zations from benzene as white prisms, m.p. 138.5° (reported[17] m.p. 137.5–139°).

Anal. Calcd. for C_6H_7NO: N, 12.84. Found: N, 12.65.

The picrate formed yellow needles from methanol, m.p. 189–190°.[25]

Reaction of V with Ammonia.—A mixture of 0.5 g. of 2-chloro-3-cyanomethylpyridine, 0.1 g. of hydrated copper sulfate and 15 ml. of concentrated ammonium hydroxide was heated in a sealed tube at 135 ± 10° for a period of 42 hours. Insoluble material was separated by filtration and the filtrate was evaporated to dryness *in vacuo* and treated with 5 ml. of cold water. The tan solid was separated and recrystallized from acetic acid–water to yield 105 mg. (19%) of white crystals, m.p. 215–217° dec. The analytical sample had m.p. 219–221.5° dec. after recrystallizations from the same solvent. The 2-aminopyridine-3-acetic acid (VIII), which is somewhat hygroscopic, was dried at 110° *in vacuo* for analysis.

Anal. Calcd. for $C_7H_8N_2O_2 \cdot {}^1/_2H_2O$: C, 52.17; H, 5.63. Found: C, 52.47; H, 5.78.

The aqueous filtrate from the crude product was treated with hydrogen sulfide, the resulting small quantity of copper sulfide separated and the filtrate evaporated to dryness *in vacuo*. The residue was extracted with hot absolute ethanol, which was evaporated. The new residue was dissolved in 5 ml. of absolute ethanol, 30 ml. of acetone was added, a small amount of insoluble material was separated and the solution was concentrated to one-third volume. The 0.11 g. of pale-orange precipitate was recrystallized from absolute ethanol–benzene and from water. There resulted 23 mg. (4.6%) of 2-hydroxypyridine-3-acetic acid, m.p. 240–241° dec. This material showed no melting-point depression on admixture with authentic compound prepared from the 2-chloropyridine-3-acetic acid (*vide infra*).

2-Hydroxypyridine-3-acetic Acid.—A solution of 0.16 g. of 2-chloropyridine-3-acetic acid in 10 ml. of 5% sodium hydroxide was heated in an autoclave at 200° for 4.5 hours. The brown mixture was filtered, acidified with concentrated hydrochloric acid and evaporated to dryness. The residue was extracted with boiling methanol and the solid from evaporation of the extract was recrystallized from water and from methanol. There was thus obtained 95 mg. (66.5%) of white crystals, m.p. 240–241° dec. The compound, which gave a red color with ferric chloride, was dried at 110° *in vacuo*.

Anal. Calcd. for $C_7H_7NO_3$: N, 9.15. Found: N, 9.12.

7-Azaoxindole (IX).—One-tenth gram of VIII was heated under nitrogen at 225° for 10 minutes, after which the residue was subjected to sublimation at 170° (10 mm.). The white needles, m.p. 175°, weighed 56 mg. (67% on the basis of the hemihydrate of VIII). The analytical sample, prepared by another sublimation, had the same melting point; Kägi[19] reported m.p. 175°.

Anal. Calcd. for $C_7H_6N_2O$: N, 20.89. Found: N, 21.18.

Absorption Spectra.—Ultraviolet spectra were determined with a Beckman model DU quartz spectrophotometer from 10^{-4} *M* solutions in cyclohexane, unless otherwise specified.

AMHERST, MASS.

(25) H. C. Chitwood, U. S. Patent 2,557,076, June 19, 1951; *C. A.*, **46**, 145 (1952), reported m.p. 187–188°.

[CONTRIBUTION FROM THE MOORE LABORATORY OF CHEMISTRY, AMHERST COLLEGE]

7-Azaindole. VI. Preparation of 5- and 6-Substituted 7-Azaindoles[1]

BY MICHAEL M. ROBISON,[2] BONNIE L. ROBISON AND FLORENCE P. BUTLER

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7-Azaindoline (2,3-dihydro-7-azaindole) behaves, as expected, like a substituted 2-aminopyridine and undergoes substitution reactions in the pyridine ring without accompanying reaction at the 3-position. Thus the substance was transformed *via* 1-nitro-7-azaindoline to the 5-nitro and 5-amino compounds, while treatment of 7-azaindoline-7-oxide with acetic anhydride produced two derivatives bearing oxygenated functions at the 5- and 6-positions, respectively. Since the substituted azaindolines could be dehydrogenated to azaindoles, these reactions provide a reasonably convenient route to this hitherto inaccessible class of substitution products.

