Found: C, 62.91; H, 7.22). The filtrate was concentrated to dryness (reduced pressure and *ca.* 25°) and a residue of 0.17 g., m.p. 130–149°, isolated. No effective separation of this mixture was accomplished.

2-Benzyl-4-carbomethoxy-2-oxazoline (XI).—To 10.0 g. (0.0643 mole) of methyl DL-serinate hydrochloride was added 10.5 g. (0.0707 mole) of freshly distilled methyl phenyliminoacetate. The suspension was stirred overnight during which time the contents of the flask were protected from light. After 20 hours of stirring the suspension was filtered. The residue collected on the filter weighed 4.80 g. (3.44 g. theoretical for ammonium chloride). The filtrate was concentrated and distilled; b.p. 120–121° (0.2 mm.), $n^{25}D$ 1.5288. The yield of 2-benzyl-4-carbomethoxy-2-oxazoline (X) was 10.78 g. (76.5% of theory).

Anal. Calcd. for $C_{12}H_{13}NO_3$: C, 65.74; H, 5.98. Found: C, 65.62; H, 5.93.

O-Phenylacetyl-DL-serine.—The saponification of 4.00 g. (0.0183 mole) of methyl 2-benzyl-4-Δ^2-oxazolinoate with 18.25 ml. of 1 *N* sodium hydroxide in 100 ml. of methanol was carried out in four hours at room temperature. Methanol was then removed under reduced pressure at 30° and after filtration the solution was acidified with 19 ml. of 1 *N* hydrochloric acid. The white solid which precipitated was filtered and, after drying in a vacuum desiccator, weighed 3.3 g. (80.5% of theory); m.p. 159–160°. A positive ninhydrin test indicated that O-phenylacetyl-DL-serine, rather than the desired 2-benzyl-4-carboxy-2-oxazoline, was obtained.

Anal. Calcd. for $C_{11}H_{13}NO_4$: C, 59.18; H, 5.87; neut. equiv., 223. Found: C, 59.14; H, 5.68; neut. equiv., 219.

All attempts to prepare 2-benzyl-4-Δ^2-oxazolinoic acid or its sodium or potassium salt failed.

Attempted Preparation of Ia *via* 2-Benzyl-4-Δ^2-oxazolinoyl Chloride.—Saponification of 2-benzyl-4-carbomethoxy-2-oxazoline in absolute methanolic potassium hydroxide gave a glassy residue which was treated with oxalyl chloride.[25] After removal of excess oxalyl chloride, the residue was dissolved in acetone and treated with L-valine in the presence of a few drops of pyridine. The product was an unidentified golden yellow solid; m.p. 238–242° dec., neut. equiv. 516, sublimed at 180° bath temperature (0.1 mm.). None of the desired product was isolated.

Methyl O-Phenylacetyl-DL-serinate Hydrochloride.—To a solution of 1.00 g. (0.00456 mole) of 2-benzyl-4-carbomethoxy-2-oxazoline in 50 ml. of purified dioxane was added 0.76 ml. (0.00912 mole) of concentrated (12 *N*) hydrochloric acid. After 90 minutes at room temperature, the solution was concentrated to dryness at reduced pressure and 30°. Two recrystallizations from dioxane–absolute ether yielded methyl O-phenylacetyl-DL-serinate hydrochloride, m.p. 142–144°.

Anal. Calcd. for $C_{12}H_{16}ClNO_4$: C, 52.65; H, 5.89; Cl, 12.95; neut. equiv., 273. Found: C, 52.66; H, 5.85; Cl, 12.9; neut. equiv., 260.

Attempted Preparation of Ia *via* 2-Benzyl-4-carboxhydrazide-2-oxazoline (XII).—By the action of anhydrous hydrazine upon 2-benzyl-4-carbomethoxy-2-oxazoline (XI) in methanol a nearly quantitative yield of a compound believed to be 2-benzyl-4-carboxhydrazide-2-oxazoline (XII), m.p. 207–208° dec., was obtained.

