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Transformation of Tobacco Alkaloids*

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This is a review of the work on the transformation of tobacco alkaloids which has been carried out at the Japan Tobacco and Salt Public Corporation in the past ten years.

Figure 1 shows the transformation of N-methylpyrrolidines and N-methylpiperidine oxides. Nicotine oxide [II] is easily formed from nicotine under natural conditions, and it is stable at room temperature. *Rayburn* (1) has shown that the oxide is rearranged to 2-methyl-6-(3'-pyridyl)tetrahydro-1,2-oxazine [III] by heating.

We found another rearrangement reaction of the oxide. Upon acyl halides' or anhydrides' being reacted with nicotine-N'-oxide by triturating it with a glass rod in a glass flask, the oxide was spontaneously rearranged giving an N-acylated butylamine derivative [IV]. When this reaction occurs under cooled conditions, then a near-theoretical yield of N'-acylated pseudooxynicotine can be obtained. When the N'-methylmyosmine [V] was heated under vacuum, nicotyrine [VI], N-methylnicotinamide [VII], other pyridine compounds and a large

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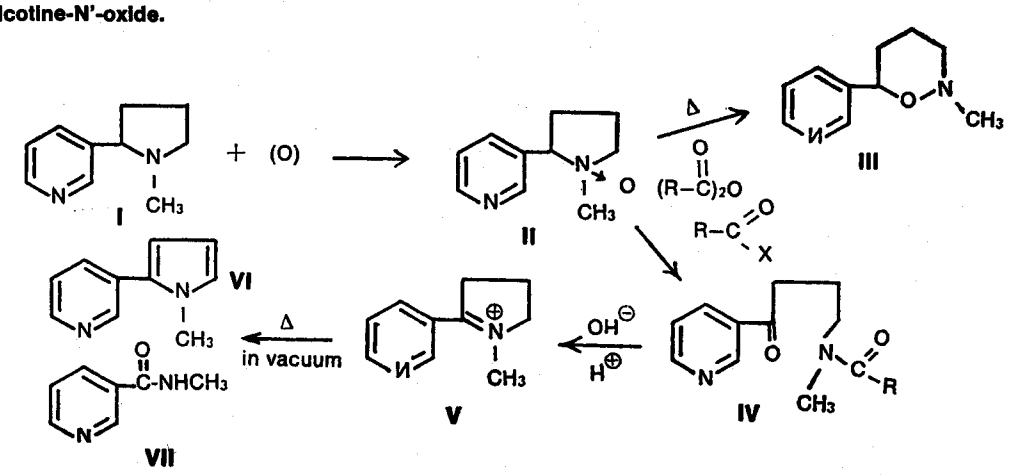
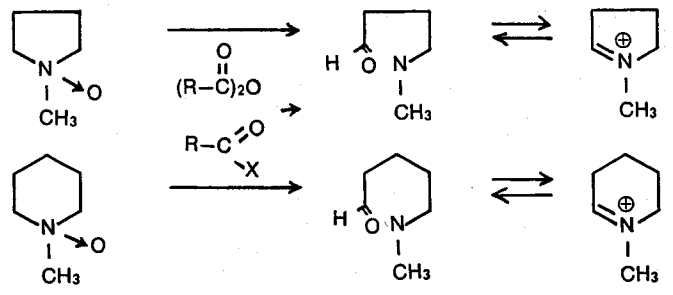
Figure 1.

Reaction of nicotine-N'-oxide.



Reaction of N-methylpyrrolidine and N-methylpiperidine oxide.



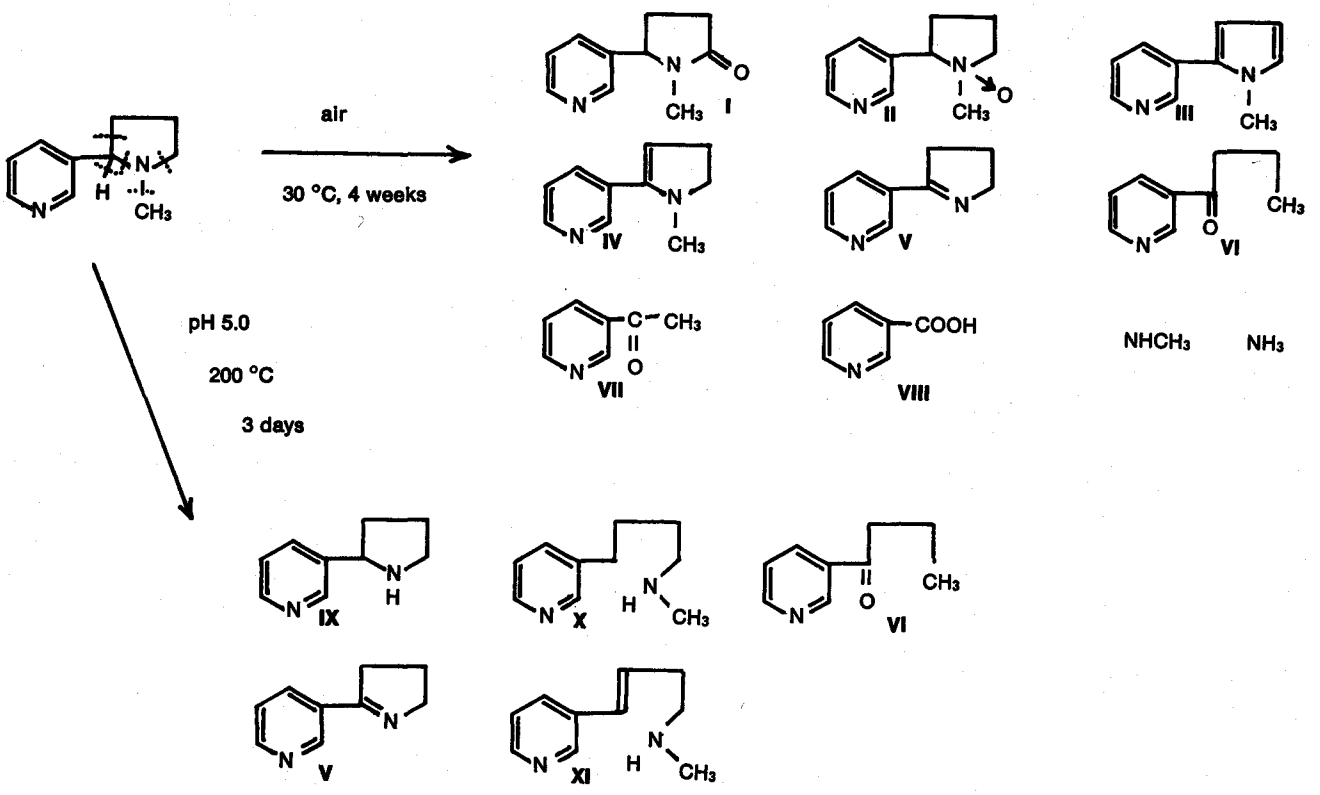
I: nicotine
II: nicotine-N'-oxide
III: 2-methyl-6-(3'-pyridyl)tetrahydro-1,2-oxazine
IV: N-acyl-N-methyl-4-oxo-4-(3'-pyridyl)butylamine
V: N'-methylmyosmine
VI: nicotyrine
VII: N-methylnicotinamide

