[CONTRIBUTION FROM THE DEPARTMENT OF PHARMACOLOGY, HARVARD MEDICAL SCHOOL]

The Synthesis and Cyclization of α-Methylamino-β-(4-carboxy-3-indole)-propionic Acid

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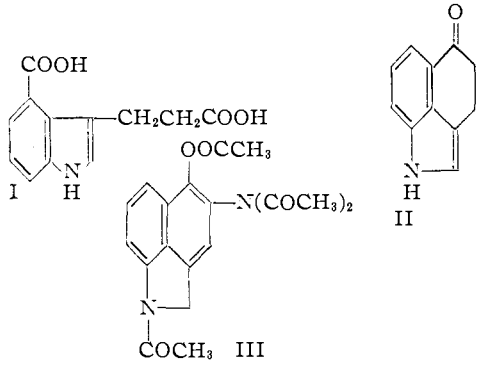
α-Methylamino-β-(4-carboxy-3-indole)-propionic acid (IX) has been prepared as an intermediate for studies in the benz(cd)indole series. The precursor of the methylamino acid, methyl α-carbomethoxy-α-acetylmethylamino-β-(4-cyano-3-indole)-propionate (VII), has been found to undergo a rather complex reaction with 40% aqueous potassium hydroxide, giving rise to an equimolar mixture of the tetrahydrocarbolinedicarboxylic acid X and 4,4′-dicarboxy-3,3′-diindolylmethane (XI). This unexpected behavior has been traced to a partial cleavage of the dimethyl ester VII to 4-cyano-3-indolemethanol (XVIII) which, in turn, suffers self-condensation, resulting in the diindolylmethane derivative XI, with concomitant release of formaldehyde to the normal hydrolysis product IX, affording the tetrahydrocarbolinedicarboxylic acid X. Certain of the properties and reactions of 4-cyano-3-indolemethanol (XVIII), prepared by hydride reduction of 4-cyano-3-indolecarboxaldehyde (XIII), have been observed. Cyclization of α-acetylmethylamino-β-(4-carboxy-3-indole)-propionic acid (XX) with acetic anhydride, in the presence of potassium cyanide, has been shown to provide 1-acetyl-4-acetylmethylamino-5-acetoxy-1,2-dihydrobenz(cd)indole (XXI). The changes associated with this tricyclic indoline derivative in both acid and alkaline media have been examined with particular reference to the propensity to dehydrogenation of the free methylaminonaphthol XXIII.

In the course of studies with 4-substituted indoles, the reaction of β-(4-carboxy-3-indole)-propionic acid (I) with acetic anhydride in the presence of potassium cyanide was demonstrated to bring about cyclization to the trinuclear ketone, 5-keto-1,3,4,5-tetrahydrobenz(cd)indole (II).[2] The α-acetamino derivative of I, on the other hand, when exposed to the same reagents, was found to give rise to the naphthalene (benzindoline) III, characterized by a bond structure tautomeric with that of the indole II.[3] This disparity of isomer, together with the rather critical experimental conditions mandatory for ring closure, underscores a certain theoretical interest in the transformation, quite apart from the question of its usefulness as a mode of entry into the ergoline system.



Moreover, the problem of indole–naphthalene interrelationship which has proved central to this work presents a number of subordinate points which require clarification and elaboration. For example, the pronounced vulnerability to dehydrogenation of 1,3,4,5-tetrahydrobenz(cd)indole primary α-amino ketones, when in the free state, has not been adequately interpreted, particularly in view of the decidedly greater stability of related tertiary amino derivatives.[4]

The development of a general procedure for the synthesis of α-methylamino acids[5] has now made possible further scrutiny of the anhydride cyclization leading to intermediates, characterized by a secondary methylamino function, appropriate to continued synthetic and illustrative work.

The starting compound for the new series was again 4-cyanogramine (IV),[2] which, with a large excess of methyl iodide at 0°, gave as the predominant quaternary salt in most repetitions bis-(4-cyanoskatyl)-dimethylammonium iodide (V).[6] The initially sought, and considerably more soluble, 4-cyanogramine methiodide (VI) was isolated from extracts obtained by trituration of the addition product with hot water. The methiodide VI, as well as the bis-compound V, when allowed to react with dimethyl acetylmethylaminomalonate sodium,[5] afforded methyl α-carbomethoxy-α-acetylmethylamino-β-(4-cyano-3-indole)-propionate (VII). Condensation of the sodium derivative with 4-cyanogramine itself, in the presence of dimethyl sulfate, was equally successful.

Attempts to hydrolyze the complex ester VII to the amino acid in the manner adopted in the case of ethyl α-cyano-α-acetamino-β-(4-cyano-3-indole)-propionate[3] led to results which were not anticipated. The experimental circumstances, the rigors of which were exacted by the difficult hydrolysis of the 4-cyano group, involved treatment of the solid compound with 40% aqueous potassium hydroxide at reflux temperature for a period of several days.

In the present instance, the dimethyl ester appeared to dissolve with great reluctance and a completely homogeneous solution was not observed after 5 days at 100°. Processing of the reaction mixture afforded, in 37% yield, a nicely crystalline, slightly soluble amino acid. This substance, however, failed to react with acetic anhydride and gave no response to the usual indole color reagents. On the basis of its general behavior and combustion values, and of supplementary information contributed by its dimethyl ester, the amino acid was assigned the tetrahydrocarboline constitution X.

(1) National Science Foundation Predoctoral Fellow 1954–1955; United States Public Health Service Fellow 1955–1956.

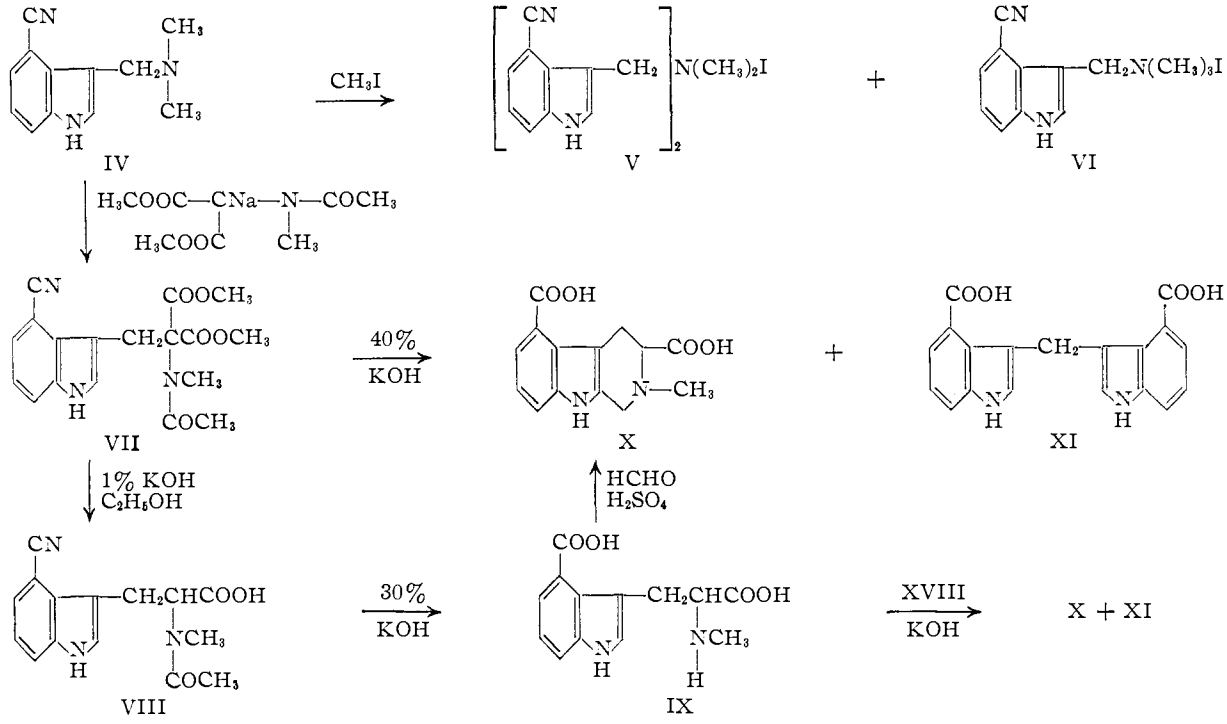
(2) F. C. Uhle, THIS JOURNAL, **71**, 761 (1949).

