Correlation of Polynuclear Aromatic Hydrocarbon Formation Between Pyrolysis and Smoking*

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INTRODUCTION

Currently, there is considerable interest in the relationship between tobacco constituents and the polynuclear aromatic hydrocarbons (PAH) of tobacco smoke. Since it is well documented that at high temperatures organic materials produce PAH (1, 2, 3), numerous pyrolytic studies have shown that the hexane or petroleum ether (PE) extract of tobacco contains major precursors of smoke PAH (4, 5, 6). Since the hexane extract is composed of numerous classes of organic compounds (7, 8), much controversy has arisen over which compound and/or classes of compounds in the extract are the most efficient PAH producers (9, 10, 11).

Many of the studies have been criticized because conditions differing from actual smoking were employed (11). We felt that such criticism could be minimized if one would use pyrolytic conditions which would produce tobacco pyrolyzate PAH distributions similar to those found in cigarette smoke condensate (CSC). By applying recently developed methods for obtaining smoke PAH profile data (12, 13, 14), it appeared possible to determine pyrolytic conditions which yield correlatable pyrolyzate-CSC PAH profiles. The use of these conditions in a systematic search for smoke PAH precursors should allow determination of the contribution of the various leaf constituents to the total PAH content of tobacco smoke. We, therefore, studied the effects of different pyrolysis temperatures on PAH levels produced by whole tobacco, its PE extract, and the residual extracted tobacco (marc). We compared the gas chromatographic PAH profiles of the pyrolyzates with those of CSC to determine the optimum pyrolytic temperature. Results of these experiments are reported, as are yields of phenols and acids in the pyrolyzates.

EXPERIMENTAL

Petroleum Ether Extraction

A sample (335 g) of finely ground (16 mesh) 1971 South Carolina flue-cured tobacco (redried, unaged) was extracted with petroleum ether (PE) (boiling point 30–60 °C) in a Soxhlet apparatus for 64 h. The extract was subsequently filtered and taken to dryness on a rotary evaporator to yield 26.5 g (7.9% yield) of material. The extracted tobacco (marc) was removed from the extraction thimble and oven-dried (90 °C, 5 h) to a weight of 308.5 g. Portions of the marc and PE extract were used for pyrolytic studies.

Solvents

All solvents used were glass-distilled Burdick and Jackson** type and were redistilled prior to use.

Pyrolysis

The pyrolyses were performed in a horizontal 5 ft. × 2 in. inside diameter Vycor tube inserted into a Lindberg Heavy-Duty furnace. Nitrogen flow through the pyrolysis system was maintained at 150 ml/min. The oven was pre-heated to the desired temperature (650, 700, 750, 800, 850 ± 5 °C at the center of the heated zone). The sample (5 g of PE extract, 10 g of tobacco or marc) contained in a Vycor boat was placed at the cool end of the pyrolysis tube. The boat was drawn in the direction of the gas flow at a rate of 33.97 cm/min by a wire attached to a kymograph (gear driven rotating drum) into the center of the heated zone. Products were flushed from the tube by the nitrogen into traps consisting of an uncooled 5 l expansion flask, an ice-water-cooled cold finger, two dry ice-cooled cold

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** Reference to a company or product name does not imply approval or recommendation by the USDA.

fingers, and a gas-washing bottle containing ethyl ether (E)/benzene (B) (1:1, v/v) and 0.5 N NaOH, in a 1:1 volume ratio.

Fractionation of Pyrolyzates

After quantitative removal of the products from the traps with the E-B solvent, the pyrolyzates were fractionated as outlined in Figure 1. The pyrolyzates were partitioned between the E-B solvent and 1 N NaOH. The aqueous layer was adjusted to pH 6.5 with concentrated HCl and phenolics were extracted with E. The aqueous layer was further adjusted to pH 2.0 and the acids were extracted with E.

The neutral fraction (NF) was divided into two portions representing 10% and 90% of the solution. The one-tenth portion was taken to constant weight for determination of the NF yield. The larger portion was reduced in volume and slurried with 40 g of silicic acid (SA) [pre-washed with methanol (M), and activated at 150 °C overnight] and 100 ml each of B and isooctane. Benzene and excess isooctane were removed on a rotary evaporator until only an isooctane-wet, SA-sample mixture remained. The NF-SA mixture was slurried with PE and poured onto a 100 g SA column. The column was eluted with a total of 500 ml of PE (which included the amount used for slurrying the NF-SA sample) to yield fraction F-PE. Paraffins, which could interfere with PAH analyses were eluted by the PE (15). The PAH-containing fraction (F-BPE) was eluted with 1500 ml of 25% B in 75% PE.

Figure 2. UV absorption curves for elutions of F-BPE and standards used in determination of limits for GF cuts GF-B and GF-C.

Elutions with 1000 ml volumes of B, E, and M yielded F-B, F-E, and F-M, respectively. The yields of NF and its subfractions are listed in Table 1.

The PAH were isolated from F-BPE by the Bio-Beads SX-12 gel filtration (GF) system, previously detailed (12, 13). For the pyrolyzates of the PE extract, concentrated F-BPE solutions in B were diluted to 10 ml. A 1 ml sample of each of these solutions was placed onto the GF system, and 2 ml were evaporated to dryness for determination of fraction weight. The F-BPE solutions of tobacco and marc pyrolyzates were transferred to 2 ml volumetric flasks. A 1 ml portion was placed on the GF system and the remainder was used for determination of fraction yield.

PAH in GF fractions were collected in two portions as determined by the elution volumes of trimethyl-

Figure 1. Pyrolyzate fractionation scheme.

Table 1. Effect of pyrolysis temperature on the yields of chromatographic fractions from pyrolyzate neutrals.

