Synthesis of Amino Acids in the Catecholamine Series

By WALTER H. HARTUNG*, ALBERT N. MATTOCKS‡, and ROBERT I. ELLIN§

Tyrosine is generally proposed as the initial precursor in the biogenesis of epinephrine and norepinephrine. Methods are presented for the preparation of amino acids which could be included in alternate mechanisms or possibly interfere with the metabolic sequence. During the course of this study the following compounds were prepared: phenylserine and its ethyl ester, *p*-hydroxyphenylserine, *O*-methyl tyrosine, and ω-aminoacetylcatechol in an attempt to prepare dihydroxyphenylserine, DOPS. An effort was made to improve on the practicality of the methods previously used. In some cases a specific adaptation of known procedures was employed; in others new syntheses were developed.

RECENT STUDIES (1, 2) tend to advance Blaschko's original hypothesis (3) concerning the role of various amino acids in the biogenesis of catecholamines: tyrosine→3,4-dihydroxyphenylalanine (DOPA)→hydroxytyramine (Dopamine)→norepinephrine. Only a few steps in the classical sequence of reactions have been clearly demonstrated. The purpose of this investigation was to prepare, by convenient methods, amino acids related to those proposed in the biogenetic sequence. The amino acids may have functions of their own or serve as intermediates in alternate metabolic pathways leading to the formation of norepinephrine. Recently, Dickenson and Thompson (4) reported that phenylalanine was necessary for virus synthesis and that L-threophenylserine was the most active of a series of compounds tested against influenza A virus. Holtz, *et al.* (5), found that all organ extracts which decarboxylated DOPA also attacked the phenylserines. During the course of this study the following compounds were prepared: phenylserine and its ethyl ester, *p*-hydroxyphenylserine, *O*-methyl tyrosine, and ω-aminoacetylcatechol (in an attempt to prepare dihydroxyphenylserine, DOPS). An effort was made to improve on the practicality of the methods previously used. In some cases a specific adaptation of known procedures was employed, and in others new syntheses were developed.

EXPERIMENTAL

The melting points reported in the paper are uncorrected and were determined by the capillary tube method.

p-Hydroxyphenacyl Cyanide (II).—In 100 ml. of alcohol was dissolved 21.5 Gm. (0.125 mole) of *p*-hydroxyphenacyl chloride (6) (Ia), and the solution was warmed to 50°. To the warm solution was added, with stirring, 26 Gm. (0.46 mole) potassium cyanide in 80 ml. water. The mixture was kept warm and stirred for an additional 30 minutes, then allowed to cool to room temperature. The solution was acidified to litmus with hydrochloric acid and allowed to cool overnight in a refrigerator. The resulting crystals were filtered and redissolved in 50% alcohol to which 3 Gm. of decolorizing charcoal had been added. The mixture was warmed for 15–20 minutes on a hot plate and filtered while hot. Upon cooling, the clear filtrate yielded 10 Gm. (60%) of *p*-hydroxyphenacyl cyanide as tan crystals. Concentration of the filtrate produced no appreciable quantities of product. The dried cyanide melted with decomposition at 172°. No further purification was considered necessary for subsequent reactions.

Anal.—Calcd. for $C_9H_7NO_2$: N, 8.7. Found: N, 8.9.

Attempted Synthesis of α-Hydroximino-β-keto-β-(p-hydroxyphenyl)-propionic Acid Ethyl Ester from p-Hydroxyphenacyl Cyanide (II).—In 30 ml. of absolute alcohol which had been previously saturated with dry hydrogen chloride, 6 Gm. (0.037 mole) of *p*-hydroxyphenacyl cyanide (II) was dissolved. The solution was placed in a stoppered flask and allowed to stand at room temperature for 5 days. To this solution 100 ml. of distilled water was added, and the mixture was warmed to 30–60° and cooled overnight in a refrigerator. A small quantity of flocculent precipitate was observed in the beaker, and the mixture was extracted with ether to isolate all the reaction product. Evaporation of solvent from the ether extracts produced dark crystals which were dissolved in hot alcohol, charcoaled, and reprecipitated by cooling. A yield of 5.5 Gm. of yellow crystals was obtained. Two repetitions of this reaction produced only dark oily substances from which no crystalline material could be obtained.

