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# **Chemical Studies on Tobacco Smoke**

III. Primary and Secondary Nitroalkanes in Cigarette Smoke\*

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We have previously presented data demonstrating an inverse relation of the nitrate content of tobacco with the number of puffs per cigarette, total particulate matter (TPM), nicotine, volatile phenols, and polynuclear aromatic hydrocarbons (PAH) in the smoke of cigarettes (6). These findings applied specifically to tobaccos of the same type, as for example for the four Burley samples in Figure 1. Using benzo(a)pyrene (BaP) values as indicators for the concentration of PAH in cigarette smoke, it appears that these aromatic hydrocarbons are selectively reduced with increasing nitrate content of tobacco.

Pyrolysis experiments in nitrogen at  $880^{\circ}$  C of tobaccos with increasing nitrate content or in an atmosphere of nitrogen admixed with NO led to an increased inhibition of the formation of BaP and other PAH as compared to pyrolysis in nitrogen with various concentrations of O<sub>2</sub> (Fig. 2).



Most stimulating, however, were the findings of the bioassays on mouse skin with the TPM from cigarette tobaccos rich in nitrate [Fig. 3, (3)].

These "tars"\*\* were found to have lower PAH content and significantly reduced tumorigenicity as well.

Attempts to interpret the mechanism of selective PAH reduction in smoke of tobaccos rich in nitrate content led to a working hypothesis, which is, in part, supported by experimental evidence (3): Alkali nitrates decompose in the hot zones of a tobacco product into alkali nitrites, and further, into oxides, oxygen and nitrogen oxide. The latter compound oxidizes to a minor extent

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\*\* The term "tar" is used throughout for convenience. It is acknowledged that it is not chemically accurate in the strictest sense.

to  $NO_2$  (5). Pyrolysis studies indicate that thermically activated nitrogen oxides may react as scavengers in the reducing environment of the hot zone. *Kallianos* et al. have demonstrated that the catechol yield in the smoke of Burley tobacco is inversely proportional to the amount of nitrate (4). The presence of 4-nitrocatechol in the smoke of cigarettes made from high-nitrate



tobaccos would be in line with our working hypothesis, although the investigators suggest its formation by nitration.\* *Sloan* and *Sublett* reported an increased yield of methyl nitrite in the smoke of nitrate rich cigarette tobaccos (8), a result supporting our concept. The inhibition of the BaP formation by the presence of nitric oxide, or addition of salts generating NO during the pyrolysis of cellulose, as reported by *Robb* et al. (7) can be interpreted similarly.



Therefore, it is suggested that during the actual pyrosynthesis of new compounds the combination of pyrolytically formed organic radicals is inhibited by their competitive reaction with nitrogen oxides. Consequently, we would expect that the yield of aromatic hydrocarbons is also reduced. The identification and increased yield of aliphatic and aromatic nitrohydrocarbons in the smoke of nitrate rich tobaccos may serve as additional support for our hypothesis of PAH-inhibition.

\* During a discussion at the 21st Tobacco Chemists' Research Conference, Kallianos stated that 4-nitrocatechol has been demonstrated only when smoke is condensed in cold traps. It has not been found present in smoke condensed on Cambridge filters. In this case we would concur with the nitration concept of catechol. This communication presents data on the isolation, identification and quantitative determination of primary and secondary nitroalkanes in cigarette smoke. In addition, evidence for the dependence of the nitroalkane yields in the smoke as a function of the nitrate content of tobacco will be discussed.

# METHODS AND MATERIAL

# Cigarette Smoking

United States blended nonfilter cigarettes, 85 mm in length, were conditioned, selected and smoked as previously reported (2). In series A on each of the 4 channels of the CSM-10, the smoke of four cigarettes is drawn through a Cambridge filter and, subsequently, through a 250 ml gas wash bottle filled with about 50 ml of water. The water in the gas wash bottle is replaced when 25 cigarettes have been smoked. For series A, 200 cigarettes are smoked. In series B, 20 cigarettes are puffed on one channel. Washings and loaded Cambridge filters are placed in distillation flasks of 1000 or 500 ml.

