wt. 11.1 g. (81%). It melted at 145–147° (Ullmann[2] reported the m.p. as 148–148.5° for the recrystallized mixture).

When the reaction time was extended to 18 hours the yield was 87%.

B. **96% Sulfuric Acid.**—The same quantities of the same reagents were used except that an equivalent volume of 96% sulfuric acid was substituted for the one of greater strength. No temperature rise occurred when the *p*-chlorotoluene was added to the mixture of the acids. The whole was heated to 30° and the reaction allowed to proceed for 90 minutes as the temperature fell gradually.

At the end of this time the mixture was processed as above. Only 3.6 g. (26%) of the thiaxanthone mixture was obtained, m.p. 137–142°. On acidification the ammoniacal extract furnished 5.1 g. of almost pure dithiosalicylic acid.

C. **100% Sulfuric Acid Saturated with Potassium Bisulfate.**—One hundred milliliters of 100% (rather than 101%) sulfuric acid was stirred for one hour with 50 g. of fused potassium bisulfate. The suspension was cooled to 21° and treated first with 8.0 g. of pure dithiosalicylic acid and then with 25 ml. of *p*-chlorotoluene. After five minutes the temperature rose to 32° and reached the maximum, 40°, after 13 minutes. After 1.5 hours the mixture was worked up in the usual way. There was obtained 5.4 g. (40%) of the thiaxanthone mixture.

The control run was carried out with the identical reagents, except that the potassium bisulfate was omitted. Prior to the addition of the dithio acid and the *p*-chlorotoluene, the sulfuric acid was stirred for one hour to simulate the above experiment as closely as possible. When the *p*-chlorotoluene was added to the stirred mixture of acids at 21° the temperature rose to 30° in 45 seconds. The whole was cooled in an ice–alcohol bath but the temperature continued to rise until it reached 35°. After 1.5 hours the solution was poured into water and processed as usual. The neutral fraction weighed 11.3 g. (83%).

Acknowledgments.—Our thanks are due to Dr. A. R. Surrey for samples of γ-(propylamino)-propylamine and γ-[N-ethyl-N-(2-hydroxypropyl)-amino]-propylamine. Miss E. Haggett prepared the γ-dibutylaminobutylamine and Mrs. M. J. Unser prepared the γ-(dialkylamino)-propylamines.

RENSSELAER, N. Y.

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

The Chemistry of Tobacco Fermentation. I. Conversion of the Alkaloids. A. The Formation of 3-Pyridyl Methyl Ketone and of 2,3′-Dipyridyl

BY WALTER G. FRANKENBURG, ALFRED M. GOTTSCHO, EDITH WOOLEVER MAYAUD AND TIEN-CHIOH TSO

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Nicotine and minor tobacco alkaloids are converted in the leaves of cigar tobacco into various 3-substituted pyridine compounds, including 3-pyridyl methyl ketone and 2,3′-dipyridyl.

Decrease of Alkaloids in Tobacco Leaves during Fermentation.—Probably the most characteristic chemical effect in the fermentation[1] of certain cigar tobaccos[2] is the substantial decrease of their alkaloid contents.[3] The nicotine initially present in these tobaccos decreases in regular fermentation by about 30 to 50% and in a recently developed, more effective, fermentation procedure by as much as 80 to 95%. Since volatilization from the leaves accounts for not more than about 8% of the total nicotine loss, considerable amounts of nicotine must be converted during fermentation into other products. Besides its industrial significance, this conversion of nicotine is of general interest in view of the mild conditions (temperature 43 to 54°, *p*H range 5.6 to 6.8) at which it occurs.

Prior to any studies of the catalytic or enzymic mechanism of this alkaloid degradation within the tobacco leaves, more detailed information is needed on its chemistry, and particularly on the transformation products derived from the alkaloids. A search was undertaken for these products[4] in our laboratory, using as a starting material well fermented[5] Pennsylvania Seedleaf tobacco.[2]

Solvent extraction of this tobacco discloses the presence of various newly formed 3-substituted pyridine derivatives in addition to small amounts of unchanged alkaloids. Although the former have not all been identified, the total amount of pyridine nitrogen in the extracted fractions can be estimated by a combination of the following methods: (1) Kjeldahl determination of the nitrogen contained in silicotungstate precipitates, (2) ultraviolet absorption measurements, and (3) oxidation to nicotinic acid and determination of the latter.

Quantitative Correlation between the Pyridine Derivatives in Tobacco Leaves before and after Fermentation.—Table I represents a survey of the pyridine derivatives found in thoroughly fermented samples and of the method used in separating them. In four fractions, obtained by extracting successively with different selective solvents, about 87% of the pyridine originally present in the leaves as a moiety of nicotine can be recovered, partly as unchanged nicotine, and partly as a component of newly formed pyridine compounds (Table I, column 10). This indicates that the pyridine ring remains practically intact during the fermentation. Of these fractions, 1 and 2 are each again separated into two subfractions by liquid–liquid extraction with organic solvents at specific *p*H's.