Five grams of the yellow crystalline product of alcoholysis were dissolved in 100 ml. of ether, and the solution was saturated with hydrogen chloride. Butyl nitrite (2.4 Gm., 0.023 mole) was added to the solution over a period of 30 minutes. After an additional 30 minutes of stirring, the reaction mixture was transferred to a distilling flask, and the solvents were removed by heating. The residue was taken up in hot toluene and reprecipitated by cooling. Four grams of light gold crystals was obtained. The product melted at 153–155°. The calculated nitrogen content for α-hydroximino-β-keto-β-(*p*-hydroxyphenyl)-propionic acid ethyl ester is 5.91%, and the nitrogen content of the product as shown by Kjeldahl analysis was 11.0%. No

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study of the structure of the product was made, but it is interesting that the corresponding amide would contain 13.5% nitrogen and that the nitroso substituted derivative of the ester desired would contain 10.5% nitrogen. Should this compound actually have been α-hydroximino-β-keto-β-(3-nitroso-4-hydroxyphenyl)-propionic acid ethyl ester, the method might serve as a synthesis of compounds of this type. The possibilities of this reaction deserve further study.

p-Hydroxyphenylglyoxylohydroxamyl Cyanide (IVa).—To a solution of 5.5 Gm. (0.027 mole) of *p*-hydroxyphenylglyoxylohydroxamyl chloride (III*a*) in 100 ml. of alcohol was added 6.5 Gm. (0.1 mole) of potassium cyanide in 100 ml. of water. The solution was heated with occasional stirring for 1 hour on a steam bath. After allowing the mixture to cool, hydrochloric acid was added until the reaction was acid to litmus, and the mixture was cooled in the refrigerator. After 1 hour of cooling, the crystals that had precipitated were filtered, dried, and weighed. Filtration gave 1.5 Gm. of brown needles and ether extraction of the filtrate followed by evaporation of solvent produced 3.5 Gm. additional product, the total yield being 5 Gm. (95%). The crude nitrile was recrystallized from dilute alcohol, and the crystals melted at 160–161° with decomposition. Repeated reactions gave yields of 90–95%.

Anal.—Calcd. for $C_{10}H_8N_2O_3$: N, 14.7. Found: N, 14.1, 14.3.

α-Hydroximino-p-hydroxybenzoylacetic Acid (V).—In 50 ml. of 10% aqueous sodium hydroxide was dissolved 3.5 Gm. (0.018 mole) of *p*-hydroxyphenylglyoxylohydroxamyl cyanide (IV*a*), and the mixture was heated in an open beaker on a steam bath for 6 hours, evolution of ammonia being completed at the time. Crushed ice (100 Gm.) was added, and the cold mixture was acidified with dilute hydrochloric acid and extracted with ether. The ether was dried over anhydrous sodium sulfate and removed on a water bath. The residue did not require further purification since it was only slightly colored. A yield of 3.6 Gm. (96%) of crude acid was obtained as yellow crystals, melting with evolution of gas at 124–125°. Attempts at recrystallization of small amounts of the acid for analysis were unsuccessful; only amorphous products were obtained. A neutral equivalent of the compound was made by dissolving a portion in excess 0.02 *N* base and residually titrating with 0.02 *N* acid.

Anal.—Calcd. for N.E. $C_9H_9NO_6$: 225. Found: N.E. 219, 228.

p-Hydroxyphenylserine Hydrochloride (VI).—In 100 ml. of absolute alcohol containing 5 Gm. hydrogen chloride was dissolved 2.1 Gm. (0.01 mole) of the α-hydroximino acid (V); 3 Gm. of 10% palladium catalyst was added. The mixture was shaken in hydrogen at a pressure of 1 Atm., and the theoretical quantity of hydrogen was absorbed in 1 hour. The solution was filtered to remove the catalyst and the filtrate concentrated under reduced pressure to about 30 ml. Ether was added until a definite turbidity was apparent, and the mixture was then cooled overnight. The crystals which had formed were filtered, washed with ether, and dried. The product was recrystallized from hot alcohol. The crystals darkened to gold at 180–185° and melted to a red oil with evolution of gas at 224–225°. This melting point behavior is similar to that reported for the free base (7).

