Photoreductions and Photocyclizations of Tryptophan

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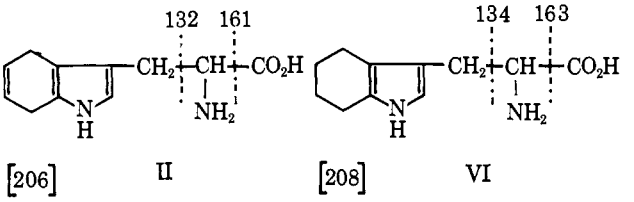
Abstract: Ultraviolet irradiation of aqueous solutions of L-tryptophan in the presence of excess sodium borohydride leads to 4,7-dihydro-L-tryptophan (10%), 2,3-dihydro-L-tryptophan (4%), indole-3-propionic acid (2%), and a trace of tryptamine. The catalytic reduction of 4,7-dihydro-L-tryptophan gives the unstable 4,5,6,7-tetrahydro-L-tryptophan, whose racemate was obtained by alkaline decarboxylation of 2-carbethoxy-4,5,6,7-tetrahydro-DL-tryptophan (ethyl ester). Birch reduction of L-tryptophan gave 4,7-dihydro-L-tryptophan, identical with the main product of the photoreduction, in 55% yield. A novel photocyclization converts N-chloroacetyl-L-tryptophan into the eight-membered lactam IX of L-tryptophan-4-acetic acid. Yields of up to 40% of IX are obtained when the photoinduced cyclization is carried out in neutral aqueous solution.

When L-tryptophan was irradiated with a high-pressure mercury lamp in the presence of sodium borohydride, the indole nucleus was gradually reduced to hydroaromatic tryptophans. In addition, partial photolytic deamination and decarboxylation were observed (Figure 1).

After 2 hr the reaction mixture was chromatographed on Dowex. Elution with pyridine–acetic acid buffer solution permitted the isolation of five fractions A–E. Fraction A was recrystallizable from water, to give colorless crystals, mp 143°, identical with indole-3-propionic acid (IV) (Chart I).

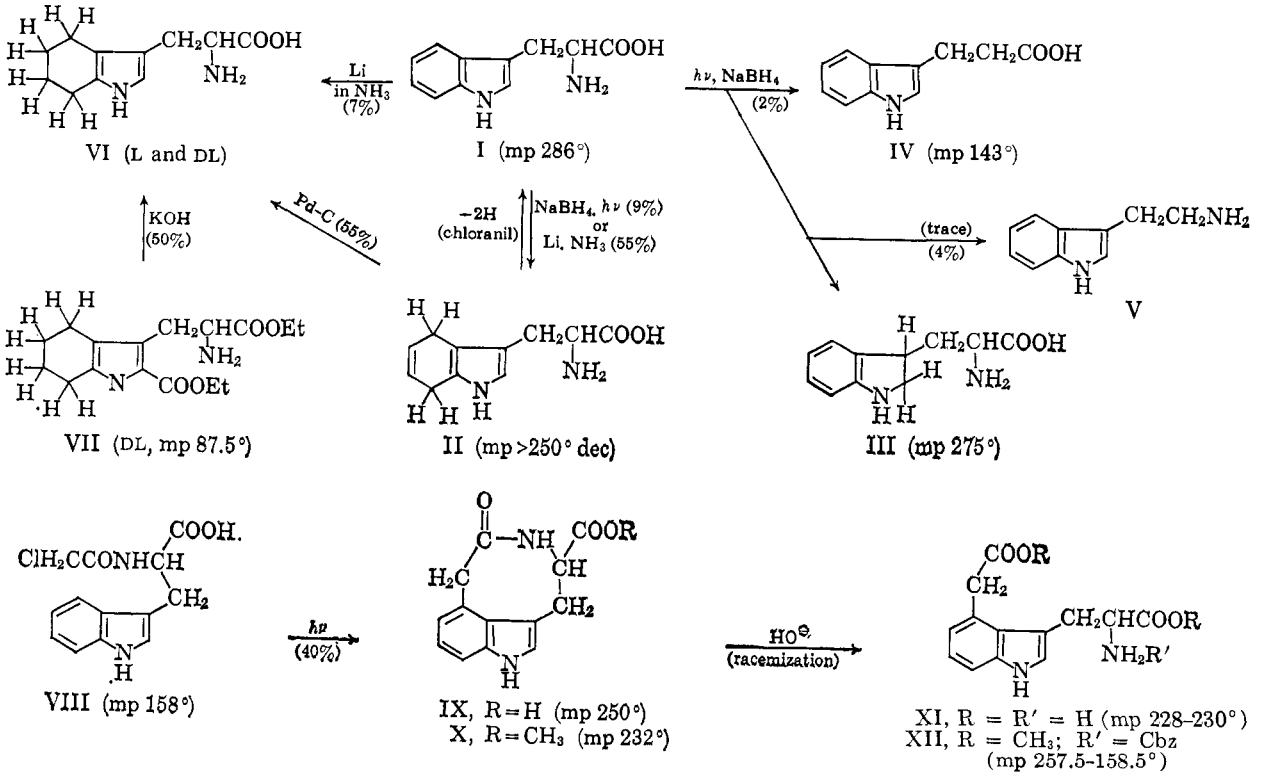
Fraction B, the main product of this photoreduction, gave positive ninhydrin and Ehrlich reactions. On the basis of the mass spectrum, 206 (M), 161 (small), and 132 (very strong), this photoproduct is a dihydrotryptophan. The presence of an intact pyrrole ring



