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The Synthesis of Labile Hydroxytryptophan Metabolites[1]

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New labile metabolites derived from tryptophan and bearing oxygen functions in positions 5 and 7 have been prepared and correlated with regard to their actual and potential significance in various biological systems (Table I). By modifications of the Nenitzescu–van der Lee synthesis 5- and 7-hydroxytryptophan, -tryptamine and -(hetero)auxin have been prepared in good yield. The availability of these synthetic substances made possible various biochemical tests and the identification of some of the compounds with new metabolites of tryptophan (Table I).

In continuation of our studies on the mechanism of oxidation of tryptophan[1,3] we required, and synthesized, 5- and 7-hydroxytryptophan and some derivatives which, in analogy to the catabolism of tryptophan, might occur as metabolites of these two hydroxyamino acids in living systems (Table I).

5-Hydroxyindoles.—We improved the synthesis of 5-benzyloxyindole,[4] the key intermediate in the preparation of all 5-hydroxyindoles reported here, to an approximate 60% over-all yield by using the procedure of Nenitzescu[5] and v. d. Lee[6] as modified by Robertson, *et al.*[7] When 2-nitro-5-benzyloxybenzaldehyde[8] was condensed with nitromethane in the presence of alcoholic potassium hydroxide, the nitroalcohol, obtained as a slowly crystallizing oil, was treated directly with sodium acetate and acetic anhydride to give the readily crystallizing 5-benzyloxy-2-ω-dinitrostyrene. Minor modifications in the original method[9] ultimately gave an almost quantitative yield of product pure enough for the next step, the reduction to 5-benzyloxyindole which apparently exists in a metastable (m.p. 96°)[4b] and a stable (m.p. 107°) form.[10] Hydrogenolysis[11] gave 5-hydroxyindole[9] in quantitative yield.

The Mannich condensation, which with indole itself proceeds in excellent yield,[12] proceeds less well with substituted indoles.[13] Only a 39% yield of 5-benzyloxygramine[14] was obtained, the rest of the starting material being converted to neutral by-products (probably the diindolylmethane[15]) which were not examined further. After some experimentation a procedure was developed using 50% acetic acid in dioxane as solvent which raised the yield of the desired gramine to 95%.

The reaction of 5-benzyloxygramine with sodium cyanide[16] in aqueous alcohol at a higher dilution gave a mixture of 5-benzyloxyindoleacetamide[14] and the nitrile.[17] The reduction of this mixture, or the pure amide, with lithium aluminum hydride gave the crystalline 5-benzyloxytryptamine[14,17] which on hydrogenation in the presence of palladium-on-carbon gave **5-hydroxytryptamine,** a sensitive oil isolated as the hydrochloride or pic-

(1) Oxidation Mechanisms. VIII. Labile Metabolites. II. *Cf.* THIS JOURNAL, **75**, 500 (1953), and *Experientia*, **8**, 377 (1952). This paper is based on a portion of a thesis submitted by Arvid Ek in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Arts and Sciences of Harvard University, January, 1952.

(2) Department of Chemistry, Bowling Green State University, Bowling Green, Ohio.

(3) *Cf.* A. Ek, H. M. Kissman, J. B. Patrick and B. Witkop, *Experientia*, **8**, 36 (1952); H. M. Kissman and B. Witkop, THIS JOURNAL, **75**, 1967 (1953).

(4) (a) H. Burton and J. L. Stoves, *J. Chem. Soc.*, 1726 (1937), 16% over-all yield; (b) W. R. Boehme, THIS JOURNAL, **75**, 2502 (1953), 30% over-all yield.

(5) C. Nenitzescu, *Ber.*, **58**, 1063 (1925).

(6) J. van der Lee, *Rec. trav. chim.*, **44**, 1089 (1925).

(7) R. J. S. Beer, K. Clarke, H. G. Khorana and A. Robertson, *J. Chem. Soc.*, 1605 (1948).

(8) F. A. Mason, *ibid.*, **127**, 1195 (1925).

(9) R. J. S. Beer, K. Clarke, H. F. Davenport and A. Robertson, *ibid.*, 2029 (1951).

(10) H. Burton and M. Leong, *Chemistry & Industry*, 1035 (1953).

(11) *Cf.* F. Bergel and A. L. Morrison, *J. Chem. Soc.*, 49 (1943).

(12) H. Kühn and O. Stein, *Ber.*, **70**, 567 (1937).

(13) Fifty-nine per cent. yield of 5-ethoxygramine [H. N. Rydon and S. Siddappa, *J. Chem. Soc.*, 2462 (1951)]; 72% yield for 5-methoxygramine and 53% for 7-methoxygramine [J. B. Bell and H. G. Lindwall, *J. Org. Chem.*, **13**, 547 (1948)].

(14) *Cf.* K. E. Hamlin and F. E. Fischer, THIS JOURNAL, **73**, 5007 (1951); no yield given there.

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

(16) H. R. Snyder and F. J. Pilgrim, THIS JOURNAL, **70**, 3770 (1948).

(17) M. E. Speeter, R. V. Heinzelmann and D. I. Weisblat, *ibid.*, **73**, 5514 (1951).

TABLE I

CHARACTERIZATION AND BIOLOGICAL ROLE OF 5- AND 7-HYDROXYINDOLES

Compound (m.p., °C.)	Biological role: Expected	Found
5-Hydroxyindole[7] (108–108.5)	In bacteria by cleavage of 5-hydroxytryptophan or by oxidn. of indole	
5-Hydroxytryptamine hydrochloride[25] (167–168)	As the biogenic amine from 5-hydroxytryptophan, precursor of 5,6-dihydroxytryptamine (6-hydroxyserotonin)	As serotonin, bis*nor*bufotenine, enteramine, thrombocytine in various organs[a]
5-Hydroxy(hetero)auxine (166)	As a metabolite of 5-hydroxytryptamine; cell-stretching hormone in the plant	Regularly excreted in urine in amts. varying from 5–10 mg./per diem in humans[b,c]; weak auxine activity in peas[d]
5-Hydroxytryptophan (293–298)	As an oxidn. prod. of tryptophan, precursor of 5-hydroxytryptamine (and (?) 5-hydroxykynurenine,[e] 5-hydroxyanthranilic acid)	To be a new important metabolite of tryptophan and new natural hydroxyamino acid, substrate for a new specific decarboxylase[24]
7-Hydroxyindole (IXb) (100–100.5)	As a possible oxidn. prod. of indole or tryptophan in bacteria or plants (*cf.* vomicine)	
7-Hydroxytryptamine (XIIb) hydrochloride (145–148)	To be a physiologically active isomer of 5-hydroxytryptamine	To have 1/3 of vasoconstrictor activity of 5-hydroxytryptamine[f]
7-Hydroxy(hetero)auxine (XIVb) (176–177)	As a possible oxidn. prod. of heteroauxine	To have weak auxine activity (pea-slit internode)[d]
7-Hydroxytryptophan (XVIb) (>330)	As a possible precursor of 3-hydroxykynurenine,[g,i] diabetogenic[k]	Active in supporting the growth of certain niacin mutants of *Neurospora*, but not of tryptophan mutants[h]

[a] *Cf.* V. Erspamer, *Rend. sci. farmitalia*, 1, 5 (1954). [b] E. Titus and S. Udenfriend, *Federation Proc.*, March (1954), and private communication from Dr. S. Udenfriend. [c] *Cf.* oxidative deamination of serotonin by guinea pig tissue [H. Blaschko, *Biochem. J.*, **52**, PX (1952)] and by guinea pig liver homogenate [W. M. Govier, B. G. Howes and A. J. Gibbans, *Science*, **118**, 596 (1953). The possibility of the introduction of a second phenolic hydroxyl group into serotonin or 5-hydroxy(hetero)auxine prompted us to start the synthesis of 5,6-dihydroxyindole derivatives in this series as well as the presumable intermediate between serotonin and 5-hydroxy(hetero)auxine, *i.e.* 5-hydroxyindole-β-acetaldehyde. [d] Dr. K. V. Thimann who kindly had these tests performed in his laboratory, found 6 and 3% of auxine activity in 5-hydroxy- and 7-hydroxyindoleacetic acid, respectively. The possibility that 5-hydroxy(hetero)auxine, in combination with coenzyme A [A. C. Leopold and F. S. Guernsey, *Proc. Nat. Acad. Sci.*, **39**, 1105 (1953)] or plant protein [S. H. Siegel and A. W. Galston, *ibid.*, **39**, 1111 (1953)] might exert a greater activity on the normal or abnormal [*cf.* F. Lembeck, *Nature*, **172**, 910 (1953)] growth of plant (and animal ?) cells must also be considered. 5-Hydroxyindoleacetonitrile is being tested. [e] 5-Hydroxykynurenine, a sample of which was kindly placed at our disposal by Prof. Katashi Makino, University of Kumamoto, was not detected by Dr. O. Hayaishi when incubating 5-hydroxytryptophan with *Pseudomonas* [Strain 6: *cf.* R. Y. Stanier, O. Hayaishi and M. Tsuchida, *J. Bacteriol.*, **62**, 355 (1951)]; a similar resistance of 5-hydroxytryptophan to the peroxidase–oxidase system from various liver homogenates [W. E. Knox and A. H. Mehler, *J. Biol. Chem.*, **187**, 419 (1950)] was noticed by Dr. A. H. Mehler; O. Wiss and H. Hellmann, *Z. Naturforschung*, 8b, 70 (1952), report the absence of 5-hydroxykynurenine on paper chromatograms from incubation experiments of L-tryptophan with rat liver homogenates. [f] I. H. Page, *J. Pharmacol. Exptl. Therap.*, **105**, 58 (1952). [g] At a time when the biogenesis of porphyrins [D. Shemin and O. S. Russel, THIS JOURNAL, **75**, 4873 (1953)] was not yet established, 7- and 6,7-hydroxylated tryptophans were visualized as compounds yielding properly substituted pyrroles on oxidative ring opening between positions 6 and 7. [h] Dr. D. M. Bonner, who kindly tested 7-hydroxytryptophan in some *Neurospora* strains, reported the following preliminary results: "The 7-hydroxytryptophan proved active in supporting the growth of certain niacin mutants, though not of tryptophan mutants. Thus, 7-hydroxytryptophan cannot be converted to tryptophan, but apparently can be converted to 3-hydroxykynurenine, which in turn can be taken on to 3-hydroxyanthranilic acid and on to niacin. My feeling about the activity of this compound is that it does not represent a new method of the biosynthesis of 3-hydroxykynurenine, but rather reflects the fact that the enzyme system which is involved in the conversion of tryptophan to kynurenine is not so specific as to preclude the utilization of 7-hydroxytryptophan as substrate." [i] 3-Hydroxykynurenine, found recently as one of the photoöxidaton products of tryptophan [Z. Yoshida and M. Kato, THIS JOURNAL, **76**, 311 (1954)] may result from kynurenine, the major oxidation product, by light-induced further oxidation rather than *via* 7-hydroxytryptophan. Solutions of tryptophan in contact with air, according to S. Udenfriend, give spots on paper chromatography identical with 5-hydroxytryptophan. Thus, the introduction of phenolic hydroxyl into the 5-position of tryptophan and into the 3-position of kynurenine (*ortho* to the $-NH_2$, *meta* to the $>CO$ group) so far seems to follow the rules of electrophilic substitution. [k] *Cf.* Y. Kotake, Jr., T. Inada and Y. Matsumura, *J. Biochem. (Japan)*, **41**, 255 (1954).

