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).

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[CONTRIBUTION FROM THE GENERAL CIGAR CO. RESEARCH LABORATORY]

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

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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).

fermented cigar tobacco[4] yielded nicotine when treated with reducing agents.

In the stepwise solvent extraction of tobacco, first described in 1948[8] and modified in 1952,[3] this substance I reducible to nicotine was obtained in the chloroform extract, fraction 2, and enriched in subfraction 2b of that fraction.

Oxynicotine can be quantitatively reduced to nicotine by any of several methods, *e.g.*, zinc and hydrochloric acid, zinc and acetic acid, sodium bisulfite,[6] and Devarda alloy in alkaline solution.[9] By subjecting aliquots of fraction 2b to reduction and simultaneous steam distillation in the presence of magnesium oxide and Devarda alloy, and by measuring the ultraviolet absorption spectrum of the nicotine obtained in the distillate, the quantity of substance I in fraction 2b was determined easily. These reduction experiments showed that the pyridine nitrogen of I amounted to 90% of the total pyridine nitrogen contained in fraction 2b and to about 50–70% of the total pyridine nitrogen originally present in fraction 2.[3]

By measuring the ultraviolet absorption spectrum of the residue of the distillation made in the presence of magnesium oxide and Devarda alloy, and by graphically subtracting this absorption spectrum from that of the same aliquot of fraction 2b prior to removal of I, a "difference spectrum" for I was obtained. It resembled that of pure oxynicotine in that its maximum of absorption was at 258 mμ.

The possibility that the reducible compound is oxynicotine was checked quantitatively by evaluating the "difference spectrum" as oxynicotine, correcting for background impurities.[10] The millimoles of I in fraction 2b calculated in this way, agreed closely with the millimoles of nicotine found in the distillate.

These spectroscopic studies and the similar behavior of both oxynicotine and the reducible substance I in fraction 2b toward many different solvents, made it very likely that I is oxynicotine. Nevertheless, it appeared desirable to isolate I for final identification.

Synthetic Alkaloid Transformation Products.—In 1947 and later in 1950, Weil[11,12] described certain alkaloid transformation products produced *in vitro* by irradiating, with visible light, an aqueous solution of nicotine containing very small quantities of methylene blue as an optical sensitizer. As pointed out at an earlier time,[8] these photochemical products possessed chemical properties[13] similar to those of the natural alkaloid transformation products found in the chloroform extractable portion (fraction 2) of certain types of fermented cigar tobacco.

Since later work by Weil's associates[14] resulted in the isolation of oxynicotine from the irradiation products of nicotine, it seemed probable that oxynicotine may be one of the components of fraction 2 obtained from tobacco.

Isolation of Oxynicotine from Fraction 2b.—Substance I in fraction 2b was purified by a detailed procedure and finally isolated as its picrate, m.p. 167–168°. This picrate changed on standing, inasmuch as melting points measured within approximately 24 hours after the preparation of the picrate were usually found to have decreased. When the fresh dipicrate of I was mixed with a dipicrate of synthetically prepared oxynicotine, a mixed melting point of 157–160° was obtained. Elementary analysis of the picrate of I showed that its composition was that of oxynicotine dipicrate.

There is no agreement in the literature on the true melting point of oxynicotine dipicrate. The values reported range from 154–158°[5] to 168°[7] and 169°.[15] Badgett and Eisner[14] reported that an oxynicotine dipicrate prepared by them showed a melting range of 163–175° with fusion occurring first at the lower temperature, but without the formation of a meniscus. At the higher temperature the melt cleared up and a meniscus formed.

Chromatography and Ultraviolet Absorption Spectrum.—Both authentic oxynicotine and substance I isolated from tobacco *via* fraction 2b were subjected to chromatographic tests on paper, using Whatman No. 1 paper and solvent mixtures of *n*-butyl alcohol:ethanol:acetate buffer of *p*H 5.6 (50:10:40); butyl acetate:methanol:ammonia (0.25%) (95:5:25)[16]; and benzene:methanol:acetate buffer of *p*H 5.6 (60:15:25). Both the authentic sample and compound I exhibited identical R_f values of 0.44, 0.0 and 0.0 in the respective solvent systems.

