# THE SYNTHESIS OF ARYLSERINES

by
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of Doctor of Philosophy

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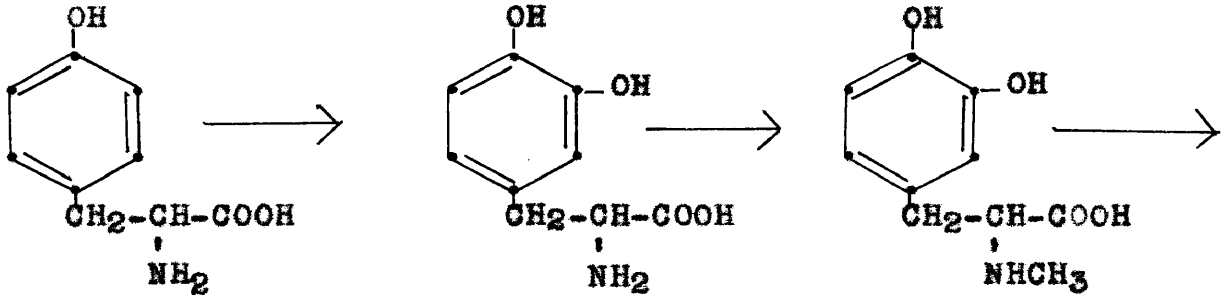
# INTRODUCTION

Arylserines have not to date been isolated from natural sources; yet they are intriguing compounds holding considerable attraction for the physiologist and biochemist. This is especially true since 3,4-dihydroxyphenylserine appears in every scheme thus far proposed for the biosynthesis of epinephrine and nor-epinephrine, hormones elaborated by the adrenal medulla (1,34,48). The arylserines have not been isolated from the tissue which produces the hormone nor is there available any direct evidence of their occurrence in the adrenal medulla. However, because of high dilution and instability, the detection and isolation of the intermediates might well be a very difficult procedure. The lack of positive evidence does not preclude the possibility that arylserines are intermediates in the biogenesis of the adrenal medullary hormones.

Dittrich (48) has shown a number of routes by which phenylalanine may be converted into epinephrine. It is likely that not all of these are possible or even plausible.

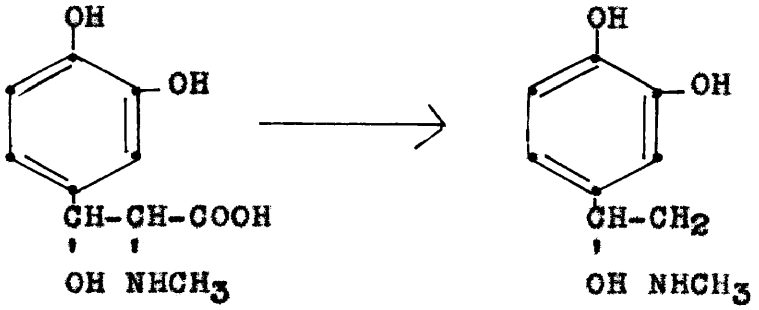
Schmidt (1) favors the following scheme, starting with tyrosine:



tyrosine

3,4-dihydroxyphenylalanine

3,4-dihydroxyphenyl-N-methylalanine

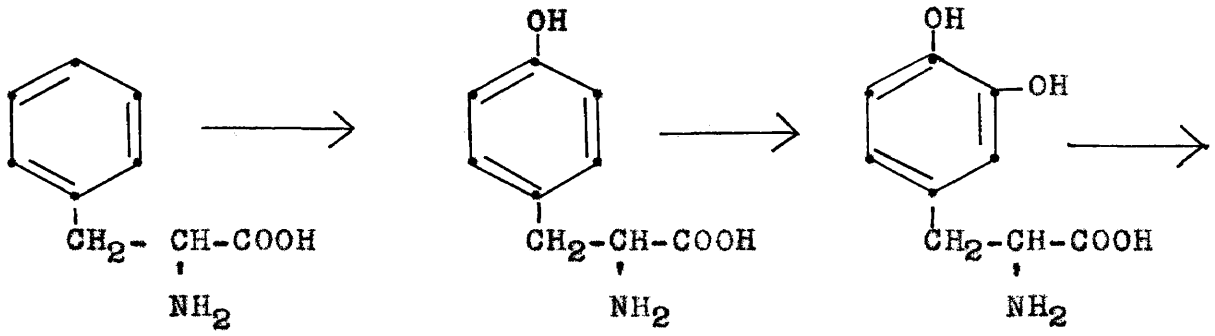


3,4-dihydroxyphenyl-N-methylserine

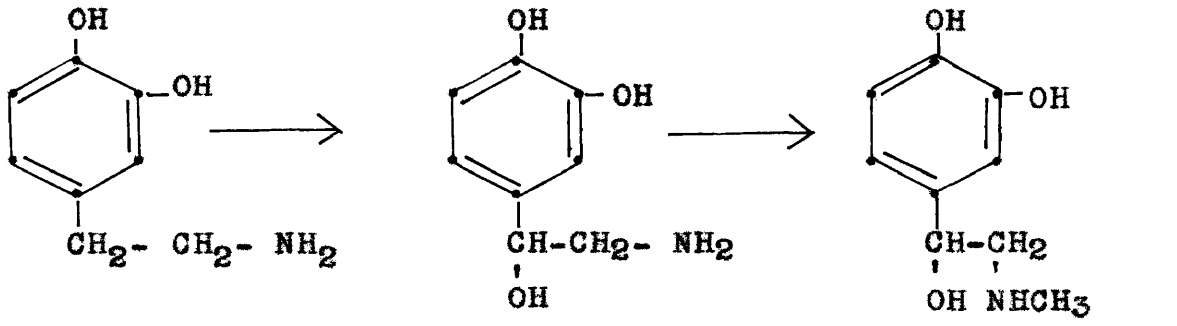
epinephrine

There are four steps necessary in the above sequence: (a) Introduction of a second phenolic hydroxyl group, (b) N-methylation, (c) Introduction of a side chain hydroxyl group, (d) Decarboxylation.

The more recent theory of Blaschko (2) outlined below has been received with more favor (3).



phenylalanine — tyrosine — 3,4-dihydroxy-phenylalanine



3,4-dihydroxy phenylethylamine — nor-epinephrine — l-epinephrine

At least five reactions occur in this process:(a)Stepwise introduction of phenolic groups (2) into the para position, (b) into the meta position, (c) Decarboxylation, (d) Introduction of a side chain hydroxyl group and (e) N-methylation. Blaschko's mechanism differs from that of Schmidt not so much in the types of reactions as in the order of their occurrence.

In the following paragraphs evidence will be presented to show that each of the above steps can take place in the human body.

The *in vitro* oxidation of phenylalanine was accomplished by Raper (4) by the action of hydrogen peroxide and ferrous sulphate on phenylalanine. Moss and Schoenheimer (5) demonstrated the biological conversion of phenylalanine to tyrosine by the addition of deutero-phenylalanine to a casein-containing stock diet of growing and adult rats. The tyrosine samples isolated from internal organs of their experimental animals were found to contain a concentration of deuterium which indicated that twenty to thirty percent was derived from deutero-phenylalanine.

Meta-hydroxylation has been shown to take place only after para-hydroxylation by means of both enzymatic and nonenzymatic oxidation experiments. Onslow and Robinson (6) suggested that the oxidation of tyrosine to 3,4-dihydroxyphenylalanine, usually referred to as DOPA, took place in the presence of a phenol oxidase system. Raper (7) confirmed this observation by isolating DOPA from tyrosine-tyrosinase systems. However, tyrosinase isolated from different sources gave different yields of DOPA. These variations were explained as resulting from the presence of contaminating enzymes, probably peroxidase or polyphenolases, in the tyrosine preparations, which could oxidize DOPA but not tyrosine. Arnow (8) produced DOPA by irradiating tyrosine with ultraviolet light. He showed that the melanin in the skin was increased by such irradiation by the production of DOPA from tyrosine and its subsequent conversion to melanin by the dopa oxidase of the melanoblasts. Melanin was also formed from

polypeptides containing tyrosine on oxidation in the presence of tyrosinase (9). Beyer indicated that the ascorbic-dehydro-ascorbic acid system is capable of oxidizing tyramine to the corresponding ortho dihydroxy compound. The amino group is not attacked and deamination does not occur since the catechol nucleus and the corresponding ortho-quinone could enter into oxidation-reduction equilibrium with the ascorbic-dehydro-ascorbic acid system. Heard and Welch (11) stated: "Ascorbic acid is the agent responsible for the maintenance of the 3,4-quinone of adrenaline in the reduced state and the inhibition of pigment formation. As long as a small amount of ascorbic acid remains present in the reduced form, diminution in pressor activity does not take place." In patients having tyrosinosis, DOPA was excreted in the urine after oral administration of tyrosine (12,13). If tyrosinosis results from incomplete metabolism of tyrosine resulting in increased excretion of DOPA, it is possible that DOPA is an intermediate in the normal metabolism of tyrosine.

