able amines by the general procedure of Westfall and Morris.[16] The color developed was measured in a Coleman model 14 spectrophotometer against a standard curve prepared from freshly prepared glacial acetic acid solutions of the corresponding aromatic amines, using the maxima of 525 mμ for aminofluorene, 505 mμ for aminobiphenyl, and 520 mμ for benzidine. Control runs of aromatic amines incubated at 37° with the tissue homogenates up to 4 hours revealed no appreciable destruction of the amines and recoveries of over 90%. The data in terms of micromoles of substrate hydrolyzed per hour per gram of wet tissue are given in Table III.

No evidence of hydrolysis up to 2 hours of incubation was observed by any of the tissue homogenates for the D-butyrinyl, D-norvalyl, D-norleucyl, D-valyl, D-leucyl, D-alloisoleucyl, D-methionyl, L- and D-S-benzylcysteinyl, L- and D-phenylalanyl, L- and D-tryptophyl and the mixed L-prolyl and L-N-benzylprolyl, derivatives of aminofluorene, nor for the D-norvalyl, D-norleucyl, D-valyl, D-leucyl, D-alloisoleucyl, D-tryptophyl, D-phenylalanyl and L- and D-S-benzylcysteinyl derivatives of aminobiphenyl, or for the di-D-alanyl, and di-L- and di-D-leucyl derivatives of benzidine. These data have therefore been omitted from Table III.

(16) B. B. Westfall and H. P. Morris, *J. Natl. Cancer Inst.*, **8**, 17 (1947); *cf.* H. M. Dyer, H. E. Ross and H. P. Morris, *Cancer Research*, **11**, 307 (1951).

Acknowledgments.—The authors are indebted to Mr. Robert J. Koegel and his staff for the elemental analyses, and to Mrs. Betty Whitaker for her work on the enzymic determinations.

BETHESDA, MARYLAND

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

Rauwolfia Alkaloids. XX.[1] 11-Methoxyalloyohimbane from Reserpine

BY C. F. HUEBNER, A. F. ST. ANDRÉ, E. SCHLITTLER AND A. UFFER

RECEIVED MAY 11, 1955

Two reserpones were obtained by hydrolysis and decarboxylation of methyl anhydroreserpate (III). They were transformed *via* their thioketals to two reserpanes. The negatively rotating reserpane was shown to be 11-methoxyalloyohimbane (IV) by an independent synthesis thus providing proof for the *cis* juncture of rings D and E in reserpine. IV was prepared by catalytic hydrogenation of 11-methoxysempervirine which in turn resulted from the reaction of the lithium derivative of harmine with isopropoxymethylenecyclohexanone.

We have demonstrated in a previous paper[1] that the stereochemistry of the pentacyclic ring skeleton of deserpidine is that of 3-epialloyohimbane. It also was reported briefly that reserpine can be degraded to a negatively rotating reserpane which was proved by synthesis to possess a *cis* fusion of rings D and E. The details of this proof are given here. The nature of this ring juncture has been confirmed recently by another group of workers who showed that methyl reserpate tosylate can be converted to a salt derived by intramolecular quaternization.[2] Since both deserpidine and reserpine and their derivatives can be epimerized at C-3 and since the two alkaloids possess virtually equivalent pharmacological activities,[3] reserpine also can be regarded as a derivative of 3-epialloyohimbane. The high degree of dependence of biological properties on steric factors is indicated by the complete inactivity of 3-isoreserpine.[1]

Two reserpones, one (I) with a negative and the other (II), with a positive rotation, are formed by the acid-catalyzed hydrolysis and decarboxylation of methyl anhydroreserpate (III). Both of these ketones have been converted to their thioketals and thence by Raney nickel desulfurization to the two corresponding reserpanes IV and V. There is little change in the magnitude of rotation in proceeding from the ketone to the hydrocarbon. Since alloyohimbane and 3-epialloyohimbane obtained from the naturally occurring alkaloid, 3-epi-α-yohimbine, show a negative and positive rotation, respectively[4] (Table I), it seemed likely that the negatively rotating reserpane was 11-methoxyalloyohimbane (IV). This supposition was proved by the synthesis of the racemic form of IV.