Anal.—Calcd. for $C_9H_{13}ClNO_4$: N, 6.01. Found: N, 5.96, 6.01.

3,4-Dihydroxyphenylglyoxylohydroxamyl Cyanide (IVb).—To 10.65 Gm. (0.05 mole) of 3,4-dihydroxyphenylglyoxylohydroxamyl chloride (6) (III*b*) dissolved in 100 ml. of alcohol was added 13 Gm. (0.3 mole) of potassium cyanide dissolved in 200 ml. of water. The mixture was heated, with occasional stirring, on a steam bath for 1 hour and allowed to cool. On acidification with dilute hydrochloric acid, a brownish mass formed which dissolved on addition of excess acid. The dark mixture was extracted with three 250-ml. portions of ether. The ether extract was dried over anhydrous sodium sulfate and evaporated to dryness on a steam bath. The resulting crude product was taken up in 50 ml. of ether, boiled with 1 Gm. of charcoal, and filtered. After the addition of 350 ml. of toluene, the solution became cloudy and was placed in a refrigerator for 2 hours. A yield of 5.5 Gm. (53%) of yellowish-green crystals was obtained, melting at 172 to 173.5°.

Anal.—Calcd. for $C_9H_9N_2O_4$: C, 52.43; H, 2.93; N, 13.6. Found: C, 52.43; H, 2.95; N, 13.5.

Attempted Preparation of α-Hydroximino-3,4-dihydroxybenzoylacetic Acid (VII).—A solution of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide (IV*b*) (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 5–6 hours of heating. On cooling to room temperature, the solution was treated with 100 Gm. 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 sulfate, filtered, and evaporated to dryness on a steam bath. Very hygroscopic brown crystals were obtained. Further purification by recrystallization from ether produced yellow-brown crystals which were also hygroscopic. An accurate analysis could not be obtained.

Preparation of ω-Aminoacetylcatechol (VIII). (Attempted Synthesis of 3,4-Dihydroxyphenylserine).—In a hydrogenation flask were placed the product obtained from the hydrolysis of 0.02 mole of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide (IV*b*), 100 ml. of absolute alcohol containing 10 Gm. of dry hydrogen chloride, and 3 Gm. of a 10% palladium-on-charcoal catalyst. The mixture was shaken on a Parr hydrogenation apparatus until hydrogen was no longer absorbed, about 0.05 mole 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 Gm. of colorless crystals. Recrystallization from absolute alcohol gave 3.6 Gm. of a hydrochloride that melted to a red oil with evolution of a gas at 244–246°.

Anal.—Calcd. for 3,4-dihydroxyphenylserine hydrochloride, $C_8H_{11}NO_3 \cdot HCl$: C, 43.29; H, 4.85; N, 5.6. Calcd. for arterenol hydrochloride, $C_8H_{11}NO_3 \cdot HCl$: C, 46.72; H, 5.9; N, 6.8. Calcd. for ω-aminoacetylcatechol, $C_8H_9NO_3 \cdot HCl$: C, 47.18; H, 4.9; N, 6.8. Found: C, 46.65; H, 4.9; N, 6.9.

The analytical data were consistent with the values calculated for ω-aminoacetylcatechol or arterenol hydrochloride. The melting point of arterenol hydrochloride was reported by Simonoff (8) as 136° with decomposition. The melting point of the amino ketone hydrochloride reported by Barger and Dale (9) 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, can be explained if the β-keto acid obtained by hydrolysis of 3,4-dihydroxyglyoxylohydroxamyl cyanide (IV*b*) underwent decarboxylation to form ω-hydroximinoacetylcatechol. The evolution of gas, presumably carbon dioxide, noted during the hydrolysis, is evidence of the possibility that decarboxylation occurred.