(3) F. C. Uhle and S. H. Robinson, *ibid.*, **77**, 3544 (1955).

(4) F. C. Uhle, *ibid.*, **73**, 2402 (1951).

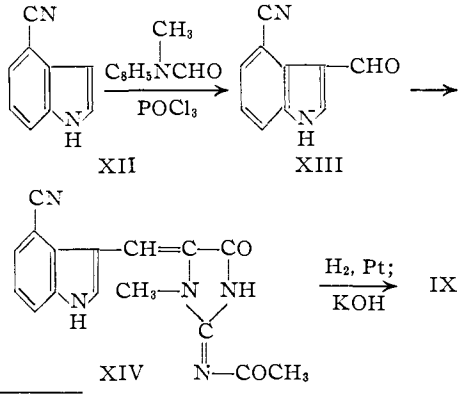
(5) F. C. Uhle and L. S. Harris, *ibid.*, **78**, 381 (1956).

(6) *Cf.* the behavior of gramine, which, under similar conditions, yields principally the methiodide; T. A. Geissman and A. Armen, *ibid.*, **74**, 3916 (1952).



The desired α-methylamino-β-(4-carboxy-3-indole)-propionic acid (IX) was secured from the ester VII by a two-stage procedure. Saponification with dilute potassium hydroxide in ethanol solution at 0° led to the isolation (95%) of α-acetylmethylamino-β-(4-cyano-3-indole)-propionic acid (VIII), unexceptional hydrolysis of which, with concentrated alkali, furnished the methylamino acid IX, separated (55%) through the copper salt.

The identity of this substance was verified by an independent preparation, albeit in unacceptable over-all yield, which represented the introductory effort toward the methylamino acid but which did not provide a crystalline product until authentic material for nucleation became available *via* the amino ester route. 4-Cyanoindole (XII)[2] had been transformed with N-methylformanilide and phosphorus oxychloride to 4-cyano-3-indolecarboxaldehyde (XIII)[7] which, with creatinine and acetic anhydride, gave the aldal XIV in practically quantitative amount. Catalytic hydrogenation in



alkaline solution in the presence of platinum, followed by hydrolysis with 5 *N* sodium hydroxide, resulted in an amino acid fraction which, when seeded, deposited *ca.* 5% of IX.[8]

α-Methylamino-β-(4-carboxy-3-indole)-propionic acid (IX), when permitted to react with formaldehyde in sulfuric acid solution[9] afforded 1,2,3,4-tetrahydro-2-methyl-9H-pyrid[3,4,-b]-indole 3,5-dicarboxylic acid (X), identical with the tertiary amino acid isolated from the attempted simple hydrolysis of the ester VII with 40% aqueous caustic potash.

In seeking to account for the direct formation of the tetrahydrocarboline from the dimethyl ester, it was recalled that the compound was accompanied in the hydrolyzate by a non-basic product, collected, as well, in 37% yield. This carboxylic acid, which melted quite sharply at 253–255°, was notable for the alacrity with which it became colored dark red in the presence of mineral acids or of oxygen. Composition figures, which, at first, were misleading because of the sensitivity of the substance under manipulations prefatory to analysis, were finally reconciled with the molecular formula $C_{19}H_{14}N_2O_4$. The ultraviolet absorption spectrum displayed only the 4-indolecarboxylic acid chromophore and indicated the presence of two such residues per molecule. Diazomethane yielded a crystalline methyl ester.

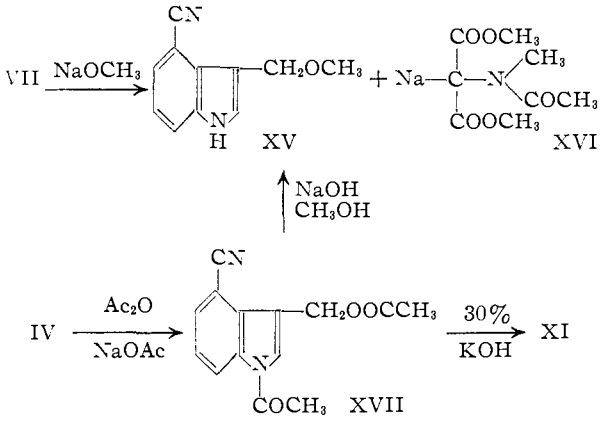
Inasmuch as the ostensibly sluggish dissolution of the solid ester VII in concentrated fixed base ap-

(7) Reaction of 4-cyanogramine with hexamethylenetetramine in acetic acid led only to highly pigmented solutions; *cf.* H. R. Snyder, S. Swaminathan and H. J. Sims, THIS JOURNAL, **74**, 5110 (1952).

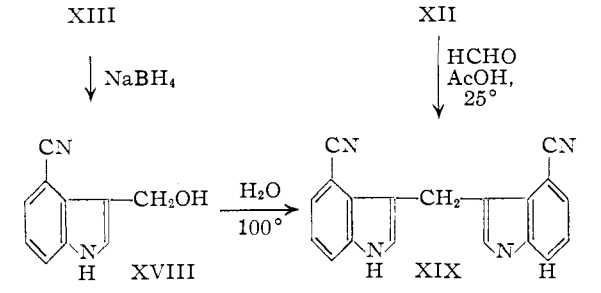
(8) The unsatisfactory issue of this hydrogenation is possibly due, in part, to the restrictions imposed by the very limited solubility of the creatinine derivative. Sodium amalgam gave only dark red amorphous material.

(9) *Cf.* the conditions for the transformation of α-amino acids to tetrahydrocarbolinecarboxylic acids reported by W. A. Jacobs and L. C. Craig, *J. Biol. Chem.*, **113**, 759 (1936), and by H. R. Snyder, C. H. Hansch, L. Katz, S. M. Parmerter and E. C. Spaeth, THIS JOURNAL, **70**, 219 (1948).

peared to be implicated in the anomalous results, it was considered that a major fraction of the starting compound had suffered a competing segmentation, prior to hydrolysis of the carbomethoxy groups.[10] Indeed, the marked lability of the ester VII in alkaline milieu was readily demonstrated. Treatment with one mole of sodium methoxide in refluxing methanol for a period of 6 hours resulted in essentially quantitative scission of the molecule to afford dimethyl acetylmethylaminomalonate sodium (XVI) and 4-cyano-3-methoxymethylindole (XV).[11] The formulation of the latter substance was corroborated by its preparation from 4-cyanogramine (IV). Boiling acetic anhydride in the presence of sodium acetate smoothly transformed the Mannich base to N-acetyl-4-cyano-3-acetoxymethylindole (XVII) which, with two equivalents of sodium hydroxide in methanol at 25°, gave the methyl ether XV.