(1) A process in which the cured tobacco leaves, moistened with selected amounts of water, are kept at temperatures of about 45° with periodical aerations. See W. G. Frankenburg, *Advances in Enzymology*, **10**, 325 (1950).

(2) Particularly "filler" tobaccos, such as Pennsylvania Seedleaf, U. S. type 41.

(3) Nicotine represents about 90% of the total alkaloids in these tobaccos.

(4) As a part of general investigations concerning the conversions of all the nitrogenous leaf components during the fermentation of cigar tobacco.

(5) The tobacco samples used had on the average nicotine contents of 4.2% before and of 0.7% after fermentation (0.72% and 0.12% nicotine nitrogen, respectively).

TABLE I[a]

FRACTIONATION OF ALKALOIDS AND ALKALOID TRANSFORMATION PRODUCTS IN WELL-FERMENTED PENNSYLVANIA SEEDLEAF CIGAR TOBACCO

Abbreviations used for solvents: Hy = hydrocarbon; E = ethyl ether; Chl = chloroform; Alc = alcohol; W = water; tert. am. = tertiary amyl alcohol.

Main fractions (1)	Obtainable by extracting fermented tobacco successively at *p*H 7.5 with Hy (2)	E (3)	Chl (4)	Alc (5)	W (6)	Sub-fractions (7)	Obtained from aqueous soln. of main fraction by extracting with[a] (8)	At *p*H (9)	Pyridine N in fraction in % of total pyridine N of alkaloids, before fermentation (10)	Compounds contained in fractions (11)
1	+[d]	+	+	+	+	1a	Hy, E, Chl	3.5	2	2,3′-Dipyridyl (80%)
										3-Pyridyl methyl ketone (20%)
						1b	Hy, E, Chl	≧8	15	Unchanged alkaloids (nicotine, nornicotine, anabasine)
2	−	−	+[d]	+	+	2a	Tert. am.	10	11	Unidentified
						2b	Extraction residue of 2a	...	18	Oxynicotine[b]
3	−	−	−	+[d]	+		E	3.0	16	Nicotinic acid[c]
4	−	−	−	−	−		.....	...	25	Unidentified "insoluble pyridine compounds"

[a] First results achieved with this scheme were reported by W. G. Frankenburg in *Science*, **107**, 427 (1948). [b] Tentatively identified. [c] W. G. Frankenburg and A. M. Gottscho, *Archiv. Biochem.*, **21**, 247 (1949). [d] The black face solvents were usually employed for the separation procedure.

The separation and analysis of the unchanged alkaloids in fraction 1b, the determination of oxynicotine in fraction 2b, and of nicotinic acid in fraction 3 will be described in later papers. The percentages found for these substances by the solvent extraction method agree well with the values obtained by an appropriate analysis of aqueous extracts of the fermented leaves.

Characteristics of Fraction 1a.—The present communication deals with the detailed investigations of fraction 1a. The substances of this fraction are initially obtained in mixture (fraction 1) with unchanged nicotine and "secondary" alkaloids, but divulge their presence by their characteristic ultraviolet absorption.

Nicotine, nornicotine and anabasine show, in acidic aqueous solutions, absorption minima located at points between 226 and 230 mμ, narrow absorption bands with peaks between 259 and 261 mμ, and steep declines toward 280 mμ, followed by "tails" with very low extinction values at longer wave lengths.[6]

Fractions 1 obtained from *unfermented* tobacco yield distinct and easily evaluated alkaloid absorption spectra, whereas fractions 1 prepared from well *fermented* tobacco exhibit spectra differing from those of the alkaloids by a considerable shift of the minimum toward longer wave lengths and by greatly increased extinction values between 270 and 290 mμ. This indicates the presence in the fermented leaves of unknown substances which superimpose their absorption on that of the alkaloids.

Separation of Fractions 1a and 1b.—Extensive preliminary experiments showed that these unknown substances resemble the alkaloids in many respects. Like the latter, they are precipitated by picric, picrolonic, styphnic, silicotungstic and phosphotungstic acids, are slowly distillable with steam from neutral or alkaline aqueous solutions, and extractible with ethyl ether.

A separation of fraction 1 into two clearly defined subfractions was finally achieved by its liquid–liquid extraction with ethyl ether at *p*H 3.5. The unknown compounds are quantitatively extracted (fraction 1a), leaving the alkaloids in the aqueous phase (fraction 1b[7]). Figure 1 demonstrates the concurrent resolution of the initial spectrum A of fraction 1 into the spectrum B of the extract (fraction 1a) and into the alkaloid spectrum C of the residue (fraction 1b).