Anal. Calcd. for $C_{11}H_{13}N_3O_2$: C, 60.26; H, 5.98. Found: C, 60.23; H, 6.09.

Attempts to form the azide in aqueous hydrochloric acid with sodium nitrite or in ethanol using amyl nitrite and sodium ethoxide were unsuccessful. However, by dissolving the hydrazide in cold glacial acetic acid and treating with sodium nitrite, there was some indication that the azide had formed. Even so, further treatment of the "azide" with DL-valine gave no evidence of a coupling product.

Attempted Aminolysis of 2-Benzyl-4-carbomethoxy-2-oxazoline.—To 2.60 g. (0.0119 mole) of 2-benzyl-4-carbomethoxy-2-oxazoline was added a chloroform solution of methyl DL-valinate (from 2.00 g. (0.0120 mole) of methyl DL-valinate hydrochloride). The solution was subjected to 100,000 p.s.i.[26] at room temperature for 16 hours. When the pressure was released the solution was light yellow in color with a trace of suspended matter. Concentration gave a viscous, clear yellow liquid which became glassy on drying at reduced pressure. This product may have been impure methyl N-(2-benzyl-4-Δ^2-oxazolinoyl)-valinate (Ib).

Anal. Calcd. for $C_{17}H_{22}N_2O_4$: CH_3O, 9.74. Found: CH_3O, 9.17.

Distillation of a small amount of this material at 225–250° (bath temperature) and 0.1 mm. was accomplished with difficulty. The distillate was a yellow gum and all attempts to obtain a crystalline material failed.

At atmospheric pressure an ether solution of 2-benzyl-4-carbethoxy-2-oxazoline and ethyl D-valinate showed no reaction after 22 hours at 0° and 24 hours at room temperature.

(25) A. L. Wilds and C. H. Shunk, THIS JOURNAL, **70**, 2427 (1948).

(26) High pressure accelerates the aminolysis reaction as will be shown in a subsequent communication from this Laboratory.

MADISON, WISCONSIN

[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY OF PRINCETON UNIVERSITY]

Desethyllycoramine

BY W. C. WILDMAN[1] AND W. T. NORTON[2]

RECEIVED AUGUST 17, 1953

Two synthetic routes to hydrophenanthridines related to the *Lycoris* alkaloids have been investigated. A synthesis of desethyllycoramine (IVa) from 4-(3-methoxyphenyl)-5-nitrocyclohexene is described.

Lycoramine, an alkaloid isolated from *Lycoris radiata* by Kondo, Tomimura and Ishiwata,[3] is considered to possess the structure represented by IVb.[4,5] In view of several anomalies in the proof of structure of lycoramine and other closely related compounds, it seemed desirable to find suitable synthetic routes to these compounds as a means of final verification of structure. This paper describes a synthesis of desethyllycoramine (IVa).

For this synthesis, 4-(3-methoxyphenyl)-5-nitrocyclohexene proved to be an ideal starting material. The double bond was in the proper position for the introduction of the vicinal glycol function and the nitro group could be converted readily to the heterocyclic nitrogen atom. Two alternate routes using this general plan were investigated to determine the point at which the hydroxyl groups could be introduced most successfully. Hydroxylation of VII with performic acid was not satisfac-

