ethyl alcohol. On cooling, 3 g. (70% yield) of the monopicrate separated. It was recrystallized from 95% ethyl alcohol, m.p. 193°.

*Anal.* Calcd. for $C_{18}H_{20}N_6O_7$: N, 19.44. Found: N, 19.22.

The picrate salt was shaken with a mixture of 25 ml. of nitrobenzene and 25 ml. of concentrated hydrochloric acid. The hydrochloric acid layer was separated and concentrated by heating under reduced pressure, and the residue was recrystallized from ethyl alcohol–ether, m.p. 195° (capillary); $\lambda_{max}$ 286 mμ, log ε 3.74.

*Anal.* Calcd. for $C_{12}H_{17}N_3 \cdot 2HCl$: C, 52.18; H, 6.93; N, 15.21. Found: C, 51.98; H, 7.20; N, 15.37.

**3-Chloromethylindazole (XVI).**—A solution of 2.2 g. (0.015 mole) of 3-hydroxymethylindazole[1] ($\lambda_{max}$ 253 mμ (log ε 3.58), 286 (3.69)) and 25 ml. of thionyl chloride was heated under reflux for 2 hours. The excess thionyl chloride was removed by heating under reduced pressure, and dry ether was added to the residue. The solid that resulted was collected and recrystallized from a thionyl chloride–ether mixture. The 3-chloromethylindazole hydrochloride weighed 1.5 g. (50% yield) and melted over a temperature range near 240°. When the salt was dried at 78° and 1 mm. pressure, hydrogen chloride was lost and 3-chloromethylindazole base was obtained, m.p. 110°.

*Anal.* Calcd. for $C_8H_7ClN_2$: C, 57.68; H, 4.24; N, 16.82. Found: C, 57.47; H, 4.36; N, 16.46.

**3-Cyanomethylindazole (XVII).**—To a solution of 50 g. of potassium cyanide and 50 ml. of water was added 20 g. (0.1 mole) of 3-chloromethylindazole hydrochloride dissolved in 500 ml. of ethyl alcohol. After stirring at room temperature for 8 hours the mixture was filtered, and the filtrate was concentrated by heating under reduced pressure. The residue was extracted with ether. After drying the ether was evaporated and a residue of crude 3-cyanomethylindazole remained. (This material was used in the preparation of 1-acetyl-3β-acetylaminoethylindazole given above.) The residue was distilled under reduced pressure, and about 5 g. (32% yield) of pure product was obtained, b.p. 220° (1 mm.). The product solidified on standing and was recrystallized from benzene–petroleum ether, m.p. 75°; $\lambda_{max}$ 253 mμ (log ε 3.61), 2.91 (3.69).

*Anal.* Calcd. for $C_9H_7N_3$: C, 68.77; H, 4.49; N, 26.74. Found: C, 68.46; H, 4.44; N, 24.48.

**α-Amino-β-(4,5,6,7-tetrahydroindazole-3)-propionic Acid (XIV).**—To a stirred mixture of 6.6 g. (0.033 mole) of diethyl formylaminomalonate, 3.3 g. (0.06 mole) of sodium methylate and 50 ml. of ethyl alcohol, cooled to 10°, was added 6.2 g. (0.03 mole) of 3-chloromethyl-4,5,6,7-tetrahydroindazole hydrochloride dissolved in 50 ml. of ethyl alcohol. Following addition the mixture was stirred at room temperature for 6 hours and then allowed to stand overnight. The salt was removed by filtration, and the filtrate was concentrated to dryness. The residue was dissolved in 300 ml. of hot ethyl acetate. This solution was concentrated to 100 ml. After cooling 4 g. (36% yield) of ethyl α-carbethoxy-α-formylamino-β-(4,5,6,7-tetrahydroindazole-3)-propionate separated, m.p. 165–167°; $\lambda_{max}$ 226 mμ, log ε 3.79.

*Anal.* Calcd. for $C_{16}H_{23}N_3O_5$: N, 12.46. Found: N, 12.58.

The ethyl α-carbethoxy-α-formylamino-β-(4,5,6,7-tetrahydroindazole-3)-propionate was heated overnight on the steam-bath with 50 ml. of concentrated hydrochloric acid. After removing the acid by heating under reduced pressure the residue that remained was dissolved in 50 ml. of ethyl alcohol, and 1 ml. of aniline was added. The α-amino-β-(4,5,6,7-tetrahydroindazole-3)-propionic acid that separated was collected and recrystallized from water, m.p. 270–272° dec.; $\lambda_{max}$ 226 mμ, log ε 3.73. The over-all yield was about 30%.

