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STEAM VOLATILE AMINES OF LATAKIA TOBACCO LEAF*

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Abstract—Ground Latakia tobacco leaf was steam distilled from a strongly alkaline solution into hydrochloric acid. The free amines were converted to their corresponding trifluoracetamides on an ion exchange column and the separated derivatives identified on five capillary columns by comparison with authentic compounds.

INTRODUCTION

IN RECENT years work on tobacco bases has been confined mainly to studies on nicotine and other alkaloids. Wider investigations into the volatile bases of tobacco and tobacco smoke have, however, been carried out by such workers as Neurath *et al.* who, by the combined use of column and paper chromatography, succeeded in identifying seventeen volatile amines from tobacco[1] of which nine were identified for the first time, and twenty-six amines from tobacco smoke[2] of which fifteen were identified for the first time. Some studies on primary and secondary amines in tobacco smoke were carried out by Pailer *et al.*[3] who separated, with the aid of gas chromatography and mass spectrometry, sixty-five amines including a number of aromatic amines which they claimed had not been previously detected.

The complexity of Latakia tobacco, the curing process for which has been previously described,[4] demands the use of capillary columns for gas chromatographic analysis. However, there are certain problems connected with the analysis of free amines by GLC, associated with the serious peak-tailing due to partial adsorption of the amine on the support material. Various solutions have been offered and these include the use of an inert support such as Teflon[5] and the addition of sodium hydroxide to the stationary phase.[6] However, for our purposes it was considered preferable to convert the amines to the corresponding trifluoroacetamides (TFA). These derivatives were chosen not only because peak-tailing is thereby eliminated, but also because they have a lower volatility than the amines from which they are derived.

RESULTS AND DISCUSSION

A quantity of ground Latakia tobacco was steam distilled from alkaline solution into hydrochloric acid. The amine hydrochlorides formed were converted to the trifluoroacetamide derivatives and the mixture was analysed by capillary column GLC.

The mixture was chromatographed on five columns coated with trixylenyl phosphate

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* Part II in the series "Constituents of Certain Tobacco Types".

[1] G. NEURATH, A. KRULL, B. PIRMANN and K. WANDREY, *Beitr. Tabakforsch.* 3, 571 (1966).

[2] G. NEURATH, D. DÜNGER, J. GEWE, W. LÜTTICH and H. WICHERN, *Beitr. Tabakforsch.* 3, 563 (1966).

[3] M. PAILER, W. J. HÜBSCH and H. KUHN, *Fachl. Mitteilg. Österr. Tabakregie* 7, 109 (1967).

[4] W. J. IRVINE and M. J. SAXBY, *Phytochem.* 7, 277 (1968).

[5] C. LANDAULT and G. GUICHAN, *J. Chromatogr.* 9, 133 (1961).

[6] J. F. O'DONNELL and C. K. MANN, *Anal. Chem.* 36, 2097 (1964).

(TXP), polyphenyl ether OS124 (PPE), *m*-bis-(*m*-phenoxyphenoxy)benzene + Apiezon L (MBMA), silicone oil (SE-30), and diethylene glycol succinate (DEGS). The last-named column was a support-coated open-tubular column while the remainder were surface-coated capillary columns. Peaks were identified by comparison of the relative retention times of unknown peaks with those of known standards.

The MBMA stationary phase separated the mixture into fifty-eight component peaks, of which thirty-nine corresponded by retention time to forty-nine known amine derivatives. A series of other compounds were also injected and found to be absent. These were the TFA derivatives of *p*-ethylaniline (relative retention 1·35), allylamine (0·23), *N*-(2-methyl)piperidine (0·82), *N*-butylisopropylamine (0·49), pyrrole (0·23 and 1·35), *m*-toluidine (1·18), 2-aminopentane (0·42), 3-aminopentane (0·35), *N*-ethyl-*n*-hexylamine (0·98), *N*-ethyl-*n*-butylamine (0·58), 2,6-diaminotoluene (0·97), *N*-(3-methyl)pyrrolidine (0·78).

By use of the PPE column thirty-nine peaks were again identified corresponding to fifty-three amines. The following compounds were not found: pyrrolidine (0·81), diallylamine (0·50), di-*n*-amylamine (1·10), *sec*-butylamine (0·25), di-isopropylamine (0·35), *N*-methyl-*n*-propylamine (0·35), *p*-ethylaniline (1·65), pyrrole (0·80), 2-aminopentane (0·41), 3-aminopentane (0·41), *N*-ethyl-*n*-butylamine (0·64).

With a 30 m column coated with SE-30 thirty-seven amines were tentatively identified in the mixture. Compounds found to be absent were: di-isopropylamine (0·43), di-isobutylamine (0·83), di-isoamylamine (1·19), *N*-(3-methyl)piperidine (0·79), *p*-toluidine (1·10) and *N*-methyl-*n*-amylamine (0·74).