The natural occurrence of 3,4-dihydroxyphenylalanine in the Broad Bean has been found by Guggenheim (14) and in the Georgia Velvet and Lyon Bean by Miller (15). Raper and Speakman (16) identified tyrosinase in the mealworm while Durham (17) reported evidence of its presence in the skin of guinea pigs and rabbits. Hageboon and Adams (18) reported the presence of a phenolase in a mouse melanoma.

That decarboxylation can take place in the mammalian

organism has been well established. In 1924 Neuberg and Gottschalk (20) showed that liver and muscle macerates could bring about decarboxylation of pyruvic acid. On addition of pyruvic acid to the above macerate, the aldehyde content was increased up to 70%. Only decarboxylation would account for these results. The decarboxylation of tyrosine to yield the pressor agent tyramine was found to take place in the kidney and pancreas through the action of a decarboxylase catalyst (2). Gram-positive organisms of the acidophilus group have been shown to possess a similar decarboxylase (21) and it is likely that decarboxylation of tyrosine in the intestines partially accounts for the tyramine eliminated by the body. Devine (22) carried out experiments in which solutions of various substrates related to tyrosine were incubated with surviving adrenal medulla tissue slices and then assayed colorimetrically for epinephrine. Of the compounds tested phenylethylamine was the most active, being converted to epinephrine in amounts up to nearly 40%. Phenylalanine was about one-fourth as active.

While most amino acids do not have any effect on arterial blood pressure, many of the corresponding amines produce arterial hypertension often equal to that produced by equal doses of epinephrine. It would be logical then to consider the role of certain amino acids in the pathogenesis of hypertension. Bing and Zucker (23) showed that pressor amines resulted only when enzymatic action took place under anerobic conditions. When oxygen was present the pressor amines were destroyed by

a second group of catalysts, forming pharmacologically inert end products. To substantiate their conclusions, DOPA was injected into a partially ischemic kidney of a cat and allowed to incubate two and one-half hours. On restoration of renal circulation, an elevation of blood pressure was obtained. They concluded that in the ischemic kidney, the oxygen tension was below that necessary for deamination of the pressor amine, and that amino acid metabolism would be arrested in such kidneys after initial decarboxylation.

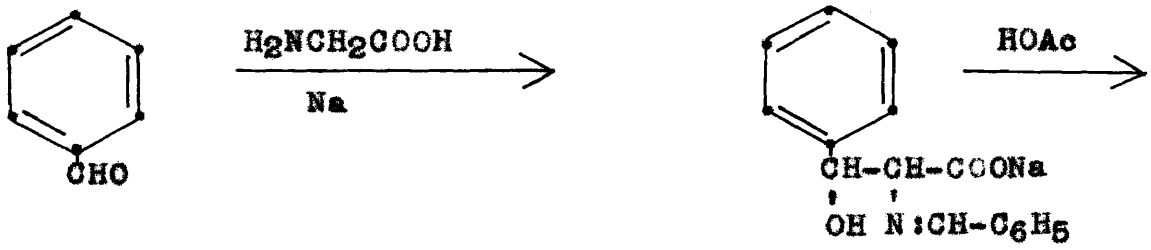
Holtz (24) showed the presence of dopa decarboxylase in practically all animals capable of producing epinephrine and demonstrated its ability to decarboxylate selectively L-3, 4-dihydroxyphenylalanine. Substantiating Bing and Zucker's work, he isolated, from DOPA, 3,4-dihydroxyphenylethylamine in the kidney and pancreas only when the organ was incubated with the amino acid in an atmosphere of nitrogen. This precaution was necessary in order to prevent oxidation of the amine by amine oxidase. Blaschko (2) determined manometrically the activity of L-dopa decarboxylase in liver and kidney extracts by measuring the formation of carbon dioxide under anaerobic conditions. In mammals the enzyme was found to be present in the extracts of all species examined, including the human kidney. Both the bacterial and mammalian enzymes were shown to be stereospecific as far as production of the naturally occurring stereoisomeride is concerned. Beyer (3) interprets the above results by concluding that "experimental

hypertension may be due to the decarboxylation of amino acid precursors of pressor amines in a damaged and ischemic kidney in which condition that organ is incapable of bringing about their deamination. To obtain such a circumstance it must be supposed that 1, the predominant site of inactivation of these amines is the kidney; 2, that a rather severe state of renal ischemia is an integral part of the hypertension, and 3, that the principal mode of inactivation of the phenolic pressor amines is by deamination."

From the evidence now available, the stage at which β-hydroxylation occurs is still not established. Raper and Heard (25) reported the formation of adrenalone when N-methyl DOPA was oxidized with tyrosinase in vitro. Similar oxidations of epinephrine and 3,4-dihydroxyphenylethylamine with tyrosinase did not produce adrenalone. From this it was concluded that side chain oxidation took place only so long as the terminal carboxyl group was retained. On the other hand perfusion of adrenalone through the adrenal gland did not result in its reduction to epinephrine. Beyer agreed with the following conclusions of Holtz (24) and Vinet (26). Holtz was able to introduce both an alcoholic hydroxyl group on the carbon atom and a methyl group on the amino nitrogen of 3,4-dihydroxyphenylethylamine, suggesting the possibility that decarboxylation preceeded these processes in the biogenesis of these hormones. His results were in agreement with those of Vinet who claimed that the adrenal medulla was capable of bringing about the C-oxidation and N-methylation of decarboxylated dihydroxyphenylalanine.

N-methylation in the body has long been recognized. His (27) in 1887 isolated N-methylpyridinium hydroxide from the urine of dogs after feeding them pyridine. Ackerman (28) showed that when nicotinic acid was fed to dogs, it was excreted as the N-methyl derivative, trigonellin, in the urine. Cannon and Rosenbleuth (29) did not accept Schmidt's view that demethylation of epinephrine took place in the body. Sympathin E is thought by many (30,31) to be identical with nor-epinephrine. Since a primary sympathomimetic amine could arise directly in the body, demethylation for the biosynthesis of this compound need not be assumed to occur. The experiments conducted by Cannon and Rosenbleuth indicated that a primary amine was formed first and that a secondary amine arose from it by N-methylation. By the use of compounds containing isotopic nitrogen, Bloch and Schoenheimer (32) elucidated the biological origin of the creatine molecule by showing that the formation of guanidoacetic acid from glycine and arginine, followed by N-methylation in the presence of methionine, formed creatine. Du Vigneaud and coworkers (33) confirmed these results. The transfer of methyl groups from methionine in the formation of creatine was demonstrated by the isolation of deuteriocholine and deuteriocreatine from the tissues of rats fed methionine containing a deuteriomethyl group.

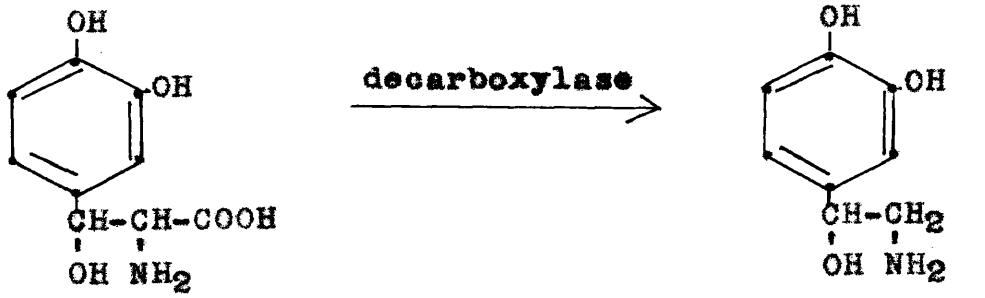
The formation of arterenol by a process in which phenylserine rather than phenylalanine is pictured as a key intermediate has been suggested by Rosenmund and Dornsaft (34) and can be indicated by the following equations:





phenylserine

p-hydroxyphenylserine



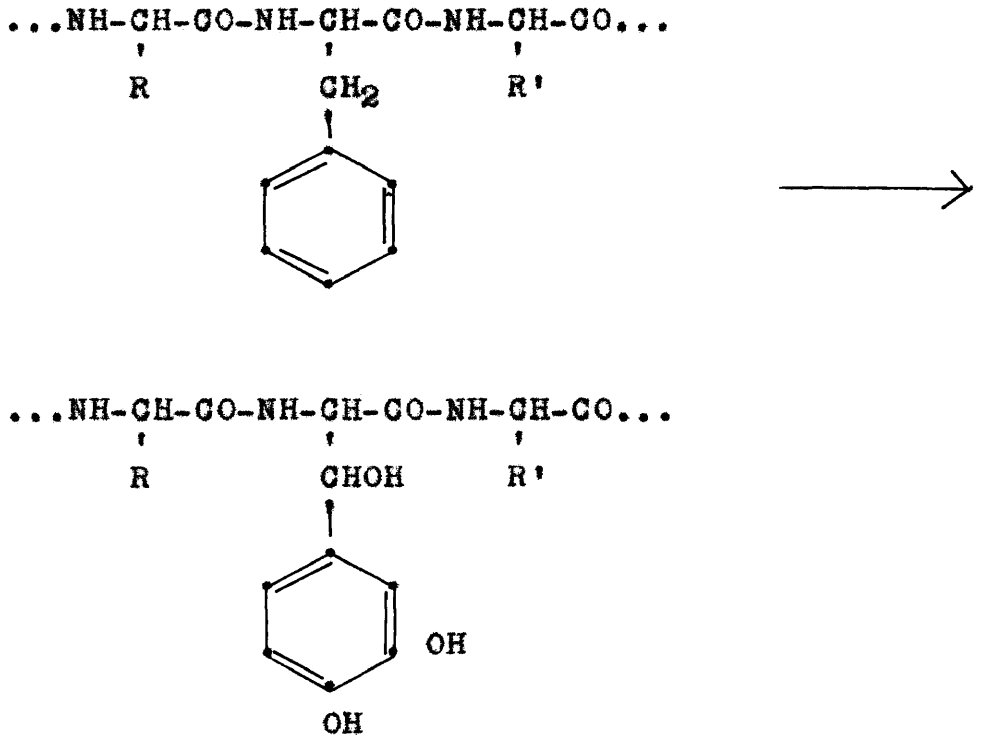

3,4-dihydroxyphenyl-serine

arterenol

This type of reaction requires only a low order of activation in vitro. It is essentially an aldol condensation, of

a type which has been suggested for many biochemical reactions. Since the chemical structure of phenylserine is closely related to that of phenylalanine, and differs only by the presence of a β-hydroxyl in the side chain, the introduction of phenolic hydroxyls into the former might reasonably be expected to take place by the methods already described for the latter. There is, however, no direct experimental evidence supporting steps 1 and 2 outlined above. Decarboxylation by a decarboxylase would then give rise to nor-epinephrine. The use of sarcosine, N-methyl glycine, in place of glycine in the above procedure would similarly give rise to epinephrine.

Hartung (19) has suggested that phenylalanine, coupled by way of peptide linkages, rather than free phenylalanine, undergoes conversion to a corresponding epinephrine precursor.



Hydroxylation in the nucleus and side chain is pictured as taking place as discussed above. The subsequent action of a polypeptidase and decarboxylase would give rise to norepinephrine. On bio-N-methylation epinephrine would be formed.

In spite of all these attractive hypotheses the fact still remains that neither phenylserine nor its derivatives has ever been isolated from natural sources. The parent amino acid, serine, has been found in human hair (35), proteins (36) and phosphatides of the brain (37). Serine has been shown to make up 3.57% of the insulin molecule (38). There are still a number of hydroxy amino acids present in insulin which have not been identified. It is impossible that one or more may be derivatives of phenylserine.

Further interest in phenylserine has recently been aroused (75) by its use as a starting material in the synthesis of chloromycetin, an important antibiotic, as indicated by the following equations:

$$C_6H_5CHOHCHNH_2COOH.HCl + CH_3OH \longrightarrow C_6H_5CHOHCHNH_2COOC_2H_5$$

$$\xrightarrow{LiAlH_4} C_6H_5CHOHCHNH_2CH_2OH \xrightarrow{Ac_2O}$$

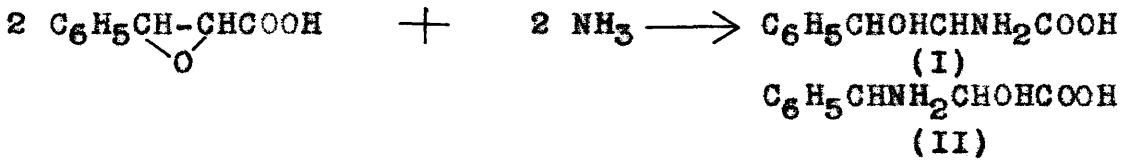
$$C_6H_5CH(OAc)-CH(NHCOCH_3)-CH_2OAc \xrightarrow{HNO_3} p\text{-}(NO_2)C_6H_5CH(OAc)-CH(NHCOCH_3)-CH_2OAc$$

$$\xrightarrow[H_2O]{CHCl_2COOR} p\text{-}(NO_2)C_6H_5CHOH-CH(NHCOCHCl_2)-CH_2OH$$

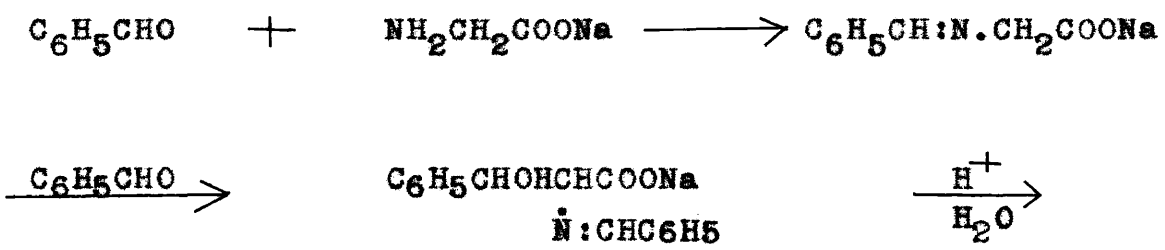
Chloromycetin

## Literature Survey of Methods of Preparing α-Amino β-Hydroxy Acids and Their Esters

Phenylserine was first synthesized by Erlenmeyer (40) who in 1894, by treatment of phenylglycidic acid with concentrated ammonia, isolated two amino acids with the same empirical formula. He reported the products to be phenyserine (I) and its isomer, phenylisoserine (II).



Erlenmeyer and Früstück (41) obtained N-benzalphenylserine by condensing two moles of benzaldehyde with the sodium salt of glycine. Hydrolysis yielded free phenylserine.



$C_6H_5CHOHCHNH_2COOH$ + $C_6H_5CHO$

phenylserine

A process has been reported in which one mole of glycine and one mole of benzaldehyde in a strongly aqueous medium of 1.1-2.5 moles of sodium hydroxide yielded phenylserine upon acidification (42). Using a similar procedure Rosenmund and Dornsaft (34) condensed benzaldehyde with glycine ethyl ester and obtained the ethyl ester of phenylserine. By an analagous reaction of glycine ethyl ester with protocatechuic aldehyde, the important 3,4-dihydroxyphenyl derivative, DOPS[1], was produced. However, not only were the yields low, but certain aldehydes, such as anisaldehyde failed to react. Employing slight modifications Dalgliesch and Mann (44) prepared N-methyl DOPS by condensing 3,4-diethylcarbonatobenzaldehyde with sarcosine. Again this type of reaction was reported to be critically affected by the nature of the substituents in the benzene ring. Van Loon and Carter (45) prepared α-amino-β-methoxy-β-phenylserine in the hope of using it as the intermediate for the preparation of phenylserine. Instead of the free amino acid, β-phenylnapthalene was isolated on acid hydrolysis. Knoop (46) allowed α-chloro-β-hydroxy-β-phenylpropionic acid to react with methylamine. The expected α-methylamino-β-hydroxy-β-phenylpropionic acid did not result. Instead the product proved to be the β-methylamino acid. It is probable that an intermediate reaction, formation of an inner ether, took place. The ether would then add methylamine as Erlenmeyer had

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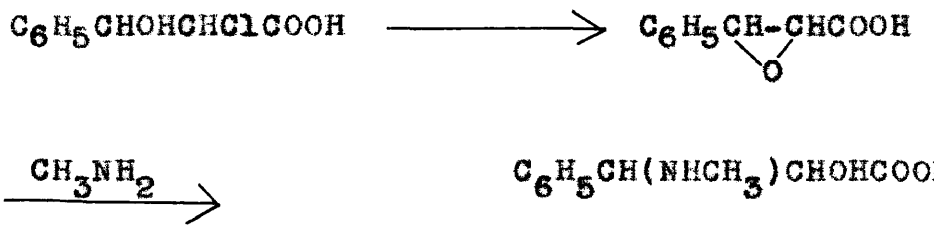
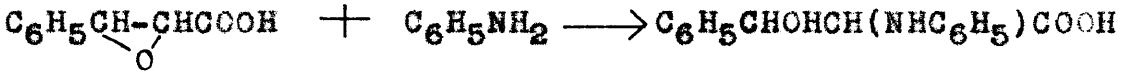
[1] In that 3,4-dihydroxyphenylalanine is often called DOPA, it has been proposed that 3,4-dihydroxyphenylserine be, in like manner, referred to as DOPS (39).

shown (40).