*Anal.* Calcd. for $C_{10}H_{15}N_3O_2$: C, 57.40; H, 7.23; N, 20.08. Found: C, 57.00; H, 7.25; N, 20.13.

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[CONTRIBUTION FROM THE LILLY RESEARCH LABORATORIES]

# The Indazole Analog of Serotonin

BY C. AINSWORTH

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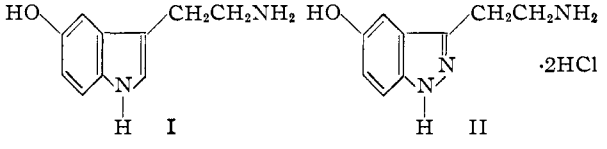
The preparation of 3β-aminoethyl-5-hydroxyindazole dihydrochloride (II) together with some preliminary pharmacological findings is described.

Serotonin (5-hydroxytryptamine, I) has been examined in some detail as a physiological agent,[1,2] and indications are that it will continue to attract further investigation.[3] A number of indole derivatives have been synthesized[4] and found to possess varying degrees of serotonin and antiserotonin action in pharmacological tests. In this Laboratory we have been interested for some time in tryptamine compounds and recently have reported[5] the indazole analog of tryptamine. The present paper describes the synthesis of compound II, which is the indazole analog of serotonin.




(1) I. H. Page, *Physiol. Rev.*, **34**, 563 (1954).

(2) V. Erspamer, *Pharmacol. Rev.*, **6**, 425 (1954).

(3) I. H. Page and J. W. McCubbin, *Circulation*, **14**, 161 (1956), entitled their editorial "Serotonin or Tenure for the Pharmacologist."

(4) For a leading reference see E. Shaw, THIS JOURNAL, **77**, 4319 (1955).

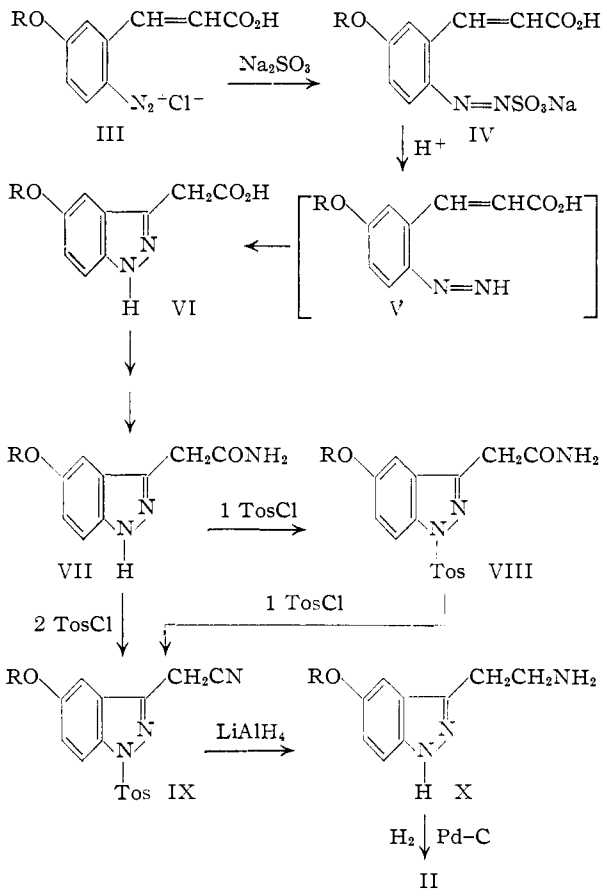
(5) C. Ainsworth, *ibid.*, **79**, 5242 (1957).

The method of preparation of compound II is illustrated by the following series of reactions where R is benzyl. The ring-closure step involving the conversion of compound IV to VI is analogous to the reaction reported by Fischer and Tafel[6] for the preparation of 3-indazoleacetic acid from sodium 2-(2-carboxyvinyl)-phenylazosulfonate.