# Internal Standard

Preliminary investigations have revealed two pertinent facts. First, the losses during the analysis are greater for nitromethane and nitroethane than for nitroalkanes with lower vapor pressure. Secondly, no significant peak could be detected in the gas chromatogram where 2-nitro-n-butane was expected. Based on these observations, two internal standards were used. For the analysis of nitromethane and nitroethane two micrograms of highly purified nitromethane-C<sup>14</sup> (195,600 dpm; International Chemical and Nuclear Corp., City of Industry, California) in 0.5 ml toluene were added. As internal standard for the less volatile nitrohydrocarbons 200  $\mu$ g of purified 2-nitro-n-butane in 1 ml ether and for series B, 20  $\mu$ g of 2-nitro-n-butane in 1 ml ether were added. In order to assure that primary and secondary nitroalkanes have comparable losses during the analysis, we occasionally used 2  $\mu$ g of 2-nitropropane-1-C<sup>14</sup> (80,000 dpm; Mallinckrodt Nuclear, Orlando, Florida) dissolved in 1 ml toluene. Prior to the analytical procedure, the solvents containing these internal standards were pipetted into the distillation flasks already filled with the loaded Cambridge filters and the contents of the gas wash bottles.

The Nuclear Chicago Scintillation System 720 counted with an efficiency of  $72-73^{0/0}$  for the unquenched C<sup>14</sup>-labelled nitromethane and 2-nitropropane when dissolved in toluene with  $0.4^{0/0}$  PPO (2.5-diphenyloxazole) and  $0.05^{0/0}$  POPOP (p-bis[2(5-phenyloxazolyl)]benzene) as scintillators. During the analyses reported here, we did not observe any significant quenching. However, this may be experienced when working with nitroparaffin concentrates exceeding  $0.05^{0/0}$  in the scintillator solution. If such quenching occurs, a correction curve with nitromethane must be established.

#### FIGURE 4

Determination of nitroalkanes in cigarette smoke



# Concentrating of Nitrohydrocarbons

After loaded Cambridge filters, washings and internal standards are placed in the distillation flasks, 50 ml of  $2-N H_2SO_4$  are added for the analysis in series A and 5 ml 2-N H<sub>2</sub>SO<sub>4</sub> for the analysis in series B. The nitrohydrocarbons are steam-distilled. In order to avoid losses by insufficient condensation, the receiving flask is connected via vacuum adapter with a gas wash bottle containing 50, respectively 20 ml of 2-N NaOH. The distillation is terminated after collecting about 500 ml distillate for series A and 200 ml for series B. The condensates are extracted 3 times with 150 or 50 ml ether respectively. The combined ether layers (450 or 150 ml) are subsequently extracted with the 2-N NaOH solution from the gas wash bottles. This extraction is repeated twice more with 50 ml or 20 ml of 2-N NaOH. The combined NaOH extracts are acidified under cooling with 5-N H2SO4 which is carefully dropped into the flask through a Liebig-condenser. The acidic solution is extracted 3 to 4 times with ether, and subsequently the combined organic phase is extracted once with saturated NaHCO3-solution. The ether layer is then dried over dessicated sodium sulfate, filtered and carefully concentrated by distillation of the solvent via a distilling head for steam distillation until a concentrate of about 5 ml, however not less than 3 ml, is obtained. The temperature of the water bath for the distillation should not exceed  $55^{\circ}$  C. The separation scheme is outlined in Figure 4.

