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EFFECTS OF FLUE-CURING AND AGEING ON THE VOLATILE BASIC CONSTITUENTS OF VIRGINIA TOBACCO*

Dedicated to Professor H. Erdtman on his 75th birthday

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Key Word Index—*Nicotiana tabacum*; Solanaceae; Virginia tobacco; volatile bases; flue-curing and ageing.

Abstract—Flue-curing and ageing of Virginia tobacco reduce the amounts of nicotine and several of its transformation products. In contrast, the concentrations of certain structurally simple pyridines and a few compounds, which are presumably products of sugar–amino acid interactions, are found to increase. Generation of several pyridines and a few other bases during the leaf processing is also observed.

INTRODUCTION

Although the typical tobacco aroma is created on curing (drying) and ageing of the leaf, little is known about the corresponding chemical changes which cause the odour differences. In view of this a study of the volatile flavour fractions derived from Virginia tobacco at selected stages of leaf processing was undertaken and we now report the result obtained for the bases.

RESULTS AND DISCUSSION

As described previously [1], the headspace vapours were collected from six samples representing the green (GN), yellowed (YE), flue-cured (FC), flue-cured and aged (6, 12 and 24 months, 6M, 12M, 24M) stages of the same tobacco using a charcoal-trapping system. The collected material was separated into neutrals, acids and bases, which were examined in detail by capillary column GC–MS. Identification of individual components was accomplished by comparison of MS and, where possible, retention times with those of authentic samples. GC peak areas were normalised on an added internal standard and recalculated on a weight per dry weight of tobacco basis to enable quantitative comparisons within a series of tobacco samples.

Flue-curing and ageing greatly affect the basic fractions and reduce their total amounts by some 50% (Table 1). The concentrations of most major bases decrease, whereas several minor components accumulate or are generated. These effects, are summarized in Table 2.‡ Thirtyfour bases were identified and most of these fall into two major groups according to their probable biogenetic origins: (i) nicotine and its transformation products, (ii) compounds formed by sugar–amino reactions.

Nicotine and its transformation products

Nicotine (**23**) is the major alkaloid of *Nicotiana tabacum*, where it co-occurs with a number of structurally closely related compounds such as nornicotine (**27**) myosmine (**28**), nicotyrine (**30**), and cotinine (**34**). It is well established that nicotine (**23**) is actively metabolised both in the intact growing plant and during the leaf processing. While it is probable that the metabolism in the intact plant, which proceeds via the pathway nicotine (**23**), nornicotine (**27**), myosmine (**28**) and nicotinic acid (**35**) [2–4], is enzyme-assisted, both enzymic and nonenzymic reactions have to be considered during the post-harvest period. Although the elucidation of the course and mechanisms of these reactions has to await the results from proper tracer experiments, it is known that the reduction of the nicotine content during curing and ageing is accompanied by the accumulation of certain other compounds.

Table 1. Weight (g) of basic fraction obtained per kg of dry tobacco

GN	YE	FC	6M	12M	24M
0.51	0.48	0.51	0.49	0.37	0.26*

* This value is somewhat uncertain due to an experimental error.

* Part 39 in the series 'Tobacco Chemistry'. For part 38 see ref. 1.

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‡ Since the amounts of compounds released during the headspace collection period do not represent those actually present in the tobacco and since the observed relative abundance within a fraction is biased by differences in volatility, a quantitative comparison can only be made with respect to a given compound between fractions. Rather than presenting more than one thousand quantitative values, we have choosen to indicate the observed changes for the individual compounds by +, − and = signs in Table 2.

Table 2. Results obtained for the analysis of the basic compounds

	Basic Compound	Detected in	Effect on Concentration*
1	Dimethylamine	GN—24 M	=
2	Pyridine	GN—24 M	+
3	2-Methylpyridine	GN—24 M	+
4	2-Methylpyrazine	GN—24 M	=
5	2,6-Dimethylpyridine	GN—24 M	+
6	3-Methylpyridine	GN—24 M	+
7	4-Methylpyridine	YE—24 M	+
8	2-Ethylpyridine	6 M—24 M	+
9	2,5-Dimethylpyrazine	FC—24 M	+
10	2,6-Dimethylpyrazine	GN—24 M	=
11	2,3-Dimethylpyrazine	GN—24 M	=
12	3-Ethylpyridine	6 M—24 M	+
13	2-Ethyl-6-methylpyrazine	GN—24 M	=
14	2,3,6-Trimethylpyridine	FC—24 M	+
15	2,3,5-Trimethylpyrazine	GN—24 M	+
16	2-Ethyl-3,6-dimethylpyrazine	GN—24 M	=
17	Tetramethylpyrazine	GN—24 M	+
18	3-(1-Propenyl)-pyridine	GN—24 M	–
19	2-Acetylpyridine	12 M—24 M	+
20	3-Cyanopyridine	6 M—24 M	+
21	Methyl nicotinate	GN—24 M	=
22	3-Acetylpyridine	GN—24 M	–
23	Nicotine	GN—24 M	–
24	N-Methylmyosmine†	GN—24 M	–
25	2-Formyl-5-methylpyrrole	YE—24 M	+
26	3,6,6-Trimethyl-5,6-dihydro-7H-2-pyrindin-7-one	FC—24 M	+
27	Nornicotine	GN—24 M	–
28	Myosmine	GN—24 M	–
29	Nicotine-N'-oxide	GN—24 M	=
30	Nicotyrine	GN—24 M	–
31	5,6,7,8-Tetrahydroindolizin-8-one	FC—24 M	+
32	2,3'-Dipyridyl	GN—24 M	=
33	N-Methylnicotinamide	GN—24 M	–
34	Cotinine	GN—24 M	–

