THE SYNTHESIS OF α-AMINO-β-HYDROXY ACIDS

By

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of the University of Maryland in partial fulfillment
of the requirements for the degree
of Doctor of Philosophy

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To Mr. Edward Scally, who so kindly prepared the draft of the chart.

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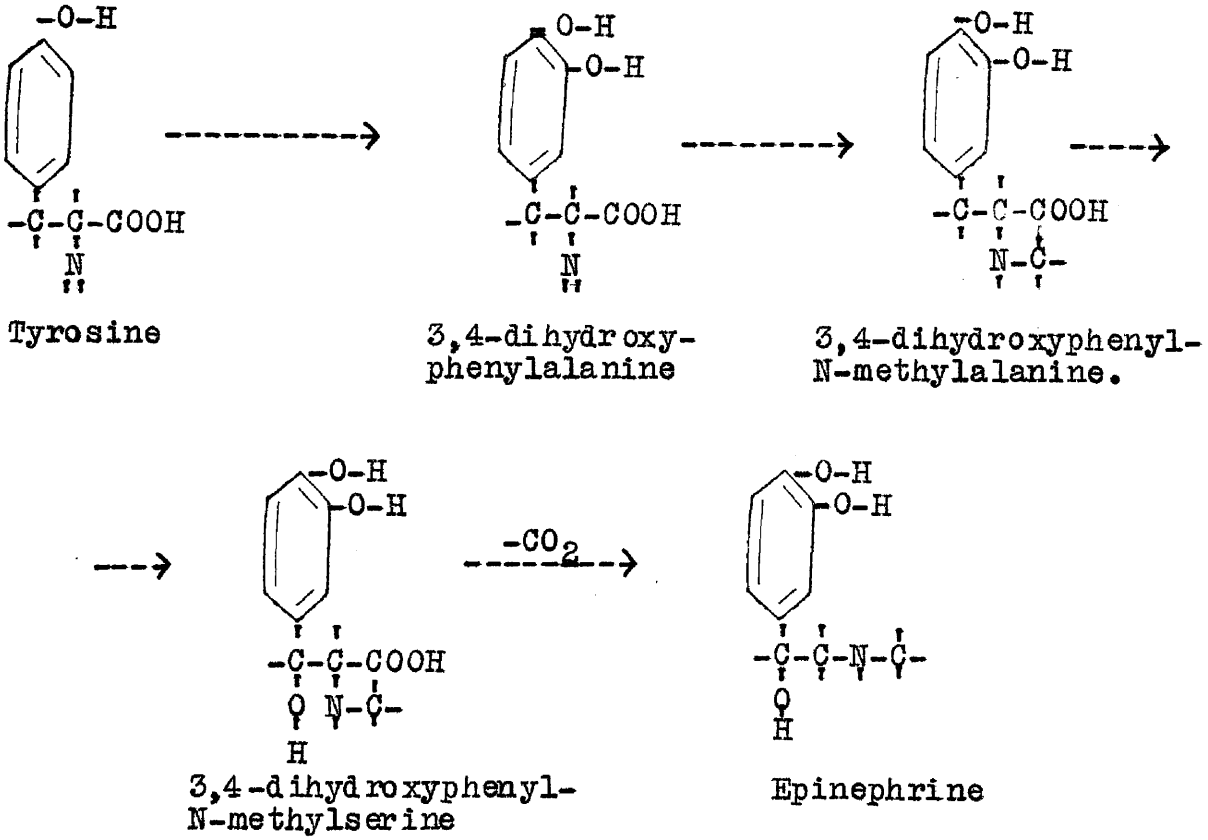
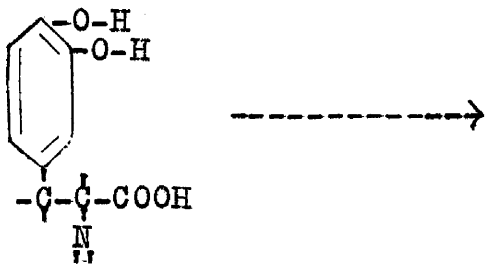
INTRODUCTION

General

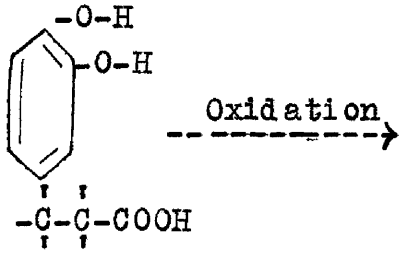
Any study of the nuclear-substituted-phenylserines would be incomplete without reference to their important position between the amino acids normally occurring in the body and epinephrine. Especially is this true of the 3,4-dihydroxyphenyl-substituted-acid, which has long been suspected of being a precursor of the adrenal medulla hormone. Among the theories advanced for the conversion of amino acids to this important product, is the one below which is representative of those presented. (65)



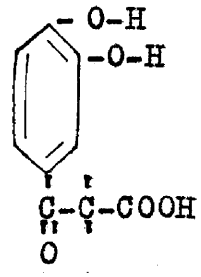
Also the theory of Rosenmund and Dornsaft (26) outlined below, for the biological formation of epinephrine postulates the formation of β -(3,4-dihydroxyphenyl) serine as an intermediate which is obtained from 3,4-dihydroxyphenylalanine as a starting material.



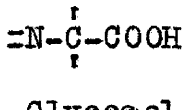
3,4-dihydroxyphenylalanine



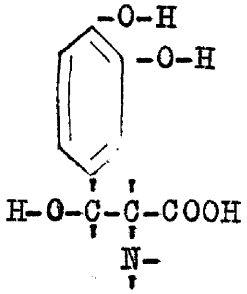
3,4-dihydroxyphenyl-propionic acid.



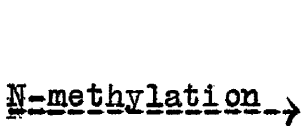
3,4-dihydroxy-benzoylformic acid

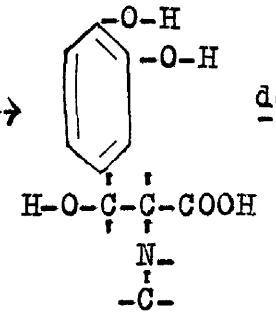


Glycocol

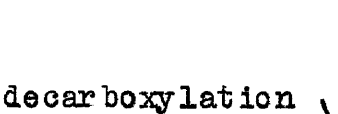


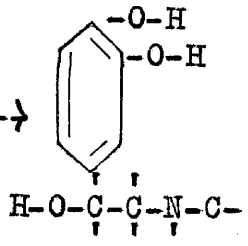
3,4-dihydroxy-phenylserine





N-methyl-3,4-dihydroxy phenylserine



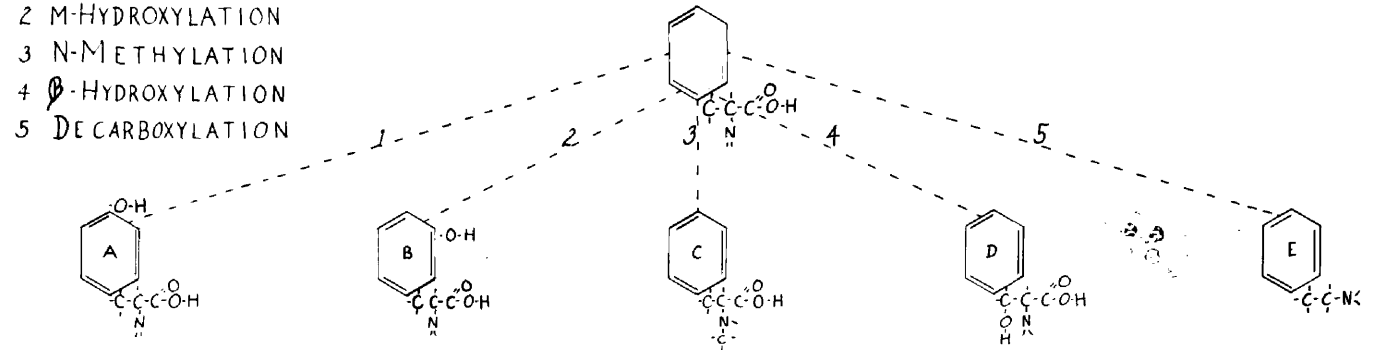


Epinephrine

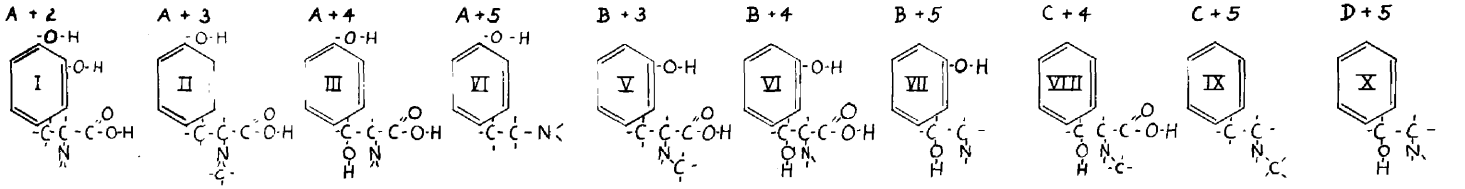
A clearer conception of the relationship and all possible hypothetical intermediates may be obtained from the chart on the next page. In this diagram phenylalanine is used as the starting material, and each of the five steps necessary for its conversion to epinephrine, is applied successively. In this manner it is possible to show not only all the possible routes, even though not all are biologically probable, but also every possible structural intermediate.

