tion to dryness gives a gum which is transformed to a crystalline powder (5 g.) by stirring with hot ethanol while recrystallization may be accomplished from a large volume of ethanol diluted with water (m.p. 240–245°, $[\alpha]_D$ +3.1° (*c* 1, chloroform)), a wholly satisfactory solvent for the recrystallization of this substance could not be found.

Anal. Calcd. for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.95; H, 7.44; N, 7.82.

Infrared absorption shows a weak band in the NH-OH region at 3183 cm.$^{-1}$ and a carbonyl band at 1734 cm.$^{-1}$.

SUMMIT, NEW JERSEY

[CONTRIBUTION FROM THE RESEARCH DEPARTMENT, CIBA PHARMACEUTICAL PRODUCTS, INC.]

Rauwolfia Alkaloids. XV. The Constitution of Reserpic Acid: Position of Substituents in Ring E

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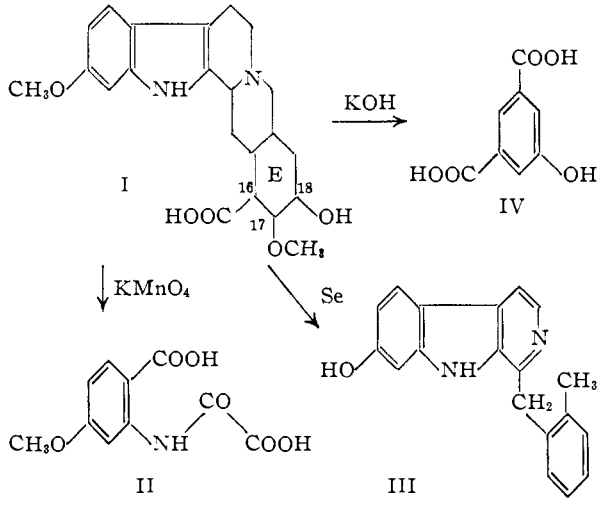
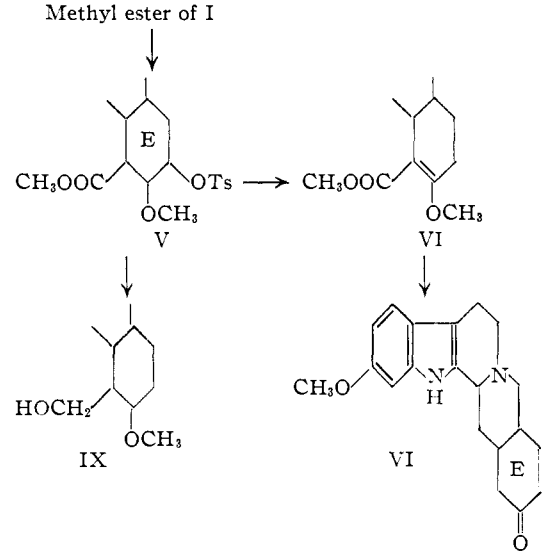
In consonance with the proposed formulation of methyl anhydroreserpate (VI) as the enol ether of a β-keto ester, it was hydrolyzed and decarboxylated by acid to the ketone VII. The carboxyl in reserpic acid has been placed in the 16-position by the sequence: reduction of the carboxyl to hydroxymethyl followed by selenium dehydrogenation of the resulting alcohol to 1-(2,6-dimethylbenzyl)-7-hydroxy-9H-pyrid[3,4-b]indole (VIII). The structure of VIII was established by synthesis of its methyl ether XIV from harmine and 2,6-dimethylcyclohexanone.

The alkaloid reserpine, the trimethoxybenzoyl ester of methyl reserpate, has acquired some importance in the therapy of hypertension[1] and more recently in psychiatric use.[2] The constitution of reserpic acid has been formulated as I. This postulate is supported by the following degradative reactions: permanganate oxidation of reserpic acid yields N-(carboxyformyl)-4-methoxyanthranilic acid (II), selenium dehydrogenation gives 7-hydroxyyobyrine (7-hydroxy-1-(2-methylbenzyl)-9H-pyrid[3,4-b]indole (III)) and by a potash fusion 5-hydroxyisophthalic acid (IV) is obtained.[3]



Since no ketoyobyrine type compound was isolated from the selenium dehydrogenation of reserpic acid, the position of the carboxyl group was not located. For biogenetic reasons, it was assumed that the carboxyl occupies the same 16-position (numbered according to Barger and Scholz[4]) as it does in yohimbine. Since reserpic acid easily lactonizes, it appeared likely that the hydroxyl group of reserpic acid is in the 18-position. Also infrared spectral evidence spoke in favor of a γ-lactone. The methoxyl group was conditionally attributed to position 17 as indicated in formula I.