Fourneau and Billeter (47) further elaborated Knoop's work by showing that ammonia and aliphatic amines reacted with β-phenylglycidic acid to give derivatives of phenylisoserine, while aromatic amines on treatment with β-phenylglycidic acid gave derivatives of phenylserine.



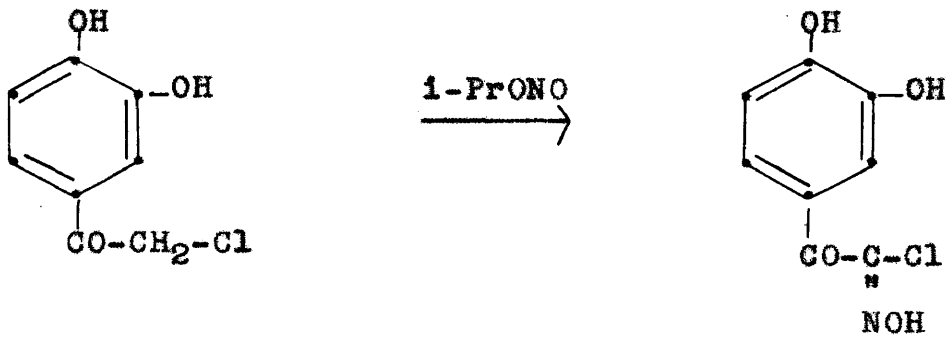
Ethyl benzoylacetate was nitrosated by Dittrich (48) in another successful attempt to prepare phenylserine. On reduction of the α-oximino acid, phenylserine ethyl ester was obtained. A number of methods have been published for the preparation of the β-keto-ester used as starting material in this synthesis. Lowman (50) first prepared ethyl benzoylacetate by condensing ethyl benzoate with ethyl oxalate. It has also been prepared by treatment of ethyl phenylpropiolate (51) or α-bromocinnamic acid (52) with concentrated sulfuric acid. Buchner and Curtius (53) obtained it by treating ethyl diazoacetate with benzaldehyde; Marguery (54) by condensing benzene with ethyl malonyl chloride and aluminum chloride; Meyer and Togel (55) by reacting benzoyl chloride with the product of the reaction between magnesium and ethyl chloroacetate and Shriner and Schmidt (56) by the hydrolysis of ethyl benzoylacetoacetate.

Object of research.-Arylserines are believed to be the *in vivo* precursors of sympathimimetic amines; for example, 3,4-dihydroxyphenylserine is the intermediate proposed in the biosynthesis of epinephrine and nor-epinephrine. More recently phenylserine was used as the starting material in the synthesis of chloromycetin. This problem was undertaken with the purpose of developing a satisfactory and economical procedure for the synthesis of arylserines, with special interest being given to the preparation of 3,4-dihydroxyphenylserine.

# EXPERIMENTAL

Preparation of 3,4-dihydroxyphenacyl chloride.-This synthesis has been described by Levin (57). In a 500 ml. round-bottomed flask containing 83.3 g. (0.4 mole) of phosphorous pentachloride was placed 42.5 g. (0.45 mole) of monochloroacetic acid in small portions. The mixture was refluxed for three hours and allowed to stand overnight. On distilling at atmospheric pressure the fraction coming over at 105-110° was collected. To the chloroacetyl chloride thus prepared were added 200 ml. of benzene and 44 g. (0.4 mole) of catechol. The mixture was refluxed over a steam bath for twenty-four hours. After distilling off the benzene, 400 ml. of boiling hot water was carefully added to the residue with rapid stirring. The rapid stirring was continued until the reaction mixture had cooled to room temperature. The slightly purple crystalline product was filtered with suction, washed with a small amount of cold water and recrystallized from hot water using 3 g. of Norite. The decolorized filtrate was placed in an icebox overnight. White crystals, weighing 32.5 g. (43%) were obtained, melting at 170-171.5° with decomposition. The melting point reported in the literature is 173° with decomposition. On repeating this reaction yields up to 52% were obtained.

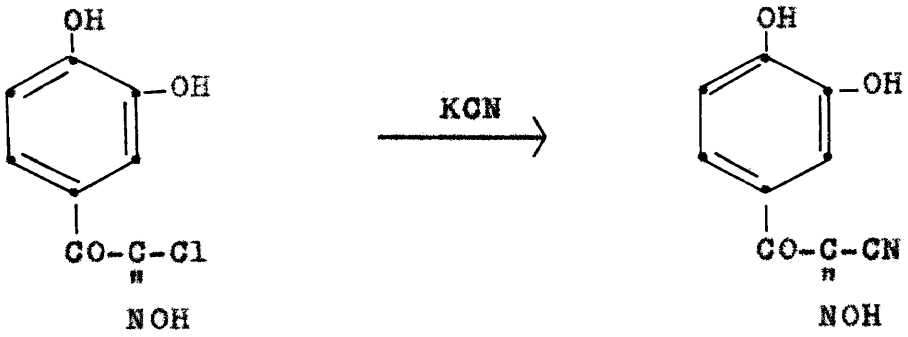
Preparation of 3,4-dihydroxyphenylglyoxylohydroxamyl chloride.-




Using Levin's (57) method, a 1-L. three-necked, round-bottomed flask was provided with a reflux condenser, mercury-sealed mechanical stirrer, an inlet tube for hydrogen chloride, a dropping funnel and gas trap. Dry hydrogen chloride was passed into 70 g. (0.38 mole) of 3,4-dihydroxyphenacyl chloride suspended in 750 ml. of ether. After five minutes 37.5 g. (0.42 mole) of freshly distilled isopropyl nitrite was dropped into the rapidly stirred mixture. The flow of hydrogen chloride was then adjusted to about five bubbles per second. The rate of isopropyl nitrite addition was so controlled that the color of the reaction mixture did not become darker than reddish-orange. After half of the isopropyl nitrite had been added, complete solution resulted. Stirring and addition of hydrogen chloride was continued for thirty minutes after the addition of the nitrite, until the color returned to a light red. The reaction mixture was allowed to stand overnight. The ether was evaporated on a

steam bath and the resulting product recrystallized from a mixture of ether and benzene, producing 65.5 g. (80%) of the isonitroso compound melting at 182-184° with decomposition. The melting point was in agreement with that found by Levin (57).

Preparation of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide.-




To 10.65 g. (0.05 mole) of 3,4-dihydroxyphenylglyoxylohydroxamyl chloride dissolved in 100 ml. of alcohol was added 13 g. (0.2 mole) of potassium cyanide dissolved in 200 ml. of water. The mixture was heated, with occasional stirring, on a steam bath for one hour and allowed to cool. On acidification with dilute hydrochloric acid a brownish mass formed; which, on addition of excess acid, dissolved. The dark mixture was extracted with three 250 ml. portions of ether. The ether extract was dried over anhydrous sodium sulphate and evaporated to dryness on a steam bath. The resulting crude product was taken up in 50 ml. of ether, boiled with one gram of Norite and filtered. After the addition of 350 ml. of toluene,

the cloudy solution was placed in an icebox for two hours. By this means 5.5 g. (53%) of yellowish-green crystals melting at 172-173.5° was obtained.

Analysis.[1] Calculated for $C_9H_9O_4N_2$: C, 52:43; H, 2.93; N, 13.59. Found: C, 52.43; H, 2.95; N, 13.45.

Mattocks (58), by a method similar to the above procedure, reported the melting point of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide to be 162-164°; however, he did not completely purify it.

Attempted preparation of ∝-hydroximino-3,4-dihydroxybenzoylacetic acid.-3,4-Dihydroxyphenylglyoxylohydroxamyl cyanide (0.02 mole) was dissolved in 100 ml. of 10% aqueous sodium hydroxide and the mixture was heated on a steam bath until ammonia was no longer liberated. The reaction required five to six hours heating. On cooling to room temperature, the solution was treated with 100 g. of crushed ice and acidified with dilute hydrochloric acid. An immediate evolution of gas was noted. The mixture was extracted with ether, and the ether extract dried over anhydrous sodium sulphate, filtered and evaporated to dryness on a steam bath. Very hygroscopic brown crystals were thus obtained. Further purification by recrystallization from ether produced yellow brown crystals which were still hygroscopic.

