CHROM. 5271

FLAME PHOTOMETRIC DETECTION OF VOLATILE SULPHUR COMPOUNDS IN SMOKE FROM VARIOUS TYPES OF CIGARETTES

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(Received January 21st, 1971)

SUMMARY

A gas chromatograph with flame photometric detection was used to detect at least thirty-seven sulphur compounds in the vapour phase of cigarette smoke. A suggestion as to their possible identity is given for many of them. According to the literature only thirteen sulphur compounds so far have been found in smoke. In a comparative study on three types of cigarettes, prepared from flue cured, air cured and sun cured tobacco, respectively, a great similarity was observed, but there were also some striking differences between air cured tobacco on one hand and flue cured and sun cured tobacco on the other. It is possible that on this basis a method may be developed for the determination of the amount of air cured tobacco in an unknown tobacco blend.

Nothing is known about the importance of sulphur compounds with respect to the health of the smoker, though one could speculate on a possible protective effect against alkylating smoke constituents.

INTRODUCTION

The flame photometric detector, based on a principle patented by Draegerwerke[1] and developed further for use in gas chromatography by BRODY AND CHANEY[2], selectively detects phosphorus or sulphur compounds in subnanogram quantities. Several applications of gas chromatographic analysis of volatile sulphur compounds have recently been reported, covering subjects such as beer volatiles[3–5], kraft mill malodors[6], North Sea natural gas[7] and SO_2 in air[8,9]. The present communication is intended to show the suitability and limitations of the detector for the direct gas chromatographic analysis of sulphur compounds in the vapour phase of native cigarette smoke.

EXPERIMENTAL

Gas chromatography

The gas chromatograph used was a Tracor Microtek MT 160 with standard Melpar flame photometric detector (FPD). A glass column, length 5.5 m, I.D. 3 mm, filled with 25% 1,2,3-tris-2-cyanoethoxypropane on Chromosorb W, AW, 60–80 mesh and fitted for on-column injection, was operated at 75°. Carrier gas was nitrogen

(80 ml/min). Flame gases were hydrogen (144 ml/min), oxygen (20 ml/min) and air (40 ml/min). Chromatograms were recorded on a Westronics 1 mV recorder. Volumes of 5.8 ml of the vapour phase of smoke were injected immediately after smoke generation, *i.e.* within 30 sec.

Smoking and sampling

Non-commercial, non-flavoured model cigarettes as well as commercial cigarette brands were studied. Model cigarettes were made (a) from flue cured tobacco, (b) from light air cured tobacco and (c) from sun cured tobacco; for these a Virginia, an American burley and a Greek Orient tobacco were chosen, respectively. Cigarettes (a) and (b) had a weight of 970–1010 mg; cigarettes (c) had to be filled to a weight of 1050–1090 mg in order to obtain a comparable density. Further cigarette make-up was identical. Two commercial cigarette brands from the Dutch market were chosen in addition, one being of the flue cured type, the other of the dark air cured type. All cigarettes were 70 mm long and had no filter. They were conditioned for at least a week over a saturated NH_4NO_3 solution. A rotating BAT smoking machine (Heinr. Borgwaldt, Hamburg, G.F.R.), accommodating 30 cigarettes, was used. One rotation on this machine takes 1 min so as to put each cigarette before the central suction opening 1 × per min for 2 sec; puffs of 35 ml were taken until butts of 23 mm were left.

Smoke samples were drawn from the suction pipe of the smoking machine with a gas tight chrome plated brass syringe through a rubber septum. A Cambridge CM 113A glass fibre filter was inserted upstream in the smoke line to retain the particulate phase of the smoke and to allow the vapour phase to pass on to be analysed. Only the last puff of the cigarettes was studied, *i.e.* the eighth, seventh and tenth puff from the flue cured, light air cured and sun cured tobacco respectively. Each sample was evenly drawn from the smoke stream while six "last puffs" from six cigarettes were moving past the sampling point one after the other in close formation. Two other "last puffs" were used for preflushing the syringe. The remaining ports of the smoking machine were fitted with unlit dummy cigarettes for negative pressure maintenance during the rotation cycle.

This manual sampling method, if carried out carefully, permits highly reproducible results to be obtained[10] without complicated instrumentation. The method has been in regular use with us—with flame ionisation detection—for filter efficiency comparisons and for investigations on the effect of the variety of the tobacco on the smoke composition.