Evidence for the validity of the assumption that all three substituents in ring E are on adjoining carbon atoms has been described briefly.[5] The experimental details of this degradation are given in the present paper. The tosyl ester of methyl reserpate (V) was subjected to detosylation with collidine yielding methyl anhydroreserpate (VI). Its strong infrared absorption bands at 1613 and 1709 cm.$^{-1}$ are known to be characteristic for the grouping ROOC—C=C—OR.[6] Further proof that VI is the enol ether of a β-keto acid was offered by its acid hydrolysis and simultaneous decarboxylation to the ketone reserpone[7] (VII).



Although these reactions indicated with some degree of certainty the relative positions of the

(1) R. W. Wilkins, *Ann. N. Y. Acad. Sci.*, **59**, 36 (1954).

(2) N. S. Kline, *ibid.*, **59**, 107 (1954).

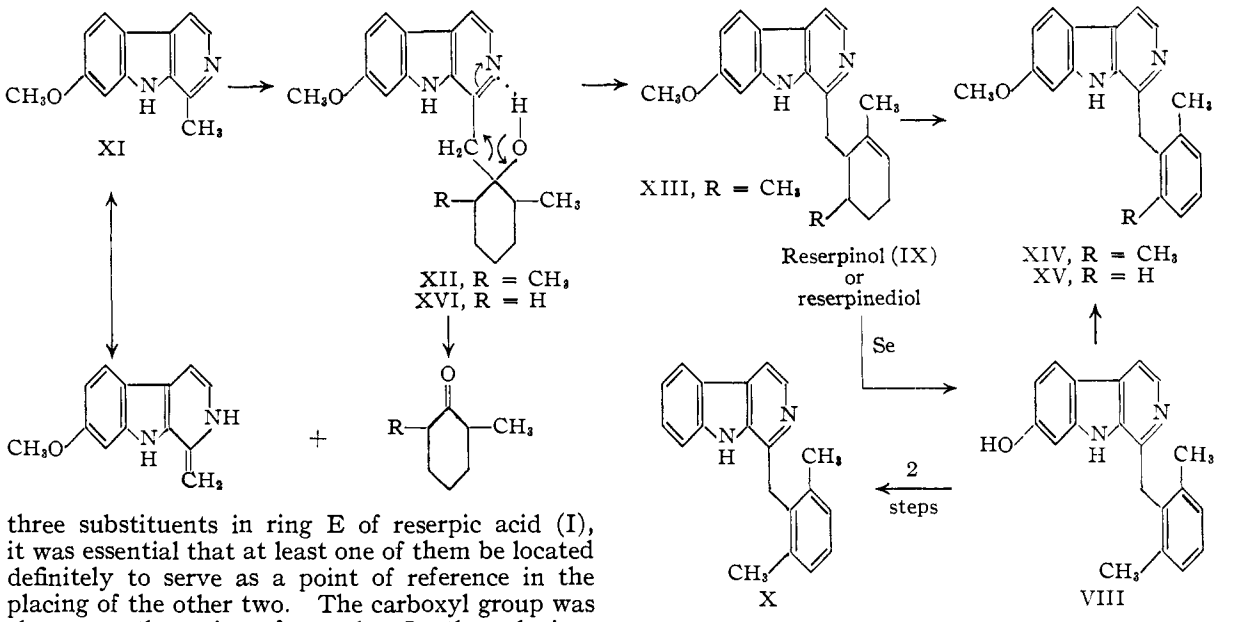
(3) L. Dorfman, A. Furlenmeier, C. F. Huebner, R. Lucas, H. B. MacPhillamy, J. M. Mueller, E. Schlittler, R. Schwyzer and A. F. St. André, *Helv. Chim. Acta*, **37**, 59 (1954).