# Gas Chromatography

Series A One to five microliters of the concentrate are injected into a gas chromatograph (Perkin-Elmer Model 800 with flame ionization detector). The separation of the primary and secondary nitroalkanes is achieved on a 2 m stainless steel column with an i. d. of 3 mm, filled with  $5^{0/0}$  Apiezon N and  $5^{0/0}$  Armeen SD on fire brick, mesh 60-80 (Applied Science Laboratories, State College, Pennsylvania). The carrier gas is helium; its inlet pressure is about 2.5 atm. The separation is started at  $50^{\circ}$  C for 2 minutes and is then linearly programmed at a rate of  $4^{\circ}$  C per minute up to  $100^{\circ}$  C and is thereafter continued isothermally. The temperature of the injection-port stays at  $180^{\circ}$  C, the detector temperature is kept at  $200^{\circ}$  C. A Leeds & Northrup recorder ranging from 0.0 to 1.0 mv is employed. With the attenuation at 50 about 1.5  $\mu$ g of nitromethane reach the full scale of the recorder chart. A typical gas chromatogram, as obtained from the nitroalkane concentrate, is shown in Figure 5. According to the retention times obtained with reference compounds (Table 1), the major peaks appear to be nitromethane, nitroethane, 2-nitropropane, 1-nitropropane, 1-nitro-n-butane and 1-nitro-n-pentane.

For the collection of material for mass spectrometry, the same gas chromatography system is used with a splitter ratio of 4:1 which allows injections of up to 100 µl ether concentrate.

Series B For the analysis of nitroalkanes in the smoke of 20 cigarettes, we employed a gas chromatograph with an electron capture detector, in this study a Varian Aerograph 1200. This detector system, specifically sensitive to nitro-compounds allows the determination of concentrations down to about  $0.1-0.01 \mu g$  nitroalkanes in 1 microliter solution. The separation was achieved on the same column as in series A with nitrogen as carrier gas and an inlet pressure



of about 2.0 atm. This chromatography was isothermal at  $80^{\circ}$  C, the injection-port and detector temperature were kept at  $160^{\circ}$  C. The recorder was the same as employed in series A. With an attenuation of 8 about 0.05 µg reach the full scale of the recorder chart. A linear dose response was obtained for 0.01–1.0 µg of a nitroalkane.

# FIGURE 5

Gas chromatogram of a concentrate of nitro-compounds from cigarette smoke

Column :	2 m, 3 mm
Phase:	5% Apiezon N + 5% Armeen SD on fire brick 60/80
Temperature :	50° for 2 min., programming: 4° per min. until 100° C
Carrier gas:	Helium
Detector:	Flame ionization

A typical gas chromatogram obtained from a nitrohydrocarbon concentrate is shown in Figure 6 with 2-nitro-n-butane as internal standard. According to the retention times and relative retention volumes (Table 1), the presence of at least 7 nitro-compounds in the concentrate is indicated. This gas chromatography system confirms the presence of the same nitroalkanes implied by the first system.

# Identification of Nitroalkanes

For the identification of the primary and secondary nitrohydrocarbons we employed basically the gas chromatography system A with 4:1 splitter. In order to collect the effluents corresponding to the maxima in the chromatogram, we tried at first to use an open U-tube fitted directly into the septum of the effluent tube. This trap was cooled by immersion in drv ice acetone. However, the system proved to be ineffective for the collection of the more volatile nitro-compounds and, thus, had to be improved. The material indicated by retention time to emerge at the peak of 1-nitro-n-pentane was collected from 10 injections resulting in an estimated effluent of about 150  $\mu$ g. After rechromatography and collection, the material was used for mass spectrometric analysis. These analyses were carried out by the Morgan-Schaffer Corp. in Montreal, Canada, with a Hitachi Perkin-Elmer RMU-6D instrument. Lacking a set-up for direct introduction of gas chromatographic effluents into a mass spectrometer, we had to develop a specific system for the more efficient collection of 50 µg and more of a nitroalkane with lower molecular weight. A U-tube with long sidearms is attached to the end of the gas chromatography column and is immersed in ice water. After each fractional collection both arms are sealed and are reopened only briefly for collection of the corresponding material from the next separation. After five such fractional collections, the closed U-tube is cooled in liquid nitrogen, one arm is opened and the tube is evacuated. Finally, the tube is heated to about 100° C and the now vaporized nitrohydrocarbon is injected directly through the capillary inlet into the mass spectrometer. Using this technique, one is able to obtain a mass spectrum for 500 µg or even less of a nitrohydrocarbon. However, for estimating the relative abundance of the fragments one has to disregard with this arrangement the ions m/e 32 and m/e 28 since some air is introduced with the sample. Otherwise the relative abundance of all other ions can be estimated within  $\pm 10\%$ .



