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

The Preparation of Hydroxyphenylserines from Benzyloxybenzaldehydes and Glycine[1]

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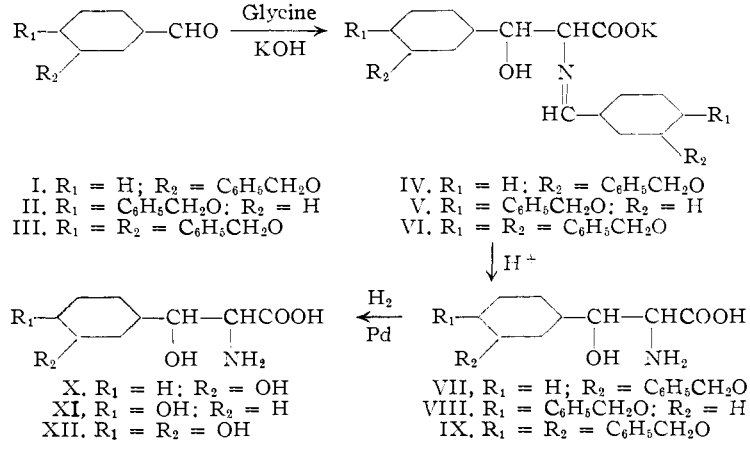
threo-β-*m*-Hydroxyphenylserine (X), *threo*-β-*p*-hydroxyphenylserine (XI) and both diastereoisomers of β-3,4-dihydroxyphenylserine (XII) have been prepared. Glycine, when allowed to react with *m*-benzyloxybenzaldehyde (I) in alcoholic potassium hydroxide, yielded *threo*-β-*m*-hydroxyphenylserine (X) after acidification and hydrogenolysis of the intermediate N-benzylidene potassium salt. Likewise, from glycine and *p*-benzyloxybenzaldehyde (II), *threo*-β-*p*-hydroxyphenylserine (XI) was prepared. The condensation of 3,4-dibenzyloxybenzaldehyde (III) with glycine yielded β-3,4-dibenzyloxyphenylserine (IX) as a mixture of diastereoisomers after acidification of the intermediate N-benzylidene potassium salt. These isomers were separated by fractional crystallization and hydrogenolyzed individually to yield both pure diastereoisomers of β-3,4-dihydroxyphenylserine (XII).

The involved, indirect procedure required for the preparation of *erythro*-β-*p*-hydroxyphenylserine from ethyl *p*-benzyloxybenzoylacetate[2] and the fact that the *threo* isomer was not obtained at all indicated that any attempt to extend this method of synthesis to the preparation of even one of the diastereoisomers of β-3,4-dihydroxyphenylserine would meet with failure. It could be expected that the intermediates in the 3,4-dihydroxy series would be even less stable than those in the *p*-hydroxy series. For these reasons, a modification of the original Erlenmeyer[3] synthesis was examined for its applicability to the preparation of various β-benzyloxyphenylserines which, on hydrogenolysis, would yield the desired β-hydroxyphenylserines. In this modification, the aqueous alkali used by Erlenmeyer for the condensation of the aldehyde with glycine, is replaced by alcoholic alkali. Such substituted serines as β-(2-thienyl)-serine,[4] β-(2-furyl)-serine[5] and β-3,4-methylenedioxyphenylserine[6] have been synthesized by this modified procedure.

In this Laboratory, benzyloxybenzaldehydes were found to react rapidly with glycine in alcoholic potassium hydroxide to give N-benzylidene-β-benzyloxyphenylserines. Acidification and hydrogenolysis yielded the desired β-hydroxyphenylserines. Initial experiments were carried out in the *m*-hydroxy series because of the ease of identification of the diastereoisomeric β-*m*-hydroxyphenylserines by melting point alone.[2]