(4) G. Barger and C. Scholz, *ibid.*, **16**, 1343 (1933).

(5) E. Schlittler, H. B. MacPhillamy, L. Dorfman, H. Furlenmeier, C. F. Huebner, R. Lucas, J. M. Mueller, R. Schwyzer and A. F. St. André, *Ann. N. Y. Acad. Sci.*, **59**, 1 (1954).

(6) F. Bader, *Helv. Chim. Acta*, **36**, 215 (1953).

(7) Trivial names are proposed for these compounds until their complete stereochemistry is certain. At that time systematic names may be assigned. See W. Klyne, *Chem. and Ind.*, 1032 (1953).



three substituents in ring E of reserpic acid (I), it was essential that at least one of them be located definitely to serve as a point of reference in the placing of the other two. The carboxyl group was chosen as the point of attack. In the selenium degradation of I, the carbon atom originally present in the carboxyl group was lost, but conversion of the carboxyl to hydroxymethyl prior to the dehydrogenation afforded a methyl substituted 7-hydroxyyobyrine (VIII) in which the entire carbon skeleton of ring E is retained. The position of the methyl in VIII should indicate the position of the original carboxyl. Reserpinediol[3] or, better, reserpinol[7] (IX), the lithium aluminum hydride reduction product of V, was used in this selenium dehydrogenation. Analogously, Karrer and co-workers[8] have obtained a methylyobyrine (1-(2,6-dimethylbenzyl)-9H-pyrid-[3,4-b]indole)(X) from yohimbyl alcohol in this manner.

The first approach to a proof of structure of VIII was an attempt to remove the phenolic hydroxyl by treatment of the tosylate with Raney nickel thereby converting it to Karrer's methylyobyrine (X). This sequence has been used successfully by us to establish the structure of 7-hydroxyyobyrine (III).[3] In the present case, however, Raney nickel treatment of the tosylate gave a mixture of substances from which it was impossible, on the small scale employed, to isolate a pure yobyrine. A persistent impurity which lowered the melting point was present although the infrared absorption spectrum of the preparation was virtually identical to that of X. In an effort to obtain a more satisfactory proof of structure of VIII, a synthesis of its methyl ether was devised.

We chose a series of reactions fashioned in part on the sempervirine synthesis of Woodward and McLamore[9] rather than the conventional yobyrine synthesis of Julian.[10] The latter, involving the preparation of 6-methoxytryptamine and 2,6-dimethylphenylacetic acid, would be excessively tedious. An excess of phenyl lithium converted harmine (XI) to its lithium derivative which was then treated with 2,6-dimethylcyclohexanone (*cis–trans* mixture) to give the carbinol XII in good yield. XII was dehydrated to the olefin XIII by the action of phosphoric anhydride in refluxing xylene. XIII is formulated as a cyclohexene derivative with the double bond out of conjugation with the harmine system rather than as the alternative cyclohexylidine because its ultraviolet spectrum was identical to that of the alcohol XII. Heating XIII with palladium on carbon at 220° for a short period gave 1-(2,6-dimethylbenzyl)-7-methoxy-9H-pyrid[3,4-b]indole (XIV) in moderate yield. XIV was identical in all respects to a sample prepared by treatment of VIII, the selenium dehydrogenation product of reserpinediol or IX, with diazomethane. Thus the experimental evidence for the assignment of the three substituents in ring E of reserpic acid is provided.

An earlier pilot experiment designed to lead to the previously known 7-methoxyyobyrine (XV) used 2-methylcyclohexanone in the reaction with the lithium derivative of harmine. It was our original intention to simultaneously dehydrate and dehydrogenate the carbinol XVI to the desired XV. We were surprised to find that treatment of XVI with palladium on carbon at 220° or merely heating at 220° resulted in the recovery of harmine and 2-methylcyclohexanone. This degradation can be explained by the indicated electronic displacement occurring within the six-membered ring formed by hydrogen bonding between the hydroxyl and the pyridine nitrogen XVI. Evidence for the existence of this ring is found in the fact that the infrared absorption of XVI shows no normal unassociated hydroxyl stretching band. To dispose of this obstacle in the synthesis, dehydration was performed separately as indicated above. In this manner XVI yielded 7-methoxy-1-(2-methylbenzyl)-9H-pyrid[3,4-b]indole (XV) which was identical in all respects to a sample prepared by the methylation of the selenium dehydrogenation

(8) P. Karrer, R. Schwyzer, A. Flam and A. Saemann, *Helv. Chim. Acta.*, **35**, 865 (1952).