rate, identical with serotonin picrate.[18] The 5-benzyloxyindoleacetic acid could also be isolated from the reaction of the gramine with sodium cyanide, and on catalytic debenzylation readily gave **5-hydroxyindoleacetic acid,** a compound identified by Udenfriend and Titus (Table I, refs. *b* and *c*) with the major oxidative breakdown product of 5-hydroxytryptamine excreted regularly and in remarkable quantity in human urine. It is possible that the metabolite which Herrmanns and Sachs[19] found in a patient with "liver cancer," and which they coupled with diazotized 2,5-dichloroaniline, is 5-hydroxyindoleacetic acid. Attempts to prepare this coupling product are still in progress.

The introduction of an alanine side chain into 5-benzyloxygramine by the use of formaminopiperidinomethylmalonate[20] proceeded only in very low yields.[21] The condensation with diethyl formami-

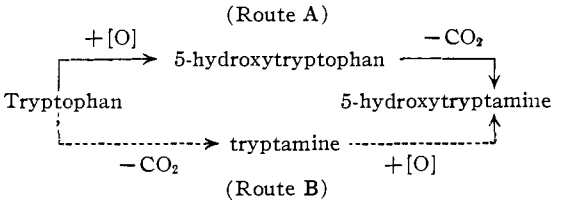
(18) M. M. Rapport, *J. Biol. Chem.*, **180**, 961 (1949).

(19) L. Herrmanns and P. Sachs, *Z. physiol. Chem.*, **114**, 79, 88 (1921).

(20) A. Butenandt, H. Hellmann and J. Renz, *ibid.*, **284**, 175 (1949).

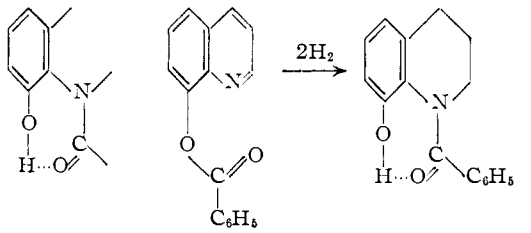
(21) *Cf.* H. Hellmann, G. Hallmann and F. Lingens, *Ber.*, **86**, 1346 (1953); H. R. Snyder, C. Y. Meyers and D. B. Kellom, THIS JOURNAL, **75**, 4672 (1953).

domalonate (prepared by catalytic hydrogenation of ethyl isonitrosomalonate in ethyl formate[22] rather than by Galat's procedure[23]) rendered the substituted malonic ester in good yield. Hydrolysis followed by decarboxylation gave an almost quantitative yield of 5-benzyloxytryptophan, which on catalytic hydrogenolysis furnished **5-hydroxytryptophan**. At this point the compound was turned over to Dr. S. Udenfriend and his associates who found that 5-hydroxytryptophan is the substrate for a highly specific new decarboxylase of probably widespread occurrence in animal and human organisms.[24] Thus, 5-hydroxytryptophan is a *new natural hydroxyamino acid*, too labile, as it turned out, to be isolable from living systems; it represents the intermediate between tryptophan and serotonin in a novel pathway (A) of oxidative catabolism of tryptophan making unlikely (or ruling out) the alternate route (B).[25]



7-Hydroxyindoles.—In order to carry out the same reactions for the preparation of 7-hydroxyindoles, 2-nitro-3-hydroxybenzaldehyde was required as a starting material. This compound had been prepared previously in a laborious fashion by nitration of *m*-hydroxybenzaldehyde[26] in about 5% yield.[27] The parent 2-nitro-3-hydroxytoluene was prepared here using the method of Gibson,[28] which proceeded in good yield, contrary to the experience of Butenandt.[29] Oxidation of this compound with chromium trioxide in a mixture of acetic anhydride and acetic acid, a method originated by Thiele and Winter,[30] furnished the desired 2-nitro-3-hydroxybenzaldehyde in acceptable yield.

7-Benzyloxyindole was obtained, starting with this aldehyde, in an over-all yield of 50% using the reactions (Chart I) employed in the 5-hydroxy series. Hydrogenolysis readily yielded the debenzylated compound with the properties of the known 7-hydroxyindole.[7,31] An attempt to prepare a sample of the known 7-acetoxyindole[7,31] *via* 3-acetoxy-2,ω-dinitrostyrene, gave a reduction product which in contrast to 5-acetoxyindole, did not form a picrate and did not give any color reaction in the Ehrlich test. The compound, which has the composition $C_{10}H_{11}NO_3$ differs from the expected 7-acetoxyindole by the elements of water, and shows two characteristic bands at 2.80 and 3.00 μ in the infrared (measured in chloroform); phenol shows similar bands at 2.76 and 3.00 μ. A strong band at 6.10 μ might be the carbonyl of an amide shifted by hydrogen-bonding in the same way as in vomicine (amide band at 6.12 μ in chloroform). The compound $C_{10}H_{11}NO_3$ may possibly have the partial structure I.



I II III

The related migration of an O-acyl group to a *basic* nitrogen has been observed in the reduction of 8-benzoyloxyquinoline (II) to N-benzoyl-8-hydroxy-1,2,3,4-tetrahydroquinoline (III, no OH-band, CO at 6.16μ).[32]

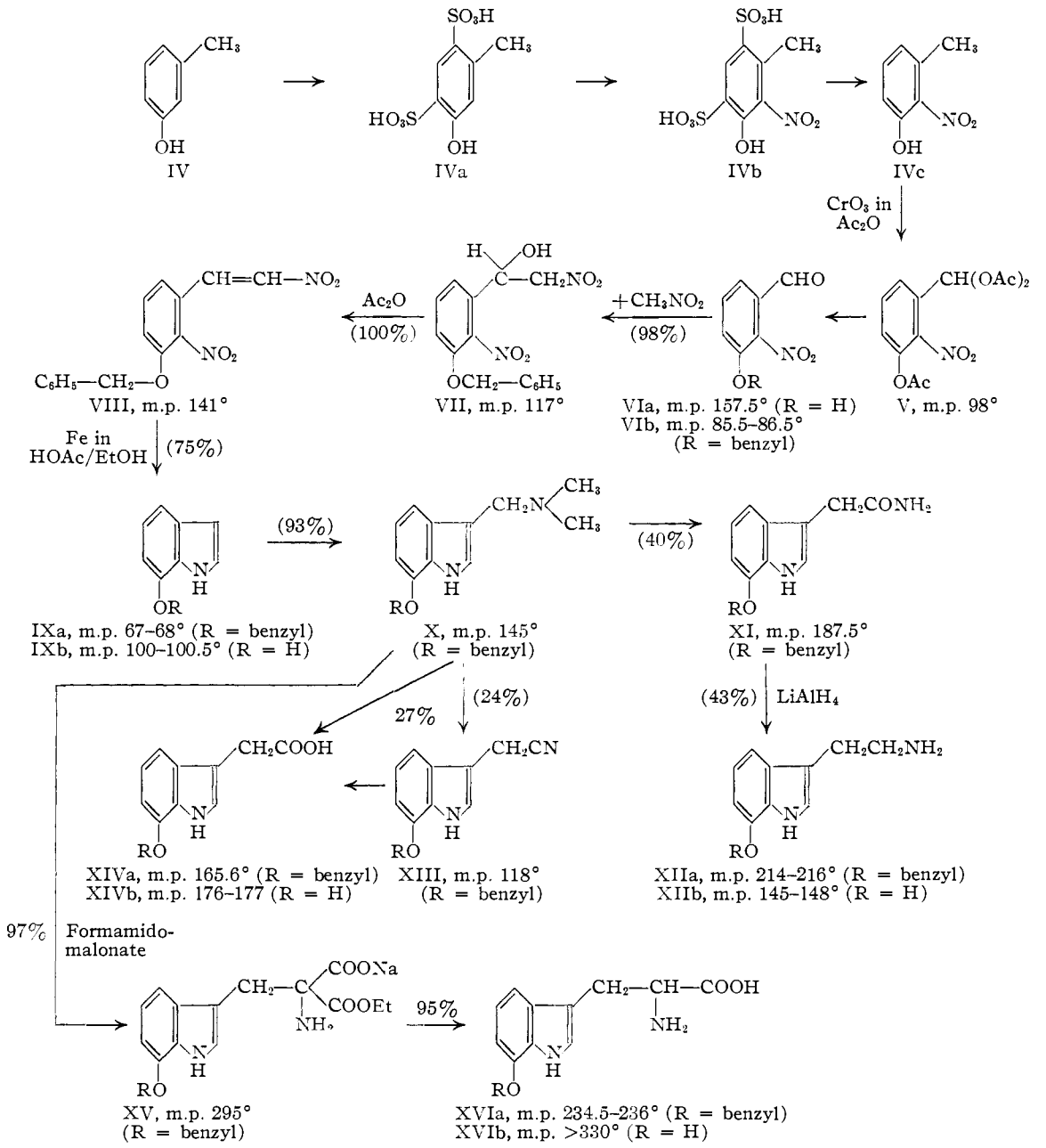
The preparation of **7-hydroxy(hetero)auxine, 7-hydroxytryptamine** ("**isoserotonin**") and **7-hydroxytryptophan** offered no difficulty and is outlined in Chart I. The use of dioxane also improved the yield in the preparation of **7-benzyloxygramine**. 7-Hydroxytryptamine proved even more sensitive than the 5-isomer; its red, high-melting picrate, was more stable. The **7-hydroxytryptophan** was obtained from the 7-benzyloxy precursor, which was difficult to purify, as colorless needles or rods on recrystallization from aqueous ethanol under nitrogen.