The condensation of two moles of *m*-benzyloxybenzaldehyde (I) with one mole of glycine was brought about in an alcoholic potassium hydroxide solution. A solid, crystalline potassium salt (IV) could be isolated, but a better over-all yield was obtained when the entire reaction mixture was acidified. Acidification of the benzylidene compound IV caused the regeneration of approximately half of the starting *m*-benzyloxybenzaldehyde (I) with the simultaneous formation of β-*m*-benzyloxyphenylserine (VII). This compound appeared to be a single diastereoisomer and its melting point did not change after a number of recrystallizations. Although no evidence of the presence of the other diastereoisomer was obtained, this does not constitute absolute proof that the reaction proceeds to yield only one isomer. Debenzylation of the β-*m*-benzyloxyphenylserine (VII) was achieved by catalytic hydrogenolysis and the product was identified by its melting point as *threo*-β-*m*-hydroxyphenylserine (X).

threo-β-*p*-Hydroxyphenylserine (XI) was prepared from *p*-benzyloxybenzaldehyde (II) in a similar manner. Two moles of *p*-benzyloxybenzaldehyde (II) were condensed with one mole of glycine and the resulting potassium benzylidene salt V was isolated as a crystalline solid in good yield. Acidification of the salt V gave β-*p*-benzyloxyphenylserine (VIII) which appeared to be a single isomer. Catalytic hydrogenolysis removed the benzyl group and *threo*-β-*p*-hydroxyphenylserine (XI) was obtained. The *threo* configuration was assigned to the product by analogy with the *m*-hydroxy series. The decomposition point of the β-*p*-hydroxyphenylserine prepared by this method is 15° lower than that reported by this Laboratory for the *erythro* isomer.[2] Holland, Jenkins and Nayler[7] have recently reported the preparation of *threo*-β-*p*-hydroxyphenylserine (m.p. 188°) from *threo*-β-*p*-nitrophenylserine.

When 3,4-dibenzyloxybenzaldehyde (III) was allowed to react with glycine in alcoholic potassium

(1) Presented at the Miniature Meeting of the Philadelphia Section of the American Chemical Society, January 29, 1953.

(2) W. A. Bolhofer, THIS JOURNAL, **75**, 4469 (1953).

(3) E. Erlenmeyer, *Ber.*, **25**, 3445 (1892).

(4) M. E. Dullaghan and F. F. Nord, THIS JOURNAL, **73**, 5455 (1951).

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

(6) S. Kanao and K. Shinozuka, *J. Pharm. Soc. Japan*, **67**, 218 (1947).

(7) D. O. Holland, P. A. Jenkins and J. H. C. Nayler, *J. Chem. Soc.*, 273 (1953).

hydroxide, the potassium salt of the N-benzylidene-β-phenylserine (VI) separated from the alcohol solution as a heavy oil. After acidification and removal of 3,4-dibenzyloxybenzaldehyde, the β-3,4-dibenzyloxyphenylserine (IX) was obtained as a powder. This product proved to be a mixture of two diastereoisomers which could be separated by fractional crystallization to give a high-melting and a low-melting isomer. The high-melting isomer was debenzylated in 50% methanol by catalytic hydrogenolysis. The product, the low-melting more-soluble isomer of β-3,4-dihydroxyphenylserine (XII), was obtained as a hydrate when the solution was concentrated.[8] The low-melting isomer of β-3,4-dibenzyloxyphenylserine (IX) was obtained as a hydrate. It was debenzylated in dilute alkali and the high-melting isomer of β-3,4-dihydroxyphenylserine (XII) was obtained. This isomer appears to be identical with the β-3,4-dihydroxyphenylserine reported by Rosenmund and Dornsaft[9] and Dalgliesh and Mann.[10]