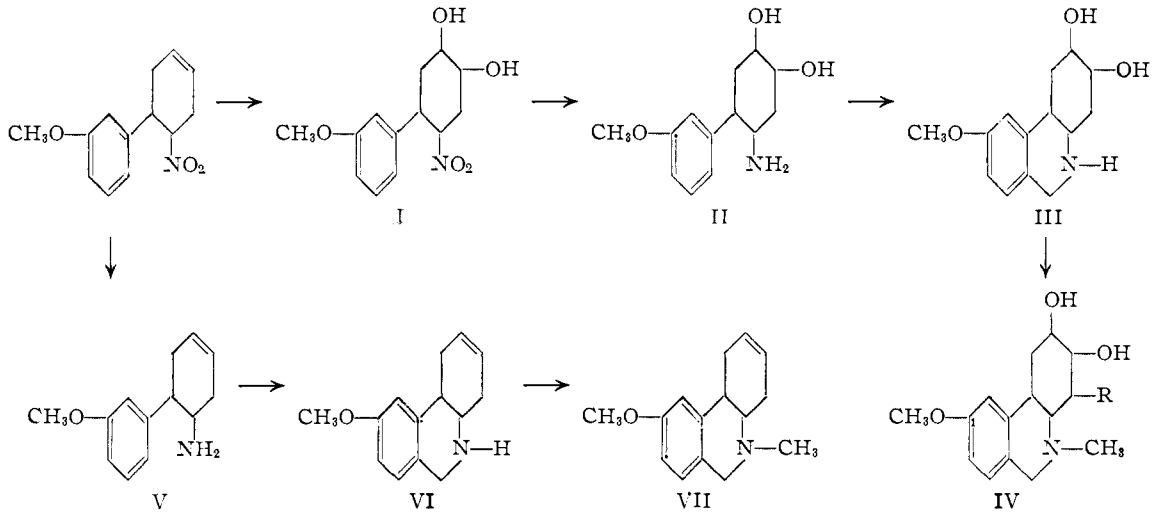
(1) National Heart Institute, National Institutes of Health, Bethesda, Maryland.

(2) Abstracted from a thesis submitted by W. T. Norton in partial fulfillment of the requirements for the degree of Doctor of Philosophy, Princeton University, September, 1953.

(3) H. Kondo, K. Tomimura and S. Ishiwata, *J. Pharm. Soc. Japan*, **52**, 51 (1932).

(4) H. Kondo and S. Ishiwata, *Ber.*, **70**, 2427 (1937).

(5) An excellent review of the alkaloids of the *Amaryllidaceae* may be found in R. H. F. Manske, "The Alkaloids," Vol. II, Academic Press, Inc., New York, N. Y., 1952, p. 331.



a, R = H
b, R = C_2H_5

tory. Since the route involving hydroxylation of 4-(3-methoxyphenyl)-5-nitrocyclohexene was successful, further studies on the conversion of VII to IVa were abandoned.

Two isomers of V were obtained upon reduction of the starting nitro compound with lithium aluminum hydride. Due to the strongly basic nature of the reducing agent, it is likely that the reduction proceeds through the *aci* form of the nitro compound and the two isomers are *cis* and *trans* forms of V. Since one isomer was isolated in very low yield (3%), it was not investigated further. Pictet–Spengler cyclization of V occurred under very mild conditions to give a 64% yield of VI. Nearly a quantitative yield of VII was obtained by Eschweiler methylation of VI. Several attempts to introduce the hydroxyl groups with performic acid were unsuccessful. It is possible that other routes to the *trans* glycol would yield the desired product. However, hydroxylation of the nitrocyclohexene occurred readily with performic acid. Catalytic reduction of I followed by cyclization and methylation gave the desired desethyllycoramine.