1 P-HYDROXYLATION
2 M-HYDROXYLATION
3 N-METHYLATION
4 β-HYDROXYLATION
5 DECARBOXYLATION

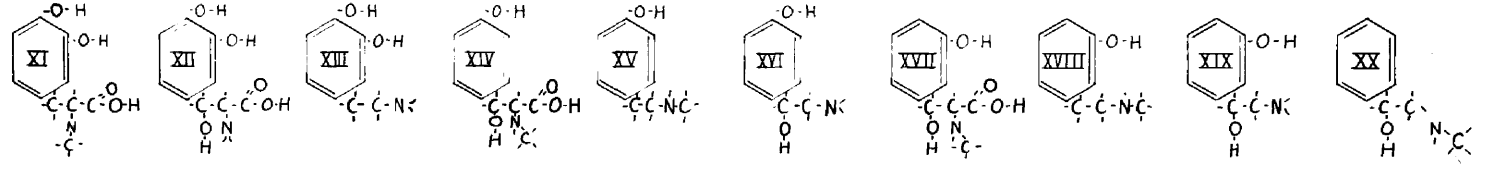
PHENYLALANINE



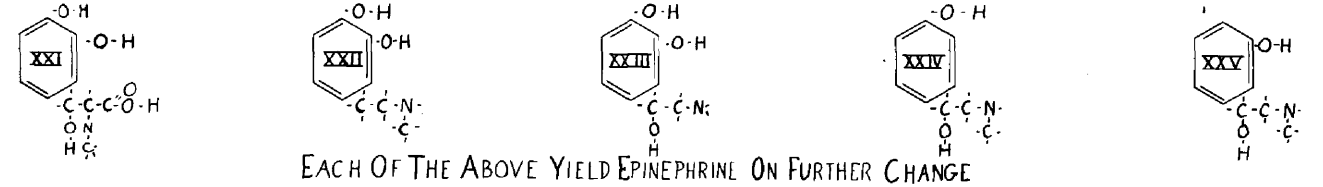
A + 2 A + 3 A + 4 A + 5 B + 3 B + 4 B + 5 C + 4 C + 5 D + 5



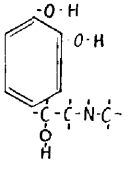
A + 2 + 3 A + 2 + 4 A + 2 + 5 A + 3 + 4 A + 3 + 5 A + 4 + 5 B + 3 + 4 B + 3 + 5 B + 4 + 5 C + 4 + 5



A + 2 + 3 + 4 A + 2 + 3 + 5 A + 2 + 4 + 5 A + 3 + 4 + 5 B + 3 + 4 + 5



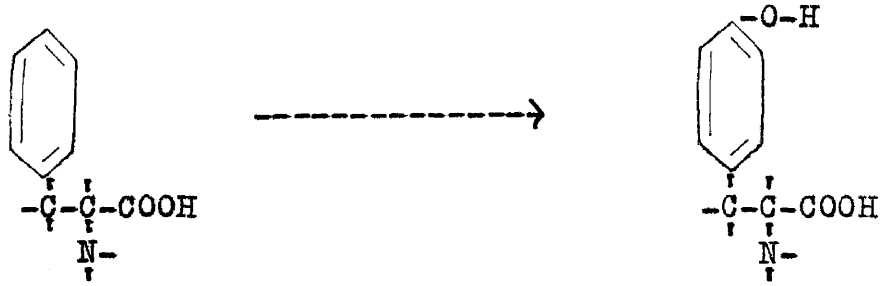
EACH OF THE ABOVE YIELD EPINEPHRINE ON FURTHER CHANGE



As shown in the preceding chart, there are five steps necessary, viz. (1) - p-hydroxylation, (2) - m-hydroxylation, (3) - N-methylation, (4) - β -hydroxylation, (5) - decarboxylation. These are numbered for simplicity. In the succeeding paragraphs references from the literature show that each step not only can, but does take place in the human body, although not necessarily on phenylalanine.

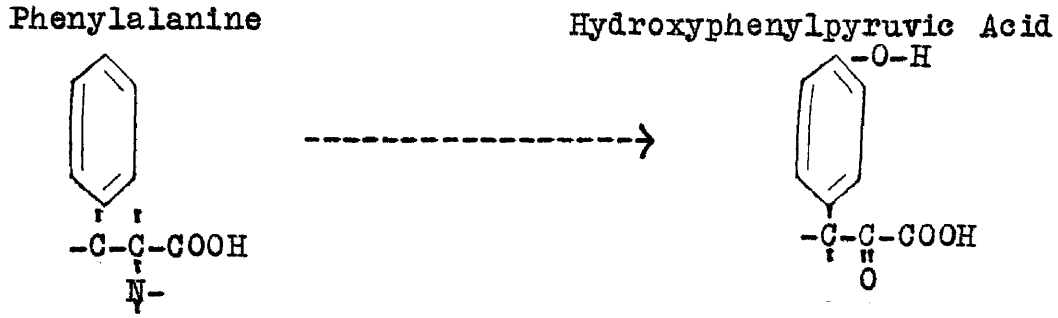
One must bear in mind one thing: in many of the examples given, after the reaction cited has taken place, the compound may be destroyed in the body and eliminated. It is not felt that this prevents the use of these examples as the body shows at many times a remarkable ability to select and control reactions which are to its advantage. Also the fact that certain of the intermediates have never been isolated, should not be taken as proof that the reaction does not occur. These hormones occur in such great dilution in the body that one could hardly expect to isolate the intermediates.

Proof of p-hydroxylation (Step #1): This was shown by Embden and Baldes (1). Their experimental work showed that surviving liver tissues perfused with dl-phenylalanine lead to an estimable increase in the tyrosine content of the liver mash. No demonstrable increase could be detected in the controls.



Phenylalanine Tyrosine

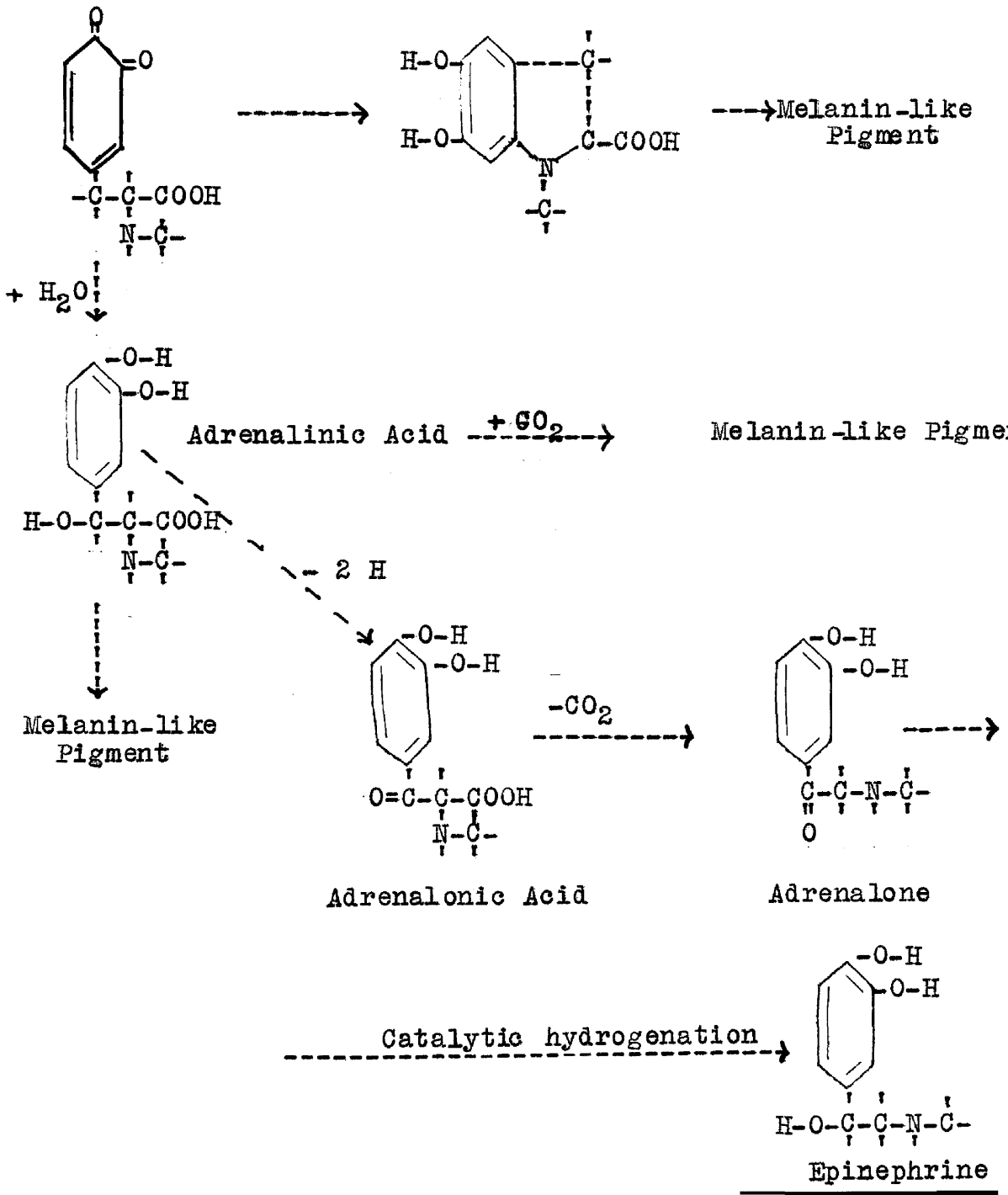
The work of Kotake, Masai and Mori (2) showed the hydroxyphenyl-pyruvic acid could be isolated from the urine of rabbits receiving phenylalanine.