Investigation of Fraction 1a.—The nitrogen content of fraction 1a as determined by Kjeldahl is small, averaging about 0.009% of the tobacco dry weight, or about 2.5% of the pyridine nitrogen of the original alkaloids in the unfermented samples. This nitrogen stays associated with absorption spectrum B, both in steam distillates and in silicotungstic acid precipitates[8] obtained from fraction 1a. The ratio of the absorbancies at the maximum to that at the minimum (A_{max}/A_{min}) is for the steam distillates about 3.1 compared with 1.8 prior to distillation, indicating a purification of the bases[9] by the distillation. Successive cuts of the distillates show a clearly perceptible shift of their absorption maxima from 276 mμ for the first, to 271 mμ for the last cuts, pointing to the presence of at least two volatile components with different absorption spectra.

For a separation and identification of these components, a larger amount of fraction 1a containing an estimated 4 to 5 g. of unknown bases, was prepared by extracting, in portions, 10 kg. of fermented tobacco leaves. The crude bases were steam distilled in the presence of MgO in aliquots of about 150 mg., the concentrated distillates adjusted to *p*H 3.5 and re-extracted with ether. The dark yellow oil left after evaporation of the ether, representing about 0.05% of the tobacco weight, was then subjected to a vacuum fractionation at about 4 mm. By this procedure, the oil was separated into two volatile components, the first a

(6) M. L. Swain, A. Eisner, C. F. Woodward and B. A. Brice, THIS JOURNAL, **71**, 1341 (1949).

(7) For a detailed study the alkaloids are re-extracted from this aqueous phase with ethyl ether at a *p*H ≦ 8 (see Table I).

(8) The bases precipitated with silicotungstic acid are recovered by dissolving the precipitates in a sufficient quantity of 2.0% NaOH, and removing the silicotungstic acid as its Ba salt.

(9) Progressive purification of tobacco alkaloids and of related compounds is usually indicated by increased ratios of the maximum/minimum absorbancies.

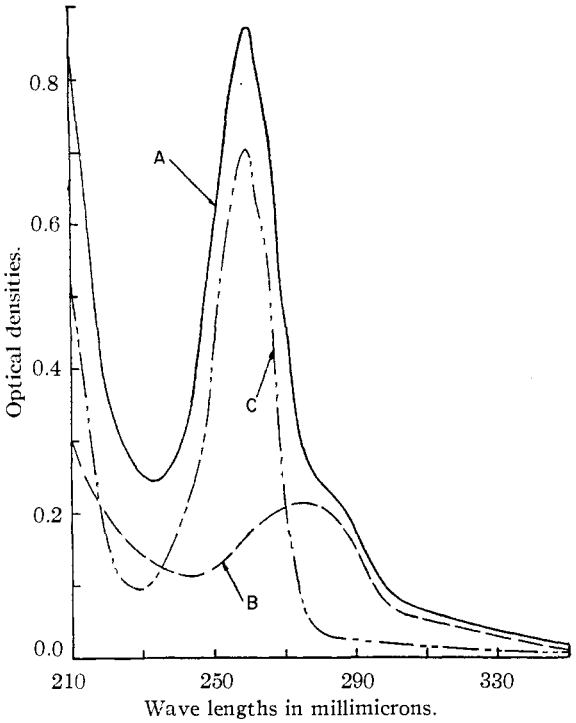


Fig. 1.—Absorption spectra of: fraction 1 (A), and of its components, fraction 1a (B) and the alkaloid fraction 1b (C); solvent, 0.3 *N* aqueous HCl. The concentration of all three fractions is that of extracts obtained from 11 g. of fermented tobacco, dissolved in one liter.

colorless liquid, volatilizing at temperatures between 0 and 35°, and the second evaporating at higher temperatures.

Identification of a Ketonic Pyridine Base in Fraction 1a.—The absorption spectra of the more volatile base in acidified and in alkaline aqueous solution, resemble closely the spectra of 3-pyridyl methyl ketone,[6] 3-pyridyl propyl ketone[10] and of the alkaloids myosmine[6,11] and N-methylmyosmine.[11,12] The latter exist, in aqueous solutions, as 3-pyridyl amino alkyl ketones.[12,13] This evidence for a 3-pyridyl alkyl ketone structure of the more volatile tobacco base was further supported by qualitative analytical tests which proved the presence of a carbonyl group and the absence of an aldehyde group. The absorption spectra of the four compounds mentioned above show almost identically located maxima and minima (a to g, Fig. 2) but deviate from each other by having distinctly different ratios between their absorption maxima and absorption minima (A_{max}/A_{min}). The corresponding ratios for the absorption spectra of the tobacco base were found to be practically identical with those of 3-pyridyl methyl ketone:

	A_b/A_c	A_d/A_c	A_e/A_f	A_g/A_f
3-Pyridyl methyl ketone	3.82	3.30	3.49	1.38
Tobacco ketone	3.87	3.26	3.54	1.36

(10) A sample of this substance was kindly furnished by Dr. R. L. Frank, Noyes Chemical Laboratory, University of Illinois.

(11) These compounds were kindly supplied by Dr. A. Eisner of the Eastern Regional Research Laboratory, U. S. Department of Agriculture, Philadelphia, Pa.

(12) P. G. Haines and A. Eisner, THIS JOURNAL, **72**, 1719 (1950).