All known Pictet–Spengler cyclizations of *m*-alkoxyphenethylamines have occurred para to the activating group.[6] Only one product was isolated from the cyclization of II. Cyclization of V gave a 64% yield of VI and a 7% yield of by-product which was not investigated further. Infrared absorption studies of I through VII substantiate para ring closure. The spectra of all compounds as Nujol mulls were recorded from 2–15 μ. In addition, those compounds which were soluble in chloroform were studied in the 5–6 μ region by the method of Young, DuVall and Wright.[7] Concentrated chloroform solutions of both VI and VII showed absorption bands at 5.35 and 5.78 μ. This is in agreement with the absorption found for 1,2,4-trisubstituted benzene derivatives and is clearly incompatible with the spectrum expected for 1,2,3-trisubstitution. A further check on the reliability of this method was found in the spectra of I and 2-(2,3-dimethoxyphenyl)-cyclohexanone oxime which showed absorption similar to that recorded for simpler *m*- and 1,2,3-trisubstituted benzene derivatives, respectively. Compounds I, II and V showed strong absorption at 12.6–12.9 μ, characteristic of *m*-substituted benzene derivatives,[8] while III, IVa, VI and VII had strong absorption bands from 12.2–12.4 μ, characteristic of 1,2,4-trisubstitution. Only VII had a band in the 13.0–13.2 μ region, characteristic of 1,2,3-trisubstitution, and since VII is derived from VI it would seem that this absorption is not due to substitution on the benzene ring.

Lycoramine possesses five asymmetric carbon atoms while desethyllycoramine has four. At the present time no information has been recorded concerning the configuration at any of these centers although a clue in this direction may be found in the work of Kondo.[4] When oxidized with cold permanganate lycoramine forms a dihydroxylactam (carbonyl at the 9-position). Oxidation of this lactam with chromic acid gives a compound that is definitely known to be an α-diketone. However, the dihydroxylactam is extremely resistant to periodate and lead tetraacetate oxidation in spite of the vicinal glycol group. Because a *trans*-glycol is known to react less readily with these reagents than the *cis* isomer, it would seem likely that lycoramine possesses the *trans* configuration.[9] This probability was taken into consideration in the present synthesis of desethyllycoramine; peroxide–formic acid hydroxylation is known to give the *trans*-glycol.[10] Some substantiation for this assumption was found in the periodate oxidation of III and IVa. Oxidation of these compounds with periodic acid under the exact conditions used by Kondo[4] proceeded to the extent of 15 and 17%, respectively. Kondo found that the oxidation of the dihydroxylactam of lycoramine proceeded to about 12% of completion.

(6) W. M. Whaley and T. R. Govindachari, "Organic Reactions," Vol. VI, John Wiley and Sons, Inc., New York, N. Y., 1951, p. 151.

(7) C. W. Young, R. B. DuVall and N. Wright, *Anal. Chem.*, **23**, 709 (1951).

(8) F. A. Miller in H. Gilman, "Organic Chemistry," Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1953, p. 123.

(9) C. C. Price and M. Knell, THIS JOURNAL, **64**, 552 (1942); R. Criegee, *Sitzber. Ges. Beförder. ges. Naturw. Marburg*, **69**, 25 (1934).

(10) D. Swern, *ibid.*, **70**, 1235 (1948).

Experimental[11,12]

4-(3-Methoxyphenyl)-5-nitro-1,2-cyclohexanediol (I).—To a stirred solution of 0.34 g. of 30% hydrogen peroxide in 15 ml. of 88% formic acid, 2.33 g. (0.01 mole) of 4-(3-methoxyphenyl)-5-nitrocyclohexene[13] was added in small portions. Heat was evolved and the nitroölefin gradually dissolved. The reaction mixture was allowed to stand overnight and then was concentrated *in vacuo* to a viscous orange sirup which was dissolved in 20 ml. of ethanol containing 3 ml. of concentrated hydrochloric acid. The solution was heated under reflux for 30 minutes, decolorized with charcoal and poured into 100 ml. of hot water. Upon cooling, 1.62 g. of brown solid, m.p. 112–116°, was isolated. Concentration of the mother liquor gave an additional 0.463 g., m.p. 115–116°, total yield 2.083 g. (78%). An analytical sample was prepared by recrystallization from water, m.p. 118–118.5°.

Anal. Calcd. for $C_{13}H_{17}NO_5$: C, 58.41; H, 6.43; N, 5.24. Found: C, 58.29; H, 6.19; N, 5.28.