For many years it was believed that tyrosine was a necessary dietary factor. However, R. S. Alcock (12) showed that animals may exist and develop normally on a diet free from tyrosine. Thus, if tyrosine is an essential dietary factor, it may be biologically derived from phenylalanine. This is in line with the general concensus of opinions of biochemists. Further evidence is seen in the work of J. Devine (21) which is cited later.

Proof of m-hydroxylation (Step #2): This step has never been shown to take place in the body until after the para position has been oxidized. After this, however, step #2 takes place rather readily and much in-vitro work has been done to demonstrate it. Raper and Haphold (3) showed that tyrosinase was able to oxidize tyrosine without deamination. Robinson and Onslow (4) suggested that the first step was the formation of 3,4-dihydroxyphenylalanine, often referred to in literature as dopa. This was later substantiated by Raper (5, 10) who isolated the compound from tyrosine-tyrosinase mixtures and studied the effects of various conditions upon the yield.

Subsequent work of Heard and Raper (11) showed that, by using N-methyl-tyrosine in place of tyrosine, the speed of melanin information could be reduced, with a corresponding increase in the yield of pressor substances. The pressor effect of these compounds were greatly increased by catalytic reduction. The first compound formed was believed to be adrenalone. The diagram below shows the proposed direction of these reactions.

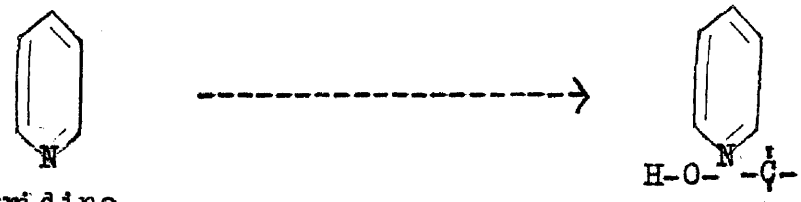


The presence of 3,4-dihydroxyphenylalanine in nature has been demonstrated by Guggenheim (6) in work on the pod of the Broad Bean, and by Miller (7) in work on the Georgia Velvet Beam. Tyrosinase, the enzyme responsible for m-hydroxylation, has been shown to be present in the meal worm by Biederman and identified by Raper and Speakman (8). Miss Dunham (9) later obtained evidence of its presence in the skin of rabbits and guinea pigs. Thus there is evidence of the oxidation of phenylalanine through tyrosine to 3,4-dihydroxyphenylalanine. In the discussion up to this point the proof of the second step has been based on the in-vitro action of tyrosinase, a substance whose presence has not been conclusively demonstrated in higher animals. This objection is overcome by the work of Raper (12) and Medes (14) who proved that tyrosine may be oxidized by iron and hydrogen peroxide to the 3,4-dihydroxy compound. Further proof of this oxidation is cited in the discussion of experiments on animal tissue.

Proof of β-Hydroxylation (Step #3): This is the most difficult of all to demonstrate in vivo. The reason for this is easily understandable, when one considers the ease with thish β-hydroxy compounds of this type are further oxidized to yield benzoic acid and its derivatives, which are eliminated as hippuric acid. From the work of Raper and Heard (11), some conception of such oxidation may be gotten, along with the importance of the carboxy group. As stated before, N-methyl tyrosine with tyrosinase in-vitro gave a greater yield of pressor substances than tyrosine itself. Attempts were then made to substitute an N-dimethyl compound

in its place. Hordenine was the material chosen. Surprisingly, however, no oxidation of the beta position took place. This seems to indicate the importance of the carboxy group for oxidation of the beta position. It may also explain the importance of the corresponding beta keto and hydroxy acids, and the lack of physiological activity of some suspected presursors of epinephrine in the body (11).

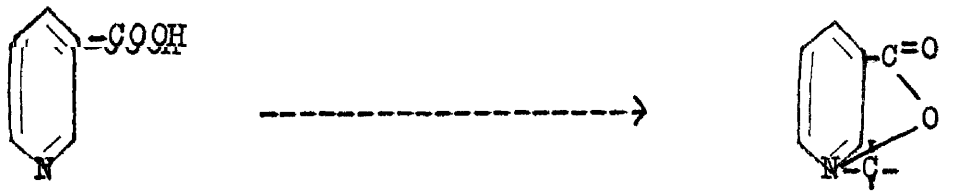
Proof of N-Methylation (Step #4): The first factor in this case is the possibility that the body obtains proteins which already possess an N-methyl group. Whether or not this occurs is not important as it is readily demonstrated that the body is capable of performing N-methylation. For example, both creatine and creatinine are N-methyl compounds occurring freely in nature. On the other hand W. His (15) showed that when pyridine was fed to dogs it was excreted as N-methyl-pyridinium hydroxide in the urine.



Pyridine

N-methyl-pyridinium hydroxide

Ackerman showed that dogs fed on nicotinic acid excreted trigonellin in the urine.



Nicotinic Acid

Trigonellin

These few examples are strongly suggestive that the animal body possesses the ability to perform N-methylation when such methylation is either advantageous or necessary. It may be well to refer again here the work of Raper and Heard (11) who showed that the use of N-methyl-tyrosine reduced side reactions which tended to cut down the yield of pressor substances. It is entirely possible that the N-methyl group in epinephrine tends to serve the same purpose in the body.

Proof of Decarboxylation (Step #5): Chemical literature abounds with proof that this takes place in the body. It seems hardly necessary to attempt to cite the references on this point. One needs only to open any volume on the study of aminoacids to convince himself easily of this. However, it appears sufficient at this point to cite some of the original work on this subject, and then follow it with the summation by Bing and Zucker (19) which appears to be rather conclusive. Neuberg and Gottschalk (17) showed that liver and muscle mascerates possessed a considerable capacity for generating acetaldehyde and that the addition of pyruvic acid increased the aldehyde content from 90 to 160%. This can only be brought about by decarboxylation. From this rather inauspicious beginning the efforts along this line continued with ever increasing proof not only of the body's ability to decarboxylate acids, but the very specificity of the enzymes involved, as summed up by Bing and Zucker (19): "There exist in the kidney numerous metabolic enzymes or specialized catalyst acting directly on the amino acids taking from each its car-

boxyl group. These carboxylases thus transform the acids to the corresponding amines. The action of these decarboxylating enzymes is readily demonstrated in aqueous solutions of the renal cortex. The resulting amines may be isolated chemically or titrated by means of their pressor effect on arterial blood pressure".

Thus far each individual step has been discussed in the conversion of phenylalanine to epinephrine. Examples have been cited showing how each step could possibly take place. Now experimental work will be cited showing that one or several of these steps can and probably do take place simultaneously in the organs of the body. The work of J. Devine (21), with slices of adrenal medulla, demonstrates this very well. He found that when phenylalanine, phenylethylamine, or tyramine was incubated with adrenal medulla there was formed an adrenalin-like substance as shown by both the colorometric and arterial pressure methods. These in-vitro experiments have shown the presence of a catechol-like substance, called by him catechol X. This substance, while not identified chemically, was capable of being converted by the tissue to epinephrine in yields of 30 to 40% of the original amounts present.

Some more recent and still more convincing work was done along this line by Bing (18) whose in vivo and in vitro work showed an increase in pressor substances by materials common to the animal body. Renal cortex was incubated with dopa (3,4-dihydroxyphenl-alanine) under aerobic and anaerobic conditions. These extracts were then injected into animals.

