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Note

Study of nitrogen-containing compounds in cigarette smoke by gas chromatography–mass spectrometry

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Nitrogen-containing compounds represent 30% of the substances identified in tobacco smoke[1,2], which may be an important factor when their effects in bioassays are considered. In one fraction (fraction II), previously isolated[3] by means of the Craig counter-current distribution technique, nitrogen-containing compounds were found and when applied to mouse skin it showed a clear tumour-promoting activity.

Therefore it appeared of interest to study this basic tar fraction, which was isolated in our laboratory by a more classical procedure. A non-filter, flue-cured Virginia tobacco cigarette was compared with an air-cured black tobacco cigarette. Gas chromatography–mass spectrometry (GC–MS) was utilized for the investigation and 59 compounds were found, ten of which were identified for the first time.

EXPERIMENTAL

Preparation of the fraction

The smoking machine utilized to obtain the fraction was a 30-channel Borgwaldt machine, supplied with a jet impaction trap to collect the smoke condensate. Cigarette smoking was performed according to the international CORESTA standards, *i.e.*, the machine drew one puff of 2 sec duration per minute, with a puff volume of 35 ml. The butt length was 23 mm.

A 100-mg amount of collected tar was dissolved in 25 ml of chloroform, 25 ml of 2 *N* sulphuric acid were added and the solution was shaken vigorously. The two phases were separated, the chloroform layer extracted with 25 ml of 2 *N* sulphuric acid and the aqueous layer was adjusted to pH 11 by adding 3 *N* sodium hydroxide solution. The free bases thus obtained were extracted with 75 ml of chloroform. The operation was repeated three times. The combined chloroform extract (225 ml) was concentrated to 1 ml under a water pump vacuum at 40°C.

The operation must be performed quickly to avoid the decomposition of some compounds, such as β-nicotyrine, which are not stable in acidic media.

With this method, quantitative extractions of numerous bases (including the main tar alkaloids) are possible. However, other bases, such as pyrazines, or neutral, water-soluble compounds cannot be extracted quantitatively, amphoteric nitrogen-containing compounds, such as hydroxypyridines, are removed.

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Gas chromatography–mass spectrometry

A Ribermag R10-10B GC–MS instrument equipped with a SIDAR 3A data system and a 65 m × 0.3 mm I.D. glass capillary column, coated with Carbowax 20M, was utilized.

The gas chromatograph was operated under a pressure of 0.6 bar of helium with a bypass of 15 ml/min. The temperature was kept at 90°C for 9 min and then programmed to 220°C at the rate of 4°C/min.

Retention times and mass spectra were compared with those of commercially available compounds; those which were not available were synthesized by conventional methods.

RESULTS

Figs. 1 and 2 show the chromatograms of the two samples and Table I lists the identified compounds and the identification procedures (GC–MS indicates that the retention time and the mass spectrum were obtained; MS means that the compound was not available and that identification was established on the basis of the mass spectrum only).

Compounds in the fraction are mostly alkaloids. Many pyridine, pyrazine and quinoline derivatives were also found. Most of them were already known to be

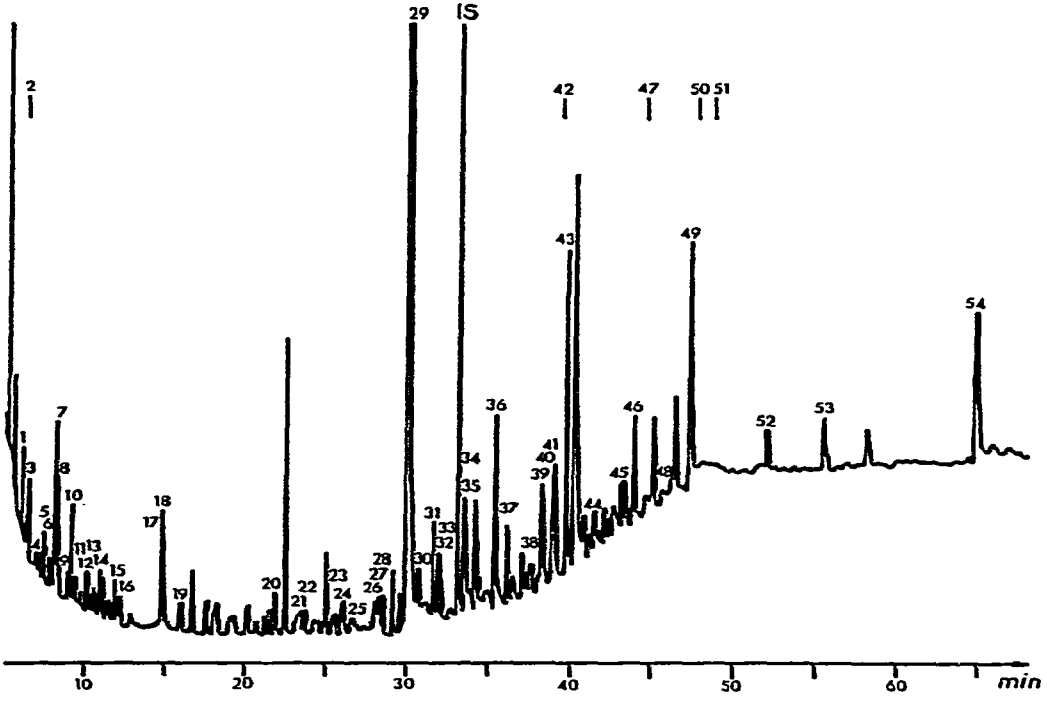

Fig. 1. Chromatogram of Virginia tobacco cigarette fraction obtained on a 65-m Carbowax 20M column. Internal standard (IS): N-methylpyrrole. For peak identification see Table I.