and an unconjugated double bond was concluded from nmr and ultraviolet spectra (λ_{max} 214 mμ). The structure of 4,7-dihydrotryptophan (II) for this product was proved by an independent synthesis, *viz.*, Birch reduction of tryptophan.[3]

Catalytic reduction of 4,7-dihydro-L-tryptophan in the

Chart I



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(3) *Cf.* S. O'Brien and D. C. C. Smith, *J. Chem. Soc.*, 4609 (1960).

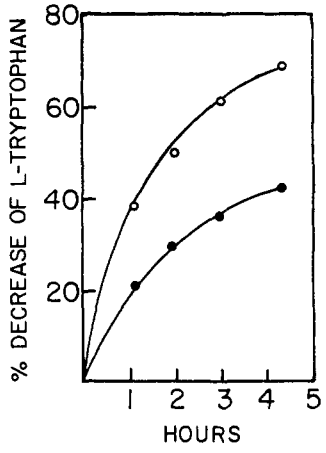


Figure 1. Decrease of the absorption of L-tryptophan at 280 mμ (O) and of the ninhydrin color absorption at 570 mμ (●) as a function of time.

presence of palladium on charcoal in alkaline solution gave (amorphous) tetrahydro-L-tryptophan (VI) with peaks in the mass spectrum at 208 (M), 163, and 134. This tetrahydrotryptophan is identical with the decarboxylation product of 2-carbethoxy-4,5,6,7-tetrahydro-DL-tryptophan ethyl ester[4] with regard to infrared spectra, thin layer chromatography, and paper electrophoresis. Dehydrogenation of II to tryptophan was easily accomplished by chloranil.

Fraction C was purified to give crystalline 2,3-dihydro-L-tryptophan, easily obtainable by a number of routes involving catalytic hydrogenation of L-tryptophan or its N,N′-bistrifluoroacetyl derivative.[5]

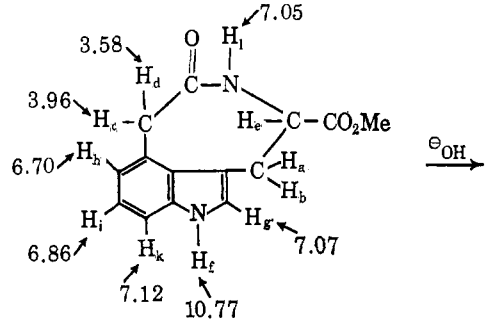
Fraction D contained tryptophan whose recovery under the conditions described approached 50% of the starting material.

Fraction E yielded a trace of tryptamine, an observation which is in accord with the well-known photodecarboxylation of amino acids.

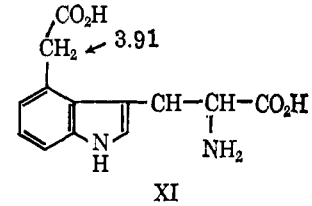
Deamination and decarboxylation of amino acids in aqueous solutions under the influence of radiation from different sources are well known.[6] These reactions have been observed in our experiments even in the presence of $NaBH_4$. *Prima facie* one would expect borohydride to decrease the concentration of free radical initiators by destroying hydroperoxides.[7] Borohydride protects, in fact, tryptophan effectively from the commonly observed photoxidation reactions.[8,9]

Photoreduction of the indole nucleus with $NaBH_4$ leads mainly to two isolable products which have either the pyrrole (II) or the benzene moiety (III) intact. There is, at present, no adequate information about the active reducing species operative in these novel reductions nor about the electronic state of tryptophan involved, and further results from esr and emission spectroscopy have to be awaited.[10] Our studies about the mechanism of the photoreduction of thymidine with $NaBH_4$ suggest the possibility that hydrogen atoms, rather than hydride ions, may be involved.[11]