The ones obtained under aerobic conditions produced depression of arterial pressure, while those obtained under anaerobic conditions produced an increase in blood pressure comparable to a corresponding amount of epinephrine. It was suggested by the author that these results were due to hydroxy-tyramine. On repetition of the work using the whole kidney, similar results were obtained suggesting that the whole kidney was involved.

Like results were obtained in vivo by Bing and Zucker (19) who injected dopa (3,4-dihydroxyphenylalanine) into the kidney parenchymas of cats. In his preliminary work clamps were placed over the renal pedicle stopping completely renal circulation. Ringer's solution containing 10 mgms. of dopa was then injected into the renal capsule. Two and one half hours later the clamps were removed restoring renal circulation. In all cases the removal of the clamps was followed by a marked increase in the arterial pressure. The average was 68 mm of mercury and the maximum was 115 mm. When the same amount of dopa was injected into kidneys under normal conditions no subsequent rise was noted. Similar experiments performed on cats with renal circulation reduced 50% yielded intermediary amines which were not destroyed by the 50% ischemic kidney (19). These pressor substances were presumed to be hydroxytryamine. This conclusion is borne out by the observations of Mason and his coworkers who found that in the ischemic kidney the ammonia production was reduced. This indicated that decarboxylation rather than deamination was brought about by anaerobic conditions.

Thus far attempts have been made to show how the body could

convert related substances to epinephrine. Practically all experiments have shown that under the conditions of the experiments certain organs were able to convert the closely related phenylalanine to epinephrine-like substances. It seemed significant that in nearly all cases it was phenylalanine which gave the best results. Another example of this is the work of R. G. Smith and C. W. Edmunds (22). They reduced the epinephrine content of the glands to 15% by the daily injection of physostigmine. The report does not describe how this diminished content was determined. They then injected tyrosine and 3,4-dihydroxyphenylalanine. Although the former did not speed up the return to normal, they suggest that the latter may have accelerated this rate.

It may be well at this point to review the compounds listed in the chart, to point out those which have been prepared and to indicate briefly their medicinal or biological importance.

Chemical names and corresponding numbers

of compounds appearing in the preceeding chart.

1			- Phenylalanine.
2	-	(A)	- Tyrosine,
3	-	(B)	- m-Hydroxyphenylalanine.
4	-	(C)	- N-Methyl-Phenylalanine.
5	-	(D)	- Phenylserine.
6	-	(E)	- Phenylethylamine.
7	-	(I)	- 3,4-Dihydroxyphenylalanine.
8	-	(II)	- Surinamin.
9	-	(III)	- p-Hydroxyphenylserine.
10	-	(IV)	- Tyramine.
11	-	(V)	- N-Methyl-m-hydroxyphenylalanine.
12	-	(VI)	- m-Hydroxyphenylserine.
13	-	(VII)	- m-Hydroxyphenylethanolamine.
14	-	(VIII)	- N-methyl-phenylserine.
15	-	(IX)	- N-methyl-phenylethylamine
16	-	(X)	- Phenylethanolamine.
17	-	(XI)	- N-Methyl-3,4-dihydroxyphenylalanine.
18	-	(XII)	- 3,4-Dihydroxyphenylserine.
19	-	(XIII)	- 3,4-Dihydroxyphenylethanolamine.
20	-	(XIV)	- N-Methyl-p-hydroxyphenylserine.
21	-	(XV)	- N-Methyl-p-hydroxyphenylethylamine.
22	-	(XVI)	- p-Hydroxyphenylethanolamine.
23	-	(XVII)	- N-Methyl-m-hydroxyphenylserine.
24	-	(XVIII)	- N-Methyl-m-hydroxyphenylethylamine.
25	-	(XIX)	- m-Hydroxyphenylethanolamine.
26	-	(XX)	- N-Methyl-phenylethanolamine.
27	-	(XXI)	- N-Methyl-3,4-dihydroxyphenylserine.
28	-	(XXII)	- Epinine.
29	-	(XXIII)	- Nor-epinephrine.
30	-	(XXIV)	- Synephrin.
31	-	(XXV)	- Neo-synephrin.
32	-		- Epinephrine.

A brief review of the amino acids appearing in the chart include the following: (No. 1) phenylalanine, the proposed starting point in the biological synthesis of epinephrine, was discovered by Schulze and Barbier (59), and is one of the commonly occurring amino acids in proteins. (No. 2) tyrosine, discovered by Leibig (60) in 1884, is also one of the normal protein acids and as already mentioned, is probably the precursor of (No. 7) 3,4-dihydroxyphenylalanine (Dopa) which is important in the formation of melanin. Of the eight N-methyl acids (Nos. 4, 8, 11, 14, 17, 20, 23, 27) N-methyltyrosine (No. 8) are reported, but nothing is given of their biological activity. No report of the other six has been found in the available literature; nor has any reference been found to either of the m-hydroxy acids (Nos. 3 and 12).

On the other hand among the amines the following: Nos. 6, 10, 13, 15, 16, 19, 22, 23, 24, 25 and 26 have been studied and the relationship of their structure to their pharmacological activity has been discussed by Barger and Dale (62), Hartung (61) and Tainter (63), and others. The most active of the group, Epinine (No. 28), Nor-epinephrine (No. 29), Synephrine (No. 30), Neo-synephrine (No. 31) are commercially available and clinically used. Epinephrine (No. 32) is the hormone of the adrenal medulla.

A cursory glance brings out one significant fact; viz. that much work has been done on the substituted ethanolamines, while very little has been accomplished on the closely related amino acids or their ethyl esters. This naturally brings up the question why. There are two chief reasons which suggest them-

selves; first, the structural relationship of these phenylserine derivatives to the pressor amines has apparently never been adequately emphasized. Second, the difficulty in obtaining both the intermediates and their end products.

The present investigation was initiated in order to permit a more careful study of the acids which are structurally intermediate between pressor compounds derived from phenylethanolamine, $C_6H_5CHOH:CH_2.NH_2$ and the corresponding derivatives of phenylserine, $C_6H_5.\underset{NH_2}{C}HOH:CH.COOH$. For this purpose it was first necessary to develop a method for their synthesis. Further interest may be attached to these B-hydroxy-A-amino acids; these, or similar, compounds may occur naturally: e.g., in the composition of the insulin molecule, of 288 units, there are as yet six unidentified hydroxy-amino acids. While these are not known at the present time, there exists the possibility that one or more may be found in this group or at least may be capable of synthesis by similar methods.

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

The preparation of phenylserine and α-amino-β-keto-acids has been limited to direct condensation methods such as that described by Erlenmeyer (30) and Erlenmeyer and Frustuck (31). In his original work Erlenmeyer succeeded in condensing the sodium salt of the inner ether of benzoylacetic acid with ammonia according to the following scheme:

$$C_6H_5CH\underset{O}{-}CHCOOEt + NH_3 \longrightarrow C_6H_5CHOHCNH_2COOEt$$

He also used the ethyl ester of glycine in this condensation. Erlenmeyer himself admitted the limitations of these methods. Later Rosenmund and Dornsaft (26) repeated these experiments using the ethyl ester of glycine. According to these workers the reaction went as follows:

$$Ph.CHO + H_2N.CH_2COOR \longrightarrow Ph.CH{:}N.CH_2COOR + H_2O$$

$$Ph.CHO + Ph.CH{:}N.CH_2COOR \longrightarrow Ph.CH(OH)\underset{\displaystyle N:CH.Ph.}{CH}.COOR$$

$$Ph.CH(OH).\underset{\displaystyle N:CH.Ph}{CH}.COOH + H_2O \longrightarrow Ph.CH(OH).CH(NH_2).COOH + Ph.CHO.$$

They succeeded in preparing not only phenylserine but also the important 3,4-dihydroxyphenylserine. Here again appeared the same limitations described by Erlenmeyer: viz. that certain aldehydes as anisaldehyde would not take part in the condensation. No yields were stated and the reaction itself does not appear particularly promising.

This lead to a search for a method which would give good yields and would be more generally applicable. Theoretically, excellent intermediates for the α-amino-β-hydroxy-acids should be found in the α-isonitroso-β-keto-acids of the general formula R-CH(O)-C(=N-O-H)-COOH or their esters. Compounds of this type are known. Nitrosation of ethyl acetoacetate in acid solution with sodium nitrite (23) forms α-isonitroso-β-ketobutyric acid. While results obtained in this reaction are suitable, Pechmann's (24) method looked more promising. He nitrosated ethyl acetoacetate using amyl nitrite in alcoholic hydrogen chloride. This reaction successfully applied to glutaric acid by Randall and Harrington (25) and McIlwain and Richardson (43) yielding α-isonitroso-β-keto-glutaric acid. Hartung and Munch (27) had reported good yields in the nitrosation of acetophenones and propionones by using isopropyl nitrite and hydrogen chloride in ether. It was finally decided to attempt a combination of both these methods in the hope of increasing the yields. Experiments soon showed that these modifications were justified.