5-Amino-4-(3-methoxyphenyl)-1,2-cyclohexanediol (II).—A solution of 2.083 g. of I in 30 ml. of glacial acetic acid was reduced at a pressure of 40 pounds of hydrogen using platinum oxide as a catalyst. The catalyst was separated by filtration. The acetic acid was removed under a nitrogen jet and finally with the water pump. Addition of about 10 ml. of 10% sodium hydroxide solution caused the precipitation of the free amine. The solid was removed by filtration, 1.81 g. (98%), m.p. 171–173°. An analytical sample was prepared by sublimation and recrystallization from water, m.p. 177.5–178°.

Anal. Calcd. for $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.73; H, 8.06; N, 6.17.

Reduction of an ethanolic solution of I using Raney nickel catalyst gave an 82% yield of the product, m.p. 173–174°.

The hydrochloride of II was prepared by passing dry hydrogen chloride into an ethanolic solution of II. Recrystallization from ethanol gave colorless prisms, m.p. 263–265° dec.

Anal. Calcd. for $C_{13}H_{20}NO_3Cl$: C, 57.07; H, 7.36; N, 5.12; Cl, 12.95. Found: C, 56.94; H, 7.18; N, 5.07; Cl, 12.93.

2,3-Dihydroxy-6-methoxy-1,2,3,4,4a,9,10,10a-octahydrophenanthridine Hydrochloride.—A solution of 716 mg. (2.61 mmoles) of the hydrochloride of II in 5 ml. of water was made basic with 10% sodium hydroxide solution. When no further precipitation occurred, 0.3 ml. of formalin was added. The reaction mixture was allowed to stand 30 minutes with occasional swirling. The aqueous solution was decanted and the gummy residue was washed with 3 ml. of water. The washings were combined with the decanted solution. The residue then was treated with 2 ml. (12 mmoles) of 1:1 hydrochloric acid. Trituration of the gum caused crystallization, and after standing one hour 204 mg. of product was isolated by filtration, m.p. 262–265° dec. The filtrate was added to the decanted solution and washings, treated with 1 ml. of concentrated hydrochloric acid, and concentrated to dryness under an air jet. The solid so obtained was refluxed 10 minutes with 6 ml. of dry methanol, and the sodium chloride was removed by filtration. Concentration of the filtrate and trituration with acetone–ether gave an additional 338 mg. of product, m.p. 260–263° dec. The total yield was 73%. A portion was recrystallized from ethanol for analysis, m.p. 274.5–275° dec.

Anal. Calcd. for $C_{14}H_{20}NO_3Cl$: C, 58.84; H, 6.70; N, 4.90. Found: C, 58.96; H, 6.82; N, 5.07.

Addition of 10% sodium hydroxide solution to an aqueous solution of the salt caused precipitation of the **trihydrate of III.** Recrystallization from water gave transparent plates, m.p. 212–213°. Water of crystallization was lost from 50–100° and the crystals became opaque.

Anal. Calcd. for $C_{14}H_{19}NO_3 \cdot 3H_2O$: C, 55.43; H, 8.31; N, 4.62; H_2O, 17.82. Found: C, 55.95; H, 8.39; N, 4.78; H_2O, 17.63.

The anhydrous base (III) was obtained by heating the hydrate *in vacuo* at 100° for 3 hours, m.p. 212–213°.

Anal. Calcd. for $C_{14}H_{19}NO_3$: C, 67.44; H, 7.68. Found: C, 67.51; H, 7.77.

Desethyllycoramine (IVa).—A solution of 416 mg. (1.46 mmoles) of the hydrochloride of III in 3 ml. of water was made basic with 10% sodium hydroxide solution and heated under reflux with 4 ml. of formalin and 6 ml. of 88% formic acid for 16 hours. The solution was concentrated under an air jet and the product was precipitated with 10% sodium hydroxide solution. Recrystallization of the crude product from water gave 350 mg. (86%) of desethyllycoramine monohydrate which melted in the range of 100–120° with gradual loss of water of crystallization.