N-Chloroacetyl-L-tryptophan (VIII) undergoes an interesting cyclization reaction under ultraviolet irradiation. This reaction is accompanied by a concomitant loss of chlorine and proceeds in the presence or absence of $NaBH_4$ (pH 9.5–10). Optimal yields of this photocyclization are obtained in aqueous neutral solution. Photocyclization may be initiated by homolysis[12] rather than heterolysis[13] of the chloroacetyl group. The product was the eight-membered lactam IX[14] of L-tryptophan-4-acetic acid, whose crystalline methyl ester X was easily obtained by the action of diazomethane. The lactam IX and its ester X have typical indole ultraviolet absorption. Their main fragments in the mass spectrogram are 199, 171, 170, 144, and 143, indicative of two methylene groups directly attached to the indole nucleus. The nmr spectrum of X was measured in deuterated dimethyl sulfoxide at 20 and 50° and in a mixture of D_2O and deuterated dimethyl sulfoxide (Figure 2). The tentative assignments for X are summarized as follows:



X



XI

Saponification of the ester X by 4.0 *N* sodium hydroxide at 120–130° gave an amino acid XI which, on the basis of electrophoretic migration, must be diacidic. In the nmr spectrum of the diacid XI a singlet at δ 3.91 corresponds to the peaks H_c and H_d of the parent lactam ester X.

An authentic sample of DL-tryptophan-4-acetic acid, made by an independent route[15] and obtained through the courtesy of Professor Plieninger, turned out to be identical with the saponification product of X with regard to thin layer chromatography, paper electrophoresis, and infrared spectra.

(4) H. M. Kissman and B. Witkop, *J. Am. Chem. Soc.*, 75, 1967 (1953).
(5) A. Mauger and B. Witkop, unpublished observations.
(6) *Cf.* A. D. McLaren and D. Shugar in "Photochemistry of Proteins and Nucleic Acids," P. Alexander and Z. M. Bacq, Ed., The Macmillan Co., New York, N. Y., 1964, pp 88–108.
(7) The question of how far borohydride will reduce radicals or undergo one-electron transfer reactions is linked with whether aqueous solutions of borohydride are a source of hydride ions and protons only (*cf.* R. E. Davis, E. Bromels, and C. L. Kibby, *J. Am. Chem. Soc.*, 84, 885 (1962)), or whether they, under conditions of radiation, are capable of generating hydrogen atoms.
(8) Z. Yoshida and M. Kato, *ibid.*, 76, 311 (1954).
(9) G. Matsuda, *Nagasaki Igakkai Zasshi*, 28, 438 (1953); *Chem. Abstr.*, 48, 6842*d* (1954).
(10) P. Douzou and M. Ptak, *J. Chim. Phys.*, 61, 1681 (1964).
(11) G. Ballé, P. Cerutti, and B. Witkop, *J. Am. Chem. Soc.*, 88, 3946 (1966).
(12) *Cf.* P. L. Lee and F. H. Westheimer, *Biochemistry*, 5, 837 (1966).
(13) H. E. Zimmerman, *Advan. Photochem.*, 1, 199 (1963).
(14) The correct name of this tricycle is 4-carboxy-6-oxo-3,4,6,7-tetrahydro-1H,5H-azocin[4,5,6-*cd*]indole.
(15) H. Plieninger, M. Höbel, and V. Liede, *Chem. Ber.*, 96, 1618 (1963).