Appendix. Attempted Routes into the 7-Hydroxyindole Series.—In order to utilize the convenient method of Warner and Moe[33] for the preparation of 7-hydroxytryptophan, *o*-benzyloxyphenylhydrazine was needed. The parent *o*-benzyloxyaniline has been obtained previously[34] by reduction of the benzyl ether of *o*-nitrophenol.[35] Repetition of these procedures gave poor results and a better synthesis was developed by starting with *o*-aminophenol which was N-acetylated in aqueous solution, then O-benzylated and finally converted, in an over-all yield of 72%, to the requisite *o*-benzyloxyaniline by removal of the acetyl group by basic hydrolysis. However, attempts to prepare *o*-benzyloxyphenylhydrazine were not successful. Reduction of the diazonium salt was tried with stannous chloride and with sodium sulfite under a variety of conditions, but the only product isolated was the hydrochloride of the starting aniline. Another blocking group was then tried, and *o*-aminophenyl *p*-toluenesulfonate[36,37] was prepared by a method analogous to the one described for *o*-benzyloxyaniline. When this aniline was diazotized and the diazonium salt reduced with stannous chloride a very

(22) Roche Products Ltd., A. Cohen and J. A. Silk, British Patent 611,600 [*C. A.*, **43**, 3445 (1949)].
(23) A. Galat, THIS JOURNAL, **69**, 965 (1947).
(24) S. Udenfriend, C. T. Clark and E. Titus, *ibid.*, **75**, 501 (1953).
(25) Route B has been favored by V. Erspamer and M. Vialli, *Ricerca Sci.*, **22**, 1420 (1952); *cf.* the comprehensive and valuable review of V. Erspamer, *Rend. sci. farmitalia*, **1**, 5 (1954).
(26) P. Friedländer and O. Schenk, *Ber.*, **47**, 3040 (1914).
(27) H. H. Hodgson and E. W. Smith, *J. Chem. Soc.*, 76 (1937).
(28) G. P. Gibson, *ibid.*, **123**, 1269 (1923).
(29) A. Butenandt, *Z. Naturforsch.*, **5B**, 444 (1950).
(30) J. Thiele and E. Winter, *Ann.*, **311**, 353 (1900).
(31) R. I. T. Cromartie and J. Harley-Mason, *J. Chem. Soc.*, 2525 (1952).
(32) C. J. Cavallito and T. H. Haskell, THIS JOURNAL, **66**, 1166 (1944); I am indebted to Drs. Cavallito and Kirchner for a sample of III.
(33) D. T. Warner and O. A. Moe, *ibid.*, **70**, 2765 (1948).
(34) A. Sieglitz and H. Koch, *Ber.*, **58**, 78 (1925).
(35) W. A. Baker, A. W. W. Kirby and L. V. Montgomery, *J. Chem. Soc.*, 2876 (1932).
(36) F. Bell, *ibid.*, 1981 (1930).
(37) L. C. Raiford and J. R. Shelton, THIS JOURNAL, **65**, 2048 (1943).

CHART I

SYNTHESIS OF 7-HYDROXYTRYPTOPHAN AND DERIVATIVES



small amount of a crystalline base was obtained which gave the correct analysis for *o*-tosyloxyphenylhydrazine. Further attempts to obtain this hydrazine were then abandoned because the *o*-tosyloxyphenylhydrazone of ethyl pyruvate, obtained by the Japp–Klingemann reaction[38] from ethyl acetopropionate and *o*-tosyloxybenzenediazonium chloride, could not be brought to cyclize.[39]

With 2-nitro-3-benzyloxyphenylpyruvic acid in hand, two other possible syntheses of 7-benzyloxyindole were also tried. By standard methods[40] the pyruvic acid was oxidized to the corresponding phenylacetic acid which on reduction gave 7-benzyloxyoxindole. Reduction with lithium aluminum hydride gave, besides unchanged starting material, a very small amount of a base isolated as its *yellow* picrate, probably that of 7-benzyloxy-2,3-dihydroindole, by analogy with earlier results.[41] 1-Benzyl-7-benzyloxyoxindole might be expected to give the corresponding indole on reduction with lithium aluminum hydride, and here both benzyl groups probably could be removed by catalytic hydro-

(38) F. R. Japp and F. Klingemann, *Ber.*, **21**, 549 (1888).

(39) A somewhat related observation has been made previously in that the *o*-nitrophenylhydrazone of pyruvic acid does not yield 7-nitroindole-2-carboxylic acid [G. K. Hughes, F. Lions and E. Ritchie, *J. Proc. Roy. Soc., New South Wales*, **72**, 209 (1939)] but remains unaffected by cyclization agents [H. N. Rydon and S. Siddappa, *J. Chem. Soc.*, 2462 (1951)].

(40) K. G. Blaikie and W. H. Perkin, *ibid.*, **125**, 296 (1924).

(41) P. L. Julian and H. C. Printy, THIS JOURNAL, **71**, 3206 (1949).

genation. An exploratory benzylation of oxindole yielded 3,3-dibenzyloxindole rather than N-benzyloxindole which has been prepared in the meantime.[42] A novel and promising approach to 5- and 7-oxygenated indoles is the light-induced ring constriction of methoxyquinoline diazoketones to methoxyindole-β-carboxylic acids.[43] An attempt to form 5-benzyloxyindole by reductive cyclization of 2-nitro-3-benzyloxyacetonitrile failed.

Experimental[44]

5-Benzyloxy-2,ω-dinitrostyrene.—2-Nitro-5-hydroxybenzaldehyde was prepared by nitration of *m*-hydroxybenzaldehyde carbonate followed by hydrolysis, according to Mason.[8] Eighteen grams of the benzyl ether, prepared by the procedure of Burton and Stoves,[4a,44a] was dissolved by warming in 360 cc. of ethanol and then cooled in ice with stirring, causing the aldehyde to separate as a finely divided crystalline powder. To this suspension 5.6 cc. of nitromethane was added and then, after cooling to −10°, an ice-cold solution of 9.8 g. of potassium hydroxide in 14 cc. of water and 140 cc. of alcohol was added dropwise with stirring in the course of 25 minutes. The temperature was kept at −10° throughout the time of addition during which the aldehyde dissolved. The mixture was then allowed to warm up to −5° and soon became a thick mush as fine colorless crystals separated. The stirring was continued for two hours while the temperature was kept at −5°, and the mixture then acidified by the careful addition of 13 cc. of concentrated hydrochloric acid diluted with 50 cc. of ice-water while the temperature was kept below 5°. The precipitate dissolved again and, on dilution with 2 l. of water, an oil separated which was extracted with several portions of ether. The extract was dried over sodium sulfate and the solvent removed. The residual oil crystallized completely overnight; yield 23 g.

The nitroalcohol was mixed with 23 g. of anhydrous sodium acetate and treated with 100 cc. of acetic anhydride. The mixture was kept close to the boiling point for five minutes and then poured into 600 cc. of cold water. An oil separated and quickly crystallized. After standing for three hours, the light yellow-brown crystals were collected, washed with water and dried. Twenty-one grams (100%) of the styrene, m.p. 143–145°, was obtained, sufficiently pure for the next step. A sample was recrystallized from a mixture of ethyl acetate and ethanol, sublimed in high vacuum at 130° and then crystallized once more from the same solvent mixture. Pure yellow crystals were obtained, m.p. 149–150°.

Anal. Calcd. for $C_{15}H_{12}N_2O_5$: C, 59.98; H, 4.03; N, 9.33. Found: C, 60.12; H, 3.84; N, 9.21.