Further substantiation that the "reducible" alkaloid transformation product isolated from fermented tobacco *via* fraction 2b is oxynicotine was presented by the ultraviolet absorption spectrum, which is practically identical with that of pure oxynicotine (maximum at 258 mμ, minimum at 227 mμ).

Experimental

Fraction 2.—Fermented tobacco powder (250 g. of Pennsylvania Seedleaf, U. S. Type 41) which had been exhaustively extracted with petroleum ether (giving fraction 1)[3] was allowed to stand for several days until the greater part of the solvent had evaporated. The sample then was extracted with chloroform at approximately the same rate as was used for the petroleum ether extraction. Completeness of the chloroform extraction was determined spectrophotometrically.[3]

The alkaloid transformation products contained in the chloroform extract were transferred into aqueous acid as follows: Aqueous hydrochloric acid (0.25 *N*, 125 ml.) was placed in a 500-ml. distillation bulb. To this was added the chloroform extract in *ca.* 250-ml. portions. Each chloroform portion was distilled off from this mixture. A brown resinous solid was filtered off and washed with 0.25 *N* HCl. The combined filtrate and washings comprise the aqueous solution of fraction 2.[3]

An estimate of the total pyridine nitrogen contained in this fraction was based on an evaluation of its ultraviolet absorption spectrum. The content of oxynicotine was determined by subjecting an aliquot of the fraction to a steam

(8) W. G. Frankenburg, *Science*, **107**, 427 (1948).

(9) A. Devarda, *Chem. Zentr.*, **61**, 1952 (1892).

(10) C. O. Willits, M. L. Swain, J. A. Connelly and B. A. Brice, *Anal. Chem.*, **22**, 430 (1950).

(11) L. Weil, *Science*, **107**, 426 (1948).

(12) L. Weil and J. Maher, *Arch. Biochem.*, **29**, 241 (1950).

(13) These properties include the composition of their silicotungstates, the identity of their reduction products and of the colored products formed from their reaction with 2,4-dinitrochlorobenzene.

(14) C. Badgett and A. Eisner, private communication.

(15) C. H. Rayburn, W. R. Harlan and H. R. Hanmer, THIS JOURNAL, **63**, 115 (1941).

(16) W. L. Porter, J. Naghski and A. Eisner, *Arch. Biochem.*, **24**, 461 (1949).

distillation in the presence of magnesium oxide and Devarda alloy.[4]

Fraction 2a.—The aqueous acidic solution of fraction 2 was concentrated on a water-bath to *ca.* 200 ml., adjusted to *p*H 10 with aqueous sodium hydroxide, and then shaken out with *t*-amyl alcohol[17] (previously saturated with water) 12 times, using 80 ml. of alcohol for each extraction. After every third extraction the *p*H of the aqueous phase was re-adjusted to 10 with sodium hydroxide. The combined extracts constitute fraction 2a.

Fraction 2b.—The aqueous residue of the *t*-amyl alcohol extraction, rich in oxynicotine, was made 1 *N* with hydrochloric acid and shaken out with ethyl ether (to remove the *t*-amyl alcohol). The aqueous residue was adjusted to *p*H 10 with sodium hydroxide and extracted with ethyl ether in a liquid–liquid extraction apparatus. The aqueous residue then was adjusted to *p*H 3 and re-extracted in a liquid–liquid apparatus with ethyl ether. All the ether extracts were discarded The residue of the third ether extraction (at *p*H 3) was fraction 2b.

Purification of Oxynicotine in Fraction 2b.—After neutralizing fraction 2b, it was heated to boiling; 4 g. of bone charcoal was added, the mixture shaken, and the charcoal was filtered off. The dark yellow filtrate was adjusted to *p*H 2 with hydrochloric acid, and a 12% solution of silicotungstic acid (STA) slowly added until no further precipitation occurred. After standing at 6° overnight, the precipitate was filtered off, and washed with hydrochloric acid (0.01 *N*).

The STA precipitate was suspended in water and 0.5 *N* sodium hydroxide was added until solution occurred. An excess of solid barium hydroxide was added and the mixture agitated intermittently for 6 to 7 hr. After the solids had settled, a few drops of supernatant liquid were tested with hydrochloric acid. The absence of a precipitate in this test indicates complete removal of the STA as the Ba salt. If a precipitate formed, the shaking with barium hydroxide was continued. When the test was negative, the solids were filtered off and discarded.

