired, and further experiments are in hand.

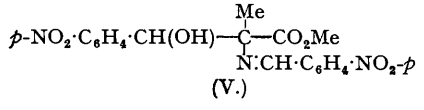
The non-catalysed condensation of an amino-acid ester with *p*-nitrobenzaldehyde is a novel reaction in which a hydrogen atom on the α-carbon atom is involved. It recalls, therefore, to some extent the observation by Haenel (*Naturwiss.*, 1950, **37**, 91) that ethyl alkylidenesuccinates

condense with aldehydes and ketones without catalyst, simply on prolonged heating at elevated temperature.

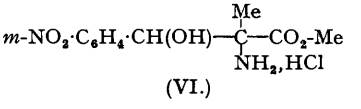
In the normal condensation of aromatic aldehydes with amino-acids, the azlactone synthesis, in which the amino-acid is employed in its *N*-acylated form and which requires acetic anhydride as condensing agent, condensation does take place at the methylene group but is accompanied by the elimination of water. In aqueous-alkaline solution, however, only the amino-group of the amino-acid anion reacts with aromatic aldehydes, and salts of *N*-arylideneamino-acids are obtained (M. Bergmann, Ensslin, and Zervas, *Ber.*, 1925, **58**, 1034). It is assumed that also in the present case one molecule of *p*-nitrobenzaldehyde reacts first with the amino-group of glycine ester; in the *N*-*p*-nitrobenzylidene derivative (IV), the methylene group is activated by the two adjacent double bonds in the same way as in ethyl acetoacetate or malonate and thus enabled to undergo the aldol-type condensation with a second molecule of the aldehyde. This hypothesis is supported by the observation that neither sarcosine methyl ester nor *NN*-dimethylglycine methyl ester condenses with *p*-nitrobenzaldehyde in methanol.

It appeared interesting to study systematically the scope of the new reaction. The following aromatic aldehydes gave a positive response: * *p*-, *o*-, and *m*-nitro-, *p*-cyano-, *p*-carbethoxyoxy-, *p*-hydroxy-, and 2 : 6-dichloro-benzaldehyde, and furfuraldehyde; but in the last case resinification occurred. The following aldehydes proved refractory: *p*-dimethylamino-, 2 : 4-dinitro-, *p*-benzyloxy-, and 3-nitro-4-hydroxy-benzaldehyde, anisaldehyde, and 2-formylthiophen. Also, benzil could not be induced to react with glycine ethyl ester. In some cases, the products corresponding to (II) were precipitated; in others, addition of alcoholic hydrochloric acid and ether led to hydrolysis of the C=N bond and precipitation of the hydrochloride of the expected substituted β-phenylserine ester. Of the amino-acid esters, those of β-phenylalanine, glutamic, and aspartic acid could not be induced to react. Alanine methyl ester, on the other hand, reacted with *p*-nitrobenzaldehyde (but not with benzaldehyde or anisaldehyde) as did glycine esters, giving a crystalline product (V) corresponding to (II). Its ester group could be hydrolysed without affecting the C=N bond. Infra-red analysis of the free acid has shown the presence of a free hydroxyl group (3400 and 1104 cm.$^{-1}$, the latter characteristic of the secondary hydroxyl group), and a broad band in the C=N absorption region, which is probably composed of the C=N band proper and the absorption of the C=O group of the carboxyl (1714 cm.$^{-1}$). In addition, a weak band at 1164 cm.$^{-1}$ was observed, most probably due to the *iso*propyl skeleton, but no trace of the characteristic oxazolidine absorption was detected.

No attempt has been made, so far, to prove the configuration of the new substituted β-phenylserines; it can be assumed that their configuration will prove to be identical with that of the parent substance and of the *p*-nitro-compound. In this respect, it was of interest to investigate whether an optically active amino-acid would given an active derivative or suffer racemisation during condensation. Optically active alanine methyl ester gave a lævorotatory product (V) with *p*-, and a dextrorotatory hydrochloride (VI) with *m*-nitrobenzaldehyde.



(V.)



(VI.)

The biological activities of the newly synthesised phenylserines, of the corresponding alcohols, and of some of their derivatives, will be described elsewhere; Buu-Hoï, Hoán, Jacquignon, and Khoï (*J.*, 1950, 2766), Bambas, Troutman, and Long (*J. Amer. Chem. Soc.*, 1950, **72**, 4445), and Dann, Ulrich, and Moeller (*Z. Naturforsch.*, 1950, **5***b*, 446) have reported recently that the halogen analogues of the natural chloramphenicol (halogen instead of nitro) have a considerable activity against *Escheria coli*, *Staphylococcus aureus*, and *Bacillus paratyphosus*.