The infrared absorption spectra of DL-threonine,[11] DL-allothreonine[11] and the racemic diastereoisomers of β-phenylserine,[12] β-*m*-hydroxyphenylserine,[2] β-*p*-hydroxyphenylserine[2] and β-3,4-dihydroxyphenylserine were determined in Nujol. The absorption curves are not absolutely conclusive in themselves, but the data can be used in conjunction with chemical evidence as an indication of stereochemical structure. Comparison of the spectra of substances of proven structure (threonine and allothreonine and *threo*- and *erythro*-β-phenylserine) shows that a band occurs regularly at 11.90–11.95 μ for those substances having the *erythro* configuration. This band was not observed in the spectra of compounds possessing the *threo* configuration. A band at 11.90 μ appeared also in the absorption spectrum of the β-*m*-hydroxyphenylserine assigned the *erythro* structure on the basis of chemical evidence alone. Likewise, examination of the spectra of the β-*p*-hydroxyphenylserines indicates that the configuration assigned each diastereoisomer is correct. The presence of a band at 11.95 μ in the spectrum of the β-3,4-dihydroxyphenylserine melting at 199–200° indicates that it has the *erythro* structure. This band is missing from the spectrum of its isomer melting at 220–225° which can therefore be assigned the *threo* configuration.

Experimental[13]

threo-β-*m*-**Benzyloxyphenylserine (VII).**—To a solution of 5.61 g. (0.1 mole) of C.P. potassium hydroxide and 3.75 g. (0.05 mole) of glycine in 75 ml. of absolute alcohol, there was added a solution of 21.2 g. (0.1 mole) of *m*-benzyloxybenzaldehyde[14] in 25 ml. of absolute alcohol. The mixture was warmed until it was clear and the solution was allowed to stand at room temperature. A brown oil separated which crystallized slowly, m.p. 125–130°. However, it was unnecessary to obtain a crystalline product at this point. After standing overnight, the alcohol was decanted and the residual oil was dissolved in a mixture of 200 ml. of 2 *N* hydrochloric acid and 50 ml. of benzene. The benzene was separated and the aqueous solution was again extracted with 50 ml. of benzene. (From these benzene extracts approximately half of the starting *m*-benzyloxybenzaldehyde was recovered.) The aqueous solution was concentrated under vacuum until all the alcohol was removed. Neutralization was effected with concentrated ammonia and, after standing overnight at 0°, crystalline *threo*-β-*m*-benzyloxyphenylserine (9.0 g., 62.7%) was obtained. After two recrystallizations from methyl alcohol, the product melted with decomposition at 185°.

Anal. Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.97; N, 4.88. Found: C, 67.08; H, 6.18; N, 4.85.

threo-β-*m*-**Hydroxyphenylserine (X).**—A solution of 14.37 g. (0.05 mole) of *threo*-β-*m*-benzyloxyphenylserine in 50 ml. of 2 *N* ammonium hydroxide and 25 ml. of methyl alcohol was hydrogenated at atmospheric pressure using 1.0 g. of 5% palladium-on-charcoal catalyst. After 24 hours, the theoretical quantity of hydrogen had been absorbed and the catalyst was removed by filtration. Water (100 ml.) was added to the filtrate and the solution was concentrated under reduced pressure to a small volume. The mixture was neutral and 7.9 g. (79.7%) of *threo*-β-*m*-hydroxyphenylserine had crystallized, m.p. 215° with dec. After two precipitations from alkali (with acid), the product melted with decomposition at 225°. This compared well with the decomposition point of 230° previously reported[2] for the *threo* compound and indicated that the method yielded product almost exclusively in the *threo* configuration.

threo-β-*p*-**Benzyloxyphenylserine (VIII).**—To a solution of 5.61 g. (0.1 mole) of C.P. potassium hydroxide and 3.75 g. (0.05 mole) of glycine in 75 ml. of absolute alcohol, there was added a solution of 21.2 g. (0.1 mole) of *p*-benzyloxybenzaldehyde[15] in 50 ml. of alcohol. The mixture was warmed until it was absolutely clear (*ca.* 60°) and the solution was allowed to stand at room temperature. A crystalline solid precipitated which was filtered after 5 hours and washed thoroughly with alcohol and ether. This product was the potassium salt of N-*p*-benzyloxybenzylidene-β-*p*-benzyloxyphenylserine and it was obtained in 95% yield, m.p. 161–163°.