The DEGS column was of the support-coated open-tubular type and 15 m in length. Thirty-six peaks were identified on this stationary phase; the only two compounds which were absent from the mixture were pyrrolidine (0·67) and 1-phenethylamine (1·83).

On the final column, coated with TXP, all compounds for which peaks had existed on previous columns were again found in the mixture and since no negative results were obtained no further columns were used.

Thirty-six primary and secondary aliphatic and aromatic amines have been identified on five stationary phases, although examination of Table 1 indicates that despite the use of highly efficient columns, a number of compounds still give coincident peaks. Thus the TFA derivatives of *n*-propylamine and *N*-methylisopropylamine are unresolved on MBMA and similarly the derivatives of *o*-ethylaniline and *n*-octylamine both possess the identical relative retention time of 1·17 on the same column. Unfortunately the pair of derivatives from 2,3-dimethylaniline and 2-phenethylamine not only possess the same retention time on MBMA but are also coincident on SE-30. The amides of 3,4-dimethylaniline and 2,4,6-trimethylaniline are coincident on PPE but are well separated on the other columns. The column coated with DEGS fails to separate the pair derived from 2-phenethylamine and 2,4,6-trimethylaniline. The TXP column fails to separate the derivatives of aniline and *o*-ethylaniline.

Generally the aromatic amines were present in much smaller amounts than the aliphatic amines, which may account for their not being found by other workers; however, 2-phenethylamine always gave rise to a large peak.

Of the heterocyclic amines, only pyrroline and piperidine were identified. Both pyrrole and pyrrolidine were proved to be absent even though the latter has been identified as a component of Virginia and Burley tobacco,[1] and both compounds have been previously found in tobacco-smoke condensate.[7,2,3] Pyrrole is unique in that the derivative produces two peaks when chromatographed because trifluoracetylation can take place in two positions.

[7] R. L. STEDMAN, *Chem. Rev.* **68**, 153 (1968).

Only nine of the thirty-six compounds listed in Table 1 had been previously identified in tobacco. Twenty-six of the amines had been identified before as components of tobacco-smoke condensate.

TABLE 1. AMINES DETECTED IN LATAKIA TOBACCO LEAF

Compound (TFA derivative)	MBMA	PPE	SE-30	DEGS	TXP
Methylamine[a,b]	0·09	0·11	0·07	0·03	0·15
Ethylamine[a,b]	0·12	0·13	0·12	0·04	0·41
*Iso*Propylamine[b]	0·12	0·11	0·23	0·27	0·33
n-Propylamine[b]	0·22	0·23	0·26	0·61	0·53
*iso*Butylamine[b]	0·29	0·27	0·32	0·65	0·58
n-Butylamine[a,b]	0·41	0·44	0·43	0·81	0·69
t-Butylamine	0·09	0·09	0·16	0·19	0·23
*iso*Amylamine[a,b]	0·54	0·65	0·52	0·90	0·79
n-Amylamine[b]	0·63	0·74	0·63	1·00	0·85
n-Hexylamine[b]	0·79	0·97	0·82	1·17	1·00
n-Heptylamine	1·00	1·13	1·00	1·41	1·18
n-Octylamine	1·17	1·17	1·18	1·56	1·42
Dimethylamine[a,b]	0·14	0·13	0·13	0·09	0·15
Diethylamine[b]	0·24	0·23	0·26	0·23	0·26
Di-*n*-propylamine	0·58	0·52	0·63	0·42	0·48
Di-*n*-butylamine	0·89	1·00	1·00	0·84	0·78
Di-*sec*-butylamine	0·71	0·78	0·86	0·59	0·61
N-Methylethylamine[a,b]	0·17	0·16	0·23	0·14	0·19
N-Methyl*iso*propylamine	0·22	0·23	0·29	0·16	0·25
N-Methyl-*n*-butylamine	0·48	0·52	0·52	0·39	0·45
N-Methyl*iso*butylamine	0·37	0·40	0·43	0·29	0·38
N-Methyl*iso*amylamine	0·58	0·62	0·65	0·50	0·52
N-*n*-Propyl*iso*propylamine[b]	0·45	0·23	0·56	0·35	0·92
Pyrroline[b]	0·65	0·69	0·52	0·66	0·56
Piperidine[a,b]	0·74	0·81	0·65	0·64	0·65
Aniline[b]	1·02	1·20	0·91	1·75	1·43
o-Toluidine[b]	1·11	1·30	1·13	1·60	1·35
o-Ethylaniline[b]	1·17	1·40	1·09	1·70	1·43
2,5-Dimethylaniline[b]	1·22	1·51	1·13	1·82	1·56
2,6-Dimethylaniline[b]	1·26	1·53	1·09	1·91	1·74
2,3-Dimethylaniline[b]	1·34	1·65	1·20	1·94	1·35
3,4-Dimethylaniline[b]	1·43	1·60	1·29	2·06	1·51
2,4,6-Trimethylaniline[b]	1·45	1·60	1·13	2·01	2·06
2-Phenethylamine[a,b]	1·34	1·69	1·20	2·01	2·04
m-Anisidine[b]	1·47	1·80	1·29	2·26	2·16
1,2,5,6-Tetrahydropyridine[a,b]	0·73	0·84	0·66	0·74	0·64

Retention times relative to the figures underlined.