TABLE I

ROTATIONS IN CHLOROFORM OF ALLO- AND 3-EPIALLOYOHIMBANE DERIVATIVES

Compound	$[\alpha]^{25}D$
Methyl anhydroreserpate	−129°
Alloyohimbane	−130
11-Methoxyalloyohimbane	−149
3-Epialloyohimbane[4]	+105
11-Methoxy-3-epialloyohimbane	+ 72

Harmine (VI) was converted to its lithium derivative with phenyllithium and treated with isopropoxymethylenecyclohexanone (VII) according to the sempervirine synthesis of Woodward and McLamore.[5] The resulting 11-methoxysempervirine (VIII) was hydrogenated over platinum oxide in the presence of base to yield *dl*-11-methoxyalloyohimbane (IV). Le Hir and co-workers[6] have shown that the reduction of sempervirine under these conditions leads to *dl*-alloyohimbane. The infrared spectrum of the synthetic *dl*-11-methoxyalloyohimbane in chloroform solution was identical to that of the negatively rotating reserpane (IV) thus proving that rings D and E are *cis* fused in reserpine. The infrared spectra of IV and the positively rotating reserpane (V) are distinctly different.

(1) Paper XIX, H. B. MacPhillamy, C. F. Huebner, E. Schlittler, A. F. St. André and P. R. Ulshafer, THIS JOURNAL, **77**, 4335 (1955).

(2) P. A. Diassi, F. C. Weisenborn, C. M. Dylion and O. Wintersteiner, *ibid.*, **77**, 2028 (1955).

(3) J. A. Schneider, A. J. Plummer, A. E. Earl, W. E. Barret, R. Moore and R. Dibble, *J. Pharmacol. Exptl. Therap.*, **114**, 10 (1955).

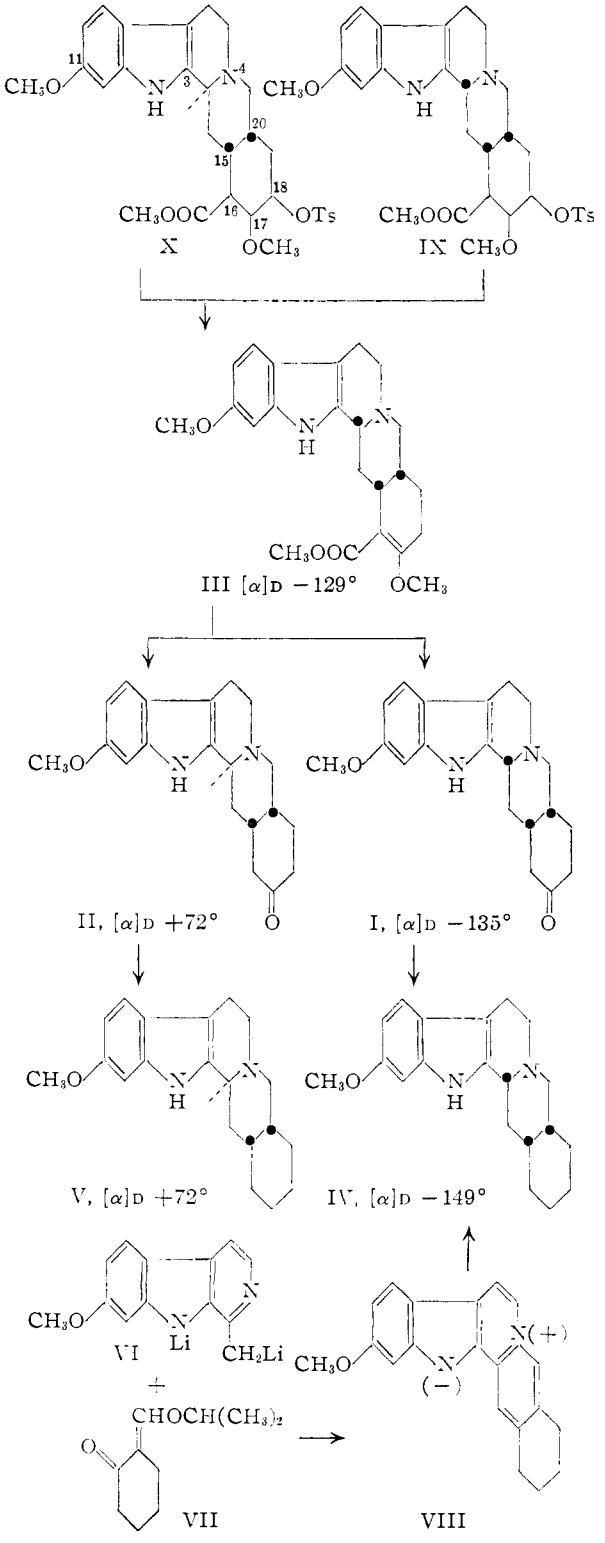
(4) F. E. Bader, D. F. Dickel, C. F. Huebner, R. A. Lucas and E. Schlittler, THIS JOURNAL, **77**, 3547 (1955).