Pyrolysis temperature Fraction	PE extract					Tobacco			Marc
	650 °C	700 °C	750 °C	800 °C	850 °C	650 °C	700 °C	750 °C	700 °C
	Yield (g/100 g pyrolyzed) ^a								
Neutrals	24.84	22.27	26.19	28.17	24.43	6.19	5.15	5.08	4.71
	% by weight of neutrals ^b								
Petroleum ether (PE)	10.2	10.6	13.1	6.9	7.8	7.3	7.3	7.5	3.1
Benzene-petroleum ether (BPE)	51.3	62.5	50.6	59.5	72.8	16.0	25.9	31.3	6.6
Benzene (B)	5.6	6.8	8.7	8.8	14.1	14.2	9.9	14.1	10.8
Ethyl ether (E)	15.8	16.2	14.5	7.7	9.5	28.1	22.9	25.4	4.5
Methanol (M)	7.2	6.8	6.0	4.1	4.4	22.8	21.6	19.7	28.3
Total	90.1	102.3	92.9	87.0	108.6	88.4	87.6	98.0	53.3

a: Based on one determination.

b: Based on total weight of neutral placed on silicic acid column.

naphthalene (GF fractions 36-39, portion GF-B) and fluoranthene (GF fractions 40-60, portion GF-C) (Figure 2).

Cigarette Smoke Condensate PAH Isolation

The Kentucky 1R1 Reference cigarettes were smoked under standard conditions (16, 17). The PAH in the CSC from 90 cigarettes were isolated according to our procedure (13). Thus, the CSC dissolved in B-M-E was extracted with water, and organic solubles subjected to SA column and GF chromatography as described above to yield PAH isolates GF-B and GF-C.

Gas Chromatographic (GC) Analysis

The PAH in GF-B and GF-C were analyzed on a Hewlett-Packard 5830 reporting gas chromatograph equipped with a 15 ft. × 1/8 in. stainless steel column packed with 5% Dexsil 300 GC on 100/120 mesh Chromosorb W-AW. Helium flow rate was 40 ml/min (measured at 90 °C). Oven temperature was held for 10 min at 90 °C after sample injection, increased to 325 °C at 2 °/min, then held at 325 °C for 45 min.

The injection port and flame ionization detector were maintained at 290 and 350 °C, respectively. Response data were obtained for the major parent PAH. Hexatriacontane (C₃₆) and pentatriacontane (C₃₅) were used as internal standards for PAH quantitation of pyrolyzates and CSC, respectively.

The gas chromatograms of all GF-B and GF-C portions were divided into the following fractions by retention times: naphthalene, relative retention time (RRT) 0.238-0.568; fluorene, RRT 0.581-0.731; phenanthrene, RRT 0.754-0.951; pyrene, RRT 0.964-1.180; chrysene, RRT 1.203-1.359; and benzopyrene, RRT 1.384-1.594. The total peak areas of each fraction from the two chromatograms were combined. Assuming detector responses identical to those of the major parent PAH, GC volatile PAH weights were then calculated for each fraction (Table 2).

Phenols were analyzed on a 6 ft. × 1/8 in. glass column containing 10% SP-2401 on 100/120 mesh Supelcoport. The injector and flame ionization detector were maintained at 215 °C. After a pre-hold at 40 °C for 3 min, the oven was temperature programmed to 200 °C at 4°/min. The helium flow rate was 35 ml/min.

Table 2. Total GC PAH fraction weights of pyrolyzate PAH.

Pyrolysis temperature PAH group	PE extract					Tobacco			Marc
	650 °C	700 °C	750 °C	800 °C	850 °C	650 °C	700 °C	750 °C	700 °C
	Amount (g/100 g) ^a								
Naphthalene	3.08	4.88	4.29	4.45	4.70	0.20	0.32	1.31	0.14
Fluorene	1.04	1.41	1.28	1.02	0.62	0.14	0.11	0.26	0.087
Phenanthrene	1.25	1.89	2.32	3.05	2.60	0.17	0.16	0.32	0.090
Pyrene	0.68	1.03	1.39	1.88	1.85	0.072	0.063	0.18	0.040
Chrysene	0.20	0.33	0.55	0.94	0.90	0.017	0.018	0.070	0.009
Benzopyrene	0.13	0.17	0.47	1.21	1.51	0.003	0.019	0.053	0.006
Total	6.38	9.71	10.30	12.55	12.18	0.60	0.69	2.19	0.33
% Benzene-petroleum ether (BPE)	25.7	43.6	39.5	44.8	55.4	10.6	48.0	48.0	106.0

a: Based on GC volatile material assuming detector response for group is identical to that of major parent PAH; average of two determinations (± 5%).

Table 3. Comparison of PAH distributions in gel filtration cut GF-C of CSC and pyrolyzates.

Peak No.	Relative retention times ^a	Compound	CSC	Pyrolyzate			
				700 °C Tobacco	700 °C Marc	700 °C PE	850 °C PE
				% Composition ^b			
1	0.238	naphthalene	3.35	9.77	13.32	9.39	19.19
2	0.292	dimethylindenes	1.22	0.32	0.48	1.14	—
3	0.300	dimethylindenes unidentified	1.03 f	0.56 c d	0.69 c d	0.98 c d	—
4	0.335	2-methylnaphthalene	2.42	5.11	3.10	9.18	1.91
5	0.350	1-methylnaphthalene	2.98	5.09	3.48	6.75	1.27
6	0.372	unidentified trimethylindene	0.38 f	0.31 f	0.30 f	0.54 f	—
7	0.396	biphenyl trimethylindene	0.47 c e	0.26 c e	0.23 f	0.54 c e	1.27 f
8	0.434	dimethylnaphthalenes 1-vinylnaphthalene	1.09 c d	0.57 c d	0.59 c c	2.55 c d	0.21 f
9	0.450	dimethylnaphthalenes 2-vinylnaphthalene	2.95 c c	2.55 c c	2.05 c d	5.68 c c	1.28 f
10	0.467	dimethylnaphthalenes	1.12	0.55	0.56	1.36	—
11	0.476	acenaphthylene dimethylnaphthalene	2.16 c c	8.71 c e	6.64 f	3.78 c e	6.54 f
12	0.503	acenaphthene dimethylnaphthalene	0.70 c c	0.50 c e	0.54 f	0.56 c e	0.41 f
13	0.526	trimethylnaphthalene dibenzfuran	1.38 d c	0.64 d c	0.87 e c	0.31 f	0.09 f
14	0.545	trimethylnaphthalene naphthofuran	0.46 e c	0.79 e c	0.85 e c	0.49 c d	0.06 f
15	0.555	methyl-2-vinylnaphthalene trimethylnaphthalenes	1.21	0.68 c e	0.79 c e	1.07 c d	—
16	0.568	methyl-2-vinylnaphthalene trimethylnaphthalene	0.80 e c	0.20 c c	0.17 c d	0.80 c c	—
17	0.581	1-methylacenaphthylene	1.36 c	2.32	2.10	1.64	0.21
18	0.588	fluorene methylacenaphthylene	5.19 c d	4.09 c c	3.69 c c	2.80 c c	2.45 c e

