phenylhydrazone[4,5] in 300 g. of polyphosphoric acid was warmed in an oil-bath with stirring. When the temperature of the mixture reached 90°, an exothermic reaction was noted. An ice-bath was used to keep the temperature below 125°. The mixture was stirred at 105–115° for 30 minutes before ice-water was added to hydrolyze the polyphosphoric acid. The solid was collected, washed thoroughly with water, and dried. Extraction with 150 ml. of ethanol removed unchanged starting material. The residue was recrystallized from ethanol to give 18.7 g. (57%) of yellow crystals, m.p. 220–223°. An analytical sample melted at 225–226°.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.64; H, 4.52; N, 11.81.

Ethyl 7-Nitroindole-2-carboxylate (IId).—A mixture of 20 g. (0.080 mole) of ethyl pyruvate *o*-nitrophenylhydrazone[4,5] and 110 g. of polyphosphoric acid was treated as described for the *p*-isomer. The crude product was extracted with 3.0 l. of boiling ligroin (b.p. 60–90°). Concentration of the ligroin solution to 350 ml. and cooling gave 12.3 g. (66%) of yellow needles, m.p. 91–93°. An analytical sample recrystallized from dilute ethanol melted at 92–93°.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.37; H, 4.09; N, 11.80.

Fischer Cyclization of Ethyl Pyruvate *m*-Nitrophenylhydrazone. A. Ethyl 4-Nitroindole-2-carboxylate (IIa).—A mixture of 21 g. (0.084 mole) of ethyl pyruvate *m*-nitrophenylhydrazone[4,5] and 100 g. of polyphosphoric acid was treated as described for the *p*-isomer. The crude product was crystallized from 800 ml. of benzene using charcoal. A yellow solid separated slowly at room temperature. After several days there was obtained 9.5 g. of crystalline product, m.p. 170–228°. Two recrystallizations from benzene gave 3.8 g. (19%) of yellow needles, m.p. 228–230°.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.18; H, 4.48; N, 12.14.

B. **Ethyl 6-Nitroindole-2-carboxylate (IIc).**—The mother liquor from which crude IIa, m.p. 170–228°, was obtained was concentrated to 200 ml. and left overnight at room temperature. A crop of 3.5 g. of yellow-orange crystals, m.p. 170–192°, separated. Two recrystallizations from benzene gave 1.6 g. (8.2%) of yellow needles, m.p. 195–197°.

Anal. Calcd. for $C_{11}H_{10}N_2O_4$: C, 56.40; H, 4.30; N, 11.96. Found: C, 56.49; H, 4.34; N, 11.85.

Further concentration of the mother liquor yielded 1.9 g. of product melting at 175–177°. Repeated recrystallizations failed to change the melting point of this material. It appears to be a mixture of the 4- and 6-isomers, since a mixture of equal parts of IIa and IIc melted at 175–177° and gave no melting point depression when mixed with the above material. A similar phenomenon has been observed for a mixture of the corresponding trifluoromethylindole esters.[10]

Nitroindole-2-carboxylic Acids (Table I).—To a solution of 9.7 g. (0.04 mole) of the ethyl nitroindole-2-carboxylate in 75 ml. of hot ethanol was added a solution of 7.5 g. (0.13 mole) of potassium hydroxide in 18 ml. of water. A copious precipitate of the potassium salt separated as the mixture stood for six hours at room temperature. The addition of 350 ml. of warm water gave a solution which was added slowly with stirring to excess dilute hydrochloric acid. The solid was collected, washed thoroughly with water and dried. The crude acids were decarboxylated without further purification. Analytical samples were prepared by crystallization from dilute ethanol.