In an attempt to avoid decarboxylation, the mixture, resulting from the heating of 3 Gm. of 3,4-dihydroxyphenylglyoxylohydroxamyl cyanide (IV*b*) with sodium hydroxide was transferred without purification to a hydrogenation bottle. The flask was shaken with 0.5 Gm. of a 10% palladium catalyst in an effort to prevent poisoning of the 3 Gm. 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 Gm. of absolute alcohol containing 5 Gm. of dry hydrogen chloride and placed in a hydrogenation bottle with 3 Gm. of a 10% palladium catalyst. After hydrogenation, ω-aminoacetylcatechol, melting with decomposition at 244–246°, was obtained.

Phenylserine and Phenylserine Ethyl Ester Hydrochloride.—In a 500-ml. round-bottomed flask were placed 8.5 Gm. (0.08 mole) of benzaldehyde, 9.5 Gm. (0.04 mole) of carbobenzyloxyglycine ethyl ester, 2 Gm. 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 24 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 refrigerator. This was treated with 50 ml. of absolute alcohol containing 3 Gm. of dry hydrogen chloride and 2 Gm. 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 Gm. (41%) of phenylserine ether ester hydrochloride, melting at 157–161°. The melting point was in agreement with those already reported for phenylserine ethyl ester hydrochloride (10).

Anal.—Calcd. for $C_{11}H_{15}NO_5 \cdot HCl$: N, 5.70. Found: N, 5.78, 5.83.

N-Carbobenzyloxyphenylserine.—In a 500-ml. round-bottomed flask were placed 8.5 Gm. (0.08 mole) of benzaldehyde, 8.4 Gm. (0.04 mole) of carbobenzyloxyglycine, 2 Gm. (0.45 mole) of sodium wire, and 75 ml. of dry ether; reaction took place as previously described. Isolation of the product by the above procedure produced 8 Gm. 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 solidified on addition of dry ether and standing in the refrigerator; it melted at 83–85°.

Anal.—Calcd. for $C_{17}H_{17}NO_6$: N, 4.44. Found: N, 4.53.

Phenylserine.—*N*-Carbobenzyloxyphenylserine (1 Gm., 0.003 mole) was dissolved in 75 ml. of alcohol containing 7 Gm. of dry hydrogen chloride and 2 Gm. of a 10% palladium catalyst. About 100 ml. of hydrogen (a slight excess of the theoretical amount) was absorbed in 15 minutes. The catalyst was filtered and the filtrate concentrated under reduced pressure. Addition of an excess of dry

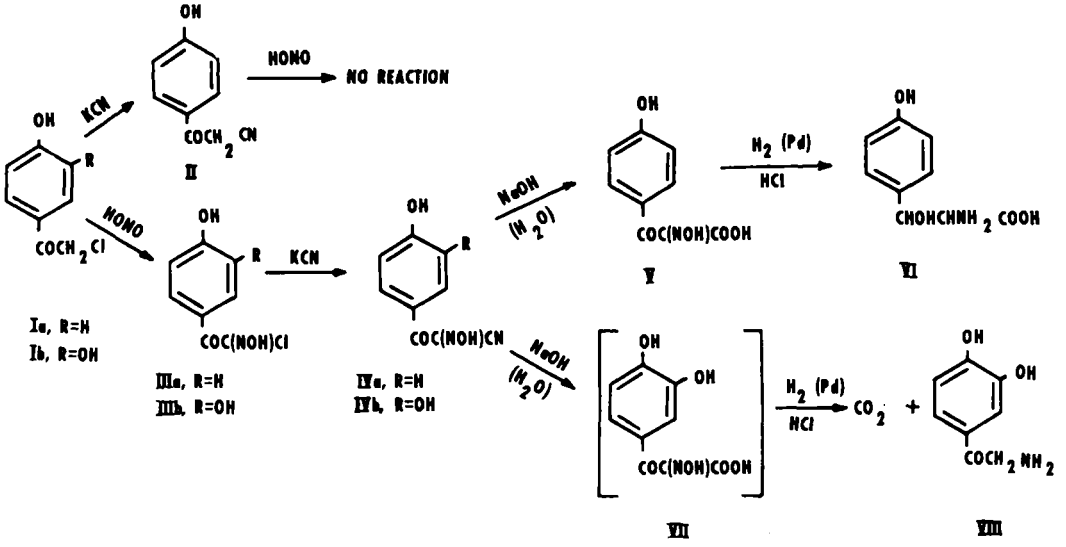

Figure 1.

ether produced 0.4 Gm. (57%) of crystals melting at 148–150°. Carrara and co-workers (11) reported the melting point of phenylserine hydrochloride to be 157°.