RESULTS AND DISCUSSION

Qualitative aspects

Fig. 1 shows the results as obtained by flame photometric detection, when the non-commercial, non-flavoured model cigarettes were compared. Four measurements were carried out on the flue cured and the sun cured tobacco and three measurements on the light air cured tobacco. The chromatograms in Fig. 1 represent average results.

In literature reports, thirteen sulphur compounds have so far been found to occur in the vapour phase of cigarette smoke[11,12]: H_2S, SO_2, CS, CS_2, COS, HSCN, thiocyanogen, methyl mercaptan, ethyl mercaptan, dimethyl sulphide, dimethyl

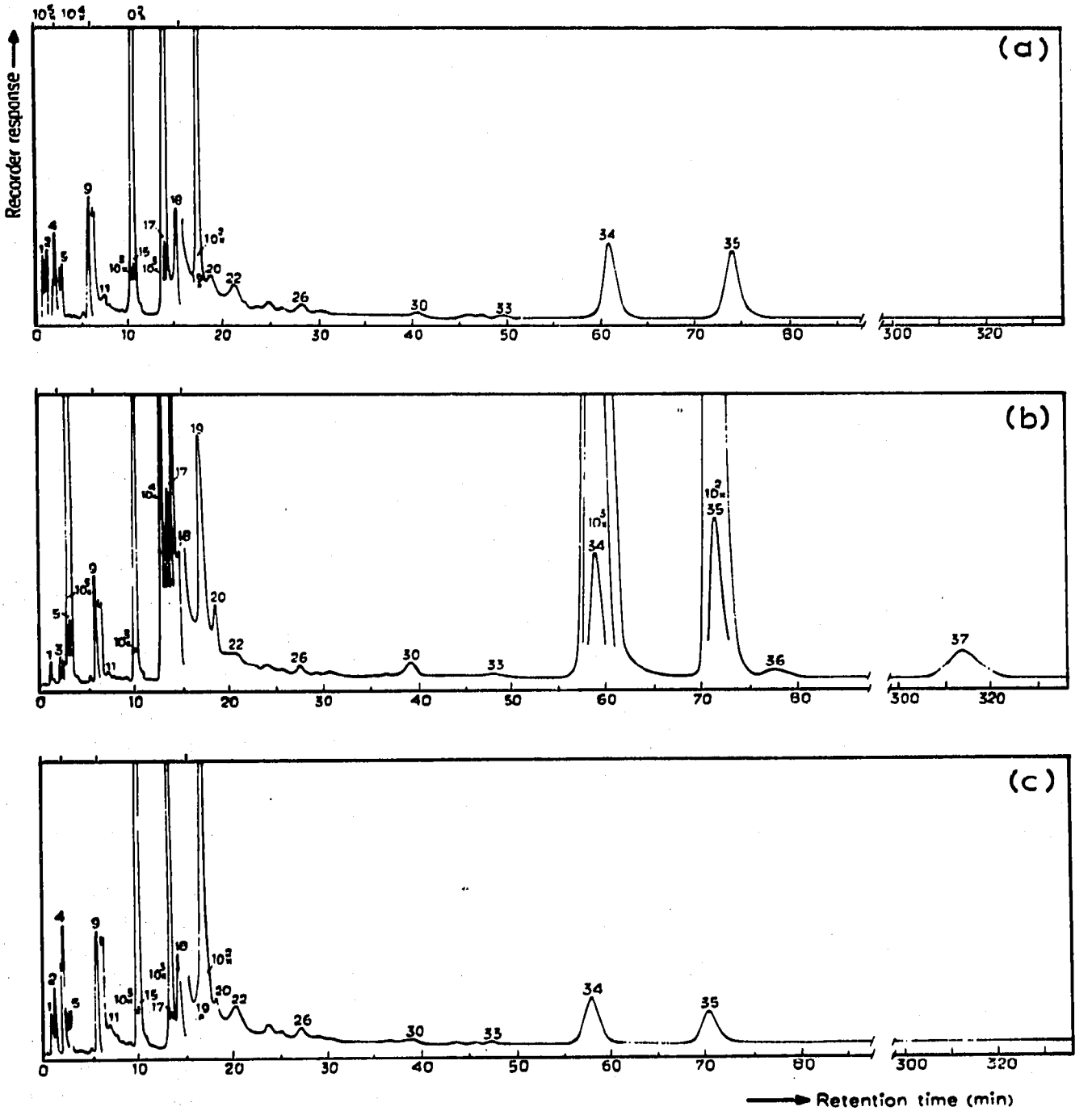

Fig. 1. Gas–liquid chromatograms of the vapour phase of smoke from non-flavoured model cigarettes: (a) flue cured tobacco; (b) light air cured tobacco; (c) sun cured tobacco. Flame photometric detection. Attenuation as indicated × 256. For GLC conditions see EXPERIMENTAL.