amount of resin were obtained. This is very suggestive of natural nicotine degradation. Nicotine-N′-oxide [II] is formed during fermentation, but it is stable at room temperature. However, a nucleophilic attack on the oxide [II] would probably occur during fermentation, so that the formed unstable N′-methylmyosmine [V] would be transformed to give various further transformed compounds. This rearrangement reaction of the N′-oxide [II] could be used for the preparation of Δ_1-pyrrolines or Δ_1-piperideines (2, 3). N′-methylmyosmine is regarded as a key compound in nicotine degradation. However, there has been no information confirming the structure of so-called "N′-methylmyosmine". We obtained N′-methylmyosmine (b.p. 90—93 °C, 2 mmHg) by refluxing pseudooxynicotine with benzene and confirmed the structure of 1-methyl-2-(3′-pyridyl)-2-pyrroline by comparing with 2-methyl-1-(2′-methyl-6′-cyclohexene-1′-yl)-pyrrolidine by means of ^{1}H-NMR (18).

Frankenburg and co-workers (4) have demonstrated a number of degradation products of nicotine in cigar tobacco. We conducted an experiment on nicotine aeration to determine the oxidation of nicotine by air at room temperature. A number of products were identified, as seen in Fig. 2. A large part of the products was nicotine-N′-oxide. N′-Methylmyosmine was first identified as a degradation product. Most compounds, except cotinine and nicotine-N′-oxide, are supposedly derived from N′-methylmyosmine. Nicotinic acid must be derived from N-methylnicotinamide which has in turn been derived from N′-methylmyosmine. When a nicotine salt solution was heated at 200 °C under vacuum in an autoclave to determine the manner of initial pyrolytic degradation of nicotine, nornicotine was obtained as a major product together with metanicotine and dihydrometanicotine. In all the detected degradation products, the C-N bonds of the pyrrolidine ring moiety were cleaved (5, 6).

In the tobacco plant, nicotine has been regarded as a secondary metabolite. However, dealkylation of nicotine commonly occurs in most wild species and some cultivars. We tried to establish a structural minimum requirement of the dealkylation system of tobacco alkaloids in tobacco plants, using a number of derivatives of tobacco alkaloids and leaves of the Cherry Red strain of the Bright yellow variety of *Nicotiana tabacum* L., as *Dawson* (7) has worked using a limited number of tobacco alkaloid derivatives. Experimental leaves were prepared by grafting the tobacco scion on a tomato stock. The substrates were fed from the end of the petiole of the leaves and analyzed. The substrates in the feeding experiment were prepared by chemical synthesis or isolation from natural sources. As shown in Table 1, among compounds analogous to nicotine, both R- and S-nicotine ($pK_{a\,1}$ = 7.9, $pK_{a\,2}$ = 3.1), N′-methylanabasine, nicotine-N′-oxide (being reduced to nicotine) and 6-hydroxynicotine were demethylated. No nicotyrine ($pK_{a\,1}$ = 4.9) and cotinine ($pK_{a\,1}$ = 4.5) resulted in the demethylated compound. From these results it was inferred that the methylated nitrogen atom is required to

Figure 2. Air oxidation and low-temperature pyrolysis of nicotine.



I: cotinine
II: nicotine-N′-oxide
III: nicotyrine
IV: N′-methylmyosmine
V: myosmine
VI: 3-pyridylpropyl ketone
VII: 3-pyridylmethyl ketone
VIII: nicotinic acid
IX: nornicotine
X: dihydrometanicotine
XI: metanicotine

Table 1. Demethylation of tobacco alkaloid analogues in tobacco leaves.