TABLE I

NITROINDOLE-2-CARBOXYLIC ACIDS

	M.p., °C.	Yield, %	Analyses, % C	H	N
4-Nitro-	317–318	91	53.03	3.27	13.22
5-Nitro-	326–328	90	52.15	3.45	13.87
6-Nitro-	304–305	92	52.45	3.04	13.38
7-Nitro-	269–272	90	52.53	3.07	13.93
	Calcd. for $C_9H_6N_2O_4$:		52.44	2.93	13.59

Nitroindoles (Table II).—A mixture of 11.3 g. (0.055 mole) of the nitroindole-2-carboxylic acid, 1.1 g. of cupric oxide and 90 ml. of quinoline was refluxed for two hours. The dark mixture was stirred into 100 ml. of concentrated hydrochloric acid and 200 g. of ice. Filtration removed a dark solid. Both the solid and filtrate were extracted with ether. The combined extracts were washed with dilute sodium bicarbonate solution and water before they were dried and evaporated. The residue was recrystallized from dilute ethanol.

TABLE II

NITROINDOLES

Nitro group	M.p., °C.	Yield, %	Analyses, %[c] C	H	N	Ultraviolet spectra[a] λ_{max}, mμ ($\epsilon \times 10^{-3}$)	λ_{min}, mμ ($\epsilon \times 10^{-3}$)
4-	205–206	51	59.76	3.85	17.02	235(10.9) 380(6.4)	283(0.5)
5-	141–142	71	59.35	3.95	16.85	265(17.7) 324(8.4)	238(6.2) 290(3.3)
6-	144–145	38	59.48	3.50	16.96	250(10.1) 326(8.4) 355–360[b] (7.7)	227(6.4) 280(1.7)
7-	95–96	78	59.28	4.03	16.78	232(9.4) 250(7.8) 365(7.2)	223(8.5) 244(7.7) 285(0.4)

[a] In 95% ethanol. [b] Inflection. [c] Calcd. for $C_8H_6N_2O_2$: C, 59.26; H, 3.73; N, 17.28.

WHEATON, ILLINOIS

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(10) J. Bornstein, S. A. Leone, W. F. Sullivan and O. F. Bennett, THIS JOURNAL, **79**, 1745 (1957).

[CONTRIBUTION FROM THE NOYES CHEMICAL LABORATORY, UNIVERSITY OF ILLINOIS]

Synthesis of Indoles by Catalytic Reduction of *o*-Nitrobenzyl Cyanides

BY H. R. SNYDER, E. P. MERICA, C. G. FORCE AND E. G. WHITE

RECEIVED MARCH 20, 1958

Numerous substituted indoles have been synthesized by the use of a recently developed method involving catalytic reduction of *o*-nitrobenzyl cyanides over palladium-on-carbon. By the same method indole has been prepared from the benzoate of *o*-nitromandelonitrile. Possible mechanisms are discussed. Attempts to prepare indole and simple substituted indoles by the Stephen reduction of *o*-nitrobenzyl cyanides have been unsuccessful. In some instances 2-aminoindoles were obtained instead.

In studies of the synthesis of certain 6-substituted indoles, an attempt was made to utilize the Stephen reaction[1] on the appropriate *o*-nitrobenzyl cyanides. When ethyl 2-nitro-4-carbethoxyphenylcyanoacetate (I) was treated with the Stephen reagent–an ethereal solution of anhydrous stannous chloride, saturated with dry hydrogen chloride–2-amino-3,6-dicarbethoxyindole (II) was isolated in 68% yield instead of the expected 3,6-dicarbethoxyindole

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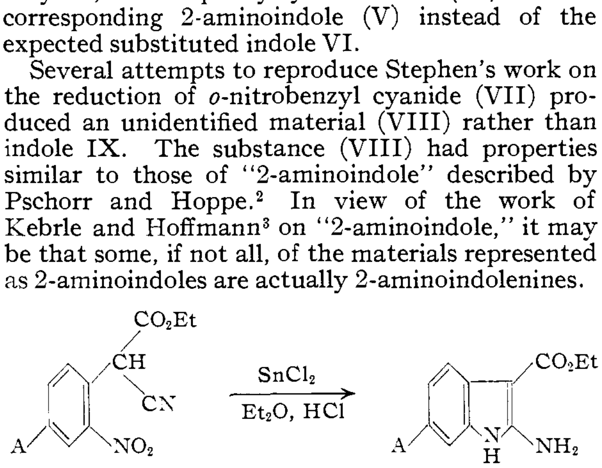
(1) H. Stephen, *J. Chem. Soc.*, **127**, 1874 (1925).

