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DETERMINATION OF α-DICARBONYL COMPOUNDS IN CIGARETTE SMOKE

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SUMMARY

The most abundant α-dicarbonyl compounds in cigarette smoke were detected after reaction with *o*-phenylenediamine and were identified by gas chromatography–mass spectrometry as quinoxaline derivatives. Diacetyl, 2,3-pentanedione, glyoxal, pyruvic aldehyde and 2-oxobutanal were found to be present, the last compound being found in cigarette smoke for the first time.

These compounds were determined by gas chromatography and high-performance liquid chromatography (HPLC). The two methods were compared and HPLC was selected for routine analysis.

The levels of α-dicarbonyl compounds in different types of cigarettes were determined, and the formation of these compounds in cigarette smoke is discussed on the basis of pyrolysis experiments.

INTRODUCTION

Two groups of α-dicarbonyl compounds have been identified in cigarette smoke, as follows.

(a) α-Diketonic compounds, represented by diacetyl, a preponderant compound the levels of which vary from 30 to 250 μg per cigarette, and 2,3-pentandione, present at lower levels of 5–50 μg per cigarette. These constituents play an important part in the aroma of the smoke, as the levels indicated, corresponding to amounts ranging from 20 to 200 ppm, are well above the threshold of olfactory or taste perception of about 10^{-2} ppm[1].

(b) Compounds with at least one aldehyde function; pyruvic aldehyde is the main representative, its level varying from 5 to 60 μg per cigarette; glyoxal has also been found, but at much lower levels. This class of compound is of little importance in the aroma, but through its properties which inhibit cell division[2] it can have a biological effect.

To study the organoleptic and biological problems connected with the presence of these compounds in cigarette smoke, it is necessary to have a routine method for their determination. This extremely complex mixture (several thousand constituents have been identified) does not easily lend itself to direct measurement. Existing methods include the analysis of the gaseous phase of the smoke by capillary column gas

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chromatography[3]; this method is difficult to use in a routine manner and does not measure non-volatile α-dicarbonyl compounds; HPLC has been used for the analysis of carbonyl compounds as their 2,4-dinitrophenylhydrazones, but dicarbonyl derivatives were not mentioned[4,5].

We directed our attention to methods for the selective derivatization of the α-dicarbonyl function, particularly the formation of nitrogenous heterocycles by condensation with an *o*-diamine[6,7]. We chose *o*-phenylenediamine, which leads to the formation of quinoxalines, which have not been identified in tar and which are suitable for spectrophotometric measurements owing to their absorption in the ultraviolet region of the spectrum.

The principle of the prosed method is as follows. Cigarette smoke is brought into contact with an aqueous solution of *o*-phenylenediamine and, after complete reaction, the analysis of the quinoxalines formed is carried out either by reversed-phase HPLC by direct injection of the reaction mixture, or by gas chromatography (GC) after extraction of the reaction mixture with chloroform. Combined GC–mass spectrometry (MS) made it possible to determine the identity and purity of the peaks analysed. After the agreement of the two methods had been checked, the HPLC technique was chosen for routine application because of its simplicity.

To study the origin of these substances in cigarette smoke, various substances were pyrolysed at temperature around the temperature of maximum combustion of a cigarette, in various gaseous atmospheres, and the α-dicarbonyl compounds were determined in the pyrolysates.

EXPERIMENTAL

Mechanical smoking of cigarettes and trapping of smoke

A single-channel Borgwaldt smoking machine is used. The particulate phase of the smoke is trapped by filtration through a Cambridge fibre-glass filter, which retains particles larger than 0.2 μm, and the water-soluble part of the gaseous phase by bubbling into 25 ml of 0.05% aqueous solution of *o*-phenylenediamine (Merck, Darmstadt, G.F.R.; puriss grade) in a Quickfit test-tube (Ref. MF/24/3/8) with a BC 11 absorption head in which the original ball is replaced with a fritted-glass cylinder (porosity 1).

The smoking of the cigarette was carried out under normal conditions (CORESTA standards). The cigarettes are conditioned for 24 h at 25°C in an atmosphere of 60% relative humidity before smoking. The machine takes puffs of 35 ml for 2 sec at the rate of one puff per minute. The analysis is carried out on the smoke from one cigarette.