The *N*-dichloroacetyl derivatives of β-phenylserine and β-*p*-nitrophenylserine have been prepared by Billet (*Compt. rend.*, 1950, **231**, 293) and Woolley (*J. Biol. Chem.*, 1950, **185**, 293); in view of the possibility that the dichloroacetylation attacks the hydroxyl, and not the amino-group, their infra-red spectra have been studied (in paraffin oil). These prove that the substances are indeed the *N*-acyl derivatives (for a detailed discussion of the spectra see the Experimental section): the absorption at 2585 cm.$^{-1}$ (β-phenylserine derivative) and 2530 cm.$^{-1}$ (nitro-compound) is characteristic of the acylamide group in *N*-acylamido-acids.

Some unsuccessful attempts may be briefly recorded, both to elucidate the configuration of β-phenylserine and its *p*-nitro-derivative, and to obtain their (*erythro*-)-

* Ethyl pyruvate gave a vigorous reaction, but no definite product could be isolated.

enantiomers, following the work of Elliott (*Nature*, 1948, **162**, 657; Attenburrow, Elliott, and Penny, *J.*, 1948, 310) and of Pfister, Robinson, Shabica, and Tishler (*J. Amer. Chem. Soc.*, 1949, **71**, 1101) in the threonine series (cf. Weijlard, Pfister, Swanezy, Robinson, and Tishler, *ibid.*, 1951, **73**, 1216). In accordance with Vogler's results (*loc. cit.*), it was found impossible, however, to prepare an oxazoline derivative. The esters of the two acids did not react with ethyl benzimidate in boiling dichloro-, tetrachloro-, or dibromo-ethane (Elliott, *J.*, 1949, 589; Johnson and Schubert, *J. Amer. Chem. Soc.*, 1950, **72**, 2187). Also, the cyclisation of *N*-benzoyl-β-phenylserine ester, according to Valco's method (U.S.P. 2,416,552) failed: in boiling *iso*propylbenzene no reaction occurred, in higher-boiling solvents resinification took place. Equally, no definite derivatives were obtained when the hydrochloride of phenylserine methyl or of *m*-nitrophenylserine ethyl ester was heated with urea (Close, *J. Org. Chem.*, 1950, **15**, 1131).

Treatment of *N*-benzoyl-β-phenylserine methyl ester with thionyl chloride gave the desired methyl α-benzamido-β-chloro-β-phenylpropionate; its treatment with silver acetate (Johnson and Schubert, *loc. cit.*; McCasland, Clark, and Carter, *J. Amer. Chem. Soc.*, 1949, **71**, 637) did not lead to any definite products, although silver chloride was formed. In general, only *threo*-compounds disclose active chlorine in this reaction, but the intractability of the product renders any conclusion as to configuration doubtful.

Experimental.

(M. p.s are uncorrected.)

β-Phenylserine was prepared according to G.P. 632,424 (Ges. fuer Kohlentechnik) in 69% yield. Recrystallisation from 10 parts of 50% alcohol is the best means of purifying it, the m. p. being 198—203° (Carrara and Weitnauer, *loc. cit.*, give 194—195°). The hydrochloride has m. p. 164—166° (decomp.).

A suspension of phenylserine (100 g.) in anhydrous alcohol (300 c.c.) was treated with gaseous hydrogen chloride until a clear solution resulted. The solvent was distilled off *in vacuo* and the crystalline ethyl ester hydrochloride washed with ice-cold alcohol and recrystallised from the same solvent (yield, 100—110 g., 75—77%); it had m. p. 164—165° (decomp.). To an ice-cold solution of the hydrochloride (50 g.) in water (100 c.c.), a cold solution of anhydrous potassium carbonate (50 g.) in water (50 c.c.) was gradually added, with cooling. The free ethyl ester crystallised at once; it was filtered, dried, and recrystallised from light petroleum (yield, 25—30 g., 60—66%; m. p. 82—84°).

The methyl ester hydrochloride, prepared analogously, had m. p. 161° (decomp.), and the free ester m. p. 62° (from light petroleum).