Before this method of synthesis could be attempted a good method of obtaining the β-keto ester was needed. One immediately turns to the work of Claisen (28) on the preparation of acetoacetic ester and to Claisen and Lowman (29) for the preparation of ethyl benzoylacetate. This proceeds according to the following general reaction:

$$C_6H_5C\begin{matrix}(OEt)_2\\ ONa\end{matrix} + CH_2COOEt \longrightarrow C_6H_5\underset{\underset{ONa}{|}}{C}:CHCOOEt + 2EtOH$$

$$C_6H_5\underset{\underset{ONa}{|}}{C}:CHCOOEt + HCl \longrightarrow C_6H_5\underset{\underset{O}{||}}{C}CHCOOEt + NaCl$$

Fischer and Bulow (32), L. Claisen (33) and James (34) prepared ethyl benzoylacetate using acetoacetic ester, sodium ethoxide and benzoyl chloride. All of the above methods are reported as giving comparatively good yields.

Other methods reported included the following: A. Michael and M. Browne (35) hydrolyzed α-brom-β-phenylacrylic acid in sulphuric acid and water to yield the ester.

$$C_6H_5\underset{\underset{Br}{|}}{C}:CHCOOEt + H_2O \xrightarrow{H_2SO_4} C_6H_5\underset{\underset{O}{||}}{C}-CH_2COOEt + HBr$$

L. Claisen (37) succeeded in preparing the ester from the benzoyl substituted mono-sodium mono-ethyl ester of malonic acid by hydrolysis with water.

$$NaOOC-\underset{\underset{C_6H_5C:O}{|}}{CH}-COOEt + H_2O \longrightarrow C_6H_5\underset{\underset{O}{||}}{C}-CH_2COOEt + Na_2CO_3 \quad CO_2$$

(38) by treating acetophenone in the presence of sodium ethoxide with diethylcarbonate.

$$C_6H_5\underset{\underset{O}{||}}{C}-CH_3 + EtO\underset{\underset{O}{||}}{C}OEt \xrightarrow{NaOEt} C_6H_5\underset{\underset{O}{||}}{C}-CH_2COOEt + EtOH$$

Buchner and Curtius (39) accomplished the same end by treating benzaldehyde with diazo-ethyl acetate in ether.

$$C_6H_5CHO + N_2CH\text{-}COOEt \longrightarrow C_6H_5\underset{\underset{O}{\|}}{C}\text{-}CH_2COOEt + N_2$$

Margurey (40) treated the mono-clor-mono-ethyl ester of malonic acid in the presence of benzene with anhydrous aluminum chloride.

$$C_6H_6 + ClOCCH_2COOEt \xrightarrow{AlCl_3} C_6H_5\underset{\underset{O}{\|}}{C}CH_2COOEt + HCl$$

R. Myer, Togel (41) reacted benzoyl bromide with monobrom ethyl acetate in the presence of magnesium to obtain ethyl benzoylacetate. A modification of the Grignard Synthesis. Thus there were available numerous methods for the preparation of ethyl benzoylacetate. Of these the Claisen Condensation appeared to promise the best results. A method based on this reaction was obtained from the Eastman Kodak Co. (36). This was the one used in the experimental work described later.

Methods had been selected thus far for the preparation of the ethyl esters of the keto acids and for their nitrosation. However, on reviewing the reduction of these β-keto-α-oximino-acids rather contradictory results were found. Randall and Harrington (25) reported the reduction of α-oximino-β-ketoglutaric acid to the corresponding hydroxy amino acid using palladinized charcoal and alcoholic HCl as the solvent. However, McIlwain and Richardson (43), using the same catalyst and solvent, could not get the reduction beyond the keto stage. When this α-amino-β-keto acid was isolated and again submitted to hydrogenation the amino acid was isolated. Adkins and Reeve (44) using Raney nickel on α-oximino-β-ketobutyric ester

could not obtain the hydroxy amino acid. However, by first converting the oximino to the ethyl ether and then submitting it to catalytic reduction with Raney nickel using alcoholic HCl as the solvent they were able to obtain the α-amino-β-hydroxy acid. Thus the outlook at this point was not too promising. After reference to the smooth reduction of the isonitroso acetophenones and propiophenones as reported by Hartung and Munch (27) it was decided to attempt the reduction in the same manner using Palladium chloride on Norite as the catalyst and absolute alcohol at least 2 N in hydrogen chloride as the solvent. This proved so successful that no further changes were needed in this step.

These results encouraged the belief that any intermediate of the structure $Ar.CO.\underset{\underset{NOH}{|}}{C}.COOEt$ may be similarly hydrogenated to the corresponding $Ar.CHOH.\underset{\underset{NH_2}{|}}{C}H.COOEt$. If this is true, then apparently, the availability of the β-keto esters of the type $Ar.CO.CH_2COOEt$. would prove the most difficult problem in this investigation.

The ethyl ester of benzoic acid is readily available for use in the Claisen Condensation, but the esters of nuclear-substituted benzoic acid are not easily obtainable. Therefore, it was decided to find first a method for synthesizing these ethyl nuclear-substituted-benzoylacetates. The procedure which, after careful consideration, seemed to merit investigation is the following:

$$Ar.CO.CH_2Cl \longrightarrow Ar.CO.CH_2.CN. \longrightarrow Ar.CO.CH_2-C\begin{matrix}=NH.HCl \\ -OEt.\end{matrix} \longrightarrow$$

$$Ar.CO.CH_2COOEt.$$

The Friedel-Craft synthesis provides good yields of alkyl, alkoxy, and halogen-nuclear-substituted derivatives of phenacyl chloride in both laboratory (45 - 46) and industry (47 - 48). Fries and Pfaffendorf (49) describe good methods for the preparation of o- and p-hydroxyphenacyl chloride. A method given in Organic Synthesis (50) and varied slightly to give better yields of 3,4-dihydroxyphenacyl chloride (42) was available. Therefore, no difficulty was anticipated in obtaining the starting materials needed for this reaction.

There are many methods of preparing nitriles, but from the phenacyl chloride there is only one of any value. This is the work of Gabreil and Eschenbach (51) and Obre'gia (52) which consists of treating the phenacyl halide with sodium cyanide under proper conditions (in a 50% alcohol solution and maintaining this at a temperature of 50° C. for about one half hour). The reaction proceeds according to the following equation:

$$Ar.CO.CH_2Cl + KCN \longrightarrow Ar.CO.CH_2CN.KCN + KCl.$$

The potassium salt of the cyanoketone remains in solution on dilution with water and the unreacted materials are separated by filtration. This clear solution is then permitted to stand with charcoal and filtered by suction. It is then rendered acid with dilute hydrochloric acid and the crystals of the nitrile which form are filtered off. These are washed with ice cold alcohol and dried. The authors report yields up

to 60% by this method. A few changes were introduced to facilitate obtaining a pure product; these are reported in the experimental portion.

The final step to be studied was the hydrolysis of the nitrile. Here there was a choice of two approaches; the nitrile could be hydrolyzed to the acid, or directly to the ester. Pinner (54) in 1883 had shown that nitriles can be hydrolyzed to the imino ether hydrochloride by treating it with alcoholic HCl. P. P. T. Sah (56) has shown that the imino ether could be changed to the ortho ester by further treatment in absolute alcohol and ether in the presence of hydrogen chloride. Finally Haller (55) has shown that benzoylaceto-nitrile can be hydrolyzed to the imino ether hydrochloride by treating it with alcoholic HCl and then to the ester by warming the salt with dilute hydrochloric acid. Other workers who have succeeded in the hydrolysis of the nitriles by this method were P. Pfeiffer and Matton (57) and P. Pfeiffer, Irma Engelhardt and Willy Alfuss (53). The last group of workers made a complete study of the effects of substitution on the phenyl ring, and cis-trans structure on the hydrolysis. They showed that if the ring was unsubstituted or if it was removed from the nitrile by one carbon atom the hydrolysis to the ester worked very well. Since the compounds to be hydrolyzed in this study were so constructed, it was justifiably believed that this hydrolysis would take place rather easily. Finally L. Speigel and H. Szydlowsky (58) obtained good results in the hydrolysis of the alkyl nitriles using alcohol and sulphuric acid. Thus

there seemed to be sufficient precedent to warrant the hope that the desired esters could be obtained directly.

Aim for research: This problem was undertaken with three purposes in mind, viz. first, to determine whether α-oximino-β-keto ester can be connected into the corresponding α-amino β-hydroxyacids or their esters; second, to develop a satisfactory procedure for the synthesis of β-keto acids or esters from which the α-oximino-β-keto esters may be conveniently obtained; third, to make available a series of nuclear substituted β-phenylserines for physiological or pharmacological examination.