Table 3 (continued)

Peak No.	Relative retention time ^a	Compound	CSC	Pyrolyzate			
				700 °C Tobacco	700 °C Marc	700 °C PE	850 °C PE
				% Composition ^b			
19	0.596	methylacenaphthylene 9-methylfluorene	1.00 c c	1.57 f	1.44 f	1.17 f	0.01 f
20	0.612	methylacenaphthene benz(e)indene	1.58 c c	1.35 c c	1.51 c c	1.16 c d	0.07 f
21	0.629	methylacenaphthene	0.22 d	1.09 f	0.99 f	0.57 f	0.01 f
22	0.636	benz(f)indene	0.93	1.78	1.88	0.79	0.19
23	0.647	methylnaphthofuran dimethylacenaphthene	0.17 c d	0.05 c d	0.56 f	0.09 d c	—
24	0.660	methylnaphthofuran dimethylacenaphthene	0.24 c d	0.07 c d	0.34 c d	0.36 d c	—
25	0.682	2- & 3-methylfluorene methylnaphthofuran dimethylacenaphthene	3.56 c d d	1.37 c d d	1.62 c d d	1.84 c f d	0.28 c c
26	0.694	1- & 4-methylfluorene dimethylacenaphthene methylidibenzofuran	2.99 c d e	0.96 c d e	1.19 c d c	1.44 c c e	0.31 c c
27	0.704	methylbenz(e)indene dimethylacenaphthylene dimethylacenaphthene	0.85 c d d	0.63 c c d	0.85 c c d	0.97 c c d	—
28	0.716	methylbenz(f)indene methylbenz(e)indene dimethylacenaphthene	1.13 c e d	0.97 c c d	1.24 c c d	1.22 c c c	0.07 c d
29	0.731	methylbenz(f)indene dimethylacenaphthene	1.29 c d	0.45 c d	0.71 c d	0.56 c d	—
30	0.754	phenanthrene	5.73	7.91	7.90	4.16	17.38
31	0.760	anthracene	2.06	1.73	—	1.15	—
32	0.843	2- & 3-methylphenanthrene	5.23	3.33	2.65	3.44	1.39
33	0.859	1- & 9-methylphenanthrene 1- & 2-methylanthracene	3.88 c d	4.87 c d	4.21 c c	3.91 c d	1.98 c d
34	0.877	9-methylanthracene 4-methylphenanthrene 2-phenylnaphthalene	0.43 c c	0.71 c d d	0.91 c d d	0.50 c d d	0.88 f
35	0.905	dimethylphenanthrene dimethylanthracene unidentified	0.61 c d	0.23 c c	0.55 c c	0.05 c e	0.03 c e

Table 3. Comparison of PAH distributions in gel filtration cut GF-C of CSC and pyrolyzates (cont'd.).

Peak No.	Relative retention time ^a	Compound	CSC	Pyrolyzate			
				700 °C Tobacco	700 °C Marc	700 °C PE	850 °C PE
				% Composition ^b			
36	0.924	dimethylphenanthrene dimethylantracene unidentifieds	0.70 c d	0.16 c d	0.32 c d	0.63 c d	0.14 c d c
37	0.941	dimethylphenanthrene dimethylantracene unidentifieds	3.44	2.04 c d	1.99 c d	2.61 c f	0.26 c f c
38	0.951	dimethylphenanthrene unidentifieds	0.84	0.60 c d	0.66 c d	0.70 f	0.11 c c
39	0.964	fluoranthene dimethylphenanthrene	2.58 c e	1.70 c e	1.58 c e	1.14 c e	4.95 c e
40	0.973	acephenanthrylene (aceanthrylene)	0.95	1.60 c	1.45 c	0.61 c	1.38 c
41	0.993	di- or trimethylphenanthrene	0.71	0.82	0.90	0.19	0.11
42	1.000	pyrene cyclopentenophenanthrene	2.90 c e	3.17 c e	2.83 f	2.22 c	7.40 f
43	1.032	trimethylphenanthrene(s) cyclopentenophenanthrene	0.98 c c	0.47 c c	0.79 c c	0.41 c c	—
44	1.044	8-methylfluoranthene trimethylphenanthrene	1.68 c e	0.59 c e	1.37 c e	0.33 c e	0.32 c e
45	1.067	benzofluorene (1,2; 2,3; 3,4) 1- & 2-methylfluoranthene	2.67 c c	2.54 c d	2.41 c d	1.01 c d	1.59 c d
46	1.080	2-methylpyrene trimethylphenanthrene	1.73 c c	1.58 c c	1.32 c c	0.91 c c	0.56 f
47	1.095	1- & 4-methylpyrene	2.26 c	2.44 f	2.87 f	1.70 f	1.05 f
48	1.124	methyl-1,2-benzofluorene dimethylfluoranthene	0.61 c c	0.20 c d	0.50 c d	0.36 c d	0.12 f
49	1.133	methylbenzofluorene dimethylpyrene dimethylfluoranthene	1.17 d c c	0.36 c d d	0.79 c d e	0.70 c d d	0.12 c c
50	1.150	dimethylpyrene dimethylfluoranthene methylbenzofluorene	0.79 c d d	0.06 c d c	0.27 c d c	0.21 c c d	—
51	1.160	dimethylpyrene benzo(c)phenanthrene methylbenzofluorene	0.93 c e d	0.53 c e c	1.05 c e c	0.61 c d e	0.66 f
52	1.169	dimethylpyrenes methylbenzofluorenes	0.72	0.16 c d	0.49 c d	0.34 c d	—