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

(6) A. Le Hir, R. Goutarel and M. M. Janot, *Compt. rend.*, **235**, 63 (1952).

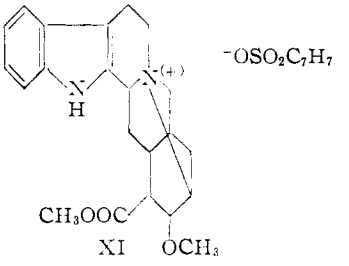
CHART I

It is to be understood that these formulas do not represent absolute configurations. The generally accepted stereochemistry for alloyohimbane is used here. See ref.6 and G. Stork and R. K. Hill, THIS JOURNAL, 76, 949 (1954).



Although it is not essential to the argument presented above, an inquiry into the configuration of methyl anhydroreserpate (III) at C-3 is of some value. The following reasons make it appear probable that III has the allo configuration. Methyl 3-isoreserpate tosylate (IX), a compound already possessing the allo configuration,[1] gives on detosylation in refluxing collidine the same methyl anhydroreserpate (II) as is obtained from the normal methyl resperpate tosylate (X). The yields of III from both sources are identical. Further, the rotation of III in which only the asymmetric centers at C-3, C-15 and C-20 remain is of the same sign and magnitude as that of alloyohimbane and 11-methoxyalloyohimbane (Table I). In contradistinction, the 3-epiallo compounds have a positive rotation. These values also illustrate the dominant influence of the asymmetric center at C-3 on rotation. The effect is most readily apparent in the compounds in which the asymmetry in ring E with its important vicinal effects at C-16, C-17 and C-18 has been destroyed. The relatively minor effect of the 11-methoxyl group on the rotation also is seen.

Epimerization at C-3 accompanies the formation of methyl anhydroreserpate (III) from X. We have shown already[1] that refluxing collidine containing a catalytic amount of *p*-toluenesulfonic acid will epimerize reserpine and its derivatives. In contrast, deserpidine and its derivatives do not epimerize under these conditions nor does methyl deserpidate tosylate yield any isolatable anhydro compound on refluxing in collidine, either with or without added *p*-toluenesulfonic acid. The inner quaternary salt (XI) is formed exclusively.



It has been found that in the preparation of methyl anhydroreserpate (III) from methyl 3-isoreserpate tosylate (IX) in refluxing collidine, a quaternary salt as a second product is not formed, as it is from the normal tosylate.[2] These facts give some insight into the mode of formation of III from X in refluxing collidine. Two competing reactions occur: the conversion of X into the quaternary salt, and the epimerization of X to IX followed by detosylation to III. We have found that the addition of *p*-toluenesulfonic acid in this reaction suppresses the formation of the unwanted quaternary compound and ensures a higher yield of III. The excess acid probably serves to increase the rate of epimerization of X to IX. The reluctance of IX to form an inner quaternary salt is probably due to the necessity of the alloyohimbane system to pass from the *trans–anti–cis* to the energetically unfavorable *cis–syn–cis* conformation in order to bring C-18 into the neighborhood of N-4 for bond formation.