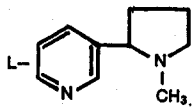
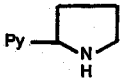
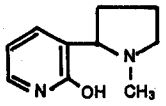
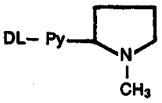
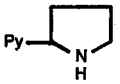
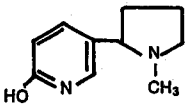
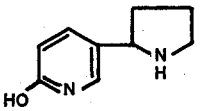
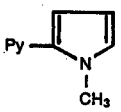
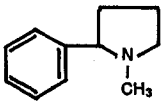
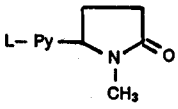
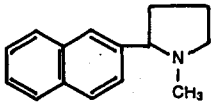
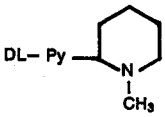
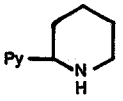
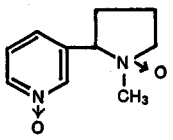
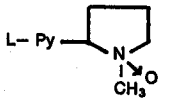
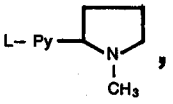
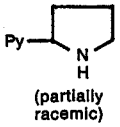
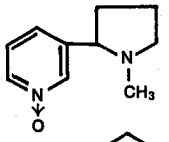
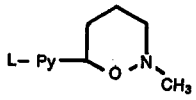
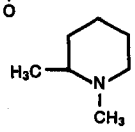
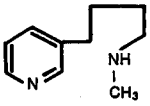
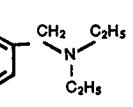
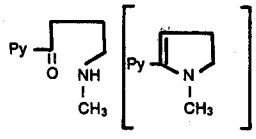
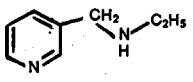
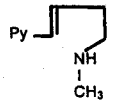
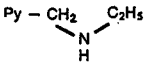
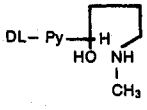
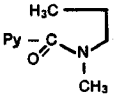
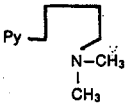
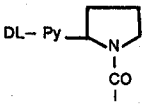
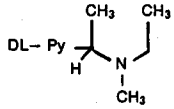
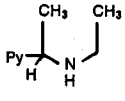
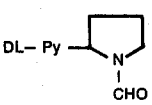
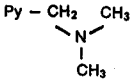
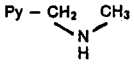
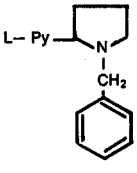
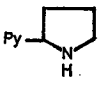
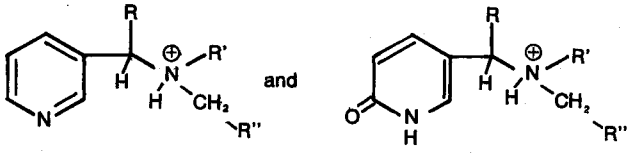
Substrate	Product	Substrate	Product
	 (partially racemic)		none
	 (D-dominant)		
	none		none
	none		none
	 (D-dominant)		none
	  (partially racemic)		none
	none		none
	none		none
	none		
	none		none
	none		none
	none		none
	 (D-dominant)		none
			

Figure 3. Minimum requirement of the structure for dealkylation of nicotine analogues in tobacco leaves.



R = or ≠ R'
R = or ≠ R''

be ionized for demethylation under physiological conditions. The anionic site would be present in the dealkylation enzyme system. No alicyclic amine such as pyrrolidine or piperidine was necessarily required for the dealkylation substrate. An appropriate distance (approx. 4.16 Å) between the nitrogen atom in the pyridine and the nitrogen atom to be dealkylated is required. The dealkylation was not confined to methyl groups. Experimentally, we could work out the structural minimum requirement of the dealkylation system in tobacco leaves as shown in Fig. 3 (8).

Tobacco alkaloids are secondary products which have been regarded as metabolically inert substances except their N-methyl group. The N-methyl group of nicotine is fairly well metabolized. L-Nicotine was demethylated to give partially racemized L-nornicotine (9). We proved further transformation of nornicotine and anabasine in tobacco leaves by feeding optically inactive ones and by measuring the optical activity of recovered alkaloids. As seen in Table 2, the optically inactive alkaloids were transformed into optically active compounds. Nicotine was predominantly demethylated in D-form, and nornicotine was predominantly dehydrogenated to myosmine in L-form (10, 11, 12). The transformation pathway of nicotine in tobacco leaves is shown in Fig. 4. Recently, myosmine was also confirmed as a metabolite in the leaves by *Leete* and his co-workers (13).

Anabasine is a principal alkaloid in *Nicotiana glauca* L. and also a common minor alkaloid in *Nicotiana tabacum* L. The anabasine isolated from the root of *Nicotiana glauca* L. showed a little levo rotation and the one in the leaf showed dextro rotation (Table 3). These facts suggest that anabasine in *Nicotiana glauca* L. is first biosynthesized in the racemic form and the D-enantiomer is predominantly degraded in the root, whereas the L-enantiomer is predominantly degraded in the leaf. The stereospecificity of the degradation of anabasine in the leaves of *Nicotiana tabacum* L. was shown in the feeding experiments to be of reverse order. The variation of stereospecificity of anabasine degradation remains to be explained. The optical rotation increased from 0 to —2.9 after a week (Table 3). This suggests that anabasine is

Figure 4. Transformation of nicotine and nornicotine in Nicotiana tabacum (Cherry Red) leaves.

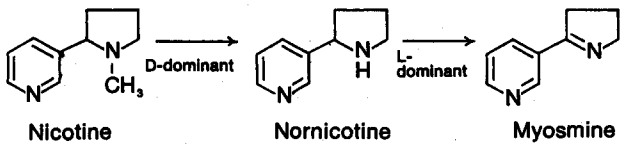
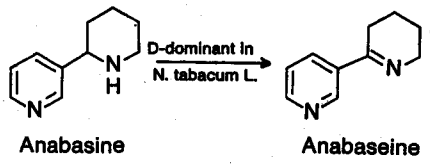


Figure 5. Transformation of anabasine in Nicotiana tabacum.



further transformed in the plant. The metabolite was identified as anabaseine by isolation (12). The transformation pathway of anabasine in tobacco leaves is shown in Fig. 5.

We have noticed the presence of a fairly large amount of an isatin-positive alkaloid (I-A) other than nornicotine in the Cherry Red strain of converter-type tobacco (14), as shown in the paper chromatogram (Fig. 6). I-A was isolated from Cherry Red leaves of *Nicotiana tabacum* L., Bright yellow, by the sequential procedure of methanol extraction, cation exchange chromatography, partition chromatography using *tert*-amylalcohol-acetate buffer and HPLC. I-A was isolated in a resinous form. This was crystallized by treatment with chloroform and purified by recrystallization from chloroform and liquid chromatography [m.p. 66—68 °C (decomposition), $[\alpha]_D^{22} = -83.80°$ (c = 1.08 in H_2O)]. A high-pressure liquid chromatogram of the methanol extract of tobacco is shown in Fig. 7. Color test, hydrolysis, and spectral data (^{1}H- and ^{13}C-NMR, MS, IR and UV) suggest that

Table 2. Transformation of nicotine and nornicotine in Nicotiana tabacum L. (Cherry Red) leaves.