disulphide, methyl thionitrite and thiophene. In the chromatograms of Fig. 1, however, thirty-seven peaks can be observed. Erroneous interpretation of even the smallest of these peaks as S compounds is unlikely, in view of the large amounts of non-S compounds that would be required to produce these same peaks. On injection of some major non-S vapour phase constituents (acetaldehyde, acetone, acrolein,

isoprene, benzene, toluene, limonene) in quantities equal to those present in the vapour phase of cigarette smoke, we obtained FPD responses that formed only an extremely small fraction of the actual responses obtained from a vapour phase analysis; in the case of some other, less abundant, non-S compounds, about which some hesitation was initially felt as well, no FPD response was observed at all. We must conclude that the vapour phase of cigarette smoke contains considerably more S compounds than have been reported before.

It was verified separately that all the peaks stem from the burning or heating of the tobacco. When an analysis of the vapour phase of "smoke" from air cured tobacco was repeated, but now with unlit cigarettes, only some very small peaks, emerging early in the chromatogram, were visible; their size was negligible when compared with the peaks in the smoke.

Of the thirteen S compounds, known from the literature, those that were available to us were injected for retention time comparison. Many other reference S compounds were chromatographed as well. Extrapolation resulted in some additional information. The relative adjusted retention values thus obtained (dimethyl disulphide = 1.00; air peak at 0.9 min) are listed in Table I, together with the values for the peaks of Fig. 1. Table I serves to show the scope of the chromatograms and to suggest the possible identity of some of the peaks.

Other compounds than those tested may also be expected to elute in the time span of the chromatogram, *e.g.* the higher straight chain and branched mercaptans, mixed sulphides and disulphides and cyclic S compounds.

Clearly, more evidence is needed for identification of the many peaks found, especially the peaks emerging later in the chromatogram, where many structural isomers are theoretically possible. Even with the sulphur specificity of the detector, the retention time on one packed column cannot be considered to be unambiguous proof of identity for higher-molecular-weight compounds.

Some striking differences are seen in Fig. 1 between the flue cured and sun cured tobacco on one hand and air cured tobacco on the other. Peaks No. 36 and 37 are present in smoke from air cured tobacco only. Four other peaks (No. 5, 17, 34 and 35) in the chromatograms of smoke from air cured tobacco (Fig. 1b) are considerably larger than the corresponding peaks of smoke from flue cured and sun cured tobacco (Figs. 1a and 1c, respectively). Additional smaller differences are present.

It can be seen from Table I that peak 5 could be ethyl or isopropyl mercaptan or dimethyl sulphide or combinations of these; peak 17 could be dimethyl disulphide or hexyl mercaptan or both; peak 34 could be dimethyl trisulphide; peak 36 could be *n*-propylallyl disulphide. No identity could be suggested for peaks 35 and 37; compounds examined but rejected were di-*n*-propyl disulphide (relative adjusted retention 3.93), ethylallyl disulphide (4.40), allyl isothiocyanate and thiocyanate (5.23 and 5.30) and furfuryl mercaptan (8.61). No signal was observed for methional under our experimental conditions.

For the two commercial cigarette brands, one thought to consist mainly of flue cured tobaccos, the other to consist mainly of (dark) air cured tobaccos, chromatograms essentially similar to those of Figs. 1a and 1b were obtained. This permits the conclusion that the differences, found between the smoke from flue cured tobacco and the smoke from air cured tobacco, are not confined to the particular tobacco types used in the model cigarettes and that light air cured and dark air cured tobacco produce smoke with a similar sulphur peak pattern.

TABLE I

RELATIVE ADJUSTED RETENTION (DIMETHYL DISULPHIDE = 1.00) OF SULPHUR COMPOUNDS IN THE VAPOUR PHASE OF CIGARETTE SMOKE AND OF SOME REFERENCE SULPHUR COMPOUNDS, SUGGESTING POSSIBLE IDENTITY

