

Chemical Studies on Tobacco Smoke

VI. The Determination of Carbazoles in Cigarette Smoke*

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Bioassays on mouse skin have revealed high carcinogenic and tumor initiating activity for the neutral subfraction BI (5, 15). This fraction constitutes about 0.6% of dry cigarette smoke condensate and contains, among other components, polynuclear aromatic hydrocarbons (6), indoles (4), chlorinated hydrocarbon insecticides (3), and carbazoles.

The identification of carbazoles in tobacco smoke was first reported in 1962 by Rodgman and Cook (11). From a large scale separation, these investigators obtained a small subfraction of smoke condensate from which crystalline carbazole (70 µg/100 Turkish cigarettes) was isolated. Methylcarbazoles were also tentatively identified in this fraction (15 µg/100 cig.). Carbazole has since been identified in cigarette smoke by several other groups (10, 14). Schmeltz et al., for instance, reported the presence of 206.6 µg of carbazole, 110.6 µg of methylcarbazole and 38.8 µg of substituted carbazoles (12) in the smoke of 100 cigarettes.

In this communication we describe a method for the quantitative determination of carbazole and alkylated carbazoles in cigarette smoke.

METHODS AND MATERIAL

Synthesis of Carbazole-10-C¹⁴

C¹⁴-labelled carbazole was not commercially available. Since it was needed as an internal standard for the quantitative analysis, the Borsche synthesis (2) was attempted with micro-equipment. After extensive tests with unlabelled phenylhydrazine, this method was chosen for the synthesis of carbazole-10-C¹⁴.

Phenyl-1-C¹⁴-hydrazine (Tracerlab, Waltham, Mass.) was purified over basic aluminum oxide (activity III, Woelm). A solution of 5.21 mg of the purified material (specific activity 1.05 mC/mM) in 100 µl of 75% ethyl alcohol and 10 µl of conc. HCl was cooled by ice water and stirred with a magnetic spinbar. Five mg of purified cyclohexanone in 20 µl of 75% alcohol was added. The solution remained at room temperature under N₂ for one hour and was then gradually heated to 80° C in a water bath and kept at this temperature for 30 minutes. The dry residue was dissolved in 1 ml of purified mesitylene, before 10 mg of powdered palladium on carbon (5%; Girdler, Louisville, Kentucky) was added. The suspension was heated gradually in an oil bath to about 185° C and refluxed for two hours. After cooling, the mixture was filtered and the Pd/C washed with mesitylene. The filtrate was placed on a column of 3 g of deactivated alumina (Woelm, neutral, activity II) and eluted with n-hexane. The active fractions were combined and rechromatographed on 3 g of alumina (act. II). The radioactive eluate with n-hexane-benzene (8:1) was evaporated to dryness and then dissolved in toluene. According to gas chromatographic analysis 4.7 mg of carbazole-10-C¹⁴ was isolated (58% yield). The purity of the compound was determined by its ultraviolet absorption spectrum and by gas chromatography. The specific activity of the carbazole-10-C¹⁴ was 1.01 mC/mM. This value did not change with further chromatography on alumina.

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Carbazole and 2-methylcarbazole were obtained from K & K Laboratories (Plainview, New York). Both were purified by chromatography on deactivated alumina and recrystallized: carbazole m. p. 244–245° C*; 2-methylcarbazole m. p. 260–261° C. Neither U.V.-spectra nor gas chromatography showed impurities for either compound.

1-Methylcarbazole was synthesized, as reported earlier (7, 12) by starting with 2.21 g (18 mM) of freshly distilled o-methylphenylhydrazine (K & K Laboratories) and 1.76 g of cyclohexanone (18 mM). The mixture was refluxed for one hour in 10 ml of acetic acid. The dry, impure 1-methyl-1,2,3,4-tetrahydrocarbazole (2.79 g) was subsequently refluxed for 1 hour with 1.0 g of 5% Pd/C in 10 ml of mesitylene. The raw 1-methylcarbazole (2.71 g) was free of carbazole as shown by gas chromatography. Our results, therefore, do not agree with those reported by Pausacker and Schubert (8). The 1-methylcarbazole was purified on deactivated alumina resulting in a 44% yield (1.44 g). After three recrystallizations, the melting point in a closed tube was 121–122° C and the gaschromatographic analysis revealed pure 1-methylcarbazole.

Similarly, 3-methylcarbazole was synthesized from p-methylphenylhydrazine and cyclohexanone (18 mM) (7, 12). Chromatography on alumina gave 1.84 g (56%) of the raw product. After three recrystallizations from cyclohexane, 3-methylcarbazole had a m. p. of 210–211° C.

In the synthesis of 4-methylcarbazole by the same procedure, gas chromatographic analysis showed the raw product to be mainly a mixture of 2- and 4-methylcarbazoles. The solvent (mesitylene) was reduced *in vacuo* to about 5 ml. At room temperature 1.12 g of colorless crystals precipitated, of which 90% were 2-methylcarbazole. The mother liquor was reduced to a volume of 2.5 ml; this concentrate yielded 350 mg of impure 4-methylcarbazole. The raw product was chromatographed on deactivated alumina and then recrystallized from mesitylene giving 160 mg of pure 4-methylcarbazole (m. p. 128–129° C).

