Gas Chromatography/Mass Spectrometric and Nuclear Magnetic Resonance Spectrometric Studies of Carcinogenic Polynuclear Aromatic Hydrocarbons in Tobacco and Marijuana Smoke Condensates

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Comparative analyses of the polynuclear aromatic hydrocarbon fractions of tobacco and marijuana smoke condensates were carried out with the combination of chromatographic and spectral methods. The constituents of selectively enriched extracts, further purified and fractionated by a combination of LC methods, were analyzed by capillary GC/MS. Many close, yet toxicologically important, isomers of alkyl derivatives were successfully resolved. Their presence in the studied mixtures was further confirmed by Fourier-transform NMR spectrometry. Some 150 polynuclear components in each smoke material type were quantitated and tentatively identified as to parent ring structures and type of alkyl substituents.

Although some scientific evidence led, several decades ago, to a correlation between smoking and lung cancer, it was not until 1953 that the first extensive production of skin cancer in mice upon application of cigarette smoke condensate was reported (*1*). Since that time, a great deal of effort has been made to identify the carcinogenic components in smoke generated by the tobacco user.

Polynuclear aromatic hydrocarbons (PAH) comprise the largest known group of chemical carcinogens, and it is this class of compounds that is credited with the major carcinogenic activity of smoke condensates. Much has been written about their carcinogenic and mutagenic properties (*2–8*). This activity has been found mainly in tri-, tetra-, penta-, and hexacyclic compounds. The activity of larger ring structures has been very little characterized because of their limited availability and the difficulty encountered in their separation and characterization in complex mixtures.

The carcinogenic activity of a particular compound is very dependent on its structure. Shape, size, and steric factors all seem to be of importance. The addition of substituent groups in favorable positions in certain PAH often have an activating influence (*1*). For example, chrysene and the 1-, 2-, 4-, and 6-methylchrysenes have moderate tumor initiating activities, whereas 3- and, especially, 5-methylchrysenes are strong tumor initiators (*9*). In some cases, alkyl substituents can also reduce the carcinogenic activity in relation to that of the parent compound. Dibenzo[*a*,*i*]pyrene is a very potent carcinogen, but 5,8-dimethyldibenzo-[*a*,*i*]pyrene exhibits no activity at all (*1*). With these great differences in carcinogenic activity of PAH with seemingly small differences in structure, and with the specificity of biological reactions, it becomes apparent that the structural elucidation of isomers and trace constituents in PAH mixtures is especially important in providing useful information for future studies concerning the mechanism of carcinogenesis and its induction by cigarette smoke. Identification of alkylated PAH has become increasingly important with the observation that only 1–3% of the activity of tobacco smoke can be explained by nonalkylated carcinogenic PAH (*10*).

The development of capillary column gas chromatography suitable for the separation of PAH (*11–16*) provides the necessary degree of resolution to separate and identify many previously unidentified trace compounds. The high resolution provided by these columns is ideal for compound identification by combined GC-MS, and a tremendous amount of useful data can be obtained by this method. There is one drawback, however, in that the mass spectra of the different alkyl-substituted isomers do not differ sufficiently for the identification of each individual compound. On the other hand, chemical shifts and splitting patterns obtained by proton NMR can often give complementary information concerning the position of substitution (*17–20*).

The concentration of interest concerning the effects of smoking on health has been previously directed toward tobacco smoking, but with the increase in popularity of marijuana smoking and the controversies involved in its legalization, the need for detailed analyses of marijuana smoke composition is apparent. A recent study (*21, 22*) reported that exposure of human lung explants to fresh smoke from marijuana or tobacco cigarettes results in alterations of DNA and chromosomal complement. It is suggestive that this change may represent an early stage preceding malignant transformation. It was also observed that the variability of cells with deviating DNA content and chromosomal numbers was markedly greater after marijuana smoke. Because of the results of these experiments, it became of interest to compare the composition of the PAH fractions from both smoke types.