5-Benzyloxyindole.—A mixture of 21 g. of crude 5-benzyloxy-2,ω-dinitrostyrene, 70 g. of iron powder, 140 cc. of ethanol and 140 cc. of acetic acid was stirred and warmed until the reduction became vigorous and cooling with ice-water was necessary to prevent the mixture from boiling over. Stirring was continued and the reaction mixture kept just below the boiling point by occasional cooling or heating. After seven minutes the mixture suddenly thickened and the color changed from brown to greenish-gray; after five more minutes it was poured into a suspension of 350 g. of sodium bisulfite in 1.75 l. of water and extracted five times with ether. It was usually necessary to centrifuge an intermediate emulsified layer after the first extraction. The combined ether extracts were washed with sodium bicarbonate solution and then with water. After drying over sodium sulfate and removal of the solvent, 15.5 g. of a brown solid remained which was dissolved in 180 cc. of benzene and 180 cc. of petroleum ether was added. The supernatant liquid was decanted from the precipitate which separated and poured onto a column of 150 g. of aluminum oxide packed under petroleum ether. The process of dissolving in benzene and adding petroleum ether was repeated four times with the precipitate. The indole was eluted completely from the column by 360 cc. of benzene–petroleum ether mixture and then 350 cc. of pure benzene. The eluate was concentrated to a small volume and petroleum ether added to the hot solution until crystallization began. By working up the mother liquor a total of 9.6 g. (61%) of almost colorless, fine needles was obtained, m.p. 106–109°. An analytical sample, prepared by recrystallization from benzene and petroleum ether, sublimation in high-vacuum at 90°, and a second recrystallization from the same solvent mixture, melted at 104–106°. The reported m.p. 95–97°,[4a] 94–96°,[4b] is that of the metastable form.[10]

Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.71; H, 5.87. Found: C, 80.51, 80.75; H, 6.60, 6.16.

The ultraviolet spectrum in absolute ethanol showed $\lambda_{max.}$ (log ε) 308 (3.42); [295 (3.58)]; 275 (3.80). The spectrum remained unchanged on addition of base or acid.

5-Benzyloxyindole Picrate.—A sample of the indole was dissolved in benzene and treated with a solution of picric acid in the same solvent. After the addition of petroleum ether a red picrate crystallized out. It was recrystallized twice from a little benzene; small red needles, m.p. 142–143°.

Anal. Calcd. for $C_{15}H_{13}NO \cdot C_6H_3N_3O_7$: C, 55.76; H, 3.57; N, 12.3. Found: C, 56.13; H, 3.62; N, 11.8.

5-Hydroxyindole.—The hydrogenolysis of 0.22 g. of 5-benzyloxyindole in 7 cc. of ethyl acetate went to completion in 45 minutes in the presence of 0.2 g. of 5% palladium-on-carbon. The catalyst was filtered off and the filtrate evaporated to dryness in a current of nitrogen. There was obtained 0.13 g. (98%) of colorless crystals, m.p. 107–108°. Recrystallization from benzene gave short, fine colorless needles, m. p. 108–108.5°, not depressed on admixture with an authentic sample.[7]

Anal. Calcd. for C_8H_7NO: C, 72.16; H, 5.30; N, 10.52. Found: C, 72.02; H, 5.64; N, 10.32.

The infrared spectrum, measured in chloroform, shows the following major bands: 2.75 (strong narrow band: > NH); 2.84, 2.92 (phenolic hydroxyls); 6.12m; 6.28s; 6.73s; 6.86s; 7.45s; 7.77m; 8.74s; 8.87m; 10.57s.

5-Benzyloxygramine.—When the Mannich condensation was carried out in glacial acetic acid, as described by Kühn and Stein,[12] only a 39% yield was obtained. The following procedure was then adopted: A solution of 8.9 g. of 5-benzyloxyindole in 40 cc. of dioxane was added dropwise, in the course of 30 minutes, to an ice-cooled, stirred mixture of 40 cc. of dioxane, 40 cc. of acetic acid, 3.2 cc. of 36% aqueous formaldehyde and 8.8 cc. of 25% aqueous dimethylamine. The clear solution was stirred and cooled for two hours and then allowed to warm up to room temperature overnight. The next day, 500 cc. of water was added, and the turbid mixture filtered after the addition of charcoal and Filter-cel. The clear filtrate was made alkaline with an excess (400 cc.) of dilute sodium hydroxide solution. The gramine separated as an oil which quickly solidified and was filtered off after cooling in the refrigerator. Washing with water, and drying gave 10.6 g. (95%) of a colorless, coarse powder, m.p. 135.5–137°. A sample was purified for analysis by two recrystallizations from a mixture of benzene and hexane. Small glittering cubes were obtained, m.p. 138.5–139.5°, reported[17] m.p. 138°. Infrared spectrum (chloroform): 2.85s (>NH); 6.14m; 6.29m; 6.75s; 6.88s; 7.26m; 7.31m; 7.75s; 8.57m; 9.18m; 9.52m; 9.95s.

5-Benzyloxyindole-3-acetamide.—A solution of 0.28 g. of 5-benzyloxygramine, 0.25 g. of sodium cyanide, 4 cc. of ethanol and 1 cc. of water was refluxed for 88 hours. The solution, which contained some precipitate, was diluted with water. The crystalline material which separated was washed thoroughly with water and dried, giving 0.18 g. of a sticky tan powder, m.p. 133–138°. It was washed with benzene and recrystallized from benzene containing a small

(42) H. Rinderknecht, H. Koechlin and C. Niemann, *J. Org. Chem.*, **18**, 979 (1953).

(43) O. Süs, M. Glos, K. Möller and H.-D. Eberhardt, *Ann.*, **583**, 150 (1953).

(44) All melting points are corrected, all boiling points uncorrected. The analyses were performed by Dr. William C. Alford and his associates, Analytical Service Laboratory, National Institutes of Health.

(44a) The yields obtained by the procedure of Burton and Stoves never exceeded 65%. In the preparation of 5,6-dibenzyloxyindole derivatives the following method, described for 5-benzyloxyindole, was found to be superior: A mixture of 0.83 g. (0.005 m.) of 2-nitro-5-hydroxybenzaldehyde, 0.63 cc. (0.0055 m.) of benzyl chloride, 0.69 g. (0.005 m.) of potassium carbonate, and 15 cc. of dimethylformamide was heated at 100° with stirring for two hours. After cooling dilution with water gave 1.25 g. (97.5%) of a light-yellow product, m.p. 68–72°, which after recrystallization from isopropyl ether yielded 1.15 g. of light yellow crystals, m.p. 70–73° (89.5% yield).

amount of methanol. A second recrystallization from the same solvent gave small, colorless needles, m.p. 158–159°, reported m.p. 158°.[14]

Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 72.66; H, 5.63; N, 10.02.

The infrared spectrum (in chloroform) showed the following major bands: 2.85; 2.93; 5.95s; 6.33w; 6.73m.

By working up the mother liquors a small amount of the corresponding nitrile was obtained as shown by its infrared absorption spectrum with a band at 4.4 μ, and no carbonyl band. In contrast to the 7-isomer, however, purification proved difficult and has not yet been achieved.

5-Benzyloxyindole-3-acetic Acid.—The basic filtrate from the crude amide, isolated above, was concentrated to remove alcohol and acidified with hydrochloric acid. The resulting precipitate was collected, yielding 40 mg. of light tan crystals, m.p. 128–131°. Recrystallization from water (using charcoal) gave colorless shiny leaflets, m.p. 133.5–134.5°. The compound was slightly contaminated by the amide. Dr. E. Titus, repeating the preparation, obtained an analytically pure sample, m.p. 149–150.5°.

Anal. Calcd. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.38; N, 4.98. Found: C, 72.56; H, 5.09; N, 5.07.

The infrared spectrum (in chloroform) showed the following major bands: 2.84s (>NH); 5.83s; 5.95m; 6.12w; 6.27s; 6.73s; 6.87s; 7.25w; 7.79w.

5-Hydroxyindole-3-acetic Acid.—The hydrogenolysis of the benzyl ether proceeded readily and in excellent yield (95% of crude product) by stirring 0.07 g. of the benzyl compound in 10 cc. of ethyl acetate with an equal weight of 5% palladium-on-carbon under one atmosphere of hydrogen for one-half hour. The theoretical amount of hydrogen was taken up and after removal of the catalyst the solvent was evaporated *in vacuo*. The residue was recrystallized twice from water (using charcoal the first time), m.p. 166°.

Anal. Calcd. for $C_{10}H_9NO_3$: C, 62.82; H, 4.74; N, 7.33. Found: C, 62.47; H, 4.87; N, 7.25.

The color reactions and R_f-values of this compound have been reported by Stowe and Thimann.[45]

5-Benzyloxytryptamine Hydrochloride.—Reduction of 0.26 g. of the amide (the crude amide-nitrile mixture serves equally well) was carried out by continuous extraction into a solution of 0.5 g. of lithium aluminum hydride in 50 cc. of ether. After the 36 hours required to complete the extraction, the excess hydride was decomposed with ethanol and then water was added to ensure complete decomposition of the precipitated complex. The ether layer was decanted and the residue was washed with fresh ether. The combined solutions were dried over solid potassium hydroxide and evaporated to dryness in a stream of nitrogen. The oily residue, 0.23 g. (93%), was taken up in ether and precipitated with hydrogen chloride gas. The colorless hydrochloride was recrystallized from ethanol–ether, m.p. 263–264°, reported m.p. 265°.[17]

A sample of the hydrochloride was hydrogenated in ethanol in the presence of an equal weight of palladium-on-carbon catalyst. One mole of hydrogen was readily taken up at room temperature and atmospheric pressure. After removal of the catalyst, the ethanol was evaporated *in vacuo* and the crystalline residue dissolved in water and treated with aqueous picric acid. The red picrate was recrystallized from water. The needles began to melt at 104° and were completely melted at 110°; after resolidifying from 125–135° they finally melted at 184–187°, confirming the observations of Hamlin and Fisher,[14] and Rapport,[18] but not of Erspamer, *et al.*[46]

Diethyl Formamidomalonate.—Since attempts to repeat the procedure of Galat[23] gave very poor results, the ester was finally prepared by a method described only in a patent.[22] Diethyl isonitrosomalonate was prepared by the method of Snyder and Smith[47] in 63% yield, b.p. 137–138° (3 mm.). A solution of 5.7 g. of the ester in 24 cc. of ethyl formate was hydrogenated at 1500 lb. pressure in the presence of 0.5 g. of palladium-on-carbon. The catalyst was filtered off, the solvent removed and the residue distilled *in vacuo*. There was obtained 5.5 g. (90%) of a rapidly crystallizing oil, b.p. 130–140° (1.5 mm.), m.p. 50–52°.