Pyrolysis of tobacco and various substances

The apparatus (Fig. 1) consists of a 70 cm × 9 mm I.D. quartz horizontal pyrolysis tube in which is placed a quartz scoop (10 cm × 4 mm diameter) containing the sample to be studied. One end of the tube is connected to a rotameter to measure the flow-rate of the pyrolysis atmosphere, which is either dry air or nitrogen. The other end is connected to the trapping system, which is the same as that described for the smoking machine. This fixed pyrolysis tube is inserted into a ring-shaped oven, 75-mm wide, which can move along the tube at a steady speed of 1 cm/min. The oven

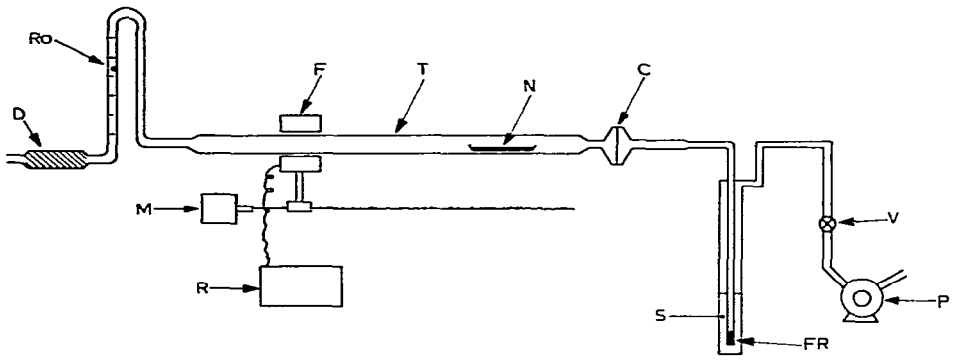


Fig. 1. Pyrolysis apparatus. M = Oven motor; T = quartz tube (70 cm × 9 mm I.D.); N = quartz scoop containing the sample; Ro = rotameter; D = dehydration cartridge; F = oven; R = oven temperature regulator; C = Cambridge filter; FR = fritted-glass cylinder; S = aqueous solution of *o*-phenylenediamine; V = flow control valve; P = pump.

temperature can be regulated from 200 to 1200°C by means of a Lindberg proportional regulator. The pyrolysis is carried out at 800°C with a gas flow-rate of 7.5 ml/min.

Preparation of sample

Immediately after smoking or pyrolysis, the Cambridge filter, covered with tar, is placed in the aqueous solution of *o*-phenylenediamine that has trapped the water-soluble part of the gaseous phase of the smoke. After the filter has been crushed, the condensation reaction is allowed to develop at room temperature, in the absence of light, for at least 2 h. The internal standards for the quantitative chromatographic analyses are then added. For HPLC 1 ml of a 1 mg/ml solution of 3-methylisoquinoline in acetonitrile and for GC 1 ml of a 125 μg/ml solution of isoquinoline in acetonitrile are used. In HPLC the aqueous reaction mixture is injected directly, but in GC the quinoxalines have to be extracted from the filtered aqueous mixture with 1 ml of chloroform.

High-performance liquid chromatography

The apparatus used is a Varian Model 8500 and the separation is carried out with a column (23 cm × 4 mm I.D.) of Partisil 10 ODS 3 (Whatman, Maidstone, Great Britain). The solvent is acetonitrile–water (35:65) at a flow-rate of 160 ml/h. The absorption of the eluate is measured at 312 nm by means of a Spectromonitor III (LDC, Riviera Beach, FL, U.S.A.); 10 μl of reaction mixture are injected.

Gas chromatography

The apparatus used is a Fractovap Model 2350 (Carlo Erba, Milan, Italy) equipped with a flame-ionization detector (FID). The column is a capillary column (80 m × 0.3 mm I.D.) of Carbowax 20M. The oven temperature is kept at 185°C and those of the detector and injector at 225°C. The pressure of the carrier gas at the head of the column is 800 g/cm^2. A volume of 1 μl is injected with a splitting ratio of 1:20.

Mass spectrometry

A Nermag R 10 10 B GC–MS system equipped with a SIDAR 4 D data system is used. The column and chromatographic conditions are as described above.

RESULTS AND DISCUSSION

Identification of α-dicarbonyl compounds in cigarette smoke by GC, HPLC and GC–MS

The sample of smoke treated as described above was analysed by GC and HPLC and compared with a mixture of synthetic products consisting of the reaction products of glyoxal (in the form of a bisulphite addition compound), pyruvic aldehyde, diacetyl and 2,3-pentanedione with *o*-phenylenediamine. The GC and HPLC results obtained from a cigarette of Virginia tobacco are shown in Fig. 2a and b and those corresponding to the synthetic products are shown in Fig. 3a and b.