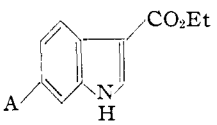
(III). Similarly, the Stephen reagent converted ethyl 2,4-dinitrophenylcyanoacetate (IV) to the corresponding 2-aminoindole (V) instead of the expected substituted indole VI.

Several attempts to reproduce Stephen's work on the reduction of *o*-nitrobenzyl cyanide (VII) produced an unidentified material (VIII) rather than indole IX. The substance (VIII) had properties similar to those of "2-aminoindole" described by Pschorr and Hoppe.[2] In view of the work of Kebrle and Hoffmann[3] on "2-aminoindole," it may be that some, if not all, of the materials represented as 2-aminoindoles are actually 2-aminoindolenines.

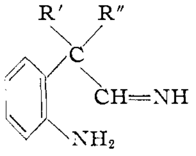


I, A = $CO_2C_2H_5$
IV, A = NO_2

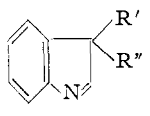
II, A = $CO_2C_2H_5$
V, A = NH_2



III, A = $CO_2C_2H_5$
VI, A = NH_2



XVIII



XIX

Work was in progress in this Laboratory on the reductive cyclization of *o*-nitrobenzyl cyanides over palladium-on-carbon catalyst when the reports of Walker[4] and of Plieninger and Nógrádi[5] appeared. Walker[4] converted various α-substituted 2-nitro-4,5-dimethoxyphenylacetonitriles (X) to the corresponding indoles XI by catalytic reduction over palladium-on-carbon. Plieninger and Nógrádi[4] reported that 5,6-dimethoxyindole could be prepared in 95% yield, compared with the 60% yield obtained by Walker, if Raney nickel were used as the catalyst.

During the present work, numerous indoles were prepared by the catalytic reduction of the appropriate *o*-nitrobenzyl cyanides in the presence of palladium-on-carbon. The reaction was utilized to convert I to III, 2-nitro-4-carbethoxybenzyl cyanide (XII) to 6-carbethoxyindole (XIII), VII to IX, and 5-benzyloxy-2-nitrobenzyl cyanide (XIV) to 5-hydroxyindole (XV), in 90, 75, 39 and 75% yield, respectively.

Because of the structural similarity between *o*-nitromandelonitrile (XVI) and *o*-nitrobenzyl cyanide, it seemed appropriate to investigate the possibility of converting XVI or one of its derivatives to indole. In order to avoid the dissociation of the cyanohydrin to the aldehyde and hydrogen cyanide, the benzoyl derivative, *o*-nitromandelonitrile benzoate (XVII), was used. Hydrogenolyses of benzyl esters are well known.[6] Catalytic reduction of XVII in ether solution over palladium-on-carbon catalyst in the presence of triethylamine and anhydrous magnesium sulfate produced indole in 60% yield.

Walker[4] concluded that the course of the catalytic reduction of *o*-nitrobenzyl cyanides was as follows. Rapid reduction of the nitro group produces the *o*-aminobenzyl cyanide, which forms an internal amidine. The amidine isomerizes, if there is at least one hydrogen atom on position 3, to the 2-aminoindole, which is converted to the corresponding indole only under vigorous reducing conditions. The present work suggests that the above scheme is incorrect. Compound II, treated with hydrogen over palladium-on-carbon under conditions more vigorous than those required to convert I to III, was recovered unchanged. Walker's mechanism requires that III be produced.