Although 7-azaindole can be prepared in about 50% yield by cyclization of 2-formamido-3-picoline,[3] the extremely harsh conditions of the cyclization render impractical attempts to extend the preparation to azaindoles containing functional groups. Further, the 3-position of 7-azaindole, like that of indole, is most susceptible to substitution reactions,[3a,4] so that the azaindole nucleus itself does not offer ready entry into its pyridine ring. The possible biological interest of azaindoles bearing substituents in the six-membered ring prompted a search for methods of introducing such groups.

The most promising approach to the problem of blocking the pyrrole ring and allowing substitution in the pyridine ring appeared to be *via* 7-azaindoline (I). The structure of this substance, which was first prepared by Kruber,[5] was not demonstrated with certainty, though ultraviolet measurements appeared to substantiate it.[6] The success of several substitution reactions involving the reduction product, however, definitely confirms the 2,3-dihydro formulation.

The reaction of I with fuming nitric acid and sulfuric acid at low temperature produces 1-nitro-7-azaindoline (II) in high yield. The position of the nitro group was indicated by a negative active-hydrogen test and by analogy to the similar reaction which takes place with 2-methylaminopyridine.[7] The nitro compound II on warming with sulfuric acid, is converted to the isomeric 5-nitro-7-azaindoline (III), the site of substitution in which was again indicated by inference from the known case.[7] Further, reduction of the benzoyl derivative of III to 1-benzoyl-5-amino-7-azaindoline and treatment of the amine with nitrous acid afforded a

(1) This investigation was supported by a research grant, number C-2574, from the National Cancer Institute of the National Institutes of Health, Public Health Service.

(2) CIBA Pharmaceutical Products, Inc., Summit, N. J.

(3) (a) M. M. Robison and B. L. Robison, THIS JOURNAL, **77**, 457 (1955); (b) **77**, 6554 (1955).

(4) M. M. Robison and B. L. Robison, *ibid.*, **78**, 1247 (1956).

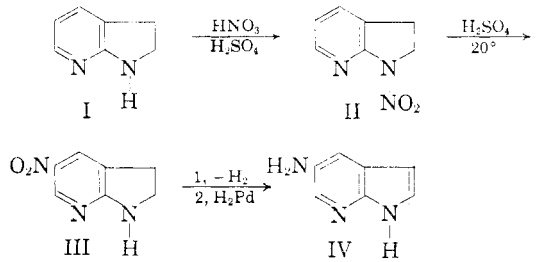
(5) O. Kruber, *Ber.*, **76**, 128 (1943).

(6) M. M. Robison, F. P. Butler and B. L. Robison, THIS JOURNAL **79**, 2573 (1957).

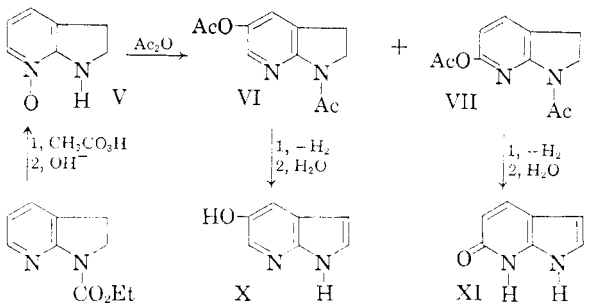
(7) A. E. Chichibabin and A. W. Kirssanov, *Ber.*, **61**, 1223 (1929).

normal diazonium salt, which could be coupled with β-naphthol. On treatment with palladium–charcoal, III can be dehydrogenated to 5-nitro-7-azaindole, which, in turn, can be hydrogenated to 5-amino-7-azaindole (IV).

For comparison, the nitration of 7-azaindole itself was investigated. It was found that the unreduced substance reacts very readily at low temperature to form an acidic mononitro compound. Consideration of other electrophilic substitutions of the nucleus together with the acidic properties of the product lead to its formulation as 3-nitro-7-azaindole.



A second approach to substitution in the pyridine ring involved reactions of 7-azaindoline-7-oxide (V). This derivative, which was prepared by treatment of 1-carbethoxy-7-azaindoline with peracetic acid and subsequent removal of the carbethoxy group, reacts with acetic anhydride to produce rearrangement products bearing acetoxy groups in the pyridine ring. Although this reaction of aromatic N-oxides with acetic anhydride is well known with unsubstituted heterocycles or their alkyl homologs, it has not, apparently, been reported for pyridines bearing functional groups in the α- or γ-position, except for 2-aminopyridine-N-oxide. In this case,[8] rearrangement does not occur