The production of both 11-methoxyalloyohimbone (I) and 11-methoxy-3-epialloyohimbone (II) in the ratio of 2 to 3 by treatment of methyl anhydroreserpate (III) with acid deserves attention, since, in contrast, the more complex reserpine de-

rivatives with substituents at C-16, C-17 and C-18 epimerize completely to the allo form. We have speculated that because the energy contents of the two yohimbanes epimeric at C-3 are very similar, the presence or absence of steric interactions caused by the substituents in ring E could well be the determining factor in the position of the equilibrium.[1] This has since been clearly demonstrated by Wenkert and Lui[7] who showed that the composition of the system 3-epialloyohimbane–alloyohimbane at equilibrium is 3.6 to 1.

We also wish to record here the details of another example of this epimerization reaction where the position of equilibrium is not entirely in favor of the allo configuration as it is with the ring E substituted reserpine derivative. When a specially purified sample of α-yohimbic acid which has the allo configuration[8] was subjected to an Oppenauer oxidation, 3-epialloyohimbone was obtained as well as the anticipated alloyohimbone. The ratio was about 1 to 9. Since these are not conditions for the attainment of a true equilibrium, the amount of the 3-epiallo compound under such conditions probably would be greater.

Acknowledgments.—It is a pleasure to acknowledge the able assistance of Mrs. D. Davis. We also express our appreciation to Mr. L. Dorfman and his associates for the microanalyses and physical measurements.

Experimental[9]

11-Methoxyalloyohimbone (I) and 11-Methoxy-3-epialloyohimbone (II) from Methyl Anhydroreserpate (III).—A solution of 1.96 g. of homogeneous methyl anhydroreserpate[10] (III) (as evidenced by paper and alumina chromatography) in 24 ml. of ethanol and 120 ml. of 12% hydrochloric acid was refluxed for 3 hours. It then was concentrated *in vacuo* to a small volume, made basic with 20% sodium hydroxide solution and exhaustively extracted with chloroform. The combined extracts were washed with water, dried with sodium sulfate and evaporated to dryness *in vacuo*. The residue (1.32 g.) was dissolved in a small amount of benzene and chromatographed on 35 g. of aluminum oxide (Woelm, Activity II–III). The material eluted with benzene and benzene–20% acetone was a crystalline mixture, 0.89 g., m.p. 210–230° dec. This was rechromatographed similarly and the first two fractions eluted with benzene–10% acetone were recrystallized from methanol and yielded 0.186 g. of I, m.p. 236–239° dec., $[\alpha]^{24}D$ −135° (chloroform). This substance is the previously reported reserpone.[11]

The following five fractions eluted from the above chromatogram with benzene–10% acetone and benzene–20% acetone yielded after recrystallization from methanol 0.299 g. of II, m.p. 240–243° dec., $[\alpha]^{24}D$ +72° (chloroform).

Anal. Calcd. for $C_{20}H_{24}N_2O_2$: C, 74.04; H, 7.46; N, 8.64. Found: C, 73.88; H, 7.57; N, 8.49.

A mixture of the two compounds showed a marked depression of the melting point. The infrared absorption curves of these two substances were different. However, both indicated the presence of a ketone and the methoxyindole moiety.

11-Methoxy-3-epialloyohimbone Ethylene Mercaptal.—A stream of dry hydrogen chloride gas was bubbled into a stirred solution of 0.240 g. of II in 10 ml. of acetic acid containing 1.5 ml. of dithioglycol at 5–10°. After 30 minutes the admission of gas was stopped and the reaction was allowed to continue at room temperature for five hours. The mixture was evaporated to dryness *in vacuo* and the residue was crystallized from methanol to yield 0.310 g. of the thioketal hydrochloride, m.p. 303–306° dec. A sample was recrystallized from methanol for analysis, m.p. 310° dec.

Anal. Calcd. for $C_{22}H_{28}N_2OS_2 \cdot HCl$: C, 60.60; H, 6.70; S, 14.71. Found: C, 60.50; H, 6.99; S, 14.57.