Table 3 (continued)

Peak No.	Relative retention time ^a	Compound	CSC	Pyrolyzate			
				700 °C Tobacco	700 °C Marc	700 °C PE	850 °C PE
				% Composition ^b			
53	1.180	dimethylpyrenes benzo(g,h,i)fluoranthene	0.89 c d	0.07 c e	0.81 c e	0.39 c e	0.43 f
54	1.203	1,2-benzanthracene	1.30	2.54	2.19	1.69	2.29
55	1.207	chrysene triphenylene	c d	f	f	f	3.49 c e
56	1.230	3,4-dimethylenepyrene trimethylpyrene	0.11 c c	0.08 c c	0.35 c c	0.17 c c	0.35 f
57	1.276	methyl-1,2-benzanthracene methylchrysene	0.53 c c	0.86 c c	0.72 c c	1.10 c c	0.60 c c
58	1.288	methyl-1,2-benzanthracene methylchrysene	0.08 d c	0.36 c c	0.60 c c	0.24 c c	0.21 c c
59	1.294	methylchrysenes	0.11 c	0.50	0.94	0.39	0.73
60	1.310	3,4-trimethylenepyrene dimethylchrysene	0.02 c d	0.12 c e	0.28 c d	0.10 c e	—
61	1.336	dimethylchrysene dimethyl-1,2-benzanthracene	0.22 c c	0.15 c c	0.81 c c	0.37 c c	
62	1.359	dimethylchrysene dimethyl-1,2-benzanthracene	0.13 c c	0.13 c c	0.09 c c	0.35 c d	
63	1.384	benzo(b,j,k)fluoranthenes	0.33	0.43	0.28	0.27	2.79
64	1.401	benzo(a)fluoranthene	0.16	0.18	0.08	0.09	0.86
65	1.429	benzo(e)pyrene benzo(a)pyrene	0.34 d c	0.75 c c	0.54 c c	0.57 c c	3.78 c c
66	1.449	perylene methylbenzofluoranthene	0.04 c c	0.16 c d	0.29 c e	0.31 c e	1.31 f
67	1.464	methylbenzofluoranthenes	0.02	0.07	0.12	0.24	1.00
68	1.484— 1.492	methylbenzo(a)pyrene methylbenzo(e)pyrene	0.10 c c	0.29 c c	0.10 c c	0.32 c c	1.06 c c
69	1.508	methylbenzo(a)pyrene methylbenzo(e)pyrene methylperylene	0.09 c c d	0.32 c c d	0.06 c c d	0.36 c c d	0.47 c c e
70	1.541	methylbenzo(a)pyrene methylbenzo(e)pyrene methylperylene	0.06 c c d	0.04 c c c	0.02 c c d	0.08 c c c	0.15 c c

Table 3. Comparison of PAH distributions in gel filtration cut GF-C of CSC and pyrolyzates (cont'd.).

Peak No.	Relative retention times ^a	Compound	CSC	Pyrolyzate			
				700 °C Tobacco	700 °C Marc	700 °C PE	850 °C PE
				% Composition ^b			
71	1.551	methylbenzo(a)pyrene methylbenzo(e)pyrene indeno(1,2,3-cd)fluoranthene dibenz(ghi)anthracene	—	0.02 c c e e	0.03 c e	0.03 c e e	0.06 c c e d
72	1.586	dimethylbenzo(a)pyrene dimethylbenzo(e)pyrene dibenz(a,h)anthracene	—	0.04 c c d	—	0.05 c c d	0.25 f
73	1.594	dimethylbenzo(a)pyrene dimethylbenzo(e)pyrene dibenz(a,h)anthracene dibenz(a,c)anthracene	—	0.10 d d c c	0.02 e e c c	0.04 d d c c	0.58 c c
74	1.612	indeno(1,2,3-cd)pyrene	0.02	0.13	0.04	0.35	2.01
75	1.691	benzo(ghi)perylene	—	0.12	0.08	0.11	0.30
76	1.703	anthanthrene methylindeno(1,2,3-cd)pyrene	—	0.02 c c	0.02 c d	0.01 c c	1.20 c c
77	1.719	methylindeno(1,2,3-cd)pyrene methyl(g,h,i)perylene	—	0.01 c c	—	0.01 c c	0.56 c c
78	1.751	methyl(g,h,i)perylene methylindeno(1,2,3-cd)pyrene dimethylindeno(1,2,3-cd)pyrene	—	0.01 c c d	—	0.01 c c d	0.08 c c d
79	1.782	methyl(g,h,i)perylene methylananthracene dimethylindeno(1,2,3-cd)pyrene	—	0.01 c d c	—	—	0.01 c d c
80	1.835	dibenzpyrene	—	0.07	—	0.04	0.04

a: Relative to pyrene ± 0.003 relative retention units.

b: Based on total peak area from RRT 0.238 through 1.835, assuming unity response.

c: Greater than 30% of peak area (based on UV intensity).

d: Accounts for 5 to 30% of peak area (based on UV intensity).

e: Less than 5% of peak area (based on UV intensity).

f: Only component appearing in UV spectra.

g: Possibly vinylphenanthrenes.