and only the diacetyl derivative of the N-oxide is produced. From the reaction of V two products were obtained, both of which had the compositions of diacetyl derivatives of hydroxyazaindolines. These were readily separated since one, 1-acetyl-5-acetoxy-7-azaindoline (VI), is soluble in dilute acid, while the other, 1-acetyl-6-acetoxy-7-azaindoline (VII), is not. The weakly basic character of VII is consistent, for it has been found in this Laboratory that 2-acetamido-6-acetoxypyridine[9] is also insoluble in cold, dilute hydrochloric acid. Further evidence for the constitution of VII was provided by hydrolysis to 2,3-dihydro-1H-pyrrolo[2,3-b]pyrid-6(7H)-one (VIII) whose infrared spectrum exhibited absorption at 1640 cm.$^{-1}$, ascribable to a pyridone carbonyl group. Hydrolysis of 1-acetyl-5-acetoxy-7-azaindoline (VI), on the other hand, produced the isomeric 5-hydroxy-7-azaindoline (IX), whose infrared spectrum showed no absorption in the carbonyl region. The ultraviolet spectra of the hydrolysis products were also markedly different, while both the infrared and ultraviolet spectra of the two diacetyl derivatives were very similar, as would be expected.

It was also observed that the diacetyl derivatives could be dehydrogenated with palladium–charcoal to the corresponding 7-azaindole compounds. These acetates were not isolated as such, but were hydrolyzed directly to 5-hydroxy-7-azaindole (X) and 1H-pyrrolo[2,3-b]pyrid-6(7H)-one (XI). The latter product, like VIII, showed carbonyl absorption in the infrared, while further evidence was obtained for the constitution of the β-substitution product by a study of the ultraviolet spectra of X in neutral and basic solutions. The latter spectrum closely resembles that of the 5-amino-7-azaindole, and the bathochromic shift of the absorption peaks observed on changing to a medium of higher *p*H is characteristic of β-hydroxypyridine derivatives.[10] A number of examples of such substitutions at a position "*meta*" to a hetero-nitrogen have been reported for these rearrangements,[10,11] but the yield of β-substitution product in this case (21% *vs.* 31% of VII) is considerably higher than usual for such anomalous reactions.

Several attempts were also made to effect a Chichibabin amination of 7-azaindole or 7-azaindoline. The desired 6-amino compounds were not isolated, however, for from the reactions in several solvents only unchanged starting material and decomposition products were obtained.

Experimental[12,13]

1-Nitro-7-azaindoline (II).—A solution of 6.0 g. of 7-azaindoline in 5 ml. of concentrated sulfuric acid was maintained at a temperature of −5° while a mixture of 5.5 g. of fuming nitric acid (d. 1.5) and 5 ml. of concentrated sulfuric acid was added with efficient stirring. The addition was completed in 1 hour, after which the yellow mixture was stirred at the same temperature for an additional 2 hours. It was then poured onto 60 g. of ice and neutralized by addition of ammonium hydroxide with external cooling. The cream-colored product, after thorough washing with water, weighed 8.06 g. (98%) and had m.p. 142–142.5° dec. The analytical sample was prepared by sublimation at 120° (0.07 mm.), by Darco treatment of a solution in dilute hydrochloric acid and reprecipitation, and by recrystallizations from ethyl acetate. The pale-yellow needles had m.p. 145.5–146.5° dec.

Anal. Calcd. for $C_7H_7N_3O_2$: C, 50.91; H, 4.27. Found: C, 50.97; H, 4.33.

5-Nitro-7-azaindoline (III).—The N-nitro compound (7.92 g.) was dissolved as completely as possible in a mixture of 2.5 ml. of water and 7.5 ml. of concentrated sulfuric acid at room temperature, and the thin slurry was added to 50 ml. of cold, concentrated sulfuric acid with efficient stirring. The reaction mixture was maintained at −10 to −15° during the 20-minute addition and the orange solution was then allowed to warm slowly to room temperature overnight, during which period it became dark-brown. It

(8) R. Adams and S. Miyano, THIS JOURNAL, **76**, 2785 (1954).

(9) O. A. Seide and A. I. Titov, *Ber.*, **69**, 1884 (1936).

(10) *Cf.* S. Okuda, *Pharm. Bull. Japan*, **3**, 316 (1955).

(11) *Cf.* M. M. Robison and B. L. Robison, *J. Org. Chem.*, **21**, 1337 (1956), and references cited therein.

(12) Analyses by Drs. Weiler and Strauss, Oxford, England, except for some nitrogen determinations which were carried out by a semimicro Kjeldahl technique in this Laboratory.

(13) Melting points are corrected.

was poured onto 200 g. of ice, the solution was treated with Darco and the product was precipitated with ammonium hydroxide. Although the nitro compound itself is somewhat soluble in strong alkali, it was expedient to wash the crude material thoroughly with 5% sodium hydroxide solution to remove some unknown, highly colored impurity. The treatment was repeated until the base-washings were only faintly colored, after which the yellow powder was washed with water, dried and sublimed at 190° (0.2 mm.) to yield 3.64 g. (46%) of product, m.p. 258–260°. The analytical sample was prepared by recrystallizations from *n*-propyl alcohol and from acetonitrile and by a final sublimation. The yellow crystals had m.p. 260.5–261.5° dec.