#### Reference Compounds

Nitromethane and nitroethane were obtained from Matheson, Coleman & Bell, Rutherford, N. J.; 1-nitropropane and 2-nitropropane from Eastman Organic Chemicals, Rochester, N. Y.; 1-nitro-n-butane, 2-nitro-n-butane, nitrocyclopentane and nitrocyclohexane from K & K Laboratories, Plainview, N. Y., and 1-nitro-n-pentane from Fluka, A. G., Buchs, S. G., Switzerland. All these reference compounds were purified by gas chromatography (System 1).

	FIGURE 6
Gas d of nitro-co	hromatogram of a concentrate mpounds from cigarette_smoke
Column:	2 m, 3 mm
Phase :	5 % Apiezon N + 5 % Armeen SD on fire brick 60/80
Temperature :	80° C
Carrier gas:	N <sub>2</sub>
Detector:	Electron capture

		Gas-chromotography-systems				
	Nitroalkanes	Flame ionization detector		Electron capture detector		
Name	Formula	Molecular weight	Relative retention time (min)	Molar retention volume	Relative retention time (min)	Molar retention volume
Nitromethane	CH <sub>3</sub> -NO <sub>2</sub>	61.04	1.0	1.0	1.0	1.0
Nitroethane	CH <sub>3</sub> -CH <sub>2</sub> -NO <sub>2</sub>	75.07	1.68	1.47	1.38	0.86
2-Nitropropane	CH₃-CH-CH₃	89.09	2.10	1.48	1.77	0.621
	NO <sub>2</sub>					
1-Nitropropane	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -NO <sub>2</sub>	89.09	3.15	1.50	2.46	0.620
2-Nitro-n-butane	CH3-CH2-CH-CH3 NO2	103.12	3.9	1.50	3.23	0.620
1-Nitro-n-butane	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -NO <sub>2</sub>	103.12	5.6	2.16	5.00	0.582
1-Nitro-n-pentane	CH <sub>3</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -CH <sub>2</sub> -NO <sub>2</sub>	117.15	7.9	2.50	10.76	1.42
Nitrocyclopentane	-NO2	115.13	8.1	2.27	11.6	1.40
Nitrocyclohexane	-NO2	129.8	11.3	2.39	14.1	1.51

Relative retention times and relative molar retention volumes of several primary and secondary nitroalkanes\*

\* Details of the gas chromatographic system, see Method Chapter.

# Quantitative Determination

In series A 1–3  $\mu$ l, in series B 1  $\mu$ l nitroalkane concentrate were injected into the gas chromatograph and three 50  $\mu$ l samples were transferred into counting vials filled with scintillator solution. The observed quenching effects did not exceed the experimental variation of the counting method, which is better than  $\pm 10/0$ .

#### RESULTS

#### Qualitative Analysis

Retention times in the two gas chromatographic systems and the relatively sensitive electron capture detector system for nitroalkanes suggested the presence of nitromethane, nitroethane, 1-nitropropane, 2-nitropropane, 1-nitro-n-butane and 1-nitro-n-pentane in cigarette smoke. Furthermore, according to the gas chromatographic pattern it appeared not unlikely that the smoke concentrate contained a second nitropentane.

