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Note

Pyrolysis–gas chromatographic estimation of tobacco alkaloids and neophytadiene*

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Alkaloids in tobacco are normally determined by the steam-distillation procedure of Griffith[1] or the automated procedures of Harvey *et al.*[2] and Davis[3]. The major alkaloid is nicotine and it comprises approximately 95% of the alkaloids in flue-cured tobacco[4]. When the major alkaloid is nornicotine, characterized by genetic instability in some cultivars or breeding lines, the cited procedures result in poor estimates of the amounts of these two important alkaloids. High levels of nornicotine produce an undesirable tobacco product[5], so that a means of quantitating the alkaloid is important for the evaluation of tobacco quality.

The most common method of evaluating the nornicotine content of tobacco involves the initial determination of total alkaloids, as nicotine, followed by steam-distillation of a sub-sample with magnesium oxide[6] to determine nicotine content. The difference between the total-alkaloid value and the result given by the second distillation represents approximately 51% of the nornicotine; from this value, the nornicotine content can be calculated.

The pyrolysis–gas chromatographic (PGC) procedure described here permits qualitative and quantitative evaluation of the alkaloids concerned. The procedure obviates any extraction stage and offers the selectivity and sensitivity of gas chromatography.

EXPERIMENTAL

Samples of tobacco were obtained from breeding lines of variable genetic background as well as from the standard cultivars currently used for commercial production in Canada. The cured tobacco was ground to pass through a 40-mesh U.S. sieve and lyophilized before analysis.

The procedure of Bush[7] was used for sample extraction in preparation for alkaloid analysis by gas chromatography. The internal standard was 6-methylquinoline.

Alkaloids were analysed on a Varian 3700 gas chromatograph equipped with a CDS-111 integrator and autosampler. The operating parameters were as follows: 6-ft. column packed with a mixed phase of 7% of Carbowax 20M, 3% of polyphenyl

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ether (6-ring) and 2% of potassium hydroxide[8] on Supelcoport (80–100 mesh); the flow-rates for the carrier gas (helium), hydrogen and air were 30, 30 and 300 ml/min, respectively; temperatures for the injector and detector were 220° and 260°, respectively, and the column temperature was programmed from 180° to 200° at 1°/min.

Pyrolysis studies were conducted with a Victoreen pyrolyzer custom-fitted to a Varian 1200 gas chromatograph and an Infotronics CRS-208 integrator. The mixed-phase column as described above was 3 ft. in length. Gas flow-rates were as above, except for that of air, which was reduced to 270 ml/min. The pyrolysis temperature was varied as required in the initial studies, but was maintained at 250° for the alkaloid determinations.

Standard alkaloids were either redistilled under vacuum, as for nicotine, or isolated from a sample of tobacco (cultivar Delhi 34 Cherry Red) in which nornicotine was the predominant alkaloid. Separation was accomplished by collecting the appropriate peaks from the Varian 3700 using thermal-conductivity detectors.

All chemical standards were checked for purity by thin-layer chromatography using co-chromatography with authentic standards in two solvent systems[9,10] and by UV spectral analysis. Neophytadiene was identified tentatively by co-chromatography with a standard.

Standard tobaccos and samples were pyrolyzed in a sequence involving approximately 10 standards reasonably spaced among the samples. The areas obtained for the standards were subjected to linear regression analysis to establish a regression formula, which was then used to estimate values for the two alkaloids from the samples.