An alternative course can be suggested for the catalytic reduction of *o*-nitrobenzyl cyanides in a non-acidic medium. After the rapid reduction of the nitro group, the addition of one mole of hydrogen to the nitrile group would form a β-*o*-aminoarylethylimine (XVIII). This compound could form an indolenine (XIX) in one of at least two ways: (a) cyclization by intramolecular loss of ammonia, or (b) by the hydrolysis of the imine to the corresponding aldehyde, which could cyclize to form XIX and water. Thus, only a catalytic amount of water would be required. The indolenine XIX could then tautomerize to the indole if either R′ or R″ is a hydrogen atom.

Experimental[7]

Ethyl 2-Nitro-4-carbethoxyphenylcyanoacetate (I).—Clean sodium (9.2 g.) was added to a solution of 150 ml. of absolute ethanol and 150 ml. of anhydrous benzene. The excess ethanol and benzene were removed under diminished pressure after the reaction was complete. Absolute ether (300 ml.) was added, and the resulting suspension was cooled to 0° in an ice-bath while 45.2 g. of ethyl cyanoacetate was rapidly added dropwise. A solution of 54.8 g. of ethyl 3-nitro-4-bromobenzoate in 500 ml. of absolute ether was slowly added dropwise. After the addition was complete, stirring was continued for 48 hr. at reflux temperature, during which time the color of the mixture changed from pink to dark red. The contents of the flask was cooled and shaken with 1500 ml. of water. The layers were separated, and the aqueous layer was extracted four times with ether. The combined ether extract was cooled in an ice-bath while 1:3 hydrochloric acid solution was added slowly until the color changed from red to white. An oil separated and solidified almost immediately. It was collected, dried, and recrystallized from ether–petroleum ether solution; m.p. 73–75°, yield 47.8 g. (78%). The analytical sample melted at 73.5–75°.

Anal. Calcd. for $C_{14}H_{14}O_6N_2$: C, 54.89; H, 4.61; N, 9.14. Found: C, 55.10; H, 4.47; N, 9.20.

Stephen Reaction on I.—A suspension of 95 g. of anhydrous stannous chloride in 750 ml. of absolute ether was stirred well while dry hydrogen chloride gas was passed through it. When the stannous chloride had dissolved and two layers were present, a solution of 30.6 g. of I in 400 ml. of absolute ether was added in one portion. The reaction was exothermic; an ice-bath was used to cool the mixture. The mixture was stirred for 4 hr. and then filtered. The precipitate was suspended in 1 l. of water and 40% sodium hydroxide was added slowly until the *p*H was 9. The suspension was warmed and stirred on a steam-bath for 10 min. and then it was cooled and filtered. The precipitate was dissolved in hot ethanol and filtered to remove a little insoluble material. Upon cooling, white crystals formed. The material weighed 13.68 g. and melted at 212–214°. When water was added to the purple filtrate, 5.02 g. of additional material was ob-

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[2] R. Pschorr and G. Hoppe, *Ber.*, **43**, 2543 (1910).

[3] J. Kebrle and K. Hoffmann, *Helv. Chim. Acta*, **39**, 116 (1956).

[4] G. N. Walker, THIS JOURNAL, **77**, 3844 (1955).

[5] H. Plieninger and I. Nógrádi, *Ber.*, **88**, 1961 (1955).

[6] W. H. Hartung and R. Simonoff, *Org. Reactions*, **7**, 263 (1953).

[7] Melting points are uncorrected unless otherwise stated.

tained. The total yield of II was 18.7 g. (68%). The material gave a positive Ehrlich test, and the infrared spectrum contained absorption bands at 2.96 and 6.1 μ. The analytical sample melted at 214.5–215.5°.

Anal. Calcd. for $C_{14}H_{16}O_4N_2$: C, 60.86; H, 5.84; N, 10.14. Found: C, 60.91; H, 5.94; N, 10.34.

The acetyl derivative, m.p., 225–226°, and the urethan (ethyl carbamyl derivative, m.p. 149°) were prepared.