Anal. Calcd. for $C_7H_7N_3O_2$: C, 50.91; H, 4.27. Found: C, 51.01; H, 4.38.

1-Benzenesulfonyl-5-nitro-7-azaindoline.—A solution of 165 mg. of III and 0.4 g. of benzenesulfonyl chloride in 3 ml. of pyridine was refluxed for 35 minutes, cooled and poured into 10 ml. of water. The tan crystalline product, after filtration and trituration with dilute hydrochloric acid, with dilute ammonium hydroxide and with water, weighed 245 mg. (80%) and had m.p. 173–177°. The analytical sample was recrystallized from 95% ethanol as white needles, m.p. 177–178°.

Anal. Calcd. for $C_{13}H_{11}N_3O_4S$: C, 51.14; H, 3.63. Found: C, 50.74; H, 3.55.

An attempt was made to oxidize the sulfonamide to the corresponding substituted nicotinic acid, as was done in the case of 7-azaindoline.[5] Difficulties were encountered in the process, however, and when the character of the diazonium salt derived from 1-benzoyl-5-amino-7-azaindole served to establish the position of the substituents, the oxidation experiments were discontinued. It had been planned to prepare the substituted nicotinic acid from 2-benzenesulfonamido-5-nitro-3-picoline for comparison. This oxidation was not carried out, but the hitherto unreported sulfonamide was prepared. 2-Amino-5-nitro-3-picoline[14] was derivatized as in the previous case except that the reflux period was extended to 8 hours, and the strongly acidic product was precipitated from the aqueous solution by addition of hydrochloric acid. The analytical sample was prepared by Darco treatment of a solution in alkali, by sublimation at 160° (0.2 mm.), and by recrystallization from ethanol; the m.p. was 162–163°.

Anal. Calcd. for $C_{12}H_{11}N_3O_4S$: C, 49.14; H, 3.78. Found: C, 49.15; H, 4.10.

1-Benzoyl-5-nitro-7-azaindoline.—A mixture of 165 mg. of the nitro compound, 0.45 g. of benzoic anhydride and one micro-drop of concentrated sulfuric acid in 10 ml. of dry benzene was refluxed for 6 hours, the benzene was evaporated and the residue was triturated thoroughly with cyclohexane, then with dilute aqueous sodium carbonate. The yellow product weighed 0.24 g. and had m.p. 200–204°. The analytical sample, prepared by recrystallizations from ethyl acetate, was obtained in the form of shiny, pale-yellow plates, m.p. 204.5–205.5°.

Anal. Calcd. for $C_{14}H_{11}N_3O_3$: C, 62.45; H, 4.12. Found: C, 62.72; H, 4.10.

Hydrogenation of the Benzoyl Derivative.—The benzamide (269 mg.) was suspended in 80 ml. of 95% ethanol and hydrogenated at atmospheric pressure using 0.25 g. of 5% palladium–charcoal catalyst. After the theoretical hydrogen was absorbed, the catalyst was separated and the solution was evaporated to produce the crude amine. This product was quite unstable and it was not purified as such, but a sample produced a heavy, red precipitate on diazotization and coupling with β-naphthol.[15] The hydrochloride of the benzamide was also unstable, but it was found that the dihydrochloride of the hydrolysis product was considerably easier to purify. The product from a hydrogenation as above was added to 5 ml. of concentrated hydrochloric acid and the mixture was refluxed 3 hours, cooled and extracted with ether. The water layer was then evaporated to a low volume under nitrogen and added to excess acetone and ether to precipitate the product. By dissolution in methanol and precipitation with ether (all operations under dry nitrogen) **5-amino-7-azaindoline dihydrochloride monohydrate** was obtained as a rather unstable solid, m.p. about 223°. The product was also prepared by hydrogenation of III, directly.

Anal. Calcd. for $C_7H_7N_3 \cdot 2HCl \cdot H_2O$: C, 37.18; H, 5.79; N, 18.57. Found: C, 37.50; H, 5.80; N, 18.47.

5-Nitro-7-azaindole.—A mixture of 1.78 g. of III, 0.8 g. of 5% palladium–charcoal and 50 g. of Dowtherm was refluxed 6 hours, cooled, diluted with 50 ml. of benzene and filtered. The filtrate was extracted with concentrated hydrochloric acid, the catalyst was washed with hydrochloric acid and the combined acid solutions were then diluted, filtered through Darco and neutralized with ammonium hydroxide. The bright-yellow product weighed 0.87 g. (50%) and melted at 275–280°. A sample was prepared for analysis by recrystallizations from acetonitrile, from nitromethane and from *n*-propyl alcohol and by sublimation at 180° (0.2 mm.), m.p. 280°.