2-Phenylindole was prepared from 3.6 g of freshly distilled phenylhydrazine and 4.0 g of acetophenone according to Shriner et al. (13). The overall yield was 48%. This material was purified by chromatography on deactivated alumina and recrystallized from 95% ethanol giving gas chromatographically pure 2-phenylindole; m. p. 189–190° C (35% yield).

3-Phenylindole was synthesized from 3.6 g of phenylhydrazine and 4.0 g of phenylacetaldehyde, in an overall yield of 34%. It was purified by chromatography on deactivated alumina and recrystallization from 95% ethanol; m. p. 88–89° C (overall yield 29%).

Cigarette Smoking

Fifty U. S. blended nonfilter cigarettes of 85 mm length and selected by weight are smoked individually by a CSM-10 smoking machine under standard conditions (4). The smoke is drawn through three 100 ml traps. The first trap is cooled with ice water, the second with dry ice-acetone and the third (a gas wash bottle) is filled with 50 ml of methanol-water (4:1). A Cambridge filter assembly is placed between the third trap and the smoking machine. The condensate from traps 1 and 2 is transferred with the 50 ml of methanol-water from trap 3 into a 500 ml separatory funnel. Fifty ml of n-hexane are used to extract the traces of particulate matter from the Cambridge filter and to rinse the traps. The same extracting and rinsing procedure is repeated with 50 ml of methanol-water (4:1) and finally with 50 ml of n-hexane.

Internal Standard

At the beginning of each analysis, 9.4 µg of carbazole-10-C¹⁴ (125,000 dpm) in 1 ml of toluene is added to the smoke condensate from 50 cigarettes. The Nuclear Chicago Scintillation System 720 is used and counts at an efficiency of 71.2% for the unquenched C¹⁴-labelled carbazole in toluene with 0.4% PPO (2,5-diphenyloxazole) and 0.005% POPOP (p-bis[2(5-phenyloxazolyl)]-benzene as scintillators.

Concentrating the Carbazoles

After a thorough shaking, the methanol-water layer is separated and the n-hexane layer is extracted twice more with 100 ml of methanol-water (4:1, pre-equilibrated with n-hexane). This

* All melting points are corrected.

Heterocyclic compound	M. P. °C	C_M / C_H	C_H / C_W
Carbazole	245–246	5.2	28.4
1-Methylcarbazole	121–122	4.1	118
2-Methylcarbazole	260–261	3.6	124
3-Methylcarbazole	210–211	2.9	315
4-Methylcarbazole	128–129	2.8	414
2-Phenylindole	189–190	2.4	>500
2-Phenylindole	88–89	2.4	>500

TABLE 1
Partition coefficients of carbazoles and phenylindoles (21° C)

C_M Concentration in methanol-water 4:1

C_H Concentration in n-hexane

C_W Concentration in methanol-water 1:2

Coefficients were obtained after spectroscopic determination (U. V.) for concentrations of about 1 mg/liter.

extracts at least 97% of the carbazoles and phenylindoles from the upper phase (Table 1). The combined methanol-water layers (300 ml) containing about 80% of the condensate are diluted with water until the methanol-water ratio changes from 4:1 to 1:2. Two extractions with equal volumes of pre-equilibrated n-hexane suffice to bring more than 99% of the carbazoles and phenylindoles into the n-hexane layers (Table 1). The combined upper phases are concentrated by vacuum distillation at 12 mm Hg and a water bath temperature below 30° C to 30–50 ml. The hexane layer (residue 10–12% of starting material) is extracted twice with 20 ml of 2 N-acetic acid, washed with water once, dried over desiccated sodium sulfate, and concentrated to about 50 ml. The residue contains 4–6% of the condensate.

Column Chromatography

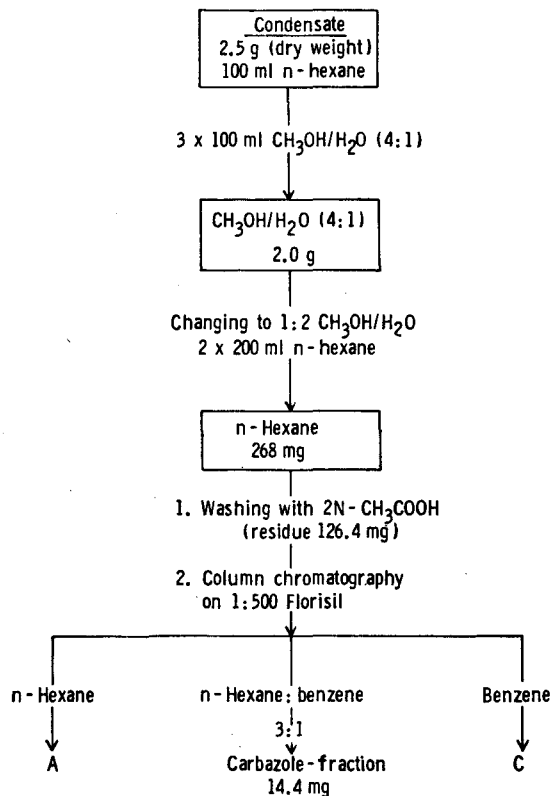
The carbazole concentrate in 5 ml of hexane is chromatographed on about 65 g of Florisil, mesh 60–100 (Fisher Scientific Co., New York) and eluted with n-hexane (200 ml) and n-hexane-benzene (3:1). The first six fractions (300 ml) are free of carbazole, fractions 7–11 (each 50 ml)

show β -activity, and the next three fractions contain still some alkylated carbazoles and phenylindoles. Fractions 7–14, are concentrated under reduced pressure, the concentrate is then transferred to a 3 ml vial and 0.2 ml toluene is added. This solution is freed from n-hexane and benzene by evaporation under N_2 at room temperature. The residue of the carbazole concentrate contains less than 1% of the starting material, see separation scheme Fig. 1.