Method of Accomplishing this end: The program as originally proposed, in substance, reads as follows: The ethyl ester of benzoylacetic acid will first be prepared by the Claisen reaction. This will then be nitrosated and reduced. The physical and chemical properties of the intermediates will be studied, and finally the ester will be hydrolyzed to phenylserine and its properties compared to those described by Erlenmeyer (30 - 31) and Rosenmund and Dornsaft (26) for that acid. If these experiments prove successful, the same compound will be prepared by hydrolysis of phenacyl cyanide and the intermediates compared to those above. If these warrant, attempts will then be made to prepare the nuclear-substituted-acids according to the reactions indicated.

EXPERIMENTAL

General

All temperatures recorded, unless otherwise specified, are uncorrected. Final temperatures reported, when corrected, are designated by "(cor.)" and were determined by Anschutz "stem-immersion" thermometers.

When "absolute alcohol" was used, it was prepared from commercial absolute alcohol (about 97% ethyl alcohol). Ten liters of this was refluxed about twenty-four hours with three pounds of fresh, unslaked lime, broken into small pieces. The alcohol was distilled off and the operations repeated, the product from the second distillation being used.

Analyses for nitrogen were carried out using the Kjeldahl-Gunning method.

Syntheses

Preparation of ethyl benzoylacetate. (According to Claisen's Method).

$$C_6H_5COOEt + 2Na + CH_3COOEt \longrightarrow C_6H_5C(O\text{-}Na):CHCOOEt + NaOEt + H_2$$

$$C_6H_5C(O\text{-}Na):CHCOOEt + HCl \longrightarrow C_6H_5C(=O).CH_2.COOEt + NaCl$$

- I -

In a 500 cc three-neck flask to which was attached a reflux condenser, and a mechanical stirrer, was placed 75 g. ($\frac{1}{2}$ mole) of ethyl benzoate (previously dried for 24 hours over anhydrous

calcium chloride and distilled at 197° C.) and 11 g. (1/8 mole) of absolute ethyl acetate (dried over anhydrous calcium chloride for 24 hours and redistilled, boiling at 77-79° C.) The solution was heated on a water bath and the temperature maintained between 75 and 80° C, and to it was added 11.5 g. (½ mole) of sodium metal cut in thin slices. This was stirred until almost all the sodium had reacted (about two hours). Then 5.5 g. (1/16 mole) more of ethyl acetate and 5.7 g. (¼ mole) of sodium were added and stirring continued for about two hours. After this a final 5.5 g. (1/16 mole) ethyl acetate and 5.7 g. (¼ mole) sodium were added and stirring continued for fifteen hours after all had reacted.

The reaction mixture was now cooled and poured through a wire gauze into a beaker containing cracked ice and 300 cc of concentrated hydrochloric acid. This was stirred until the reaction was complete, when a heavy oil separated out. The oily layer was separated and the aqueous layer extracted with two successive portions of 150 cc each of benzene. The benzene extracts were added to the oil and the whole neutralized with a solution of sodium bicarbonate. This was then dried with anhydrous sodium sulphate. It was then placed in a distilling flask and the benzene removed under the reduced pressure of a water pump; from the residue all distilling above 140° C. at 10 mm. pressure was collected. In this first run the yield was negligible due to decomposition on distillation.

- II -

In this case the ethyl benzoate used was made in the laboratory, by refluxing benzoyl chloride with ethyl alcohol until hydrogen chloride no longer came off. The product was then distilled and the fraction coming over at 197° C. used. This was dried over anhydrous calcium chloride and redistilled. Ethyl acetate was dried over anhydrous calcium chloride and that boiling 77-78° C. was used. The same quantities and method as in #1 were used except that after the final amount of sodium had been added the temperature was permitted to rise slowly and maintained at 95° C. for twelve hours. Then the same proceedure as outlined above was followed. In this case the yield was 61 Gms. or 63.5% of crude ethyl benzoylacetate calculated on the amount of ethyl benzoate used.

- III -

The same method and quantities were used as in I except that the temperature was permitted to rise above the melting point of sodium before any ethyl acetate was added. A violent reaction took place, which was permitted to continue and no ethyl acetate was added. After completion the product was treated as in I and II and distilled. The distillate was a solid and though it had a definite odor of benzaldehyde, the major portion proved to be benzoic acid melting point 121-122°.

Nitrosation of ethyl benzoylacetate. The reaction proceeds as follows:

$$C_6H_5\underset{\underset{O}{\|}}{C}CH_2COOEt + CH_3\overset{\overset{O.N:O}{|}}{C}HCH \xrightarrow{HCl} C_6H_5\underset{\underset{O}{\|}}{C}-\underset{\underset{N.OH}{|}}{C}-COOEt + CH_3CHOHCH_3$$

In a three-neck flask, fitted with a reflux condenser and a suitable hydrogen chloride trap was placed 50 g. (.26 mole) ethyl benzoylacetate. An inlet for hydrogen chloride, and a dropping funnel were attached. The flask was placed on a cold water bath to prevent overheating. Hydrogen chloride was passed in for about twenty minutes and then 22 g. ($\frac{1}{4}$ mole) of isopropyl nitrite was dropped in. The rapidity of addition was determined by the color and controlled so that the color never became darker than a light orange. The passage of hydrogen chloride was continued after the addition of the nitrite until the color returned to light yellow. This usually required twenty to thirty minutes. The mixture was then placed in a distilling flask and the alcohol removed under reduced pressure. The product was recrystalized from hot toluene. Yield was 48.9 g. or 85% melting point 117.5 to 118.5° C. (cor.)

When the nitrosation experiment was repeated and the conditions and proportions of reagents used, as described above, it gave a yield of 51 g. of isonitroso compound or 88.7% melting point 117.5 to 118.5° C. (cor.) Analysis for nitrogen gave these results:

$C_{11}H_{11}O_4N$. Calculated N, 6.33%: found N, 6.35% and 6.29%.

Preparation of phenacyl chloride. In a 1 liter, three-neck, round-bottom flask, fitted with a sealed mechanical stirrer, a separatory funnel, and a reflux condenser connected to a gas absorption trap, were placed 102 g. (0.77 mole) anhydrous alluminum chloride and 265 cc benzene. Then 53.1 cc (0.70 mole) chloracetyl chloride was allowed to drop in during the course of about one half hour. After all the chloracetyl chloride had been added the reaction mixture was refluxed on a water bath until evolution of hydrogen chloride had ceased. Then the mixture was allowed to cool and poured upon a mixture of equal parts of ice water and concentrated hydrochloric acid. The organic layer was then separated and washed with water, and dried with anhydrous calcium chloride. The excess benzene was removed by distillation on a water bath and the residue distilled under reduced pressure. The product distilling at 120-135° C. at 4 mm of pressure was redistilled. Yield 80.5 g. or 74%: melting point 57 to 58° C.

Preparation of phenacyl cyanide. The reaction proceeds as follows:

$$C_6H_5COCH_2Cl + NaCN \longrightarrow C_6H_5COCH_2CN + NaCl$$

Thirty grams (1/5 mole) of phenacyl chloride was dissolved in 90 cc of alcohol in a 500 cc beaker at a temperature of 40° C. In a separate 250 cc beaker 30 grams of sodium cyanide was dissolved in 90 cc water. This was added to the above solution and stirred until all dissolved. The temperature was then maintained at 50° C. for about a half hour. After

this cold water was added until no more precipitation was noticed. The crystals were allowed to settle and then filtered on a suction filter until clear. Then sufficient dilute hydrochloric acid was added to make the solution acid to litmus. The crystals which formed were permitted to settle and filtered off. The precipitate was dissolved in a minimum of water to which had been added an amount of sodium cyanide equal to the weight of the crystals. To this was added 1 g. of Norite and filtered on a suction filter and the filtrate made acid to litmus with dilute hydrochloric acid. The crystals were filtered off and dried. Weight 24.5 g. This was then taken up in boiling 60% alcohol 3 g. of Norite added and boiled for about fifteen minutes. This was filtered while hot and set in an ice chest and cooled for about two hours. The crystals were filtered off, washed with cold dilute alcohol and dried. Yield 17.5 g. or 61.8% Melting point 79° C.

Repetition of the experiment above, using the conditions and proportions of reagents described above gave yields of phenacyl cyanide, varying from 55 to 65 percent.

Recrystalization of the product from alcohol gave crystals melting 80° C. (uncor.)

Hydrolysis of phenacyl cyanide. The reaction proceeds as follows:

$$C_6H_5COCH_2CN + CH_3CH_2OH \text{ (absolute)} + HCl \longrightarrow$$

$$C_6H_5CC(=N-H\ HCl)CH_2C(-OEt)$$

$$C_6H_5COCH_2C\begin{matrix}=N\text{-}H\ HCl\\ -OEt\end{matrix} + H_2O \longrightarrow C_6H_5COCH_2COOEt + NH_4Cl$$

In a glass stoppered separatory funnel was placed 150 cc absolute alcohol saturated with hydrogen chloride. In this was dissolved 15 g. (.1 mole) phenacyl cyanide and the solution set aside for five days in a cool place. The crystals which formed were filtered off on a suction filter. These were redissolved in a minimum of water, 5 cc of dilute hydrochloric acid added, the solution brought to boiling and allowed to cool. A clear colorless liquid settled out. The alcohol, from the mother liquor of the original hydrolysis, was then evaporated, the resulting crystals dissolved in a minimum of water, 10 cc of dilute hydrochloric acid added and brought to boiling. In this case also a clear colorless liquid settled out. The two oily layers were combined and the two aqueous portions extracted with ether and the oils and ethereal extracts combined and dried with anhydrous sodium sulphate. The ether was removed by means of a current of air until the odor of ether was no longer observed. The residual liquid suspected of being crude ethyl benzoylacetate weighed 10 g. Yield of crude product 52 percent.