DL-nicotine feeding		DL-nornicotine feeding	
hours	$[\alpha]_D^{24}$	weeks	$[\alpha]_D^{24}$
0	0	0	0
64	−4.57	2	+0.98
112	−6.76	4	+2.62
160	−10.12		

Nicotine and nornicotine were recovered at the indicated time after feeding DL-nicotine and DL-nornicotine.

Table 3. Transformation of anabasine in Nicotiana glauca L. and Nicotiana tabacum L.

	Anabasine $[\alpha]_D^{24}$		
in N. glauca L.*	88 days	115 days	145 days
Root	−2.09	−0.23	−3.91
Stalk	−2.13	+0.28	−1.12
Leaf	−1.02	+1.36	+2.12
in N. tabacum L.**	0 hours	116 hours	164 hours
Leaf	0.00	−2.08	−2.90

* Anabasine was isolated from the root, stalk and leaf of Nicotiana glauca L. at the indicated time after sowing.
** Anabasine was recovered at the indicated time after feeding DL-anabasine.

Figure 6. Paper chromatogram of methanol extract of Cherry Red strain of converter-type tobacco.

Samples were developed in descending order on Whatman No. 1 dried after impregnation with 0.2 N sodium acetate in the solvent system of the upper layer [*tert*-amyl alcohol : 0.2 N sodium acetate (pH 5.6) = 1 : 1]

Color development:
1. *p*-aminobenzoic acid / BrCN
2. isatin

Shadowed spots were positive with the isatin reagent.

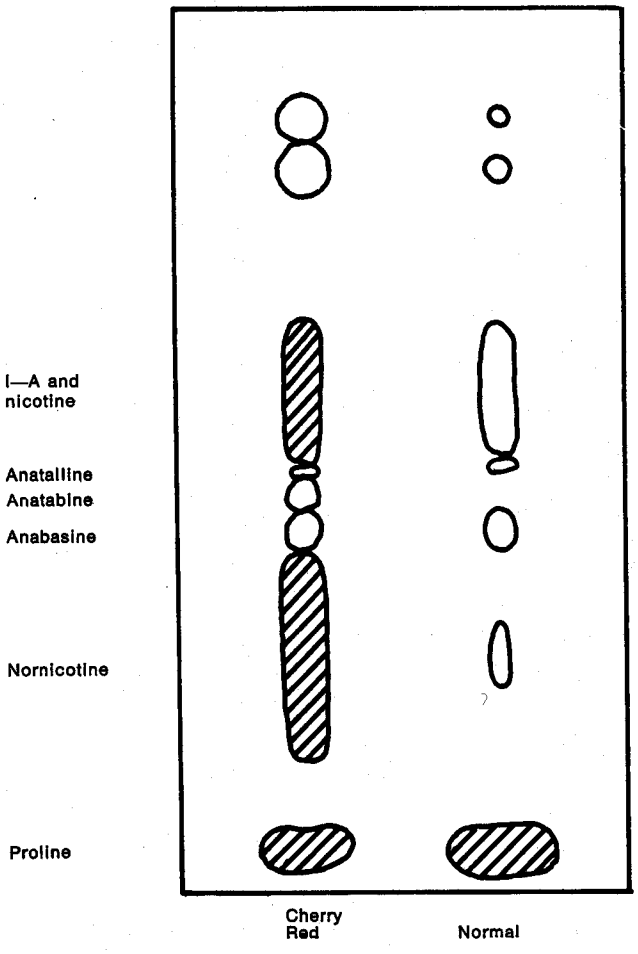


Figure 7. High-pressure liquid chromatography of methanol extract of Cherry Red tobacco.

WATERS HPLC ALC 201
column: μ-Bondapak C_{18} (4 × 300 mm)
elution conditions: 50 % methanol (2 ml / min)

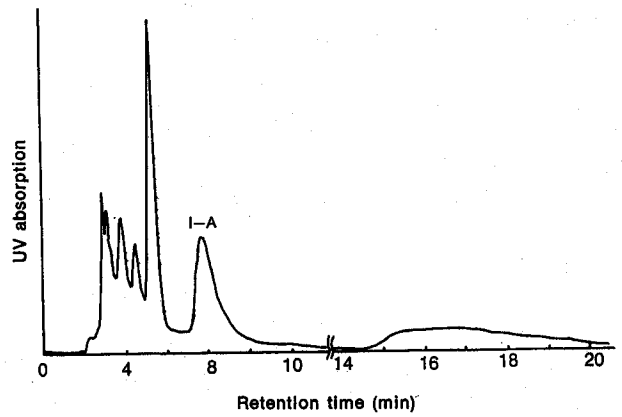
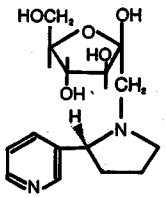


Figure 8. Structure of the isatin-positive alkaloid (I-A).



1-(1'-2'(S)-nornicotino)-1-deoxy-β-D-fructofuranoside

Figure 9. Changes of alkaloid content in the course of flue-curing.

The isatin-positive alkaloid (I—A) was determined by liquid chromatography, nicotine and nornicotine by gas chromatography.