FIGURE 1

Separation scheme for carbazoles from cigarette smoke condensate

The weights given are average values from several separations.



Gas Chromatography

Two to three microliters of the concentrate are injected into a gas chromatograph, which, in this case, was a Perkin-Elmer Model 800 with hydrogen flame ionization detector. The carbazoles are separated on a 6 m stainless steel column (i. d. 3 mm), filled with 10% SE-30 on Chromosorb W (acid washed, mesh 60–80; Applied Science Laboratories, State College, Penn.) with helium as carrier gas (inlet pressure 2.5 atm.). The separation is completed isothermally at 230° C with injection port and detector temperatures of 280° C. A Leeds & Northrup recorder ranging from 0.0–1.0 mV is employed with an attenuation of 100. About 1.0 μ g carbazole reach the full scale of the recorder chart with this setting. A typical gas chromatogram from a carbazole

FIGURE 2

Gas chromatogram of carbazole concentrate from cigarette smoke for quantitative analysis

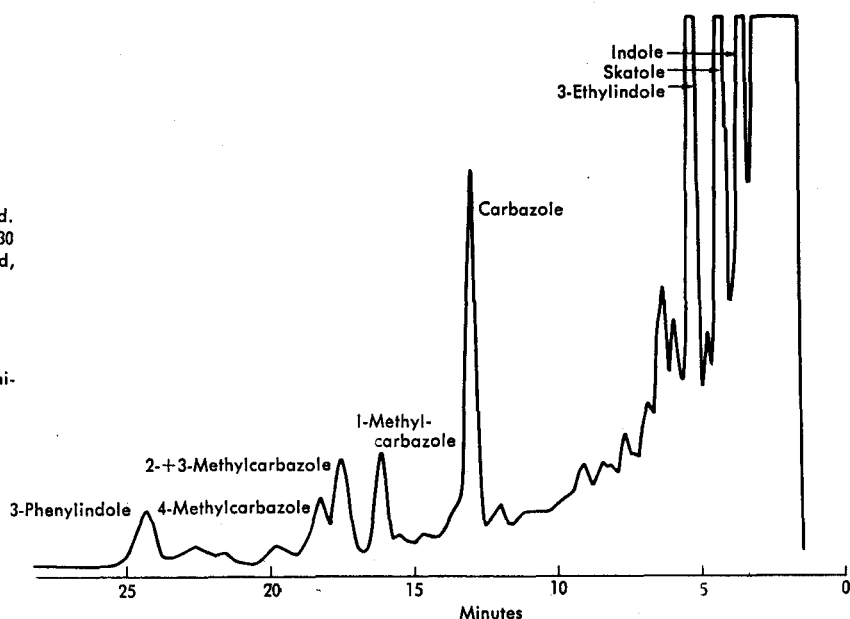
Conditions:

6 m stainless steel column, i. d. 3 mm, filled with 10% SE-30 on Chromosorb W (acid washed, mesh 60-80)

Column temperature: 230° C

Carrier gas: helium (2.5 atm.)

Detector: hydrogen flame ionization detector



concentrate is shown in Fig. 2. According to the retention times, the major peaks in the carbazole fraction are carbazole, 1-methylcarbazole, 2- and 3-methylcarbazole, 4-methylcarbazole and 3-phenylindole (Table 2).

Identification of Carbazoles

A large-scale separation beginning with 200 g of condensate was carried out in order to isolate and identify the components of the carbazole fraction. The procedure was accomplished without an internal standard. After column chromatography on Florisil, the carbazole concentrate determined by gas chromatographic analysis was rechromatographed on neutral alumina (Woelm, activity II). The concentrate ($\sim 0.05\%$) was separated by gas chromatography into individual components. Basically, however, the outlined setting was used with a 4:1 splitter and low detector sensitivity. Under these conditions up to 3 μ l of the carbazole concentrate could be injected for each separation (Fig. 3).

Eighty per cent of the injected carbazoles, which were emitted via the gas stream splitter before the detector, were trapped in capillary tubes. In some instances up to 10 separations were required in order to collect 30-50 μ g of a component. When the mass spectrum demonstrated that a single component had been isolated, the collection of this material was repeated, the capillary tube sealed on both ends, and a melting point was taken.

Infrared absorption spectra can be helpful in identifying individual methylcarbazoles in a mixture of carbazole and various monomethylcarbazoles (9). We therefore collectively trapped all components that left the gas chromatography column in the period between 12.8 and 19.2 minutes (see Fig. 3). The collected crystalline material was thoroughly mixed with KBr to a 0.5% concentration, pressed into a pellet, and a spectrum in the range from 720-920 cm^{-1} was recorded.