The potassium salt (41.6 g., 0.08 mole) was stirred vigorously with 400 ml. of 1 *N* hydrochloric acid and the mixture was filtered immediately. The solid *p*-benzyloxybenzaldehyde was washed with 200 ml. of 0.5 *N* hydrochloric acid and then with water. It weighed 19.7 g. (theory 17.0 g.) and probably contained some β-*p*-benzyloxyphenylserine. The acid filtrates were combined and, on neutralization, β-*p*-benzyloxyphenylserine crystallized, 17.7 g. (77.0%). A sample, recrystallized from a mixture of alcohol (6 parts), water (2 parts) and dimethylformamide (2 parts) melted at 190–192° dec.

Anal. Calcd. for $C_{16}H_{17}O_4N$: C, 66.88; H, 5.97; N, 4.88. Found: C, 66.91; H, 5.98; N, 4.86.

threo-β-*p*-**Hydroxyphenylserine (XI).**—To 100 ml. of 0.5 *N* sodium hydroxide there was added 4.0 g. of *threo*-β-*p*-benzyloxyphenylserine and 0.25 g. of 5% palladium-on-charcoal catalyst. The benzyloxy compound did not dissolve completely. The mixture was hydrogenated at atmospheric pressure and, when absorption of hydrogen ceased (305 ml., theory 330 ml.), there was no undissolved material present except the catalyst. After removal of the catalyst by filtration, the filtrate was neutralized with hydrochloric acid and then concentrated under reduced pressure to 30 ml. On cooling, a crystalline product weighing 1.8 g. (65.5%) was obtained. This product was recrystallized from water and washed until chloride free, m.p. 195–205° dec.

Anal. Calcd. for $C_9H_{11}O_4N$: C, 54.82; H, 5.62; N, 7.11. Found: C, 54.89; H, 5.73; N, 7.06.

β-**3,4-Dibenzyloxyphenylserine (IX).**—A warm (70°) solution of 89.0 g. (0.28 mole) of 3,4-dibenzyloxybenzaldehyde[16] was added to a solution of 15.7 g. (0.28 mole) of potassium hydroxide and 10.5 g. (0.14 mole) of glycine in 140 ml. of alcohol. A crystalline solid formed rapidly but this soon

(8) This isomer has presumably been prepared by G. Fodor and J. Kiss (*Acta Univ. Szeged. Chem. et Phys.*, **3**, 26 (1950)), who isolated it as a non-crystalline hydrochloride.

(9) K. W. Rosenmund and H. Dornsaft, *Ber.*, **52**, 1734 (1919).

(10) C. E. Dalgliesh and F. G. Mann, *J. Chem. Soc.*, 658 (1947).

(11) Commercial sample.

(12) W. A. Bolhofer, THIS JOURNAL, **74**, 5459 (1952).

(13) The microanalyses were carried out by Mr. Kermit Streeter and his staff. All melting points are uncorrected. The infrared spectra were determined by the Microanalytical Laboratory of the Massachusetts Institute of Technology.

(14) W. S. Rapson and R. Robinson, *J. Chem. Soc.*, 1533 (1935).

(15) E. Worner, *Ber.*, **29**, 142 (1896).

(16) H. S. Mahal, H. S. Rai and K. Venkataraman, *J. Chem. Soc.*, 866 (1935).