Anal. Calcd. for $C_7H_5N_3O_2$: C, 51.54; H, 3.09. Found: C, 51.46; H, 2.95.

5-Amino-7-azaindole (IV).—A suspension of 355 mg. of the nitroazaindole and 0.2 g. of 5% palladium–charcoal in 50 ml. of 95% ethanol was stirred with hydrogen at atmospheric pressure until the theoretical hydrogen was absorbed. Filtration and evaporation of the solvent followed by recrystallization of the residue from dry benzene afforded 0.27 g. (93%) of amine, m.p. 124–129°. Recrystallizations from dry benzene and sublimation at 110° (0.3 mm.) produced the white, crystalline analytical sample, m.p. 130.5–131.5°. The substance gives a deep-purple color on treatment with sodium nitroprusside and alkali, in a manner similar to that of 7-azaindole,[3a] while diazotization of the amine gives a product which couples readily with β-naphthol. An ultraviolet spectrum determined from a cyclohexane solution of the amine exhibited maxima at 280 (log ε 3.70) and 326 mμ (3.62), while minima were observed at 254 (3.35) and 304 mμ (3.45).

Anal. Calcd. for $C_7H_7N_3$: C, 63.14; H, 5.30; N, 31.56. Found: C, 63.16; H, 5.11; N, 31.31, 31.56.

3-Nitro-7-azaindole.—To 10 ml. of fuming nitric acid (d. 1.5), maintained at 0°, 1.18 g. of 7-azaindole was added over a period of 20 minutes with shaking. The solution was kept at the same temperature for 1 hour, then poured into 100 g. of ice and water and neutralized with solid sodium bicarbonate. The product, after drying and recrystallization from acetonitrile, weighed 1.35 g. (83%). Further recrystallizations from the same solvent produced small, white needles which began to decompose at 260°, but did not melt by 300°. The product, unlike 5-nitro-7-azaindole, is readily soluble in 5% sodium hydroxide solution, but is insoluble in 5% hydrochloric acid. The acidity of azaindole derivatives bearing an electron-withdrawing substituent at the 3-position has been noted previously.[4]

Anal. Calcd. for $C_7H_5N_3O_2$: C, 51.54; H, 3.09. Found: C, 51.45; H, 3.18.

3-Amino-7-azaindole Dihydrochloride.—One-half gram of 5% palladium–charcoal was added to a suspension of 815 mg. of 3-nitro-7-azaindole in 150 ml. of 95% ethanol, and hydrogenation was carried out at atmospheric pressure. After the gas-uptake was complete, the light-orange solution was filtered directly into 5 ml. of concentrated hydrochloric acid, and the solution was evaporated to dryness *in vacuo*. The resulting 1.01 g. of crude material was purified for analysis by repeated dissolution in hot methanol (Darco) and precipitation by addition of hot acetonitrile. All operations were carried out under nitrogen. The melting point of the salt, which varies with rate of heating, is approximately 253° dec.

Anal. Calcd. for $C_7H_7N_3 \cdot 2HCl$: C, 40.80; H, 4.40; N, 20.39. Found: C, 40.35; H, 4.36; N, 20.36.

The substance gives an immediate, dark-red color with ferric chloride and the test solution, on standing, becomes purple and deposits a black solid. Similar phenomena have been observed on oxidation of 3-aminoindole with this reagent.[16]

1-Carbethoxy-7-azaindoline.—A mixture of 13.2 g. of 7-azaindoline, 8.70 g. of pyridine and 110 ml. of dry benzene was stirred at room temperature while a solution of 11.95 g. of ethyl chloroformate in 110 ml. of dry benzene was added dropwise over a period of 1 hour. The mixture containing

(14) O. Seide, *Ber.*, **57**, 1802 (1924).

(15) R. L. Shriner, R. C. Fuson and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., 1956, New York, N. Y., p. 127.

(16) W. Madelung, *Ann.*, **405**, 92 (1914).

bright-pink salts in suspension was heated at reflux 4 hours, then filtered hot. The precipitate was washed with dry ether and the washings combined with the benzene. After filtration through Darco the solution was evaporated to dryness *in vacuo* and the residue was dried over sulfuric acid to remove pyridine. After washing with low-boiling petroleum ether, the pink product weighed 10.9 g. and melted at 80–83°. Further purification by recrystallization from cyclohexane or *n*-hexane produced white cubes, m.p. 84–85.5°. In the first reactions, however, the product was obtained in a different crystalline modification which melted at 67–68°. It was this sample which was analyzed, after a similar purification. The melting point of the 68° material was unchanged on storage, but on admixture with the 84° modification the substance melted sharply at 83–85°.

Anal. Calcd. for $C_{10}H_{11}N_2O_2$: C, 62.48; H, 6.29. Found: C, 62.28, 62.23; H, 6.43, 6.46.