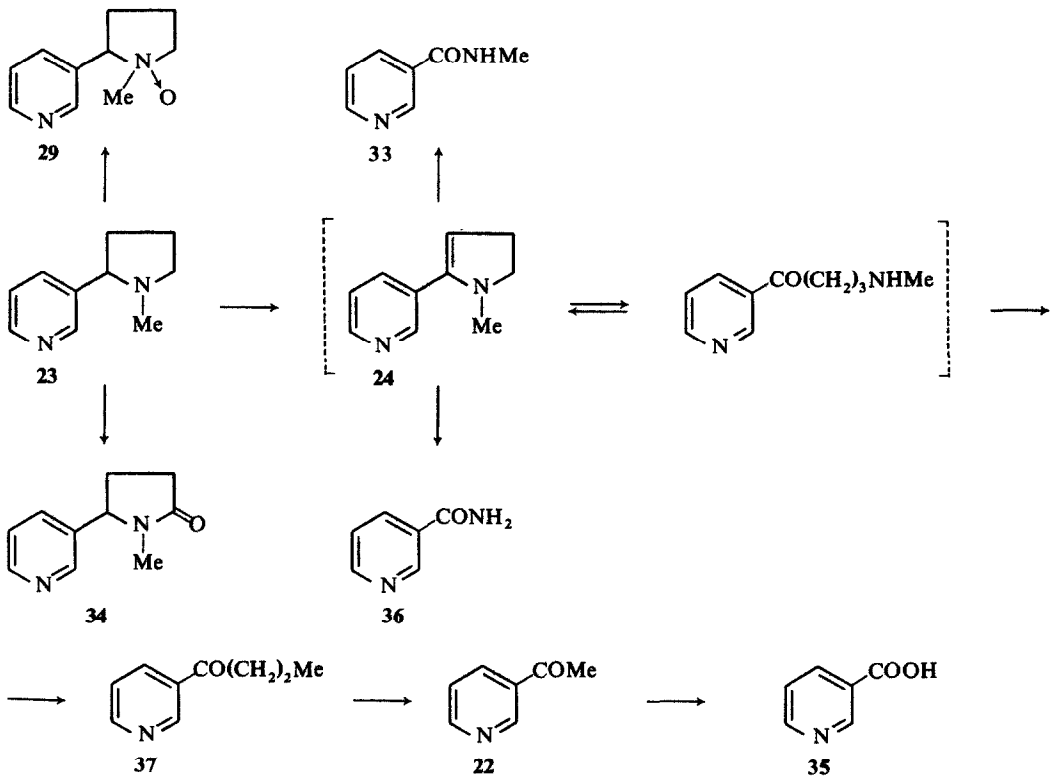
* +/– Means increase/decrease of the concentration of a compound (weight/dry weight of tobacco) on curing and ageing: = means that this concentration remained unchanged.
† Tentative assignment.

Thus, Frankenburg *et al.* [5–9] isolated a series of compounds, all retaining the pyridine ring, from a fermented cigar-filler tobacco, whose nicotine content had undergone a considerable reduction (30–50%) as a result of the post-harvest treatment. Whitout specifying the mechanisms involved, it was suggested that these compounds are formed from nicotine (**23**) as shown in Scheme 1.

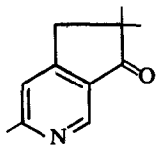
Nicotine (**23**) is degraded to *N*-methylnicotinamide (**33**) and nicotinamide (**36**) via the hypothetical intermediate *N*-methylmyosmine (**24**). The hydrated form of the latter (**24**) is converted to 3-pyridylpropyl ketone (**37**), which is degraded further with the formation of 3-acetylpyridine (**22**) and nicotinic acid (**35**). Nicotine (**23**) may also undergo oxidation to nicotine *N'*-oxide (**29**) and cotinine (**34**).