Anal. Calcd. for $C_{16}H_{18}O_5N_2$: C, 60.37; H, 5.69; N, 8.80. Found: C, 60.14, H, 5.46; N, 8.93. Calcd. for $C_{17}H_{20}O_6N_2$: C, 58.61; H, 5.79; N, 8.04. Found: C, 58.80; H, 6.02; N, 8.18.

Catalytic Reduction of I to 3,6-Dicarbethoxyindole (III).—A solution of 6.12 g. of I in 60 ml. of freshly distilled ethyl acetate was placed in a pressure bottle with 1 g. of 30% palladium-on-carbon. Hydrogenation was begun at 56 p.s.i. at 22°. Approximately 3 moles of hydrogen per mole of I was absorbed during the first 15 min. The temperature was raised to 85–90° and another mole of hydrogen was absorbed during the next 2 hr. The product, insoluble in ethyl acetate, was collected by filtration along with the catalyst. The product was dissolved in ethanol, filtered to remove the catalyst, and recrystallized twice from ethanol–water solution. It melted at 134–136°. A sample purified by sublimation melted at 139.4–139.9°. The yield was 4.70 g. (90%). The product gave a positive Ehrlich test, and the infrared spectrum contained absorption bands at 3.11 and 6.15 μ.

Anal. Calcd. for $C_{14}H_{15}O_4N$: C, 64.35; H, 5.79; N, 5.36. Found: C, 64.69; H, 6.19; N, 5.55.

Ethyl 2,4-Dinitrophenylcyanoacetate (IV).—Clean sodium (23 g.) was cut in small pieces and added to a solution of 300 ml. of absolute ethanol and 200 ml. of anhydrous benzene. When the reaction was complete, the excess ethanol and benzene were removed under diminished pressure. Absolute ether (800 ml.) was added and the suspension was cooled to 0° in an ice-bath while 113 g. of freshly distilled ethyl cyanoacetate was added rapidly through a dropping funnel. A thick white paste formed. A solution of 101 g. of 2,4-dinitrochlorobenzene in 300 ml. of absolute ether was added slowly dropwise and, after the addition was complete, the resulting red mixture was stirred for 18 hr. at room temperature. The solid material was collected by filtration and washed with absolute ether and then dissolved in 2 l. of water. Ether (500 ml.) was added and the solution was carefully acidified in an ice-bath with about 200 ml. of 1:3 hydrochloric acid solution. The ether layer was evaporated on a steam-bath, leaving an oil which solidified on being permitted to stand overnight. After the material was recrystallized twice from ethanol, the melting point was 64–65°. The yield was 111.3 g. (80%).

Stephen Reaction on IV.—A suspension of 30.4 g. of anhydrous stannous chloride in 250 ml. of absolute ether was saturated with dry hydrogen chloride. Compound IV (5.6 g.) was added to the mixture in small portions with vigorous shaking. When the addition was complete, the mixture was allowed to stand for 3 hr.; two layers formed. The lower layer was separated and made alkaline with 10% aqueous sodium hydroxide in an ice-bath. A white solid formed and was collected by filtration. The crude product melted at 170° dec. Two recrystallizations from water produced no change in the melting point. The infrared spectrum contained absorption bands at 2.97 and 6.02–6.08 μ. The product presumably is V.

Anal. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.25; H, 5.97; N, 19.13. Found: C, 60.51; H, 6.25; N, 19.00.

Stephen Reaction on *o*-Nitrobenzyl Cyanide (VII).—A suspension of 24 g. of anhydrous stannous chloride in 200 ml. of absolute ether was saturated with dry hydrogen chloride gas. A solution of 4.05 g. of *o*-nitrobenzyl cyanide in 200 ml. of absolute ether was added all at once. A precipitate formed immediately; stirring was continued overnight. The lower layer, containing the solid, was separated and dissolved in water. The *p*H was raised to 5 with 10% sodium hydroxide, and the mixture was extracted several times with ether. Evaporation of this extract produced no residuum. The *p*H of the aqueous layer was raised to 8. A white precipitate formed. The suspension was steam distilled until 2 l. of distillate had been collected. The distillate was extracted with ether, and the ether extract was dried over anhydrous magnesium sulfate and evaporated. Only a trace of red solid remained. The aqueous residue from the steam distillation developed a purple color on exposure to air. Only highly colored impure materials could be isolated from it.