The fragmentation pattern of the effluent of the major last peak of the gas chromatogram was identical with that of synthetic 1-nitro-n-pentane [Figure 7, (1)]. The materials corresponding to the other maxima in the gas chromatogram were separately collected with the second system



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## Six nitroalkanes in cigarette smoke – series A\* (ug per cigarette)

Number of analyses**	Nitromethane	Nitroethane	2-Nitropropane	1-Nitropropane	1-Nitro-n- butane	1-Nitro-n- pentane
1	0.561	1.04	1.03	0.780	0.720	0.221
2	0.518	1.12	1.14	0.690	0.732	0.234
3	0.519	1.12	1.05	0.742	0.703	0.199
4	0.541	1.08	1.09	0.689	0.728	0.228
5	0.478	0.97	1.12	0.740	0.684	0.196
Average (m)	0.523	1.08	1.08	0.728	0.713	0.215
Standard variations (Sv)	0.031	0.07	0.05	0.039	0.020	0.017
Variation coefficient $\left(\frac{Sv \times 100}{m}\right)$	5.9 °/o	5.7 %	4.3 °/o	5.2 %	2. <b>8 º/</b> o	8.1 %

\* 85 mm U.S. blended cigarette without filter tip smoked under standard conditions.

\*\* Each analysis is completed with 200 cigarettes with internal standards nitromethane-C<sup>14</sup> and 2-nitro-n-butane.

and the fragmentation patterns of the effluents compared with those of authentic reference compounds (Fig. 8). Despite the fact that we could not utilize possible ions m/e 32 and m/e 28, the other fragments sufficed for the identification of nitroparaffins. With the exception of nitromethane which gave its molecular ion m/e 61, the other nitroalkanes did not show molecular ions or, if so, only with low intensity. This observation and the relative abundance of fragmentation ions which are formed by  $\alpha$ -cleavage of the nitroalkanes and the occurence of fragments with m/e 27 and m/e 30 from the tested nitroparaffins are in agreement with data from the API reference (1). Figure 8 shows the fragmentation pattern of 5 isolated nitroparaffins from cigarette smoke and allows the identification of nitromethane, nitroethane, 1-nitropropane, 2-nitropropane and 1-nitro-n-butane. The fragmentation pattern from material collected from the peak before 1-nitro-n-pentane is suggestive of an isomeric nitropentane, and **possibly of 2-nitro-n-pentane**. However, this identification must remain tentative, since we lack the corresponding reference compound at this time.

#### Quantitative Analysis

The results of five quantitative analyses of the mainstream smoke of a U. S. 85 mm cigarette without filter tip for the six identified nitroalkanes are summarized in Table 2. The variation coefficients range from  $2.8^{0/0}$  to  $8.1^{0/0}$ . Assuming that the unidentified nitrohydrocarbon is a nitropentane other than 1-nitro-n-pentane, we estimate its concentration in the smoke of one cigarette to be about 0.06 µg.

Table 3 presents the data based on  $3 \times 20$  cigarettes analyzed in the series B. These results are in agreement with those of the series A, for which we smoked 200 cigarettes each. Although the individual values appear somewhat higher for series B than for series A, this difference may be due to obtaining the same brand of cigarettes on the open market at different times.

Number of analyses**	Nitromethane	Nitroethane	2-Nitropropane	1-Nitropropane	1-Nitro- n-butane	1-Nitro- n-pentane
1	0.612	1.12	1.24	0.821	0.791	0.224
2	0.584	1.11	1.18	0.782	0.784	0.218
3	0.582	1.19	1.22	0.761	0.843	0.212
Average (m)	0.592	1.14	1.21	0.788	0.806	0.218
Series A Average (m)	0.523	1.08	1.08	0.728	0.713	0.215

TABLE 3

Six nitroalkanes in cigarette smoke – series B\* (ug per cigarette)

\* 85 mm U.S. blended cigarette without filter tip smoked under standard conditions.

\*\* Each analysis is completed with 20 cigarettes with internal standards nitromethane-C14 and 2-nitro-n-butane.

