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

The Preparation and Cyclization of 4-Carboxy-DL-tryptophan

BY FREDERICK C. UHLE AND STEPHEN H. ROBINSON

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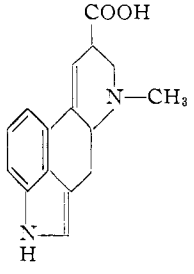
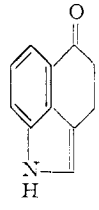
4-Cyanogramine (III) has been allowed to react with ethyl acetaminocyanoacetate in the presence of dimethyl sulfate to yield ethyl α-acetamino-α-cyano-β-(4-cyano-3-indole)-propionate (IV) alkaline hydrolysis of which has afforded 4-carboxy-DL-tryptophan (V). Cyclization of V with acetic anhydride in the presence of potassium cyanide has led to the production of 1-acetyl-4-diacetamino-5-acetoxy-1,2-dihydrobenz[cd]indole (VII).

A preparative endeavor which has been pursued in this Laboratory as a part of a program of study directed toward the total synthesis of lysergic acid (I) has been concerned with efforts to construct the N-methyltetrahydropyridine nucleus of the tetracyclic ergoline ring system as an elaboration of the tricyclic 3,4-trimethyleneindole derivative, 5-keto-



I



II

1,3,4,5-tetrahydrobenz[cd]indole (II). This primary intermediate was first prepared by a specifically developed procedure involving the cyclization of β-(4-carboxy-3-indole)-propionic acid with acetic anhydride at reflux temperature in the presence of potassium cyanide.[1] In succeeding stages of the general synthetic scheme, the α-bromoketone derived from the N-acetyl conjugate of II was allowed to react with appropriate, secondary methylamines to yield tertiary derivatives suitable for final intramolecular condensation.[2]

As an ancillary approach, designed to contribute to a delineation of the scope of the fundamental annulation reaction, as well as to permit an evaluation of an alternative route to the α-amino ketones, the cyclization procedure has been studied with β-(4-carboxy-3-indole)-propionic acid derivatives in which an amino function had been introduced into the α-position prior to ring closure with acetic anhydride. 4-Cyanogramine[1] (III) was allowed to react with the sodium derivative of ethyl acetaminocyanoacetate, in the presence of dimethyl sulfate, to yield the complex ethyl propionate IV. Vigorous alkaline hydrolysis of this cyano ester led to the production of 4-carboxy-DL-tryptophan (V) which was isolated as the hydrochloride or as the slightly soluble copper derivative. Acetylation of the amino acid with acetic anhydride in aqueous solution afforded the N-acetyl derivative VI which crystallized from water as a monohydrate.

When this acetylamino acid was allowed to react with refluxing acetic anhydride in the presence of potassium cyanide for a period of 48 hours, according to the conditions developed for the preparation of 5-keto-1,3,4,5-tetrahydrobenz[cd]indole (II), a nicely crystalline, neutral product was obtained in 58% yield. This substance did not give the color tests characteristic of the indole nucleus and was found to be capable of transformation, with dilute potassium hydroxide solution, to an alkali-soluble substance. Acetyl determination disclosed the presence in the molecule of four acyl functions, cleavage of which, in acid solution, led to the production of a dihydrobromide. On the basis of these properties, as well as of spectroscopic evidence, the compound has been assigned the dihydroazaacenaphthylene formulation VII.

When the ring closure reaction was conducted for shorter periods of time, *e.g.*, for two hours, the tetraacetate VII, which in these instances was separated only by careful, and repeated, fractional crystallization, was accompanied by a second product which proved to be the related triacetyl derivative IX in which the 4-acetamino group had undergone no further attack by the cyclization reagent.[3]

Hydrolysis of the tetraacetate VII with a mixture of acetic and 48% hydrobromic acids led to complete scission of the four acetyl functions to produce the dihydrobromide of 4-amino-5-hydroxy-1,2-dihydrobenz(cd)indole (VIII). The base, which was precipitated in crystalline condition when an aqueous solution of the hydrobromide was treated with sodium acetate, was transformed with acetic anhydride in alkaline, aqueous solution to the triacetate IX, identical with the substance derived from the two-hour cyclization reaction. Treatment of the tetraacetate VII with dilute, aqueous sodium hydroxide in ethanol solution at 0° led to cleavage of the 5-acetoxy-, as well as of the second 4-acetamino-, linkage, to produce 1-acetyl-4-acetamino-5-hydroxy-1,2-dihydrobenz(cd)indole (X).[4]