Anal. Calcd. for $C_{13}H_{21}NO_3 \cdot H_2O$: C, 64.03; H, 8.24; N, 4.98. Found: C, 64.06; H, 8.21; N, 5.36.

The hydrochloride of IVa was prepared by removing the water of crystallization azeotropically with dry benzene. The anhydrous gum was dissolved in absolute ethanol and treated with dry hydrogen chloride. The product was separated by filtration and recrystallized from ethanol, m.p. 223–225° dec.

Anal. Calcd. for $C_{15}H_{22}NO_3Cl$: C, 60.09; H, 7.40; N, 4.67. Found: C, 59.88; H, 7.46; N, 4.58.

The methiodide crystallized from water as colorless prisms which melted at 210–212°, solidified and then decomposed at 227°.

Anal. Calcd. for $C_{16}H_{24}NO_3I$: C, 47.41; H, 5.97; N, 3.46. Found: C, 47.58; H, 5.99; N, 3.65.

Periodate Oxidation of III and IVa.—Samples of 0.1596 g. of III and 0.1488 g. of IVa were oxidized by the procedure of Kondo.[4]

Anal. Calcd. for $C_{14}H_{17}NO(OH)_2 \cdot 3H_2O$ (III): 10.52 ml. of 0.1 *N* sodium thiosulfate. Found: 1.60 ml.

During the oxidation of III the sparingly-soluble acid sulfate crystallized from solution, m.p. 299–300° dec.

Anal. Calcd. for $C_{15}H_{19}NO(OH)_2 \cdot 1H_2O$ (IVa): 10.58 ml. of 0.1 *N* sodium thiosulfate. Found: 1.86 ml.

5-Amino-4-(3-methoxyphenyl)-cyclohexene (Va and Vb).—To a slurry of 9.0 g. (0.24 mole) of lithium aluminum hydride and 300 ml. of anhydrous ether was added dropwise a solution of 10.0 g. (0.043 mole) of 4-(3-methoxyphenyl)-5-nitrocyclohexene[13] in 170 ml. of anhydrous ether. After the reaction had subsided, the mixture was heated under reflux for 2.5 hours. The reaction mixture was cooled in an ice-bath and hydrolysis was effected with 5% sodium hydroxide solution. Hydrolysis was stopped at the point where the combined hydroxides of lithium and aluminum suddenly precipitated. The ether was decanted and the precipitated hydroxides were washed several times with ether. The combined ether layers were dried and evaporated to yield 7.79 g. (89%) of yellow oil. This oil was dissolved in ether and a gummy hydrochloride was precipitated with dry hydrogen chloride. The gum was dried to a powder in a vacuum desiccator. Trituration with acetone yielded 5.67 g. (55%) of the hydrochloride of Va, m.p. 170–190° dec. A sample was recrystallized four times from methanol–ether to give fine needles, m.p. 215–217° dec.

Anal. Calcd. for $C_{13}H_{18}NOCl$: C, 65.12; H, 7.57; N, 5.84. Found: C, 65.41; H, 7.59; N, 5.88.

The hydrochloride of Vb was obtained from the mother liquor, 0.81 g. (8%), m.p. 140–160° dec. A sample was recrystallized four times from methanol–ether to give small prisms, m.p. 150–152° dec.

Anal. Calcd. for $C_{13}H_{18}NOCl$: C, 65.12; H, 7.57; N, 5.84. Found: C, 65.48; H, 7.57; N, 5.85.

The benzamide of Va was prepared in quantitative yield by the Schotten–Baumann method. Two recrystallizations from benzene–petroleum ether gave long, silky needles, m.p. 142–142.5°.

Anal. Calcd. for $C_{20}H_{21}NO_2$: C, 78.15; H, 6.89; N, 4.56. Found: C, 78.46; H, 7.00; N, 4.58.