α - Hydroximino - β - (p - methoxyphenyl)-propionic Acid.—A 5.3 Gm. (0.024 mole) quantity of *p*-methoxybenzylmalonic acid was dissolved in 50 ml. of ether, and 4.85 Gm. (0.047 mole) of butyl nitrite was added. The mixture was placed in an ice bath and stirred while hydrogen chloride was passed into the beaker until none of the gas was absorbed. Stirring and cooling were continued for 20 minutes, and the reddish reaction mixture was warmed on a hot water bath until the ether had evaporated. A residue of slightly colored solid remained; this was dissolved in warm alcohol and reprecipitated by the addition of cold water to yield 4.95 Gm. (96%) of colorless crystals of α-hydroximino-β-(*p*-methoxyphenyl)-propionic acid. The recrystallized product melted at 156°. Hamlin and Hartung (12) reported a melting point of 156–157° for this compound. Subsequent reactions gave yields of 90–95%.

O-Methyltyrosine.—To a solution of 4 Gm. (0.019 mole) of the α-hydroximino-β-(*p*-methoxyphenyl)-propionic acid in 100 ml. ethyl alcohol was added 3 Gm. of a 10% palladium catalyst. To the mixture was then added 10 ml. of 36% hydrochloric acid; the mixture was shaken with hydrogen at a pressure of 10 Atm. The theoretical quantity of hydrogen was absorbed in 3.5 hours, and the mixture was filtered to remove the catalyst. The solvent was removed under reduced pressure on a hot water bath, and the residue was dissolved in 15 ml. of warm distilled water and titrated to neutral with 0.1 *N* sodium hydroxide using methyl red indicator. Cooling of the neutral solution precipitated 3.5 Gm. (93%) of *O*-methyltyrosine. The melting point, 295–296° with decomposition, agreed with the value reported (13, 14).

DISCUSSION

Phenylglyoxylohydroxamyl cyanide (II) was prepared by nitrosating phenacylchloride and reacting the resulting isonitroso compound with potassium cyanide. Both keto and cyano groups are known to promote nitrosation of adjacent aliphatic carbon atoms. Indeed, Pasquale (15) obtained good yields of II by nitrosating phenacyl cyanide. We were not, however, able to prepare *p*-hydroxyphenylglyoxylohydroxamyl cyanide (IV*a*) by nitrosating *p*-hydroxyphenacylcyanide, nor are reports available indicating that 3,4-dihydroxyphenylglyoxylohydroxamylcyanide (IV*b*) can be prepared by nitrosating dihydroxyphenacylcyanide. This would indicate that substituents adjacent to carbonyl groupings drastically affect nitrosation reactions.

The potential of these compounds as enzyme inhibitors or alternate precursors of norepinephrine were not investigated; however, a group of the National Institutes of Health (16, 17) reported recently on the activity of a number of related compounds as substrates and inhibitors of dopamine-β-oxidase.

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Assay Methods for Some *Vinca rosea* Alkaloids II

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Colorimetric and fluorometric methods are presented for quantitative determination of some *Vinca* alkaloids. The identification is accomplished with a new color reagent—ceric ammonium sulfate in phosphoric acid—and two thin-layer chromatographic systems.

THE ALKALOIDS from *Vinca rosea* Linn. represent a new approach in the chemotherapeutic treatment of a variety of human neoplasms (6).

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In Part I a colorimetric method which was very specific for vincaleukoblastine[1] (vinblastine) was described (5). Since that time, only leurocristine[1] (vincristine)—another dimeric indole-indoline compound isolated later (8, 10)—has been shown to give the same reaction as vincaleukoblastine. By the vincaleukoblastine method (5), leurocristine exhibits the same color curve as

[1] The A.M.A. Council on Drugs has approved vinblastine and vincristine as generic names for these alkaloids. Vincaleukoblastine is marketed as Velban (vinblastine sulfate) by Eli Lilly and Co., Indianapolis, Ind.