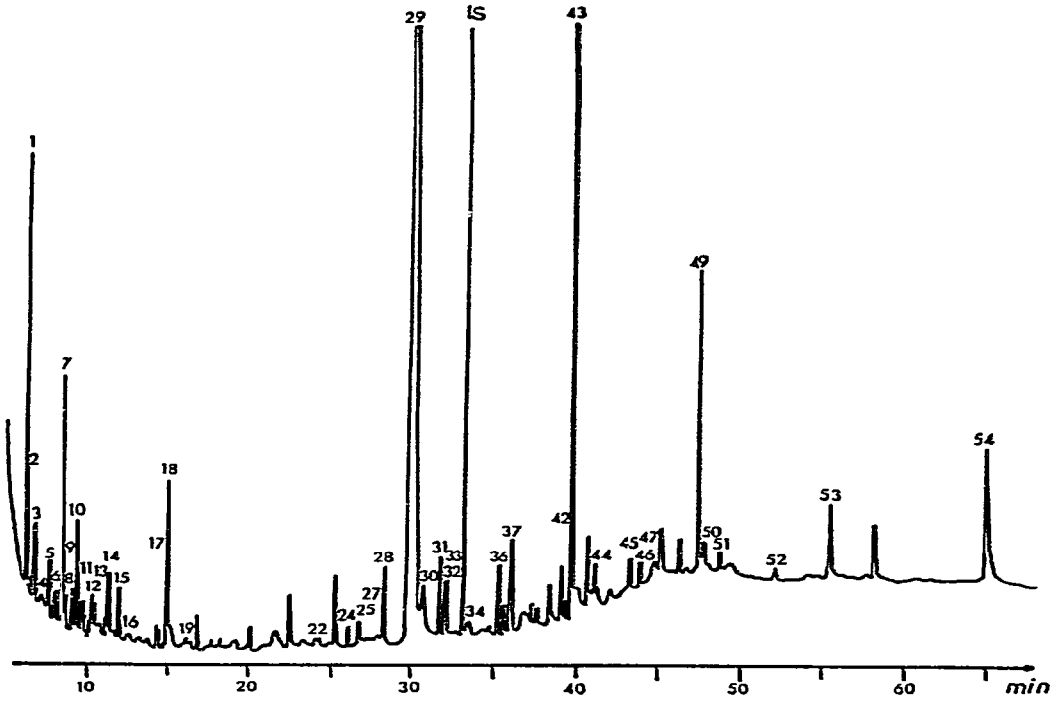

Fig. 2. Chromatogram of dark air-cured tobacco cigarette fraction obtained on a 65-m Carbowax 20M column. Internal standard (IS): N-methylpyrrole. For peak identification see Table I.

constituents of cigarette smoke[1], but ten minor components had not previously been mentioned in the literature (Table II).

Among the latter, two aliphatic amides and three imidazole derivatives were found. Of these two classes of compounds, about twenty were shown to be present in tobacco smoke by Schumacher *et al.*[7], who investigated the water-soluble fraction. On the other hand, no pyridine amino derivatives have previously been reported in cigarette smoke. In addition, we identified 4,4′-bipyridyl. In addition to 2,3′-bipyridyl, identified in tobacco and smoke, 2,2′-bipyridyl[10] and 3,3′-bipyridyl[7] have previously been identified as smoke components.

The qualitative differences between the two cigarettes affect only the minor components (Figs. 1 and 2 and Table I). Imidazole derivatives and several amides were missing in the black tobacco tar fraction. On the other hand, indole derivatives and pyrazine were present in this sample only. None of these compounds could be quantitatively extracted so that it is impossible to state whether they are actually missing from the tar, or this fraction only (indole compounds, for instance, are partly destroyed by the fractionating procedure).

The presence of 4,4′-bipyridyl in black tobacco tar and of 5-methyl-2,3′-bipyridyl in flue-cured Virginia tobacco tar could characterize these two brands. It is difficult to explain the origin of these compounds. Neither of them had previously been found in tobacco, which leads us to believe that they are produced during the pyrolysis.

TABLE I

NITROGEN-CONTAINING COMPOUNDS IDENTIFIED IN CIGARETTE SMOKE

D = dark air-cured tobacco cigarette; V = Virginia tobacco cigarette.