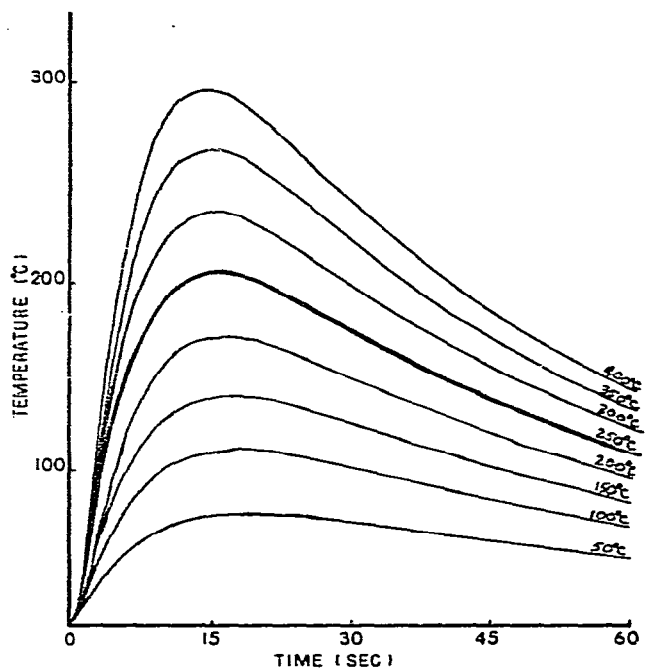

Fig. 1. Temperature attained within the pyrolysis chamber to which the sample was exposed. Pyrolysis at 250° was repeated four times to indicate temperature-profile reproducibility.

TABLE I

RELEASE OF SPECIFIC ALKALOID COMPONENTS AND NEOPHYTADIENE DURING PYROLYSIS–GAS CHROMATOGRAPHY OF A TOBACCO SAMPLE

Amounts (each the average of four determinations) are expressed as peak area per mg of tobacco pyrolysed.

Component	*Pyrolysis temperature, °C*					
	100	*150*	*200*	*250*	*300*	*350*
Nicotine	61,552	74,250	81,124	84,228	84,869	50,698
Nornicotine	—	59,757	69,941	75,588	76,239	72,731
Neophytadiene	24,787	25,341	26,470	26,795	16,154	2430

RESULTS AND DISCUSSION

The Victoreen pyrolyzer reproduced constant pyrolysis-temperature profiles in the sample chamber (Fig. 1). The temperature trace at 250° was repeated four times. The temperature profiles at 50° to 400° are included to illustrate the conditions within the sample chamber to which the sample was exposed. Maximum temperatures were attained within 15 to 18 sec except at settings below 150°.

Nicotine was relatively volatile and readily released by pyrolysis, even at 100° (Table I). Nornicotine, being less volatile, was not noticeably degraded at temperatures up to 350°, but, in multiple trials, it showed maximum release by pyrolysis at

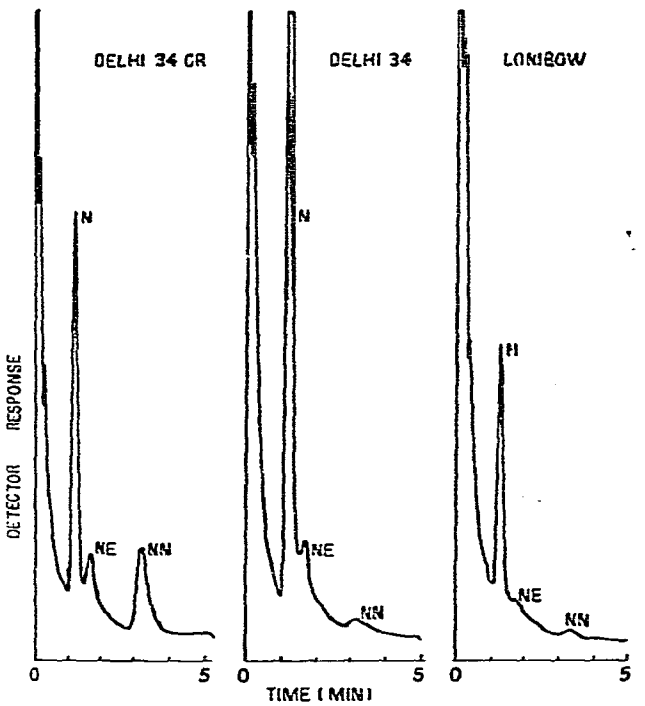

Fig. 2. Qualitative comparison of PGC investigation of three tobacco cultivars with different major alkaloid components; *ca.* 1 mg of tobacco tissue was used to produce each pyrogram. N, nicotine; NE, neophytadiene; NN, nornicotine.