In this paper, we report the detailed analysis and identification of some 150 PAH isolated from both marijuana and tobacco smoke condensates. A discussion of the methodology employed in this study has been previously published (*13*). Our present emphasis, therefore, will be directed toward the identification of mixture constituents.

EXPERIMENTAL

Smoke Condensate Collection. Cigarettes prepared from 2000 g each of Mexican marijuana (obtained from the National Institute of Mental Health, Rockville, Md.; content of Δ^9-tetrahydrocannabinol: 2.8%) and standard tobacco cigarettes (from the Tobacco-Health Research Institute, University of Kentucky, Lexington, Ky.) were smoked with a smoking machine (*23*) under conditions so as to simulate as closely as possible the smoking habits of the

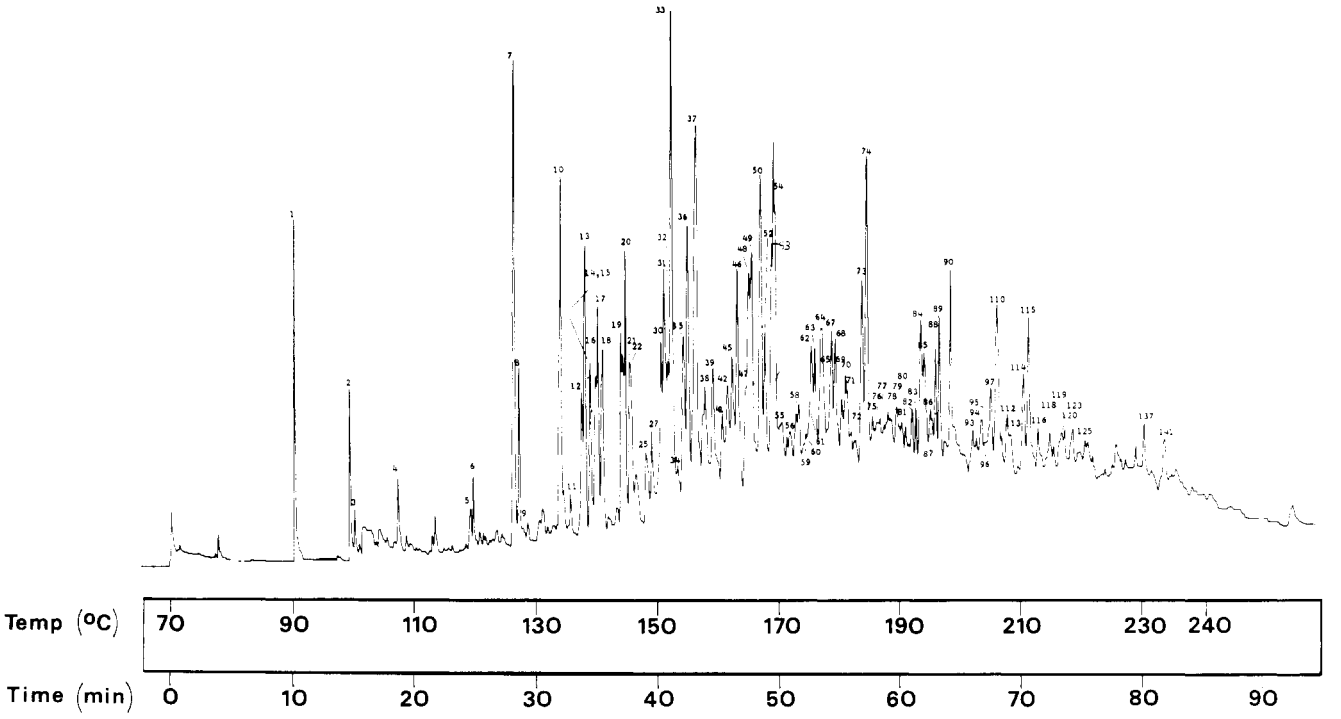


Figure 1. Capillary-column gas chromatogram of the polynuclear aromatic hydrocarbon fraction of smoke condensate from 100 g of marijuana

Column: 11.0 m × 0.26 mm i.d., glass capillary coated with SE-52 methylphenylsilicone stationary phase. Amount injected: 4 μl from a total volume of 400 μl. Key: see Table II