5-Benzyloxy-2-nitrobenzaldehyde was prepared according to the procedure reported by Ek and Witkop.[7] It was converted to the corresponding nitrocinnamic acid by the standard malonic acid method, and this was reduced with ferrous hydroxide to give 2-amino-5-benzyloxycinnamic acid. The amine was diazotized, and the resulting salt III was treated with sodium sulfite to form compound IV. The azo compound IV was warmed with dilute hydrochloric acid, and the indazole VI was formed in good yield. Compound V is proposed as an intermediate in the formation of compound VI from IV. It has the feature of being in the same oxidation state as compound VI.

(6) E. Fischer and J. Tafel, *Ann.*, **227**, 303 (1885).

(7) A. Ek and B. Witkop, THIS JOURNAL, **76**, 5579 (1954), footnote 44a.




The indazoleacetic acid VI was converted to the ethyl ester and this in turn to the amide VII. The amide was not reduced by lithium aluminum hydride even when the mixture was heated for a long period of time. Attempts to dehydrate the amide VII to the corresponding nitrile were not successful when standard reagents were used. However, the arylsulfonyl chloride method of Stephens, Bianco and Pilgrim[8] using two moles of tosyl chloride in pyridine brought about a smooth conversion of compound VII to IX. When one mole of tosyl chloride was used compound VIII was formed, and this in turn with another mole of tosyl chloride was converted to IX.[9] By treatment of compound IX with lithium aluminum hydride the nitrile grouping was reduced to the amine and the tosyl was cleaved[10] to give the aminoethylindazole X. The benzyl grouping of compound X was hydrogenolyzed using 5% palladium-on-carbon to form the desired indazole II.

3β-Aminoethyl-5-hydroxyindazole dihydrochloride (II) has been tested for pharmacological action; preliminary results indicate that it behaves much like serotonin. On a weight basis it is about one-fourth to one-half as active as 5-hydroxytryptamine creatinine sulfate (serotonin) on the isolated rat uterus. The stimulating action is blocked by a 1:200,000,000 dilution of lysergic acid diethylamide. Compound II is 80% as active as serotonin on the isolated guinea pig ileum, both agents being blocked by atropine but not by "Histadyl" (Thenylpyramine, Lilly). In cats under chloralose anesthesia, compound II, administered intravenously, showed about one-tenth the depressor response of serotonin.

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## Experimental[11]

**5-Benzyloxy-2-nitrocinnamic Acid.**—A solution of 405 g. (1.6 moles) of 5-benzyloxy-2-nitrobenzaldehyde,[7] 330 g. (3.2 moles) of malonic acid, 12 ml. of piperidine and 474 g. (6 moles) of dry pyridine was heated on the steam-bath for 2 hours and then under reflux for 15 minutes. It was added to 3 l. of ice-water containing 500 ml. of concentrated hydrochloric acid. The solid that separated was collected, washed with water and air-dried. The product was recrystallized from about 4 l. of 95% ethyl alcohol, and 350 g. (71% yield) of 5-benzyloxy-2-nitrocinnamic acid was obtained, m.p. 180°; $\lambda_{max}$ 259 mμ (log ε 4.29), 309 (3.96).

*Anal.* Calcd. for $C_{16}H_{13}NO_5$: C, 64.21; H, 4.38; N, 4.68. Found: C, 64.67; H, 4.36; N, 4.52.

**2-Amino-5-benzyloxycinnamic Acid.**—In a 5-l. 3-neck flask equipped with a stirrer and heating mantle was placed 370 g. (1.3 moles) of ferrous sulfate heptahydrate, 3 l. of hot water, 50 g. (0.16 mole) of 5-benzyloxy-2-nitrocinnamic acid dissolved in 100 ml. of 5 *N* ammonium hydroxide and 250 ml. of concentrated ammonium hydroxide. After heating near boiling for 30 minutes the black mixture was filtered through a previously heated large büchner funnel containing a thin layer of Filter-Cel. The filter cake was heated to boiling with 2 l. of 1 *N* sodium hydroxide, and the mixture was filtered. The combined filtrates were treated with 100 ml. of acetic acid and then with 6 *N* hydrochloric acid to *p*H 4. After cooling, the yellow solid that separated was collected, washed with water, slurried with 500 ml. of ethyl alcohol and then air-dried. The 2-amino-5-benzyloxycinnamic acid weighed 40 g. (93% yield) and melted at 200°. A sample was recrystallize l from ethyl alcohol, m.p. 205–206° dec.; $\lambda_{max}$ 236 mμ (log ε 4.18), 282 (4.06), 380 (3.71); $pK'_a$ about 2.5 and 6.7 (66% dimethylformamide).