TABLE 2

Retention volumes and retention times*

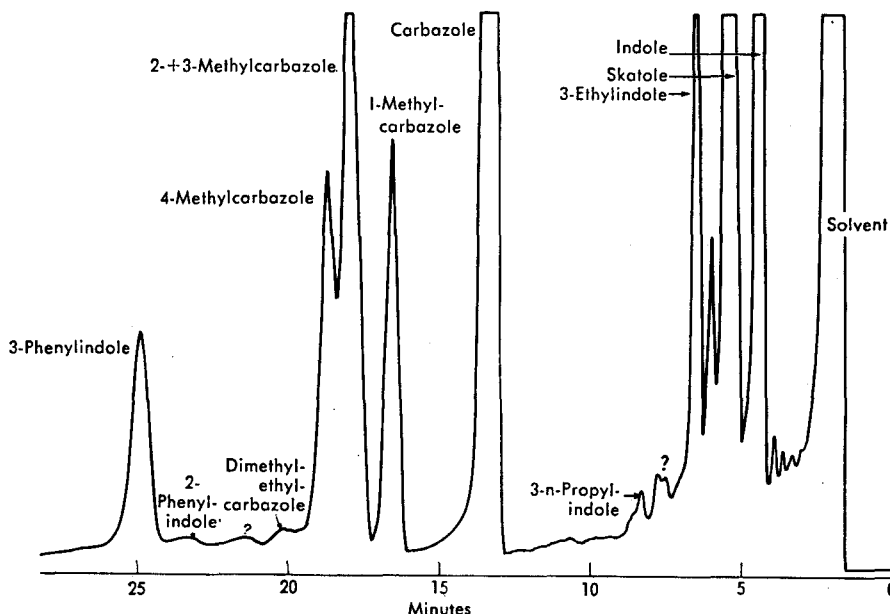
* Gas Chromatography System: 230° C isotherm, 6 m. column, i. d. 3 mm; liquid phase 10% SE-30 on Chromosorb W (acid washed, mesh 60-80). Carrier gas: helium.

** The effective carbon number is calculated from the molar retention volume of n-eicosane (ret. time 1.52) of which the effective carbon number was set at 1.0.

Heterocyclic compound	Relative retention time	Relative molar retention volume	Effective carbon number**
Carbazole	1.0	1.0	0.71
1-Methylcarbazole	1.25	1.4	0.91
2-Methylcarbazole	1.35	0.96	0.62
3-Methylcarbazole	1.35	0.96	0.62
4-Methylcarbazole	1.41	0.96	0.62
2-Phenylindole	1.83	0.95	0.58
3-Phenylindole	1.90	0.94	0.57

FIGURE 3

Gas chromatogram of carbazole concentrate from a large scale separation of cigarette smoke condensate
Conditions: See legend Fig. 2.



The infrared absorption spectra were recorded on a Perkin-Elmer Infrared Spectrophotometer, Model 21. The mass spectra were carried out by the Morgan-Schaffer Corporation in Montreal, Canada, with a Hitachi Perkin-Elmer RMU-6D instrument.

Quantitative Determination

From the final carbazole concentrate with internal standard three 2–4 μ l samples are injected into the gas chromatograph and three 20–50 μ l samples are placed into counting vials with scintillator solution. The quenching effect remains within the experimental variation of the counting method which is better than $\pm 1\%$. The amount of carbazole originally present in the smoke (X) is calculated as follows:

$$X = \frac{a(c-b)}{b}$$

a = carbazole- C^{14} added at the beginning of the analysis,
 b = carbazole- C^{14} isolated,
 c = carbazole + carbazole- C^{14} isolated.

RESULTS

Qualitative Analysis

The material which corresponds to the retention time of authentic carbazole in the chromatogram of the carbazole concentrate (Fig. 3) was collected from several runs. Its melting point in a closed capillary tube was 244–245° C (ref. 245–246° C) and its mass spectrum (Fig. 4) is identical to that reported in an earlier publication (1). The isolated component corresponding to 1-methylcarbazole had a melting point of 119–120° C (ref. 121–122° C) and a mass spectrum identical to that of an authentic specimen (Fig. 5). The component with the retention time of 2- and 3-methylcarbazole was a mixture, as revealed by its melting point (180–195° C) (ref. see Table 1) and mass spectrum (Fig. 6). The isolated 4-methylcarbazole gave a mass spectrum comparable with that of a synthesized specimen (Fig. 7). However, the isolated material contained a 10% impurity as seen by the parent ion m/e 211, the doubly charged ion m/e 105.5 and the fragments m/e 196, 168 and 130. Since a limited quantity of starting material was available for the gas chromatographic separation of the unknown (m/e 211), we did not attempt to elucidate the structure of this impurity.

TABLE 3

Carbazoles and 3-phenylindole in cigarette smoke*
($\mu\text{g}/\text{cigarette}$)

N-heterocyclic hydrocarbon	Number of analyses**					Average (m)	Variation coefficient %
Carbazole	0.95	1.01	1.08	0.97	1.03	1.00	5.0
1-Methylcarbazole	0.24	0.22	0.21	0.25	0.22	0.23	7.2
2- + 3-Methylcarbazole	0.19	0.20	0.22	0.18	0.18	0.19	9.0
4-Methylcarbazole	0.090	0.100	0.107	0.103	0.094	0.098	7.2
3-Phenylindole	0.30	0.33	0.30	0.31	0.32	0.31	4.2

* 85 mm nonfilter U. S. blended cigarette; smoking conditions: one puff per minute; 35 ml puff volume, 2 seconds puff duration, butt length 23 mm.