(9) (a) R. B. Woodward and W. M. McLamore, THIS JOURNAL, **71**, 379 (1949); (b) B. Witkop, *ibid.*, **75**, 3361 (1953).

(10) P. L. Julian, N. J. Karpel, A. Magnani and E. W. Meyer, *ibid.*, **70**, 180 (1948).

product III. The one possible point of ambiguity in the synthesis of XIV, that of rearrangement of a methyl group during dehydration of the cyclohexanol derivative or the succeeding dehydrogenation, may be resolved. Carlin and Constantine[11] gave rigorous proof that there is no rearrangement in the dehydration and dehydrogenation of 1-(2,4-dimethylphenyl) - 2,6 - dimethyl - 1 - cyclohexanol. Further we have shown that there is no rearrangement in the sequence XVI to XV.

Although assignment of configuration to the six asymmetric centers in reserpic acid could now be made with a fair degree of assurance on the basis of the probable stereochemical course of its reported reactions, we prefer to postpone this until more positive evidence is at hand.

Because of the present widespread attention to reserpine, it is interesting to note a recent claim of A. J. Steenhauer[12] that reserpine is identical with Alkaloid B isolated by van Itallie and Steenhauer[13] in 1932. Originally, similarity of Alkaloid B with serpentinine was claimed, but with the exception of a few color reactions and a melting point no details were given at that time. In her recent paper, Steenhauer gives an empirical formula $C_{36}H_{48}N_2O_{12}$ for Alkaloid B and states that by alkaline hydrolysis an acid with m.p. 170° was obtained. The 1932 paper does not give a detailed description of how Alkaloid B was prepared. By following the process described by van Itallie and Steenhauer in 1932 and as amended in the 1954 paper, we have isolated reserpine. (We are indebted to Dr. J. Mueller, CIBA Limited, Basle, for these experiments.) The authors in the 1932 paper recognized neither the chemical nature nor the pharmacologic properties of their Alkaloid B.

We are indebted to Mr. Louis Dorfman for invaluable aid in the interpretation of spectral data and to his staff for the microanalyses. We wish also to acknowledge the capable assistance of Mmes. Jean Colville, Alice Davis and Diana Rosen.

Experimental

Reserpone (VII).—Methyl anhydroreserpate (VI)[3] (480 mg.) was refluxed for three hours in a mixture of 6 ml. of ethanol and 30 ml. of 10% aqueous hydrochloric acid. The solution was concentrated to half its original volume, made basic with 20% aqueous sodium hydroxide and extracted with chloroform. The chloroform solution was evaporated to dryness after washing with water and drying over anhydrous sodium sulfate. The residue crystallized on the addition of a few drops of methanol. It was desirable, however, to further purify the ketone by chromatography on 10 g. of alumina (acid-washed activity II to III). The material was put on the column in benzene, and crystallization of the fraction eluted with benzene–acetone (4:1) from ethyl acetate gave 160 mg. of VII, m.p. 241–242°.

Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 73.87; H, 7.45; N, 8.62.

The infrared absorption of VII exhibited a strong carbonyl band (Nujol mull) at 1704 cm.$^{-1}$.

Reserpinol (IX).—A solution of 11.37 g. of O-tosylmethyl reserpate (V) in 125 ml. of anhydrous tetrahydrofuran was added dropwise over 25 minutes to a stirred solution of 7.0 g. of lithium aluminum hydride in 290 ml. of tetrahydrofuran. The mixture was stirred under reflux for five hours and allowed to stand overnight at room temperature. Water (500 ml.) was slowly added with stirring and cooling after which the tetrahydrofuran was distilled off. The mixture of IX and inorganic salts was filtered off. After washing with water, the organic fraction was extracted four times with 300-ml. portions of hot acetone. IX, which separated during the concentration of the acetone, was recrystallized from about 400 ml. of acetone; yield 5.5 g., m.p. 248° with softening and resolidification at about 200°. This alcohol, in common with reserpinediol and yohimbyl alcohol, solvates with varying amounts of solvent. Because of this tendency, the acetate of IX is described below as a characteristic derivative. Sublimation of an analytical sample at 0.001 mm. gave IX in an unsolvated form.