*Anal.* Calcd. for $C_{16}H_{15}NO_3$: C, 71.36; H, 5.61; N, 5.20. Found: C, 71.15; H, 5.76; N, 5.36.

The hydrochloride was formed in dilute hydrochloric acid and was recrystallized from water, m.p. 180° dec.

*Anal.* Calcd. for $C_{16}H_{15}NO_3 \cdot HCl$: C, 62.85; H, 5.28; N, 4.58. Found: C, 62.72; H, 5.38; N, 4.68.

**5-Benzyloxy-3-indazoleacetic Acid (VI).**—To a suspension of 9 g. (0.03 mole) of 2-amino-5-benzyloxycinnamic acid hydrochloride, 5 ml. (0.03 mole) of 6 *N* hydrochloric acid and 70 ml. of water at 20° was added 2 g. (0.03 mole) of sodium nitrite. The mixture was cooled to 0° and a tan solid separated. A sample of 4-benzyloxy-2-(2-carboxyvinyl)-benzenediazonium chloride (III) was obtained from water, m.p. 130–140°; $\lambda_{max}$ 256 mμ (log ε 4.35), 325 (4.31); infrared maxima (Nujol mull) 4.50m (N≡N), 5.87m (C=O), 6.11w (C=C). The analytical sample of IV, dried under reduced pressure at 56° for 4 hours, analyzed for a monohydrate.

*Anal.* Calcd. for $C_{16}H_{13}ClN_2O_3 \cdot H_2O$: C, 57.40; H, 4.52; N, 8.37. Found: C, 57.46; H, 4.79; N, 8.54.

To the cold diazonium salt mixture was added 9.5 g. (0.075 mole) of sodium sulfite. The temperature rose

(8) C. R. Stephens, E. J. Bianco and F. J. Pilgrim, THIS JOURNAL, **77**, 1701 (1955).

(9) The ultraviolet absorption spectra of VIII and IX are similar. The assignment of the tosyl grouping to the 1-position is based on the work of K. von Auwers, *Ber.*, **58**, 2081 (1925), who established the 1-isomer as the stable form in the tosylindazole series.

(10) M. Kulka and R. H. F. Manske, *J. Org. Chem.*, **17**, 1501 (1952), reported a similar finding in the carbazole series.

(11) Unless otherwise indicated the melting points were determined with a Fisher–Johns assembly and are reported as read. The ultraviolet absorption data were obtained in methanol.

about 5°, and an orange colored solution resulted. Stirring was continued in the ice-bath for 15 minutes and a yellow solid separated. After 30 minutes 20 ml. of acetic acid was added, and the mixture was heated on the steam-bath to effect solution. The solution was filtered, and on cooling sodium 2-(2-carboxyvinyl)-4-benzyloxyphenylazosulfonate (IV) was deposited. A sample was recrystallized from water, m.p. 155° dec.; $\lambda_{max}$ 324 mμ (log ε 4.23), 273 (4.36); infrared maxima (Nujol mull) 5.86s (C=O), 6.12w (C=C); $pK'_a$ 6.8 (66% dimethylformamide).

*Anal.* Calcd. for $C_{16}H_{13}N_2NaO_6S$: C, 49.99; H, 3.41; N, 7.29. Found: C, 49.41; H, 3.81; N, 7.27.

Compound IV was dissolved in 100 ml. of hot water, and 10 ml. of 6 *N* hydrochloric acid was added. The solution was allowed to cool slowly, and 6 g. of product was obtained. It was recrystallized from 50% aqueous ethyl alcohol, and after drying at 100° about 4.5 g. (53% yield) of 5-benzyloxy-3-indazoleacetic acid (VI) was obtained, m.p. 162°; $\lambda_{max}$ 254 mμ (log ε 3.69), 308 (3.69); infrared maxima (Nujol mull) 3.05m (bonded NH), 5.91m (C=O); $pK'_a$ 6.9 (66% dimethylformamide).

*Anal.* Calcd. for $C_{16}H_{14}N_2O_3$: C, 68.07; H, 5.00; N, 9.92. Found: C, 67.91; H, 5.22; N, 9.87.