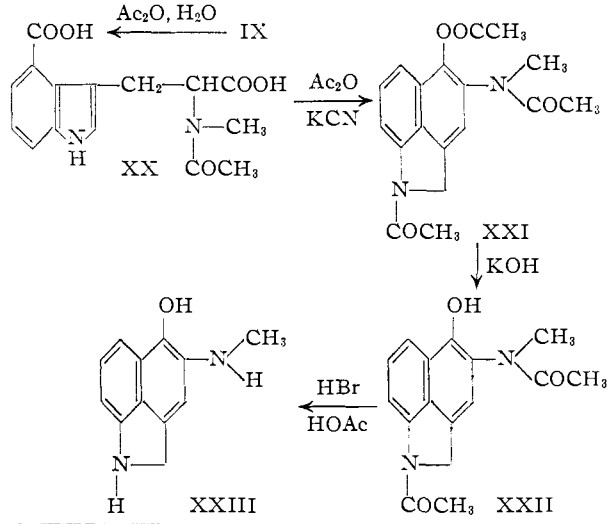
Since cleavage in this sense in aqueous solution presupposes the formation of the corresponding hydroxy compound, attention was directed to the preparation of 4-cyano-3-indolemethanol (XVIII) which was acquired by reduction of 4-cyano-3-indolecarboxaldehyde (XIII) with sodium boro-



hydride in pyridine[12] as well as with lithium aluminum hydride in tetrahydrofuran.[13]

In accord with recent experience with 3-indolemethanol,[14] the alcohol XVIII was found to exemplify a rather mutable arrangement. At elevated temperature,[15] or in the presence of alkali, self-condensation with extrusion of the elements of formaldehyde rapidly developed, leading to 3,3'-diindolylmethane derivatives.[16] In aqueous solution under reflux for a prolonged period, 4-cyano-3-indolemethanol gave rise to 4,4'-dicyano-3,3'-diindolylmethane (XIX), a compound also derived from 4-cyanoindole (XII) and formaldehyde in acetic acid at 25°. With 5.5 *N* aqueous potassium hydroxide at 100°, the carbinol, as well as N-acetyl-4-cyano-3-acetoxymethylindole (XVII), afforded 4,4'-dicarboxy-3,3'-diindolylmethane (XI), identical with the carboxylic acid first isolated following vigorous alkaline modification of the dimethyl ester VII.

It was concluded, therefore, that the genesis of the tetrahydrocarbolinedicarboxylic acid X from the ester VII had been dependent upon surrender of formaldehyde by the labile indolemethanol derivative XVIII to that segment of the reaction product which had escaped side chain fragmentation and thereby represented the normal hydrolysis



(10) As a result of the findings with the ester VII, the hydrolysis of methyl α-carbomethoxy-α-acetylmethylamino-β-(3-indole)-propionate to α-methylamino-β-(3-indole)-propionic acid[5] was re-examined. When an experiment involving concentrations identical with those which, in the present work, led to the isolation of X was carried out, the ester had entirely dissolved after a few minutes at reflux temperature. Because of the appreciable water solubility of the expected amino acid, the entire reaction mixture, after adjustment of *p*H, was allowed to react with potassium cyanate. The hydantoin of α-methylamino-β-(3-indole)-propionic acid was isolated in 50% yield. No tertiary amino acid was found.

(11) In a parallel experiment at 25° for 15 hours, 50% of the methyl ether XV was isolated; 40% unchanged VII was recovered. Ethyl α-acetamino-α-cyano-β-(4-cyano-3-indole)-propionate[3] with sodium methoxide in methanol at 65° for 6 hours gave 17% of XV.

(12) *Cf.* the preparation of 3-indolemethanol from 3-indolecarboxaldehyde with sodium borohydride in methanol, R. M. Silverstein, E. E. Ryskiewicz and S. W. Chaikin, THIS JOURNAL, **76**, 4485 (1954), and from gramine methiodide with sodium hydroxide in the presence of ether.[14]

(13) *Cf.* the finding that 3-indolecarboxaldehyde, 3-indolecarboxylic acid and ethyl 3-indolecarboxylate, when allowed to react with lithium aluminum hydride in ether under various conditions, gave only skatol.[14] Obtention of the carbinol with lithium aluminum hydride in the present work is doubtless contingent on prompt deposition from the medium of the metal complex.

(14) E. Leete and L. Marion, *Can. J. Chem.*, **31**, 775 (1953).

(15) The instability of the compound at higher temperature is foreshadowed by the imprecise nature and broad range of its melting point. A similar behavior was observed with 4-cyanogramine.[2]

(16) *Cf.* the formation of dipyrrylmethanes from pyrrolemethanol derivatives, H. Fischer and C. Nenitzescu, *Ann.*, **443**, 114 (1925), and A. H. Corwin, W. A. Bailey, Jr., and P. Viohl, THIS JOURNAL, **64**, 1267 (1942); *cf.* further the production of 1,1'-dimethyl-3,3'-diindolylmethane as a by-product in the alkylation of ethyl cyanoacetate (as well as of ethyl acetaminomalonate and of tricarbethoxymethane) with 1-methylgramine, and as the principal end result of the reaction of 1-methylgramine methiodide with sodium hydroxide, H. R. Snyder and E. L. Eliel, *ibid.*, **71**, 663 (1949).

component. In confirmatory event, when the methylamino acid IX was admitted to reaction with two molecular equivalents of 4-cyano-3-indolemethanol (XVIII) in 30% aqueous caustic potash at reflux temperature, the tricyclic tertiary amino acid X was produced in virtually quantitative yield.

The methylamino acid IX, with acetic anhydride in aqueous solution, gave the N-acetylmethylamino acid XX which crystallized as the monohydrate. This substance,[17] when allowed to react with acetic anhydride in the presence of potassium cyanide under the conditions defined[18] in the case of 4-carboxy-DL-tryptophan,[3] afforded N-acetyl-4-acetylmethylamino-5-acetoxy-1,2-dihydrobenz(cd)indole (XXI). Mild hydrolysis with basic reagents led to the alkali-soluble N-acetyl-4-acetylmethylamino-5-hydroxy-1,2-dihydrobenz(cd)-indole (XXII), while severance of both ester and amide linkages with a combination of 48% hydrobromic and glacial acetic acids produced the dihydrobromide of 4-methylamino-5-hydroxy-1,2-dihydrobenz(cd)indole (XXIII).[19,32]

In an endeavor to assess the capacity of the methylaminonaphthol XXIII to withstand dehydrogenation, the base obtained by neutralization of the dihydrobromide with sodium acetate was given the opportunity to react with molecular oxygen in ethanol solution under brisk reflux for a period of one hour. The sole product recovered, following acetylation in aqueous ethanol, was the diacetylmethylaminonaphthol XXII; no keto-1,5-dihydrobenz(cd)indole derivative was isolated in crystalline form. The methylamino homolog therefore appears to exhibit a substantially greater measure of resistance to oxidation than does the primary relative 4-amino-5-hydroxy-1,2-dihydrobenz(cd)indole[3,20] in the situations thus far devised.

Experimental[21]

4-Cyano-3-indolecarboxaldehyde (XIII).—A mixture of 31 g. (0.23 mole) of N-methylformanilide and 36 g. (0.23 mole) of phosphorus oxychloride was allowed to remain at ordinary temperature for 15 minutes.[22] After 150 ml. of ethylene dichloride had been added, the solution was cooled to 0°. To this mixture was added, in portions, 14.2 g. (0.10 mole) of 4-cyanoindole.[2] After the solution had been allowed to stir for 45 minutes, 40 g. of calcium carbonate was added and the mixture was maintained at reflux temperature for 30 minutes. The whole was added to a solution of 150 g. of sodium acetate in 2500 ml. of water and the ethylene dichloride was removed by steam distillation. The calcium salts were separated by filtration. The crystalline product, which deposited from the hot aqueous filtrate, was recrystallized from water; yield 14.1 g. (83%), m.p. 224–226°.

Anal. Calcd. for $C_{10}H_6N_2O$ (170.17): C, 70.57; H, 3.55; N, 16.46. Found: C, 70.20; H, 3.62; N, 16.53.

The semicarbazone was prepared in ethanol and was recrystallized from the same solvent; m.p. > 300°.

Anal. Calcd. for $C_{11}H_9N_5O$ (227.22): C, 58.14; H, 3.99; N, 30.82. Found: C, 57.87; H, 4.11; N, 30.61.

1-Methyl-2-acetimino-5-(4-cyanoskatylidene)-4-imidazolidinone (XIV).—A mixture of 1.7 g. (0.10 mole) of 4-cyano-3-indolecarboxaldehyde (XIII), 1.7 g. (0.15 mole) of creatinine, 2.5 g. of anhydrous sodium acetate, 25 g. of acetic acid and 2.5 g. of acetic anhydride was maintained at reflux temperature for 30 minutes.[23] The bright orange deposit was collected by filtration and was recrystallized from acetic acid; yield 3.0 g. (98%); m.p. > 300°.