To study the interferences due to water-soluble constituents in the smoke, we prepared a sample of the latter by trapping in an aqueous solution containing no

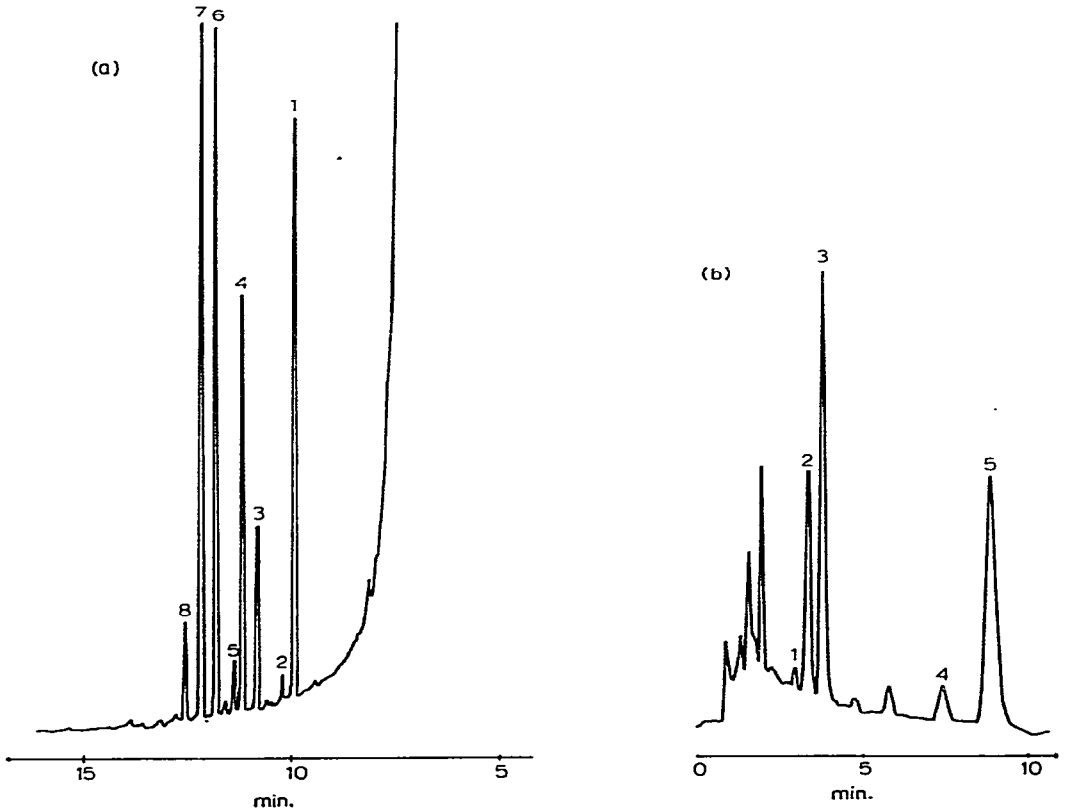


Fig. 2. Chromatograms of Virginia filter cigarette smoke. (a) GC: 1 = nicotine; 2 = glyoxal; 3 = pyruvic aldehyde; 4 = isoquinoline (internal standard); 5 = 2-oxobutanal; 6 = triacetin; 7 = diacetyl; 8 = 2,3-pentanedione. (b) HPLC: 1 = glyoxal; 2 = pyruvic aldehyde; 3 = diacetyl; 4 = 2,3-pentanedione; 5 = 3-methylisoquinoline (internal standard).