Peak number	*Sulphur compounds in the vapour phase of cigarette smoke*	*Reference compounds*[a]
1		
2		H_2S[b]
3	0.08	CS_2[b] 0.08
4	0.10	Methyl mercaptan[b] 0.10
5	0.16	Ethyl mercaptan[b] 0.14; dimethyl sulphide[b] 0.14; isopropyl mercaptan 0.14
6	0.21	Methyl ethyl sulphide 0.22; SO_2 0.20 + tail
7	0.24	*n*-Propyl mercaptan 0.23; isobutyl mercaptan 0.27
8	0.35	Allyl mercaptan 0.33; methyl *n*-propyl sulphide 0.33; diethyl sulphide 0.34
9	0.39	Isoamyl mercaptan 0.37; *n*-butyl mercaptan 0.38
10	0.43	
11	0.51	Methyl *n*-butyl sulphide 0.50; ethyl *n*-propyl sulphide 0.50; methyl allyl sulphide 0.51
12	0.56	
13	0.61	*n*-Amyl mercaptan 0.62
14	0.66	
15	0.74	Thiophene[b] 0.74; di-*n*-propyl sulphide 0.74
16	0.80	Diisobutyl sulphide 0.81
17	1.00	Dimethyl disulphide[b] 1.00; *n*-hexyl mercaptan 1.02
18	1.10	*n*-Propyl allyl sulphide 1.07; *n*-propyl *n*-butyl sulphide 1.12
19	1.29	
20	1.40	Methyl ethyl disulphide 1.43
21	1.48	Diallyl sulphide 1.47
22	1.58	
23	1.75	Di-*n*-butyl sulphide 1.75
24	1.85	Diethyl disulphide 1.89; 2,4-dimethyl thiophene 1.89
25	1.96	
26	2.12	Methyl *n*-propyl disulphide 2.14
27	2.28	
28	2.37	
29	2.90	
30	3.06	Methyl allyl disulphide 3.02
31	3.40	
32	3.56	
33	3.77	
34	4.65	Dimethyl trisulphide 4.65
35	5.64	
36	6.14	*n*-Propyl allyl disulphide 6.20
37	24.8	

[a] Values for some major non-S compounds of the vapour phase are: isoprene 0.06; acetaldehyde 0.20; methanol, acetone, acrolein, benzene 0.43–0.49; toluene 0.80; limonene 1.03.

[b] Sulphur compounds already known to be present in cigarette smoke[11,12]; CS, COS, HSCN, thiocyanogen and methyl thionitrite were also found before, but were not available to us.

Quantitative aspects

The quantitative interpretation of the differences in peak height needs caution, as the FPD used in the form specific for sulphur responds to about the square of the concentration[2,4,7,9,13], due to the emission by S_2 formed in the flame. These reports were confirmed under the conditions prevailing in our experiments, even though a disulphide was used for calibration. We obtained a nearly linear relation on a log–log scale between peak height and amount of dimethyl disulphide injected, with a slope of 1.9–2.0 over the range from 0.1 ng up to 50 ng of S, injected into the gas chromatograph. From the results of DREWS *et al.*[4] a slope of 1.8 can be calculated for diisopropyl sulphide, from those of GRICE *et al.*[13] a slope of 1.9 was found for thiophene, while the results of BRODY AND CHANEY[2] for parathion show a slope of 2.0 at the highest concentrations and of 1.6 at the lowest. GOODE[7] obtained a slope of 1.8 at high concentrations for several sulphides, thiophenes and a disulphide, and 1.0 at low concentrations.

Above 50 ng of S the slope of the response curve decreases and becomes very small for amounts over 1 μg. At about 10 μg of S we observed peak top reversal, apparently due to detector overloading.

In the linear range good reproducibility was obtained. Seven measurements showed a relative standard deviation of 2.4% for the amount of S.

The absolute detection limit for dimethyl disulphide under the conditions of our experiment was about 1 ng. GRICE *et al.*[13] report a detection limit of 0.2 ng S (0.5 ng thiophene); GOODE[7], with an improved FPD detector, reports a detection limit of 0.02 ng per injection for C_2 and C_4 sulphides, but for diethyl disulphide this value is about 0.1 ng. Since the detection limit of the FID detector for C compounds is of the same order of magnitude and since the FID detector is capable of responding to sulphur compounds in a manner which does not differ greatly from that for other groups of compounds[14], we conclude that the main advantage of the FPD detector over the FID detector is not a considerably higher sensitivity to S compounds, but rather an extreme insensitivity to non-S compounds.