The excess barium was removed from the filtrate with sulfuric acid. The filtrate was precipitated once more with STA and the STA precipitate decomposed with barium hydroxide as already described. The final filtrate from this second purification *via* STA and barium hydroxide was neutralized and evaporated to dryness *in vacuo* at 40–50°. The solid residue was extracted with ethyl acetate.

Isolation of Oxynicotine as the Dipicrate.—The ethyl acetate extract was mixed with a saturated aqueous picric acid solution and ethyl acetate was allowed to evaporate off at room temperature, leaving yellow crystals in the aqueous phase. After standing for at least 24 hr. at 6°, these crystals were collected and recrystallized twice from boiling water, m.p. 167–168°, mixed m.p. 157–160°.

Anal. Calcd. for $C_{22}H_{20}O_{15}N_8$: C, 41.51; H, 3.17; N, 17.61. Found: C, 41.76; H, 3.24; N, 17.64.

Decomposition of Oxynicotine Dipicrate into its Component Parts.—Six hundred and fifty-one mg. of picrate was dissolved in hydrochloric acid (2.5 *N*) and extracted quantitatively with ethyl ether in a liquid–liquid extraction apparatus. The picric acid was extracted from the ether by aqueous sodium hydroxide and its concentration in this aqueous solution after acidification was determined spectrophotometrically. The ultraviolet absorption spectrum of the aqueous acidic residue of the liquid–liquid ether extraction was measured. In agreement with the spectrum of authentic oxynicotine, it showed a maximum at 258 mμ, and a minimum at 227 mμ. From the weight of the original picrate taken and the picric acid recovered, the weight of oxynicotine in this residue was computed. From this data the absorptivity at the absorption maximum of 258 mμ of the isolated oxynicotine was determined: calcd. from measurements of authentic oxynicotine: 31.4; found, 29.8.

The composition of the picrate was also computed: Calcd. for oxynicotine dipicrate, picric acid: 72.0%; found, picric acid, 72.4% (average of 2 determinations).

Reduction of Oxynicotine to Nicotine.—An aliquot containing 138 mg. of oxynicotine, evaluated spectrophotometrically, was steam distilled in the presence of magnesium oxide and Devarda alloy.[4] The nicotine in the distillate was determined spectrophotometrically.[10]

Calcd. for 100% reducibility of oxynicotine: 125 mg. of nicotine; found: 121 mg. of nicotine.

A picrate of the alkaloid which appeared in the distillate was prepared, m.p. 220–221°. A mixed m.p. with authentic nicotine dipicrate produced no depression.

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(17) The use of this solvent for this purpose was called to the authors' attention by Dr. A. Eisner, Eastern Utilization Research Branch, U. S. Department of Agriculture, Philadelphia 18, Pa.

[CONTRIBUTION FROM THE RESEARCH LABORATORY, GENERAL CIGAR CO., INC.]

The Chemistry of Tobacco Fermentation. I. Conversion of the Alkaloids. C. The Formation of 3-Pyridyl Propyl Ketone, Nicotinamide and N-Methylnicotinamide

BY W. G. FRANKENBURG, A. M. GOTTSCHO, A. A. VAITEKUNAS AND R. M. ZACHARIUS

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Additional nicotine degradation products formed during the fermentation of cigar filler tobacco are 3-pyridyl propyl ketone, nicotinamide and N-methylnicotinamide. An additional and major degradation product of nicotine was detected by paper chromatography, but not identified.

The chloroform fraction 2 obtained during the successive solvent extractions of fermented cigar tobacco leaves[1] (Pennsylvania Seedleaf, U. S. Type 41) contains a large proportion of the degradation products of nicotine formed during the fermentation[2] process. From the absorbance at *ca.* 260 mμ of the aqueous solution of this fraction 2, it is estimated that its content of these newly formed pyridine compounds can be as high as 30% of the nicotine content present in the leaves prior to fermentation.[1,2]

The chloroform fraction 2 is fractionated into two subfractions 2a and 2b.[2,3] Oxynicotine (I), a major component of fraction 2, is found enriched in the subfraction 2b. This paper deals with the further

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