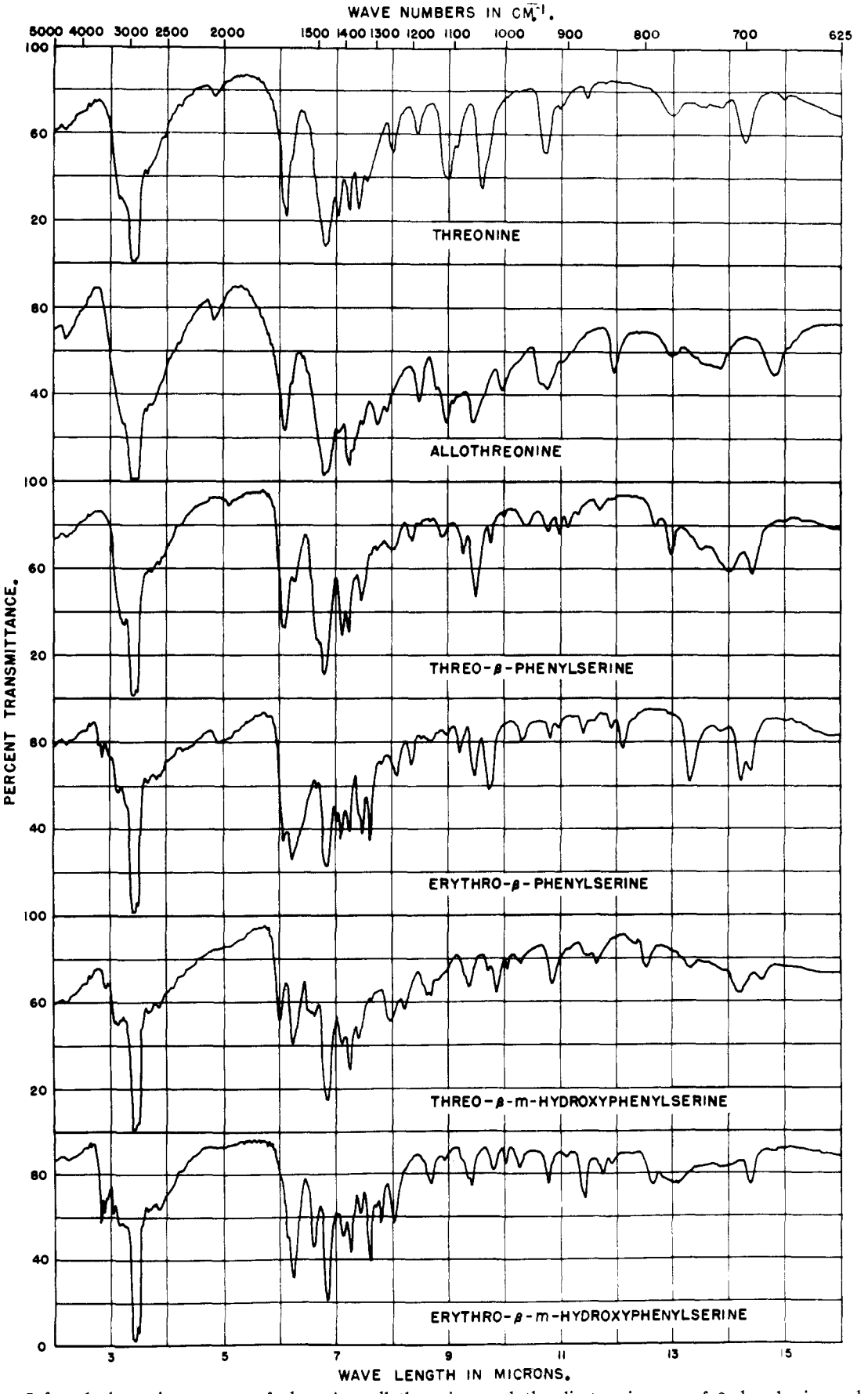


Fig. 1.—Infrared absorption spectra of threonine, allothreonine, and the diastereoisomers of β-phenylserine and β-*m*-hydroxyphenylserine in a mineral oil mull.[13]