(13) P. G. Haines, A. Eisner and C. F. Woodward, *ibid.*, **67**, 1258 (1945).

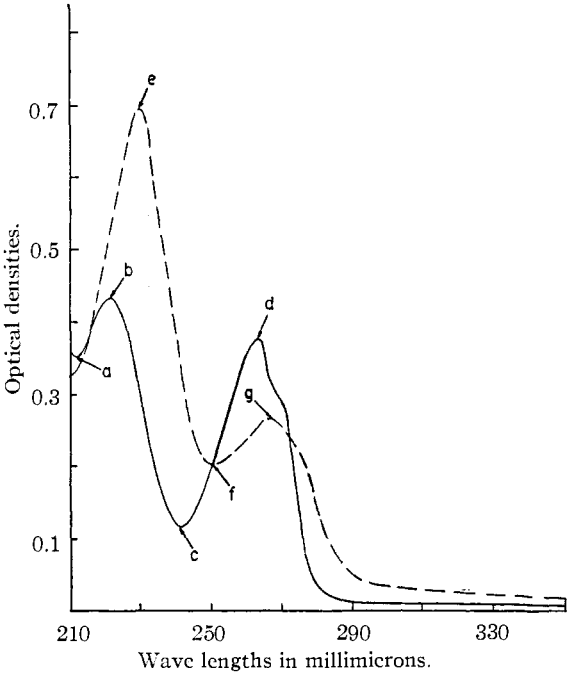


Fig. 2.—Absorption spectra of 3-pyridyl methyl ketone obtained from fermented tobacco: ——, in 0.3 *N* aqueous HCl; - - - - - - -, in 0.02 *N* aqueous NaOH; concentration, 9.2 mg. of ketone in one liter. The maxima and minima are coded in conjunction with the text.

Final confirmation of the identity of the ketonic tobacco base with 3-pyridyl methyl ketone was obtained by a comparison of the picrates, styphnates, 2,4-dinitrophenylhydrazones and mercuric chloride addition compounds of both substances. The total quantity of 3-pyridyl methyl ketone found in our samples is about 0.01% of the dry leaf weight, or about 20% of the bases of fraction 1a.

Isolation and Identification of a Second Base in Fraction 1a.—The second component of fraction 1a

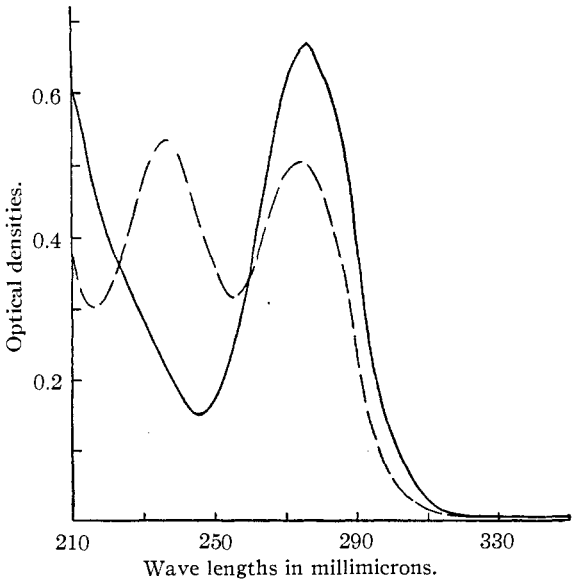


Fig. 3.—Absorption spectra of 2,3'-dipyridyl, obtained from fermented tobacco: ——, in 0.3 *N* aqueous HCl; - - - - - -, in 0.2 *N* aqueous NaOH; concentration, 7.2 mg. of dipyridyl in one liter.

volatilized at temperatures between 45 and 120° and condensed as a pale yellow oil, soluble in aqueous acid. Its absorption spectra in acid and alkaline water (Fig. 3) differed markedly from that of the known tobacco alkaloids and 3-pyridyl alkyl ketones. Qualitative tests with this substance failed to show the presence of any functional groups except the pyridine ring. Contrary to the behavior of most pyridyl derivatives, the substance resisted oxidation with boiling alkaline permanganate, yielding only after very prolonged treatment small amounts of nicotinic acid and other products. The unusually high absorbancy of the substance at its absorption maximum of 277 mμ (in acid) indicated a specific electronic configuration of the pyridine ring, presumably caused by its conjugation with additional double bonds. Molecular weight determination by the freezing point method with benzene as a solvent and by the Rast camphor method gave an average molecular weight of 156. Kjeldahl determinations of the unpurified base had indicated a nitrogen content of approximately 16%.[14]

These observations were compatible with a structure containing two pyridine rings combined in such a way that the double bonds of both rings are in conjugation with each other, *i.e.*, a dipyridyl structure. To substantiate this conjecture, the absorption spectra of 2,2′-dipyridyl and of 3,3′-dipyridyl,[11] two compounds readily available, were measured. Neither one was identical with the absorption of the tobacco base, but there was an unmistakable resemblance, especially in regard to both the high absorbancies at the maxima and the pronounced shift of the maxima on alkalinization. Since 3-substitution of the pyridine ring is a common characteristic of all the pyridine derivatives that have, so far, been found in tobacco, 2,3′-dipyridyl was prepared according to Skraup and Vortmann[15] using the modification of Smith.[16] Its absorption spectra in acid and in alkaline solutions were in every respect identical with the spectra of the second base of fraction 1a. The identity of both substances was further confirmed by a comparison of the mono- and dipicrates, and of the monostyphnates of both compounds.