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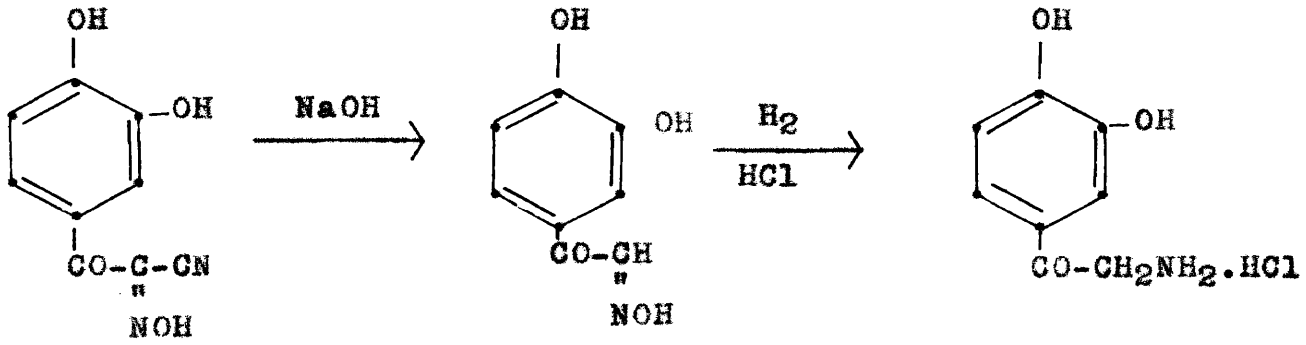
[1] Analyses were obtained through the courtesy of Sharp and Dohme, Inc.

An accurate analysis could not be obtained. Mattocks (58) obtained a light brown powder as the product of this reaction, and, likewise, offered no analytical data in support of its constitution.

Preparation of ω-aminoacetycatechol. Attempted synthesis of 3,4-dihydroxyphenylserine.-Mattocks (58) reported the




synthesis of 3,4-dihydroxyphenylserine by a procedure analogous to the one described below; however, only ω-aminoacetylcatechol could be obtained in the present investigation.

In a hydrogenation flask were placed the product obtained from the hydrolysis of 0.02 mole of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide, 100 ml. of absolute alcohol containing 10 g. of dry hydrogen chloride and 3 g. of a 10% palladium on charcoal catalyst (73). The mixture was shaken on a Parr hydrogenation apparatus until hydrogen was no longer absorbed, about 0.05 moles being taken up. The catalyst was filtered and the filtrate concentrated in vacuo

to 30 ml. Addition of 100 ml. of dry ether precipitated 4 g. of colorless crystals. Recrystallization from absolute alcohol gave 3.6 g. of a hydrochloride that melted to a red oil with evolution of a gas at 244-246°, in agreement with the melting point reported by Mattocks, 240-241° (58).

Analysis. Calculated for 3,4-dihydroxyphenylserine hydrochloride, $C_9H_{11}O_5N.HCl$: C, 43.29; H, 4.85; N, 5.62.

Calculated for arterenol hydrochloride, $C_8H_{11}O_3N.HCl$: C, 47.18; H, 4.95; N, 6.88.

Calculated for ω-aminoacetyl catechol, $C_8H_9O_3N.HCl$: C, 47.18; H, 4.95; N, 6.88.

Found: C, 46.65; H, 4.95; N, 6.88.

The analytical data was consistent with the values calculated for ω-aminoacetylcatechol or arterenol hydrochloride. The melting point of arterenol hydrochloride was reported by Simonoff (59) as 136° with decomposition. The melting point of the amino ketone hydrochloride reported by Barger and Dale (60) was 252° with decomposition. From the above data it may be assumed with reasonable certainty that the compound formed was ω-aminoacetylcatechol rather than arterenol or 3,4-dihydroxyphenylserine.

The apparent formation of ω-aminoacetylcatechol by this series of reactions, rather than 3,4-dihydroxyphenylserine, might be explained if the beta-keto acid obtained by hydrolysis of 3,4-dihydroxyglyoxylohydroxamyl cyanide underwent decarboxylation to form ω-hydroximinoacetylcatechol. The evolu-

tion of a gas, presumably carbon dioxide, noted during the hydrolysis, is evidence of the possibility that decarboxylation did occur.

In an attempt to avoid decarboxylation, the mixture, resulting from the heating of 3 g. of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide with sodium hydroxide was transferred without purification to a hydrogenation bottle. The flask was shaken with 0.5 g. of a 10% palladium catalyst in an effort to prevent poisoning of the 3 g. of additional catalyst which was later added. A negligible amount of hydrogen was taken up, indicating incomplete hydrogenation. The solution was acidified and extracted with ether. On evaporation of the ether, effervescence was again observed. The residue was dissolved in 100 g. of absolute alcohol containing 5 g. of dry hydrogen chloride and placed in a hydrogenation bottle with 3 g. of a 10% palladium catalyst. After hydrogenation, ω-aminoacetylcatechol, melting with decomposition at 244-246° was obtained.

Attempted alcoholysis of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide.-To 50 ml. of a solution containing 11.5 g. (0.25 mole) ethyl alcohol, 9.1 g. (0.25 mole) of dry hydrogen chloride and enough ether to make up 500 ml., was added 2 g. (0.01 mole) of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide. The mixture was placed in a refrigerator for ten days. At the end of this time 35 ml. of hot water was added. The solution was extracted with ether. Evaporation of the ether

produced a brownish-green oil which solidified on standing in a desiccator over phosphoric anhydride, to brownish-green crystals melting at 167-169°.

Analysis. Calculated for $C_{11}H_{11}O_6N$; N, 5.5; Found: N, 12.6.

Alcoholysis apparently did not take place, and the starting material was recovered as indicated by the following: 1) Analytical data was in agreement with that calculated for 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide; 2) The melting point was only four degrees below that of the starting material; 3) A mixed melting point showed no appreciable depression.

Preparation of phenylglyoxylohydroxamyl cyanide.-To a solution of 9.2 g. (0.05 mole) of phenylglyoxylohydroxamyl chloride, prepared by Levin's procedure (57), in 100 ml. of alcohol, was added 200 ml. of water containing 13 g. (0.2 mole) of potassium cyanide. The dark reaction product was heated on a steam bath with stirring for thirty minutes. The mixture was cooled, acidified with dilute hydrochloric acid, and again cooled before extracting with ether. The ethereal solution was dried over anhydrous sodium sulphate. Removal of the ether on a steam bath gave a 40% yield of phenylglyoxylohydroxamyl cyanide. After recrystallization from an ether-benzene mixture, it melted at 119-121°. The reported melting point is 120-121°.

Preparation of phenacylcyanide.-According to Dittrich's directions (48), 31 g. (.02 mole) of phenacyl chloride dissolved in 90 ml. of alcohol at a temperature of 40° was added

to 30 g. (0.6 mole) of sodium cyanide dissolved in 90 ml. of water. After keeping the temperature at 50° for 30 minutes, additional water was added to complete precipitation. The solid was filtered off and the filtrate acidified with dilute hydrochloric acid. The resulting crystals were filtered and dissolved in a minimum amount of water containing an amount of sodium cyanide equal to the weight of the crystals. To this solution was added 1 g. of Norite. The mixture was filtered and the filtrate again acidified with dilute hydrochloric acid. The crystalline precipitate was taken up in hot 60% alcohol and treated with 2 g. of Norite. On filtration and cooling, 16 g. (55%) of pure phenacyl cyanide was obtained, melting at 78-79°.

Nitrosation of phenacyl cyanide.-To a 500 ml. three-necked, round-bottomed flask provided with reflux condenser, mercury-sealed stirrer, an inlet tube for hydrogen chloride and a dropping funnel was placed 15 g. (0.1 mole) of phenacyl cyanide dissolved in 100 ml. of ether. After passing dry hydrogen chloride slowly through the solution, 15 g. (0.17 mole) of freshly distilled isopropyl nitrite was dropped into the flask with rapid stirring. The rate of hydrochloric acid addition was adjusted to four to five bubbles per second, and the rate of isopropyl nitrite addition was so controlled that the color of the reaction mixture did not become darker than orange. Stirring and addition of hydrogen chloride was continued twenty minutes after the complete addition of nitrite.

The ether was evaporated on a steam bath, leaving 11 g. (63%) of brown crystals of phenylglyoxylohydroxamyl cyanide. After recrystallization from an ether-benzene mixture, the cyanide melted at 119-121°.

Analysis. Calculated for $C_9H_6O_2N_2$: N, 16.1. Found: N, 15.4, 15.8.