The m. p., given in the abstract (*Chem. Abs.*, 1950, **44**, 7268) of Carrara and Weitnauer's paper (*loc. cit.*) for the esters are those of the hydrochlorides.

threo-3-*Acetoxy*-2-*amino*-1-*phenylpropyl Acetate*.—To lithium aluminium hydride (6 g.) in dioxan (200 c.c.) was added slowly, with vigorous stirring at 100°, a solution of β-phenylserine ethyl ester (12·2 g.) in dioxan (30 c.c.). Water was added to destroy the excess of hydride, and the solid was filtered off and extracted four times with boiling alcohol. The dioxan filtrate and the alcoholic extracts were combined and evaporated, and acetic anhydride (20 c.c.) was added to the residue. The clear solution which resulted after 30 minutes at room temperature was evaporated *in vacuo* and the residue triturated with warm anhydrous alcohol. The mass was cooled and filtered. The diacetyl derivative (9 g.), when triturated with alcohol and recrystallised from the same solvent, had m. p. 165° (lit., 168—169°; *erythro*-form, 110—111°) (Found: N, 5·6, 5·7. Calc. for $C_{13}H_{17}O_4N$: N, 5·6%). The corresponding triacetyl derivative also had the correct m. p. (84°) of the *threo*-compound.

N-p-*Nitrobenzylidene*-β-p-*nitrophenylserine Ethyl Ester*.—When glycine ethyl ester (4 g.) and *p*-nitrobenzaldehyde (12 g.) were dissolved in methanol (30 c.c.) by brief heating, an intensely yellow solution resulted which soon began to deposit crystals of this *ester* which, crystallised from methanol, had m. p. 144° (Found: N, 10·7, 11·0. $C_{18}H_{17}O_7N_3$ requires N, 10·9%).

β-p-*Nitrophenylserine Ethyl Ester Hydrochloride*.—The reaction mixture, prepared as above, or the mother-liquor from the crystals of m. p. 144°, when treated with concentrated alcoholic hydrochloric acid and an excess of ether, gave a thick precipitate. Recrystallisation from butyl alcohol gave the salt, m. p. 189° (decomp.) (Found: N, 9·8. Calc. for $C_{11}H_{15}O_5N_2Cl$: N, 9·6%).

β-p-*Nitrophenylserine Methyl Ester Hydrochloride*.—From glycine methyl ester (4 g.) and *p*-nitrobenzaldehyde (13 g.), dissolved in methanol and kept at room temperature for 6 hours, the ester (III; R = Me) (7 g.) was obtained by cooling at 0°. Treatment of the total reaction mixture with an excess of methyl-alcoholic hydrochloric acid (2 mols. per mol. of ester) and double the volume of ether, gave the *hydrochloride* (6·3 g., 50%), m. p. 183° (decomp.) (from methanol–ether) (Found: N, 10·1. $C_{10}H_{13}O_5N_2Cl$ requires N, 10·1%).

β-m-*Nitrophenylserine Methyl Ester Hydrochloride*. The condensation product (as III) from glycine methyl ester (2·3 g.) and *m*-nitrobenzaldehyde (7·6 g.) in methanol, separated as a viscous oil. Both the oil and the supernatant liquid, when treated with methanolic hydrochloric acid and ether, gave the same *hydrochloride*, which after recrystallisation from butyl alcohol, had m. p. 131° (Found: N, 10·0, 10·2; Cl, 12·4, 12·4. $C_{10}H_{13}O_5N_2Cl$ requires N, 10·1; Cl, 12·7%).

The reaction mixture from glycine ethyl ester (5·2 g.) and *m*-nitrobenzaldehyde (15 g.) in ethyl alcohol (50 c.c.) was treated with alcoholic hydrochloric acid (9 c.c.) and ether (200 c.c.) and cooled at 0°. The yield of *β-m-*nitrophenylserine ethyl ester hydrochloride was 7·4 g. after 24 hours at room temperature, and 7·0 and 7·2 g. after 64 and 110 hours, respectively. When 10·4 g. of ester and 100 c.c. of ethyl alcohol were used, the yield was 10·7 g. The m. p. was 180—184° (decomp.).

β-m-*Nitrophenylserine*.—To *m*-nitrophenylserine ethyl ester hydrochloride (10·9 g.), wetted with a few drops of methanol, N-sodium hydroxide (75 c.c.) was added. After 1 hour at room temperature, the mixture was neutralised with N-hydrochloric acid (37·5 c.c.). Immediately, needles began to separate. *β*-m-*Nitrophenylserine* crystallised from water and had m. p. 203° (decomp.) (6·5 g.) (Found: N, 12·7. $C_9H_{10}O_5N_2$ requires N, 12·4%).