Anal. Calcd. for $C_{16}H_{13}N_5O_2$ (307.30): C, 62.53; H, 4.26; N, 22.79. Found: C, 62.47; H, 4.48; N, 22.96.

Bis-(4-cyanoskatyl)-dimethylammonium Iodide (V).—To a solution of 4.0 g. (0.02 mole) of 4-cyanogramine (IV)[2] in 25 ml. of methanol was added 28.4 g. (0.20 mole) of methyl iodide. After 3 hours at 0°, the precipitate was collected by filtration. The filtrate was concentrated to dryness under reduced pressure. The residue was extracted with 3 successive quantities of 10 ml. of water at 100°. The water-insoluble material was combined with the precipitate from the methanol solution to yield a total of 2.1 g. (44%), very slightly soluble in all of the common organic solvents tested; m.p. 194–195°.

Anal. Calcd. for $C_{22}H_{20}N_5I$ (481.35): C, 54.89; H, 4.19; N, 14.55. Found: C, 53.87; H, 4.52; N, 14.10.

4-Cyanogramine Methiodide (VI).—The crystalline precipitate which deposited at 0° from the 30 ml. of aqueous extract obtained in the preparation of bis-(4-cyanoskatyl)-dimethylammonium iodide as described above was collected by filtration and was recrystallized from water; yield 2.7 g. (40%), m.p. 165–168°.

Anal. Calcd. for $C_{13}H_{16}N_3I$ (341.21): C, 45.76; H, 4.73; N, 12.31. Found: C, 45.33; H, 5.01; N, 12.24.

Methyl α-Acetylmethylamino-α-carbomethoxy-β-(4-cyano-3-indole)-propionate (VII). A. From 4-Cyanogramine Methiodide (VI).—To a suspension of 2.7 g. (0.0079 mole) of 4-cyanogramine methiodide in 15 ml. of absolute methanol was added 1.78 g. (0.0079 mole) of dimethyl α-acetylmethylaminomalonate sodium.[5] After the mixture had been maintained at reflux temperature for 5 hours, the methanol was distilled under reduced pressure. The residue was dissolved in chloroform and was extracted with dilute hydrochloric acid and with water. The remainder from the dried ($MgSO_4$) chloroform solution was recrystallized from methanol; yield 1.98 g. (70%), m.p. 179–181°.

Anal. Calcd. for $C_{18}H_{19}N_3O_5$ (357.35): C, 60.50; H, 5.36; N, 11.76. Found: C, 60.36; H, 5.44; N, 12.08.

B. From Bis-(4-cyanoskatyl)-dimethylammonium Iodide (V).—A mixture of 4.81 g. (0.01 mole) of V, 2.25 g. (0.01 mole) of dimethyl α-acetylmethylaminomalonate sodium[5] and 10 ml. of absolute methanol was maintained at reflux temperature for 15 hours. The methanol was distilled under reduced pressure. The residue was dissolved in chloroform and was extracted with dilute hydrochloric acid and with water. The remainder from the dried ($MgSO_4$) chloroform solution was recrystallized from methanol; yield 2.85 g. (80%), m.p. and mixed m.p. with VIIA, 179–181°. The hydrochloric acid extract was basified with dilute sodium hydroxide solution to yield 1.49 g. (75%) of 4-cyanogramine.

C. From 4-Cyanogramine (IV).—To a solution of 1.67 g. (0.0084 mole) of 4-cyanogramine and 2.25 g. (0.01 mole) of dimethyl acetylmethylaminomalonate sodium[5] in 30 ml. of methanol was added dropwise 2.14 g. (0.017 mole) of dimethyl sulfate. After 20 hours at ordinary temperature, the methanol was distilled under diminished pressure.

(17) Preparation of the α-acetylmethylamino acid was found to be obligatory for successful cyclization. Exposure of the methylamino acid IX to the ring closure reagents failed to lead to a crystalline product.

(18) The significance of these conditions for the general course of the reaction will be commented on in greater detail in a forthcoming publication.

(19) The N-methylacetamino function of XXI was split with signally greater difficulty[32] than was the case with the primary amino analog.[3] In the Freudenberg microanalytical procedure, degradation with *p*-toluenesulfonic acid for a period of 8 hours was required to give correct results; after 3 hours, the equivalent of only 2 acetyl residues had been accounted for.

(20) A. Stoll and J. Rutschmann, *Helv. Chim. Acta*, **35**, 141 (1952); C. A. Grob and B. Hofer, *ibid.*, **36**, 847 (1953).

(21) Microanalyses and spectroscopic determinations by Dr. S. M. Nagy and associates of the Massachusetts Institute of Technology, Cambridge, Mass. Melting points were observed on a calibrated Kofler micro hot-stage.

(22) *Cf.* the conditions for the preparation of 3-indolecarboxaldehyde developed by A. C. Shabica, E. E. Howe, J. B. Ziegler and M. Tishler, THIS JOURNAL, **68**, 1156 (1946).

(23) *Cf.* the conditions of E. Erlenmeyer, *Ann.*, **284**, 49 (1895), and of B. H. Nicolet and E. D. Campbell, THIS JOURNAL, **50**, 1155 (1928), and the demonstration by H. R. Ing. *J. Chem. Soc.*, 2047 (1932), that these reagents lead to acetylation of the imino group.

The residue was dissolved with a mixture of methylene chloride and water. The organic layer was washed with dilute hydrochloric acid and with water. The remainder from the dried ($MgSO_4$) methylene chloride solution was crystallized from methanol; yield 1.95 g. (65%), m.p. and mixed m.p. with VIIA, 179–181°.

4,4′-Dicarboxy-3,3′-diindolylmethane (XI). A. From Methyl α-Carbomethoxy-α-acetylmethylamino-β-(4-cyano-3-indole)-propionate (VII).—To a solution of 4.2 g. (0.075 mole) of potassium hydroxide in 10 ml. of water was added 2.67 g. (0.0075 mole) of VII. The solid ester entered into solution only very slowly. After 5 days at reflux temperature, the solution was diluted with 10 ml. of water and was clarified by filtration (60 mg. of insoluble material, washed with an additional 10 ml. of water). To the filtrate was added 12.5 ml. of 6 *N* hydrochloric acid. After 20 hours at 0°, the precipitate was collected by filtration and was washed with 25 ml. of water. The substance was purified by repeated reprecipitation with dilute hydrochloric acid from its solution in dilute ammonium hydroxide. Unless kept in the dark in an evacuated desiccator, the compound rapidly became colored bright red. For analysis, it was dried *in vacuo* over phosphorus pentoxide at ordinary temperature; yield 480 mg. (37%), m.p. 253–255° [24]; ultraviolet λ_{max} 233 mμ, log ε/2 4.37; 305 mμ, log ε/2 3.79; λ_{min} 257 mμ, log ε/2 3.04.[25]

Anal. Calcd. for $C_{19}H_{14}N_2O_4$ (334.32): C, 68.25; H, 4.22; N, 8.38. Found: C, 67.44; H, 4.29; N, 8.60.

B. From N-Acetyl-3-acetoxymethyl-4-cyanoindole (XVII).—To a solution of 1.5 g. of potassium hydroxide in 5 ml. of water was added 256 mg. (0.001 mole) of XVII. After 2 weeks at reflux temperature the mixture was diluted with water and was clarified by filtration (50 mg. of insoluble material, m.p. 185–195°). The filtrate was acidified with hydrochloric acid; yield 120 mg. (72%), m.p. 253–255°.