Catalytic Reduction of VII to Indole IX.—A solution of 3.24 g. of *o*-nitrobenzyl cyanide in 60 ml. of ethyl acetate was placed in a pressure bottle with 1 g. of 30% palladium-on-carbon. Hydrogenation was begun at 60 p.s.i. at room temperature. About 4 moles of hydrogen per mole of VII was taken up in the first 10 minutes. The temperature was raised to 80–90° for 3 hr., but there was no further change. When the bottle was opened, the effluent gases had the odor of ammonia. The mixture was filtered and the filtrate was distilled at 45° at 30 mm. until no more distillate was obtained. The remaining brown liquid was dissolved in ethanol and treated with Darco. Water was added, and crystals formed when the solution was cooled. A sample purified by sublimation melted at 50–51°. The yield was 0.92 g. (39%). The infrared spectrum was identical with the spectrum of an authentic sample of indole.

Anal. Calcd. for C_8H_7N: N, 11.97. Found: N, 11.96.

Ethyl *p*-Bromomethylbenzoate.—A solution of 108.9 g. of ethyl *p*-toluate (b.p. 136° at 55 mm.) in 450 ml. of Merck reagent grade carbon tetrachloride was heated to the boiling point in a 2-l. 3-necked flask fitted with a stirrer, a condenser and a Gooch tube attached to an erlenmeyer flask containing 124 g. of purified N-bromosuccinimide and 1 g. of Eastman grade benzoyl peroxide, well mixed. The N-bromosuccinimide-peroxide mixture was added to the boiling solution in small portions. The reaction was vigorous with considerable foaming. Refluxing was continued for 3 hr., after which the succinimide which had separated was removed by filtration and the carbon tetrachloride was removed by distillation at 60 mm. When the residue was cooled to 0°, it solidified. The crude material weighed 102.2 g. (100%). The material was used in the preparation of the cyanide without further purification. The melting point was 35° (lit.[8] 35–36°).

Ethyl *p*-Cyanomethylbenzoate.—A solution of 60.6 g. of ethyl *p*-bromomethylbenzoate in 70 ml. of ethanol was added dropwise to a solution of 15.0 g. of potassium cyanide in 25 ml. of hot water at such a rate as to maintain gentle reflux. Refluxing was continued for 4 hr. As much alcohol as possible was removed at 40 mm., and the remaining liquid was poured into 800 ml. of water. The oil which separated was taken up in ether. The ether solution was dried over magnesium sulfate and distilled. The product distilled at 105–108° at 0.1 mm. The yield was 32.0 g. (74%). A sample purified by redistillation boiled at 107–108° at 0.1 mm. and melted at 28°.

4-Carbethoxy-2-nitrobenzyl Cyanide (XII).—A mixture of 3.15 g. of fuming nitric acid and 18 ml. of concentrated sulfuric acid was maintained at 5–10° while 3.05 g. of ethyl *p*-cyanomethylbenzoate was added dropwise. Stirring was continued while the temperature was kept at 5–10° for 4 hr. The orange solution was then poured into 100 ml. of ice-water; a solid formed. It was collected by filtration and recrystallized from ether–petroleum ether solution. White needles were obtained which melted at 75.5–76.5°, yield 2.21 g. (59%).