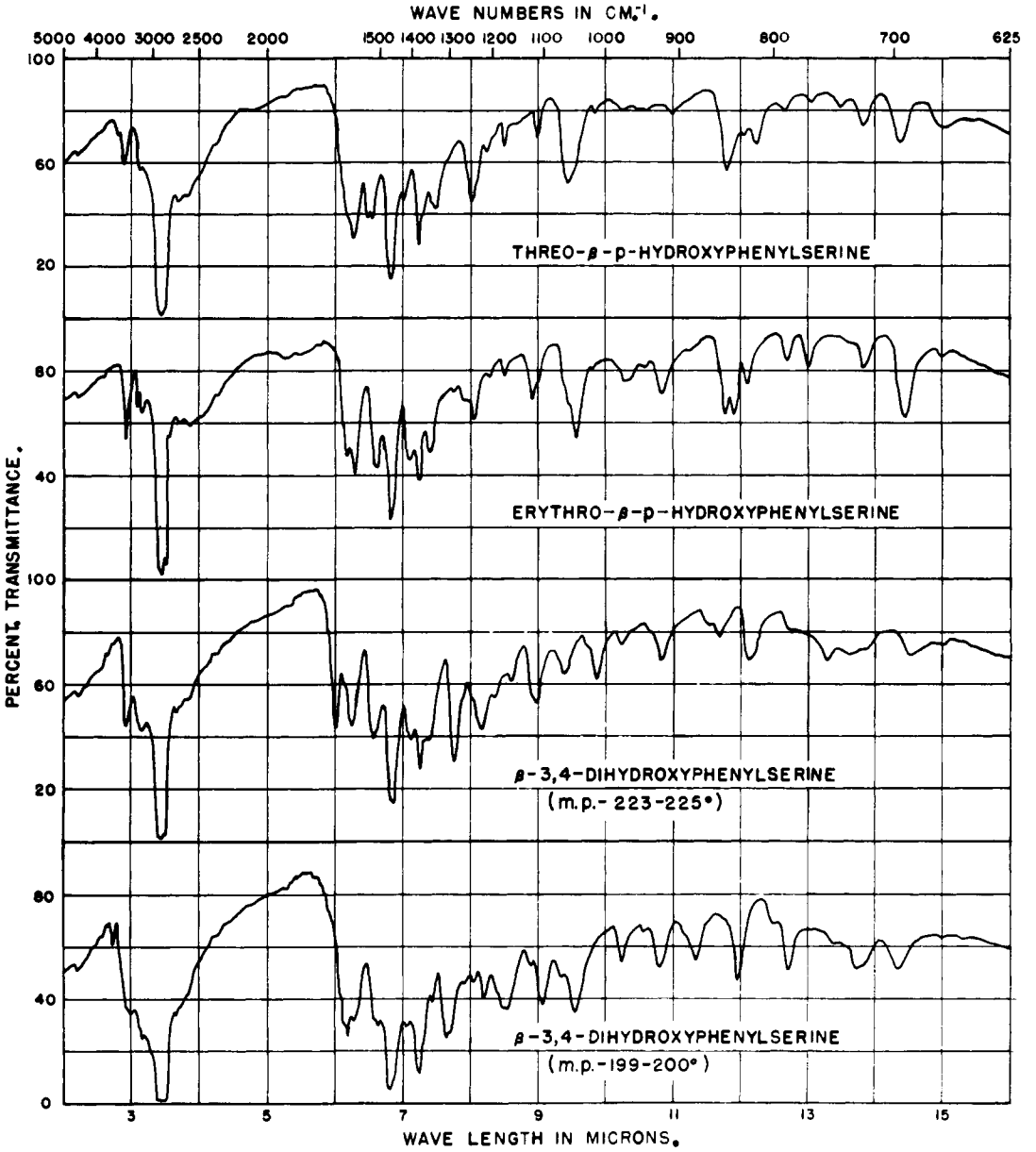


Fig. 2.—Infrared absorption spectra of the diastereoisomers of β-*p*-hydroxyphenyserine and β-3,4-dihydroxyphenylserine in a mineral oil mull.[13]

dissolved and an oil separated from solution. After standing overnight at 0°, the alcohol was decanted from the viscous oil.

The oil was dissolved in 350 ml. of carbon tetrachloride and the clear solution was acidified with 15 ml. of glacial acetic acid. Potassium acetate was removed by filtration and the filtrate was stirred vigorously with 500 ml. of water for two hours. The water was decanted and the carbon tetrachloride suspension was washed by decantation with four 500-ml. portions of water. The carbon tetrachloride slurry was allowed to evaporate in a flat tray and a dry yellow powder remained. After extraction of the powder with three portions of 500 ml. of ether, 30.5 g. (55.4%) of insoluble β-3,4-dibenzyloxyphenylserine remained. Evaporation of the ether solution yielded 51 g. of 3,4-dibenzyloxybenzaldehyde.