The pink salts from the reaction mixture were dissolved in water and the solution was made strongly basic with potassium carbonate and extracted with ether. Evaporation of the dried extracts and removal of pyridine *in vacuo* over sulfuric acid left 7-azaindoline. This, after washing with low-boiling petroleum ether and with a small amount of water, weighed 4.6 g. (35%) and melted at 81–85°, undepressed on admixture with starting material.

7-Azaindoline-7-oxide (V).—A mixture of 10.0 g. of the urethan and 13.6 g. of 40% peracetic acid was warmed to about 60° to initiate an exothermic reaction. The solution was cooled as necessary to maintain the temperature at 50–65°, then, after the reaction had moderated in about 1.5 hours, was heated at 65°. After a total reaction period of 5 hours, the mixture was evaporated *in vacuo* below 40°, water was added and the evaporation was repeated. The product was hydrolyzed by adding 52 ml. of 10% aqueous sodium hydroxide and refluxing 1 hour, then neutralizing with acetic acid and evaporating to dryness at a temperature below 40°. The residue was extracted with five 65-ml. portions of boiling chloroform and the extracts were filtered through Darco and evaporated to dryness. The residue, after extraction with 130 ml. of boiling cyclohexane in four portions, consisted of 4.66 g. of fairly pure 7-azaindoline-7-oxide, m.p. 145–152°. Recrystallization from ethyl acetate (Darco) produced 3.25 g. (46%) of thick, cream-colored needles, m.p. 154–156.5°. The analytical sample had m.p. 155–156°. The substance gives a dark-green color with ferric chloride.

Anal. Calcd. for $C_7H_8N_2O$: C, 61.75; H, 5.92; N, 20.58. Found: C, 61.51; H, 6.12; N, 20.46.

Treatment of V with Acetic Anhydride.—Water (0.22 g.) was added to 24.4 g. of acetic anhydride and, after reaction had taken place, 3.25 g. of the oxide V was added and the solution was left at room temperature overnight. The mixture was then refluxed 5 hours and evaporated to dryness *in vacuo*, after which the dark residue was dissolved as completely as possible in ether and the solution was filtered through Darco. The ether was extracted with two 80-ml. portions of cold 5% hydrochloric acid, then washed with a few milliliters of 5% sodium bicarbonate solution and dried. The cold acid layer was neutralized with solid sodium bicarbonate and extracted with ether. Evaporation of this dried ether extract produced a sticky material which was transformed to 1.08 g. of crisp solid, m.p. 114–125°, on repeated extraction with hot, low-boiling petroleum ether. Recrystallizations from dry cyclohexane produced the analytical sample, white needles, m.p. 127.5–129°. The ultraviolet spectrum of the **1-acetyl-5-acetoxy-7-azaindoline (VI)** exhibited maxima at 250 (log ϵ 4.21) and 309 mμ (3.93) and minima at 222 (3.40) and 273 mμ (3.12). The infrared spectrum showed strong absorption bands at 1750 (ester $>C=O$), at 1215 (ester $-\overset{|}{C}-O-$) and at 1650 cm.$^{-1}$ (amide $>C=O$).

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49; N, 12.72. Found: C, 60.19; H, 5.72; N, 12.76.

The above acid-extracted ether solution was evaporated to yield 1.65 g. (31%) of VII, m.p. 94–95.5°. The analytical sample, obtained by recrystallization from dry *n*-hexane, formed white filaments, m.p. 95.5–96°. The ultraviolet spectrum of the **1-acetyl-6-acetoxy-7-azaindoline (VII)** had absorption maxima at 249 (log ϵ 4.12) and 303 mμ (4.02), while minima were observed at 222 (3.48) and 268 mμ (3.12). The ester bands in the infrared were located at 1750 and 1205 cm.$^{-1}$, while the amide carbonyl was found at 1660 cm.$^{-1}$.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.49. Found: C, 60.13; H, 5.61.

5-Hydroxy-7-azaindoline (IX).—One-fourth gram of VI was heated with 1.5 ml. of 5% hydrochloric acid under nitrogen at steam-bath temperature for 40 minutes. The gold solution was made slightly alkaline with ammonium hydroxide and immediately evaporated *in vacuo* at less than 40°. The yellow product was recrystallized from absolute ethanol under nitrogen to produce gold crystals, m.p. 231–233.5° dec., in 76% yield. The analytical sample, prepared by further recrystallizations and by sublimation at 145° (0.09 mm.), was obtained in the form of yellow crystals, m.p. 233.5–235° dec. No explanation was found for the color of IX. It may be noted that both the diacetyl precursor and 5-hydroxy-7-azaindole (*vide infra*) are white, as would be expected. The rather unstable product, which gives an orange ferric chloride test, is soluble in alkali with immediate darkening. The ultraviolet maxima occur at 239 (log ϵ 3.93) and 330 mμ (3.67) and the minima at 225 (3.83) and 270 mμ (2.99), when measured from 95% ethanol solution. In addition, end absorption is noted extending above 400 mμ. The infrared spectrum showed a strong band at 3400 cm.$^{-1}$ and a band at 1590 cm.$^{-1}$ ascribable to the pyridine ring, but no absorption in the carbonyl region above the 1590 cm.$^{-1}$ band.