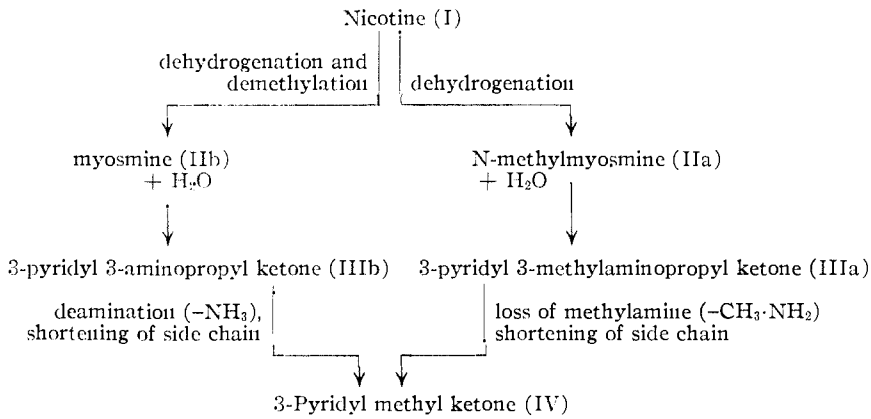
Späth and Zajic[17] isolated very small amounts of 2,3′-dipyridyl from a tobacco concentrate. We estimate that the amounts found by these authors correspond to only approximately 0.5% of the quantities found by us in our tobacco samples. The significance of this discrepancy, however, should not be overestimated since the tobacco used in Späth's work deviates both in type and in its previous treatment from fermented cigar tobacco.

Shortly after this identification of our second base was accomplished, Krumholz[18] published the absorption spectra of 2,3′-dipyridyl and of its hydrochloride. Within the limits of error, these spectra coincide with those measured by us for the synthetic 2,3′-dipyridyl and for the second base prepared from tobacco fraction 1a.

Discussion

No literature data were found on the presence of 3-pyridyl methyl ketone or of any other ketone bases in tobacco tissues. Tobacco smoke contains, according to Schöller[19] small amounts of 3-pyridyl *ethyl* ketone.

Since N-methylmyosmine (IIa) and myosmine (IIb) in the presence of water form 3-pyridyl 3-aminopropyl ketones, the 3-pyridyl methyl ketone (IV) found in the leaves may be derived from nicotine (I) as



The conversion (I) → (IIb) *in vitro* has been described by Woodward, Eisner and Haines,[20] and the close relationship between (I) and (IIa) has been demonstrated in synthesis work in various laboratories.[12,21,22]

The transformation of (IIIa) or of (IIIb) into (IV) involves a shortening of the side chain accompanied by liberation of ammonia or methylamine. Pyridine derivatives with shortened side chain in the 3-position have been observed among the products formed in the pyrolysis of nicotine. Methylamine was found as another product of this pyrolysis as well as of other decomposition reactions of nicotine and nicotine derivatives.[23,24]

With the exception of myosmine,[25] none of the substances contemplated here as intermediates (IIa, IIIa and IIIb) seem to be present in easily detectable quantities in our fermented tobacco samples. The absence of N-methylmyosmine may be explained by the tendency of this base to form, spontaneously, higher molecular polymerization products. Conceivably, the "insoluble pyridine derivatives" (Table I, fraction 4) discovered by us

(14) The presence of non-nitrogenous impurities and of traces of water in the crude base interfered with accurate nitrogen determinations at this point.

(15) Z. H. Skraup and G. Vortmann, *Monatsh.*, **3**, 599 (1882).

(16) C. R. Smith, THIS JOURNAL, **52**, 397 (1930).

(17) E. Späth and E. Zajic, *Ber.*, **69**, 2448 (1936).

(18) P. Krumholz, THIS JOURNAL, **73**, 3487 (1951).

(19) R. Schöller, *Fachl. Mitt. Oesterr. Tabak Regie*, **3**, 7 (1935).

(20) C. F. Woodward, E. Eisner and P. G. Haines, THIS JOURNAL, **66**, 911 (1944).

(21) J. P. Wibaut and J. Th. Hackmann, *Rec. trav. chim.*, **51**, 1157 (1932).

(22) E. Späth, J. P. Wibaut and F. Kesztler, *Ber.*, **71**, 100 (1938).

(23) A. Pinner, *ibid.*, **27**, 2861 (1894).

(24) R. L. Frank and C. Weatherbee, THIS JOURNAL, **70**, 3482 (1948).