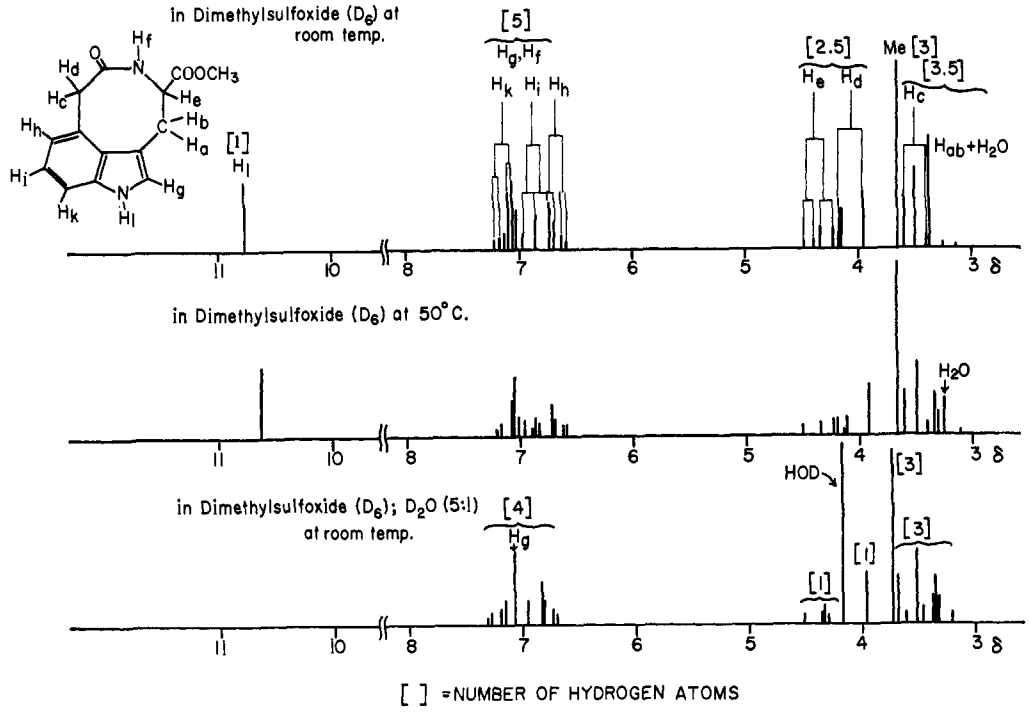
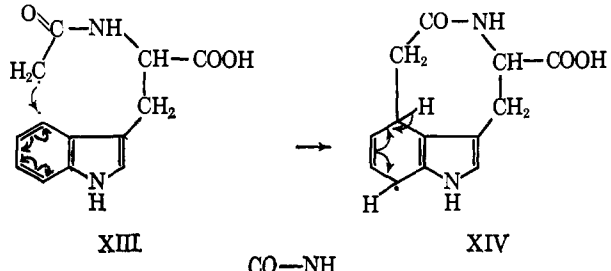


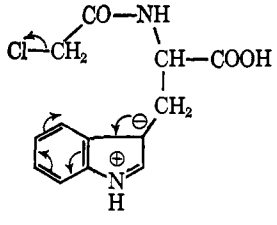
Figure 2. Nmr spectra of ester X in pure and aqueous dimethyl sufoxide at 20 and 50°.

Both compounds were converted to identical dimethyl N-carbobenzyloxy-DL-tryptophan-4-acetate (XII) which gave superimposable infrared and mass spectra, identical thin layer chromatograms, melting points, and mixture melting points. Ring opening of the ester X by alkali is, therefore, accompanied by racemization.

Photolytic homolysis of the halogen–carbon bond and intramolecular aromatic substitution by the free radical XIII at C-4 of the indole nucleus (XIV) are likely to be basic steps in this novel photocyclization.

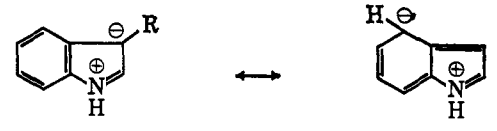


XIII XIV



XV

However a concerted intramolecular cyclization with extrusion of chloride ion XV may also have to be considered as a possible alternative, especially so since the contribution of an anionic species (XVI ⇄ XVII) seems to be more important in the excited state than in the ground state of the indole moiety.[16,17]

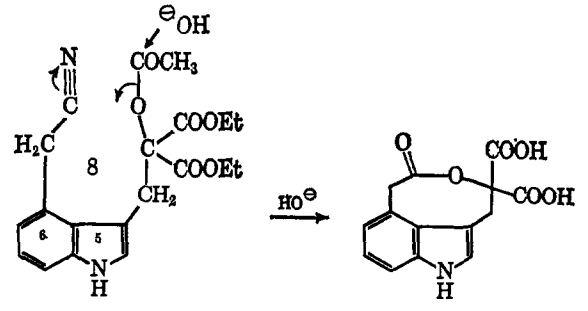


XVI XVII

Presently we are studying the scope and limitations of this novel photocyclization by varying the nature of the halide, the length of the haloacyl group, and the character of the (substituted) aromatic system. Apart from mechanistic insights these studies will provide preparative approaches to interesting new heterocyclic compounds.