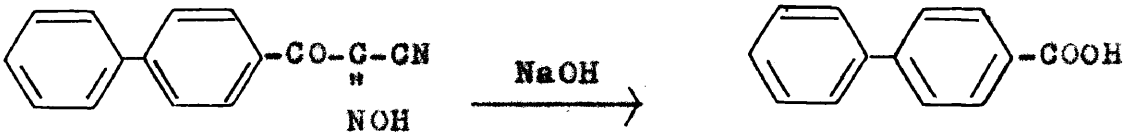
Hydrolysis of Phenylglyoxylohydroxamyl cyanide. Attempted synthesis of α-hydroximinobenzoylacetic acid.-In 75 ml. of aqueous 10% sodium hydroxide was dissolved 3.5 g. (0.02 mole) of phenylglyoxylohydroxamyl cyanide. The mixture was heated on a steam bath in an open beaker. Ammonia began to come off immediately, and continued to be evolved for more than eighteen hours, indicating that some side reaction might be taking place. The solution was cooled, treated with 100 g. of crushed ice and acidified with dilute hydrochloric acid. A white solid, containing no nitrogen and melting at 121-2°, precipitated. A mixed melting point with sublimed benzoic acid gave no melting point depression, indicating that oxidation of the side chain had occurred.

Preparation of p-phenylphenylglyoxylohydroxamyl cyanide.-To 7.02 g. (0.027 mole) of p-phenylphenylglyoxylohydroxamyl chloride prepared by Levin (57) in 150 ml. of alcohol was added 6.5 g. (0.1 mole) of potassium cyanide in 100 ml. of water. On heating for one hour on a steam bath the solution turned red. This mixture was cooled to room temperature and

acidified with dilute hydrochloric acid, the color changing to a light brown. It was then extracted with ether and the ethereal solution dried over anhydrous sodium sulphate. On removal of the ether on a steam bath 5.5 g. (82%) of product melting at 161-163° was obtained.

Analysis. Calculated for $C_{15}H_{10}O_2N_2$: N, 11.20. Found: N, 10.73, 10.73.

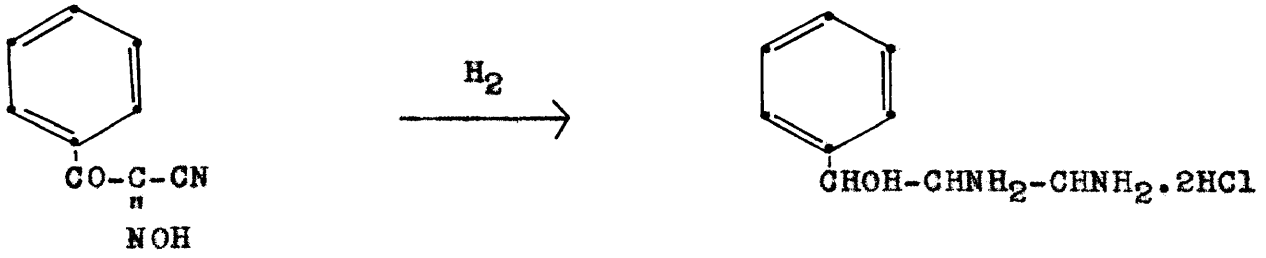
Hydrolysis of p-phenylphenylglyoxylohydroxamyl cyanide.-




To 75 ml. of aqueous 10% sodium hydroxide was added 5.0 g. (0.02 mole) of p-phenylphenylglyoxylohydroxamyl cyanide. On heating this mixture on a steam bath, ammonia was liberated. Heating was continued for ten hours until no more ammonia came off. To the cooled solution was added 100 g. of ice and then dilute hydrochloric acid until the mixture was acid to litmus. A white solid, containing no nitrogen, precipitated. The product melted at 224-5° and probably consisted of impure p-biphenylcarboxylic acid, m. p. 228°. As in the previous experiment, complete oxidation of the side chain occurred, forming p-biphenylcarboxylic acid.

Preparation of 3-phenyl-3-hydroxy-1,2-propanediamine

dihydrochloride.-In a hydrogenation flask were placed one gram




of phenylglyoxylohydroxamyl cyanide, 50 ml. of absolute alcohol containing five grams of dry hydrogen chloride and 0.3 g. of a 10% palladium on charcoal catalyst. In one hour 0.22 mole of hydrogen was absorbed. The catalyst was filtered and the filtrate concentrated to 25 ml. by distillation under reduced pressure. On addition of dry ether and a small amount of dry benzene, 0.32 g. (20%) of colorless crystals melting at 226-8° was obtained.

Analysis. Calculated for $C_9H_{14}ON_2.2HCl$: N, 11.8. Found: N, 13.28, 13.05.

Duschinsky and Dolan (61) prepared the above diamine, melting point 224-225°, by hydrolysis of 4-α-hydroxylbenzyl-2-imidazolidone. They reported a similarly high nitrogen analysis.

Preparation of 3-(3,4-dihydroxyphenyl)-3-hydroxy-1,2-propanediamine dihydrochloride.-Hydrogenation of 2 g. (0.01 mole) of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide, dissolved in 50 ml. of absolute alcohol containing five grams

of dry hydrogen chloride, was complete after 0.05 moles of hydrogen had been taken up. The mixture was filtered and transferred to a distilling flask. Vacuum distillation was used to concentrate the solution to 25 ml. To the residue an excess of dry ether and a small amount of dry benzene was added. A 37% yield, 1 g., of the diamine dihydrochloride melting at 187-9° was obtained.

Analysis. Calculated for $C_9H_{14}O_3N_2.2HCl$: N, 10.41. Found: N, 10.47, 10.55.

Preparation of 3-biphenyl-3-hydroxy-1,2-propanediamine dihydrochloride.- One gram (0.004 mole) of p-phenylphenylglyoxylohydroxamyl cyanide was hydrogenated and the product isolated in the manner described above. The yield was 0.35 g. (28%) of colorless dihydrochloride melting at 212-214°.

Analysis. Calculated for $C_{15}H_{18}ON_2.2HCl$: N, 8.94; Found: N, 8.15, 8.05.

Preparation of the monopotassium salt of ethyl hydrogen malonate.-By a modification of Freund's method (63), 25 g. (0.15 mole) of ethyl malonate dissolved in 100 ml. of commercial absolute alcohol was slowly added to 8.7 g. (0.15 mole) of potassium hydroxide in 100 ml. of absolute alcohol. After standing three hours, the alcoholic solution was heated to boiling and filtered while hot. A small amount of the dipotassium salt remained on the filter paper and the filtrate on cooling solidified to a white mass of monopotassium salt. The precipitate was filtered and combined with an additional amount obtained by concentration of the mother liquor. A total yield of 18.5 g. (72%) was obtained.

Preparation of ethyl hydrogen malonate.-To a chilled solution of 136 g. (0.8 mole) of the monopotassium salt of ethyl hydrogen malonate dissolved in 80 ml. of water (immersed in an ice bath) was slowly added with stirring 71 ml. of concentrated hydrochloric acid. A minimum of water was then added to dissolve the potassium chloride which formed. This was extracted four times with a total of 300 ml. of ether and the ether dried over anhydrous sodium sulphate. The residue, after removal of the ether on a water bath, was let stand overnight in a vacuum desiccator, distilled, and 100 g. (95%) of ethyl hydrogen malonate was collected at 147°/21 mm. (62,63).

Preparation of ethyl malonyl chloride.-To a 500 ml. three-necked, round-bottomed flask provided with a reflux condenser,

mercury-sealed stirrer, dropping funnel and calcium chloride drying tube was added 240 g. (1.13 mole) of phthalyl chloride. The flask was then immersed in an oil bath which had been previously heated to 105°. With vigorous stirring, 124 g. (0.94 mole) of ethyl hydrogen malonate was added through the dropping funnel in thirty minutes, during which time a vigorour evolution of hydrogen chloride took place. After the addition was complete, stirring was continued for two hours and the temperature was maintained at 100-110°. The mixture was then cooled and distilled under reduced pressure, producing 80 g. (64%) of ethyl melonyl chloride, collected at 88°/20 mm. Repetition of the above experiment gave yields varying between 63 and 68%.