C. From 4-Cyano-3-indolemethanol (XVIII).—See experiment X-C below.

4,4′-Dicarbomethoxy-3,3′-diindolylmethane.—To a well agitated mixture of 10 ml. of ether and 3 ml. of 40% aqueous potassium hydroxide at 0° was added, in portions, 1.0 g. of N-methyl-N-nitroso-N′-nitroguanidine.[26] After 3 minutes, the ether phase was decanted and was dried over solid potassium hydroxide pellets. This diazomethane solution was added to a stirred suspension, at 0°, of 167 mg. (0.0005 mole) of 4,4′-dicarboxy-3,3′-diindolylmethane (XI) in 10 ml. of ether. As the acid entered into solution, the ester deposited in crystalline form. After 15 minutes, the ether was allowed to evaporate in the hood. The residue was recrystallized from methanol; yield 100 mg. (55%), m.p. 237–239°.[27]

Anal. Calcd. for $C_{21}H_{18}N_2O_4$ (362.37): C, 69.60; H, 5.01; N, 7.73. Found: C, 69.30; H, 5.11; N, 7.79.

1,2,3,4-Tetrahydro-2-methyl-9H-pyrid[3,4-b]indole-3,5-Dicarboxylic Acid (X). A. From Methyl α-Carbomethoxy-α-acetylmethylamino-β-(4-cyano-3-indole)-propionate (VII).—The filtrate from the separation of 4,4′-dicarboxy-3,3′-diindolylmethane described above in experiment XIA was neutralized with sodium acetate. Crystallization promptly ensued. After 25 hours at 0°, the precipitate was collected by filtration. The filtrate was concentrated under diminished pressure to 25 ml. to afford a second crop. The compound was purified by reprecipitation with sodium acetate from its solution in dilute hydrochloric acid and, for analysis, was recrystallized from a large volume of water; yield 770 mg. (37%), m.p. 265–285°; ultraviolet λ_{max} 231 mμ, log ε 4.38; 305 mμ, log ε 3.67; λ_{min} 252 mμ, log ε 2.61.

Anal. Calcd. for $C_{14}H_{14}N_2O_4$ (274.27): C, 61.30; H, 5.22; N, 10.22. Found: C, 61.04; H, 5.48; N, 10.14.

The substance did not give a color reaction with *p*-dimethylaminobenzaldehyde and sulfuric acid.

B. From (IX) α-Methylamino-β-(4-carboxy-3-indole)-propionic Acid.—To a mixture of 65 mg. (0.00025 mole) of the methylamino acid IX and 0.25 ml. of *N* sulfuric acid in 1 ml. of water was added 0.25 ml. of 37% aqueous formaldehyde.[9] The original suspension slowly dissolved when the mixture was brought to 60°. After 1 hour at reflux temperature, a crystalline deposit had separated from the medium. Dilute ammonium hydroxide was added and the basic solution was maintained at reflux temperature for an additional hour. The solution was acidified with hydrochloric acid and was clarified by filtration. The filtrate was neutralized with sodium acetate. After 15 hours at 0°, the crystalline precipitate was collected by filtration; yield 45 mg. (66%), m.p. and mixed m.p. with XA 265–285°; infrared spectrum identical with that given by XA.

C. From α-Methylamino-β-(4-carboxy-3-indole)-propionic Acid (IX) and 4-Cyano-3-indolemethanol (XVIII).—To a refluxing solution of 262 mg. (0.001 mole) of the methylamino acid IX and 1.1 g. (0.02 mole) of potassium hydroxide in 4 ml. of water was added 380 mg. (0.0022 mole) of 4-cyano-3-indolemethanol (XVIII). The suspended material entered into solution only very slowly.[28] After 5 days at 100°, the mixture was diluted with water and was clarified by filtration (65 mg. of insoluble substance removed). The filtrate was acidified with 3.3 ml. (0.02 mole) of 6 *N* hydrochloric acid. The precipitate of 4,4′-dicarboxy-3,3′-diindolylmethane (XI) was collected by filtration and was washed with water; yield 320 mg. (87%), m.p. 253–255°. The filtrate was concentrated under reduced pressure to a volume of 4 ml. and was neutralized with sodium acetate. After 15 hours at 0°, the crystalline deposit was collected by filtration; yield 260 mg. (95%), m.p. 265–285°.

Dimethyl 1,2,3,4-Tetrahydro-2-methyl-9H-pyrid[3,4-b]-indole-3,5-dicarboxylate.—To a solution of 1.5 g. of sulfuric acid in 10 ml. of methanol was added 100 mg. (0.00036 mole) of the tetrahydrocarbolinecarboxylic acid X. After 15 hours at reflux temperature, the solution was concentrated under reduced pressure. The residue was extracted with ether and dilute aqueous potassium bicarbonate. The remainder from the washed and dried ($MgSO_4$) ether solution was recrystallized from methanol; yield 60 mg. (55%), m.p. 172–174°.

Anal. Calcd. for $C_{16}H_{18}N_2O_4$ (302.32): C, 63.56; H, 6.00; N, 9.23. Found: C, 63.77; H, 6.11; N, 9.19.

α-Acetylmethylamino-β-(4-cyano-3-indole)-propionic Acid (VIII).—To a solution of 1.43 g. (0.004 mole) of methyl α - acetylmethylamino - α - carbomethoxy - β - (4 - cyano-3-indole)-propionate (VII) in 64 ml. of ethanol was added a solution of 0.90 g. (0.016 mole) of potassium hydroxide in 16 ml. of water. After the mixture had been maintained at 0° for 75 hours, the ethanol was distilled under reduced pressure. Water was added and the solution was clarified by filtration. The filtrate was acidified with 5.5 ml. of 6 *N* hydrochloric acid. After 15 hours at 0°, the crystalline deposit was collected by filtration and was recrystallized from water; yield 1.07 g. (94%), m.p. 210–211°.

Anal. Calcd. for $C_{15}H_{15}N_3O_3$ (285.29): C, 63.15; H, 5.30; N, 14.73. Found: C, 63.26; H, 5.57; N, 14.65.

α-Methylamino-β-(4-carboxy-3-indole)-propionic Acid (IX). A. From α-Acetylmethylamino-β-(4-cyano-3-indole)-propionic Acid (VIII).—A solution of 1.14 g. (0.004 mole) of VIII and 1.5 g. of potassium hydroxide in 5 ml. of water was maintained at reflux temperature for 120 hours. The solution was acidified with 4.5 ml. of 6 *N* hydrochloric acid and solid sodium acetate was added to *p*H 5. The mixture was concentrated to dryness under diminished pressure. The residue was extracted with several successive quantitites of ethanol at reflux temperature. The ethanol was decanted and was distilled under reduced pressure. The residue from this distillation was dissolved in 5 ml. of

(24) The melting point of this dicarboxylic acid, although quite sharp and reproducible, is rather surprisingly low in view of the m.p. of the dimethyl ester and of the corresponding dinitrile XIX.

(25) *Cf.* the ultraviolet spectrum of indole-4-carboxylic acid: λ_{max} 229 mμ, log ε 4.42; 312 mμ, log ε 3.92; λ_{min} 250 mμ, log ε 2.56.

(26) A. F. MacKay and G. F. Wright, THIS JOURNAL, **69**, 3028 (1947).

(27) Attempts to prepare the dimethyl ester with methanol and sulfuric acid at ordinary, or at reflux, temperature, led to fracture of the molecule. The sole crystalline product isolated, in small amount, from the complex reaction mixture was 4-carbomethoxyindole, the m.p. of which (65–66°) was previously erroneously recorded[2] as 146–147°.

(28) Inasmuch as 4-cyano-3-indolemethanol (XVIII) is fairly soluble in hot water, the abundant precipitate in evidence at this point doubtless consists for the most part of 4,4′-dicyano-3,3′-diindolylmethane (XIX) which is formed quite rapidly from XVIII in alkaline solution. The difficult hydrolysis of the very slightly soluble dinitrile then requires several days at these concentrations.

water. To this solution was added 10 ml. of 5% aqueous cupric acetate. The apple green precipitate was collected by filtration and was washed with water. The filtrate was concentrated under reduced pressure to yield an additional precipitate which was collected.[29] The combined copper salts were suspended in water and treated with hydrogen sulfide. The copper sulfide was removed by filtration. The filtrate was concentrated under diminished pressure. The residue was recrystallized from 5 ml. of water[30]; yield 580 mg. (55%), m.p. 258–263°; ultraviolet λ_{max} 228 mμ, log ε 4.36; 302 mμ, log ε 3.81; λ_{min} 250 mμ, log ε 2.90.