The volatile acids (C₁–C₇) were analyzed on a 6 ft. × 1/8 in. glass column containing 10% SP-1200 – 12% H₃PO₄ on 80/100 mesh Chromosorb W-AW. After a pre-hold at 40 °C for 3 min, the oven was programmed at 4°/min to 140 °C. The injector and flame ionization detector were maintained at 160 °C, and helium flow rate was 35 ml/min.

The volatile acids and phenols were analyzed on a Varian 2100 gas chromatograph and peak areas were measured with a Hewlett-Packard 3380A integrator. Using GC quantitation data obtained from analytical standards, areas for all identified components were corrected for differences in detector response.

After treatment with diazomethane, the non-volatile acids (C₁₂–C₃₆) were analyzed with a Hewlett-Packard 5750 gas chromatograph equipped with 10 ft. × 1/8 in.

stainless steel column containing 10% Apolar 10-C on 100/120 Chromosorb W-AW. Temperature was programmed at 2°/min from 100 to 230 °C, and helium flow rate was 40 ml/min. Injector and flame ionization detector were maintained at 200 and 250 °C, respectively. Detector response data from a series of fatty acid methyl esters (C₁₂–C₃₂) were plotted vs. relative retention times (relative to C₁₁ methyl ester). Response factors used to calculate weight yields from methyl esters were obtained from this plot. Peak areas were measured by an Autolab System IV integrator.

Identification

The phenols and acids (volatile and non-volatile) were identified by GC retention data only. The PAH in GF-C from the tobacco, PE extract, and marc pyrolyzed at

700 °C, and from the PE extract pyrolyzed at 850 °C were subjected to preparative GC collection on a thermal conductivity Hewlett-Packard 5750 gas chromatograph, under conditions identical to those used to obtain analytical data. UV data were obtained on each collected GC peak. The PAH for the pyrolyzates (Table 3) were identified by UV and GC retention data (12, 13). When GC retention data were not known, the UV identifications were verified by mass spectral data. The PAH present in the other pyrolyzates were identified by GC retention data only. CSC PAH were identified according to *Snook et al.* (12).

RESULTS AND DISCUSSION

Since the majority of the PAH pyrolytic precursor studies on tobacco and its components have been performed at high temperatures, the PE extract was initially pyrolyzed at 850 °C. The pyrolyzate NF was subjected to the PAH analysis scheme used to analyze CSC PAH (13, 14). The gas chromatograms of PAH present in GF-C (containing parent PAH, most of their methyl derivatives, and some multi-methyl compounds of the higher members) from CSC of Kentucky 1R1 Reference cigarettes (Figure 3) and the 850 °C pyrolyzate of the PE extract (Figure 4) differed greatly in the PAH distribution (Table 3). Although higher levels of PAH material in the pyrolyzate NF allowed for more quantitative recovery of naphthalene and other small ring PAH (13), a significant reduction of methylated compounds was apparent in pyrolyzate vs. CSC. As expected, pyrolysis at 850 °C favored the production of the more thermally stable parent PAH as well as larger ring systems. It can, therefore, be concluded, that even though the burning cone in a cigarette reaches and exceeds 850 °C (9), most of the PAH in cigarette smoke are produced at lower temperatures.

Table 4. Selected PAH levels in tobacco PE extract pyrolyzates as a function of temperature.

PAH	Amount (mg/100 g) ^a				
	Pyrolysis temperature (°C)				
	650	700	750	800	850
Naphthalene	348	630	800	1460	2500
Methylnaphthalenes	709	1230	864	917	478
Fluorene	188	258	292	492	328
Phenanthrene-anthracene	212	326	664	1530	1780
Methylphenanthrenes-methylanthracenes	389	582	736	760	378
Fluoranthene	56	70	116	302	328
Pyrene	102	132	232	534	686
Methylpyrenes	119	155	220	240	136
1,2-Benzanthracene-chrysene-triphenylene	82	108	208	194	226
				288	336
Benzo(e)pyrene-benzo(a)pyrene	32	38	114	304	373
Methylbenzopyrenes	32	44	74	126	124

a: Corrected for differences in detector response; methyl PAH assumed to respond identical to parent; average of two determinations.

Figure 5. Effects of pyrolytic temperatures on the levels of the phenanthrene fraction (PH Fr.), phenanthrene-anthracene (PH-A), and methylphenanthrenes-anthracenes (Me PH-A) in PE extract pyrolyzates.

Since the PAH distribution in the 850 °C pyrolyzate did not favorably compare with that in CSC, the PE extract was also pyrolyzed at 650, 700, 750, and 800 °C. The yields of GC PAH fractions and of individual, selected PAH are listed in Tables 2 and 4, respectively, as functions of pyrolytic temperatures. The following general trends in product distributions were observed. The level of parent PAH increased with temperature. The level of methyl components increased until 800 °C, then declined with higher pyrolytic temperatures. The relative amounts of the high ring compounds increased with increased pyrolytic temperatures. For example, at 650 °C, the phenanthrene-anthracene to benzopyrenes ratio was about seven to one and dropped to less than five to one at 850 °C.

Examples of results can be more clearly illustrated graphically. The effect of pyrolytic temperature on phenanthrene-anthracene and the corresponding phenanthrene fraction levels is given in Figure 5. The yield of the GC phenanthrene fraction increased almost linearly with pyrolytic temperatures up to 800 °C, then decreased rapidly. The yield of parent compounds increased over

Table 5. Selected methyl PAH to parent PAH ratios in CSC and tobacco pyrolyzates.

PAH	Methyl PAH to parent PAH ratios			
	CSC (1R1)	Pyrolysis temperature (°C)		
		650	700	750
Phenanthrene-anthracene	2.79	1.52	1.83	0.94
Pyrene	1.30	1.36	1.30	0.92
Benzo(e)pyrene-benzo(a)pyrene	0.74	0.78	0.63	0.89

Figure 3. Gas chromatogram of PAH in gel filtration cut GF-C from condensate of 1R1 Reference cigarettes.