6-Methoxy-1,4,4a,9,10,10a-hexahydrophenanthridine (VI).—A solution of 5.15 g. (21.5 mmoles) of the pure hydrochloride of Va in water was made basic with 5% sodium hydroxide solution and extracted with ether. The ether layer

(11) All melting points are corrected. We are indebted to Mrs. Iris Siewers of the National Heart Institute, Bethesda, Maryland, for infrared spectra of several of these compounds. The numbering of the phenanthridine ring system is in accord with that found in the 4th edition of Beilstein's "Handbuch der organischen Chemie," Vol. XX, p. 466.

(12) Analyses by Clark Microanalytical Laboratories, Urbana, Illinois.

(13) W. C. Wildman and R. B. Wildman, *J. Org. Chem.*, **17**, 581 (1952).

was concentrated to give the oily free amine, and 4.0 ml. of formalin was added to form the Schiff base with evolution of heat. The mixture was warmed on the steam-bath for 20 minutes with trituration and extracted with benzene. The benzene solution was concentrated to the free Schiff base to which 12 ml. of 1:1 hydrochloric acid was added at room temperature. Heat was evolved and crystals of the hydrochloride formed in a few minutes. After standing overnight, the crystals were removed by filtration, 3.48 g. (64%), m.p. 215–230° dec. A second crop of crystals precipitated from the mother liquor, 0.396 g. (7%), m.p. 222–228° dec. The latter crop contained a second isomer that was not purified or further investigated. The material from the first crop was recrystallized three times from methanol–ether to give fine needles, m.p. 240–242° dec.

Anal. Calcd. for $C_{14}H_{18}NOCl$: C, 66.79; H, 7.21; N, 5.57. Found: C, 66.87; H, 7.21; N, 5.68.

The phenylthiourea of VI was prepared by the method of Shriner and Fuson[14] in 81% yield. This compound showed polymorphism; it melted at 158°, resolidified, and melted again at 176–178°. Two recrystallizations from methanol gave fine needles, m.p. 176–178°.

Anal. Calcd. for $C_{21}H_{22}N_2OS$: C, 71.96; H, 6.33; S, 9.15. Found: C, 72.18; H, 6.33; S, 8.86.

(14) R. L. Shriner and R. C. Fuson, "The Systematic Identification of Organic Compounds," 3rd ed., John Wiley and Sons, Inc., New York, N. Y., 1948, p. 179.

6-Methoxy-10-methyl-1,4,4a,9,10,10a-hexahydrophenanthridine (VII).—This compound was prepared by the N-methylation procedure of Icke, Wisegarver and Alles.[15] A solution of 1.33 g. (5.3 mmoles) of 6-methoxy-1,4,4a,9,-10,10a-hexahydrophenanthridine hydrochloride in water was made basic with 5% sodium hydroxide solution and extracted with ether. The ether extracts were concentrated to give the free amine. To this amine was added 1.5 ml. of 88% formic acid and 1.5 ml. of formalin. The reaction mixture was heated under reflux. During the first 15 minutes strong evolution of gas was noted. After 19 hours of heating, 2 ml. of 1:1 hydrochloric acid was added and the mixture was concentrated to a sirupy oil. Crystallization of the hydrochloride was induced by trituration with acetone, 1.36 g. (96%) of white powder, m.p. 199–207° dec. Two recrystallizations from isopropyl alcohol and one from methanol–ether gave colorless prisms, m.p. 211.5–213° dec.

Anal. Calcd. for $C_{15}H_{20}NOCl$: C, 67.78; H, 7.59; N, 5.27. Found: C, 67.72; H, 7.47; N, 5.29.

Acknowledgment.—This work was made possible by a grant to Princeton University from the Eugene Higgins Fund.

(15) R. N. Icke, B. B. Wisegarver and G. A. Alles, *Org. Syntheses*, **25**, 89 (1945).