** Each analysis was completed with 50 cigarettes.

From the three minor constituents of the carbazole fraction, which are represented by maxima at retention times of 20.1, 21.4 and 23.3 minutes (Fig. 3), the first peak shows a parent ion m/e 195 (Fig. 8) which indicates the presence of a dimethylcarbazole or an ethylcarbazole. Peak 3 contained 2-phenylindole among other unidentified components.

The effluent from the column which corresponded in retention time to 3-phenylindole gave a mass spectrum corresponding to that of the authentic compound (Fig. 9). However, a parent ion m/e 207 also appeared and most likely originates from a methylphenylindole. It amounts to about 6–8% of the total column effluent with the 3-phenylindole retention time.

Quantitative Analysis

Table 3 presents the results of five quantitative analyses of cigarette smoke for carbazole, 1-methylcarbazole, 2- and 3-methylcarbazole, 4-methylcarbazole and 3-phenylindole. The variation

coefficients of the quantitative analysis ranged from 4–9%. The yield of the carbazoles in the final concentrate varied between 88 to 95%. Table 4 lists the concentration of the carbazoles and 3-phenylindole in fraction BI and the percentage of these agents in BI from the overall yield in the smoke condensate.

TABLE 4

Carbazoles in neutral subfraction BI

N-heterocyclic hydrocarbon	In BI %	Per cent of carbazoles from total smoke condensate*
Carbazole	0.32	60
1-Methylcarbazole	0.07	54
2- + 3-Methylcarbazole	0.07	67
4-Methylcarbazole	0.02	50
3-Phenylindole	Traces	—

* Calculated from the data of Table 3.

FIGURE 4

Mass spectrum of isolated carbazole

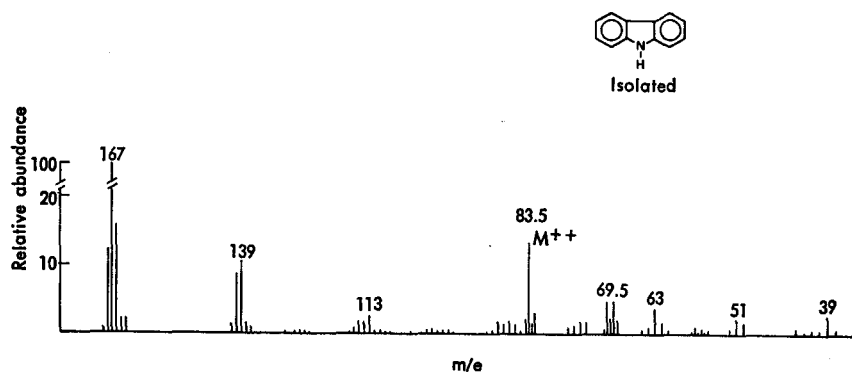


FIGURE 5
Mass spectra of synthetic and isolated 1-methylcarbazole

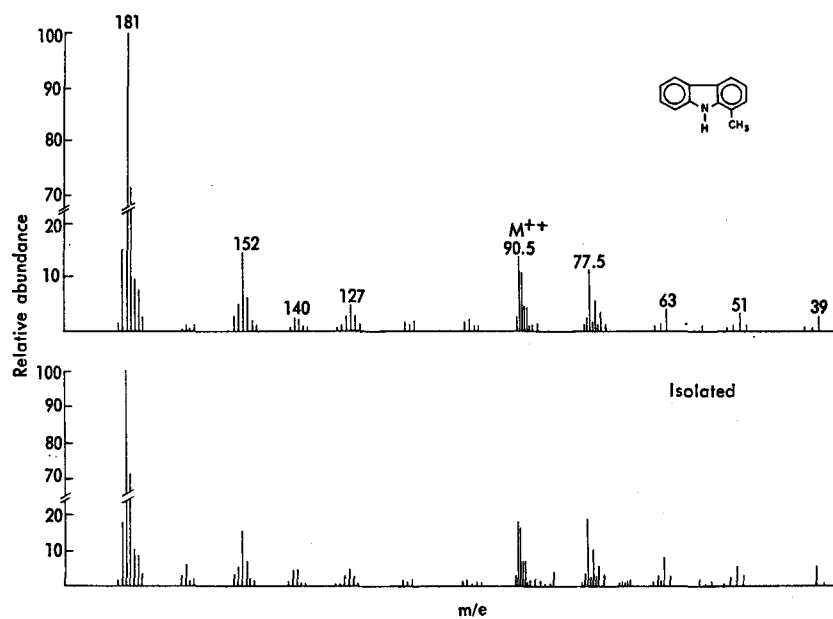


FIGURE 6
Mass spectra of synthetic 2- and 3-methylcarbazole and of a mixture of 2- and 3-methylcarbazole isolated from cigarette smoke