Preparation of ethyl benzoylacetate.-In a 1-L. three-

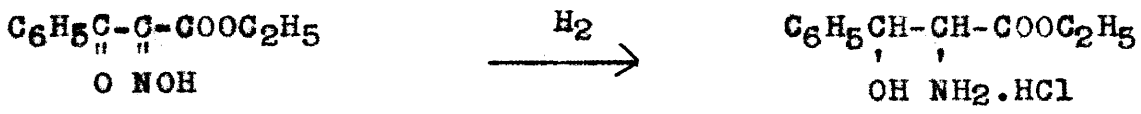
$$C_6H_6 + Cl\text{-}CO\text{-}CH_2COOC_2H_5 \xrightarrow{AlCl_3} C_6H_5COCH_2COOC_2H_5$$

necked, round-bottomed flask fitted with mercury-sealed stirrer, condenser and drying tube, was placed a solution of 30 g. (0.2 mole) of ethyl malonyl chloride in 125 ml. of benzene (64). With vigorous stirring, 37 g. (0.27 mole) of finely powdered anhydrous aluminum chloride was added in small portions. After a few additions, a vigorous evolution of hydrogen chloride took place. After complete addition of the aluminum chloride, the reaction flask was heated to 50° on a steam bath for thirty minutes. The flask was cooled and the mixture poured on crushed ice mixed with concentrated hydrochloric acid. After standing for one hour, two layers separ-

ated. The benzene layer was washed successively with water, dilute hydrochloric acid, again with water and then dried over anhydrous sodium sulphate. The residue, obtained on removal of the excess benzene by distillation under reduced pressure, was distilled and 12 g. (31% yield) of ethyl benzoylacetate was collected at 160-170°/21 mm. The reported boiling point is 165-170/20 mm.

Nitrosation of ethyl benzoylacetate.-In 150 ml. ether was dissolved 20 g. (0.1 mole) of ethyl benzoylacetate, and nitrosation was carried out as previously described under the nitrosation of phenacyl cyanide. From the reaction product were obtained tan crystals of impure ethyl ∝-oximinobenzoylacetate, which on recrystallization from hot toluene, produced 18 g. (82%) of product melting at 116-117.5°. This value agreed with that reported by Dittrich (48).

Preparation of phenylserine ethyl ester hydrochloride.-In



a hydrogenation flask were placed 11 g. (0.05 mole) of ethyl ∝-oximinobenzoylacetate, 100 ml. of absolute alcohol containing 10 g. of dry hydrogen chloride and 3 g. of 10% palladium on charcoal catalyst. The flask was attached to the hydrogenator and shaken at a pressure of 50 pounds. In 20 minutes 3.3-L. (0.15 mole) of hydrogen was taken up. The catalyst was filtered

and the filtrate concentrated under reduced pressure until crystals appeared on the sides of the filtration flask. The solution was heated on a steam bath and then poured into an Erlenmeyer flask. The flask was cooled in an icebox over night and the resulting 9 g. (75%) of white crystals were filtered. Recrystallization from dilute alcohol produced a product melting at 161-3°. Dittrich (48) reported its melting point as 162-5°.

Preparation of ethyl p-methoxybenzoylacetate.-Following the procedure outlined above, 40 g. (0.3 mole) of anhydrous aluminum chloride was slowly added in small portions to 30 g. (0.2 mole) of ethyl malonyl chloride dissolved in 170 g. (1.6 mole) of anisole. A smaller amount of hydrogen chloride was evolved than in the preceding experiment. The mixture, after complete addition of the aluminum chloride, was heated forty-five minutes on an oil bath to complete the reaction. After cooling, the mixture was poured on crushed ice containing concentrated hydrochloric acid. After one hour's standing the mixture was not entirely homogeneous; it was, therefore, extracted with ether. The ethereal solution was washed with water, dilute hydrochloric acid and dried over anhydrous sodium sulphate. The ether extract, when evaporated to remove the ether, yielded 8.5 g. (25%) of ethyl p-methoxybenzoylacetate, distilling at 140-146°/4 mm. with some decomposition. The boiling range agreed with the values reported in the literature (65).

Preparation of ethyl 3,4-dimethoxybenzoylacetate.-Twenty-five grams (0.17 mole) of ethyl malonyl chloride, 120 g. (0.87 mole) of veratrole, and 34.5 g. (0.26 mole) of anhydrous aluminum chloride were allowed to react in a manner similar to that described above. At the completion of the aluminum chloride addition, the reaction flask was heated thirty minutes on an oil bath. On pouring into a mixture of crushed ice and concentrated hydrochloric acid, the excess veratrole solidified. Gentle heating on a steam bath produced a homogeneous mixture. From the reaction product, 8.2 g. (19.6%) ethyl 3,4-dimethoxybenzoylacetate, boiling at 150-165°/9 mm., was isolated. The boiling range was the same as that reported in the literature (66).

Attempted preparation of ethyl 3,4-dihydroxybenzoylacetate.-Following the procedure outlined above 80 g. (0.6 mole) of anhydrous aluminum chloride was added to 30 g. (0.2 mole) of ethyl malonyl chloride and 33 g. (0.3 mole) of catechol dissolved in 250 ml. of carbon disulfide. After distillation of the ether and carbon disulfide, 28 g. (62%) of a viscous yellow liquid that had a boiling point of 110-120°/4 mm. was obtained.

The product was then nitrosated, following the procedures previously described. For each mole of ester 1.4 mole of isopropyl nitrite was used. Dry hydrogen chloride was bubbled into the reaction flask for ten minutes before the nitrite was added. Four hundred ml. of ether was used as solvent. The

red color that formed on the first addition of isopropyl nitrite did not disappear even on standing. Total nitrite addition over a period of forty minutes produced no color change, indicating, most likely, that a reaction did not take place. Evaporation of the ether produced a liquid which was found to have the same boiling point as the starting material.

Preparation of α-bromo-β-hydroxy-β-phenylpropionic acid.- Following Riiber and Berner's method (67), 30 g. (0.2 mole) of cinnamic acid and 28 g. (0.2 mole) of potassium carbonate were dissolved in 400 ml. of water and the solution was cooled to 4° in an ice bath. To the stirred cold solution was added a mixture of 32 g. (0.2 mole) of bromine and 57 g. (0.55 mole) of sodium carbonate in 500 ml. of water. After addition was complete, the mixture was stirred one-half hour, carefully acidified with dilute hydrochloride acid and allowed to stand overnight. Unchanged cinnamic acid was then filtered off and the clear filtrate extracted with ether. The ether was dried over anhydrous sodium sulphate, filtered, and distilled over a steam bath. The solid residue remaining was recrystallized from chloroform, producing 24 g. (50%) of colorless crystals melting at 123-5°. The reported melting point is 124° (67).

Preparation of phenylisoserine.-To a stirred cold solution

$$C_6H_5CHOHCHBrCOOH \longrightarrow C_6H_5CH\text{-}CHCOOH \quad (O)$$

$$\xrightarrow{NH_3} C_6H_5CHNH_2CHOHCOOH$$

of 5 g. (0.02 mole) α-bromo-β-hydroxy-β-phenylpropionic acid in

100 ml. of alcohol was added 35 ml. of concentrated ammonium hydroxide over a period of thirty minutes. The solution was allowed to stand three days. To remove some of the excess ammonia, the mixture was allowed to stand for three more days in a desiccator over sulfuric acid. Acidification with sulfuric acid yielded 2.1 g. (60%) of a compound melting at 278-282°. The mother liquor was then evaporated. The residue after recrystallization from alcohol yielded 0.6 g. (17%) of crystals melting at 230-2°. These two values checked with the two isoserines isolated by Fourneau and Billeter (68). They reported that ammonia and aliphatic amines react with β-phenylglycidic acid to give derivatives of phenylisoserine. With aromatic amines, derivatives of phenylserine were obtained. Erlenmeyer's fundamental phenylserine experiments (40,41) fall in line with the above hypothesis.

What probably occurred in the reaction of α-bromo-β-hydroxy-β-phenylpropionic acid with ammonium hydroxide was: first, t he formation of glycidic acid, second, addition of the amino group across the epoxide intermediate, to give the phenylisoserine isomers.

Preparation of the dipotassium salt of nitroacetic acid.- This was prepared by a modification of Steinkopf's method (69). In a 3-L. three-necked, round-bottomed flask provided with a condenser, stirrer and dropping funnel, was placed 1-L. (13.5 mole) of freshly prepared 50% potassium hydroxide. The flask was immersed in an ice bath and 200 g. (3.27 mole) of nitromethane was added dropwise so that the temperature of the

solution did not rise above 65°. After the one and one-half hours required for the addition of the nitromethane, the stirrer and condenser were removed and the reddish brown mixture was heated until crystals first appeared. After cooling, the potassium salt was filtered and washed with cold methanol. Concentration of the mother liquor yielded a second crop of crystals. A total of 195 g. (66%) of the dipotassium salt was obtained (69).