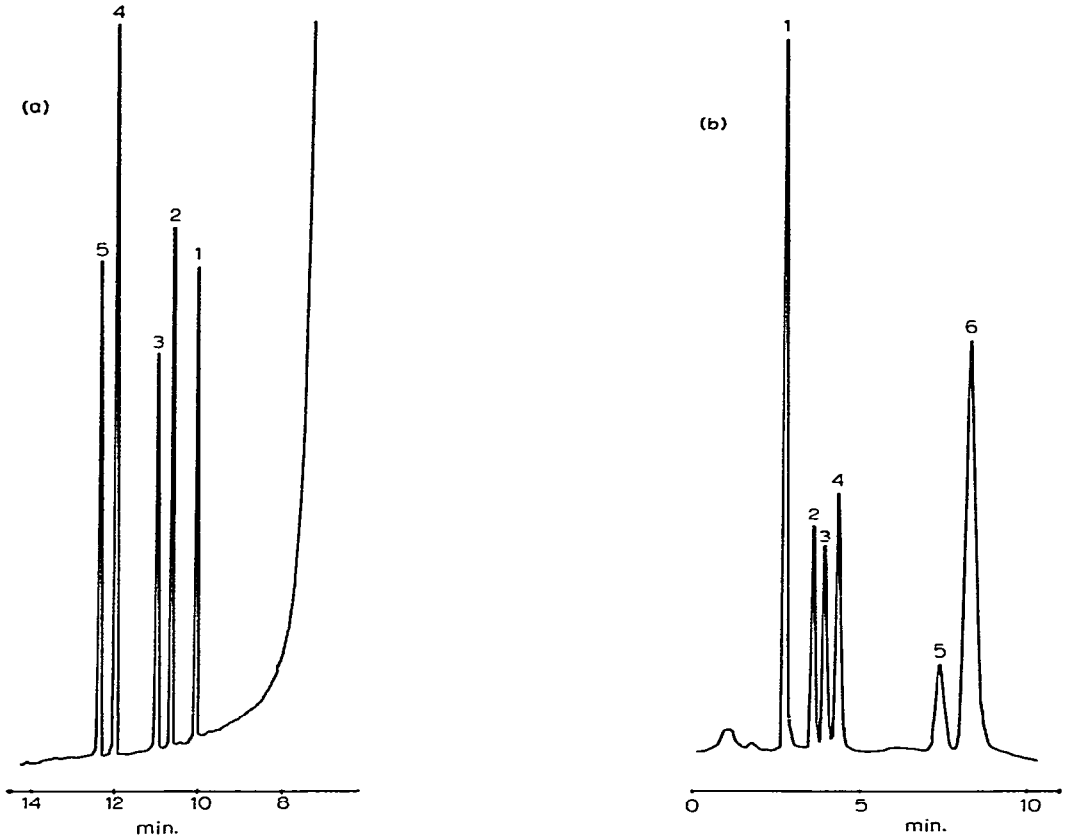


Fig. 3. Chromatograms of reference compounds. (a) GC: 1 = glyoxal; 2 = pyruvic aldehyde; 3 = isoquinoline (internal standard); 4 = diacetyl; 5 = 2,3-pentanedione. (b) HPLC: 1 = *o*-phenylenediamine (reagent); 2 = glyoxal; 3 = pyruvic aldehyde; 4 = diacetyl; 5 = 2,3-pentanedione; 6 = 3-methylisoquinoline (internal standard).

reagent; the GC and HPLC results are shown in Fig. 4a and b. Only two non-α-dicarbonyl constituents were detected in appreciable amounts by GC. In HPLC with selective detection at 312 nm, the absorption maxima of quinoxalines in this part of the spectrum do not allow the detection of impurities in the quinoxalines elution zone. All of the synthetic compounds were found in the smoke.

GC–MS made it possible to identify the non-carbonyl products nicotine and triacetin; the latter is used as a plasticizer in certain filters and is partly transferred into the smoke during smoking. The presence of quinoxalines was confirmed as follows, in decreasing order of importance: (1) 2,3-dimethylquinoxaline (diacetyl); (2) 2-methylquinoxaline(pyruvicaldehyde);(3)2-ethyl-3-methylquinoxaline(2,3-pentanedione); (4) 2-ethylquinoxaline (2-oxobutanal); and (5) quinoxaline (glyoxal).

2-Ethylquinoxaline was identified from its mass spectrum only. The mass spectra of the quinoxalines obtained are shown in Fig. 5. These spectra are the same as those published elsewhere[8,9]. 2-Oxobutanal was identified in cigarette smoke for the first time.