Support for this degradation pathway was obtained from oxidation reactions carried out *in vitro*. Thus, treatment of nicotine with hydrogen peroxide afforded nicotine *N'*-oxide (**29**) and cotinine (**34**) as the major products, whereas *N*-methylnicotinamide (**33**), nicotinamide (**36**), 3-pyridylpropylketone (**37**), 3-acetylpyridine (**22**) and nicotinic acid (**35**) were minor components. Furthermore, the latter compounds were also obtained when *N*-methylmyosmine (**24**) was reacted with hydrogen peroxide. As expected, nicotine *N'*-oxide (**29**) and cotinine (**34**) were not formed in this case [7,9].

Autoxidation of nicotine (**23**) was found to give nicotine *N'*-oxide (**29**), cotinine (**34**), nicotyrine (**30**), myosmine (**28**), nicotinic acid (**35**), methylamine and ammonia [10], whereas photo-oxygenation of nicotine gave a complex mixture from which nicotine *N'*-oxide (**29**), cotinine (**34**) and nicotyrine (**30**) were isolated [11].



Scheme 1.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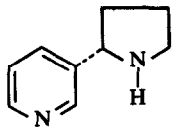
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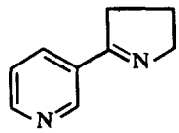
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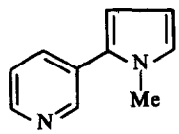
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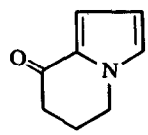
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Our studies demonstrate that nicotine (**23**) as well as a number of other nitrogen-containing compounds are already present in GN tobacco.* Of these, *N*-methylmyosmine (**24**), nornicotine (**27**), myosmine (**28**), nicotyrine (**30**), *N*-methylnicotinamide (**33**) and cotinine (**34**) are fairly abundant. Flue-curing and ageing lead to a reduction in the concentrations of all these compounds, whereas roughly constant levels are observed for nicotine *N'*-oxide (**29**), 3-(1-propenyl)-pyridine (**18**), methyl nicotinate (**21**) and 3-acetylpyridine (**22**).

This result may still be in harmony with the degradation pathway outlined above, if it is assumed that the intermediates are metabolised further on curing and ageing of the Virginia tobacco. This view receives some support from the fact that a few simple pyridine derivatives accumulate during leaf processing. Thus, the concentrations of pyridine (**2**) and 3-methylpyridine (**6**) increase significantly and 3-ethylpyridine (**12**) and 3-cyanopyridine (**20**) are generated on storage of the tobacco.

Compounds formed in sugar–amino acid reactions

Recent examinations have demonstrated that a fair number of furans, pyrroles and pyrazines, presumably generated in Maillard reactions between sugar and aminoacids, are present in tobacco [12]. The formation of these compounds is likely to take place, or to be accelerated, on leaf processing, a view which has been substantiated by our findings for the neutral furans and pyrroles [1].

Eight pyrazines were identified as minor components of the basic fractions from Virginia tobacco. While the concentrations of five of these (**4**, **10**, **11**, **13**, **16**) were retained at virtually constant levels throughout the post-harvest treatment, the relative contents of tri- and tetramethylpyrazine (**15**, **17**) increase and one compound, 2,5-dimethylpyrazine (**9**) is generated on flue-curing.

5,6,7,8-Tetrahydroindolizin-8-one (**31**), previously obtained in model experiments on heating of L-proline with D-glucose [13], is a minor component detected only in the basic fractions from FC and aged tobacco.

A few pyridine derivatives, which are substituted at positions 2 or 4, were present as minor components. Two of these, 4-methyl- and 2,3,6-trimethylpyridine (**7**, **14**) are generated during the flue-curing procedure, whereas 2-ethyl- and 2-acetylpyridine (**8**, **19**) arise during the storage of the tobacco. The concentrations of the remaining two, 2-methyl- and 2,6-dimethylpyridine (**3**, **5**), which were detected in GN tobacco, increase throughout the leaf processing. These compounds, which are not likely to emanate from degradation of nicotine (**23**), are probably products of Maillard-type of reactions [14,15].

Other compounds

3,6,6-Trimethyl-5,6-dihydro-7H-2-pyrindine-7-one (**26**) was only detected in the fractions from FC and aged tobacco, where it occurs in minute quantities. This compound, previously isolated from Burley tobacco, has been postulated to arise from a carotenoid precursor [16].

2,3'-Dipyridyl (**32**), a minor component, whose concentration undergoes virtually no alteration on curing and ageing, may be formed by dehydrogenation of anabasine [5].

EXPERIMENTAL

Experimental data have been given elsewhere [1].

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* It should be pointed out that GN tobacco had been subjected to tissue damage, which may have initiated some of the post-harvest catabolic processes.