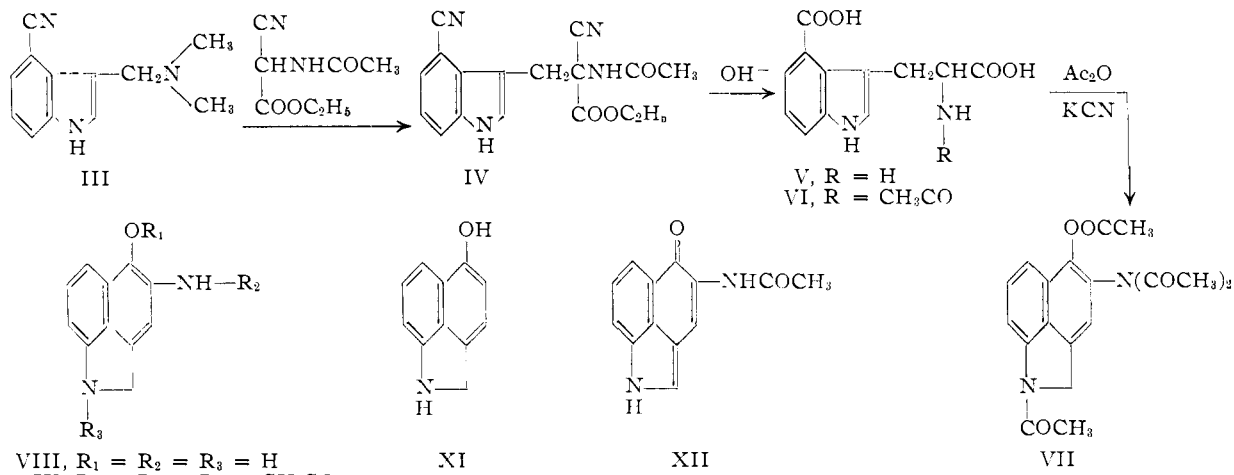
The rearrangement of indoline derivatives of the skeletal type of VIII, characterized by fully aromatic naphthalene nuclei, to the corresponding 1,3,4,5-tetrahydrobenz(cd)indole isomers, has been studied in other laboratories. While the transformation of

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

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

(3) N,N-Diacyl derivatives of aromatic primary amines are not particularly well known substances inasmuch as the relatively mild conditions conventionally employed for acylation lead uniformly to the monosubstituted product. However, it has been demonstrated in a number of instances that prolonged treatment with acetic anhydride results in the introduction of two acetyl functions, one of which, in general, proves more labile under conditions of hydrolysis. *Cf.* F. Ulffers and A. von Janson, *Ber.*, **27**, 93 (1894), J. J. Sudborough, *J. Chem. Soc.*, 533 (1901); K. Fries, R. Walter and K. Schilling, *Ann.*, **516**, 276 (1935).

(4) This compound has been obtained by A. Stoll and J. Rutschmann, *Helv. Chim. Acta*, **35**, 141 (1952), following acetylation of a dithionate reduction product of the azo derivative prepared by treatment of 1-acetyl-5-hydroxy-1,2-dihydrobenz[cd]indole with benzenediazonium chloride.



III IV V, R = H VI, R = CH_3CO

VIII, $R_1 = R_2 = R_3 = H$
IX, $R_1 = R_2 = R_3 = CH_3CO$
X, $R_1 = H$, $R_2 = R_3 = CH_3CO$

XI XII VII

5-hydroxy-1,2-dihydrobenz(cd)indole, XI, to 5-keto-1,3,4,5-tetrahydrobenz(cd)indole (II), was effected with palladium and tetralin in refluxing xylene in the presence of hydrogen,[5] the N-acetyl derivative proved to be stable to conditions favorable for rearrangement in the case of the unacylated molecule.[6] Furthermore, attempts to rearrange more complex, tetracyclic structures isomeric with the total ergoline ring system, have not succeeded.[7,8] It would appear, therefore, that the minimum requirements for indoline to indole rearrangement encompass a free, secondary nitrogen, together with the presence, at position 5, of a phenolic function.