PRINCETON, NEW JERSEY

[CONTRIBUTION FROM THE CENTRAL RESEARCH INSTITUTE OF THE JAPAN MONOPOLY CORPORATION]

Degradation of Nicotine by Soil Bacteria[1]

BY EINOSUKE WADA[1a] AND KYOJI YAMASAKI

RECEIVED FEBRUARY 10, 1953

Pseudooxynicotine (III) and the previously unknown 3-nicotinoylpropionic acid (IV) were isolated from microbial degradation products of a medium containing nicotine.

In connection with studies of tobacco alkaloids, several investigations have been made on the biochemical and chemical decomposition of nicotine.[2]

Frankenburg and co-workers[3–5] have shown that considerable amounts of the nicotine contained in cigar tobacco leaves are converted, during fermentation of the leaves, into other 3-substituted pyridine compounds such as oxynicotine, 3-pyridyl methyl ketone, 2,3′-dipyridyl and nicotinic acid.

Wenusch[6] isolated N-methylmyosmine and a purple crystalline substance from a nicotine phosphate medium as a degradation product of nicotine by bacteria. Bucherer[7] also reported the isolation of a purple crystalline substance from a nicotine-containing culture medium after incubation with three species of bacteria: *Bacterium nicotinophagum*, *B. nicotinovorum* and *B. nicotinobacter*.

Neither Wenusch nor Bucherer, however, supplied exact details concerning the substances obtained.

In studying the decomposition of nicotine by livers of rabbits and guinea pigs, etc., Werle, *et al.*,[8] considered that N-methylmyosmine and nicotyrine may be intermediate products of nicotine degradation, and that they may be decomposed further.

In our research the microbial degradation of nicotine, employing a species of bacteria isolated from the soil was examined. The present paper deals with some investigation on the mechanism of degradation of nicotine with special reference to transformation products produced by bacteria.

We have isolated the previously unknown 3-nicotinoylpropionic acid (IV), pseudooxynicotine (III) and a purple crystalline substance as decomposition products of nicotine. No evidence of the presence of nicotinic acid was found.

Although Frankenburg and Gottscho have isolated nicotinic acid from processed cigar tobacco,[5] the authors of this paper are unaware of any other evidence for the presence of nicotinic acid among the degradation products of pure nicotine in biological systems. In a study on dogs, Larson,[9,10] however, noted that following nicotine administration the urine contained a compound that yielded a red color with cyanogen bromide and further noted that the compound was not extractable with ether from alkaline urine, thus suggesting the existence of a carboxyl group at the end of the side chain.

Haines and Eisner[11] prepared pseudooxynicotine

(1) A preliminary note on this topic was published in *Science*, **117**, 152 (1953).

(1a) Department of Botany, Columbia University, N. Y.

(2) W. G. Frankenburg, *Advances in Enzymology*, **10**, 429 (1950).

(3) W. G. Frankenburg, A. M. Gottscho, E. W. Mayaud and Tien-Chioh Tso, THIS JOURNAL, **74**, 4369 (1952).

(4) W. G. Frankenburg and A. M. Gottscho, *Ind. Eng. Chem.*, **44**, 301 (1952).

(5) W. G. Frankenburg, *Archiv. Biochem.*, **21**, 247 (1949).

(6) A. Wenusch, *Z. Lebensm. Untersuch. Forsch.*, **84**, 498 (1942).

(7) H. Bucherer, *Zentralb. Bact.*, Abt. II, Bd. **105**, 166, 445 (1942).

(8) E. Werle and K. Koekbe, *Ann.*, **562**, 60 (1949).

(9) P. S. Larson, *Ind. Eng. Chem.*, **44**, 279 (1952).

(10) P. S. Larson and H. B. Haag, *J. Pharmacol. Exptl. Therap.*, **76**, 240 (1942).

(11) P. G. Haines and A. Eisner, THIS JOURNAL, **72**, 1720 (1950).