Anal. Calcd. for $C_{22}H_{30}N_2O_3$: C, 71.32; H, 8.16. Found: C, 71.19; H, 7.87.

Reserpinol Acetate.—A solution of 200 mg. of IX in 3 ml. of pyridine and 1.5 ml. of acetic anhydride was allowed to stand overnight at room temperature. The residue remaining after evaporation of the liquid reactants was dissolved in a small amount of water and made basic with ammonia. The crystalline acetate which separated was filtered and recrystallized from a methanol–water mixture; yield 120 mg. This substance also solvates, as indicated by the melting point (146–150° with sintering beginning at 120° followed by partial solidification). An analytical sample dried at 0.01 mm. for 3 days at room temperature showed the presence of one mole of water of crystallization. Prolonged drying at 100° caused melting of the sample.

Anal. Calcd. for $C_{24}H_{32}N_2O_4 \cdot H_2O$: C, 66.95; H, 7.96; N, 6.51. Found: C, 66.81; H, 8.06; N, 6.28.

1-(2,6-Dimethylbenzyl)-7-hydroxy-9H-pyrid[3,4-b]indole (VIII) from Reserpinediol.—An intimate mixture of 10 g. of reserpinediol and 12 g. of amorphous red selenium was heated for 15 minutes in a bath held at 260–270° in four portions. The combined reaction mixtures were pulverized, mixed with Hyflo and continuously extracted for 24 hours with 1 l. of acetone. The acetone was reduced in volume to about 400 ml. and chromatographed on 100 g. of alumina (unwashed activity II to III). The column was eluted with 400-ml. portions of acetone. Crystalline picrates (m.p. 210–217°) were prepared by the addition of methanolic picric acid to the yellow, oily residues of the second and third acetone eluates. Regeneration of the base from these combined picrates (0.6 g.) by passage of an acetone solution over alumina gave 260 mg. of crude crystalline VIII. Two recrystallizations from ethyl acetate gave 200 mg. of needles of VIII, m.p. 268–270° (*in vacuo*). The m.p. of a mixture with 7-hydroxyyobyrine (III), the selenium dehydrogenation product of methyl reserpate (m.p. 268–270°), was depressed to 251–261°. The infrared absorption spectra of the two compounds are distinctly different. The ultraviolet absorption spectra of III and VIII are almost identical, as would be expected since nearly identical chromophoric systems are present in each compound. VIII in ethanol exhibits maxima at 242 mμ (log ε 4.68) and 302 mμ (log ε 4.32) and minima at 224 mμ (log ε 4.37) and 272 mμ (log ε 3.51).

Anal. Calcd. for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.27. Found: C, 79.59; H, 5.99; N, 9.51.

VIII from Reserpinol (IX).—A mixture of 5.2 g. of IX and 7.0 g. of selenium was divided in equal portions and heated for 15 minutes in a bath held at 265–270°. Extraction with acetone as described above gave 5 g. of dark red anhydrous material. This was dissolved in 75 ml. of benzene–acetone (4:1), filtered from a small amount of insoluble material and chromatographed on 100 g. of alumina (acid-washed activity II to III). Elution was carried out with benzene–acetone (4:1), fractions of 75 ml. being collected. Addition of a few drops of ethyl acetate to the residues from fractions 9 through 15 caused crystallization. This crystalline material (620 mg.) was collected with ethyl acetate and rechromatographed on 12 g. of alumina, eluting with benzene–acetone. The crystalline material obtained in this manner was recrystallized from ethyl acetate giving 540 mg. of VIII, m.p. 272–274° *in vacuo*, and showing an identical infrared absorption spectrum to that of the sample obtained from reserpinediol.