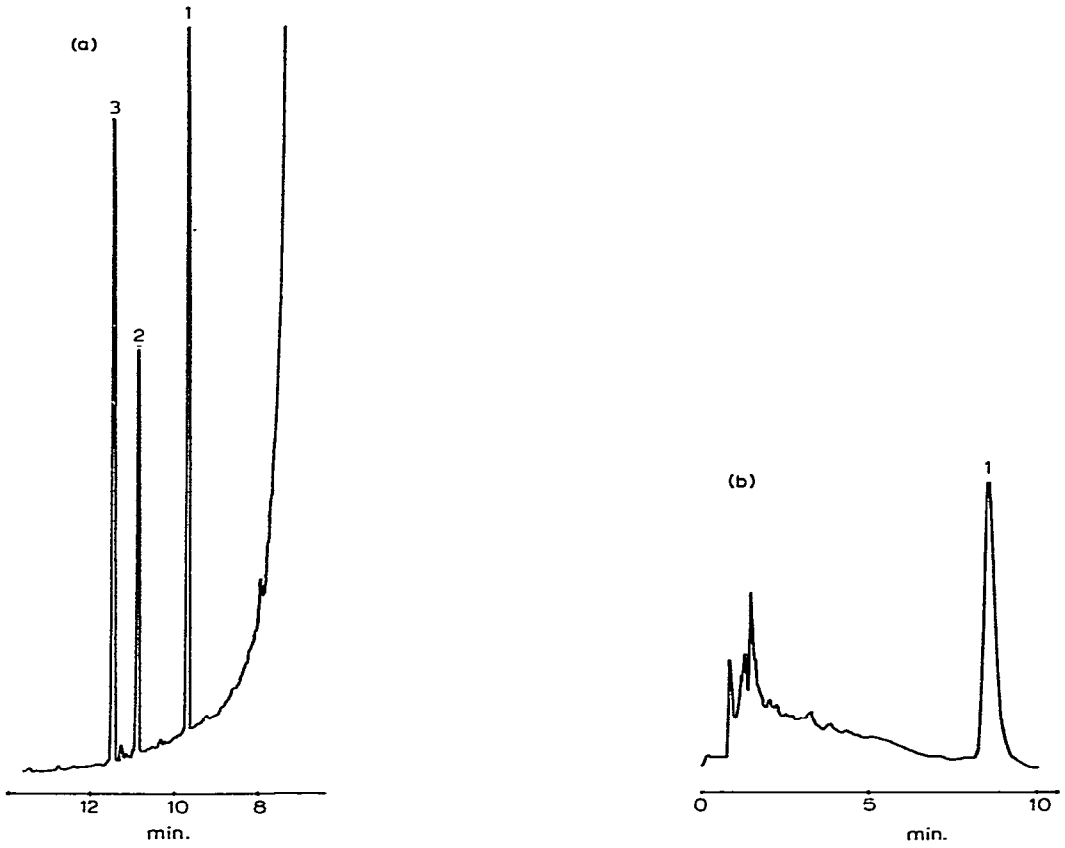


Fig. 4. Chromatograms of cigarette smoke without reagent. (a) GC: 1 = nicotine; 2 = isoquinoline (internal standard); 3 = triacetin. (b) HPLC: 1 = 3-methylquinoline (internal standard).

Quantitative study of the three main α-dicarbonyls in cigarette smoke

Fig. 2a and b show that trace amounts of glyoxal are found in cigarette smoke. The quantitative study was therefore carried out more precisely on diacetyl, 2,3-pentanedione and pyruvic aldehyde. 2-Oxobutanal was not determined quantitatively because of the lack of a reference compound.

Kinetics of the derivatization reaction. We studied the kinetics of the reaction directly on the smoke solution. HPLC analyses of the reaction mixture were carried out immediately after the end of the smoking of the cigarette, then at regular intervals. The kinetics concerning the reaction that produces quinoxalines from diacetyl, methylglyoxal and 2,3-pentanedione are shown in Fig. 6. We noted a slower reaction rate for the ketoaldehydic product than for the diketones. The reactions were completed in 2 h. We checked that products formed are stable in the reaction mixture for at least 24 h. The stability of the products formed makes it possible to use these analyses in a routine manner.

Calibration. The GC and HPLC calibration graphs (Figs. 7 and 8) were obtained for α-dicarbonyl compounds subjected to reaction with *o*-phenylenediamine