Anal. Calcd. for $C_7H_8N_2O$: C, 61.75; H, 5.92. Found: C, 61.96; H, 5.88.

2,3-Dihydro-1H-pyrrolo[2,3-b]pyrid-6(7H)-one (VIII).—The hydrolysis of the acid-insoluble diacetate was carried out by the procedure described for VI. Decomposition was more serious in this case and the yield of recrystallized material, m.p. 222.5° dec., was only 49%. The hydrolysis product was soluble in dilute acid. The analytical sample, prepared by further recrystallizations from absolute ethanol under nitrogen and sublimation at 150° (0.1 mm.), was obtained as white crystals of the same melting point. The ultraviolet spectrum showed maxima at 238 (log ϵ 3.84) and 355 mμ (4.19) and a minimum at 280 mμ (2.68). The infrared spectrum contained absorption bands at 3350, 1570 and 1600 cm.$^{-1}$ and a further strong band, ascribable to the pyridone carbonyl at 1640 cm.$^{-1}$.

Anal. Calcd. for $C_7H_8N_2O$: C, 61.75; H, 5.92. Found: C, 61.92; H, 5.99.

5-Hydroxy-7-azaindole (X).—Compound VI (1.090 g.), 0.6 g. of 5% palladium–charcoal and 24 g. of Dowtherm were mixed and refluxed 2.75 hours under nitrogen. The mixture was cooled and filtered, the catalyst was washed with 25 ml. of benzene and the benzene and Dowtherm were combined and extracted with 150 ml. of cold 5% hydrochloric acid, in 5 portions. The water layer was neutralized with ammonium hydroxide and evaporated below 40°, and the dried residue was extracted with three 100-ml. portions of boiling acetonitrile. Evaporation of the acetonitrile left a sticky solid which was hydrolyzed by heating with 3.5 ml. of 5% hydrochloric acid on the steam-bath for 40 minutes. Neutralization of the cooled solution afforded 390 mg. of crude product. Extraction of the catalyst with four 40-ml. portions of boiling 95% ethanol and evaporation of the solvent followed by a hydrolysis as above afforded 67 mg. of additional, crude 5-hydroxy compound. The combined materials were recrystallized from acetonitrile (Darco) to yield 359 mg. (54%) of 5-hydroxy-7-azaindole, m.p. 202.5–203.5° dec. The analytical sample, which was recrystallized from acetonitrile under nitrogen and sublimed at 150° (0.05 mm.), was obtained as large, white cubes, m.p. 206.5–207.5° dec. The product gave a light-red color with ferric chloride and a dark purple color with sodium nitroprusside and base. The infrared spectrum was similar to that of IX. Absorption bands were found at 3400 cm.$^{-1}$ and at 1590 and 1500 cm.$^{-1}$, but no absorption was noted in the carbonyl region. The ultraviolet spectrum of a 95% ethanol solution exhibited maxima at 218 mμ (log ϵ 4.23), and at 288 mμ (3.85) and a minimum at 242 mμ (2.90). In addition there was a shoulder extending from 300 to 310 mμ (3.80). The spectrum of an ethanol solution 0.05 *M* in potassium hydroxide had maxima at 283 (3.88) and 340 mμ (3.85) and minima at 255 (3.57) and 310 mμ (3.50). This curve closely resembles that of 5-amino-7-azaindole.

Anal. Calcd. for $C_7H_6N_2O$: C, 62.67; H, 4.51; N, 20.89. Found: C, 62.49; H, 4.89; N, 21.02.

1H-Pyrrolo[2,3-b]pyrid-6(7H)-one (XI).—The acid-insoluble diacetyl derivative (1.052 g.) was dehydrogenated by the method used for VI. In this case only polymeric materials were obtained on extraction of the catalyst with ethanol. The Dowtherm–benzene layer was extracted with four 30-ml. portions of *concentrated* hydrochloric acid, in this preparation, and the acid was cooled and neutralized with ammonium hydroxide. Evaporation at low temperature, extraction of the dried residue with acetonitrile and evaporation of this solvent left a sticky solid which, after washing with water and drying, weighed 256 mg. (40%) and melted at 208.5–213° dec. This crude material was purified for analysis by recrystallizations from acetonitrile (Darco) under nitrogen and sublimation at 150° (0.1 mm.). The white filaments had m.p. 226–226.5° dec. This substance, which is less stable than X, gives a dark-purple color with sodium nitroprusside and base, a dark purple color with ferric chloride and a violet color, apparently due to decomposition, with base alone. The ultraviolet maxima (ethanol solution) were found at 227 (log ε 4.17) and 332 mμ (3.93) and the minimum was found at 258 mμ (3.08). In the infrared, the compound absorbed at 3400, 1610 and 1650 cm.$^{-1}$. The last band is attributed to the pyridone carbonyl.