1-[(1-Hydroxy-2,6-dimethylcyclohexyl)-methyl]-7-methoxy-9H-pyrid[3,4-b]indole (XII).—A suspension of 4 g. of finely ground harmine (XI) in 200 ml. of anhydrous ether vigorously stirred in an atmosphere of dry nitrogen with 25

(11) R. B Carlin and D. A. Constantine, THIS JOURNAL, **69**, 50 (1947).

(12) A. J. Steenhauer, *Pharm. Weekblad*, **89**, 161 (1954).

(13) L. van Itallie and A. J. Steenhauer, *Arch. Pharm.*, **270**, 313 (1932).

ml. of an approximately 1.5 *N* ethereal solution of phenyllithium for one hour. Since a withdrawn sample of a few ml. showed a negative Michler ketone test for excess phenyllithium, another 25 ml. of phenyllithium reagent was added and stirring continued for two more hours. An excess of phenyllithium was noted at this point. The suspension of XI had become converted to its finely divided yellow lithium derivative. A solution of 9.5 g. of 2,6-dimethylcyclohexanone[14] in 25 ml. of dry ether was added dropwise and stirring was continued for 24 hours. Solution of the lithium derivative of harmine gradually took place. At the end of the reaction, ice-water was added with stirring. The ether was extracted with water, 100 ml. of 3% aqueous acetic acid (which failed to extract any appreciable amount of basic material) and 100 ml. of 5% hydrochloric acid. The hydrochloric acid caused the separation of an insoluble gummy salt. This was filtered off and converted to the free base by extraction with 250 ml. of ethyl acetate in the presence of ammonia. The ethyl acetate was dried over anhydrous sodium sulfate and evaporated to dryness *in vacuo* leaving a crystalline residue of the desired cyclohexanol. It was recrystallized from ethanol–water to give 2.1 g. of XII, m.p. 200–202°. The ultraviolet absorption spectrum of XII in ethanol shows maxima at 242 mμ (log ε 4.64) and 302 mμ (log ε 4.29) and minima at 221 mμ (log ε 4.40) and 272 mμ (log ε 3.42).

Anal. Calcd. for $C_{21}H_{26}N_2O_2$: C, 74.52; H, 7.74; N, 8.28. Found: C, 74.66; H, 7.69; N, 8.42.

1-(2,6-Dimethyl-1-cyclohexenylmethyl)-7-methoxy-9H-pyrid[3,4-b]indole (XIII).—XII (0.5 g.) was refluxed in 35 ml. of dry xylene with 1.5 g. of phosphorus pentoxide for four hours. The solids were filtered and shaken with ice-water. Excess ammonia was added and the base extracted with ethyl acetate. The ethyl acetate was dried over anhydrous sodium sulfate and evaporated to dryness. The crystalline residue was recrystallized from ethanol to give 150 mg. of XIII, m.p. 210–212°. The ultraviolet absorption spectrum of XIII in ethanol shows maxima at 242 mμ (log ε 4.52) and 300 mμ (log ε 4.32) and minima at 222 mμ (log ε 4.22) and 272 mμ (log ε 3.23).

Anal. Calcd. for $C_{21}H_{24}N_2O$: C, 78.71; H, 7.55; N, 8.74. Found: C, 78.80; H, 7.52; N, 8.94.

1-(2,6-Dimethylbenzyl)-7-methoxy-9H-pyrid[3,4-b]indole (XIV).—XIII (150 mg.) and an equal weight of 30% palladium on carbon[15] was intimately mixed and heated at 220° for 5 minutes. Since hydrogen evolution was negligible, formation of the completely aromatic yobyrine derivative must proceed by disproportionation of the cyclohexene XIII. Extending the time of heating yielded little additional hydrogen and caused only unwanted decomposition to black, tarry materials. The melt was extracted with hot acetone and evaporated to dryness. The residue was redissolved in the minimum of hot acetone, and the crystals appearing on cooling were recrystallized twice from ethanol, in which solvent they have only moderate solubility. In this manner 40 mg. of XIV was obtained as elongated plates, m.p. 254–256°. A mixture of this material with that obtained by methylation of the selenium dehydrogenation product of reserpinol (IX) (see below) melted at 254–255°. Infrared absorption spectra of both materials in Nujol mull were identical. Bands at 826 and 771 cm.$^{-1}$ are probably due to the 1,3,4- and 1,2,3-trisubstituted benzene systems respectively.