The β-3,4-dibenzyloxyphenylserine obtained by this method was a mixture of stereoisomers which were separated by fractional crystallization from 50% *t*-butyl alcohol. The high-melting (180–185°) isomer was the more insoluble substance and it could be obtained in an almost pure state by eluting the more-soluble, low-melting (146–147°) isomer from the mixture with minimal quantities of boiling 50% *t*-butyl alcohol. Fairly pure isomer melting at 146–147° could be recovered by cooling the hot *t*-butyl alcohol extracts to room temperature. The original crude mixture consists of about 30% of isomer melting at 180–185° and about 50–60% of the isomer melting at 146–147°. The high-melting isomer was obtained pure by recrystallizing it from 50% *t*-butyl alcohol (1 l. for 8 g.), m.p. 180–185 dec.

Anal. Calcd. for $C_{23}H_{23}O_5N$: C, 70.21; H, 5.89; N, 3.56. Found: C, 70.11; H, 5.85; N, 3.56.

The low-melting isomer was obtained pure by recrystallizing the fairly pure material from 50% *t*-butyl alcohol (20–25 g. per l.) without scratching or stirring the solution or cooling it below room temperature. Any small quantity of the high-melting isomer that is present remains in solution. However, after the low-melting isomer was filtered, the high-melting isomer in the mother liquor could be crystallized by cooling the solution to 0°. This technique also served as a check on the purity of the low-melting isomer. Analyses showed that the low-melting isomer was a hydrate, m.p. 146–147° dec.

Anal. Calcd. for $C_{23}H_{23}O_5N \cdot H_2O$: C, 67.14; H, 6.13; N, 3.41. Found: C, 67.20; H, 6.11; N, 3.46.

β-3,4-Dihydroxyphenylserine (XII). A. From High-melting β-3,4-Dibenzyloxyphenylserine.—A suspension of 3.94 g. (0.01 mole) of β-3,4-dibenzyloxyphenylserine (m.p. 180°) in 50 ml. of 50% methyl alcohol was hydrogenated at atmospheric pressure using 0.2 g. of 5% palladium-on-charcoal catalyst. In the course of the reduction, it was necessary to add 0.2 g. of fresh catalyst. After eight hours, 465 ml. of hydrogen had been absorbed (theory 480 ml.) and the reduction was stopped. Product had crystallized but it redissolved on warming and the catalyst was removed by filtration. The alcohol was removed by vacuum concentration and the aqueous solution was treated with acid-washed Darco. The almost colorless filtrate was concentrated to 5 ml. and 15 ml. of ethyl alcohol was added slowly. After standing at −20° for 18 hours, the crystalline product was collected and washed with 50% alcohol. It weighed 1.83 g. (85.9%) and melted with decomposition at 199–200°. Analyses showed that the compound was hydrated.

Anal. Calcd. for $C_9H_{11}O_5N \cdot H_2O$: C, 46.75; H, 5.67; N, 6.06. Found: C, 46.84; H, 5.66; N, 6.06.

B. From Low-melting β-3,4-Dibenzyloxyphenylserine.—A solution of 12.22 g. (0.03 mole) of β-3,4-dibenzyloxyphenylserine monohydrate (m.p. 146°) in a mixture of 30 ml. of water, 30 ml. of ethyl alcohol and 15 ml. of 2 *N* lithium hydroxide was hydrogenated at atmospheric pressure using 2.0 g. of 5% palladium-on-charcoal catalyst. After 1490 ml. of hydrogen had been absorbed (theory 1470 ml.), the reduction ceased and 6 ml. of concentrated hydrochloric acid was added to the reaction mixture. After filtration through acid-washed Darco, the light yellow filtrate was neutralized with 2 *N* lithium hydroxide (22 ml.). Crystallization was rapid and, after cooling the mixture at 0° for 24 hours, 5.94 g. (93.0%) of product was obtained. Due to the insolubility of the compound, it was purified by dissolving it in alkali and then reprecipitating by the addition of acid. The product had a gray tinge and melted with decomposition at 220–225°.