(25) W. G. Frankenburg and A. M. Gottscho, *Arch. Biochem.*, **23**, 333 (1949).

in fermented tobacco are, at least partly, polymerization products of N-methylmyosmine.

An obvious way in which 2,3′-dipyridyl may be formed in the leaf tissue is that of a dehydrogenation of anabasine (3-pyridyl-2-piperidine).[26] This reaction has been achieved *in vitro* by Orechoff and Menschikoff.[27] "Anabasine," if present in green leaves of Pennsylvania tobacco[28] shows, during fermentation, a decrease large enough to explain the increase in amounts of 2,3′-dipyridyl in the fermented tobacco. Quantitative data on the increase of dipyridyl and of pyridyl methyl ketone in tobacco leaves caused by their fermentation will be given elsewhere. It may suffice to mention here that green Pennsylvania tobacco leaves contain amounts of dipyridyl as small as those found by Späth in cigarette tobacco extracts, and that the quantity of dipyridyl and of the ketone increase during curing and fermentation by a factor of about 250.

Further analytical studies of tobacco samples, including precise determinations of their anabasine contents throughout the stages of processing will be required in order to decide whether 2,3′-dipyridyl is exclusively formed from anabasine, or by a more complicated sequence of reaction also from nicotine.

Experimental

All spectrophotometric measurements were made with a Beckman DU spectrophotometer, Model No. 2398, after calibration of the instrument, correction of the cells, etc. The water for the absorption measurements was redistilled in the presence of H_2SO_4 and $KMnO_4$ in an all glass apparatus.

All nitrogen analyses were made by using a special Kjeldahl procedure modified for the quantitative determination of heterocyclic nitrogen. This procedure will be described in detail elsewhere.

The final method adopted for the extraction and isolation of 3-pyridyl methyl ketone and of 2,3′-dipyridyl was as follows:

Preparation of Fraction 1.—Two hundred and fifty grams of well-fermented Pennsylvania Seedleaf tobacco (1947 crop, nicotine content below 0.6%), air-dried and ground to a coarse powder (passing a 2-mm. mesh) are mixed in an ice-cooled tray with 40 g. of MgO (U.S.P. Heavy) and with a fourth of its volume of acid-washed asbestos fibers. Distilled water (*ca.* 150 to 250 ml.) is added gradually, saturating the powder without showing any excess "surface water." The mixture is transferred into the extraction thimble (stainless steel gauze, 100 mesh, wire diam. 0.114 mm.) and the latter placed into the extraction chamber of an all glass "Giant Soxhlet Extraction" apparatus (Ace Glass, Inc., 3-liter boiling flask) heated by an electric mantle. The boiling rate of the petroleum ether[29] (boiling range 30 to 60° reagent grade) used as extractant is regulated to about three siphons (750 ml. each) per hour. Under these conditions, the components of fraction 1 are completely extracted during 35 hr.

From the petroleum ether extract, usually amounting to 1.8 liters,[30] the organic bases are transferred into aqueous acid as follows: To 125 ml. of 0.5 *N* aqueous HCl in a one-liter distilling flask, about 300 ml. of the petroleum ether extract is added, the flask is shaken, and the petroleum ether completely distilled off, on a water-bath at 80°. The remainder of the petroleum ether extract is added and also distilled off in successive 300-ml. portions from the aqueous phase. Yellowish-brown resinous and waxy components settling out during this operation are removed from the aqueous phase by filtration while the liquid is still hot. The filtrate, containing all the hydrocarbon-soluble nitrogenous bases of the tobacco is fraction 1. An aliquot of this solution shows a spectrum[31] of the type illustrated by curve A, Fig. 1.

Preparation of Fraction 1a.—Fraction 1 is brought to *p*H 3.5 (Beckman Model B *p*H meter) with aqueous NaOH and extracted in a liquid–liquid extractor with ethyl ether, boiling so that about 4.5 ml. of ether passes per minute through the aqueous solution (*ca.* 250 ml.). After 21 hr. all substances of fraction 1a are extracted, and are transferred into 75 ml. of 0.3 *N* aqueous HCl as described above for the petroleum ether extract. The resulting slightly yellowish aqueous solution of nitrogenous bases is made up to 100 ml. and shows an absorption corresponding to Curve B, Fig. 1. This is fraction 1a.

For purification of this fraction, two aliquots, each derived from 250 g. of tobacco, are united, brought to a *p*H between 6 and 7 with NaOH, and steam distilled in presence of 5 g. of MgO (U.S.P. Light) for 10 hr. at a rate yielding approximately one liter of distillate per hour, maintaining the volume of liquid in the distilling flask constant at *ca.* 200 ml. The distillate is collected in two liter fractions, each containing 8 milliequivalents of HCl. All the substances responsible for the characteristic ultraviolet absorption of fraction 1a are distilled by means of this procedure. The distillate is concentrated to *ca.* 400 ml., adjusted to *p*H 3.5 with aqueous NaOH, and extracted with ethyl ether for 21 hr. as described for the previous extraction of fraction 1.