Ethyl β-(5-Benzyloxyindolyl-3)-α-carbethoxy-α-formamidopropionate.—A mixture of 0.56 g. of 5-benzyloxygramine, 0.5 g. of diethyl formamidomalonate, 0.04 g. of powdered sodium hydroxide, and 5 cc. of toluene was heated to reflux for 2.5 hours while a vigorous stream of nitrogen was bubbled through. The evolution of dimethylamine began at once and had ceased at the end of the refluxing period. The light yellow solution, filtered while hot, solidified on cooling to a mush of colorless crystals, which were collected and washed first with toluene and then with benzene. After drying, there was obtained 0.68 g. (78%) of colorless, very small, fine needles, m.p. 139.5–141°. A sample recrystallized twice from aqueous ethanol gave fine, shining needles, m.p. 146.5–147.5°.

The infrared spectrum, measured in chloroform, showed the following major bands: 2.84 (>NH of indole); 2.91 (>NH of amide); 5.72s (carbonyl ester); 5.90s (amide carbonyl); 6.13w; 6.29w; 6.73s; 6.86m; 7.30s; 7.74s; 9.15s; 9.37s; 9.93m; 10.63m.

Anal. Calcd. for $C_{24}H_{26}N_2O_6$: C, 65.74; H, 5.98; N, 6.39. Found: C, 65.96; H, 6.17; N, 6.32.

5-Benzyloxytryptophan.—A mixture of 2.04 g. of the formamidoester, 2 g. of sodium hydroxide and 20 cc. of water was refluxed for six hours. The ester dissolved soon after boiling began. At the end of the reflux period the addition of 4 cc. of *concentrated hydrochloric acid*[48] precipitated the aminomalonic acid in crystalline form. Another 2.5 cc. of 2 *N hydrochloric acid*[48] was then added and the refluxing was resumed. The solid went almost completely into solution with the evolution of gas. The theoretical amount of carbon dioxide was collected within 15 minutes, when crystallization of the product began. After cooling, the amino-acid was collected, washed with water and dried. There was obtained 1.35 g. (93.5%) of a slightly pinkish, crystalline powder which began to turn yellow at 260° and melted very suddenly with evolution of gas at 279°.

Buffering the mother liquor with sodium acetate gave another 0.08 g. of colorless product, rendering the total yield almost the theoretical.

A sample of the main crop was recrystallized by dissolving in water and diluting with alcohol after treatment with charcoal. A second recrystallization gave fine white needles or rods which, when inserted in the bath at 270°, melted with vigorous evolution of gas at 280° after previous yellowing.

Anal. Calcd. for $C_{18}H_{18}N_2O_5 \cdot H_2O$: C, 65.86; H, 6.16. N, 8.54. Found: C, 66.21; H, 6.40; N, 8.62.

5-Hydroxytryptophan.—A suspension of 0.5 g. of 5-benzyloxytryptophan and 0.3 g. of 5% palladium-on-charcoal in 10 cc. of water and 10 cc. of ethanol was hydrogenated at room temperature and atmospheric pressure. The amino acid quickly went into solution and the uptake of hydrogen stopped with the absorption of the theoretical volume after one-half hour. The filtrate from the catalyst was initially colorless, but turned yellow very quickly. The evaporation *in vacuo* was discontinued when crystallization started, which was increased by the addition of 60 cc. of absolute ethanol. When the mother liquor was treated in a similar manner a small second crop was obtained. The total yield of 5-hydroxytryptophan was 0.28 g. (79%). A sample was purified for analysis with considerable loss by dissolving it in a very small amount of water (in which it is very soluble), treating the solution with charcoal, diluting with several volumes of absolute alcohol and evaporating in a stream of nitrogen until crystallization commenced. A second recrystallization gave colorless rods, m.p. 293–298° dec.

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.50; N, 12.73. Found: C, 59.88; H, 5.71; N, 12.62.

The R_f value of the amino acid in 80% aqueous pyridine was 0.61, in 70% aqueous propanol 0.31.

2-Nitro-3-hydroxybenzaldehyde (VIa).—A mixture of 565 cc. of acetic anhydride (technical grade is adequate), 570 cc. of glacial acetic acid, 70.8 g. of 2-nitro-3-acetoxytoluene[28] and 85 cc. of concentrated sulfuric acid was cooled with stirring to 5°. In the course of 70 minutes 100 g. of chro-

(45) B. B. Stowe and K. V. Thimann, *Arch. Biochem. and Biophys.*, in press.

(46) B. Asero, V. Colò, V. Erspamer and A. Vercellone, *Ann.*, **576**, 73 (1952).

(47) H. R. Snyder and C. W. Smith, THIS JOURNAL, **66**, 350 (1944).

(48) In a previous experiment in which acetic acid had been used (*cf.* ref. 20) decarboxylation did not take place and the monosodium salt of the aminomalonic acid (m.p. 295° dec.) was obtained.

mium trioxide was added in small portions while the temperature was kept between 5 and 10°.[49] After the addition was complete, stirring was continued for six hours at the same temperature and the dark green mixture was then poured into ice-water. Stirring was continued for one-half hour whereupon the reaction product which had solidified was collected and washed with cold water until colorless. The precipitate was then digested and stirred mechanically for one-half hour with 500 cc. of cold 2% sodium carbonate solution. The bright red mixture was filtered and the solid washed with water. After drying there was obtained 38.5 g. of colorless material which was hydrolyzed by refluxing for one-half hour with 136 cc. of concentrated hydrochloric acid, 225 cc. of water and 80 cc. of ethanol. The mixture was then steam distilled until the distillate no longer came over cloudy. The residue was treated with charcoal and filtered through a sintered glass funnel giving, after cooling, filtering, washing and drying, 7.7 g. of light yellow crystalline powder, m.p. 154–155°. The steam distillate, from the hydrolysis, was extracted with benzene which on evaporation left 15.5 g. (28%) of 2-nitro-3-hydroxytoluene.

A sample of the aldehyde was prepared for analysis by two recrystallizations from ethanol; m.p. 157–157.5°, reported[27] 157°. Infrared spectrum (chloroform): 3.06 (broad band of a strongly bonded phenolic hydroxyl); 5.85s (aldehyde carbonyl); 6.20s; 6.52s; 6.88.

Anal. Calcd. for $C_7H_5NO_4$: C, 50.30; H, 3.02; N, 8.38. Found: C, 50.13; H, 3.11; N, 8.20.

2-Nitro-3-hydroxytoluene-α,α-diol Triacetate (V).—In another oxidation the crude product, after the removal of acid with sodium carbonate solution, was washed, dried, and recrystallized five times from ethanol. Well-shaped, colorless rods were obtained, m.p. 97–98°. Infrared spectrum (chloroform): 3.30m; 5.85s (very strong band with shoulders at 5.90 and 5.93, signifying the two types of carbonyls); 6.47s; 7.30s; 8.85w; 9.23m; 9.93s.

Anal. Calcd. for $C_{13}H_{13}NO_8$: C, 50.16; H, 4.21; N, 4.50. Found: C, 50.28; H, 4.26; N, 4.48.

2-Nitro-3-hydroxybenzoic Acid.—The bright red sodium carbonate extract (see above) was allowed to stand overnight. It was then made strongly acid and extracted five times with ethyl acetate. The extract was evaporated to dryness leaving 7.2 g. (10.8%) of yellow crystals, m.p. 178–180°. A sample was recrystallized from *N* hydrochloric acid and then twice from acetic acid giving colorless crystals which became yellow and opaque on drying; m.p. 185–185.5°, reported m.p. 180.8–181.5°.[50] Infrared spectrum (very broad band of strongly banded phenolic hydroxyl); 5.81s (carbonyl of aromatic carboxyl); 6.20s; 6.48m; 6.90s.

Anal. Calcd. for $C_7H_5NO_5$: C, 45.92; H, 2.75; N, 7.65. Found: C, 46.07; H, 2.89; N, 7.72.

2-Nitro-3-benzyloxybenzaldehyde (VIb).—A mixture of 11.1 g. of the hydroxy aldehyde, 8.5 cc. of benzyl chloride, 2.95 g. of sodium hydroxide dissolved in 17 cc. of water, and 100 cc. of ethanol was boiled under reflux for eight hours. The alcohol was distilled off on the steam-bath and the residue poured into 250 cc. of water with stirring. The dark oil which separated crystallized readily and was filtered off, washed with water and dried. A brown powder weighing 15 g. was obtained. Repeated extraction with isopropyl ether gave, after working up the mother liquors, 12 g. (70%) of light-yellow crystals, m.p. 83–85°. A sample recrystallized from the same solvent and then from benzene–ligroin gave glass-clear platelets, m.p. 85.5–86.5°.

Anal. Calcd. for $C_{14}H_{11}NO_4$: C, 65.36; H, 4.31. Found: C, 65.91; H, 4.37.

2,4-Dinitrophenylhydrazone.—The compound, obtained by standard methods, crystallized from xylene in orange, glittering leaflets, m.p. 249–250°.

Anal. Calcd. for $C_{20}H_{15}N_5O_7$: C, 54.92; H, 3.46; N, 16.01. Found: C, 55.23; H, 3.68; N, 15.59.

***p*-Nitrophenylhydrazone.**—The compound was recrystallized from ethanol containing a little ethyl acetate; orange needles, m.p. 210–212°.