N-o-*Nitrobenzylidene-β-*o-*nitrophenylserine Ethyl Ester*.—This ester crystallised spontaneously from the reaction mixture of glycine ethyl ester (5·2 g.) and *o*-nitrobenzaldehyde (15 g.); the mother-liquor, on treatment with hydrochloric acid, gave very little precipitate. The *ester* separated from propanol in prisms, m. p. 160° (Found: N, 10·7. $C_{18}H_{17}O_7N_3$ requires N, 10·9%).

When the total reaction product was treated directly with hydrogen chloride (1·85 g.) in alcohol (8·7 c.c.) and an excess of ether, *β-*o-*nitrophenylserine ethyl ester hydrochloride* (3·6 g.) crystallised. Recrystallised from *iso*propyl alcohol, it had m. p. 141° (Found: N, 9·8. $C_{11}H_{15}O_5N_2Cl$ requires N, 9·6%).

β-Phenylserine methyl ester hydrochloride was obtained analogously in small yield from glycine methyl ester and benzaldehyde and had m. p. 161° (decomp.) (from butyl alcohol) (Found: N, 6·2; Cl, 14·8, 14·9. Calc. for $C_{10}H_{14}O_3NCl$: N, 6·1; Cl, 15·1%).

N-(2 : 6-*Dichlorobenzylidene*)-*β*-2 : 6-*dichlorophenylserine Methyl Ester*.—A solution of glycine methyl ester (2·3 g.) and 2 : 6-dichlorobenzaldehyde (8·8 g.) in alcohol was kept at room temperature for 3 days and cooled at 0°. The condensation *product* (1·7 g.) separated and after recrystallisation from butyl acetate had m. p. 128° (Found: N, 3·5, 3·6; Cl, 33·7, 33·9. $C_{17}H_{13}O_3NCl_4$ requires N, 3·3; Cl, 33·8%).

Treatment of the alcoholic mother-liquor with hydrogen chloride and ether gave *β*-2 : 6-*dichlorophenylserine methyl ester hydrochloride*, prisms (from butanol), m. p. 198° (1·4 g.) (Found: C, 40·1; H, 4·2; Cl, 35·4. $C_{10}H_{12}O_3NCl_3$ requires C, 40·0; H, 4·0; Cl, 35·3%).

N-p-*Cyanobenzylidene-β-*p-*cyanophenylserine Methyl Ester*.—The methanolic solution of glycine methyl ester (0·1 g.) and *p*-cyanobenzaldehyde (0·2 g.) (E. Bergmann and Pinchas, *J. Org. Chem.*, 1950, **15**, 1184, have stressed the similarity in electronic structure and chemical reactivity of *p*-nitro- and *p*-cyano-benzaldehyde) yielded crystals after a few hours. Crystallised from butyl alcohol, this *benzylidene* derivative had m. p. 278° (decomp.) (Found: C, 68·4; H, 4·6; N, 12·8. $C_{19}H_{15}O_3N_3$ requires C, 68·5; H, 4·5; N, 12·6%).

4-*Hydroxy*-3-*nitrobenzaldehyde* (cf. Paal, *Ber.*, 1895, **28**, 2407).—A mixture of *p*-hydroxybenzaldehyde (20 g.), acetic acid (80 c.c.), and nitric acid (*d* 1·42; 11 c.c.) was very cautiously heated to 40°. At this point, a violent reaction set in, which raised the temperature to 80°. The mass was cooled rapidly with running water, and water (80 c.c.) was added. After 12 hours, the crystals of 4-hydroxy-3-nitrobenzaldehyde were collected and recrystallised from *iso*propyl alcohol (yield, 13·5 g., 50%); they had m. p. 141—142°.

Glycine methyl ester (3 g.) and this aldehyde (9·9 g.) in methanol gave an orange-red solution which after 4 days at room temperature was treated with hydrochloric acid in the customary manner. A resinous product separated at 0°, which was triturated with methanol and recrystallised from *iso*propyl alcohol as yellow prisms, m. p. 222°, which were chlorine-free. This *substance* had approximately the same composition as the starting material and was not further investigated (Found: C, 50·8; H, 3·3; N, 8·2. $C_7H_5O_4N$ requires C, 50·3; H, 3·0; N, 8·4%).