Anal. Calcd. for $C_9H_{11}O_5N$: C, 50.70; H, 5.20; N, 6.57. Found: C, 50.15; H, 5.36; N, 6.51.

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The Synthesis of L-Histidyl Peptides[1]

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L-Histidyl peptides have been synthesized for the first time, using a modified carbobenzoxy azide peptide synthesis procedure.

In spite of the widespread occurrence of L-histidine in proteins, the synthesis of L-histidyl peptides has not previously been described.[2] Presumably this is due to the presence of the imidazole ring in the histidine side chain, which would complicate many peptide synthesis procedures. The imidazole ring is weakly basic, with pK_a approximately 6, and under certain conditions undergoes ring opening or substitution reactions. The reaction conditions which can be used in peptide synthesis are, therefore, limited. For example, it is unlikely that the histidine carboxyl could be converted to an acid chloride without having the imidazole ring protected in some way.

Two DL-histidyl peptides have been described, but the procedures used in their preparation are not applicable to the synthesis of L-histidyl peptides. Bergmann and Zervas[3] prepared DL-histidylglycine by way of an oxazolone, obtained from acetyl-DL-histidine and acetic anhydride, which reacted with glycine ethyl ester to give acetyl-DL-histidylglycine ethyl ester. Hydrolysis of the latter with dilute hydrochloric acid gave a low yield of DL-histidylglycine. Presumably this procedure could be used for the preparation of other DL-histidyl peptides, but it is not applicable to the synthesis of L-histidyl peptides since the intermediate oxazolone would be racemized. Fischer and Suzuki[4] prepared histidylhistidine by mild alkaline hydrolysis of L-histidine anhydride (3,6-di-(4-imidazolemethyl)-2,5-piperazinedione). As would be expected, the hydrolysis was accompanied by racemization.[5]

Of the various peptide synthesis procedures which might be applicable to the synthesis of L-histidyl peptides, it seemed to us that the carbobenzoxy azide procedure was the most promising, although difficulties were anticipated in the isolation of the basic carbobenzoxy-L-histidyl azide and there was the possibility of reaction of the azide with the imidazole ring present in the azide molecule.

Carbobenzoxy-L-histidine methyl ester was prepared by acylation of L-histidine methyl ester with carbobenzoxy chloride in chloroform containing triethylamine. The ester, an oil, was converted to the crystalline hydrazide by treatment with hydrazine hydrate in absolute ethanol. Potentiometric titration indicated the presence of two basic groups, pK_a 6.1 and 2.5, corresponding to the imidazole ring and the hydrazide, respectively.

In the standard carbobenzoxy azide procedure, the hydrazide is converted to the azide by treatment with nitrous acid in acid solution, and the azide is extracted into an inert organic solvent. Carbobenzoxy-L-histidyl azide, however, is basic, and is not extractable from acid solution. This difficulty was overcome by preparation of the azide in aqueous acid, followed by basification of the solution. The azide, obtained in high yield as an oil, was extracted into an inert organic solvent, and was allowed to react with an amino acid ester. The carbobenzoxy-L-histidylamino acid esters were obtained in good yield. There was no indication of reaction of the azide with the imidazole ring present within the azide molecule.

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(2) J. S. Fruton, *Advances in Protein Chemistry*, **5**, 1 (1949).

(3) M. Bergmann and L. Zervas, *Z. physiol. Chem.*, **175**, 154 (1928).

(4) E. Fischer and U. Suzuki, *Ber.*, **38**, 4173 (1905).

(5) E. Abderhalden and F. Leinert, *Fermentforschung*, **15**, 324 (1937), hydrolyzed L-histidine anhydride with acid, which would be expected to cause less racemization, but no product was isolated.