Anal. Calcd. for $C_{13}H_{14}N_2O_4$ (262.26): C, 59.53; H, 5.38; N, 10.71. Found: C, 59.82; H, 5.41; N, 10.40.

B. From 1-Methyl-2-acetimino-5-(4-cyanoskatylidine)-4-imidazolidinone (XIV).—A solution of 307 mg. (0.001 mole) of XIV in 100 ml. of water containing 80 mg. (0.002 mole) of sodium hydroxide was shaken with hydrogen in the presence of platinum obtained by catalytic reduction of 300 mg. of platinum oxide. After 26.7 ml. of hydrogen had been absorbed during 8 hours (theory 24.8 ml.), the catalyst was removed by filtration and the filtrate was concentrated under reduced pressure to a volume of 4 ml. A quantity of 1.2 g. of potassium hydroxide was added and the mixture was maintained at reflux temperature for 75 hours. The solution was acidified with 3.5 ml. of 6 *N* hydrochloric acid and was brought to *p*H 4 with sodium acetate. The mixture was concentrated to dryness under diminished pressure. The residue was dissolved in water and was clarified by filtration. The copper derivative, obtained by treatment of the filtrate with 5% cupric acetate solution, was decomposed with hydrogen sulfide. The filtrate from the copper sulfide was concentrated *in vacuo* to a volume of 1 ml. After 15 hours at 0°, the crystalline precipitate was collected and was recrystallized from water; yield 15 mg. (5%),[8] m.p., mixed m.p. and infrared spectrum identical with that given by the product derived from procedure IXA.

C.—When a suspension of 3.07 g. (0.01 mole) of the creatinine derivative XIV in 20 ml. of water was shaken with 46 g. of 2% sodium amalgam and the filtrate from the mercury submitted to prolonged hydrolysis at 100° with 30% aqueous potassium hydroxide solution, 2.7 g. of a water-insoluble copper derivative was obtained after treatment of the neutralized solution with cupric acetate. The aqueous filtrate from the copper sulfide resulting from hydrogen sulfide decomposition of the copper derivative failed to crystallize when seeded with the methylamino acid obtained by procedures IXA and IXB.

1-Methyl-5-(4-carboxyskatyl)-hydantoin.—To a solution of 65 mg. (0.00025 mole) of α-methylamino-β-(4-carboxy-3-indole)-propionic acid (IX) in 1 ml. of water was added 324 mg. (0.004 mole) of potassium cyanate. After 1 hour at 100°, the solution was acidified with hydrochloric acid and was maintained at 100° for an additional hour. The crystalline deposit was collected by filtration. The substance was insoluble in the common organic reagents tested and was purified by repeated reprecipitation, with acetic acid, from its solution in dilute ammonium hydroxide; yield 60 mg. (83%), m.p. > 340°.

Anal. Calcd. for $C_{14}H_{13}N_3O_4$ (287.26): C, 58.53; H, 4.56; N, 14.63. Found: C, 58.04; H, 4.49; N, 14.41.

α-Acetylmethylamino-β-(4-carboxy-3-indole)-propionic Acid (XX).—To a solution of 105 mg. (0.0004 mole) of α-methylamino-β-(4-carboxy-3-indole)-propionic acid (IX) in 0.27 ml. of 3 *N* aqueous potassium hydroxide was added at 0°, over a period of 20 minutes, in 10 successive equal portions, a total of 204 mg. (0.002 mole) of acetic anhydride, each addition of which was followed, after 1 minute, by 0.12 ml. (0.00036 mole) of 3 *N* aqueous potassium hydroxide. The solution was acidified with 0.76 ml. of concentrated hydrochloric acid. After 15 hours at 0°, the precipitate was collected by filtration and was recrystallized from water; yield 115 mg. (90%), m.p. 135–137°.

Anal. Calcd. for $C_{15}H_{16}N_2O_5 \cdot H_2O$ (322.31): C, 55.89; H, 5.63; N, 8.69. Found: C, 55.92; H, 5.87; N, 8.77.

1-Acetyl-4-acetylmethylamino-5-acetoxy-1,2-dihydrobenz(cd)indole (XXI).—To a quantity of 5 ml. of acetic anhydride at reflux temperature and in the absence of light was added 65 mg. of potassium cyanide and 322 mg. (0.001 mole) of α-acetylmethylamino-β-(4-carboxy-3-indole)-propionic acid hydrate (XX). After 15 hours at reflux temperature, the acetic anhydride was distilled under diminished pressure. The residue was extracted with 25 ml. of benzene and the black insoluble material was removed by filtration. After 50 ml. of petroleum ether had been added to the filtrate, the solution was again clarified by filtration. The solvents were distilled under reduced pressure. The residue was recrystallized from ethyl acetate; yield 212 mg. (65%), m.p. 189–191°; ultraviolet spectrum λ_{max} 243 mμ, log ε 4.55; 320 mμ, log ε 4.09; 334 mμ, log ε 4.05; λ_{min} 275 mμ, log ε 3.36, 330 mμ, log ε 4.04.[31]

Anal. Calcd. for $C_{18}H_{18}N_2O_4$ (326.34): C, 66.24; H, 5.56; N, 8.59; CH_3CO, 39.57. Found: C, 65.94; H, 5.75; N, 8.71; CH_3CO, 39.13.[19]

The compound gave a yellow color with dilute potassium hydroxide solution but did not give a response with *p*-dimethylaminobenzaldehyde and sulfuric acid.

1-Acetyl-4-acetylmethylamino-5-hydroxy-1,2-dihydrobenz(cd)indole (XXII). A. From 1-Acetyl-4-acetylmethylamino-5-acetoxy-1,2-dihydrobenz(cd)indole (XXI).—To a solution of 50 mg. of XXI in 10 ml. of ethanol at 0° was added 0.1 ml. of 3 *N* aqueous potassium hydroxide. After 4 hours at 0°, the ethanol was distilled under reduced pressure. The residue was dissolved in 1 ml. of water and the clear solution was acidified with acetic acid. The precipitate was collected by filtration and was recrystallized from methanol; m.p. 236–238°.

Anal. Calcd. for $C_{16}H_{16}N_2O_3$ (284.31): C, 67.59; H, 5.67; N, 9.85. Found: C, 67.46; H, 5.61; N, 9.94.

B. From 4-Methylamino-5-hydroxy-1,2-dihydrobenz(cd)-indole Dihydrobromide (XXIII).—To a solution of 240 mg. (0.00066 mole) of the dihydrobromide XXIII in a mixture of 5 ml. of ethanol and 3 ml. of water was added 210 mg. (0.0026 mole) of anhydrous sodium acetate. The mixture was maintained at reflux temperature for 1 hour. To the cooled solution was added 0.5 ml. of acetic anhydride. The mixture was concentrated under reduced pressure and was extracted with methylene chloride. The residue from the washed and dried ($MgSO_4$) methylene chloride solution was recrystallized from methanol; yield 47 mg. (25%), m.p. 236–238°.

4-Methylamino-5-hydroxy-1,2-dihydrobenz(cd)indole Dihydrobromide (XXIII).—A mixture of 270 mg. (0.00083 mole) of 1-acetyl-4-acetylmethylamino-5-acetoxy-1,2-dihydrobenz(cd)indole (XXI), 0.3 ml. of acetic acid and 0.5 ml. of 48% hydrobromic acid was maintained at reflux temperature for 6 hours.[19] The dark purple solution was diluted with 2 ml. of acetic acid. After 15 hours at 0°, the precipitate was collected by filtration and was washed with acetic acid and with anhydrous ether. It was recrystallized from a mixture of 48% hydrobromic acid and glacial acetic acid[32]; yield 180 mg. (60%), m.p. >300°.