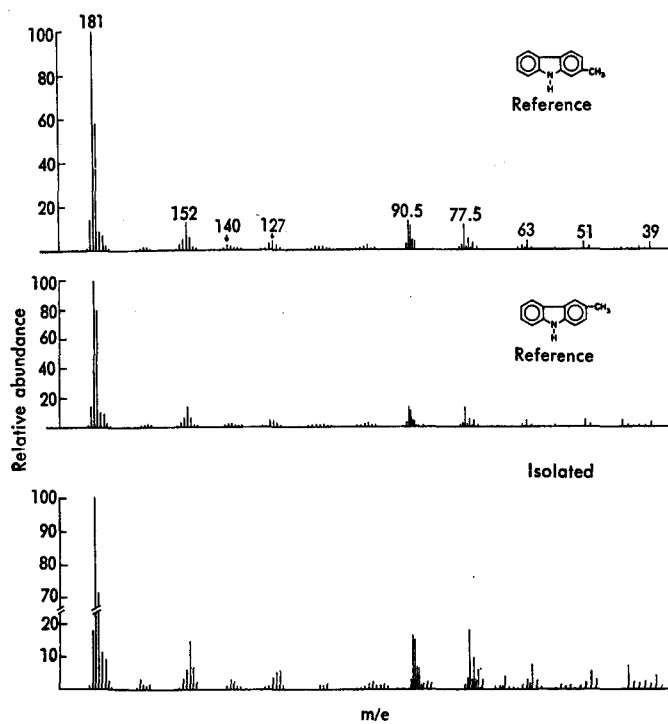


FIGURE 7
Mass spectra of synthetic and isolated 1-methylcarbazole

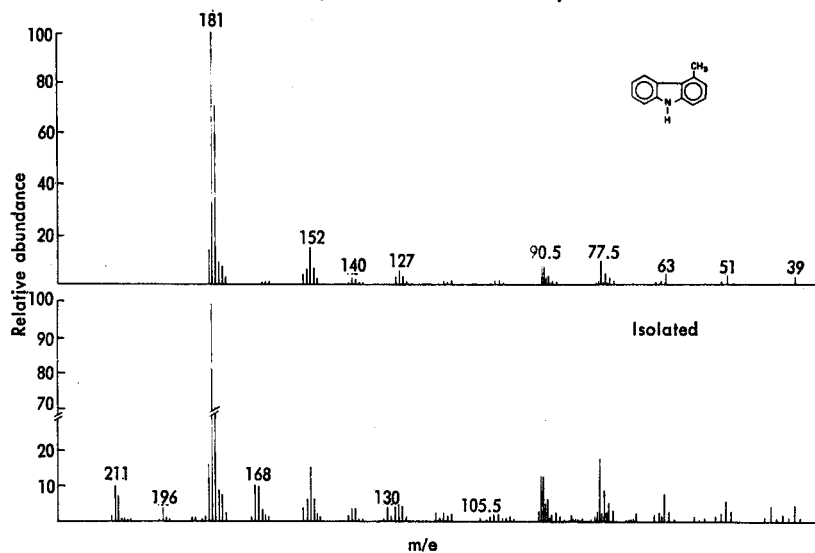


FIGURE 8
Mass spectrum of an unknown mixture of carbazoles containing a dimethyl- or ethylcarbazole