Figure 4. Gas chromatogram of PAH in gel filtration cut GF-C in 850 °C pyrolyzate of PE extract of flue-cured tobacco (divided into "PAH fractions").

Figure 6. Effects of pyrolytic temperatures on the levels of the pyrene fraction (P Fr.), pyrene (P), and methylpyrenes (Me P) in PE extract pyrolyzates.

the whole range, with largest increases occurring between 700 and 800 °C. The level of methyl components increased very slowly to 800 °C and then dropped off rapidly. Note the relative methyl PAH to parent PAH levels (Figure 5) and compare them with those of CSC (Table 5). For condensate, the methyl phenanthrene-anthracene levels were considerably larger than those of their parent compounds. Therefore, the data indicated that in order to obtain a pyrolysis PAH profile similar to that for CSC, pyrolysis temperatures lower than 750 °C should be employed. Corresponding data for the pyrene fraction are illustrated in Figure 6. Like the phenanthrene fraction, the yield of the pyrene GC fraction began to decrease at temperatures higher than 800 °C. In CSC, the methylpyrene levels were higher than those of the parent (Table 5). These data also indicated that to obtain a pyrolysis PAH profile similar to that in CSC, pyrolytic temperature should be below 750 °C. In contrast to the three- and four-ring PAH, the level of the benzopyrene fraction increased markedly with pyrolytic temperature (Figure 7). The level of methylbenzopyrenes relative to that of benzopyrenes decreased with increasing pyrolytic temperature. Because the levels of methylbenzopyrenes in CSC were about 75% of the parent PAH (Table 5), the benzopyrene data also suggested an optimum pyrolytic temperature of about 700 °C for the PE extract.

The temperature study clearly showed that pyrolysis of the PE extract at a temperature in the range of 650 to

Figure 7. Effects of pyrolytic temperatures on the levels of the benzopyrene fraction (BP Fr.), benzopyrenes (BP), and methylbenzopyrenes (Me BP) in pyrolyzates of PE extract.

750 °C in our system would yield a PAH profile most similar to that of condensate. However, the PAH components of CSC result from total reactions of all the constituents in burning tobacco. Therefore, whole tobacco was pyrolyzed at 650, 700 and 750 °C, and the resulting PAH compared with CSC PAH of 1R1 cigarettes. The methyl PAH to parent PAH ratios for the tobacco pyrolyzates were compared to the ratios for CSC (Table 5). Again, condensate results agreed best with the 650 and 700 °C data. The relative amounts of

Table 6. Comparison of relative abundances of selected PAH in CSC and tobacco pyrolyzates.

PAH	Relative amount ^a			
	CSC (1R1)	Pyrolyzates		
		650 °C	700 °C	750 °C
Phenanthrene-anthracene	3.3	2.4	4.2	3.5
Methylphenanthrenes-methylanthracenes	9.2	3.6	7.7	3.2
Fluoranthene	0.8	0.5	0.5	0.6
Pyrene	1.0	1.0	1.0	1.0
Methylpyrenes	1.3	1.4	1.3	0.9
1,2-Benzanthracene-chrysene-triphenylene	0.5	0.9	0.8	0.5
Benzo(e)pyrene-benzo(a)pyrene	0.2	0.1	0.3	0.3

a: Relative to pyrene; corrected for difference in detector response.

Figure 8. Gas chromatogram of PAH constituents in gel filtration cut GF-C from 700 °C tobacco pyrolyzate.

selected PAH in condensate and in tobacco pyrolyzates are compared in Table 6. At all three temperatures, the fluoranthene level was lower in all the pyrolyzates than in CSC. In agreement with the data on the pyrolyzate of the PE extract, the methyl PAH levels in the 750 °C pyrolyzate of whole tobacco were also lower than those in CSC. The best overall compromise appeared to be pyrolysis at 700 °C. Subsequently, peak by peak comparison of the corresponding gas chromatograms showed that the GC profile of the 700 °C tobacco pyrolyzate (Figure 8) was the most similar to that of smoke condensate (Figure 3).

Unlike the chromatogram of the 850 °C PE extract pyrolyzate (Figure 4), that of GF-C from the tobacco pyrolyzed at 700 °C (Figure 8) showed qualitatively all of the alkyl PAH. In the 700 °C tobacco pyrolyzate, the dimethylnaphthalene levels (Peaks 8, 9, and 10) were lower in CSC and acenaphthylene (Peak 11) was a major component. Further comparisons showed that the 700 °C tobacco pyrolyzates contained lower levels of fluorene (Peak 18) and of fluoranthene (Peak 39). In the 700 °C pyrolyzate, there was a decrease in the amount of benzo(b,j, and k)fluoranthenes (Peak 63) relative to the benzopyrenes (Peak 65), probably due to the lower fluoranthene levels in the pyrolyzate. However, it was apparent that the PAH distributions in GF-C from both condensate and pyrolyzate were similar. The gas chromatograms of the small ring, mono-, and multi-methyl PAH contained in GF-B from the CSC and 700 °C tobacco pyrolyzate are illustrated in Figures 9 and 10, respectively.

Again, a peak by peak comparison indicates the presence of the same alkyl PAH components.

Thus, in our system, a temperature of 700 °C produced a pyrolytic PAH profile for both the whole tobacco and its PE extractables similar to that of CSC. However, the PAH from the PE extract were somewhat higher in alkyl PAH than that from tobacco. This indicated that the PE extractable material from tobacco produces not only parent PAH but also their alkyl derivatives. In order to substantiate this conclusion, we pyrolyzed the PE-extracted tobacco, or marc, at 700 °C. The levels of GC PAH fractions in the 700 °C pyrolyzates from tobacco, PE extract, and marc are compared in Table 7.

Table 7. Comparison of PAH fraction levels in 700 °C pyrolyzates from tobacco, PE extract, and marc.