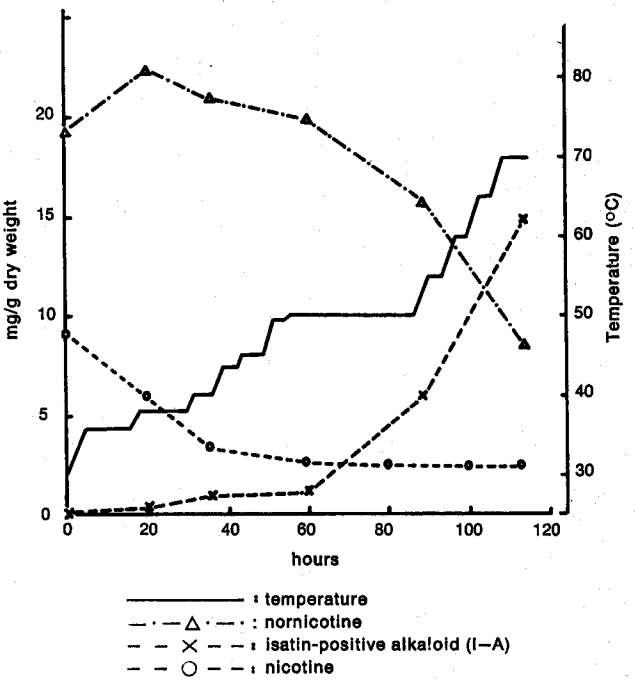


Figure 10. High-pressure liquid chromatogram of microbial metabolites of 2'(S)-nicotine-1'-N-oxide in 4 days' culture.

WATERS HPLC ALC 201
column: μ-Bondapak C_{18} (4 × 300 mm)
elution conditions: 0.02 M NH_4HCO_3 : methanol (1 : 1) (2 ml / min)

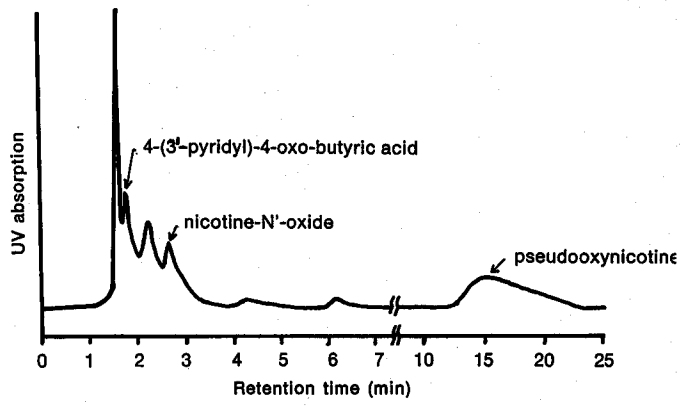
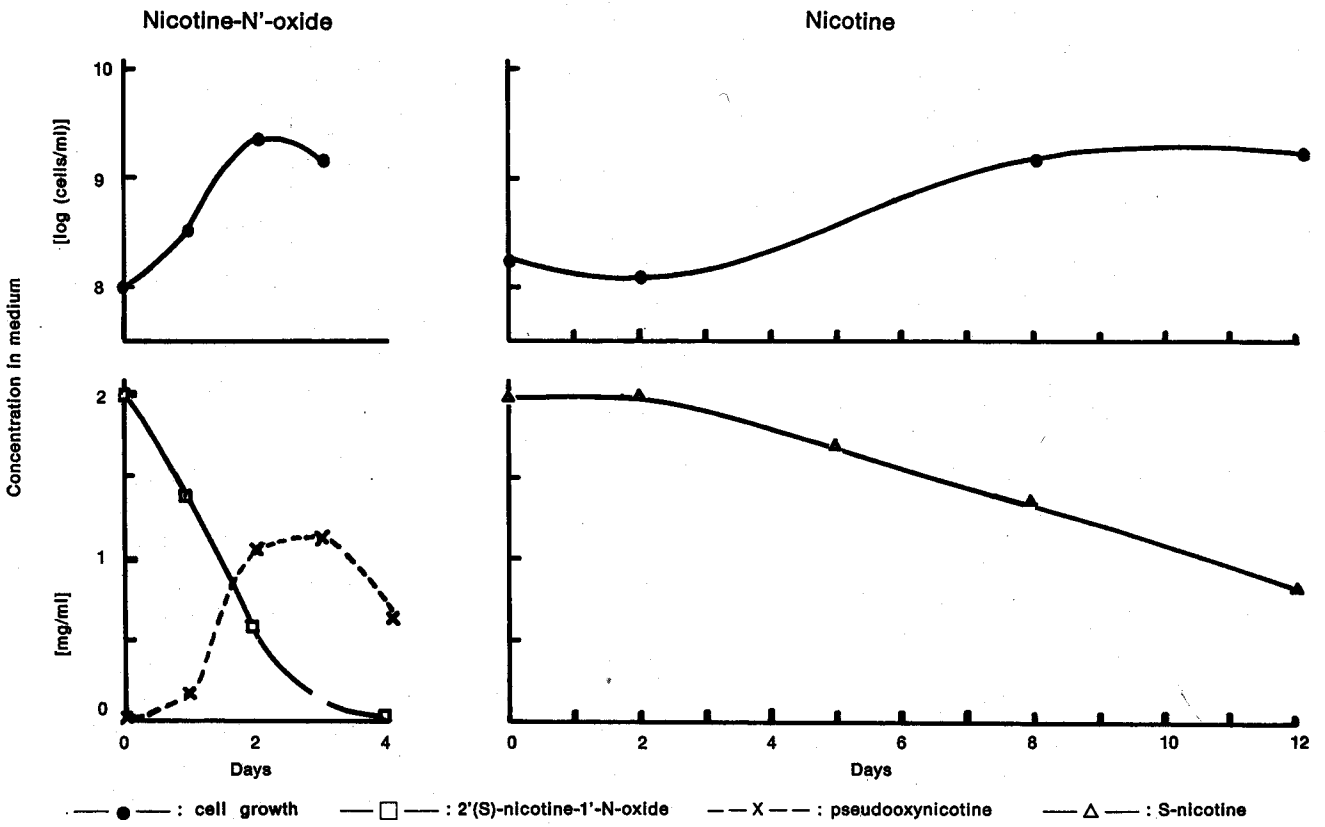


Figure 11. Cell growth and microbial degradation of 2′(S)-nicotine-1′-N-oxide and S-nicotine.