The photoactivation and participation of the 4 position of indoles and tryptophan has theoretical and practical connotations for the synthesis and biosynthesis of the ergot alkaloids.[18]

A precedent for the photocyclization of an α-halo ketone may be the photoinduced rearrangement of *p*-hydroxyphenacyl chloride (XVIII) to ethyl *p*-hydroxyphenylacetate (XX) which presumably proceeds through the bicyclic intermediate spiroketone XIX.[19]



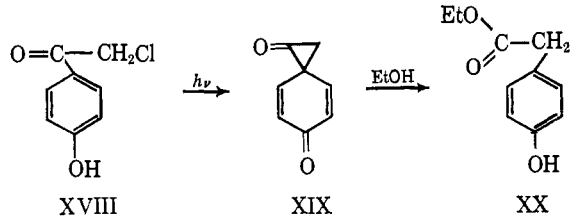
i ii

(16) *Cf.*, G. Weber, "Light and Life," W. D. McElroy and B. Glass, Ed., The Johns Hopkins Press, Baltimore, Md., 1961, p 87.

(17) The formation of an eight-membered ring attached to the 3 and 4 positions of indole has a precedent in the easy lactonization of i to ii: H. Plieninger and W. Müller, *Chem. Ber.*, 93, 2024 (1960).

(18) *Cf.* F. Weygand and H. G. Floss, *Angew. Chem.*, 75, 783 (1963).

(19) J. C. Anderson and C. B. Reese, *Tetrahedron Letters*, 1, 1 (1962).



XVIII XIX XX

Experimental Section

Methods. All melting points are uncorrected. The light source for reductive photolysis was a high-pressure Hg lamp (Hanovia, 200 W, Type S, No. 654A-36 from Hanovia Lamp Division, Engelhard Hanovia, Inc., Newark, N. J.) with Vycor filter (Hanovia, Vycor 7910). The amino acid solution was irradiated inside a cooling jacket surrounded by two semicircular irradiation chambers made of quartz. During irradiation a steady stream of nitrogen was bubbled through the solution.

The ultraviolet spectra were measured with a Cary recording spectrometer Model 14. Infrared spectra were determined with a Perkin-Elmer 237B grating infrared spectrophotometer. Proton magnetic resonance spectra were measured with a Varian Associates Model A-60 instrument. Mass spectra were determined with the AEI Model MS-9.

For thin layer chromatography the systems 1-butanol–acetic acid–water (6:2:2 by volume) and ethanol–chloroform–water (20:20:3) on silica gel G (E. Merck AG) were used.

Reductive Photolysis of L-Tryptophan. An aqueous solution (200 ml) of L-tryptophan (I, 0.408 g) and sodium borohydride (0.8 g) was divided into two semicircular quartz chambers and irradiated for 2 hr. Four batches totaling a volume of 800 ml were combined and brought to pH 6.6 through the addition of Dowex 50-X8 (RH form). The mixture was filtered and the resin was washed with 5% aqueous pyridine solution. The pale brown residue which remained after removal of the aqueous pyridine was freed from boric acid by addition and evaporation of methanol. The viscous material (1.560 g) was dissolved in 0.375 *M* pyridine–acetic acid buffer solution (pH 6.0) and chromatographed on a buffered Dowex 50-X8 column (5 × 77 cm). Elution with the same buffer solution gave fractions A and B. After adjustment of the pH of the buffer solution to 7.0 fractions C and D were separated. Finally fraction E was eluted with 0.375 *M* pyridine solution.

Fraction A was crude indole-3-propionic acid (IV, 29 mg, 1.9%) which was recrystallized from water, mp 143°, and identified with an authentic sample by melting point infrared spectra.

Fraction B was crude 4,7-dihydro-L-tryptophan (II, 159 mg, 9.4%). After rechromatography on a silica gel column (1.5 × 10 cm), elution with the solvent (chloroform–ethanol–water, 20:20:3), and evaporation, the amino acid crystallized from 40% ethanol in the form of pale yellow leaflets which did not melt but charred progressively at >250°. The molecular weight of 206 was ascertained by mass spectrum, $[\alpha]^{20}D$ −3.5 ± 3.0° (*c* 1.15, 1.0 *N* NaOH).

Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.05; H, 6.84; N, 13.58. Found: C, 63.80; H, 6.55; N, 13.51.

4,7-Dihydro-L-tryptophan gave a mauve-pink color with Ehrlich's reagent, a blue-green coloration with the modified Ehrlich's reagent,[20] and a purple color with ninhydrin reagent. The ultraviolet spectrum in 0.1 *N* NaOH showed λ_{max} 214 mμ (ε 6100); infrared spectrum (in Nujol): 2.95 μ (pyrrole NH), 6.35 (carboxylate anion), 14.85 (*cis*-CHC=H–). The nmr spectrum of the sodium salt of II in D_2O showed peaks at δ 6.68 (intensity 1), 5.97 (2), 3.62 (1), 3.22 (4), 2.83 (2).