Anal. Calcd. for $C_{12}H_{14}N_2OBr_2$ (362.08): N, 7.74. Found: N, 7.56.

N-Acetyl-3-acetoxymethyl-4-cyanoindole (XVII).—A mixture of 0.925 g. (0.00465 mole) of 4-cyanogramine (IV),[2] 2.0 g. of anhydrous sodium acetate and 10 ml. of acetic anhydride was maintained at reflux temperature for 4 hours. The mixture was added to 50 ml. of water. After 2 hours at 0°, the crystalline deposit was collected by filtration and recrystallized from ethanol; yield 1.14 g. (96%), m.p. 162.5–163.5°.

(29) The methylamino acid consistently failed to crystallize without isolation through the copper derivative as described. In order to minimize sacrifice of product due to the appreciable water solubility of the copper derivative, the total solution volume was that required to just dissolve the inorganic salts present.

(30) The product was generally readily soluble in water at this point and deposited only slowly at ordinary, or at low, temperature, even after seeding. However, if the solution were brought toward the boiling point, essentially complete crystallization invariably, and promptly, ensued.

(31) *Cf.* the ultraviolet spectrum of 1-acetyl-4-diacetamino-5-acetoxy-1,2-dihydrobenz(cd)indole[3]: λ_{max} 240 mμ, log ε 4.45; 320 mμ, log ε 3.97; 335 mμ, log ε 3.94; λ_{min}. 275 mμ, log ε 3.16; 330 mμ, log ε 3.91.

(32) This dihydrobromide is much more soluble and more difficult to recrystallize than is the corresponding salt in the primary series[3] which begins to deposit from the refluxing reaction mixture (of comparable concentration) after one-half hour.

Anal. Calcd. for $C_{14}H_{12}N_2O_3$ (256.26): C, 65.61; H, 4.72; N, 10.93. Found: C, 65.61; H, 4.94; N, 10.76.

3-Methoxymethyl-4-cyanoindole (XV). A. From N-Acetyl-3-acetoxymethyl-4-cyanoindole (XVII).—To a solution of 256 mg. (0.001 mole) of XVII in 25 ml. of methanol was added 0.8 ml. (0.002 mole) of 10% aqueous sodium hydroxide. After 5 hours at ordinary temperature, the solution was diluted with water and the methanol was distilled under reduced pressure. The crystalline product was collected by filtration and was recrystallized from a mixture of ethyl acetate and petroleum ether or from a mixture of methanol and water; yield 150 mg. (86%), m.p. 119–120°.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$ (186.21): C, 70.95; H, 5.41; N, 15.05. Found: C, 70.74; H, 5.72; N, 15.18.

B. From Methyl α-Carbomethoxy-α-acetylmethylamino-β-(4-cyano-3-indole)-propionate (VII).—A solution of 357 mg. (0.001 mole) of VII and 23 mg. (0.001 mole) of sodium in 5 ml. of methanol was maintained at reflux temperature for 6 hours. The solution was concentrated under diminished pressure to a volume of 2.5 ml. After 20 hours at 0°, the crystalline deposit, dimethyl acetylaminomalonate sodium,[5] was collected by filtration; yield 200 mg. (89%), m.p. *ca.* 320°.

The sodium derivative was dissolved in methanol and was neutralized with the equivalent quantity of hydrochloric acid. The sodium chloride was removed by filtration and the methanol was distilled under diminished pressure. The residue was extracted with 3 successive quantities of methylcyclohexane from which deposited, at 0°, 100 mg. (56%) of dimethyl acetylmethylaminomalonate, m.p. 62–63°.[5]

The filtrate from the sodium derivative was diluted with water. After 20 hours at 0°, the precipitate was collected by filtration; m.p. and mixed m.p. with XVA, 119–120°, yield 160 mg. (86%).

C. From Ethyl α-Cyano-α-acetamino-β-(4-cyano-3-indole)-propionate.[3]—To a solution of 46 mg. (0.002 mole) of sodium in 10 ml. of methanol was added 648 mg. (0.002 mole) of the cyano ester. After 6 hours at reflux temperature, the solution was diluted with water and the methanol was distilled under reduced pressure. The crystalline precipitate which separated after 24 hours at 0° was collected by filtration and was recrystallized from a mixture of ethanol and water; yield 65 mg. (17%), m.p. and mixed m.p. with XVA 119–120°.

4-Cyano-3-indolemethanol (XVIII). A. With Sodium Borohydride.—To a suspension of 680 mg. (0.004 mole) of 4-cyano-3-indolecarboxaldehyde (XIII) in 0.5 ml. of pyridine was added a solution of 40 mg. (0.001 mole) of sodium borohydride in 1 ml. of pyridine. After the initial evolution of gas had ceased, a second quantity of 40 mg. (0.001 mole) of sodium borohydride was added. The mixture was stirred for 20 minutes and was diluted with 20 ml. of water. After 15 hours at 0°, the crystalline deposit was collected by filtration and was recrystallized from ethyl acetate[33]; yield 600 mg. (82%), m.p. 140–146°[15]; infrared (KBr) 3460, 3230.

Anal. Calcd. for $C_{10}H_8N_2O$ (172.18): C, 69.75; H, 4.68; N, 16.27. Found: C, 69.97; H, 4.91; N, 16.34.

B. With Lithium Aluminum Hydride.—To a suspension of 340 mg. (0.002 mole) of 4-cyano-3-indolecarboxaldehyde (XIII) in 2 ml. of tetrahydrofuran was added dropwise a solution of 37 mg. (0.001 mole) of lithium aluminum hydride in 1 ml. of tetrahydrofuran. A yellow precipitate at once deposited from the medium. A second quantity of 37 mg. (0.001 mole) of lithium aluminum hydride in 1 ml. of tetrahydrofuran was added and the whole was stirred for 10 minutes. Ethanol was added to discharge unreacted hydride. The solution was diluted with water and the low boiling solvents were distilled under reduced pressure. The residue was extracted with ether and was washed with water. The remainder from the dried ($MgSO_4$) ether solution was recrystallized from water; yield 220 mg. (64%), m.p. and mixed m.p. with XVIIIA 139–145°.

(33) This substance exhibited a curious behavior on recrystallization in that it often deposited from ethyl acetate as a mixture of well defined hexagonal plates and long needles. Manual separation of the two forms and recrystallization of the plates alone generally gave exclusively needles. From water, the predominant form appeared to be plates.

4,4′-Dicyano-3,3′-diindolylmethane (XIX). A. From 4-Cyano-3-indolemethanol (XVIII).—A solution of 172 mg. (0.001 mole) of XVIII in 50 ml. of water was maintained at reflux temperature for 45 hours. The insoluble product which separated from the hot solution was collected by filtration and was recrystallized from a mixture of ethanol and water; yield 90 mg. (61%), m.p. 265–270°.

Anal. Calcd. for $C_{19}H_{12}N_4$ (296.32): C, 77.01; H, 4.08; N, 18.91. Found: C, 76.92; H, 4.39; N, 18.94.

When this experiment was repeated in the presence of 140 mg. (0.001 mole) of the formaldehyde acceptor 5,5-dimethylcyclohexane-1,3-dione, a heavy white deposit was formed almost at once. The mixture was kept at reflux temperature for 30 minutes. After 15 hours at 0°, the precipitate was collected by filtration and was suspended in water. The mixture was basified with 3 *N* potassium hydroxide solution. The insoluble material was collected by filtration, was washed with water and was recrystallized from a mixture of ethanol and water; yield 45 mg. (30%), m.p. 265–270°. The filtrate was acidified with hydrochloric acid to yield 210 mg., m.p. *ca.* 250–260°, presumably consisting of a mixture of the dimedon formaldehyde derivative and cross-condensation products.