Inasmuch as the aminonaphthol VIII of the present work falls within this classification, the substance may be formally regarded as a potential precursor of the indole tautomer. However, the marked propensity to dehydrogenation characteristic of primary amino derivatives of both naphthalene and indole isomers of this series, when liberated from their salts or acyl conjugates,[4,9] would be expected to impose rather critical experimental conditions for the actual achievement of the transformation. In fact, initial attempts to rearrange the crystalline base VIII, derived from the dihydrobromide, have led to the isolation of the oxidation product, 5-keto-4-acetamino-1,5-dihydrobenz(cd)-indole[4,10] (XII), as the principal end result of the manipulative sequence.

The finding that the acetic anhydride–potassium cyanide cyclization reaction has proceeded, with the aminopropionic acid derivative, to yield the naphthalene structure, as opposed to the formation of the tautomeric indole in the case of the unsubstituted dibasic acid, presents interesting problems for study in this unusual series of substances.

(5) C. A. Grob and J. Voltz, *Helv. Chim. Acta*, **33**, 1796 (1950).
(6) C. A. Grob and P. Payot, *ibid.*, **36**, 839 (1953).
(7) A. Stoll and T. Petrzilka, *ibid.*, **36**, 1125 (1953).
(8) F. R. Atherton, F. Bergel, A. Cohen, B. Heath-Brown and A. H. Rees, *Chemistry and Industry*, 1151 (1953).
(9) A. Stoll, J. Rutschmann and T. Petrzilka, *Helv. Chim. Acta*, **33**, 2257 (1950).
(10) C. A. Grob and B. Hofer, *ibid.*, **36**, 847 (1953).

Experimental[11]

Ethyl α-Acetamino-α-cyano-β-(4-cyano-3-indole)-propionate (IV).—To a solution of 1.38 g. (0.06 mole) of sodium in 50 ml. of absolute ethanol was added a solution of 10.2 g. (0.06 mole) of ethyl acetaminocyanoacetate in 120 ml. of absolute ethanol, followed by 10.0 g. (0.05 mole) of 4-cyanogramine.[1] To this stirred and cooled suspension was added, dropwise, over a period of 1 hour, 12.6 g. (0.10 mole) of redistilled dimethyl sulfate, and the whole allowed to remain at ordinary temperature for a period of 48 hours. Water was then added and the ethanol was distilled under diminished pressure. The precipitate was collected by filtration, washed with water and recrystallized from ethanol; yield 13.4 g. (82.7%), m.p. 238–240°.

Anal. Calcd. for $C_{17}H_{16}N_4O_3$ (324.33): C, 63.08; H, 4.97; N, 17.28. Found: C, 62.67; H, 5.19; N, 17.21.

4-Carboxy-DL-tryptophan (V).—To a solution of 15.8 g. (0.28 mole) of potassium hydroxide in 36 ml. of water was added, in 10 portions, over a period of 3 hours, 9.28 g. (0.028 mole) of the ester IV and the whole allowed to remain at reflux temperature for a period of 60 hours. To the cooled solution was then added 23.2 ml. (0.28 mole) of concentrated hydrochloric acid. When the resulting mixture had been clarified by filtration, crystallization commenced at once. After the solution had been allowed to stand at 0° for 24 hours, the precipitate was collected by filtration and was found to weigh, when dry, 5.42 g. The filtrate was concentrated under diminished pressure and extracted with 30 ml. of ethanol. The residue from distillation of the ethanol yielded, from water, an additional quantity of 1.35 g., affording a total of 6.77 g. (83%) of the crystalline hydrochloride which was recrystallized from dilute hydrochloric acid solution; m.p. 200–225°.

Anal. Calcd. for $C_{12}H_{13}N_2O_4Cl$ (284.70): N, 9.84; Cl, 12.45. Found: N, 9.62; Cl, 12.40.

The salt was then dissolved in water, neutralized with sodium acetate, and converted to the slightly soluble copper derivative by the addition of a saturated aqueous solution of cupric acetate. After 24 hours at 0°, the green deposit was collected by filtration, washed with cold water, resuspended in water and treated with hydrogen sulfide. After the copper sulfide had been removed by filtration, the filtrate was concentrated to dryness under diminished pressure. The residue, which consisted of the nearly pure amino acid, was recrystallized from water; m.p. 280–300°.

Anal. Calcd. for $C_{12}H_{12}N_2O_4$ (248.24): C, 58.06; H, 4.87; N, 11.29. Found: C, 58.03; H, 4.65; N, 11.65.