Anal. Calcd. for $C_7H_6N_2O$: C, 62.67; H, 4.51; N, 20.89. Found: C, 62.34; H, 4.54; N, 20.75.

Absorption Spectra.—Ultraviolet spectra were determined with a Beckman model DU quartz spectrophotometer from solutions of 10^{-4} to 5×10^{-5} *M* concentration. The solvent was cyclohexane unless otherwise specified. Infrared spectra were determined on a Baird spectrophotometer (KBr disk) by Dr. S. M. Nagy and associates at the Microchemical Laboratory, Massachusetts Institute of Technology.

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The Interaction of α-Chymotrypsin with α-N-Carbethoxy-D- and L-tyrosinmethylamide[1]

BY DAVID T. MANNING AND CARL NIEMANN[2]

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It has been shown that the α-chymotrypsin-catalyzed hydrolysis of acetyl-L-tyrosinhydroxamide, in aqueous solutions at 25° and *p*H 7.6 and 0.27 *M* in the amine component of a THAM–HCl buffer, is competitively inhibited by α-N-carbethoxy-D- and L-tyrosinmethylamide and that the K_I values parallel the K_I and K_S values previously obtained for α-N-carbethoxy-D- and L-tyrosinamide. These observations have been interpreted as providing support for the contention that $K_S \doteq k_2/k_1$ for the system α-chymotrypsin-α-N-carbethoxy-L-tyrosinamide.

Where the dependence of the initial rate of an enzyme-catalyzed reaction upon the initial specific substrate concentration may be represented by equation 1 and where the constant $K_S = (k_2 + k_3)/k_1$ may be evaluated on the basis of equation

$$E_f + S_f \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E_f + P_f \qquad (1)$$

2 the question frequently arises as to whether the value of K_S obtained for a particular system ap-

$$-d[S]/dt = d[P]/dt = k_3[E][S]/(K_S + [S]) \qquad (2)$$

proximates that of one of the two possible limits, *i.e.*, whether $K_S \doteq k_2/k_1$ or $K_S \doteq k_3/k_1$. When first faced with this question[3] it was realized that a general and unambiguous answer was beyond reach but that one arrived at on the basis of knowledge of the behavior of specific substrates and competitive inhibitors that were structurally similar could provide support for the contention that K_S did or did not approximate the limiting value given by k_2/k_1.[3] In the initial study[3] values of K_S obtained for systems involving α-chymotrypsin and acetyl- or nicotinyl-L-tryptophanamide were compared with K_I values obtained for comparable systems involving the competitive interaction of α-chymotrypsin with the hydrolysis products, *i.e.*, acetyl- or nicotinyl-L-tryptophanate ion, and the enantiomorphs of the above specific substrates, *i.e.*, acetyl-, or nicotinyl-D-tryptophanamide. Subsequently the same procedure was employed with respect to the interpretation of K_S values obtained for systems involving α-chymotrypsin and acetyl- or nicotinyl-L-tyrosinamide,[4,5] chloroacetyl- or trifluoroacetyl-L-tyrosinamide[6,7] and acetyl- or nicotinyl-L-phenylalaninamide.[8] In order to provide additional support for the contention that $K_S \doteq k_2/k_1$ in all of the systems referred to above, and particularly for the first pair, Huang and Niemann[9] turned to a comparison of the K_I values of enantiomorphic pairs of competitive inhibitors in which the L-isomer either did not lead to determinable reaction products with the analytical procedure employed or was hydrolyzed so slowly as to justify its evaluation as a competitive inhibitor rather than as a specific substrate. The pairs considered were acetyl-D- and L-tryptophanate ion, D-and L-tryptophanamide and acetyl-D- and L-tryptophanmethylamide.[9]

In all of the above cases the argument that $K_S \doteq k_2/k_1$ was based upon the supposition that when $K_S \doteq k_2/k_1$ the value of K_S will exhibit approximately the same dependence upon the nature of the specific substrate as is seen in the dependence of the value of K_I upon the nature of related competitive inhibitors, particularly when similar dependencies also are observed for comparable pairs of enantiomorphic competitive inhibitors. It is evident that the reliability of an interpretation of the above

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