Fraction C weighed 67 mg (4%) and consisted of **2,3-dihydro-L-tryptophan** (III) which was recrystallized from 70% ethanol to form colorless crystals, mp 275° dec, $[\alpha]^{20}D$ −10.7 ± 2.0° (*c* 1.4, 1.0 *N* NaOH).

Anal. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.05, H, 6.84; N, 13.58. Found: C, 63.75; H, 6.69; N, 13.31.

2,3-Dihydro-L-tryptophan gave a yellow color with Ehrlich's reagent, a pink coloration with modified Ehrlich's reagent, and a strong purple color with ninhydrin reagent. It was identical with an authentic sample prepared by another route with regard to mixture melting point, thin layer chromatography, paper electrophoresis (pH 1.9 and 6.5), and infrared spectra.

(20) Th. Wieland and H. Merz, *Chem. Ber.*, 85, 731 (1952).

Fraction D consisted of 804 mg (49%) of **recovered tryptophan** (I) which was recrystallized from dilute ethanol to give colorless leaflets, mp 286° dec, identical with an authentic sample by mixture melting point, thin layer chromatography, paper electrophoresis, and infrared spectra.

Fraction E was a trace of **tryptamine** (V) which was confirmed by simultaneous paper electrophoresis (pH 1.9 and 6.5) with an authentic sample of tryptamine.

Birch Reduction of L-Tryptophan. To a solution of 3.06 g of L-tryptophan in 500 ml of dry liquid ammonia was added (stirring, Dry Ice–acetone cooling) 4.0 g of lithium gradually in small pieces. After 40 min methanol was run in slowly until the blue color disappeared and then 44 g of ammonium acetate was added. The whole mixture was allowed to evaporate at room temperature overnight. The residue was dissolved in 0.06 *M* pyridine–acetic acid buffer solution (pH 6.0) and chromatographed on a buffered Dowex 50-X8 column (4 × 30 cm). Elution with the same buffer solution gave first 220 mg (7%) of crude 4,5,6,7-L-tetrahydrotryptophan (VI) and as a subsequent fraction 1.70 g (55%) of crude 4,7-dihydrotryptophan (II).

The crude dihydro compound II was recrystallized three times from 50% methanol to give 1.02 g of pale yellow leaflets, mp >250°. The identity of this dihydrotryptophan with the analogous product of the control was established by thin layer chromatography, paper electrophoresis, and ultraviolet, infrared, and nmr spectra.

Dehydrogenation of 4,7-dihydro-L-tryptophan (II) to L-Tryptophan. To 10 mg of 4,7-dihydro-L-tryptophan in 2.5 ml of 80% ethanol was added 13 mg of chloranil with agitation. The mixture was refluxed for 10 min. The dehydrogenation product was homogeneous and was shown by paper electrophoresis and thin layer chromatography to be identical with tryptophan.

4,5,6,7-Tetrahydro-L-tryptophan. A. The crude tetrahydrotryptophan VI was rechromatographed on a column (1.5 × 20 cm) of IRC-50 ($H^{\oplus}$ form). After elution with water, lyophilization gave 120 mg of VI as a colorless powder, which so far has failed to crystallize. Tetrahydrotryptophan gave a pink color with Ehrlich's reagent, a green color with *p*-dimethylaminocinnamaldehyde, and a purple color with ninhydrin reagent.

The ultraviolet spectrum (H_2O) showed λ_{max} 212 (ε 8100); infrared spectrum (Nujol): 3.0 μ (pyrrole NH), 6.23 μ (carboxylate anion); nmr spectrum: δ 6.63 (intensity 0.5 ?), 3.83 (1), 2.95 (2), 2.46 (4), 1.72 (4); mass spectrum: 208 (molecular peak for $C_{11}H_{16}N_2O_2$), 134 (characteristic 4,5,6,7-indole-3-methylene fragment).

4,5,6,7-Tetrahydro-L-tryptophan is identical with the synthetic DL compound VI by the criteria of infrared, nmr, and mass spectra, thin layer chromatography, and paper electrophoresis.

B. By Catalytic Hydrogenation of 4,7-Dihydro-L-tryptophan (II) in Alkaline Medium. To a solution of 200 mg of 10% palladium on charcoal, prereduced with hydrogen, was added 412 mg of 4,7-dihydro-L-tryptophan (II) in 8 ml of 0.5 *N* sodium hydroxide solution and the hydrogenation continued. After 1 hr the solution was filtered and chromatographed on a buffered Dowex 50-X8 column (2.5 × 30 cm). Elution with 0.06 *M* pyridine–acetic acid buffer solution (pH 6.0) gave 309 mg (74%) of crude 4,5,6,7-tetrahydro-L-tryptophan (VI). The strength of the buffer solution was now increased to 0.375 *M* pyridine–acetic acid (pH 7). Elution with this buffer gave 24 mg (5.7%) of L-tryptophan which was recrystallized from aqueous ethanol to give colorless leaflets, mp 275° dec. The tryptophan was identified with an authentic sample by melting point and infrared spectra.