Anal. Calcd. for $C_{20}H_{16}N_4O_5$: C, 61.24; H, 4.11; N, 14.29. Found: C, 61.05; H, 4.22; N, 14.20.

(49) The procedure is that of *Org. Syntheses*, **24**, 75 (1944).

(50) P. H. Beyer, *Rec. trav. chim.*, **40**, 621 (1921).

3-Benzyloxy-2,ω-dinitrostyrene (VIII).—The condensation of the aldehyde with nitromethane was carried out as described for the 5-benzyloxy isomer using 11.5 g. of 2-nitro-3-benzyloxybenzaldehyde and 3.6 cc. of nitromethane in 180 cc. of ethanol, and adding, with stirring, 5.0 g. of potassium hydroxide dissolved in 8.9 cc. of water and 89 cc. of ethanol. The temperature was kept at −5° during the addition which required 25 minutes and then for an additional time of 45 minutes. The aldehyde dissolved quickly, but no precipitation took place until the mixture was acidified with 8 cc. of concentrated hydrochloric acid in 100 cc. of water. The crystallization of the nitro alcohol VII was rendered complete by the addition of 300 cc. of water. The product VII was washed with water, collected and dried; it weighed 14 g. (98.5%), m.p. 115–117° (with evolution of gas). Treatment with sodium acetate and acetic anhydride as before gave 13.2 g. (98% based on aldehyde) of VIII, a light yellow powder, m.p. 135–138°. A sample was recrystallized twice from ethyl acetate containing a little ethanol; short, light yellow rods and needles, m.p. 140–141°. Infrared spectrum (in chloroform): 6.06w; 6.21m; 6.32s; 6.51s; 6.75w; 6.87w; 7.33s; 7.42s; 7.78s; 9.24m; 9.51m; 10.50m; 11.77m.

Anal. Calcd. for $C_{16}H_{12}N_2O_5$: C, 59.98; H, 4.03; N, 9.33. Found: C, 59.63; H, 4.23; N, 9.10.

7-Benzyloxyindole (IXa).—The reduction of the 3-benzyloxy-2,ω-dinitrostyrene, carried out as described for the preparation of 5-benzyloxyindole, proceeded less vigorously. From 18 g. of the styrene VIII reduced with 60 g. of iron powder in 120 cc. of ethanol and an equal quantity of acetic acid, there was obtained by the method described above 10.0 g. (75%) of almost colorless product, m.p. 67–68°. Two recrystallizations from ligroin gave colorless platelets with the same melting point.

Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.71; H, 5.87; N, 6.27. Found: C, 80.73; H, 5.95; N, 6.37.

The ultraviolet spectrum in absolute ethanol showed $\lambda_{max.}$ (log ε) 288 (3.61); [278 (3.71)]; 266 (3.88). The infrared spectrum in chloroform had the following major bands: 2.86s; 6.13w; 6.23w; 6.31s; 6.71m; 6.87w; 6.95m; 7.08s; 7.25w; 7.36m; 7.48s; 7.77s; 9.05m; 9.22s; 9.38s; 10.32m.

7-Benzyloxyindole Picrate.—With picric acid in benzene the indole gave a picrate which crystallized immediately. Two crystallizations from equal parts of benzene and petroleum ether gave orange-red, fine needles, m.p. 149–150°.

Anal. Calcd. for $C_{15}H_{13}NO \cdot C_6H_3N_3O_7$: C, 55.76; H, 3.57. Found: C, 55.79; H, 3.84.

7-Hydroxyindole (IXb).—When 0.22 g. of 7-benzyloxyindole was hydrogenated as described for the 5-isomer, the absorption of hydrogen proceeded nearly twice as fast. Evaporation *in vacuo* gave 0.13 g. (98%) of crystals, m.p. 97–99°. Sublimation in high vacuum at 75° followed by crystallization from benzene–petroleum ether gave shining needles, m.p. 100–100.5°, reported m.p. 96°.[7] Infrared spectrum (in chloroform): 2.76 (very sharp and narrow imino band of indole); 2.85 (sharp band) and 2.93 (broad band of phenolic hydroxyl); 6.10m; 6.29s; 6.45s; 6.90s; 7.08s; 7.38s; 7.74s; 8.66m; 9.09w; 9.43w; 9.83m.

Anal. Calcd. for C_8H_7NO: C, 72.16; H, 5.30. Found: C, 72.34; H, 5.48.

7-Hydroxyindole Picrate.—This compound was prepared in the usual way. Two crystallizations from benzene gave well-formed, dark red needles, m.p. 176° dec. An unsatisfactory analysis was obtained possibly due to instability rather than lack of purity.

Anal. Calcd. for $C_8H_7NO \cdot C_6H_3N_3O_7$: C, 46.42; H, 2.78. Found: C, 47.24; H, 3.29.

3-Acetoxy-2,ω-dinitrostyrene.—A solution of 0.7 g. of potassium hydroxide in 10 cc. of ethanol was cooled to −10° and added in small portions to a solution of 1 g. of 2-nitro-3-hydroxybenzaldehyde and 0.36 cc. of nitromethane in 5 cc. of ethanol, cooled to the same temperature. The mixture immediately became red, and a dark red crystalline precipitate soon separated. When approximately one-half of the base had been added the crystals disappeared and a dark red oil appeared which persisted until all of the base had been added and acidification with 6.5 cc. of cold 2 *N* hydrochloric acid was under way. The oil then redissolved and a light crystalline precipitate appeared. The acidified mixture was allowed to warm up to room temperature and then

diluted with 130 cc. of water. The clear solution was extracted five times with ether; the extract was dried over sodium sulfate and the solvent removed. The solid residue (1.3 g.) was mixed with 2 g. of anhydrous sodium acetate and 5 cc. of acetic anhydride and kept just below the boiling point for 15 minutes. Cooling and dilution with 40 cc. of water gave an oil which rapidly crystallized. After filtering, washing with water and drying, 1.2 g. (80%) of reddish tan crystals was obtained, m.p. 120–122°. Two recrystallizations from ethanol gave a light yellow product, m.p. 126–127.5°. Infrared spectrum (in chloroform): 5.58s (unusually low carbonyl of phenyl ester); 6.05m (>C=C<); 6.31s; 6.52s; 7.43s; 7.84s; 8.50s; 8.85w; 9.80s; 10.51s; 10.98m; 11.46s; 11.82s.

Anal. Calcd. for $C_{10}H_8N_2O_5$: C, 47.62; H, 3.20; N, 11.11. Found: C, 47.82; H, 3.36; N, 10.65.

Compound $C_{10}H_{11}NO_3$ from the Reduction of 3-Acetoxy-2,ω-dinitrostyrene.—A mixture of 0.9 g. of the styrene, 3.6 g. of iron powder, 7.2 cc. of ethanol and 7.22 cc. of acetic acid, was heated until the reaction started. It quickly became vigorous and was tempered by occasional cooling. After 11 minutes the mixture was filtered quickly, the basic iron salts were washed several times with hot ethanol and the combined filtrate and washings neutralized with 100 cc. of saturated sodium bicarbonate solution. The mixture was extracted five times with ether. The extracts were dried over sodium sulfate and evaporated to dryness in a stream of nitrogen. The greenish-brown crystalline residue (0.64 g.) was recrystallized twice from 10 cc. of benzene, giving long, slightly colored rods, m.p. 141.5–142.5°.

Anal. Calcd. for $C_{10}H_{11}NO_3$: C, 62.17; H, 5.74; N, 7.25. Found: C, 62.22; H, 5.72; N, 7.36.

The ultraviolet spectrum in absolute ethanol, $\lambda_{max.}$ (log ϵ) 293 (3.51); 261 (3.83); 220 (4.37) is related to N-acetylaspidosine (des-O-methylaspidospermine), $\lambda_{max.}$ (log ϵ): 293 (3.32); 260 (3.73). The infrared spectrum (in chloroform) has the following major bands: 2.80 (sharp) and 3.00 (somewhat broader, both probably bands of a bonded phenolic hydroxyl); 6.10s (bonded carbonyl of amide) 6.22s; 6.77s; 7.0s; 7.23s; 7.35s; 7.95m; 8.63m; 8.97m; 9.43m; 10.03m.

7-Benzyloxygramine (X).—The gramine was prepared by the method already described for the 5-isomer. From 2.2 g. of the indole IXa in 10 cc. of dioxane, added to a mixture of 10 cc. of dioxane, 10 cc. of acetic acid, 2.2 cc. of dimethylamine (25%, aqueous) and 0.8 cc. of 37% formaldehyde, there was obtained 2.56 g. (93.5%) of fine, white powder, m.p. 143–144.5°. Recrystallization from hexane gave colorless needles, m.p. 144.5–145°.

Anal. Calcd. for $C_{18}H_{20}N_2O$: C, 77.11; H, 7.19; N, 9.99. Found: C, 77.24; H, 7.36; N, 9.91.

7-Benzyloxygramine Picrate.—A sample of the gramine was dissolved in dilute hydrochloric acid and precipitated with aqueous picric acid. The picrate was washed thoroughly with water, dried and recrystallized from ethanol; orange needles, m.p. 172–173° after previous darkening.

Anal. Calcd. for $C_{18}H_{20}N_2O \cdot C_6H_3N_3O_7$: C, 56.58; H, 4.55; N, 13.75. Found: C, 56.40; H, 4.57; N, 13.62.

7-Benzyloxyindole-3-acetamide (XI).—A solution of 2 g. of 7-benzyloxygramine and 1.7 g. of sodium cyanide in 28 cc. of ethanol and 7 cc. of water was refluxed for 84 hours. The clear solution was then concentrated *in vacuo* which led to the crystallization of a white solid. The mixture was cooled and the solid collected and washed with water. After drying there was obtained 1.17 g. of a colorless solid, m.p. 172–175°. Recrystallization from benzene and a little ethanol gave (in two crops) 0.8 g. (40%) of the amide, m.p. 186–187.5°. Recrystallization from the same solvent raised the melting point to 187.5–188°; infrared absorption of amide carbonyl at 6.05 μ (chloroform).