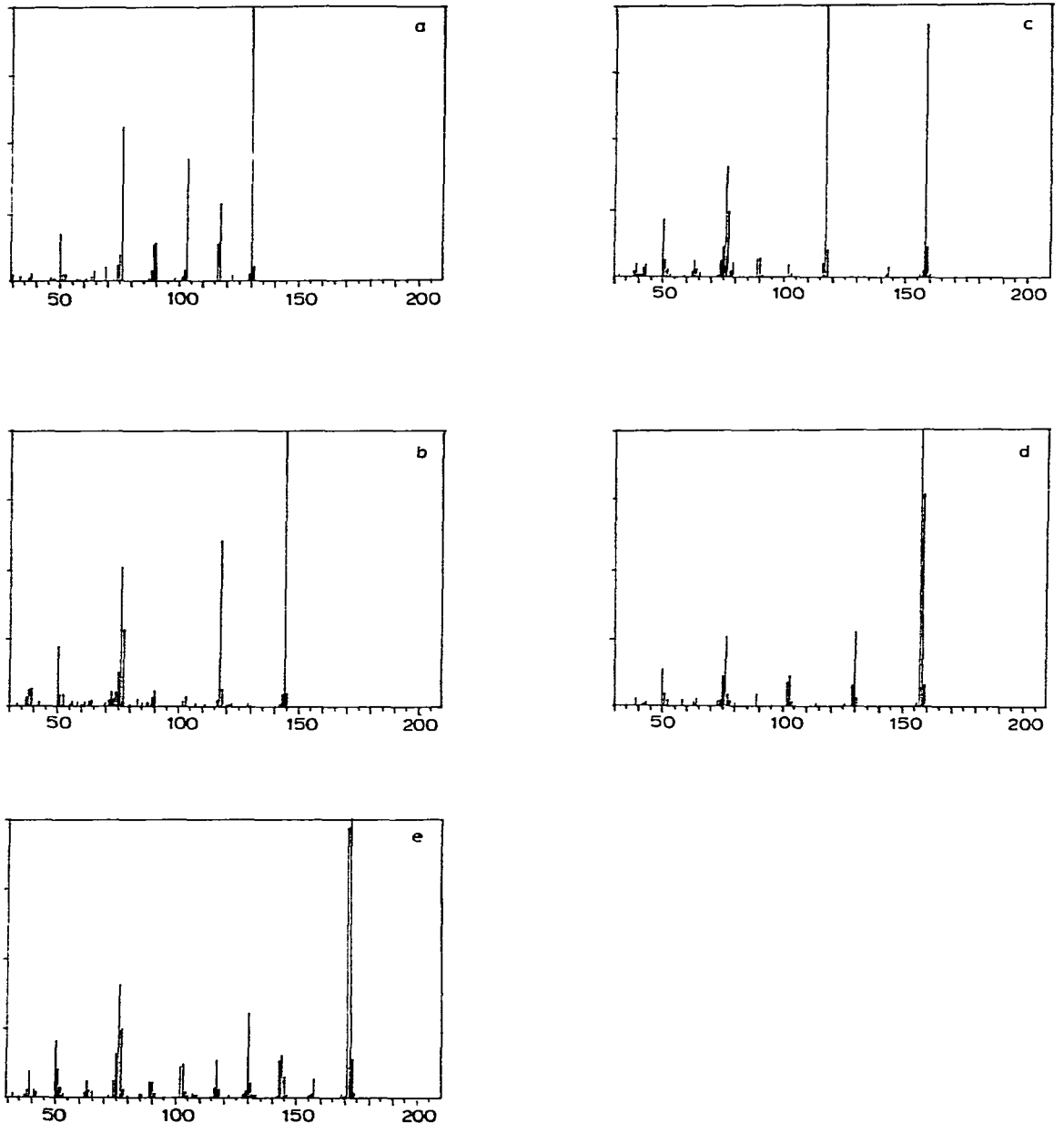


Fig. 5. Mass spectra of derivatives: (a) quinoxaline; (b) 2-methylquinoxaline; (c) 2,3-dimethylquinoxaline; (d) 2-ethylquinoxaline; (e) 2-ethyl-3-methylquinoxaline.

under the experimental conditions described above; the products were dissolved in 25 ml of a 0.05% aqueous solution of the reagent except for the glyoxal, the calibration graph of which was obtained directly from quinoxaline.

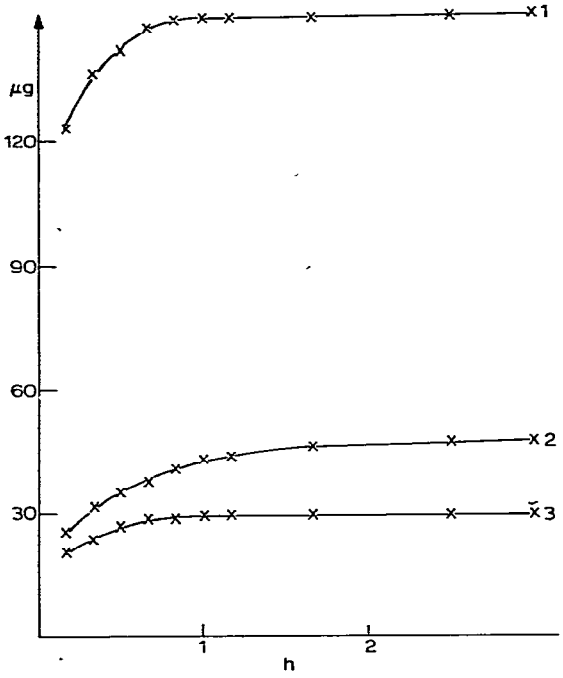
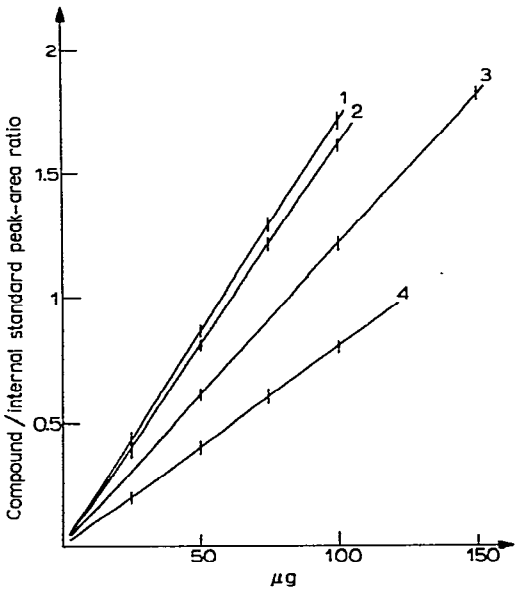


Fig. 6. Kinetics of quinoxaline formation by reaction of *o*-phenylenediamine on: 1 = diacetyl; 2 = pyruvic aldehyde; 3 = 2,3-pentanedione.