PAH group	Amount ^a		
	Tobacco (mg/100 g)	PE extract ^b (mg/8 g)	Marc ^c (mg/92 g)
Naphthalene	320	390	130
Fluorene	110	110	86
Phenanthrene	160	150	78
Pyrene	63	82	35
Chrysene	18	26	8
Benzopyrene	19	14	5
Total	690	772	342

a: Based on GC volatile material, assuming detector response for the group identical to that of major parent PAH.

b: PE extract represents about 8% of tobacco weight.

c: Marc represents about 92% of tobacco weight.

Figure 9. Gas chromatogram of PAH constituents in gel filtration cut GF-B of smoke condensate of 1R1 cigarettes.

Figure 10. Gas chromatogram of PAH constituents in gel filtration cut GF-B of 700 °C tobacco pyrolyzate.

Table 8. Comparison of the phenol yields in 700 °C pyrolyzates of tobacco, PE extract, and marc.

Phenol	Yield ^a		
	Tobacco (mg/100 g)	PE extract ^b (mg/8 g)	Marc ^c (mg/92 g)
Phenol	361	5	262
o-Cresol	75	5	63
m/p-Cresol	162	5	118
Ethyl- and dimethyl-phenols	91	13	70
1-Naphthol	16	3	11
2-Naphthol	14	2	11

a: Corrected for differences in GC detector response based on one determination.

b: PE extract represents about 8% of tobacco weight.

c: Marc represents about 92% of tobacco weight.

The data are based on the relative amount of each fraction present in whole tobacco. Thus, 8 g of PE extract, 92 g of marc, and 100 g of tobacco are mutually equivalent. In agreement with previous work (4), PE extract produced the most PAH. The 8 g of extract yielded two to three times more PAH than the 92 g of the marc. The sum of the weights of PAH produced separately by the PE extract and the marc was considerably higher than the amount produced by the whole tobacco. This is readily explainable by the mechanisms of a series of bimolecular reactions that occur in the pyrolytic synthesis of the PAH, as proposed by *Badger et al.* (1, 2, 3). Concentrating the major PAH precursors into the PE extract greatly increased the concentration of the PAH-producing intermediates in the gas phase. Since a higher concentration of reactive species greatly increases the rate of a bimolecular reaction, the pyrolysis of the PE extract would be expected to produce substantially higher levels of PAH, as observed. When tobacco is pyrolyzed, the PAH-producing intermediates, resulting from the decomposition of PE solubles, are diluted with other non-PAH producing products so that the probability of a PAH-forming bimolecular collision decreases. As a result, the amount of PAH produced during tobacco pyrolysis decreases.

The previous discussion on the PAH distribution in the pyrolyzates is based on the comparison of GC retention data with those PAH known to be present in CSC (12, 13). To verify that the pyrolyzates contained the same PAH components, we first fractionated, by preparative GC, the PAH in GF-C of the 700 °C tobacco, marc, and PE extract and the 800 °C PE extract pyrolyzates. To facilitate the identification of individual components, we collected as many cuts as practical for each GC peak. The cuts were subjected to UV analysis, and the resulting spectra compared with those obtained for CSC PAH. The resulting component identifications are given in Table 3.

The data in Table 3 substantiated all of the previous discussions. At 700 °C, a tobacco pyrolyzate PAH composition was very similar to that of CSC. The low level of methylated PAH in the marc pyrolyzate clearly shows that the PE extract is not only the major PAH

Table 9. Comparison of the acid yields in 700 °C pyrolyzates of tobacco, PE extract, and marc.

Acid	Yield ^a		
	Tobacco (mg/100 g)	PE extract ^b (mg/8 g)	Marc ^c (mg/92 g)
Volatile acids C ₁ —C ₇ ^d	33	14	23
Non-volatile acids C ₁₁ —C ₂₄ ^e	1.2	1.1	0.4

a: Based on GC volatile material in fraction (one determination).

b: PE extract represents about 8% of tobacco weight.

c: Marc represents about 92% of tobacco weight.

d: Analyzed on 10% SP-1200/2% H₃PO₄.

e: Methyl esters analyzed on 10% Apolar 10-C.

precursor, but also produces most of the methylated PAH. At 850 °C, the methyl components were not as readily formed and/or decomposed to produce high levels of the more thermally-stable parent PAH. The 700 °C marc data indicates that PE-insoluble materials are the primary precursors of oxygenated PAH, i.e. dibenzofuran (Peak 13) and naphthofuran (Peak 14) found in both the tobacco pyrolyzate and CSC.

Since the biological effects of CSC have been focused on the relationship of PAH with phenols and acid constituents (18, 19), it was also of interest to determine the patterns of formation of phenols and acids at the various pyrolytic temperatures. Table 8 lists the GC volatile phenols formed by the 700 °C pyrolysis of tobacco, PE extract, and marc. In good agreement with previous work (5), the components of the marc were the major precursors of the volatile phenols. The sum of the phenolic yields from the PE extract and marc roughly equalled the yield from tobacco. Therefore, phenols appear to be formed by thermal decomposition mechanisms other than those proposed for the PAH.

Corresponding yield data for acids are presented in Table 9. Marc constituents were the major precursors of the volatile acids. Most of the high molecular weight fatty acid materials originated in the PE extract. By GC retention data, the major identifiable non-volatile acid in the tobacco and PE extract pyrolyzates was palmitic acid. The components in the marc pyrolyzate could not be readily identified by GC retention data.

The temperature studies showed that the phenol fraction yields reached a maximum at 700 °C and then decreased rapidly with increasing temperature. GC analyses revealed that phenol, cresol, ethyl phenols, and naphthol levels also reached maximum values at 700 °C, then decreased rapidly. The acid levels remained relatively constant from 650 to 750 °C, and then dramatically decreased. According to GC retention data, the major non-volatile acids in the 650 to 750 °C pyrolyzates were the palmitic and stearic acids. Only small amounts of the naturally occurring unsaturated acids were distilling unchanged. Above 750 °C, identification by retention data was not possible. This indicated that above 750 °C essentially all of the naturally occurring acids in the PE extract were undergoing some degree of thermal decomposition.