A repetition of the above hydrolysis was 25 g. of phenacyl cyanide gave 15.5 g. of product (suspected of being crude ethyl benzoylacetate) yield 47%.

Nitrosation of ethyl benzoylacetate obtained from the nitrile: 10 grams of the product obtained by hydrolysis of phenacyl cyanide was nitrosated as already described under nitrosation of ethyl benzoylacetate. From this was obtained 7 grams of product which on purification melted at 117.5 to 118.5° C. (cor.) Analysis: Nitrogen content calculated on the formula $C_{11}H_{11}NO_4$ is 6.33%: found N 6.33% and 6.31%.

This product mixed with the isonitroso compound obtained by nitrosation of ethyl benzoylacetate purchased from Eastman Kodak Company showed no significant lowering of the melting point. The mixture softened at 116° and was completely melted at 117° C. (uncor.)

This looked like satisfactory evidence that both these compounds were identical.

Reduction of α-isonitroso β-phenyl β-keto ethyl propionate: The catalyst was prepared .3 g. of palladium chloride and 3 g. of Norite were added to 100 cc of distilled water and shaken on a hydrogenator. This adsorbed 69 cc of hydrogen. This was filtered on a suction filter and dried.

In a 250 cc round-bottom flask were placed 10 g. (1/22 mole) α-isonitroso β-phenyl β-keto ethyl propionate, 100 cc of alcohol, 10 g. hydrogen chloride and the catalyst described above. This was attached to the hydrogenator and shaken until no further hydrogen was taken up (about two hours and twenty-five minutes). 3350 cc of hydrogen were absorbed. Theoretical amount was 3040 cc. After hydrogenation, the

above mixture was brought to boiling on a water bath and sufficient boiling water added to bring the ethyl ester of phenylserine into solution and the mixture was filtered on a suction filter while hot. This clear solution was set in an ice chest for about three hours. The resulting white crystals were filtered off and recrystallized from boiling dilute alcohol. Yield 8 g. 72%.

Repetition of the above reduction gave yields varying between 88.4 and 93.6%. Melting point 162° to 165° C. (cor.) Analysis: Nitrogen content calculated on the formula $C_{11}H_{16}NO_3Cl$ is 5.71%: found N, 5.71% and 5.63%.

This compound is soluble in water, not so soluble in alcohol. An aqueous solution is precipitated on the addition of ammonium hydroxyde. On standing, this mixture gives no acid insoluble precipitate, and shows no discoloration (no pyrazine formed thus showing the absence of a ketone).

Preparation of phenylserine from the hydrochloride of the ethyl ester: Ten grams (1/24 mole) of the ethyl ester was dissolved in a minimum of water. Sufficient 10% solution of sodium hydroxide was added to render it basic to litmus. This was then warmed about ten minutes, after all had dissolved. The solution was then neutralized with concentrated hydrochloric acid (concentrated acid was used to prevent too great increase in the volume of liquid). It was carefully brought to neutral point with ammonium hydroxide and an equal volume of alcohol added. This was then cooled in an

ice chest for about one hour and the white crystals of phenylserine filtered off and washed with 95% alcohol. These were then washed with ether and dried over sulphuric acid for 24 hours. Melting point 188° to 192° C with decomposition. However, they were exposed for about an hour before the next melting point was run. This showed 184° to 188° C. (probably due to absorption of moisture). This compound had been reported by Rosenmund and Dornsaft (26) as melting at 192° C. with decomposition.

The acid, when crystallized from alcohol and water, forms white, flocculent, soft crystals which are insoluble in alcohol and ether, soluble in dilute hydrochloric acid.

Preparation of the ethyl ester of phenylalanine from phenylalanine: To 25 cc of absolute alcohol in a two-neck flask fitted with a reflux condenser and a hydrogen chloride inlet was added 10 g. of phenylalanine and brought to boiling while passing in hydrogen chloride from a generator. From time to time more phenylalanine was added until no more would dissolve. This was then heated for about twenty five minutes, cooled and filtered. About double the volume of water was added (50 cc) and made neutral with ammonium hydroxide and set aside to cool. The ethyl ester settled out as an oily liquid. This was extracted with ether. The ethereal solution was dried with anhydrous sodium sulphate. Dry hydrogen chloride was passed into the ether solution and the ethyl ester precipitated as the hydrochloride. This was filtered off and washed with ether. They were recrystallized from boiling dilute alcohol and dried. Melting point 123 to

124° C. (cor.) The hydrochloride of the ethyl ester of phenylalanine melts at 124° C.

Preparation of p-chlorophenacyl chloride: In a liter, round-bottom, three-neck flask fitted with a sealed mechanical stirrer, a dropping funnel and a reflux condenser to which was attached a hydrogen chloride trap was placed 112 g. (1 mole) chlorbenzene and 400 cc of carbon disulphide. To this was added 145 g. (1.1 moles) of anhydrous aluminum chloride. Then 112 g. (1 mole) of chloracetyl chloride was slowly dropped in at a rate which did not cause too violent refluxing of the carbon disulphide. After the addition of the chloracetyl was completed the mixture was refluxed until no more evolution of hydrogen chloride was noted. The mixture was then cooled and poured on a mixture of equal parts of ice water and concentrated hydrochloric acid. The organic layer was separated and washed with water. This was then dried over anhydrous calcium chloride and the carbon disulphide distilled off under reduced pressure. The residue was taken up in boiling alcohol and recrystallized. This was again recrystallized from boiling alcohol. Yield 125 g. (66.8%) m.p. 100º C.

Preparation of p-chlorophenacyl cyanide: To 60 cc of alcohol at 55° C. was added 15 g. (.08 mole) p-chlor phenacyl chloride and the mixture stirred until all was dissolved. To this was added 15 g. sodium cyanide dissolved in 40 cc warm water. The mixture was stirred until all

was dissolved and placed on a water bath maintained at 55° C. for about 3/4 hour. To this was then added cold water until no more precipitation occurred. The mixture was then set aside and the solid allowed to settle out. This solid was not unchanged p-chlorophenacyl chloride. The mixture was then filtered on a suction filter until clear. Dilute hydrochloric acid was added to the filtrate until it was acid to litmus. Then it was set aside to settle. The resulting crystals were filtered off and dissolved in a minimum of water to which had been added an amount of sodium cyanide equal to the weight of the crystals. One gram of Norite was added and the mixture was filtered on a suction filter until clear. It was then rendered acid with dilute hydrochloric acid and set aside for about one hour. The crystals were removed by filteration and dried. They were purified by boiling in 70% alcohol with 3 g. of Norite and filtered until clear. The solution was cooled in an ice chest for about two hours and the crystals which formed were filtered and dried. Yield 9.5 g. 66.8%. Melting point 127-128° C.

Repetition of the experiment gave yield varying from 63 to 66%. Pure p-chlorophenacyl cyanide melts at 128°.

Hydrolysis of p-chlorophenacyl cyanide: In a glass stoppered separatory funnel was placed 100 cc absolute alcohol saturated with hydrogen chloride and 23 g. (.13 moles) of p-chlorophenacyl cyanide, and allowed to stand at room temperature for five days. The crystals which formed were filtered and dried. Yield 7 g. The mother liquors were

removed under reduced pressure. The residue and crystals were then taken up in 20 cc of dilute hydrochloric acid and warmed for about five minutes and then allowed to cool. An amber colored liquid settled out. This was separated and the aqueous layer extracted with two 25 cc portions of ether. The oil obtained above and the ethereal extracts were combined and dried over anhydrous sodium sulphate. The ether was removed by a stream of air and the residue placed in an ice chest for about one hour and filtered. The filtrate weighed 15 g. representing a yield of 50% of the crude ester.

The same quantities of all materials were used in a succeeding experiment and the mixture was refluxed slowly for about two hours while passing in hydrogen chloride. The remainder of the proceedure was the same. The yield in this case was 66.6% of the crude ester.

Nitrosation of the ethyl ester of p-chlorobenzoylacetate: The nitrosation of this ester was carried out after the manner already described for the nitrosation of ethyl benzoylacetate.