# Six nitroalkanes in cigarette smoke\* (ug per cigarette)

\* 85 mm U. S. blended cigarette without filter tip smoked under standard conditions. Each analysis was completed with 200 cigarettes. A-represents the average value of 5 analyses with nitromethane-C<sup>14</sup> and 2-nitro-n-butane as internal standards. B-analyses, internal standard 2-nitropropane-1-C<sup>14</sup>.

Furthermore, as we and others experienced, results for trace components from a limited number of cigarettes lack reproducibility within the accepted analytical limits. Nevertheless, it could be shown that the electron capture detector system is an excellent analytical tool for the analysis of nitroalkanes. Despite great precautions in evaporating ether, we obtained a concentrate of nitroalkanes in which the loss of these compounds during the eight analyses was estimated to range from 10 to  $30^{\circ}/_{\circ}$  with the highest losses for nitromethane and nitroethane. However, we found later that dry-freezing (dry ice-acetone cooling) for 24 hours under a vacuum of 0.1 mm Hg leads to an oily residue and losses of only less than  $10^{\circ}/_{\circ}$  for nitromethane.



Data from a smoke analysis with 2-nitropropane-1-C14 as internal standard are given in Table 4. As to be expected, the values for nitromethane and nitroethane are too low since the internal standard 2-nitropropane-1-C<sup>14</sup> is less volatile than the internal nitromethane-C<sup>14</sup> standard. However, the values obtained for the other nitroalkanes with 2nitropropane-1-C14 as internal standard are in agreement with those of 2-nitro-nbutane as internal indicating standard, no different losses for primary and secondary nitroalkanes.

# FIGURE 8

Mass spectra of isolated and reference compounds

# Six nitroalkanes in the smoke of various cigarettes\* (ug per cigarette)

Cigarette	% NO3	Nitromethane	Nitro- ethane	2-Nitropropan <del>e</del>	1-Nitropropane	1-Nitro-n- butane	1-Nitro-n- pentane
Virginia	0.24	0.186	0.270	0.220	0.183	0.189	trace**
Blend A	0.35	0.523	1.08	1.08	0.728	0.713	0.215
Blend B	0.5	0.596	1.07	1.19	0.703	0.773	0.239
Burley I	2.15	0.709	1.48	1.43	1.10	0.940	0.295
Burley II	2.6	0.730	1.33	1.54	1.18	1.20	0.315
Burley V	2.75	0.790	1.48	1.49	0.80	0.70	0.330
Burley III	3.46	0.885	1.71	1.79	1.26	1.23	0.320
Burley IV	4.62	1.05	2.20	2.18	1. <b>42</b>	1.41	0.390
Blend B + 8.3% NaNO3	6.1	1.45	2.58	2.42	1.58	1.58	0.515
Blend B + 5% Cu (NO3) 2	3.8	0.962	1. <b>79</b>	1.80	1.36	1.10	0.415

All cigarettes were 85 mm long without filter tips and were smoked under standard conditions. The data for Blend A are average values from 5 analyses; all other results are average values from 2 analyses. For each analysis 200 cigarettes were smoked.

Less than 0.05

# Nitroalkanes in the Smoke of Different Cigarettes

In order to evaluate our working hypothesis that the nitrogen oxides, deriving from the alkali nitrates in the tobacco, may react in the hot zones as scavengers with the pyrolytically formed radicals, the mainstream smoke of eight cigarettes and two experimental cigarettes were analyzed for nitroalkanes. The results are presented in Table 5 and demonstrate an interdependence of the concentration of nitroparaffins in the smoke with the nitrate concentration of cigarette tobacco.