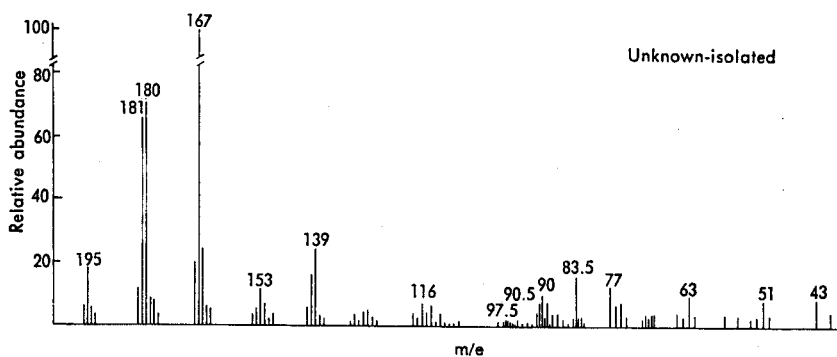


FIGURE 9
Mass spectra of synthetic and isolated 3-phenylindole

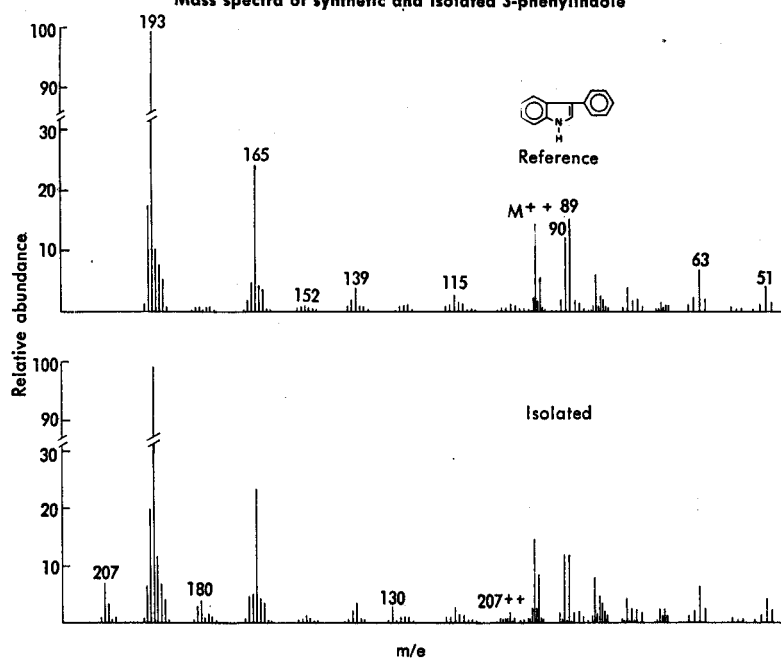
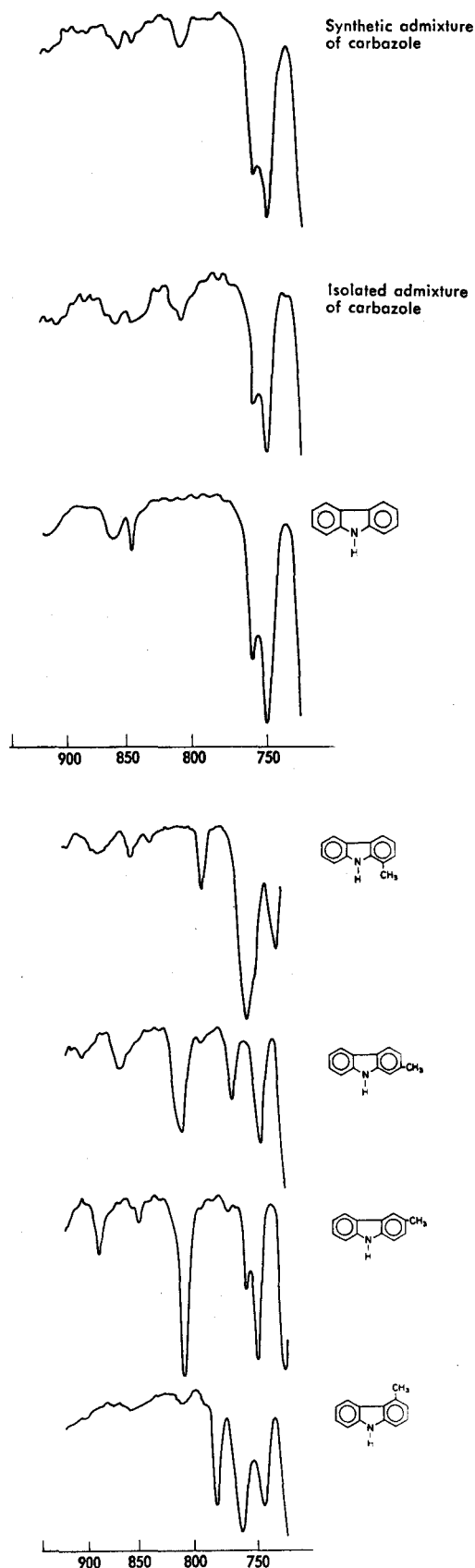


FIGURE 10



Infrared absorption spectra between 720–920 cm⁻¹ of synthetic and isolated mixture of carbazole with the 4 methylcarbazoles in a ratio of 100:23:10:10:10; and spectra in the same region of synthetic carbazole and the 4 methylcarbazoles (Preparation see "Identification of carbazoles").

DISCUSSION

This communication describes the isolation and identification in cigarette smoke of carbazole, 1-methylcarbazole, 4-methylcarbazole, 3-phenylindole, and a mixture of 2- and 3-methylcarbazole. Trace amounts (< 1.0 µg per 100 cigarettes) of a dimethylcarbazole, or an ethylcarbazole and 2-phenylindole were also tentatively identified.

The carbazole value reported here (100 µg/100 cigarettes) is in reasonable agreement with that reported by Rodgman and Cook [70 µg/100 cigarettes; (11)], especially when one considers that we compared the isolated amounts from the smoke of an 85 mm blended cigarette with a 70 mm cigarette made exclusively from Turkish tobacco. The data reported by Schmeltz et al. (12) for carbazole (206.6 µg), methylcarbazole (110.6 µg) and substituted carbazoles (38.8 µg) are not in line with our findings. One possible reason for this significant difference could be that changes have occurred in the "tar" and its fractions. A good indication of such changes is the skatole-indole ratio which, in fresh cigarette smoke of various tobacco types, we have so far always found to be about 1:1 (4). Since the main precursor for these smoke indoles is tryptophan, a constant ratio of skatole to indole is expected. However, when an aged "tar" is analyzed for skatole-indole, a ratio of up to 1:10 in favor of the chemically more stable skatole is found. In the analysis by Schmeltz et al., the ratio was about 1:17 and, therefore, one may suspect that such changes had occurred as these were also reflected in the carbazole values found by these authors.

Since we were not able to separate 2- and 3-methylcarbazole, we tried the approach of Richards (9), using the infrared spectroscopic method for distinguishing alkylated carbazoles.

A crystalline mixture of carbazole and its four methyl derivatives was isolated by gas chromatography from the carbazole concentrate of cigarette smoke. Its infrared absorption spectrum between 720–920 cm⁻¹ is presented in Fig. 10 and compares five carbazoles and a synthetic mixture of carbazole with 1- and 4-methylcarbazole in the ratio of 100:23:10:10:10. This ratio was found to exist in cigarette smoke (Table 3). The comparison of the

spectra suggests that the infrared method is not helpful in distinguishing individual methylcarbazoles, especially 2- and 3-methylcarbazole, at least as far as the analysis of tobacco smoke is concerned.