From the crude ethyl p-chlorobenzoylacetate, obtained from hydrolysis of the cyanide, yields of 57% and 62.5% were obtained. The product, after recrystallization from hot toluene melted at 135 to 136° C. (cor.) Analysis: Nitrogen content calculated for the formula $C_{11}H_{10}O_4NCl$ is 5.49%: found N, 5.45%.

Reduction of ethyl α-oximino-β-p-chlorophenyl-β-ketopropionate: The catalyst used was the same as that described previously in the reduction of α-isonitroso β-phenyl β-keto ethyl propionate. In a 250 cc round-bottom flask was placed 5 g. (1/51 mole) of ethyl α-oximino-β-p-chlorophenyl-β-ketopropionate, 50 cc of 95% alcohol, 5 g. of hydrogen chloride and the catalyst mentioned above. This was attached to a hydrogenator and shaken. 820 cc hydrogen were absorbed rapidly, then the absorption stopped completely, so 50 cc of alcohol and 25 cc of water were added and shaking continued. Absorption was still practically negligible so .1 g. of palladium chloride was added and hydrogenation resumed. When this did not produce results a jet of steam was directed at the flask while shaking. Absorption speeded up until 560 cc of hydrogen were taken up, after which absorption stopped completely. Total hydrogen taken up was 1380 cc. The theoretical amount of hydrogen needed for reduction to the amino alcohol is 1318 cc. The flask was then disconnected and the mixture brought to boiling and sufficient boiling water added to bring the compound into solution (very little was needed). The catalyst was filtered off from the hot solution on a suction filter. This clear liquid was placed in an ice chest for twenty-four hours and the crystals filtered off and dried. The supernatant liquid was then permitted to evaporate spontaneously in a vacuum descicator under reduced pressure and the residue was crystallized from a minimum of boiling alcohol. These crystals were added to

those obtained above and the whole recrystallized from boiling alcohol. They were washed with a little ice cold alcohol and dried over sulphuric acid in a descicator for 24 hours. Yield 4.2 g. 77%.

Repetition of the experiment using 7 g. (1/36 mole) ethyl α-oximino-β-keto-β-p-chlorophenylpropionate in 175 cc of alcohol and 20 g. of hydrogen chloride and the catalyst previously described absorbed 2080 cc of hydrogen. Theoretical amount of hydrogen needed for the reduction to the amino alcohol is 1848 cc. The yield was 6.3 g. or 82.5%. Melting point 168-170° C. (cor.). Analysis: Nitrogen content calculated for the formula $C_{11}H_{15}O_3NCl_2$ is 5.02%: Found N, 5.19% and 5.3%.

One half gram of the hydrochloride of ethyl β-p-chlorophenyl β-hydroxy α-amino propionate was treated with ammonium hydroxide filtered and the precipitate washed on the filter until the washings gave no test for chlorides with silver nitrate. The precipitate was then dried and subjected to sodium fusion. It gave a position test for chlorides.

One gram was dissolved in water and potassium permanganate added and boiled until no further reaction took place. This solution was extracted with ether. The ether layer was extracted with a solution of sodium hydroxide. This was acidified and the precipitate washed with cold water. A small portion was added to a solution of sodium bicarbonate. Effervescence (free acid). The remainder was dried in a vacuum descicator over night. Melting point 228 to 229° C. (p-chlorobenzoic acid). This melting point and the slightly high nitrogen content suggests a slight loss of chlorine in hydrogenation.

One half gram added to ammonium hydroxide gave no yellow color or acid insoluble precipitate. (no free ketone).

Preparation of 3,4-dihydroxyphenacyl chloride: In a 500 cc three-neck, round-bottom flask fitted with a sealed mechanical stirrer, a reflux condenser to which was attached a hydrogen chloride trap was placed 83.3 g. (0.4 mole) of phosphorus pentachloride. To this was added carefully in small portions 42.5 g. (0.45 mole) of monochloracetic acid. This was refluxed for two hours and permitted to stand over night. To this was added 200 cc of benzene and 44 g. (0.4 mole) of catechol. The mixture was refluxed for fifteen hours. The benzen was then recovered by distillation and the residue treated with 400 cc of boiling water. This was rapidly filtered on a suction flask and cooled. After cooling, the crystals were separated on a suction filter and washed with a minimum of cold water. The resulting nearly white crystals were then taken up in a minimum of boiling water and boiled with 5 g. Norite for fifteen minutes. This was filtered until clear and allowed to recrystallize in an ice chest over night. The crystals were filtered off on a suction filter and dried. Yield 35 g. (46%) m.p. decomposition to a reddish brown liquid at 170° C. (reported melting point was decomposition at 173° C. (25).

Repetition of the above experiment gave yields varying between 50.6% and 54%.

Preparation of 3,4-dihydroxyphenacyl cyanide: Difficulty was anticipated in the conversion of the 3,4-dihydroxyphenacyl chloride to the cyanide due to the ease with which the 3,4-dihydroxy compound is oxidized in alkaline medium. However, the conversion was attempted in the usual manner. As was expected only a dark tarry mass was obtained which gave very poor yield of dark brown crystals when boiled with Norite. This did not promise to be of any value.

It was then attempted to prevent oxidation by the addition of 1 g. of sodium sulphite to each 15 g. of 3,4-dihydroxyphenacyl chloride used. These experiments yielded brown crystals, which on boiling with Norite, yielded light yellow crystals which were recrystallized from boiling water and dried. When a small portion of the above crystals was dissolved in water and a solution of ferric chloride added, a deep green color developed indicating the presence of the free phenol groups. One half gram of the crystals were subjected to sodium fusion and checked with silver nitrate in acid solution. The precipitate proved to be silver sulphide and a strong odor of hydrogen sulphide was observed. This could not be accounted for, so the method was given up.

It was then decided to attempt to prevent oxidation by the addition of hydrogen. In a 250 cc flask was placed 100 cc of water. This was heated to boiling and hydrogen passed in while the solution cooled to 40° C. To this was added 15 g. (,08 mole) of 3,4-dihydroxyphenacyl chloride and 15 g. of sodium cyanide. It was stoppered immediately and the stopper

sealed with paraffin. This was permitted to stand at room temperature for one hour. The stopper was removed and the solution rendered acid with dilute hydrochloric acid. It was then placed in an ice chest over night and filtered. The crystals were boiled with 2 g. of Norite and filtered 5.5 g., 40% yield of light yellow crystals were obtained. M.P. was 218-218° C. with decomposition. Analysis: Nitrogen content calculated for the formula $C_9H_7O_3N$ is 7.99%. Found N. 7.78%.

This nitrogen content was a little low so further attempts at purification were tried. The material was purified by boiling with Norite several times until a white flocculent crystals were obtained. This was dried for twenty-four hours in a vacuum desciccator. Melting Point 220-223° C. with decomposition. This final product was 3,4-dihydroxyphenacyl cyanide.

Other attempts at obtaining the cyanide include the following: Various solvents tried for the conversion were, ethylene glycol, benzene, pyridine and acetone. None of these proved successful; finally an attempt was made to convert the 3,4-dihydroxyphenacyl chloride to the dibenzyl ether by treating the disodium salt of 3,4-dihydroxyphenacyl chloride with benzyl iodide. This also proved unsuccessful but results were sufficiently promising to warrant further study.

Preparation of p-methoxyphenacyl cyanide: Eight grams (.047 mole) of p-methoxyphenacyl chloride was treated as previously described under the preparation of phenacyl cyanide. Yield was 5.4 grams or 71% of p-methoxyphenacyl cyanide. Melting Point 128% C. Analysis: Nitrogen content calculated for the formula $C_{10}H_9O_2N$ is 8%. Found nitrogen, 7.91% and 7.84%.

Preparation of p-bromphenacyl cyanide: Thirty grams of p-bromphenacyl bromide (.067) mole was treated as previously described under the preparation of phenacyl cyanide. Yield was 12.6 grams of the crude cyanide. Most of this compound was lost in an attempt to purify it by recrystallation from hottoluene. There was no opportunity to obtain an analysis of this product; a small portion recovered melted at 243-244° with decomposition.

SUMMARY

1. The synthesis of phenacyl cyanide and its nuclear substituted derivatives from the corresponding phenacyl chlorides has been studied.

2. The conversion of the phenacyl cyanide into the corresponging β-aryl-β-keto propionic esters is described.

3. The β-keto esters may be converted in good yield to the corresponding ester of β-aryl-β-keto-α-oximinopropionic acid.

4. These α-oximino-β-keto esters have been hydrogenated to the corresponding phenylserine derivatives.

5. In the general procedure:

$$Ar.CO\text{-}CH_2Cl \longrightarrow Ar.CO\text{-}CH\ Cn \longrightarrow Ar.CO\text{-}CH_2\text{-}COOEt \longrightarrow$$

$$Ar.CO\text{-}\underset{\underset{NOH}{\|}}{C}\text{-}COOEt \longrightarrow Ar.CHOH\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOEt$$

The yields at all stages are satisfactory and encourage the belief that the method is available for general synthesis of β-hydroxy-α-amenoacids.

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