To 0.260 g. of the hydrochloride suspended between water and chloroform just enough ammonium hydroxide was added to make the system basic. The free base then was extracted several times with chloroform. The chloroform fractions were combined, washed with water and dried over anhydrous sodium sulfate. The thioketal of II obtained as an oily residue on the evaporation of the chloroform was used directly for desulfurization since the crystalline base was unstable.

11-Methoxy-3-epialloyohimbane (V).—To 0.250 g. of the thioketal of II in 80 ml. of 95% ethanol was added 0.500 g. of Raney nickel catalyst and the mixture was refluxed with stirring for six hours after which time it was filtered, washed well with hot ethanol and the filtrate concentrated to dryness *in vacuo*, m.p. 190–200° dec., weight 0.140 g. Recrystallization twice from methanol yielded 0.024 g. of V, m.p. 238–241° dec., *in vacuo* 244–245.5°, $[\alpha]^{24.5}D$ +72° (chloroform). The infrared spectra showed no carbonyl absorption.

Anal. Calcd. for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.48; N, 9.03. Found: C, 77.65; H, 8.26; N, 8.91.

11-Methoxyalloyohimbane (IV).—The isomeric ketone I formed on the acid hydrolysis of methyl anhydroreserpate (III) was treated in the manner described above with dithioglycol to yield the thioketal hydrochloride, m.p. 286–291° dec. The free base in turn was desulfurized similarly with Raney nickel catalyst and the product obtained after chromatography over alumina and recrystallization from methanol (IV) melted at 244–244.5° (*in vacuo*); $[\alpha]^{25}D$ −149° (chloroform). The m.p. of a mixture with the 3-epiallo compound (V) showed a sharp depression. The infrared spectrum in chloroform was identical to that of a sample of synthetic *dl*-11-methoxyalloyohimbane (see below) and distinctly different from the spectrum of the positively rotating reserpane (V).

Anal. Calcd. for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.48. Found: C, 77.29; H, 8.42.

11-Methoxysempervirine (VIII).—To a vigorously stirred suspension of 4 g. of harmine (VI) in 200 ml. of anhydrous ether under an atmosphere of dry nitrogen was added 40 ml. of an approximately 1.5 *N* ethereal solution of phenyllithium. After two hours a solution of 10 g. of isopropoxymethylenecyclohexanone (VII)[5] in 50 ml. of ether was added dropwise. The yellow suspension of the harmine lithium derivative gradually dissolved to yield a dark red solution. After 12 hours of continued stirring, the reaction mixture was acidified with concentrated hydrochloric acid. After two hours the semi-solid dark red tar was filtered. Trituration with a small amount of ethanol gave the crude hydrochloride. It dissolved in hot water and precipitated as the anhydronium base with sodium hydroxide. The orange gum was filtered and rubbed with a small amount of ethanol to convert it to the yellow crystalline base. It was then dissolved in ethanol and converted to its hydrochloride by the addition of ethanolic hydrogen chloride. One gram of the purified hydrochloride was obtained in this manner. It was recrystallized from 90% ethanol containing an excess of hydrochloric acid to yield fine light yellow needles of the hydrochloride of VIII, m.p. 312–315°.

Anal. Calcd. for $C_{20}H_{19}ClN_2O \cdot 1^1/_3H_2O$: C, 66.18; H, 6.04; N, 7.72. Found: C, 66.17; H, 6.03; N, 7.90.

The purified hydrochloride was converted to the anhydronium base and recrystallized from ethanol in stout yellow-orange needles, m.p. 230–235°.

Anal. Calcd. for $C_{20}H_{18}N_2O$: C, 79.44; H, 6.00; N, 9.27. Found: C, 79.03; H, 5.92; N, 9.44.

***dl*-11-Methoxyalloyohimbane (IV).**—Two normal potassium hydroxide in methanol was added to a suspension of 0.4 g. of VIII hydrochloride in 25 ml. of methanol until solution of the base occurred and the *p*H was about 10.