Anal. Calcd. for $C_{21}H_{20}N_2O$: C, 79.71; H, 6.37; N 8.85. Found: C, 79.46; H, 6.81; N, 8.49.

1-(2,6-Dimethylbenzyl)-7-methoxy-9H-pyrid[3,4-b]indole (XIV) from VIII.—An excess ethereal solution of diazomethane was added to a solution of 30 mg. of VIII in 1 ml. of methanol and 10 ml. of ether. After standing overnight the solution was evaporated to dryness and the residue recrystallized from ethanol. XIV (10 mg.) separated in elongated plates, m.p. 254–255°.

Anal. Calcd. for $C_{21}H_{20}N_2O$: C, 79.71; H, 6.37. Found: C, 79.98; H, 6.44.

1-[(1-Hydroxy-2-methylcyclohexyl)-methyl]-7-methoxy-9H-pyrid[3,4-b]indole (XVI).—Harmine (4 g.) was converted to its lithium derivative and treated with 8.4 g. of 2-methylcyclohexanone as described above (see XII). Isolation of the cyclohexanol in the same manner yielded 4.0 g. of XVI after recrystallization from ethanol–water, m.p. 169–170° with a sintering and resolidification at 120–130° caused by loss of solvent of recrystallization.

Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.65. Found: C, 73.58; H, 7.32; N, 8.65.

Harmine (XI) and 2-Methylcyclohexanone from XVI.—One hundred mg. of XVI was heated at 240° for 20 minutes in a 15-cm. test-tube. Droplets of 2-methylcyclohexanone soon began to appear in the cooler portions of the test-tube. The tube was cooled and 10 ml. of ether added. The solid contents of the tube were filtered and recrystallized from ethanol, m.p. 260–262°. The m.p. of a mixture of this material with harmine showed no depression.

Anal. Calcd. for $C_{13}H_{12}N_2O$: N, 13.20. Found: N, 13.25.

The ether filtrate was concentrated to dryness on the water-bath and the 2,4-dinitrophenylhydrazone prepared from the oily ketonic residue. Forty mg. of a hydrazone was obtained, which melted at 135–136°. A mixture of this material and an authentic sample of 2-methylcyclohexanone 2,4-dinitrophenylhydrazone also melted 135–136°.

7-Methoxy-1-(2-methylbenzyl)-9H-pyrid[3,4-b]indole, XV.—XVI (200 mg.) was refluxed in 25 ml. of xylene for 4 hours with 1 g. of phosphorus pentoxide. The solids were filtered and shaken with ice water. Excess ammonia was added and the base extracted with ethyl acetate. The ethyl acetate was dried over anhydrous sodium sulfate and evaporated to a few ml. and transferred to a 15-cm. test-tube. One hundred mg. of a 30% palladium-on-carbon dehydrogenation catalyst was added and the ethyl acetate completely evaporated. The mixture was heated for 30 minutes at 230°, cooled and extracted with hot acetone. The dark gummy residue remaining after removal of the acetone was dissolved in 10 ml. of benzene–methanol (9:1) and chromatographed on 5 g. of alumina (activity I). The column was developed with benzene, and the fastest moving band which fluoresced strongly under ultraviolet light was collected. Evaporation of the benzene gave 20 mg. of colorless needles of XV which was recrystallized from xylene, m.p. 230–232°. The m.p. of a mixture with a sample of XV obtained by methylation of III,[3] the selenium dehydrogenation product of methyl reserpate, was 230–232°. The infrared absorption spectra of the two samples are identical.

Anal. Calcd. for $C_{20}H_{18}N_2O$: N, 9.27. Found: N, 9.82 (1.34 mg. sample).

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(14) 2,6-Xylenol was reduced over platinum oxide in acetic acid at 50 p.s.i., the cyclohexanol oxidized and the ketone worked up as described by R. B. Carlin, THIS JOURNAL, **67**, 928 (1945).

(15) R. P. Linstead and S. L. S. Thomas, *J. Chem. Soc.*, 1127 (1940).