**Ethyl 5-Benzyloxy-3-indazoleacetate.**—A solution of 16 g. (0.057 mole) of 5-benzyloxy-3-indazoleacetic acid and 200 ml. of ethyl alcohol containing 8 ml. of concentrated sulfuric acid was heated overnight under reflux. The solution was concentrated to about 50 ml. by heating under reduced pressure, and the residue was then added to 200 g. of an ice–water mixture. The solid that formed on standing was collected and washed several times with water. The product was recrystallized from 50% aqueous ethyl alcohol and was obtained as needles, m.p. 103°; $\lambda_{max}$ 254 mμ (log ε 3.69), 308 (3.71). The yield of ethyl 5-benzyloxy-3-indazoleacetate was 14 g. (80%).

*Anal.* Calcd. for $C_{18}H_{18}N_2O_3$: C, 69.66; H, 5.85; N, 9.03. Found: C, 69.46; H, 5.91; N, 8.99.

**5-Benzyloxy-3-indazoleacetamide (VII).**—A solution of 10 g. (0.032 mole) of ethyl 5-benzyloxy-3-indazoleacetate, 100 ml. of methyl alcohol, 0.5 ml. of water and 25 ml. of liquid ammonia was placed in a pressure vessel and heated at 90° for 12 hours. After cooling, the mixture was concentrated to dryness by heating under reduced pressure. The resulting solid was slurried with 50 ml. of ethyl acetate and collected by filtration. The 5-benzyloxy-3-indazoleacetamide weighed 7 g. (77% yield). A sample was obtained from a relatively large volume of ethyl acetate, m.p. 185–187°; $\lambda_{max}$ 255 mμ (log ε 3.68), 309 (3.71).

*Anal.* Calcd. for $C_{16}H_{15}N_3O_2$: C, 68.31; H, 5.38; N, 14.94. Found: C, 68.36; H, 5.55; N, 14.67.

The hydrochloride was formed in ethyl alcohol and recrystallized from an ethyl alcohol–ether mixture, m.p. 186°; $\lambda_{max}$ 254 mμ (log ε 3.69), 309 (3.72).

*Anal.* Calcd. for $C_{16}H_{15}N_3O_2 \cdot HCl$: N, 12.82. Found: N, 13.05.

5-Benzyloxy-3-indazoleacetamide hydrochloride and thionyl chloride or phosphorus pentoxide gave a black residue. Starting material was recovered when phosphorus trichloride was used, and an unidentified product formed with a phosphorus trichloride–phosphorus oxychloride mixture.

**Attempted Reduction of 5-Benzyloxy-3-indazoleacetamide.**—To a suspension of 3.8 g. (0.1 mole) of lithium aluminum hydride and 100 ml. of tetrahydrofuran was added 4.5 g. (0.016 mole) of 5-benzyloxy-3-indazoleacetamide. A reddish colored solution resulted. After heating under reflux for 36 hours and allowing to stand at room temperature for 24 days, 4 ml. of water, 3 ml. of 20% sodium hydroxide and 14 ml. of water were added. The insoluble material was removed by filtration, and the tetrahydrofuran solution was concentrated. The residue was dissolved in ethyl alcohol and treated with ether containing hydrogen chloride. About 2 g. of salt separated. The infrared spectrum of this salt was identical with that of 5-benzyloxy-3-indazoleacetamide hydrochloride.

**5-Benzyloxy-1-*p*-tosyl-3-indazoleacetamide (VIII).**—A mixture of 1.4 g. (0.005 mole) of 5-benzyloxy-3-indazoleacetamide, 0.95 g. (0.005 mole) of *p*-toluenesulfonyl chloride and 1 ml. of pyridine was heated on the steam-bath for 1 hour. The mixture was treated with 25 ml. of water, and the solid was collected and washed with water. The product was recrystallized from 95% ethyl alcohol, and 1.1 g. (50% yield) of 5-benzyloxy-1-*p*-tosyl-3-acetamide was obtained, m.p. 197°; $\lambda_{max}$ 250 mμ (log ε 4.23), 310 (3.73); infrared maxima (chloroform) 2.86w, 2.97w (NH), 5.92s (C=O), 7.25s, 8.54s ($SO_2$).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_4S$: C, 63.43; H, 4.86; N, 9.65. Found: C, 63.50; H, 5.06; N, 9.35.