Anal. Calcd. for $C_{17}H_{16}N_2O_2$: C, 72.84; H, 5.75; N, 9.99. Found: C, 73.11; H, 5.84; N, 9.70.

7-Benzyloxyindole-3-acetonitrile (XIII).—When the washings from the crude amide were mixed with the basic filtrate, another precipitate separated. After adding water to make the precipitation complete, the solid was collected and washed with water. The dry material weighed 0.46 g., m.p. 115–145°. In contrast to the amide it was easily soluble in pure benzene. Concentration of such a solution to a small volume, and removal of a trace of the amide by filtration, led to slow crystallization of crude nitrile, giving a total of 0.45 g. (24%). Attempts to purify a sample by crystallization or by sublimation at 120° in high vacuum were not successful, but filtration of a benzene solution through alumina followed by washing with the same solvent gave readily crystalline, colorless material, m.p. 117–118°. Recrystallization for analysis did not change the melting point.

Anal. Calcd. for $C_{17}H_{14}N_2O$: N, 10.44. Found: N, 10.63.

7-Benzyloxyindole-3-acetic Acid (XIVa).—Acidification of the filtrate from the crude nitrile gave a colorless solid, which after washing and drying yielded microscopic leaflets, 0.54 g. (27%), m.p. 163–165°. A sample was recrystallized from dilute ethanol yielding shining leaflets, m.p. 164.5–165°.

Anal. Calcd. for $C_{17}H_{15}NO_3$: C, 72.58; H, 5.38; N, 4.98. Found: C, 72.88; H, 5.66; N, 5.19.

The infrared spectrum (Nujol mull) shows a sharp band at 2.93 (>NH of indole shifted by intermolecular bonding); 5.86s (carbonyl of carboxyl).

7-Hydroxyindole-3-acetic Acid (XIVb).—A solution of 0.28 g. of 7-benzyloxyindole-3-acetic acid in 15 cc. of ethyl acetate was hydrogenated in the presence of 0.28 g. of palladium catalyst. One mole of hydrogen was taken up in ten minutes. The filtrate from the catalyst on evaporation to dryness *in vacuo* gave 0.19 g. (100%) of colorless crystals, m.p. 175–176° (vigorous evolution of gas).

Anal. Calcd. for $C_{10}H_9NO_3$: C, 62.82; H, 4.75; N, 7.34. Found: C, 62.56; H, 5.19; N, 7.16.

The infrared spectrum (Nujol mull) shows a sharp band at 2.90 (>NH); 5.85s (carboxyl).

The color reactions and R_f values of this compound have been reported by Stowe and Thimann.[45]

7-Benzyloxytryptamine (XIIa).—Reduction of 0.74 g. of the amide in a filter thimble by continuous extraction into a solution of 1.3 g. of lithium aluminum hydride in 150 cc. of ether required one week. At the end of this time ethanol was added and then potassium sodium tartrate solution. The ether layer was separated and the aqueous phase was extracted three times with more ether. The combined extracts were washed with water, dried over solid potassium hydroxide and evaporated to dryness. The amine began to crystallize while there was still much ether left. Recrystallization from benzene gave 0.3 g. (43%) of colorless crystals, m.p. 167.5–168.5°. A second recrystallization did not change the melting point.

Anal. Calcd. for $C_{17}H_{18}N_2O$: C, 76.66; H, 6.81; N, 10.52. Found: C, 77.13; H, 6.88; N, 9.92.

7-Benzyloxytryptamine Hydrochloride.—The salt was prepared by passing hydrogen chloride gas into a solution of the amine in benzene. Two recrystallizations from ethanol-ether gave glittering leaflets, m.p. 214–216°. The hydrochloride is not very stable and a satisfactory analysis has not yet been obtained, but the results indicate the presence of a hydrate.

Anal. Calcd. for $C_{17}H_{18}N_2O \cdot HCl \cdot H_2O$: C, 63.75; H, 6.36. Found: C, 64.52; H, 6.70.

7-Benzyloxytryptamine Picrate.—The picrate was prepared by adding aqueous picric acid to a solution of the above hydrochloride in water. After two recrystallizations from ethanol shiny orange platelets were obtained, m.p. 241–242° dec.

Anal. Calcd. for $C_{23}H_{21}N_5O_8$: C, 55.76; H, 4.27; N, 14.14. Found: C, 56.07; H, 4.34; N, 13.74.

7-Hydroxytryptamine Hydrochloride (XIIb).—The catalytic debenzylation of 0.15 g. of 7-benzyloxytryptamine hydrochloride in ethanol solution in the presence of 0.15 g. of 5% palladium-on-carbon, was complete in 15 minutes. The initially colorless solution which was obtained after removal of the catalyst was concentrated *in vacuo*, but even under these conditions appreciable darkening occurred. When only a small amount of ethanol was left the cautious addition of ether produced a crystalline precipitate which was crystallized once more in the same fashion, yielding 0.08 g. (76%) of a microcrystalline powder rapidly acquiring a purplish tinge, m.p. 145–148°.

Anal. Calcd. for $C_{10}H_{12}N_2O \cdot HCl$: C, 56.47; H, 6.16; N, 13.17. Found: C, 56.39; H, 6.26; N, 12.79.

7-Hydroxytryptamine Picrate.—The picrate was obtained by treating an aqueous solution of the hydrochloride with

picric acid. Two crystallizations from water gave small, stubby, ruby-red needles. The salt charred slowly from 210 to 340°, but did not melt.

Anal. Calcd. for $C_{10}H_{12}N_2O \cdot C_6H_3N_3O_7$: C, 47.40; H, 3.73. Found: C, 47.66; H, 4.04.

7-Benzyloxytryptophan (XVIa).—A mixture of 0.12 g. of powdered sodium hydroxide, 1.5 g. of diethylformamidomalonate, 1.68 g. of 7-benzyloxygramine and 20 cc. of toluene was refluxed under nitrogen. After 35 hours the evolution of dimethylamine had ceased, and the solution was cooled and washed three times with dilute hydrochloric acid, once with water, with sodium bicarbonate solution and then again with water. After drying, the organic phase was concentrated, leaving a light-colored gum which could not be induced to crystallize. The condensation product which weighed 2.4 g. (97.5%) was hydrolyzed directly by boiling with a solution of 2 g. of sodium hydroxide in 20 cc. of water. The ester slowly dissolved, and after eight hours the colorless solution was acidified with 30 cc. of 2 *N hydrochloric acid*[51] and boiled for another 45 minutes. The white, crystalline precipitate which had separated on addition of the acid, slowly dissolved again while carbon dioxide was evolved. When almost all had dissolved, crystallization began once more and was complete after refluxing for 30 more minutes. The cool solution was filtered and the amino acid washed with water and dried, yielding 1.62 g. (95.5%) of colorless, fine crystalline powder. The product was almost insoluble in water. A sample was purified for analysis by acidification of a hot dilute sodium carbonate solution with acetic acid. 7-Benzyloxytryptophan crystallized then in colorless glittering leaflets, m.p. 234.5–236° (dark viscous melt).

Anal. Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.76; H, 6.04; N, 9.07.

7-Hydroxytryptophan (XVIb).—A suspension of 0.5 g. of 7-benzyloxytryptophan, 0.25 g. of 5% palladium-on-charcoal in 10 cc. of water and 10 cc. of ethanol was hydrogenated at room temperature and atmospheric pressure. The amino acid slowly dissolved and the theoretical amount of hydrogen was taken up in about an hour. The catalyst was filtered off and the filtrate evaporated *in vacuo* until crystallization commenced. A tthis point 50 cc. of absolute alcohol was added and the solution evaporated in a stream of nitrogen until crystallization was well under way. After standing overnight the product was collected. After washing with absolute alcohol and drying 0.23 g. (65%) of nearly colorless nicely crystalline 7-hydroxytryptophan was obtained. A sample was purified for analysis as described above for the 5-isomer, giving colorless needles or rods, m.p. >330° (chars).

Anal. Calcd. for $C_{11}H_{12}N_2O_3$: C, 59.99; H, 5.50; N, 12.73. Found: C, 59.87; H, 5.58; N, 12.66.

The R_f value in 80% aqueous pyridine was 0.72, in 70% aqueous propanol 0.34.