B. From 4-Cyanoindole (XII).—A solution of 142 mg. (0.001 mole) of 4-cyanoindole and 0.04 ml. (0.0005 mole) of 37% aqueous formaldehyde in 1.4 ml. of acetic acid was kept at ordinary temperature for 60 hours. The precipitate was collected by filtration and was recrystallized from a mixture of ethanol and water; yield 30 mg. (20%), m.p. 265–270°.

α-Acetylmethylamino-β-(3-indole)-propionic Acid.—To a solution of 332 mg. (0.001 mole) of methyl α-acetylmethylamino-α-carbomethoxy-β-(3-indole)-propionate[6] in 16 ml. of ethanol was added a solution of 224 mg. (0.004 mole) of potassium hydroxide in 4 ml. of water. After the mixture had been maintained at 0° for 72 hours, the ethanol was distilled under diminished pressure. The residue was diluted with water and was clarified by filtration. To the filtrate was added 0.66 ml. of 6 *N* hydrochloric acid. After 15 hours at 0°, the precipitate was collected by filtration and was recrystallized from water; yield 210 mg. (76%), m.p. 80–82°.[34]

Anal. Calcd. for $C_{14}H_{16}N_2O_3 \cdot H_2O$ (278.30): C, 60.42; H, 6.52; N, 10.07. Found: C, 60.33; H, 6.44; N, 10.05.

1-Methyl-5-skatylhydantoin.—To a solution of 560 mg. (0.01 mole) of potassium hydroxide in 1.4 ml. of water was added 332 mg. (0.001 mole) of methyl α-acetylmethylamino-α-carbomethoxy-β-(3-indole)-propionate.[6] The solid ester had entirely dissolved after 15 minutes at reflux temperature. After 96 hours at 100°, the solution was acidified with 1.66 ml. of 6 *N* hydrochloric acid solution. The precipitate (70 mg.) was collected by filtration and was washed with water. To the filtrate, which was neutralized with solid sodium acetate, was added 110 mg. (0.01 mole) of potassium cyanate. The solution was maintained at reflux temperature for 30 minutes. Hydrochloric acid was added and the acidified solution was kept under reflux for an additional 30 minutes. After 15 hours at 0°, the precipitate was collected by filtration and was recrystallized from water; yield 120 mg. (50%), m.p. 209–211°[35]; infrared spectrum identical with that given by the hydantoin prepared from a specimen of naturally occurring L-abrine isolated from *Abrus precatorius L.*[36]

Anal. Calcd. for $C_{13}H_{13}N_3O_2$ (243.25): C, 64.18; H, 5.39; N, 17.27. Found: C, 64.09; H, 5.66; N, 17.34.

(34) The only previous mention of this acetyl derivative appears to be the account of W. M. Cahill and R. W. Jackson, *J. Biol. Chem.*, **126**, 29 (1938), who describe the isolation of a product melting at 171° following treatment with ketene of a specimen of the DL-amino acid obtained by racemization of L-abrine with barium hydroxide. It is difficult to reconcile this report with the present finding unless it be assumed that the acetyl derivative fortuitously crystallized in anhydrous form in the earlier work. In this Laboratory, α-acetamino-β-(4-carboxy-3-indole)-propionic acid, α-acetylmethylamino-β-(4-carboxy-3-indole)-propionic acid and α-acetylmethylamino-β-(3-indole)-propionic acid have been isolated from water only as well crystalline monohydrates.

(35) E. J. Miller and W. Robson, *J. Chem. Soc.*, 1910 (1938).

(36) N. Ghatak and R. Kaul, *J. Indian Chem. Soc.*, **9**, 383 (1932); T. Hoshino, *Ann.*, **520**, 31 (1935).

Ethyl α-carbethoxy-α-nitro-β-(4-cyano-3-indole)-propionate was prepared for study as a possible precursor of α-amino substituted β-(4-carboxy-3-indole)-propionic acid derivatives. To a solution of 23 mg. (0.001 mole) of sodium in 3 ml. of absolute ethanol was added 205 mg. (0.001 mole) of ethyl nitromalonate[37] and 341 mg. (0.001 mole) of 4-cyanogramine methiodide (V). After the mixture had been maintained at reflux temperature for 15 hours, the ethanol was distilled under reduced pressure. The residue was dissolved in ether and was washed with dilute hydrochloric acid and with water. The remainder from the dried ($MgSO_4$) ether solution was recrystallized from absolute ethanol; yield 170 mg. (47%), m.p. 131–133°.

Anal. Calcd. for $C_{17}H_{17}N_3O_6$ (359.33): C, 56.82; H, 4.77; N, 11.70. Found: C, 56.75; H, 4.48; N, 11.57.

(37) D. I. Weisblat and D. A. Lyttle, THIS JOURNAL, **71**, 3079 (1949).

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BOSTON 15, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, CORNELL UNIVERSITY MEDICAL COLLEGE]

Oxypressin, a Synthetic Octapeptide Amide with Hormonal Properties[1]

BY PANAYOTIS G. KATSOYANNIS

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A cyclic octapeptide amide containing a cyclic pentapeptide amide moiety identical with the one existing in vasopressin and a tripeptide amide side chain identical with the one existing in oxytocin has been synthesized and tested for biological activity. It was found to possess oxytocic, avian depressor and pressor activities to quantitatively different degrees and in different ratios from those existing in oxytocin and vasopressin.

Oxytocin, the principal uterine-contracting and milk-ejecting hormone of the posterior pituitary gland, has been obtained in highly purified form[2–4] in this Laboratory. Structure I was suggested for

CyS—Tyr—Ileu—Glu(NH_2)—Asp(NH_2)—CyS—Pro—
Leu—Gly(NH_2)

I

the hormone on the basis of degradative studies[5–7] and confirmed by synthesis.[7] Arginine vasopressin, the principal pressor and antidiuretic hormone of the beef posterior pituitary gland, has also been isolated in highly purified form[8] and degradative studies have resulted in the postulation of structure II to represent this hormone.[9–11] It

CyS—Tyr—Phe—Glu(NH_2)—Asp(NH_2)—CyS—Pro—
Arg—Gly(NH_2)

II

will be noted that both oxytocin and vasopressin are octapeptide amides composed of a cyclic pentapeptide amide moiety linked to a tripeptide amide side chain. Six amino acids are common to both hormones and they differ from each other only in the other two amino acids; phenylalanine, present in vasopressin, is replaced by isoleucine in oxytocin and the arginine of vasopressin is replaced by leucine in oxytocin. However, in spite of their closely related composition and structures, oxytocin and vasopressin differ considerably in their biological properties.

It is therefore of interest to find out what effect changes in their structures will have on the biological properties of the hormones, with the aim of possibly correlating these changes in chemical structure with changes in biological properties. This paper describes the synthesis and purification of such a "modified hormone" which we have tentatively named "Oxypressin." This compound is the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. It contains a cyclic pentapeptide amide portion identical with the one existing in vasopressin, linked to the tripeptide amide side chain that is present in oxytocin. In other words oxypressin, III, can be con-

CyS—Tyr—Phe—Glu(NH_2)—Asp(NH_2)—CyS—Pro—
Leu—Gly(NH_2)

III

sidered either as oxytocin with phenylalanine replacing isoleucine in the ring or as vasopressin with leucine replacing arginine in the side chain.

The synthesis of this octapeptide followed the pattern introduced in this Laboratory for the synthesis of the posterior pituitary hormones, in that it involved the preparation of a protected nonapeptide (in this case, S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide), cleavage of the protecting groups with sodium in liquid ammonia and oxidation of the resulting sulfhydryl nonapeptide to the cyclic octapeptide amide. The synthesis of the

(1) This work was supported in part by grants from the National Heart Institute, Public Health Service, Grant H-1675, and Lederle Laboratories Division, American Cyanamid Co.

(2) A. H. Livermore and V. du Vigneaud, *J. Biol. Chem.*, **180**, 365 (1949).

(3) J. G. Pierce and V. du Vigneaud, *ibid.*, **182**, 359; **186**, 77 (1950).

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