The mass spectra of the methylcarbazoles and 2- and 3-phenylindoles show fragmentation patterns comparable with those observed earlier for carbazole (1). In the case of the seven components analyzed, the first major fragment formed by the loss of HCN is a benzotropylium ion. Two C_2 fragments are consecutively split off. Furthermore, the mass spectra show all the small fragments m/e 63, 51 and 39. It is indicated that mass spectra can be quite helpful in identifying carbazoles and 2- and 3-phenylindoles.

SUMMARY

A method was developed for the enrichment of carbazoles from cigarette smoke. It involves a distribution between two solvent systems, column chromatography, and, finally, separation of a carbazole concentrate into individual components by gas chromatography. The major components in the chromatogram were collected after the final separation and were then identified by mass spectra. Since the latter method showed similar fragmentation patterns for five carbazoles and 2- and 3-phenylindoles, mass spectra appeared especially suitable for their identification. Carbazole, 1-methylcarbazole, 4-methylcarbazole, 3-phenylindole, a mixture of 2- and 3-methylcarbazole and, tentatively, a dimethyl- or ethylcarbazole and 2-phenylindole were identified in cigarette smoke.

The relevancy of mass spectra for the identification of carbazoles and 2- and 3-phenylindoles is discussed.

For the quantitative analysis of carbazoles and 3-phenylindole, carbazole-10- C^{14} was employed as internal standard. The radioactive compound was synthesized on a microscale from phenylhydrazine-1- C^{14} by the *Borsche* method with a 58% yield.

The smoke of 100 U.S. nonfilter cigarettes of 85 mm length contains 100 μg of carbazole, 23 μg of 1-methylcarbazole, 19 μg of 2- and 3-methylcarbazole, 9.8 μg of 4-methylcarbazole and 31 μg of 3-phenylindole. The concentrations of the tentatively identified dimethyl-, or ethylcarbazole and 2-phenylindole were below 1.0 μg .

ZUSAMMENFASSUNG

Zur Bestimmung von Carbazolen im Zigarettenrauch wurde eine analytische Methode entwickelt. Diese besteht vornehmlich aus Verteilungen zwischen zwei Lösungspaaen, Säulenchromatographie und Gaschromatographie. Die gaschromatographische Analyse führte zur Identifizierung von Carbazol, 1-Methylcarbazol, 4-Methylcarbazol, 3-Phenylindol sowie einem Gemisch aus 2- und 3-Methylcarbazol. Fernerhin scheint Zigarettenrauch auch Spuren eines Dimethylcarbazols oder Äthylcarbazols sowie 2-Phenylindol zu enthalten.

Die Bedeutung der massenspektrographischen Analyse für die Identifizierung von Carbazolen und 2- und 3-Phenylindolen wird besprochen.

Zur quantitativen Bestimmung von Carbazolen und 3-Phenylindol wurde Carbazol-10- C^{14} synthetisiert und als Markierungsverbindung gewählt. Der Hauptstrom von 100 U.S. filterlosen Zigaretten von 85 mm Länge enthält 100 μg Carbazol, 23 μg 1-Methylcarbazol, 19 μg 2- und 3-Methylcarbazol, 9,8 μg 4-Methylcarbazol und 31 μg 3-Phenylindol. Die Konzentrationen des Dimethylcarbazols oder Äthylcarbazols und des 2-Phenylindols lagen unterhalb von 1,0 μg .

RÉSUMÉ

Une méthode analytique a été développée pour la détermination de carbazols dans la fumée de cigarette. Elle consiste en la partition entre deux systèmes de solvants, la chromatographie sur colonne et, enfin, la séparation du concentré de carbazols en composants individuels par chromatographie gazeuse. Les majeurs composants du chromatogramme sont recueillis après la séparation finale et, ensuite, identifiés par spectrophotométrie de masse. Puisque les diagrammes de frag-

mentation fournis par cinq carbazols ainsi que par les 2- et 3-phénylindols se sont montrés similaires, les spectres de masse se prêtent particulièrement bien à leur identification. On a identifié les carbazol, 1-méthylcarbazol, 4-méthylcarbazol, 3-phénylindol et un mélange de 2- et 3-méthylcarbazols, et il est probable que la fumée de cigarette contient, au surplus, des traces d'un diméthylcarbazol ou d'un éthylcarbazol ainsi que le 2-phénylindol.

On discute de l'utilité de septres de masse pour l'identification de carbazols ainsi que de 2- et 3-phénylindols.

La validité des résultats quantitatifs a été vérifiée en utilisant le carbazol-10-C¹⁴ comme standard interne. Le composé radio-actif a été synthétisé à partir de la phénylhydrazine-1-C¹⁴ à l'aide de la méthode de Borsche avec un rendement de 58 %.

Dans la fumée de 100 cigarettes américaines de 85 mm, sans filtre, on a trouvé 100 µg de carbazol, 23 µg de 1-méthylcarbazol, 19 µg de 2- et 3-méthylcarbazols, 9,8 µg de 4-méthylcarbazol et 31 µg de 3-phénylindol. Les teneurs en diméthyl- ou éthylcarbazols et en 2-phénylindol, dont on a obtenu l'identification probable, doivent être inférieures à 1,0 µg.

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