# DISCUSSION

The described analytical method permitted the identification of nitroalkanes in cigarette smoke. This group of compounds has not so far been reported to be present in tobacco smoke (3,9). Our specific interest in the pyrosynthesis of polynuclear aromatic hydrocarbons, especially those which are known animal carcinogens, led to the observation that these compounds can be reduced in the smoke when nitrate rich tobaccos are selected or when alkali nitrates are



FIGURE 9 Benzo(a)pyrene, nitromethane and nitroethane in the mainstream smoke of 100 g cigarette tobacco

added to tobacco. The reduction of the PAH is selective compared to the reduction of total particulate matter. The smoke condensate from nitrate rich tobaccos could be demonstrated to have significantly reduced tumorigenicity to mouse skin (3). The latter results are in line with our previous observations that a selective reduction of PAH, especially of benzo(a)pyrene, parallels a reduction of the tumorigenicity of the "tars" (3,9).

Based on these experimental observations we became interested in an exploratory study of the mechanism leading to the reduction of PAH in cigarette smoke. For nitrate rich tobaccos we hypothesized that the pyrolytically formed nitrogen oxides react with organic radicals which are formed by pyrolysis of non-volatile organic tobacco constituents. This reaction tends to inhibit the pyrosynthesis of aromatic hydrocarbons. The quantitative data of the newly identified nitroalkanes strongly suggest a correlation between the nitrate concentration in the tobacco and yield of these compounds in the smoke. Since one may assume that nitrohydrocarbons are formed in the hot zones from C,H-radicals and nitrogen oxides, the findings of the present study fall in line with our concept that nitrogen oxides tend to inhibit the pyrosynthesis of polynuclear aromatic hydrocarbons in tobacco smoke. Figure 9 shows an inverse relation between the yield of BaP on the one hand and nitromethane and nitroethane on the other hand. However, since the pyroformations in tobacco smoke of PAH, as well as nitroalkanes are most complex, a quantitative correlation can be established only by analyzing the burning cone of a cigarette directly for NO, NO<sub>2</sub> and nitroalkanes and indirectly for PAH.

# SUMMARY

An analytical method for the isolation and identification of nitroalkanes in cigarette smoke was developed. The analytical procedure includes water steam-distillation of cigarette smoke condensate and several extraction steps. The final concentrate of the nitroparaffins is separated into individual components by gas chromatography on  $5^{0}/_{0}$  Apiezon N and  $5^{0}/_{0}$  Armeen SD on fire brick. Utilizing a flame ionization detector system for the final analysis, 200 cigarettes had to be smoked. When an electron detector system was utilized the smoking of 20 cigarettes sufficed for the analysis of six nitroalkanes. These were identified by relative retention times and volumes, and the fragmentation patterns of the individual nitroalkanes. The presence of a seventh nitroalkane was ascertained and it was tentatively identified as a nitropentane. The smoke of one 85 mm blended United States cigarette without filter tip contained 0.53 µg nitromethane, 1.1 µg nitroethane, 1.1 µg 2-nitropropane, 0.73 µg 1-nitropropane, 0.71 µg 1-nitron-butane and 0.22 µg 1-nitro-n-pentane.

The analysis of the smoke of 10 different cigarettes demonstrated a correlated increased yield of six nitroparaffins with increased nitrate content of the cigarette tobacco. This result supports our working hypothesis that nitrogen oxides formed during the pyrolysis of alkali nitrate in tobaccos react as scavengers with organic radicals in the hot zones of burning cigarettes and tend to inhibit the synthesis of aromatic hydrocarbons, including those with known carcinogenic effects to experimental animals.

## ZUSAMMENFASSUNG

Die vorliegende Studie beschreibt eine Methode zur qualitativen und quantitativen Analyse von primären und sekundären Nitroalkanen im Rauch von Cigaretten. Die Methode umfaßt Wasserdampfdestillation, Anreicherung der primären und sekundären Nitroparaffine in einer schwach sauren Fraktion und gaschromatographische Auftrennung. Zur Identifizierung der Nitroverbindungen dienten gaschromatographische und massenspektrometrische Daten. Die Verwendung eines Gaschromatographen mit F.I.D. bedingt das Abrauchen von 200 Cigaretten pro Analyse, die Verwendung eines Instrumentes mit Elektronendetektor jedoch das Abrauchen von nur 20 Cigaretten.