Culture medium: 2 g of nicotine-N′-oxide or nicotine; 1 g, KH_2PO_4; 0.5 g, $MgSO_4 \cdot 7\ H_2O$; 0.3 ml of 1 % $FeSO_4$ solution; 0.3 ml of 1 % $CaCl_2$ solution; trace of $MnSO_4$ in 1 liter of distilled water (pH 6.4). Analysis was carried out by HPLC.

Bacteria: Arthrobacter globiformis JTS*-6 (shaking the culture at 28 °C)



the structure of the compound in the solid state is 1-(1′-2′(S)-nornicotino)-1-deoxy-β-D-fructofuranoside (Fig. 8). The infra-red absorption spectrum of this compound was identical to that of the one synthesized from S-nornicotine and D-glucose in the presence of malic acid. It must be formed from glucose and nornicotine via N′-D-glucopyranosyl-2′(S)-nornicotine by Amadori-rearrangement.

Fig. 9 shows the rise and fall of the alkaloid content during flue-curing of Cherry Red tobacco. As the curing temperature of tobacco leaves was increased, nicotine decreased first. Nornicotine increased first and then decreased. 1-(1′-2′(S)-nornicotino)-1-deoxy-β-D-fructofuranoside increased up to 15 mg / g dry weight, which indicates a non-enzymatic process. It has been reported that the converter-type tobacco appears in a frequency of 0.8 % in natural back mutation. Therefore, the crop of nicotine-type tobacco must be contaminated with the converter-type tobacco at that rate. The converter-type tobacco is generally regarded as a tobacco with light but unpleasant taste. No isolated compound gave an unpleasant taste. As a flavouring agent for tobacco, on the contrary, it had an improving effect on inferior tasting tobacco. 1-(1′-2′(S)-nornicotino)-1-deoxy-β-D-fructofuranoside was easily broken down and displayed a red black color in the presence of moist air after isolation. This must be one of the reasons for the red color development of Cherry Red tobacco leaves during the curing and redrying process.

Finally, the bacterial transformation of nicotine oxide is described. Nicotine oxide is known to be formed during fermentation. Along with the chemical degradation of this compound, microbial degradation might occur during fermentation. The bacteria degrading the oxide and the manner of the degradation of this compound are not yet known. We isolated the bacteria JTS*-5 and JTS-6 which degrade the oxide from "Nambu" Japanese cigar tobacco leaves. Both strains were identified as *Arthrobacter globiformis* by morphological, physiological and biochemical comparison with the type strain ATCC 8010 (15). The guanine and cytosine content of the DNA in the bacteria also supported the identification.

Fig. 10 shows a liquid chromatogram of the degradation product of 2′(S)-nicotine-1′-N-oxide. Pseudooxynicotine was isolated as a principal product. 4-Oxo-4-(3′-pyridyl)butyric acid was also identified as one of the products. As shown in Fig. 11, after a 3-day culture period almost all of the oxide was degraded and the pseudooxynicotine had accumulated to a maximum level. The maximum rate of the cell multiplication came a little earlier. On the other hand, nicotine was degraded slowly. Degradation products of nicotine by the bacteria were

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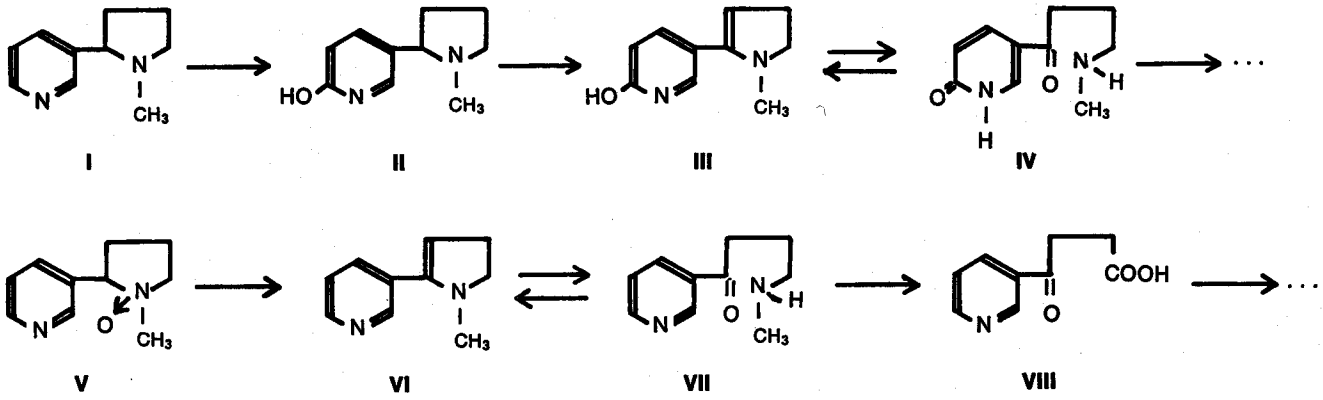
Figure 12. Degradation pathway of nicotine and nicotine-N′-oxide by Arthrobacter globiformis JTS*-6.



I: nicotine
II: 6-hydroxynicotine
III: 6-hydroxy-N′-methylmyosmine
IV: N-methyl-4-oxo-4-(3′-pyridonyl)butylamine
V: nicotine-N′-oxide
VI: N′-methylmyosmine
VII: 3-pyridyl-N-methylaminopropyl ketone
VIII: 4-(3′-pyridyl)-4-oxobutyric acid