(7) E. Wenkert and L. H. Lui, *Experientia*, **11**, 302 (1955).

(8) A. Chatterjee, A. K. Bose and S. Pakrashi, *Chemistry and Industry*, 491 (1954).

(9) All melting points recorded here are uncorrected.

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

(11) C. F. Huebner, H. B. MacPhillamy, A. F. St. André and E. Schlittler, THIS JOURNAL, **77**, 472 (1955).

Platinum oxide (0.2 g.) was added and the mixture hydrogenated at 700 p.s.i. for two hours. The catalyst was removed, methanolic hydrogen chloride added until acid to congo red and the methanol evaporated *in vacuo*. The residue was shaken with 25 ml. of water giving a suspension of the rather insoluble hydrochloride of IV. It was converted to the base and after recrystallization once from ethanol–water and from methanol, 150 mg. of elongated plates of *dl*-11-methoxyalloyohimbane (IV) was obtained. On the hot-stage it melted at 195–198° with a change to opaque crystals at 100°. The melting point in an evacuated capillary was 203–205°.

Anal. Calcd. for $C_{20}H_{26}N_2O$: C, 77.38; H, 8.44; N, 9.03. Found: C, 76.90; H, 8.22; N, 9.35.

Methyl Anhydroreserpate (III) from Methyl 3-Isoreserpate Tosylate (IX).—A mixture of 2.0 g. of IX, 0.10 g. of *p*-toluenesulfonic acid monohydrate and 12 ml. of collidine was refluxed for three hours, the solvent was removed by distillation *in vacuo* and the residue taken up in chloroform and water containing ammonium hydroxide. The aqueous layer was extracted several times with chloroform, the organic fractions combined, washed neutral with water and dried over anhydrous sodium sulfate. After filtration the solvent was evaporated and the residue on trituration with methanol formed crystals, m.p. 252.5–254.5° dec., weight 0.314 g. Recrystallization from methanol afforded 0.270 g., m.p. 261–262.5° dec. A sample recrystallized from ethyl acetate for analysis melted at 267–272° dec., $[\alpha]^{22}D$ −143° (pyridine), −129° (chloroform).

Anal. Calcd. for $C_{23}H_{28}N_2O_4$: C, 69.67; H, 7.12; N, 7.07. Found: C, 69.35; H, 7.22; N, 7.02.

The infrared spectrum was identical with that of the methyl anhydroreserpate obtained by the detosylation of methyl reserpate tosylate (X).

Oppenauer Oxidation of α-Yohimbic Acid.[12]—A mixture of 3.0 g. of pure α-yohimbic acid (rauwolscinic acid), 15.0 g. of aluminum phenolate, 75 ml. of freshly distilled cyclohexanone and 75 ml. of dry xylene was refluxed for 40 hours. It then was cooled and extracted with 300-, 150-, 100-, 75- and 75-ml. portions of 2 *N* sulfuric acid. The combined acid extracts were washed twice with benzene and then made alkaline with 40% potassium hydroxide solution. The precipitate was filtered, dried, mixed with Hyflo and continuously extracted in a Soxhlet extractor with methanol for 16 hours. The methanolic extract was concentrated, filtered through norite, and the solvent further removed until crystals formed on cooling. In this way 0.79 g. of material was obtained, m.p. 236–239° dec., $[\alpha]^{25}D$ −152° (chloroform), −97° (pyridine) [reported[13] for alloyohimbone, m.p. 241–242°, $[\alpha]^{30}D$ −104° (pyridine)].

Anal. Calcd. for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N, 9.52. Found: C, 77.49; H, 7.58; N, 9.61.

The filtrate from the above crystallization was evaporated to dryness *in vacuo*. The residue was dissolved in benzene containing 25% chloroform and then chromatographed over aluminum oxide (Woelm, Activity II–III). The material eluted with 50% benzene–chloroform yielded 0.134 g. of material, which was recrystallized from methanol, m.p. 247–250° dec., 269–271° (*in vacuo*), $[\alpha]^{24}D$ +80° (chloroform). This substance proved to be identical with epialloyohimbone.