300°. Thus, a pyrolysis temperature of 300° would have been suitable for the determination of both alkaloids; however, 250° was selected, owing to the release of neophytadiene. Neophytadiene, a constituent associated with the carcinogenicity of tobacco and a measure of the terpenoid group of compounds[11], was also readily volatilized at pyrolysis temperatures up to 250°; it was not quantitated, but was identified. PGC provided a potentially rapid means of monitoring neophytadiene change in tobaccos.

The qualitative differences in the PGC responses of three cultivars varying greatly in their alkaloid content could be readily observed (Fig. 2). The nornicotine content in Delhi 34 CR appeared less than the nicotine because of its lower volatility and relative detector response; Delhi 34 CR was a high alkaloid (primarily nornicotine) cultivar. Delhi 34 was representative of a commercial flue-cured cultivar, and Lonibow was typically a very low alkaloid cultivar. For rapid screening of genetic material and breeding lines, even the qualitative aspect could be sufficient for detecting specific tobaccos containing high levels of nornicotine.

The repeatability of the results of pyrolysis of tobacco was determined by comparing the area responses from ten samples. The standard errors for the areas obtained for nicotine and nornicotine were $\pm$ 1.26 and $\pm$ 3.67%, respectively. The sample contained, per gram, 5.08 mg of nicotine and 16.79 mg of nornicotine, determined by gas chromatography (GC). For a standard tobacco containing the normal

TABLE II

COMPARISON OF NICOTINE AND NORNICOTINE ON TWO REPLICATES

Values (each the average of duplicate determinations) are expressed as mg of alkaloid per gram of tobacco.

Treatment No.	*Nicotine*		*Nornicotine*		
	GC	*PGC*	*GC*	*PGC*	*Steam-distillation procedure*
Replicate No. 1					
1	5.48	7.32	9.94	9.64	16.3
2	20.71	26.15	0.24	1.05	2.4
3	23.73	29.03	0.35	0.15	2.0
4	25.14	31.73	0.32	0.15	3.9
5	27.38	30.02	0.33	1.12	7.3
6	33.01	35.26	0.74	0.98	6.7
7	20.47	25.80	0.67	0.23	5.9
8	13.46	16.70	0.29	0.01	3.5
9	1.86	0.36	0.46	0.83	2.6
10	3.84	3.75	8.40	6.57	12.2
Replicate No. 2					
1	4.72	6.70	8.37	8.90	13.5
2	44.59	40.20	0.50	0.50	1.6
3	32.53	31.00	0.22	0.24	3.3
4	27.33	19.15	0.73	1.30	1.2
5	27.27	26.03	1.45	1.60	5.1
6	34.67	37.41	0.30	0.30	2.8
7	28.63	22.11	0.42	1.20	3.3
8	23.41	24.29	0.17	0.50	2.7
9	8.23	5.02	0.08	0.10	1.6
10	4.25	3.60	10.41	12.00	21.2

proportions of alkaloids, a similar error was obtained for nicotine, but that for nornicotine was *ca.* 5%. The increase in variability when estimating nornicotine was due to its low concentration in normal tobacco, which made it more difficult to obtain a good estimate.

Results for the two alkaloids in tobaccos analyzed by either solvent extraction with subsequent GC or by PGC showed remarkable correspondence (Table II). Samples characteristically high in nornicotine were readily detected by PGC (treatments 1 and 10). Similarly, samples characteristically high in nornicotine and low in nicotine were readily observed. Treatment 9 was characterized by a low content of total alkaloids, again a property readily detected by PGC.

Correlation coefficients between nicotine and nornicotine, as determined by GC and PGC were 0.948 and 0.983, respectively (18 degrees of freedom); the association in each was highly significant.

The steam-distillation procedure for nornicotine tended to over-estimatd the content of that alkaloid. Results by the PGC procedure agreed with those of GC; it could therefore be assumed that PGC was a better method for estimating nornicotine than was the steam-distillation procedure.

PGC offers a more accurate estimate of nicotine and nornicotine in tobacco than does steam distillation, and, unlike the latter procedure, does not over-estimate nornicotine. Thus, PGC offers a reliable and rapid means of estimating nornicotine in tobacco without extraction.

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