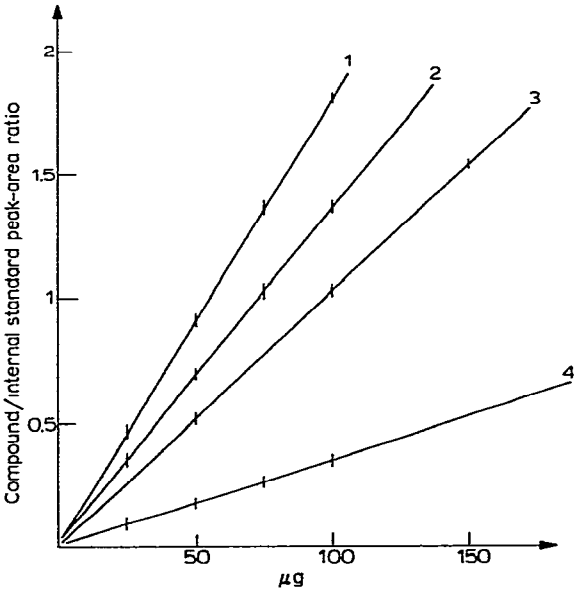


Fig. 7. Calibration graphs for GC method: 1 = glyoxal; 2 = pyruvic aldehyde; 3 = diacetyl; 4 = 2,3-pentanedione.

Fig. 8. Calibration graphs for HPLC method: 1 = glyoxal; 2 = pyruvic aldehyde; 3 = diacetyl; 4 = 2,3-pentanedione.

Results of quantitative analyses of a standard cigarette of dark air-cured tobacco by both techniques. By repeated smoking of the same kind of reference cigarette, without a filter, we carried out two series of fifteen measurements by both HPLC and GC, and the results are given in Table I.

TABLE I

COMPARISON OF HPLC AND GC RESULTS (μg/CIGARETTE) ON A REFERENCE CIGARETTE

HPLC			*GC*		
Diacetyl	*2,3-Pentanedione*	*Pyruvic aldehyde*	*Diacetyl*	*2,3-Pentanedione*	*Pyruvic aldehyde*
224	39,5	35	196	33	41
174	33	29	182	30	32
200	33	37	231	44	38
139	39,5	36	221	43	38
197	36	38	233	45	44
212	43	37	184	32	32
180	39	37,5	200	38	31
202	40	33	238	47	46
198	33	35	198	39	38
215	44	42	236	40	43
195	33	30	204	39	41
232	43	37	190	36	35
190	36	31	188	39	32
208	39	32	231	40	38
223	46	37	195	39	36
Average 205.9	38.4	35.1	208.5	38.9	37.6
Standard deviation 18.5	4.35	3.5	20.7	4.77	4.71

The average results for both series are comparable. A spread of the results can be observed, probably owing more to the cigarette sample than to the measurements themselves. The standard deviations, however, are systematically greater with the GC than the HPLC technique. The calculation of the experimental Student's t values between the two methods gives $t = 0.36$ for diacetyl, $t = 0.3$ for 2,3-pentanedione and $t = 1.64$ for pyruvic aldehyde. The Student–Fisher table gives $t = 2.048$ at 95% confidence level and at 28 degrees of freedom. In all three instances the calculated t value is less than the corresponding t value in the table, so that one can conclude that the probability of the results obtained by the two methods for the three compounds being the same is 95%.

For routine measurements we chose the HPLC method, which has the following advantages over GC:

(a) Sensitivity and simplicity. Reversed-phase HPLC makes it possible to inject aqueous solutions. The sensitivity of the detector (nanogram amounts of carbonyl compounds are detectable with a sensitivity range of 0–0.02 absorbance unit) makes possible measurements on the reaction mixture without pre-concentration. In GC with FID, pre-concentration is necessary.

(b) Selectivity. In HPLC selectivity is ensured by the choice of a detection wavelength of 312 nm. In GC a nitrogen-selective detector could have been used, but then nicotine interferes.

(c) Speed. An analysis by HPLC takes 9 min whereas GC takes 13 min.

The validity of the method was tested by studying the relationship between the amounts of the various products found and the number of cigarettes smoked and by comparison with the levels of synthetic products recovered from to the smoke solution.

Results of quantitative analyses carried out on various kinds of cigarettes. Cigarettes with and without filters, representative of the main kinds of commercial blends, were analysed. We chose cigarettes with similar condensate yields within the group with filters and within the group without filters. The results are given in Table II.