β-p-*Carbethoxyoxyphenylserine Ethyl Ester Hydrochloride*.—A mixture of glycine ethyl ester (3·1 g.) and *p*-carbethoxyoxybenzaldehyde (12 g.) (Rosenmund and Dornsaft, *loc. cit.*) (b. p. 172—174°/23 mm.) in alcohol reacted slightly exothermally but gave no precipitate. Addition of alcoholic hydrochloric acid and ether gave the desired *salt* (6·5 g.) which was purified by precipitation from its methanolic solution with anhydrous ether and had m. p. 157° (decomp.) (Found: C, 50·0; H, 6·4; N, 4·5, 4·5. $C_{14}H_{20}O_6NCl$ requires C, 50·4; H, 6·0; N, 4·2%).

β-p-*Hydroxyphenylserine Ethyl Ester Hydrochloride*.—When *p*-hydroxybenzaldehyde (12 g.) and glycine ethyl ester (5·2 g.) were kept at room temperature in alcohol, no crystalline product separated. Addition of an alcoholic solution of hydrogen chloride (1·85 g.) and ether yielded the *salt* (1·3 g.) which was reprecipitated repeatedly from methanol by addition of anhydrous ether. It melted at 128° (Found: N, 5·5. $C_{11}H_{16}O_4NCl$ requires N, 5·6%).

β-p-*Hydroxyphenylserine*.—The foregoing ester (1·0 g.) was hydrolysed at room temperature by N-potassium hydroxide (7·5 c.c.); addition of N-hydrochloric acid (3·75 c.c.) precipitated the substituted serine. From a mixture of water and *tert.*-butanol, it crystallised in fine needles, which began to decompose at 197° (Found: N, 7·1. Calc. for $C_9H_{11}O_4N$: N, 7·1%). According to Rosenmund and Dornsaft (*loc. cit.*), the amino-acid discolors at 190° and decomposes gradually when heated to 217°.

Methyl (±)-*β-Hydroxy-α-*p-*nitrobenzylideneamino-β-*p-*nitrophenylisobutyrate*.—When DL-alanine methyl ester (5 g.) and *p*-nitrobenzaldehyde (15 g.) were kept in methanol (50 c.c.) (colour: orange-red), the condensation product (2 g.) was precipitated. A second crop was obtained by evaporation

of the methanolic mother-liquor. The *ester*, recrystallised from methanol or ethyl alcohol, had m. p. 142° (yield, 9 g., 46%) (Found : N, 10·9, 10·9. $C_{18}H_{17}O_7N_3$ requires N, 10·9%). The hot solution in butyl alcohol is rose-coloured.

The methyl ester (1·4 g.) was kept in N-sodium hydroxide for 1 hour at room temperature. The colour changed from violet-brown to yellowish. The *acid* was precipitated by N-hydrochloric acid (4·8 c.c.) and recrystallised from slightly acidified water; it formed long yellowish needles, m. p. 105° (Found : C, 54·5; H, 3·7; N, 10·8. $C_{17}H_{15}O_7N_3$ requires C, 54·7; H, 4·0; N, 11·2%).

Optically Active Methyl β-Hydroxy-α-p-nitrobenzylideneamino-β-p-nitrophenylisobutyrate.—The product from L-alanine methyl ester (0·4 g.) and *p*-nitrobenzaldehyde (1 g.) crystallised spontaneously. From *iso*propyl alcohol, this *ester* formed crystals, m. p. 138—139°, $[\alpha]_D$ −2·2° ± 0·4° (*c*, 2·25 in chloroform) (Found : C, 55·4; H, 4·7; N, 10·6. $C_{18}H_{17}O_7N_3$ requires C, 55·8; H, 4·4; N, 10·9%).

Optically Active Methyl β-Hydroxy-α-m-nitrobenzylideneamino-β-m-nitrophenylisobutyrate Hydrochloride.—L-Alanine methyl ester (1 g.) and *m*-nitrobenzaldehyde (3 g.) in a little methanol were kept at room temperature for 48 hours, and excess of methanolic hydrochloric acid and ether added. Scratching precipitated the *hydrochloride*. It was purified by addition of anhydrous ether to its filtered methanolic solution and had m. p. 144° (decomp.), $[\alpha]_D$ 10·4° ± 2·4° (*c*, 1·25 in acetic acid) (Found : N, 9·5. $C_{11}H_{15}O_5N_2Cl$ requires N, 9·7%).