N-Acetyl-4-carboxy-DL-tryptophan (VI).—To a solution of 1.14 g. (0.004 mole) of 4-carboxy-DL-tryptophan hydrochloride in 20 ml. of water was added 6 ml. (0.012 mole) of 2 *N* aqueous sodium hydroxide solution. To this stirred and cooled solution was then added, over a period of 2 hours and in 10 equal, successive portions, a total of 2.04 g. (0.02

(11) Microanalyses and spectroscopic determinations by Dr. S. M. Nagy and associates of the Mass. Institute of Technology, Cambridge, Mass. The melting points were carried out on the micro-hot-stage and are corrected.

mole) of acetic anhydride, each addition of which was followed, after 10 minutes, by 1.8 ml. (0.0036 mole) of 2 *N* aqueous hydroxide solution. The mixture was then treated with 3.8 ml. of concentrated hydrochloric acid solution and allowed to remain at 0° for 36 hours. The crystalline deposit was collected by filtration and recrystallized from water; yield 1.0 g. (83%), m.p. 146–147°.

Anal. Calcd. for $C_{14}H_{14}N_2O_5 \cdot H_2O$ (308.29): C, 54.54; H, 5.23; N, 9.09. Found: C, 54.21; H, 5.24; N, 8.81.

The hydrate was transformed to the anhydrous substance by repeated evaporation, under diminished pressure, of its solution in a mixture of absolute ethanol and benzene; m.p. 228–229°.

Anal. Calcd. for $C_{14}H_{14}N_2O_5$ (290.27): C, 57.93; H, 4.86; N, 9.65. Found: C, 57.93; H, 5.02; N, 9.46.

1-Acetyl-4-diacetamino-5-acetoxy-1,2-dihydrobenz(cd)indole (VII).—To a quantity of 125 ml. of acetic anhydride at reflux temperature and in the absence of light, was added, in ten portions, over a period of 2 hours, a finely ground mixture of 3.08 g. (0.01 mole) of N-acetyl-4-carboxy-DL-tryptophan monohydrate and 0.65 g. (0.01 mole) of potassium cyanide. After the nearly black solution had been maintained at reflux temperature for a period of 45 hours, the acetic anhydride was distilled under diminished pressure and the residue was extracted with several successive quantities of refluxing benzene. The benzene solution was clarified by filtration and concentrated *in vacuo*. The residue was crystallized from absolute ethanol; yield 2.05 g. (58%), m.p. 190–192°.

Anal. Calcd. for $C_{19}H_{18}N_2O_5$ (354.45): C, 64.50; H, 5.12; N, 7.93; CH_3CO, 48.57. Found: C, 64.38; H, 4.98; N, 8.08; CH_3CO, 48.57.

Ultraviolet absorption spectrum in 95% ethanol, λ_{max} (log ϵ): 240 (4.45), 320 (3.97), 335 (3.94); λ_{min} (log ϵ): 275 (3.16), 330 (3.91).

Cf. the spectrum exhibited by 1-acetyl-5-hydroxy-1,2-dihydrobenz(cd)indole, λ_{max} (log ϵ): 231 (4.63), 320 (4.13), 336 (4.11); λ_{min} (log ϵ): 270 (2.90), 330 (4.10).[5]

The substance gave a yellow color with dilute aqueous sodium hydroxide solution but did not give a color with *p*-dimethylaminobenzaldehyde and sulfuric acid.

1-Acetyl-4-acetamino-5-acetoxy-1,2-dihydrobenz(cd)indole (IX). **A.**—To a quantity of 25 ml. of acetic anhydride at reflux temperature and in the absence of light, was added, in 8 successive portions, over a period of 2 hours, a mixture of 0.616 g. (0.002 mole) of N-acetyl-4-carboxy-DL-tryptophan monohydrate and 0.13 g. (0.002 mole) of potassium cyanide. The nearly black solution was then concentrated to dryness under reduced pressure, the residue was extracted with several successive quantities of refluxing benzene, the solution was clarified by filtration and the benzene was distilled *in vacuo*. The residue was dissolved in a small quantity of methanol at 40° and allowed to stand at 0° for a period of 12 hours. A crystalline deposit which had separated at this time was collected by filtration; weight 70 mg.; m.p. 150–200°. The mother liquor was then concentrated, under nitrogen, to one-half its volume and allowed to remain at 0° for 36 hours, after which a second crop, 215 mg., m.p. 135–195°, was obtained. The combined material, 285 mg., was then dissolved in methanol at 40° and, after 1 hour at 0°, the deposit was collected to yield a product which melted at 237–243°. This material was twice recrystallized from ethanol to afford a substance melting at 253–255°.