In summary, we found that pyrolysis of tobacco and

its PE extract at 700 °C produced PAH profiles comparable to those found in CSC. Our data confirmed that the components in the PE extract were the major precursors of the PAH in smoke and that smoke phenols are primarily formed from materials not extractable with PE.

SUMMARY

Tobacco, its petroleum ether (PE) extract, and the residual extracted tobacco (marc) were pyrolyzed at 650–750 °C, 650–850 °C, and 700 °C, respectively. Analyses of the polynuclear aromatic hydrocarbons (PAH) produced showed that the pyrolysis of the tobacco and the PE extract at 700 °C produced PAH profiles comparable to those found in cigarette smoke condensate (CSC). The data indicated that most of the alkyl PAH and the major PAH in cigarette smoke are derived from the PE extractables of tobacco. The constituents of the marc were the major precursors for phenols, oxygenated PAH, and low molecular weight acids; and those of PE extract were the major producers of high molecular weight acids.

ZUSAMMENFASSUNG

Tabak, dessen Petrolätherextrakt (PE) sowie der zurückbleibende extrahierte Tabak wurden bei Temperaturen von 650–750 °C, 650–850 °C bzw. 700 °C pyrolysiert. Die Analysen der entstandenen polycyclischen aromatischen Kohlenwasserstoffe (PAH) zeigten, daß die Pyrolyse des Tabaks und des Petrolätherextraktes bei 700 °C für die polycyclischen aromatischen Kohlenwasserstoffe Profile ergibt, die den im Zigarettenrauchkondensat (CSC) gefundenen vergleichbar sind. Die Ergebnisse deuten darauf hin, daß die meisten der polycyclischen aromatischen Kohlenwasserstoffe mit Alkylgruppe und die hauptsächlich polycyclischen aromatischen Kohlenwasserstoffe des Zigarettenrauchs auf den mit Petroläther extrahierbaren Teil des Tabaks zurückzuführen sind. Die Bestandteile des Extraktionsrückstandes waren die hauptsächlich Vorläufer der Phenole, der oxidierten polycyclischen aromatischen Kohlenwasserstoffe und der Säuren mit niedrigem Molekulargewicht; diejenigen des Petrolätherextraktes waren vorwiegend die Ursache der Säuren mit hohem Molekulargewicht.

RÉSUMÉ

On a pyrolysé du tabac, l'extrait de tabac à l'éther de pétrole (PE), et le résidu de tabac extrait à 650–750 °C, 650–850 °C et 700 °C respectivement. L'analyse des hydrocarbures polynucléaires aromatiques (PAH) produits a montré que la pyrolyse du tabac et de l'extrait PE à 700 °C donne des courbes PAH similaires à celles du condensat de fumée (CSC). Les données recueillies indiquent que la plupart des PAH alkylés et les princi-

paux PAH dans la fumée de cigarette sont dérivés des produits extractibles au PE du tabac. Les composants du résidu de tabac extrait sont les précurseurs principaux des phénols, des PAH oxygénés et des acides à bas poids moléculaire. Les constituants de l'extrait PE sont les précurseurs principaux des acides à haut poids moléculaire.

REFERENCES

1. Badger, G. M., J. K. Donnelly, and T. McL. Spotswood: *Aust. J. Chem.* 15 (1962) 605.
2. Badger, G. M., R. W. L. Kimber, and J. Novotny: *Aust. J. Chem.* 15 (1962) 616.
3. Badger, G. M., J. K. Donnelly, and T. McL. Spotswood: *Aust. J. Chem.* 18 (1965) 1249.
4. Schlottzauer, W. S., and I. Schmeltz: *Beitr. Tabakforsch.* 4 (1968) 176.
5. Schlottzauer, W. S., O. T. Chortyk, H. C. Higman, and I. Schmeltz: *Tob. Sci.* 11 (1967) 31.
6. Wynder, E. L., G. F. Wright, and J. Lam: *Cancer* 11 (1958) 1140.
7. Stedman, R. L., A. P. Swain, and W. Rusaniwskyj: *Tobacco* 154 (1962) 20.
8. Cook, C. E., M. E. Twine, C. R. Tallent, I. Harper, G. Heunisch, J. B. Lewis, and M. E. Wall: *Phytochem.* 8 (1969) 1025.
9. Wynder, E. L., and D. Hoffmann: *Tobacco and tobacco smoke — Studies in experimental carcinogenesis*; Academic Press, New York, 1967.
10. Tso, T. C.: *Preventive Medicine* 3 (1974) 294.
11. Jenkins, R. W., Jr., R. H. Newman, R. D. Carpenter, and T. S. Osden: *Beitr. Tabakforsch.* 6 (1970) 295.
12. Snook, M. E., R. F. Severson, H. C. Higman, R. F. Arrendale, and O. T. Chortyk: *Beitr. Tabakforsch.* 8 (1976) 250.
13. Severson, R. F., M. E. Snook, O. T. Chortyk, and R. F. Arrendale: *Beitr. Tabakforsch.* 8 (1976) 273.
14. Severson, R. F., M. E. Snook, R. F. Arrendale, and O. T. Chortyk: *Anal. Chem.* 48 (1976) 1866.
15. Chortyk, O. T., R. F. Severson, and H. C. Higman: *Beitr. Tabakforsch.* 8 (1975) 204.
16. Pillsbury, H. C., C. C. Bright, K. J. O'Connor, and I. W. Irish: *J. Assoc. Offic. Agr. Chem.* 52 (1969) 458.
17. Rothwell, K. (ed.): *Standard methods for the analysis of tobacco smoke*; Research Paper II, Tobacco Research Council, London, 1972.
18. Bock, F. G., A. P. Swain, and R. L. Stedman: *J. Natl. Cancer Inst.* 44 (1970) 1305.
19. Hoffmann, D., and E. L. Wynder: *J. Natl. Cancer Inst.* 52 (1971) 613.

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