[a] Amines previously identified in tobacco.

[b] Amines previously identified in tobacco smoke.

Certain amines which were found to be absent in Latakia have been previously reported by other investigators to be present as components of tobacco or tobacco-smoke condensate. Pyrrolidine and pyrrole have already been mentioned. Of the remainder, only *N*-methyl-*n*-propylamine had been found before in tobacco.[1] Allylamine, *p*-ethylaniline, *m*-toluidine and *sec*-butylamine have been reported elsewhere as components of tobacco-smoke condensate.[3]

A preliminary investigation on the amines of Virginia tobacco by the same procedure suggests that many of the amines listed in Table 1 are either absent or present in only very small concentrations. Although only a few amines have been reported in wood-smoke condensate[8] there can be little doubt that a large number of the reported amines are derived from the curing process, rather than from the leaf.

EXPERIMENTAL

Distillation

2 kg of ground Latakia tobacco were steam distilled with 8 l. 5 N NaOH. The distillate was collected in a receiver containing 500 ml 3 N HCl. After 5 l. had been collected the distillation was discontinued and the distillate extracted with CH_2Cl_2 to remove any oily material. Water was then removed from the distillate on a rotary evaporator when 40 g of amine hydrochlorides were obtained, a large proportion of which consisted of ammonium chloride and nicotine hydrochloride.

Preparation of Derivatives

The free amines were liberated from the hydrochlorides with 5 N NaOH and extracted into ether.[3] After drying, the ethereal solution was passed down a column of Zeo-Carb 226 (SRC 45) ion-exchange resin on which the amines were adsorbed. The primary and secondary amines were then converted to their corresponding trifluoroacetamides by addition of trifluoroacetic anhydride to the column. After the reaction had ceased the derivatives were washed from the column with ether and excess trifluoroacetic anhydride removed by washing with $NaHCO_3$ solution. The extract was dried and the ether removed by evaporation.

Identification of Compounds

The majority of amines used in this investigation were purchased commercially. The remainder, all secondary amines, were synthesized in the laboratory. The methyl substituted secondary amines were prepared by reacting benzaldehyde with the appropriate primary amine and methylating the product with methyl iodide in a pressure bomb.[9] The following amines were prepared by this method: *N*-methylethylamine, b.p. 34–35°, *N*-methyl*iso*propylamine, b.p. 47°; *N*-methyl-*n*-propylamine, b.p. 62–64°; *N*-methyl-*iso*butylamine, b.p. 90°; *N*-methyl-*n*-propylamine, b.p. 88–90°; *N*-methyl-*iso*amylamine, b.p. 106–107°; *N*-methyl-*n*-amylamine, 116–118°.

Gas Chromatography

All work was carried out on a Perkin-Elmer F.11 gas chromatograph fitted with a flame ionization detector. All columns were of stainless steel and were of the following types. (i) Open-tubular column (50 m × 0·25 mm, i.d.) coated with the mixed phase, *m*-bis(*m*-phenoxyphenoxy)benzene + Apiezon L (4:1); (ii) open-tubular column (50 m × 0·25 mm, i.d.) coated with polyphenyl ether OS124; (iii) open-tubular column (30 m × 0·25 mm, i.d.) coated with silicone oil SE-30; (iv) support-coated open-tubular column (15 m × 0·5 mm, i.d.) containing diethylene glycol succinate; and (v) open-tubular column (50 m × 0·25 mm, i.d.) coated with trixylenyl phosphate. A carrier gas pressure of 20 p.s.i.g. was used for all open-tubular columns, whereas a pressure of 10 p.s.i.g. was employed for the support-coated column. In all experiments an injection block temperature of 230° was employed. All columns except the one coated with TXP were programmed from 50–180° at 3°/min, the latter column being programmed from 100–200° at 5°/min.

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[8] N. I. NIKITIN, *The Chemistry of Cellulose and Wood*, translated by J. SCHMORAK, Daniel Davey, New York (1966).

[9] J. J. LUCIER, A. D. HARRIS and P. S. KOROSEC, *Org. Syn.* **44**, 72 (1964).