TABLE II

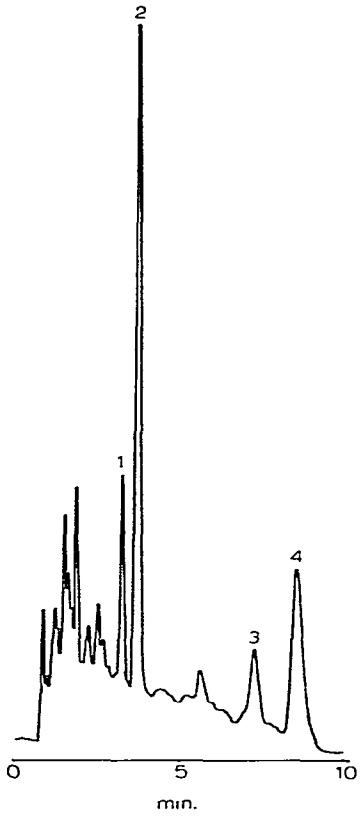
LEVELS OF α-DICARBONYL COMPOUNDS IN COMMERCIAL BRANDS (μg/CIGARETTE)

Compound	*Dark air-cured tobacco blend*		*American blend*		*Virginia tobacco blend*	
	Non-filter cigarette	*Filter cigarette*	*Non-filter cigarette*	*Filter cigarette*	*Non-filter cigarette*	*Filter cigarette*
Diacetyl	207	190	157	165	230	200
2,3-Pentanedione	32	30	23	29	45	35
Pyruvic aldehyde	33	19	60	38	70	40

The levels of α-diketones vary little from one kind of tobacco to another and the filters do not make much difference. The levels of ketoaldehydic compounds are greater in the American blends than in the dark air-cured tobaccos, and the effect of the filter is considerable.

One can explain the difference in behaviour with respect to the filters of both groups of carbonyls by the fact that the diketones occur mostly in the gaseous phase of the smoke whereas the ketoaldehydes are found in the particulate phase.

Search for substances from which α-dicarbonyl compounds may be obtained. To search substances from which α-dicarbonyl compounds of cigarette smoke may be obtained, we pyrolysed various known tobacco constituents and applied the method described to the pyrolysates. Cellulose, glucose and fructose were thus pyrolysed in atmosphere of air and nitrogen. The patterns obtained are qualitatively similar, the only observable differences being quantitative. With cellulose and sugars pyruvic aldehyde is the major product, in contrast to tobacco, from which diacetyl is the major product (see Figs. 9 and 10 and Table III). It can therefore be concluded that cellulose and sugars contribute to the formation of α-dicarbonyl compounds in cigarette smoke, particularly that of pyruvic aldehyde, but that these precursors alone do not explain the relative levels observed in the smoke and there are probably other tobacco constituents that play a part in the formation mechanism. Pyrolysis experiments carried out on mixtures of cellulose and amino acids show a profound change in the relative levels of α-dicarbonyl compounds in comparison with those observed in the pyrolysis of pure cellulose.



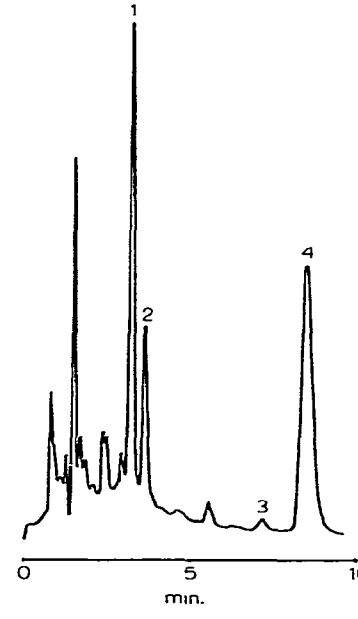


Fig. 9. Chromatogram of tobacco pyrolysate obtained by HPLC. 1 = Pyruvic aldehyde; 2 = diacetyl; 3 = 2,3-pentanedione; 4 = 3-methylisoquinoline (internal standard).

Fig. 10. Chromatogram of cellulose pyrolysate obtained by HPLC. 1 = Pyruvic aldehyde; 2 = diacetyl; 3 = 2,3-pentanedione; 4 = 3-methylisoquinoline (internal standard).

TABLE III

LEVELS OF α-DICARBONYL COMPOUNDS IN PYROLYSATES (μg/g OF PYROLYSED SUBSTANCE)

Material	*Air*		*Nitrogen*	
	Pyruvic aldehyde	*Diacetyl*	*Pyruvic aldehyde*	*Diacetyl*
Tobacco	153	600	150	850
Cellulose	3600	1120	5300	1120
Fructose	4600	1000	3200	173
Glucose	3500	800	3650	800
Cellulose + 10% asparagine	220	145	1800	1400
Cellulose + 10% aspartic acid	–	–	1200	1400

CONCLUSION

The method of derivatization by formation of nitrogenous heterocycles proposed for the determination of α-dicarbonyl compounds is sufficiently selective and sensitive to be applied directly to the complex cigarette smoke mixture. One would expect that it could also be applied successfully to the complex mixtures encountered in food and feed production in which these constituents are important for organoleptic reasons.

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