The concentration ratios (air cured/flue cured) have been listed in Table II for the peaks with major height difference of Figs. 1a and 1b; they were calculated from the respective peak heights, using the 1.9-th power root as a general approximation. Apart from peaks 36 and 37 which are present in smoke from air cured tobacco only, peaks 5, 17, 34 and 35 are present in smoke from air cured tobacco in concentrations 3.8 ×; 5.5 ×; 15.3 × and 5.4 × as high, respectively, as in smoke from flue cured tobacco. Peak No. 17, if consisting of dimethyl disulphide only, corresponds to about 965 ng in the last puff from the air cured cigarette and to about 180 ng in the last puff from the flue cured cigarette.

These results are remarkable, since in earlier experiments[10], using a conventional FID detector, we obtained chromatograms for the vapour phases of air cured and flue cured tobacco which were almost indistinguishable qualitatively and only slightly different in quantitative respect. (A similar statement has been given by GROB[15]). Furthermore, we observed that the few concentration ratios (air cured/flue cured) which deviated notably from unity among the 180 peaks observed, were in the reverse direction, *i.e.* the higher peaks were consistently found in the smoke from the flue cured tobacco with one peak ratio of 0.2 (air cured/flue cured) as the extreme value. An exception to this rule had already been noticed earlier in the FID chro-

TABLE II

CONCENTRATION RATIOS (AIR CURED/FLUE CURED) FOR THE PEAKS WITH MAJOR HEIGHT DIFFERENCES IN Figs. 1a AND 1b, CALCULATED FROM THEIR HEIGHTS

Peak number	*Peak height (h) in mm and attenuation factor*		$\frac{h_{air\ cured}}{h_{flue\ cured}}$	$\frac{Concentration_{air\ cured}{}^{a}}{Concentration_{flue\ cured}}$
	Flue cured	*Air cured*		
2	60 ($10^6 \times 256$)	3 ($10^5 \times 256$)	0.05	0.2
5	45 ($10^4 \times 256$)	58 ($10^5 \times 256$)	12.9	3.8
17	68 ($10^3 \times 256$)	173 ($10^4 \times 256$)	25.4	5.5
34	65 (10×256)	115 ($10^3 \times 256$)	177	15.3
35	60 (10×256)	146 ($10^2 \times 256$)	24.4	5.4
36	0 (10×256)	7 (10×256)	only in smoke from air cured tobacco	
37	0 (10×256)	24 (10×256)	only in smoke from air cured tobacco	

[a] Calculated as $\sqrt[1.9]{\frac{h_{air\ cured}}{h_{flue\ cured}}}$.

matograms; through cochromatography using the FID detector, this peak was now found to coincide with dimethyl disulphide. The other differences could not be seen in the crowded FID peak pattern. In the FPD chromatograms of Fig. 1 and in Table II one S compound can be seen to have a concentration ratio (air cured/flue cured) considerably smaller than one: the concentration of H_2S, peak number 2, in the smoke from air cured tobacco is 5 × as low as it is in the smoke from flue cured tobacco. This could be caused by the alkaline character of the former type of smoke.

The possibility of identifying a tobacco type by the composition of the vapour phase of its smoke, has been questioned by GROB[15]. We believe that the use of the FPD detector may shed some new light (be it monochromatic) on this subject; of course, it remains to be shown whether the effects observed are valid for all specimens of the three types of tobacco studied here. If so, the method as described in this communication, with or without definitive identification of the characteristic differences in smoke composition, might be developed further to allow of the content of air cured tobacco in blends to be determined. Determination of the exact geographic origin of a particular tobacco in a blend, however, is not likely to become possible with this method.

Initially this study was started because, in discussions on smoking-and-health, it is sometimes suggested that a correlation exists between the type of tobacco smoked by a person and his risk of getting lung cancer[16], and likewise a correlation between the lung cancer rate in a particular country and the type of tobacco mostly smoked there[17]. Smoking of flue cured tobacco is thought to be associated with a high lung cancer risk, while smoking of air cured tobacco is being associated with a low risk[16,17]. Comparison of tobacco varieties as regards composition of the smoke produced might provide important chemical information on this theme. The amount smoked and the degree of inhaling, however, are equally important parameters which could, moreover, depend on the type of tobacco themselves.

As yet there is no indication whatsoever that the differences in smoke com-

position found in this study for different tobaccos have toxicological implications, though one is tempted to speculate on the possible protective effect of the S compounds in the smoke against the influence of reactive alkylating substances, simultaneously present in the smoke.

ACKNOWLEDGEMENT

This work was supported by a grant from the research fund of the Wetenschappelijke Adviesraad "Roken en Gezondheid" (Scientific Advisory Committee "Smoking and Health"); this fund has been established by the "Stichting Nederlandse Sigarettenindustrie" (Dutch Cigarette Industry Foundation).

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