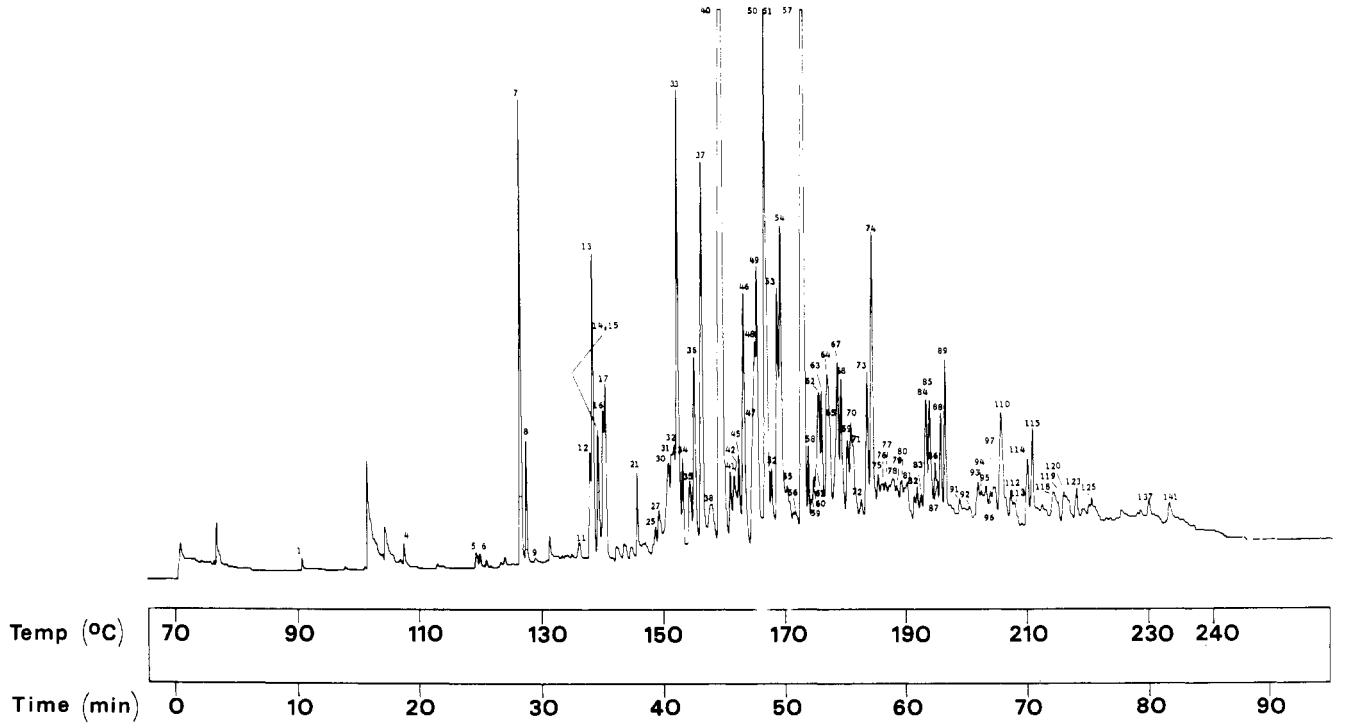


Figure 2. Capillary-column gas chromatogram of the polynuclear aromatic hydrocarbon fraction of smoke condensate from 100 g of standard research tobacco

Column: Same as in Figure 1. Amount injected: Same as in Figure 1. Key: See Table II

average tobacco cigarette smoker. The smoke condensate was collected in an acetone trap cooled with dry ice/acetone. Approximately 2000 cigarettes from each source were used in this analysis. The acetone was then removed in vacuo at 40 °C leaving a dark brown oil weighing 63.2 g and 166.5 g for tobacco and marijuana, respectively.