Quaternary Salt from Methyl Deserpidate Tosylate.—Methyl deserpidate tosylate (1.0 g.) in 6.0 ml. of collidine was refluxed for three hours. After about 1.5 hours a precipitate was formed which was filtered off at the end of the reaction time; m.p. 296–298° dec., weight 0.283 g. Recrystallization twice from methanol yielded a substance, m.p. 310–312° dec.

Anal. Calcd. for $C_{29}H_{34}O_6N_2S$: C, 64.66; H, 6.36; N, 5.20. Found: C, 64.49; H, 6.28; N, 5.33.

The collidine filtrate after the usual manner of work-up did not yield any crystalline material except some unchanged starting material. All attempts to prepare methyl anhydrodeserpidate gave the above results.

Quaternary Salt from Methyl 18-Iodo-18-desoxydeserpidate.—When 150 mg. of the above tosyl salt was dissolved in 50 ml. of acetonitrile and treated with an equivalent of sodium iodide in acetonitrile an immediate white precipitate of sodium *p*-toluenesulfonate appeared, which was filtered off and the filtrate evaporated to dryness. The residue, after washing with water to remove any excess sodium iodide, was twice recrystallized from methanol, m.p. 245.5–247° dec., $[\alpha]^{25.5}D$ +69° (methanol).

Anal. Calcd. for $C_{22}H_{27}N_2O_3I$: C, 53.44; H, 5.51; N, 5.68. Found: C, 53.54; H, 5.84; N, 5.55.

(12) A. Mookerjee, *J. Indian Chem. Soc.*, **18**, 33 (1941).

(13) A. Le Hir, M. M. Janot and R. Goutarel, *Bull. soc. chim.*, **20**, 1027 (1953).

SUMMIT, NEW JERSEY

[CONTRIBUTION FROM THE GENERAL CIGAR CO. RESEARCH LABORATORY]

The Chemistry of Tobacco Fermentation. I. Conversion of the Alkaloids. B. The Formation of Oxynicotine

BY W. G. FRANKENBURG AND A. M. GOTTSCHO[1]

RECEIVED MAY 9, 1955

An appreciable amount of the nicotine which disappears during the fermentation of cigar filler tobacco appears in the fermented leaves as oxynicotine. It may be isolated as its dipicrate by means of a series of fractionated solvent extractions. Oxynicotine can be determined analytically by reducing it to nicotine.

In previous publications from this Laboratory,[2–4] dealing with the transformation products of nicotine present in fermented cigar filler tobacco (U. S. Type 41), an oxygen-containing substance, reducible to nicotine, was mentioned. It was tentatively assumed to be oxynicotine. This paper deals with the isolation and identification of this compound from fermented cigar filler tobacco.

Oxynicotine, first obtained by Pinner,[5,6] is described by Ciamician and Silber[7] as an amine oxide of nicotine with the oxygen attached to the pyrrolidine nitrogen atom.

Oxynicotine in Tobacco.—The presence of oxynicotine in fermented cigar filler tobacco leaves was first suspected when it was found that a nicotine-free fraction L obtained from a water extract of

(1) From a thesis presented to the Committee on Graduate Studies, Franklin & Marshall College, Lancaster, Pennsylvania, in partial fulfillment of the requirements for the degree of Master of Science.

(2) W. G. Frankenburg and A. M. Gottscho, *Ind. Eng. Chem.*, **44**, 303 (1952).

(3) W. G. Frankenburg, A. M. Gottscho, E. W. Mayaud and T. C. Tso, THIS JOURNAL, **74**, 4309 (1952).

(4) W. G. Frankenburg, A. M. Gottscho, S. Kissinger, D. Bender and M. Ehrlich, *Anal. Chem.*, **25**, 1784 (1953).

(5) A. Pinner and R. Wolffenstein, *Ber.*, **25**, 1428 (1892).

(6) A. Pinner, *ibid.*, **28**, 456 (1895).

(7) G. Ciamician and P. Silber, *ibid.*, **48**, 181 (1915).