Der Rauch einer 85 mm langen und filterlosen US-Cigarette enthält 0,53  $\mu$ g Nitromethan, 1,1  $\mu$ g Nitroäthan, 1,1  $\mu$ g 2-Nitropropan, 0,73  $\mu$ g 1-Nitropropan, 0,71  $\mu$ g 1-Nitro-n-butan und 0,22  $\mu$ g 1-Nitro-n-pentan. Als siebentes Nitroalkan wurde ein Nitro-i-pentan unbekannter Struktur nachgewiesen.

Die quantitativen Daten für die Nitroparaffine im Rauche von 10 Cigaretten aus Tabaksorten mit verschiedenen Nitratgehalten demonstrieren eine Abhängigkeit der Konzentration von Nitroalkanen vom Nitratgehalt des Cigarettentabaks. Dieses Ergebnis steht im Einklang mit unserer Arbeitshypothese zur Reduktion von aromatischen Kohlenwasserstoffen (K.W.) im Rauch nitratreicher Tabake. Aromatische K.W. bilden sich in den heißen Zonen eines brennenden Tabakproduktes in mindestens zwei getrennten Stufen, der Pyrolyse, Bildung von C,H-Radikalen, und der Pyrosynthese, der Bildung von aromatischen K.W. aus C,H-Radikalen. Ein kleiner Anteil dieser pyrosynthetisierten Verbindungen sind aromatische Polycyclen, die sich als krebserregend im Tiertest erwiesen haben. Während der eigentlichen Pyrosynthese erscheint eine erhöhte Konzentration von thermisch aktivierten Stickstoffoxiden durch Konkurrenzreaktion mit C,H-Radikalen die Bildung von Aromaten zu reduzieren. Die Identifizierung von Nitroalkanen und ihre quantitative Abhängigkeit vom Nitratgehalt des Tabaks stehen in Übereinstimmung mit früheren Ergebnissen.

# RÉSUMÉ

Une méthode a été développée pour l'isolement et l'identification de nitroalcanes primaires et secondaires dans la fumée de cigarette. Le procédé analytique consiste en la distillation à la vapeur d'eau du condensat de la fumée et en plusieurs étapes d'extraction. Le concentré final des nitroparaffines est séparé en composants individuels par chromatographie gazeuse avec colonne garnie de  $5^{0/0}$  Apiézon N et  $5^{0/0}$  Armeen SD sur brique réfractaire. La détection par ionisation de flamme nécessite le fumage de 200 cigarettes, tandis que le fumage de 20 cigarettes suffit pour l'analyse de 6 nitroalcanes à l'aide d'un détecteur à capture d'électrons. Les nitroalcanes sont identifiés individuellement par étude de leur temps et volume de rétention relatifs et de leur diagramme de fragmentation. On a pu mettre en évidence la présence d'un septième nitroalcane regardé, en attendant, comme un nitropentane. Dans la fumée d'une cigarette américaine de 85 mm de longueur, sans filtre, on a trouvé 0,53 µg de nitrométhane; 1,1 µg de nitroéthane, 1,1 µg de 2-nitropropane, 0,73 µg de 1-nitropropane, 0,71 µg de 1-nitro-n-butane et 0,22 µg de 1-nitro-n-pentane.

L'analyse quantitative de la fumée de dix cigarettes différentes indique d'une manière évidente que le rendement en nitroalcanes s'élève à mesure que la teneur du tabac en nitrates croît. Ce résultat appuie l'hypothèse de travail selon laquelle les oxides d'azote formés au cours de la pyrolyse des nitrates alcalins du tabac réagissent comme fixateurs (scavengers) de radicaux organiques dans la zone d'incandescence de la cigarette brûlante et ont ainsi tendance à inhiber la synthèse d'hydrocarbures aromatiques, y compris ceux dont l'activité cancérogène chez les animaux en expérience est connue.

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