Peak number (Fig. 1)	*Compound*	*Sample where found*	*Identification confirmation*	*Ref. to previous identification in smoke*
1	Pyridine	D, V	GC–MS	2
2	Pyrazine	D	GC–MS	4
3	2-Methylpyridine	D, V	GC–MS	2
4	2,6-Dimethylpyridine	D, V	GC–MS	2
5	Methylpyrazine	D, V	GC–MS	4
6	2-Ethylpyridine	D, V	GC–MS	9
7	3-Methylpyridine	D, V	GC–MS	2
8	4-Methylpyridine	D, V	GC–MS	2
9	2,5-Dimethylpyrazine	D, V	GC–MS	5
10	2,6-Dimethylpyrazine	D, V	GC–MS	4
	2,5-Dimethylpyridine	D, V	GC–MS	5
11	Ethylpyrazine	D, V	MS	6
12	2,4-Dimethylpyridine	D, V	GC–MS	2
13	2,3-Dimethylpyrazine	D, V	GC–MS	5
	2,3-Dimethylpyridine	D, V	GC–MS	2
14	3-Ethylpyridine	D, V	GC–MS	2
15	Trimethylpyrazine	D, V	GC–MS	4
16	N,N-Dimethylacetamide	V	GC–MS	—
	4-Ethylpyridine	D, V	GC–MS	—
17	3-Propylpyridine	D, V	MS	6
18	3,5-Dimethylpyridine	D, V	GC–MS	2
	3-Vinylpyridine	D, V	MS	2
19	3,4-Dimethylpyridine	D, V	GC–MS	2
20	N-Ethylacetamide	V	GC–MS	—
21	1-Methylimidazole	V	GC–MS	7
22	N-Methyl-2-pyrrolidone	D, V	GC–MS	7
23	1,2-Dimethylimidazole	V	GC–MS	—
24	3-Cyanopyridine	D, V	GC–MS	2
25	Acetamide	D, V	GC–MS	8
26	Isobutyramide	V	GC–MS	7
27	Propionamide	D, V	GC–MS	8
28	3-Acetylpyridine	D, V	GC–MS	2
29	Nicotine	D, V	GC–MS	2
30	2-Aminopyridine	D, V	GC–MS	—
	Butyramide	D, V	GC–MS	7
31	Isovaleramide	D, V	GC–MS	7
32	Quinoline	D, V	GC–MS	4
33	2-Methylquinoline	D, V	GC–MS	1
34	Isoquinoline	D, V	GC–MS	4
35	1-Methylisoquinoline	V	GC–MS	—
36	2-Pyrrolidone	D, V	GC–MS	7, 9
37	7-Methylquinoline	D, V	GC–MS	1
38	4-Methylquinoline	V	GC–MS	1
39	2-Methylimidazole	V	GC–MS	—
40	3-Aminopyridine	V	GC–MS	—
41	2-Ethylimidazole	V	GC–MS	—
42	Nicotine-1′-oxide	D	MS	2
43	Myosmine	D, V	GC–MS	2
44	3-Phenylpyridine	D, V	GC–MS	5

(Continued on p. 192)

TABLE I *(continued)*

Peak number (Fig. 1)	*Compound*	*Sample where found*	*Identification confirmation*	*Ref. to previous identification in smoke*
45	β-Nicotyrine	D, V	GC–MS	2
46	Nicotinamide	D, V	GC–MS	2
47	Indole	D	GC–MS	2
48	7-Azaindole	V	GC–MS	5
49	2,3′-Bipyridyl	D, V	GC–MS	2
50	3-Methylindole	D	GC–MS	2
51	4,4′-Bipyridyl	D	GC–MS	—
52	5-Methyl-2,3′-bipyridyl	V	MS	7
53	N-Methylnicotinamide	D, V	GC–MS	7
54	Cotinine	D, V	GC–MS	2

TABLE II

COMPOUNDS NEWLY IDENTIFIED IN CIGARETTE SMOKE

Compound	*Mass spectrum*							
4-Ethylpyridine	107(100)	106(91)	39(24)	51(21)	65(17)	79(14)	92(14)	52(12)
N,N-Dimethylacetamide	44(100)	43(44)	42(22)	45(21)	87(14)	72(6)	41(3)	40(1)
N-Ethylacetamide	43(100)	44(85)	87(30)	42(23)	72(10)	41(5)	45(5)	59(3)
1,2-Dimethylimidazole	42(100)	54(92)	55(45)	40(38)	96(33)	41(26)	95(20)	52(19)
2-Methylimidazole	82(100)	54(86)	42(53)	81(52)	41(49)	40(29)	55(19)	52(13)
2-Ethylimidazole	81(100)	41(99)	95(74)	54(72)	39(44)	42(56)	40(55)	96(47)
1-Methylisoquinoline	143(100)	115(57)	116(18)	128(14)	142(12)	144(12)	51(11)	39(9)
2-Aminopyridine	67(100)	94(66)	41(57)	39(57)	40(34)	38(23)	66(19)	51(17)
3-Aminopyridine	94(100)	41(89)	39(81)	67(64)	40(40)	38(33)	66(20)	37(15)
4,4′-Bipyridyl	156(100)	155(40)	51(40)	50(30)	123(17)	102(16)	76(15)	103(13)

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