N-*Dichloroacetyl-β-phenylserine.*—This was obtained according to Billet (*loc. cit.*) but isolated by keeping the reaction product at low temperature for several hours. Crystallised from water containing a little acetic acid, or from toluene, it had m. p. 158° (decomp.) (Billet gives m. p. 170°) (Found : Cl, 23·9, 24·0. Calc. for $C_{11}H_{11}O_4NCl_2$: Cl, 24·3%). The quantity of dichloroacetyl chloride prescribed by Woolley (*loc. cit.*) is too small; one needs 8·1 g. for 9 g. of the amino-acid. The infra-red spectrum revealed the presence of the following bands : (1; sample from water) 3320, 2585, 1724, 1674, 1536, 1274, 1223, 719 cm.$^{-1}$; (2; sample from toluene) 3320, 2565, 1714, 1672, 1534, 1273, 1224, 719 cm.$^{-1}$. That the substance is the *N*-dichloroacetyl derivative is shown by the bands : 3320 (hydroxyl, both alcoholic and of the carboxyl group), 2585 (acylamido-acid) (Randall *et al.*, " Infra-red Determination of Organic Substances," New York, 1949, pp. 134, 135, 141), 1724 (C=O of the carboxyl group), 1674 (C=O of the amide group), 1536 (NH in amides), 1274 (secondary hydroxyl group) (Colthup, *J. Opt. Soc. Amer.*, 1950, **40**, 397), 1223 (C–N in =C–N–C) (Randall *et al.*, *op. cit.*, p. 127, show, *e.g.*, for α-acetamido-ββ-dimethylacrylic acid a band at 1230 cm.$^{-1}$), and 719 (CCl_2).

N-*Dichloroacetyl-β-p-nitrophenylserine.*—Obtained analogously and crystallised from water, this formed faintly yellowish needles, m. p. 176° (Found : N, 8·4. Calc. for $C_{11}H_{10}O_6N_2Cl_2$: N, 8·3%). The infra-red spectrum showed that the substance was the *N*-dichloroacetyl compound. 3375 (free alcoholic hydroxyl), 3240 (hydroxyl of the carboxyl group), 2530 (acylamido-acid), 1738 (C=O of the carboxyl group), 1683 (C=O of the amide group), 1609 (phenyl), 1563 (NH in amides), 1521 (substituted phenyl), 1229 (C–N in =C–N–C), 753 (substituted phenyl), 721 (CCl_2).

N-*Benzoyl-β-phenylserine.*—A solution of β-phenylserine (36·2 g.) and sodium hydroxide (8·0 g.) in water (100 c.c.) and a solution of benzoyl chloride (28·0 g.) in benzene (100 c.c.) were stirred together, and sodium hydroxide (8 g.) in water (100 c.c.) was added slowly. Addition of N-sulphuric acid (200 c.c.) precipitated the *benzoyl* derivative. Washed with ether and recrystallised from 30% ethanol, it (45 g., 80%) had m. p. 160—161° (Found : C, 67·4; H, 5·1; N, 5·1. $C_{16}H_{15}O_4N$ requires C, 67·4; H, 5·3; N, 4·9%).

The acid (14·3 g.) and diazomethane (4·0 g.) in ether (150 c.c.) gave the *methyl* ester (13 g., 87%), m. p. 123—124° (from dilute alcohol) (Found : N, 4·8. $C_{17}H_{17}O_4N$ requires N, 4·7%).

In contradistinction to free amino-acids, the *N*-acyl derivatives are smoothly methylated by diazomethane (Herzig and Landsteiner, *Biochem. Z.*, 1920, **105**, 111; cf. Biltz and Paetzold, *Ber.*, 1922, **55**, 1066; Kuhn and Brydowna, *ibid.*, 1937, **70**, 1333).

Methyl α-Benzamido-β-chloro-β-phenylpropionate.—The foregoing product (16 g.) and freshly distilled, ice-cold thionyl chloride (6 c.c.) were kept at room temperature for 2 hours. Ether (150 c.c.) precipitated the *chloro*-compound which, recrystallised from dilute alcohol, had m. p. 133—134° (2·3 g.). From the ethereal mother-liquor, a further 1·2 g. were obtained (Found : N, 4·7; Cl, 10·8. $C_{17}H_{16}O_3NCl$ requires N, 4·4; Cl, 11·0%). The infra-red spectrum (0·0054 g. in 1 c.c. of carbon tetrachloride; cell thickness, 2 mm.) showed the amide-carbonyl band at 1677 cm.$^{-1}$ and the amide-NH band at 3430 cm.$^{-1}$.

The infra-red spectra were measured and interpreted by Dr. S. Pinchas, Optics Department, Weizmann Institute of Science.

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