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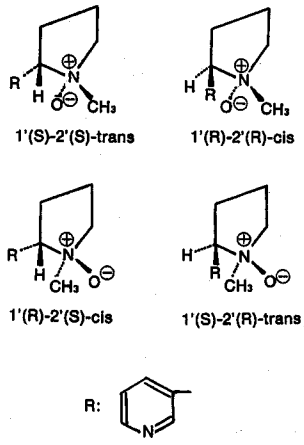
6-hydroxynicotine and 6-hydroxy-N′-methylmyosmine, but not pseudooxynicotine (16). In this organism the degradation of nicotine and nicotine-N′-oxide follows different pathways: nicotine through 6-hydroxynicotine, nicotine-N′-oxide through N′-methylmyosmine (Fig. 12). *Wada* and *Yamasaki* (17) of our Corporation have already demonstrated the nicotine degradation pathway through pseudooxynicotine by the micro-organism isolated from a tobacco field. The micro-organisms that we have worked with evidently degraded nicotine-N′-oxide directly to pseudooxynicotine without reducing it back to nicotine. The N′-oxides of S- and R-nicotine have 2 stereoisomers each with respect to the nitrogen atom: 1′(S)-2′(S)-trans and 1′(R)-2′(S)-cis; 1′(R)-2′(R)-cis and 1′(S)-2′(R)-trans, as seen in Fig. 13. The usual forms are: 1′(S)-2′(S)-trans and 1′(R)-2′(S)-cis. By this micro-organism 2′(R)-nicotine-1′-N-oxide was more efficiently degraded than 2′(S)-nicotine-1′-N-oxide. When the mixture of 1′(S)-2′(S)-trans and 1′(R)-2′(S)-cis isomers of 2′(S)-nicotine-1′-N-oxide was metabolized for 7 days, the $[\alpha]_D$-value changed from +30° to +90° (Fig. 14), which indicates that this micro-organism degraded the 1′(R)-2′(S)-cis isomer more efficiently than the 1′(S)-2′(S)-trans isomer.

Figure 13. Configurations of the four possible nicotine-N′-oxides.



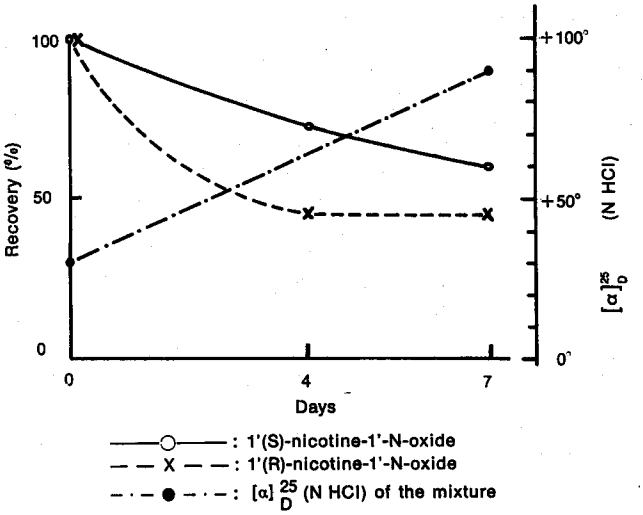
SUMMARY

Chemical transformation: In air oxidation of nicotine at room temperature, N′-methylmyosmine, which is supposed to be an active intermediate of degradation, cotinine, nicotine-N′-oxide, nicotyrine, myosmine, 3-pyridylpropyl ketone, 3-pyridylmethyl ketone, nicotinic acid, methylamine and ammonia were isolated. N′-Methylmyosmine was first characterized by ^{1}H-NMR. When N′-methylmyosmine was heated, N-methylnicotinamide and nicotyrine were obtained in addition to a large amount of polymerized resinous substances. 2′(S)-nicotine-1′-N-oxide was rearranged to acetyl pseudooxynicotine by reaction with acetyl chloride or acetyl anhydride. This rearrangement could be generally useful for the preparation of Δ_1-pyrrolines or Δ_1-piperideines. When appropriate acetyl groups were used, the products were effective in improving tobacco taste.

Phytochemical transformation: Transformation of alkaloids in the tobacco plant was investigated by measuring

Figure 14. Time course of microbial degradation of diastereomers.

Culture condition: 10 ml broth in Erlenmeyer flask (shaking the culture at 28 °C). Inoculation: 2.2 X 10^7 cells / ml.



their optical rotatory power, from which it was presumed that nicotine is biosynthesized in the S-form. The nornicotine formed in the leaves is synthesized from S-nicotine, but the one formed in the root is synthesized in the racemic form, indicating a route different from that found in the leaves. Secondary amine alkaloids such as anabasine and anatabine are in the racemic form. From Cherry Red tobacco, a transformation product of nornicotine, 1-(1'-2'(S)-nornicotino)-1-β-D-fructofuranoside (m.p. 66—68 °C), was isolated for the first time. The structure was confirmed physico-chemically and finally by synthesis. This compound increased markedly during curing, especially at the drying stage, suggesting formation through a non-enzymatic process.

Microbial transformation: 2'(S)-nicotine-1'-N-oxide, which is the most common natural oxidation product of nicotine, was degraded by bacteria abundant on the tobacco leaf surface and in the tobacco field soil. The isolated micro-organisms belong to genus *Arthrobacter*. Degradation pathway was: nicotine-N'-oxide → N'-methylmyosmine (60 %—70 % yield) → 4-oxo-4-(3'-pyridyl)butyric acid, whereas nicotine degraded slowly by a different route: S-nicotine → 6-hydroxynicotine → 6-hydroxy-N'-methylmyosmine. No analogous and homologous oxides tested were degraded by the bacteria. 1'(R)-2'(S)-Nicotine-1'-N-oxide was preferentially degraded, compared to 1'(S)-2'(S)-nicotine-1'-N-oxide.

ZUSAMMENFASSUNG

Chemische Umwandlung: Bei der Oxidation des Nikotins an der Luft bei Raumtemperatur wurden folgende Verbindungen isoliert: N'-Methylmyosmin (das für ein aktives intermediäres Abbauprodukt gehalten wird), Cotinin, Nikotin-N'-oxid, Nikotyrin, Myosmin, 3-Pyridylpropylketon, 3-Pyridylmethylketon, Nikotinsäure, Methylamin und Ammoniak. N'-Methylmyosmin wurde dabei durch ^{1}H-NMR zum ersten Male identifiziert. Bei dem Erhitzen von N'-Methylmyosmin wurden neben einer großen Menge polymerisierter harzartiger Substanzen N-Methylnikotinamid und Nikotyrin erhalten. 2'(S)-Nikotin-1'-N-oxid wurde bei Reaktion mit Acetylchlorid oder Acetylanhydrid zu Acetylpseudooxynikotin umgelagert. Diese Umlagerung könnte allgemein von Nutzen für die Synthese von Δ_1-Pyrrolinen oder Δ_1-Piperideinen sein. Bei Verwendung geeigneter Acetylgruppen erwiesen sich diese Substanzen als wirksam hinsichtlich der Verbesserung des Tabakgeschmacks.