2-Benzyloxyaniline.—*o*-Aminophenol was acetylated in aqueous solution[52] and the crude *o*-hydroxyacetanilide, obtained in 93% yield, used directly in the next step.[53] A mixture of 315 g. of *o*-hydroxyacetanilide, 295 g. of anhydrous potassium carbonate, 396 g. of benzyl bromide and 2650 cc. of dry acetone was refluxed for 58 hours with stirring and the exclusion of moisture. The mixture was then distilled to remove approximately 1500 cc. of acetone, and the residue transferred to a beaker with the aid of 1 l. of 10% sodium hydroxide. The mixture was heated on the steam-bath until most of the acetone had been driven off and then allowed to cool. An oil separated and solidified. The crystalline cake was broken up, collected and washed with dilute sodium hydroxide and water. The crude, wet *o*-benzyloxyacetanilide (560 g.) was refluxed with 2 l. of a 20% solution of potassium hydroxide in ethanol for 17 hours. At this point 1700 cc. of ethanol were removed by distillation; 1500 cc. of water was added to the residue and the distillation continued until the distillate became cloudy. The residue was cooled and the product, a dark oil, was separated. The aqueous phase was extracted twice with ether. The oil and the ether extracts were combined, washed once with water, and dried over potassium carbonate. The ether was distilled off and the remainder distilled at 115–120° (0.001 mm.), giving 353 g. (78% based on *o*-aminophenol) of colorless liquid. The product crystallized readily and completely at room temperature. Recrystallized from ligroin, it melted at 38–39° alone or mixed with an authentic sample.[35]

Attempted Preparation of *o*-Benzyloxyphenylhydrazine.—A solution of 7 g. of *o*-benzyloxyaniline in 3.5 cc. of concentrated hydrochloric acid and 35 cc. of water was filtered, treated with 35 cc. more of concentrated hydrochloric acid and cooled. The resulting suspension of the amine hydrochloride was diazotized by the addition of a solution of 2.6 g. of sodium nitrite in 5.5 cc. of water while the temperature was kept between 0 and 5°. The clear solution of the diazonium salt was added to an ice-cold solution of 24 g. of stannous chloride in 35 cc. of concentrated hydrochloric acid. A rose-colored precipitate separated, quickly turned brown forming a gum, which was treated with dilute sodium hydroxide solution and extracted with benzene. The dark-colored extract was washed with water and dried over sodium sulfate. When hydrogen chloride gas was passed into the benzene solution a precipitate separated. It was centrifuged, washed with ether and recrystallized twice from ethanol and ether to give the hydrochloride of the starting aniline, m.p. 152–154° not raised by recrystallization. Repetitions of the experiment, varying the conditions or using sodium sulfite as reducing agent,[54] were equally unsuccessful.

Anal. Calcd. for $C_{13}H_{13}NO \cdot HCl$: N, 6.00. Found: N, 6.27.

***o*-Tosyloxyaniline.**—Crude *o*-hydroxyacetanilide, prepared by the acetylation of 145.6 g. of *o*-aminophenol hydrochloride, was dissolved in 500 cc. of 2 *N* sodium hydroxide and treated with 210 g. of *p*-toluenesulfonyl chloride. The mixture was warmed on the steam-bath with frequent stirring for 45 minutes until the supernatant liquid showed acid reaction. It was then cooled and the product, *o*-tosyloxyacetanilide, collected, washed with water and pressed as dry as possible. The crude product was hydrolyzed directly by boiling with 1 l. of 15% hydrochloric acid and 100 cc. of ethanol. The solution was clear after one-half hour and, after refluxing for one hour, was set aside and allowed to stand overnight. After treatment with charcoal the solution of the aniline hydrochloride was made basic with sodium hydroxide and extracted with benzene. The extract was dried over sodium sulfate, the benzene boiled off on the steam-bath and the residue distilled *in vacuo*. The *o*-tosyloxyaniline distilled at 150–175° (0.01 mm.). After crystallization from benzene 208 g. (79% based on *o*-aminophenol) of colorless material was obtained, m.p. 99–102°, reported 98.5°.[37]

Hydrochloride.—The mother liquors, from the crystallization of the free base, were combined and treated with hydrogen chloride gas. An oil separated and crystallized from ethanol and much ether in the form of fine needles, m.p. 176–178°.

Anal. Calcd. for $C_{13}H_{14}NO_3S \cdot HCl$: N, 8.90. Found: N, 9.35.

Ethyl Pyruvate *o*-Tosyloxyphenylhydrazone.—A solution of *o*-tosyloxybenzenediazonium chloride was prepared by diazotizing 13.2 g. of the *o*-tosyloxyaniline in 20 cc. of concentrated hydrochloric acid with 3.5 g. of sodium nitrite in 10 cc. of water and diluting with 25 cc. of water. The solution was cooled to −5° and added rapidly with stirring to an ice-cold solution of 7.2 g. of α-acetopropionic acid ethyl ester in 50 cc. of ethanol to which 17 cc. of an ice-cold 50% aqueous potassium hydroxide solution and 100 cc. of ice-water had just been added. A dark, reddish-brown oil separated immediately which was extracted with ether after stirring for five minutes. The combined ether extracts were washed twice with 5% sodium hydroxide solution and several times with water. After drying over sodium sulfate, the solvent was removed, first on the steam-bath and then in a vacuum desiccator overnight. The crude hydrazone was dissolved in 60 cc. of commercial absolute ethanol, and hydrogen chloride gas was passed through this solution for four hours. The mixture, which became hot from the

(51) Again (*cf.* ref. 48) the use of acetic acid at this stage (ref. 20) yielded the sodium salt of the aminomalonic acid XV, no m.p. but charring at 250–300° when heated from room temperature, melting with decomposition at 295° when immersed in a bath at 290°.

(52) *Cf.* L. F. Fieser, "Experiments in Organic Chemistry," D. C. Heath and Co., Boston, Mass., 1941, p. 165.

(53) *Cf. Org. Syntheses*, **25**, 9 (1945).

(54) *Org. Syntheses*, Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 442.

heat of solution of the gas, deposited no ammonium chloride. After standing for 20 hours under exclusion of moisture the clear solution was refluxed for one-half hour. Some hours later an appreciable amount of crystalline material had separated, and the mixture was poured into ice-water, and extracted with ether. The oily, dark-colored solid was not very soluble in ether and it was brought into solution with a little benzene. The extract was washed once with dilute sodium hydroxide and several times with water. Drying over sodium sulfate and removal of the solvent left a dark sirup which crystallized overnight in the refrigerator; it was filtered, pressed well and washed repeatedly with ice-cold methanol. A bright yellow, crystalline product was obtained which was recrystallized twice from alcohol giving light yellow, irregular platelets, m.p. 127–128°. The yield was 6 g.

Anal. Calcd. for $C_{18}H_{20}N_2O_5S$: C, 57.41; H, 5.36; N, 7.58. Found: C, 56.97; H, 5.36; N, 7.45.

Only starting material could be recovered in attempts to cyclize this hydrazone by the several methods used by Hughes, Lions and Ritchie.[33]

7-Benzyloxyoxindole.—A 70% yield of the oxindole was obtained by dissolving 0.9 g. of 2-nitro-3-benzyloxyphenylacetic acid in a mixture of 4.5 cc. of concentrated aqueous ammonia and 1.5 cc. of water and adding a hot solution of 5.5 g. of ferrous sulfate in 6 cc. of water. The mixture was heated for an hour with frequent shaking, Filter-cel added, and the ferrous hydroxide collected and washed several times with hot, dilute ammonia. The combined filtrate and washings were acidified, but only a small amount of material precipitated. The mixture was then heated to boiling to effect cyclization, and now much more product crystallized on cooling. The oxindole was collected and washed with ice-cold water. After drying and recrystallization from benzene, colorless needles were obtained, m.p. 153–154°.

Anal. Calcd. for $C_{15}H_{13}NO_2$: N, 5.86. Found: N, 5.93.

7-Benzyloxyindoline.—Two-tenths of a gram of lithium aluminum hydride was powdered under nitrogen and suspended in 25 cc. of anhydrous ether. A solution of 1.2 g. of 7-benzyloxyoxindole in 20 cc. of tetrahydrofuran was added in the course of 15 minutes. The mixture was refluxed for three hours and allowed to stand overnight. The next day excess reducing agent was decomposed by the addition of ethanol and the precipitated complex decomposed by the addition of potassium sodium tartrate solution. The ether–tetrahydrofuran layer was separated and the aqueous phase was extracted three times with benzene. The combined extracts were washed three times with dilute hydrochloric acid and then with water, saturated sodium bicarbonate solution and water again. After drying over sodium sulfate, the solvent was evaporated and the residue crystallized from benzene. Only starting material (oxindole) was obtained and the mother liquors gave no red precipitate or red coloration with picric acid in benzene. The acid extracts became turbid, and a very small amount of an oil separated, when treated with an excess of potassium hydroxide. The base was extracted with ether which was then boiled off; the residue was treated with dilute hydrochloric acid which caused the formation of a crystalline hydrochloride. The amount was so small, however, that it was not isolated, but brought into solution by warming and precipitated with an aqueous solution of picric acid. The oily yellow picrate crystallized readily; it was centrifuged, washed with cold water and recrystallized from ethanol after drying; yellow crystals, m.p. 128–129°.

Anal. Calcd. for $C_{15}H_{15}NO \cdot C_6H_3N_3O_7$: N, 12.33. Found: N, 11.88.

2-Nitro-3-benzyloxyphenylacetonitrile.—The oxime of 2-nitro-3-benzyloxyphenylpyruvic acid was prepared in almost quantitative yield, by dissolving 0.95 g. of the pyruvic acid in 3 cc. of 10% sodium hydroxide solution and adding 0.21 g. of hydroxylamine hydrochloride dissolved in a small amount of water. The dark red color of the mixture started to fade immediately and after 30 minutes the solution had a brownish-orange color which did not change further. After standing for a total of one hour the colorless oxime was precipitated by acidification, filtered off, washed with water and recrystallized twice from dilute methanol. Almost colorless prisms were obtained which began to decompose and melt at 175°, reported m.p. 190–191°.[7] The nitrile was obtained by adding in small portions 1 g. of oxime to 2 cc. of acetic anhydride kept at 110°. Reaction took place with the evolution of gas. When all of the oxime had been added, the mixture was heated to boiling and then allowed to cool. The light yellow reaction mixture was poured on ice and allowed to stand. The oily nitrile separated and crystallized on standing; it was collected, washed with water and dried to give 0.77 g. of a light yellow, nicely crystalline powder, m.p. 111–113°. Recrystallization from ethanol gave small prisms melting at 113–114°.

Anal. Calcd. for $C_{15}H_{12}N_2O_3$: C, 67.15; H, 4.51. Found: C, 67.26; H, 4.73.

An attempt to reduce this nitrile by the method described by Stephen[55] gave only tars.

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(55) H. Stephen, *J. Chem. Soc.*, **127**, 1874 (1925).