3-Pyridyl Methyl Ketone.—Several samples of the purified fraction 1a are combined and subjected to a vacuum evaporation in an apparatus so constructed as to permit a fractional condensation. This was accomplished by the use of condensing surfaces kept at room temperature on the one hand, and of Dry Ice traps, for the condensation of the more volatile component, on the other hand.

The ether solution of purified fractions 1a is transferred in small portions into this apparatus, the ether evaporated at room temperature until the residue, a dark yellow oil of an odor reminiscent of myosmine, is almost free of ether. The apparatus is then evacuated to a pressure of 4 mm., keeping the oil in an ice-bath. After several hours at 0°[32] the temperature is slowly raised until 35° is reached. During this period of evaporation, the condensate in the Dry Ice traps is collected intermittently[33] by rinsing the traps with HCl water. The combined washings are made up to volume and examined spectroscopically, as a control for the homogeneity of the collections. When the spectroscopic examination shows very low absorption values as well as indications for the appearance of very small amounts of other absorbing substances, the evaporation of the ketonic base is considered to be complete.

An authentic sample of 3-pyridyl methyl ketone (m.p. 14°) prepared from ethyl nicotinoacetate hydrochloride[11] following the procedure of Strong and McElvain[34] and purified by vacuum distillation, showed in acid and in alkaline aqueous solution a spectrum identical with that of the ketonic base obtained from fraction 1a of our tobacco samples (see Fig. 2).

For the preparation of derivatives from the tobacco base and of 3-pyridyl methyl ketone, the free bases were recovered from their aqueous solutions by renewed extraction with ethyl ether at *p*H 3.5.

3-Pyridyl Methyl Ketone Picrate.—Twenty-five milligrams of ketonic base in ethyl ether solution is added to 5 ml. of saturated aqueous picric acid solution. As the ether evaporates at room temperature, yellow crystals form at the ether–water interface. The picrate is recrystallized from hot water, m.p. 130°, mixed m.p. 131°.

(26) Indications for the presence of anabasine in Pennsylvania Seedleaf tobacco have been reported by W. L. Porter, J. Naghski and A. Eisner, *Archiv Biochem.*, **24**, 461 (1949).

(27) A. Orechoff and G. Menschikoff, *Ber.*, **64**, 266 (1931).

(28) That part of the alkaloids which exceeds the sum of nicotine and nornicotine in our samples was calculated as "anabasine."

(29) Neohexane used in initial experiments was later replaced by the equally efficient but less expensive petroleum ether.

(30) About 700 ml. of the solvent remained absorbed by the tobacco in the extraction chamber.

(31) This serves for an estimate of the petroleum ether soluble pyridine nitrogen components in any new tobacco samples, and also for checking the completeness of the petroleum ether extraction on subsamples of a given tobacco powder.

(32) Previous small scale experiments had shown that the ketone evaporates *in vacuo* at a moderate rate at this temperature.

(33) Usually after every 6 hr.

(34) F. M. Strong and S. M. McElvain, THIS JOURNAL, **55**, 816 (1933).

Anal. Calcd. for $C_{13}H_{10}O_3N_4$: N, 16.01. Found: N, 16.5, 15.7.

3-Pyridyl Methyl Ketone Mercuric Chloride.—Forty milligrams of tobacco ketone in ethyl ether solution is added to 2 ml. of aqueous saturated $HgCl_2$ solution diluted to 6 ml. On evaporation of the ether at 5°, white needles form which are recrystallized from hot water, containing a small amount of $HgCl_2$, m.p. 161°.[35] Mixed melting points with the similarly prepared derivative of 3-pyridyl methyl ketone give no depression.

2,3'-Dipyridyl.—During the vacuum evaporation of fraction 1a, as the temperature is raised to 30° and above, a yellowish oil becomes visible at a short distance above the heated part of the evaporator. After all of the ketone has been collected, the temperature is raised to 60°. At this temperature, successive portions of condensate are collected intermittently from the condensing surfaces by rinsing with acidified water. These fractions are examined spectroscopically for homogeneity and also for estimating the yield. The absorption spectra in acid and in alkali of these fractions, shown in Fig. 3, later proved to be that of 2,3'-dipyridyl. When the evaporation becomes slower, the temperature of the bath is gradually raised to a maximum of 120° at which temperature the evaporation is continued until spectroscopic checks indicate a lack of homogeneity. At this end point, *ca.* 15% of the initial weight of fraction 1a remains as a brown tar in the evaporator.

An authentic sample of 2,3'-dipyridyl is prepared from *m*-phenanthroline[15,16] with the modification that the decarboxylation of the intermediate dicarboxylic acid is made *in vacuo*. For the preparation of derivatives, the aqueous solutions of the authentic sample as well as that of the second base of fraction 1a are extracted at *p*H 3.5, with ethyl ether.