Preparation of ethyl nitroacetate.-A suspension of 60 g. (0.33 mole) of the dipotassium salt of nitroacetic acid and 5 g. of anhydrous potassium sulphate in 160 g. (3.5 mole) of absolute alcohol was cooled to -5° and saturated with dry hydrogen chloride. The reaction mixture was kept at 0° for six hours. The potassium sulphate was filtered off and the excess ethyl alcohol evaporated at reduced pressure. The oily residue was diluted with ether and neutralized with solid sodium carbonate. The mixture was filtered, dried over anhydrous potassium sulphate and the ether evaporated. Vacuum distillation of the residue yielded 11 g. (25%) of ethyl nitroacetate boiling at 93-5°/15 mm. (70).

Attempted condensation of ethyl nitroacetate with benzaldehyde.-Following Kamlet's (71) procedure for preparing nitroalkanols, 10.6 g. (0.1 mole) of benzaldehyde was vigorously agitated with a solution of 11.0 g. (0.15 mole) of sodium bisulfite in 100 ml. of water. Ethyl nitroacetate, 13.5 g. (0.1 mole) was dissolved in a solution of 4.5 g. of sodium

hydroxide in 300 ml. of water and added with vigorous stirring to the aldehyde-bisulfite addition product. The mixture was then heated to 100° on a steam bath, with occasional stirring, and allowed to cool to room temperature. The mixture, which had separated into two layers, was then placed overnight in the refrigerator. After acidification with dilute hydrochloric acid, the mixture was extracted with ether. On evaporation of the ether nothing but unreacted benzaldehyde could be isolated. Repeated experiments with slight modifications gave the same results.

Preparation of carbobenzyloxyglycine.-Following the method of Carter and coworkers (72), a 3-L. round-bottomed flask was fitted with a rubber stopper carrying an exit tube and a delivery tube which extended to the bottom. The tubes were equipped with stopcocks so that the reaction flask could be disconnected. In the flask was placed 500 g. of dry toluene and the apparatus weighed. The flask was cooled in an ice bath, and phosgene was bubbled into the toluene until 109 g. (1.1 mole) had been absorbed. The exit gasses were passed through a flask containing toluene to remove any escaping phosgene and then through a calcium chloride tube to a gas trap. After the absorption of phosgene was complete, the connection to the phosgene cylinder was replaced by a separatory funnel. The reaction mixture was shaken while 108 g. (1 mole) of freshly distilled benzyl alcohol was rapidly added through the separatory funnel. The flask was allowed to stand in the ice bath for one-half hour and at room

temperature for two hours. The solution was then concentrated under reduced pressure, at a temperature not exceeding 60° in order to remove hydrogen chloride, excess phosgene, and the major portion of the toluene. The residue weighed 210 g. and contained about 75% carbobenzyloxy chloride.

A solution of 7.5 g. (0.1 mole) of glycine in 50 ml. of 2 N sodium hydroxide was placed in a three-necked flask fitted with a mechanical stirrer and two dropping funnels. The flask was cooled in an ice bath, and 17 g. (0.1 mole) of carbobenzyloxy chloride (22 g. of the solution obtained above) and 25 ml. of 4 N sodium hydroxide were added simultaneously to the vigorously stirred solution over a period of twenty minutes. Stirring was continued for an additional ten minutes. The toluene layer was separated and the aqueous layer extracted with ether. The aqueous solution was cooled in an ice bath and acidified to Congo red with concentrated hydrochloric acid. The precipitate was filtered, washed with cold water and dried. By this means was obtained 18 g. (86%) of carbobenzyloxyglycine melting at 119-120°.

Preparation of carbobenzyloxyglycine ethyl ester.- In a glass-stoppered bottle was placed 21 g. (0.1 mole) of carbobenzyloxyglycine dissolved in 140 ml. (2.5 mole) of absolute ethyl alcohol. To the flask was added 10 g. of concentrated sulfuric acid. After standing two days in the icebox, the mixture became cloudy. An excess of water was then added and the

compound usually precipitated as an oil. However, crystallization was readily induced by cooling and scratching, and 19 g. (80%) of pure carbobenzyloxyglycine ethyl ester melting at 34.5-36° was obtained. The above ester prepared from carbobenzyloxy chloride was reported by Barkdall and Ross (74) to melt at 35.5-36.5°.

Preparation of phenylserine ethyl ester hydrochloride.-

$$2\ C_6H_5CHO + C_6H_5CH_2OCONHCH_2COOC_2H_5 \xrightarrow{Na}$$

$$C_6H_5CHONaCH(NHOCOCH_2C_6H_5)\text{-}COOC_2H_5 \xrightarrow[HCl]{H_2} C_6H_5CHOHCH(NH_2.HCl)COOC_2H_5$$

In a 500 cc. round-bottomed flask were placed 8.5 g. (0.08 mole) of benzaldehyde, 9.5 g. (0.04 mole) of carbobenzyloxyglycine ethyl ester, 2 g. (0.045 mole) of sodium wire and 75 ml. of dry ether. The sodium wire was soon coated with a yellow layer from which it was freed by vigorous shaking. The sodium disappeared after standing twenty-four hours. The solution was filtered and the resulting solid washed with dry ether. The crystals were dissolved in a minimum of water and acidified with dilute acetic acid. Upon evaporation of the solvent under reduced pressure, a viscous oil was obtained which solidified to a gel in the icebox. This was treated with 50 ml. of absolute alcohol containing 3 g. of dry hydrogen chloride and 2 g. of a 10% palladium on charcoal catalyst. On hydrogenation 250 ml. of hydrogen was absorbed. The catalyst was filtered and the solution concentrated to 35 ml. under reduced pressure.

Addition of dry ether produced 4 g. (41%) of phenylserine ethyl ester hydrochloride melting at 157-161°. The melting point was in agreement with those already reported for phenylserine ethyl ester hydrochloride (48, 58).

Analysis. Calculated for $C_{11}H_{15}O_3N.HCl$: N, 5.70. Found: N, 5.78, 5.83.

Preparation of N-carbobenzyloxyphenylserine.-In a 500 ml. round-bottomed flask were placed 8.5 g. (0.08 mole) of benzaldehyde, 8.4 g. (0.04 mole) of carbobenzyloxyglycine, 2 g. (0.45 mole) of sodium wire and 75 ml. of dry ether, and reaction took place as previously described. Isolation of the product by the above procedure produced 8 g. of the sodium salt of N-carbobenzyloxyphenylserine. The crystals were dissolved in a minimum of water and acidified with dilute acetic acid. Evaporation of the solvent produced an oil which, on addition of dry ether and standing in the icebox, solidified. It melted at 83-85°.

Analysis. Calculated for $C_{17}H_{17}O_5N$: N, 4.44. Found: N, 4.53.

Preparation of phenylserine.-One gram (0.003 mole) of N-carbobenzyloxyphenylserine was dissolved in 75 ml. of alcohol containing 7 g. of dry hydrogen chloride, and 2 g. of a 10% palladium catalyst. About 100 cc. of hydrogen (a slight excess of the theoretical amount) was absorbed in fifteen minutes. The catalyst was filtered and the filtrate concentrated under reduced pressure. Addition of an excess of dry ether produced 0.4 g. (57%) of crystals melting at 148-150°. Carrara and coworkers (75) reported the melting point of phenylserine hydrochloride to be 157°.

# SUMMARY

In an attempt to prepare aryl-α-hydroximino-β-keto acids, which on hydrogenation would yield the corresponding arylserine, phenylglyoxylohydroxamyl cyanide, p-phenylphenylglyoxylohydroxamyl cyanide, and 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide were hydrolyzed with sodium hydroxide. Benzoic acid, p-biphenylcarboxylic acid, and ω-hydroximinoacetylcatechol were formed, indicating that either complete side chain oxidation or decarboxylation took place.

Reduction of phenylglyoxylohydroxamyl cyanide, p-phenylphenylglyoxylohydroxamyl cyanide and 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide gave good yields of the corresponding diaminoalcohol.

The synthesis of β-keto esters, by treating substituted aromatic hydrocarbons with ethyl malonyl chloride and aluminum chloride, was studied. Ethyl benzoylacetate, ethyl p-methoxybenzoylacetate, and ethyl 3,4-dimethoxybenzoylacetate were prepared. However, attempts to prepare ethyl 3,4-dihydroxybenzoylacetate were unsuccessful.

Ethyl benzoylacetate, on nitrosation and reduction, gave good yields of phenylserine ethyl ester.

Attempts to condense ethyl nitroacetate with benzaldehyde were not successful.

Condensation of carbobenzyloxyglycine ethyl ester and carbobenzyloxyglycine with benzaldehyde, followed by hydrogenolysis, gave good yields of phenylserine ethyl ester and phenylserine respectively.

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