Anal. Calcd. for $C_{17}H_{16}N_2O_4$ (312.32): C, 65.37; H, 5.16; N, 8.97; CH_3CO, 41.36. Found: C, 65.25; H, 5.56; N, 8.89; CH_3CO, 41.02.

The methanol mother liquor from the original separation of this higher melting component yielded a product melting at 163–180°, which, after repeated recrystallization from absolute ethanol, afforded a substance, m.p. 190–192°, identical with VII.

B.—To a solution of 50 mg. of the dihydrobromide of VIII in 1 ml. of water was added a few drops of acetic anhydride, followed by an aqueous solution of sodium acetate. The crystalline deposit which separated after a few minutes was dissolved by the addition of ethanol, the solution was brought to *p*H 10 with dilute sodium hydroxide solution and acetic anhydride was again added dropwise. After 15 minutes, the solution was diluted with water, the precipitate was collected by filtration and recrystallized from absolute ethanol; m.p. and mixed m.p. with IX, 253–255°.

4-Amino-5-hydroxy-1,2-dihydrobenz(cd)indole Dihydrobromide (VIII).—To a mixture of 1.5 ml. of glacial acetic acid and 2.5 ml. of 48% hydrobromic acid was added 354 mg. (0.001 mole) of the tetraacetate VII and the whole maintained at reflux temperature for a period of 1 hour. A crystalline deposit began to separate after one-half hour. After 20 hours at 0°, the precipitate was collected by filtration; m.p. > 360°; yield 280 mg. (80%).

Anal. Calcd. for $C_{11}H_{12}N_2OBr_2$ (348.07): C, 37.96; H, 3.48; N, 8.05. Found: C, 37.93; H, 3.72; N, 7.89.

1-Acetyl-4-acetamino-5-hydroxy-1,2-dihydrobenz(cd)indole (X).—To a solution of 106 mg. (0.0003 mole) of the tetraacetate VII in 20 ml. of ethanol at 0° was added 1 ml. (0.003 mole) of 3 *N* aqueous sodium hydroxide. After the solution had been maintained at 0° for 4 hours it was acidified with acetic acid, the ethanol was distilled under diminished pressure, the crystalline solid was collected by filtration with water and recrystallized from ethanol; yield 70 mg. (87%), m.p. 249–251°.[4]

Anal. Calcd. for $C_{15}H_{14}N_2O_3$ (270.28): C, 66.65; H, 5.22; N, 10.37. Found: C, 66.61; H, 5.63; N, 10.26.

Attempted Rearrangement of VIII.—To a solution of 200 mg. of the dihydrobromide of VIII in 1 ml. of water at 0° was added 1 ml. of a saturated aqueous solution of sodium acetate. The base, which began to crystallize within a few minutes, was collected after 1 hour at 0° and washed with a small quantity of water; yield 100 mg., m.p. 135–140°. This material, which presented a light tan appearance after 1 hour in a vacuum desiccator, was added, in small quantities, during a period of 1 hour, to a refluxing mixture of 15 ml. of redistilled tetralin and 25 ml. of xylene in which was suspended 200 mg. of 10% palladium on Norite and through which a rapid stream of hydrogen was passed. The period of reflux was terminated after 5 hours, the catalyst was removed by filtration, and the whole was extracted with several successive portions of 0.1 *N* hydrochloric acid solution. Acetic anhydride was added to the aqueous extract, followed by a saturated solution of sodium acetate. The yellow crystalline deposit, which began to separate within a few minutes and which proved to be **4-acetamino-5-keto-1,5-dihydrobenz(cd)indole,**[4,10] was collected after 1 hour at 0°, washed with water, recrystallized from ethyl acetate, and, finally, from methanol; yield 40 mg., m.p. 254–256°.

Anal. Calcd. for $C_{13}H_{10}N_2O_2$ (226.22): C, 69.01; H, 4.45; N, 12.37. Found: C, 68.81; H, 4.40; N, 12.40.

This experiment was repeated with the exception that, directly after the period of reflux had been discontinued, and while hydrogen was still admitted to the solution, 0.5 ml. of acetic anhydride was added to the mixture. The catalyst was removed by filtration, the xylene was distilled under diminished pressure and petroleum ether was added to the residue. The orange deposit which separated and which was collected by filtration yielded, from methanol, a few milligrams of a substance melting at 262–263° which has not been identified. The mother liquors from this material proved to be composed principally of the dehydrogenation product XII.

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BOSTON 15, MASS.