The crude tetrahydro-L-tryptophan was rechromatographed on a column (1.5 × 20 cm) of IRC-50 (RH form), eluted with water, and lyophilized to give the pure tetrahydro compound VI as a colorless powder, identical with the synthetic racemic compound by the criteria of infrared and nmr spectra.

C. By Catalytic Hydrogenation of 4,7-Dihydro-L-tryptophan in Acidic Medium. To a solution of 7.0 mg of 10% palladium on charcoal, presaturated with hydrogen in 10 ml of water, was added a solution of 309 mg of 4,7-dihydro-L-tryptophan in 2 ml of 1.0 *N* hydrochloric acid and 35 ml of water. After 2 hr, 19.5 ml (58% of 1 mmole) of hydrogen was absorbed. The solution was filtered and chromatographed on a column (4 × 23 cm) of Dowex 50-X8. Elution with a 0.375 *M* pyridine–acetic acid buffer solution gave 172 mg (55%) of the crude tetrahydro compound VI, together with a trace of a contaminant reminiscent in its basicity of 2,3-dihydrotryptophan. In addition there was obtained 1.23 mg (40%) of crude L-tryptophan. Purification and confirmation followed the methods described above.

4,5,6,7-Tetrahydro-DL-tryptophan by Decarbethoxylation of 2-Carbethoxy-DL-tryptophan. A suspension of 924 mg of 2-carbethoxy-DL-tryptophan ethyl ester[4] (VII) was suspended in 10 ml of 10% potassium hydroxide and refluxed for 26 hr. The red solution was fractionated on a buffer column (2 × 40 cm; Dowex 50-X8) and eluted with 0.6 *M* pyridine–acetic acid buffer solution (pH 6.0) to give 316 mg (50%) of crude tetrahydrotryptophan as a light brown powder, which was rechromatographed on IRC-50 (RH form), eluted with water, and lyophilized to give a homogeneous colorless powder.

The infrared spectrum (Nujol) showed 3.0 μ (pyrrole NH), 6.23 μ (COO^-); nmr spectrum (D_2O): δ 6.63 (intensity 0.4?), 3.83 (1), 2.15 (2), 2.46 (4), 1.72 (4); mass spectrum: 208 (molecular peak), 134 (4,5,6,7-tetrahydroindole-3-methylene fragment).

Photocyclization of N-Chloroacetyl-L-tryptophan. 4-Carboxy-6-oxo-3,4,6,7-tetrahydro-1H,5H-azocin[4,5,6-*c*,*d*]indole (IX). Method A. A solution of 561 mg of N-chloroacetyl-L-tryptophan and 800 mg of sodium borohydride in 200 ml of water was irradiated for 30 min. The solution was then adjusted to pH 2 by the addition of 10% hydrochloric acid, under ice cooling, and extracted with ethyl acetate. The ethyl acetate extract was dried over anhydrous sodium sulfate and evaporated to leave 489 mg of a pale yellow residue which was triturated with 15 ml of ethanol to give 89 mg (18%) of an insoluble colorless powder which was collected by filtration. Recrystallization from aqueous methanol gave fine needles, mp 250° dec, of the lactam IX.

Anal. Calcd for $C_{13}H_{12}N_2O_3$: C, 63.92; H, 4.95; N, 11.47. Found: C, 63.76; H, 5.27; N, 11.74.

The infrared spectrum showed 2.93 μ, 2.97, 5.38, 6.25, 13.40; ultraviolet spectrum: λ_{max} 280 mμ (ϵ 5400); mass spectrum: 244 (M), 199, 171, 170, 144, 143. The compound gave a blue color with Ehrlich's reagent.

Methyl Ester. The lactam IX was esterified with diazomethane in ether–methanol. After evaporation of the solvent, the residual colorless powder was recrystallized from methanol to give X as fine needles, mp 232° dec, $[\alpha]^{20}D$ −55 ± 3° (*c* 0.35, ethanol).

Anal. Calcd for $C_{14}H_{14}N_2O_3$: C, 65.10; H, 5.46; N, 10.85. Found: C, 64.58; H, 5.66; N, 10.62.

The infrared spectrum showed 3.00 μ, 3.09, 5.76, 6.06, 13.30; ultraviolet spectrum: λ_{max} 280 mμ (ϵ 5500); mass spectrum: 259 (M + 1), 258 (M), 199, 198, 171, 170, 144, 143.