**5-Benzyloxy-1-*p*-tosyl-3-indazoleacetonitrile (IX).**—A mixture of 1.4 g. (0.005 mole) of 5-benzyloxy-3-indazoleacetamide, 1.9 g. (0.01 mole) of *p*-toluenesulfonyl chloride and 3 ml. of dry pyridine was heated on the steam-bath for 30 minutes. The dark colored solution was added to 50 g. of ice-water, and after standing the solid that separated was collected and washed with water. The crude product was recrystallized from 25 ml. of hot ethyl alcohol to which was added 10 ml. of water. It was then recrystallized from an ethyl acetate–petroleum ether mixture. A final recrystallization from ethyl acetate gave 0.7 g. (44% yield) of 5-benzyloxy-1-*p*-tosyl-3-acetonitrile, m.p. 180°; $\lambda_{max}$ 250 mμ (log ε 4.27), 312 (3.70); infrared maxima (chloroform) 4.44vw (C≡N), 7.25s, 8.52s ($SO_2$).

*Anal.* Calcd. for $C_{23}H_{19}N_3O_3S$: C, 66.17; H, 4.59; N, 10.07. Found: C, 66.42; H, 4.80; N, 10.03.

A mixture of molar equivalents of 5-benzyloxy-1-*p*-tosyl-3-indazoleacetamide and *p*-toluenesulfonyl chloride in warm pyridine also gave 5-benzyloxy-1-*p*-tosyl-3-indazoleacetonitrile.

**3β-Aminoethyl-5-benzyloxyindazole (X).**—A solution of 3.2 g. (0.01 mole) of 5-benzyloxy-1-*p*-tosyl-3-indazoleacetonitrile and 100 ml. of dry tetrahydrofuran was added to a suspension of 3.8 g. (0.1 mole) of lithium aluminum hydride and 300 ml. of tetrahydrofuran. After heating overnight under reflux the mixture was treated successively with 4 ml. of water, 3 ml. of 20% sodium hydroxide and 14 ml. of water. The mixture was filtered, and the filtrate was concentrated by heating on the steam-bath. The resulting residue was extracted with 200 ml. of ethyl acetate, and after evaporating the ethyl acetate an oil remained that was induced to solidify. A sample was recrystallized from ethyl acetate–petroleum ether, and 3β-aminoethyl-5-benzyloxyindazole was obtained, m.p. 125–126°; $\lambda_{max}$ 254 mμ (log ε 3.64), 308 (3.68).

*Anal.* Calcd. for $C_{16}H_{17}N_3O$: C, 71.88; H, 6.41; N, 15.72. Found: C, 72.05; H, 6.18; N, 16.04.

The 3β-aminoethyl-5-benzyloxyindazole was dissolved in ethyl alcohol and treated with ether containing hydrogen chloride. The resulting dihydrochloride was recrystallized from ethyl alcohol, m.p. 265°; $\lambda_{max}$ 254 mμ (log ε 3.76), 308 (3.79); $pK'_a$ about 2.0 and 9.8 (66% dimethylformamide) The yield was 1.0 g. (30%).

*Anal.* Calcd. for $C_{16}H_{17}N_3O \cdot 2HCl$: C, 56.48; H, 5.63; N, 12.35. Found: C, 56.19; H, 5.59; N, 12.10.

**3β-Aminoethyl-5-hydroxyindazole Dihydrochloride (II).**—A mixture of 0.34 g. (0.01 mole) of 3β-aminoethyl-5-benzyloxyindazole dihydrochloride, 0.34 g. of 5% palladium-on-carbon and 100 ml. of ethyl alcohol was hydrogenated at room temperature and atmospheric pressure during a 6-hour period. The catalyst was removed by filtration, and the solvent was concentrated to a volume of 10 ml. Dry ether containing a small amount of hydrogen chloride was added, and the solid that separated was collected. It was recrystallized from an ethyl alcohol–ether mixture and gave 0.15 g. (60% yield) of 3β-aminoethyl-5-hydroxyindazole dihydrochloride, m.p. 235° (capillary); $\lambda_{max}$ 254 mμ (log ε 3.58), 313 (3.69); $pK'_a$ about 2.0, 9.6 and 13.2 (66% dimethylformamide). The infrared spectrum obtained in Nujol mull showed three broad bands at 2.9, 3.1–3.7 and 3.7–4.5 μ, and the following weak-medium intensity bands 6.27, 6.45, 7.86, 8.18, 8.25, 8.84, 9.16, 9.84, 12.15 and 12.36 μ.

*Anal.* Calcd. for $C_9H_{11}N_3O \cdot 2HCl$: C, 43.21; H, 5.24; N, 16.80. Found: C, 43.51; H, 5.41; N, 16.58.

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