Catalytic Reduction of XII to 6-Carbethoxyindole (XIII).—A solution of 1.84 g. of XII in 50 ml. of ethyl acetate was treated with hydrogen in the presence of 0.3 g. of 30% palladium-on-carbon at 67 p.s.i. About 2.5 moles of hydrogen was consumed per mole of XII in the first 15 min. The temperature was raised to 85–90° and the bottle was flushed occasionally and recharged with hydrogen until no more ammonia was given off when the bottle was opened. The catalyst was removed by filtration and the filtrate was evaporated to dryness on a steam-bath at 25 mm. The residue was recrystallized from ethanol–water solution. The yield was 1.12 g. (75%).

Anal. Calcd. for $C_{11}H_{11}O_2N$: C, 69.82; H, 5.86; N, 7.41. Found: C, 69.44; H, 5.62; N, 7.43.

5-Benzyloxy-2-nitrobenzyl Cyanide (XIV).—2-Nitro-5-benzyloxyphenylpyruvic acid was prepared by the method of Stoll and his co-workers,[9] which is a modification of the classic method of Blaikie and Perkin,[10] using the Koelsch

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(8) A. F. Titley, *J. Chem. Soc.*, 2571 (1928).

(9) A. Stoll, *et al.*, *Helv. Chim. Acta*, **38**, 1452 (1955).

(10) K. G. Blaikie and W. H. Perkin, Jr., *J. Chem. Soc.*, **125**, 296 (1924).

method of preparing the intermediate nitrocresol.[11] The substituted phenylpyruvic acid was then converted to XIV *via* the sodium derivative by the method of Snyder and Williams.[12]

Catalytic Reduction of XIV to 5-Hydroxyindole (XV).—Recrystallized XIV (1.000 g.) was dissolved in 83 ml. of ethyl acetate and treated with hydrogen at 40 p.s.i. for 2 hr. at 80° in the presence of 1.64 g. of 25% palladium-on-carbon catalyst. The hot solution was filtered and concentrated to dryness. The colored material was purified by sublimation at 0.1 mm., and produced 0.374 g. (75%) of material melting at 103–107° (cor., Kofler). The infrared spectrum in Nujol, and the ultraviolet spectrum in ethanol corresponded with those published by Stoll, *et al.*,[9] for 5-hydroxyindole. Recrystallization of the sublimed product from benzene gave material which melted at 107.5–108° (cor., Kofler).

***o*-Nitromandelonitrile Benzoate (XVII).**—*o*-Nitromandelonitrile (XVI) was prepared from *o*-nitrobenzaldehyde *via* the bisulfite addition compound by the method of Reissert and Hessert.[13] The benzoate XVII was prepared from XVI by the method of Heller.[14]

Catalytic Reduction of XVII to Indole IX.—*o*-Nitromandelonitrile benzoate (XVII, 1.88 g.), 0.8 ml. of triethylamine, 1.0 g. of 30% palladium-on-carbon catalyst and 2.5 g. of anhydrous magnesium sulfate were washed into a hydrogenation bottle with 25 ml. of anhydrous ether. The vessel was flushed and pressurized to 45.0 p.s.i. with hydrogen gas. Most of the uptake, corresponding to 3.7 moles of hydrogen per mole of XVII, occurred in the first 1.5 min. of agitation. The vessel was vented and recharged four times at convenient periods during the course of the reaction. After 25.5 hr., the reaction was terminated, and the ether solution was filtered. The filtrate was extracted with two 5-ml. portions of 3% aqueous sodium hydroxide solution, two 2.5 ml. portions of water, two 5-ml. portions of 3% aqueous hydrochloric acid and two 2.5-ml. portions of water. The residual ether solution was dried over anhydrous magnesium sulfate and filtered, and the solvent was evaporated. A white crystalline material formed which was sublimed, producing 0.47 g. of glistening white plates melting at 51–52.5°. A mixture of the product with authentic indole melted at 51–52.7°, and the infrared spectra of the product and authentic indole were identical. The yield was 60%.