2,3'-Dipyridyl Monostyphnate.—Thirty milligrams of tobacco dipyridyl in ether solution is added to 5 ml. of an aqueous saturated styphnic acid solution diluted to 10 ml. Yellow crystals form immediately in the ether phase, settling out as the ether evaporates. After recrystallization from hot water, a melting point of 190–191° (dec.) is obtained; a mixed m.p. with the styphnate prepared from the authentic sample of 2,3'-dipyridyl gives an identical result.

The composition of styphnate was proved by dissolving 8.2 mg. of the derivative in 2.5 *N* HCl, extracting the liberated styphnic acid with ethyl ether, transferring the latter into aqueous solution, and measuring spectrophotometrically the amounts of dipyridyl and of styphnic acid contained in the extraction residue and in the extract, respectively.

(35) C. Engler, *Ber.*, **24**, 2539 (1891), reports a m.p. of 158° for this compound.

Anal. Calcd. for dipyridyl monostyphnate: dipyridyl, 38.9; styphnic acid, 61.1. Found: dipyridyl, 37.4; styphnic acid, 62.1.

2,3'-Dipyridyl Monopicrate.—The picrate obtained by a procedure similar to that described for the styphnate, gives prior to recrystallization a dipicrate, m.p. 164°; the same result is obtained with the picrate of synthetic 2,3'-dipyridyl. Orechoff and Menschikoff[27] and Krumholz[36] report a m.p. of 166–168° for the dipicrate of 2,3'-dipyridyl. However, on repeated recrystallization of the picrates from hot water, a constant m.p. of 152° is obtained for both; mixed m.p. 152°. In agreement with the authors mentioned, these recrystallized picrates are the 2,3'-dipyridyl monopicrate:

Anal. Calcd. for $C_{16}H_{11}O_7N_5$: N, 18.2. Found: N (average of 4 determinations), 17.7.

The composition of this monopicrate is confirmed by the same procedure as described for the styphnate. Five different picrates were analyzed in this manner:

Anal. Calcd.: dipyridyl, 40.5; picric acid, 59.5. Found: dipyridyl, 40.3 ± 1.0; picric acid, 59.1 ± 1.0.

The nitrogen content of the dipyridyl was found indirectly from its picrate, using the very pure dipyridyl fraction obtained from the decomposition of the picrate:

Anal. Calcd. for $C_{10}H_8N_2$: N, 18.0. Found: N, 17.5, 17.8.

Determination of Standard Nitrogen Absorbancy[37] of Purified 2,3'-Dipyridyl.—The spectrum of the dipyridyl fraction as obtained from the vacuum evaporation apparatus agreed both in acid and in alkaline solutions with that reported by Krumholz[18] for 2,3'-dipyridyl. For a quantitative corroboration of the identity of Krumholz's and our compound, we purified the dipyridyl obtained from tobacco over its monopicrate in the manner described above and measured its absorption spectrum. The purity of the base was verified by the fact that the ratio A_{max}/A_{min} agreed within experimental limits with the value of 4.87 calculated from Krumholz's curve. Using the nitrogen content of several aliquots of this solution as found by our modified Kjeldahl procedure, the standard nitrogen absorbancy at 277 mμ of our substance was determined.

Calcd. from Krumholz's curve: 73.0. Found: 73.8, 74.8, 75.2.

(36) P. Krumholz, *Selecta Chimica*, **8**, 1 (1949).

(37) Defined as the absorbancy of a solution, containing in one liter 10 milliequivalents of the substance based on its nitrogen content.

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The Preparation of a Pyrimidine Analog (Isostere) of Promizole and Other Substituted Pyrimidines

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α-4-Aminophenyl-β-2-pyrimidylurea, *p*-aminophenyl-2-amino-5-pyrimidyl sulfone, 2-sulfanilamido-5-phenylmercaptopyrimidine and other 5-mercapto substituted pyrimidines have been synthesized. Pharmacological tests carried out with these compounds indicated that most of them were ineffective or but slightly active. Only a few showed activity comparable to that of sulfanilamide.

In seeking further light upon the relationship between chemical structure and physiological action it is common practice to modify certain structural features of substances of known activity in the hope of getting better correlations and guides for further investigation. In the first of the three substances whose preparation we are describing, the sulfonamide group linking the homocyclic and heterocyclic parts of sulfadiazine has been replaced by a carbamide group. In the second compound, which is an isomer of sulfadiazine, we have, instead of a sulfonamide, a sulfone which may also be looked upon as an isostere of Promizole.[2]

Particular attention may be called to the fact that in this compound and in the intermediates used in obtaining it, sulfur is directly attached in two different states of valence to position 5 of the pyrimidine nucleus. Our search of the literature

(1) Taken from a thesis submitted by A. Nuri Sayin in partial fulfillment of the requirement for the degree of Doctor of Philosophy.

(2) L. L. Bambas, U. S. Patent 2,389,126 (Nov. 20, 1945); *C. A.*, **40**, 991 (1946); THIS JOURNAL, **67**, 668 (1945).