Phytochemische Umwandlung: Als Ergebnis der Untersuchung der Umwandlung der Alkaloide in der Tabakpflanze durch Messung ihres Rotationsvermögens wird angenommen, daß Nikotin sich in lebendem Gewebe in der S-Form bildet. Das in den Blättern entstehende Nornikotin bildet sich aus S-Nikotin; jenes Nornikotin jedoch, das in den Wurzeln entsteht, bildet sich in der razemischen Form, wodurch sich ein Stoffwechselweg anzeigt, der von dem in den Blättern verschieden ist. Alkaloide mit sekundären Amingruppen wie Anabasin und Anatabin liegen in der razemischen Form vor. Aus Cherry-Red-Tabak wurde 1-(1'-2'(S)-Nornikotin)-1-β-D-fructofuranosid (Schmelzpunkt: 66–68 °C), ein Umsetzungsprodukt von Nornikotin, zum ersten Male isoliert. Die Struktur konnte physikochemisch und schließlich durch Synthese bestätigt werden. Der Gehalt an dieser Verbindung nahm während der Röhrentrocknung deutlich zu, insbesondere am Ende des Trocknungsprozesses beim Austrocknen des Blattgutes, was auf die Entstehung in einem nichtenzymatischen Prozeß schließen läßt.

Mikrobielle Umwandlung: 2'(S)-Nikotin-1'-N-oxid, das häufigste natürliche Oxidationsprodukt des Nikotins, wurde durch Bakterien abgebaut, die auf der Oberfläche des Tabakblattes und im Ackerboden des Tabakfeldes reichlich vorkommen. Die isolierten Mikroorganismen gehören zum Genus *Arthrobacter*. Die Abbauroute war folgende: Nikotin-N'-oxid → N'-Methylmyosmin (Ausbeute: 60–70 %) → 4-Oxo-4-(3'-pyridyl)-buttersäure. Nikotin hingegen wurde langsam auf einem anderen Weg abgebaut: S-Nikotin → 6-Hydroxynikotin → 6-Hydroxy-N'-methylmyosmin. Die Bakterien zersetzten keine der untersuchten analogen und homologen Oxide. Im Vergleich zu 1'(S)-2'(S)-Nikotin-1'-N-oxid wurde 1'(R)-2'(S)-Nikotin-1'-N-oxid vorzugsweise abgebaut.

RÉSUMÉ

Transformation chimique: À l'oxidation à l'air de la nicotine à température ambiante, on a isolé les composés suivants: la N'-méthylmyosmine (considérée comme intermédiaire actif de dégradation), la cotinine, le nicotine-N'-oxide, la nicotyrine, la myosmine, la 3-pyridylpropylcétone, la 3-pyridylméthylcétone, l'acide nicotinique, la méthylamine et l'ammoniac. La N'-méthylmyosmine a été identifiée pour la première fois par ^{1}H-NMR. En chauffant la N'-méthylmyosmine, on a obtenu de la N-méthylnicotinamide et de la nicotyrine en plus d'une grande quantité de substances résineuses polymérisées. Par réaction avec le chlorure d'acétyle ou l'anhydride acétique, on a obtenu le réarrangement du 2'(S)-nicotine-1'-N-oxide en pseudo-oxynicotine acétique. Ce réarrangement pourrait être utile en général pour la synthèse des Δ_1-pyrrolines ou Δ_1-pipéridéines. Lors de l'utilisation de groupes acétiques appropriés, ces substances se sont révélées efficaces pour l'amélioration du goût du tabac.

Transformation phytochimique: On a examiné la transformation des alcaloïdes dans la plante du tabac en mesurant leur pouvoir rotatoire. On en a déduit que la nicotine est bio-synthétisée dans la forme S. La nornicotine qui se forme dans les feuilles est synthétisée à partir de la S-nicotine, mais celle qui se forme dans les racines est synthétisée sous la forme racémique, indiquant ainsi un métabolisme différent de celui des feuilles. Des alcaloïdes à fonction amine secondaire comme l'anabasine et l'anatabine ont été trouvés sous

la forme racémique. Un produit de transformation de la nornicotine, le 1-(1'-2'(S)-nornicotine)-1-β-D-fructofuranoside (point de fusion 66—68 °C), a été isolé pour la première fois dans le tabac Cherry Red. La structure en a été confirmée par des méthodes physico-chimiques et finalement par synthèse. La quantité de ce composé a augmenté significativement pendant le «curing», particulièrement au stade du séchage, suggérant ainsi une formation par un procédé non enzymatique.

Transformation microbienne: Le 2'(S)-nicotine-1'-N-oxide, qui est le produit d'oxidation naturel de la nicotine le plus répandu, a été dégradé par des bactéries abondantes à la surface des feuilles de tabac et dans le sol des champs de tabac. Les micro-organismes isolés appartiennent au genre *Arthrobacter.* La dégradation passe par les stades suivants: nicotine-N'-oxide → N'-méthylmyosmine (rendement 60 — 70 %) → acide 4-oxo-4-(3'-pyridyl) butyrique. La dégradation de la nicotine, par contre, est lente et passe par les étapes suivantes: S-nicotine → 6-hydroxynicotine → 6-hydroxy-N'-méthylmyosmine. Aucun des oxides analogues et homologues examinés n'a été dégradé par les bactéries. Le 1'(R)-2'(S)-nicotine-1'-N-oxide a été dégradé de préférence au 1'(S)-2'(S)-nicotine-1'-N-oxide.

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