Method B. The photoreduction of 561 mg of N-chloroacetyltryptophan was extended to 50 min under the conditions described above. The solution was neutralized (pH 7.5) by the addition of 10% hydrochloric acid and concentrated to 40 ml. After further addition of 10% hydrochloric acid (pH 2.0), a white powder precipitated which was collected by filtration. The filtrate was extracted with ethyl acetate. The precipitate and the pale yellow powder which remained after evaporation of ethyl acetate were combined, dissolved in methanol, and treated with diazomethane in ether three times.

After evaporation of the solvent the residual pale yellow powder was triturated with a small volume of methanol. The insoluble powder was collected and washed with methanol to give 55 mg, mp 228–230° dec, identical with the above methyl ester. The filtrate was chromatographed on a column of silica gel. Elution with ether–methanol (20:1) gave 158 mg (30.5%) of N-acetyl-L-tryptophan (XI) and 43 mg of crude methyl ester which was recrystallized from aqueous methanol to give colorless crystals, mp 232° dec. N-Acetyl-L-tryptophan was identified with an authentic sample by infrared spectra and thin layer chromatography (methanol–ether 1:20).

Method C. Omission of Borohydride, Alkaline Solution. The solution of 280.5 mg (1 mmole) of N-chloroacetyl-L-tryptophan in 1 ml of 2.5 *N* sodium hydroxide and 99 ml of water was irradiated for 45 min. The yellow reaction mixture was brought to pH 7 by the addition of 10% hydrochloric acid, evaporated under reduced pressure to 15 ml, and acidified (congo red acidic) with 10% hydrochloric acid. The pale orange powder which precipitated was collected by filtration, dissolved in a small volume of methanol, and treated with diazomethane in ether. After evaporation of the solvents, recrystallization from methanol gave 45 mg (17.6%) of crude X, mp 225–229°. Two more recrystallizations from methanol gave fine needles of the ester X, mp 230–232°.

Method D. Neutral Aqueous Solution. The solution of 280.5 mg of N-chloroacetyl-L-tryptophan in 100 ml of water was irradiated for 45 min. The resulting colorless solution was treated as described above. There was obtained 203 mg (40%) of crude ester X, mp 226–230°. Two more recrystallizations gave fine needles, mp 230–232°.

Tryptophan-4-acetic Acid (XI). A solution of 80 mg of the lactam IX in 2 ml of 4.0 *N* sodium hydroxide was heated at 120–130° in a sealed tube. After 3 hr the pale yellow solution was chromatographed on a column (1 × 25 cm) of Dowex 50-X8. Elution with 0.5% aqueous pyridine, evaporation, and recrystallization from 50% methanol gave 41 mg of *rac*-tryptophan-4-acetic acid (X) as a fine colorless powder, mp 228–230° dec, identical with an authentic sample of racemic X[15] by the criteria of thin layer chromatography, paper electrophoresis, and infrared spectra. The ninhydrin color value (570 mμ) corresponds to 84% of that for tryptophan.

Dimethyl N-Carbobenzyloxytryptophan-4-acetate (XII). To a solution of 30 mg of tryptophan-4-acetic acid (XI) in 0.085 ml of 2.0 *N* sodium hydroxide was slowly added 34 mg of carbobenzyloxy chloride and 0.13 ml of 2.0 *N* sodium hydroxide with stirring and ice cooling. After 2 hr at room temperature, the mixture was extracted with ether; the aqueous layer was acidified (congo red acidic) with 10% hydrochloric acid and extracted with ethyl acetate. The ethyl acetate extract yielded 42 mg of the crude N-carbobenzyloxy derivative of XI as an amorphous powder. When 30 mg of this product in 1 ml of ethyl acetate was methylated with excess ethereal diazomethane, the usual work-up gave 31 mg of crude XII. Recrystallization from methanol gave fine colorless needles, mp 157.5–158.5°.

Anal. Calcd for $C_{23}H_{24}N_2O_6$: C, 65.08; H, 5.70; N, 6.60. Found: C, 64.80; H, 5.61; N, 6.80.

The infrared spectrum (in Nujol) showed 2.98 μ (indole NH), 3.00 (amide NH), 5.77 (ester), 5.88 (amide I), 6.53 (amide II); mass spectrum: 424 (M), 202, 144, 143.

This dimethyl ester of N-carbobenzyloxytryptophan-4-acetic ester (XII) was identical with the same derivative prepared from an authentic sample of synthetic IX by carbobenzyloxylation and methylation as described above, with regard to mixture melting point, thin layer chromatography, infrared spectra, and mass spectra.