Attempted Catalytic Reduction of 2-Amino-3,6-dicarbethoxyindole (II) to 3,6-Dicarbethoxyindole (III).—A solution of 2.76 g. of II in 60 ml. of ethyl acetate was shaken with 1.0 g. of 30% palladium-on-carbon under 65 p.s.i. of hydrogen at 100–105° for 3 hr. When the bottle was opened, no ammonia odor was detected. The warm suspension was filtered, and the filtrate was replaced in the pressure bottle with 1.0 g. of fresh palladium-on-carbon catalyst. The mixture was treated with hydrogen under the above conditions for 4 hr. When the bottle was opened, no ammonia odor was detected. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was II.

Acknowledgment.—We are grateful to Mr. J. Nemeth, Mrs. E. Fett, Mrs. L. Chang, Mrs. M. Benassi, Mrs. M. Stingl, Mrs. R. Ju, Miss C. Higham and Mr. R. Nesset for the microanalyses; Messrs. J. Brader, B. Clooman, and P. McMahon and Mrs. L. Giffing for the infrared analyses; and Miss G. Meerman and Mr. M. S. Chao for the ultraviolet analyses. E. G. W. was granted a Fellowship by the College of Medical Evangelists, Loma Linda, Calif., E. P. M. and C. G. F. held Research Assistantships under a grant [AT-(11-1)314] from the Atomic Energy Commission.

URBANA, ILL.

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(11) C. F. Koelsch, THIS JOURNAL, **66**, 2019 (1944).

(12) H. R. Snyder and J. K. Williams, *ibid.*, **76**, 1298 (1954).

(13) A. Reissert and K. Hessert, *Ber.*, **57**, 964 (1924).

(14) G. Heller, *ibid.*, **39**, 2334 (1906).

[CONTRIBUTION FROM THE PIONEERING RESEARCH DIVISION, QUARTERMASTER RESEARCH AND ENGINEERING CENTER]

Enzyme Action on Partition Chromatographic Columns

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This paper describes a method of obtaining an essentially continuous enzyme reaction on a two-phase column utilizing partition chromatography. In this method the enzyme, dissolved in an aqueous phase, is retained as the stationary phase on a column of hydrophilic solid such as cellulose. The substrate, moving through the column in the solvent phase, diffuses into the stationary aqueous phase where reaction with the enzyme occurs. Products of the reaction then diffuse back into the mobile phase and pass down the column. We have successfully used this principle for the reaction of β-glucosidase on several β-glucosides and invertase on sucrose. In some cases products have been obtained which result from the transfer of glycosyl groups to the alcohols used as the mobile phase. The enzymes used remain active under the conditions described for a number of weeks. While only hydrolysis and transfer reactions are reported here, it is believed that the principle involved is applicable to a wide variety of systems.

Introduction

Our original aim in using enzymes on columns was to devise a method for obtaining intermediates in enzyme reactions. In early work using adsorbed enzymes it was found that only a very small portion of the activity was retained. Such a procedure has been granted a patent (Stone)[1] but the claims indicate a slow and inefficient process. Using a two-phase column it was found that the enzyme remains free in the aqueous phase and retains high activity. This method approaches an ideal system with new substrate constantly entering from the solvent phase, and products being constantly removed. The enzyme action is continuous. There is neither inhibition by products, nor reduction in rate due to depletion of substrate. Further advantages are the absence of enzyme in the reaction products, the ability to obtain short-lived intermediates; the possibility of accomplishing a series of reactions by placing various enzymes at different levels in the column, and the ability to obtain synthetic products by transfer reactions. The process thus appears to have wider applicability than originally envisioned.

Methods

A. **Preparation of Column.**—The aqueous phase and the solvent phase were shaken together and allowed to stand overnight so as to become mutually saturated. The hydrophilic substance, cellulose (Solka floc, Brown Co.), was prepared as a slurry in the aqueous phase (0.01 *M* citrate *p*H 5.4) and dropped into the solvent phase to form a column (80 × 15 mm.). The column was then washed with the

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(1) I. Stone, U. S. Patent 2,717,852 (1955).