Solvent Partition. The concentrates were subjected to the same solvent partition scheme outlined previously (*13*), to remove acids, phenols, bases, aliphatics, etc. The PAH were concentrated in the final nitromethane extract. The dry weight of the extract was 3.8 g and 15.7 g for tobacco and marijuana, respectively. All solvents used in this study were of spectroquality and further analyzed by GLC to verify the absence of contamination. All flasks and columns containing PAH were covered with aluminum foil to minimize exposure to light.

Column Chromatography. Twelve short columns containing 2

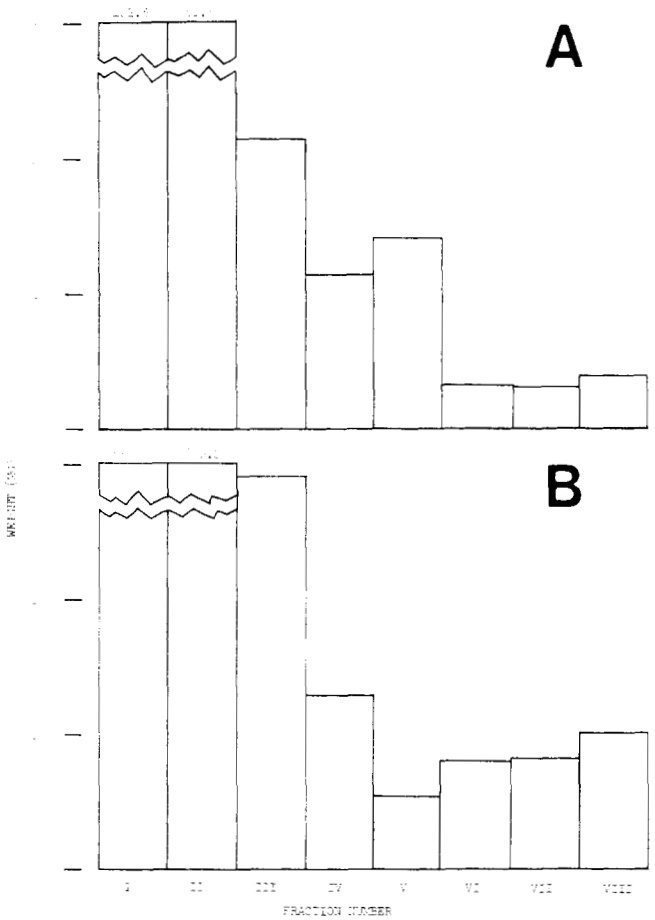


Figure 3. Histograms showing the comparison of fraction weights resulting from Sephadex LH-20 chromatography according to Table I.

(*A*) Tobacco. (*B*) Marijuana

g each of silicic acid were prepared and the nitromethane extracts of marijuana and tobacco condensates were each divided into six aliquots and transferred to the columns by prior adsorption onto 0.5 g of silicic acid. One hundred milliliters of *n*-hexane were passed through each column and collected. This step was found necessary for the removal of pigments and other uncharacterized compounds which survived the solvent partitioning steps. The six fractions of each were then recombined and evaporated to dryness in vacuo. The final weights were 4 g and 0.4 g for marijuana and tobacco, respectively.

The separation of PAH according to ring number was accomplished by Sephadex LH-20 column chromatography on a 115 cm × 1.5 cm i.d. column with isopropanol as the mobile phase (*13*). One-hour fractions were collected and bulked according to the predetermined retention volumes of PAH standard compounds (Table I). This resulted in a total of 8 fractions each for tobacco and marijuana which were subsequently evaporated to dryness in vacuo and weighed.

High-Resolution Liquid Chromatography. A commercially available bonded-phase packing (OPN/Porasil C, 37–75 μ particle

Table I. Bulking Scheme for Fractions Obtained through Sephadex LH-20 Chromatography

Fraction No.	Standard	Retention volume, ml	Bulking volume, ml
I	...	...	0–258
II	Naphthalene	300	259–336
III	Anthracene	372	337–402
IV	Fluoranthene	432	403–480
V	Triphenylene	534	481–558
VI	Benzo[*a*]pyrene	588	559–660
VII	Dibenz[*a*,*c*]anthracene	732	661–810
VIII	...	...	811–1500

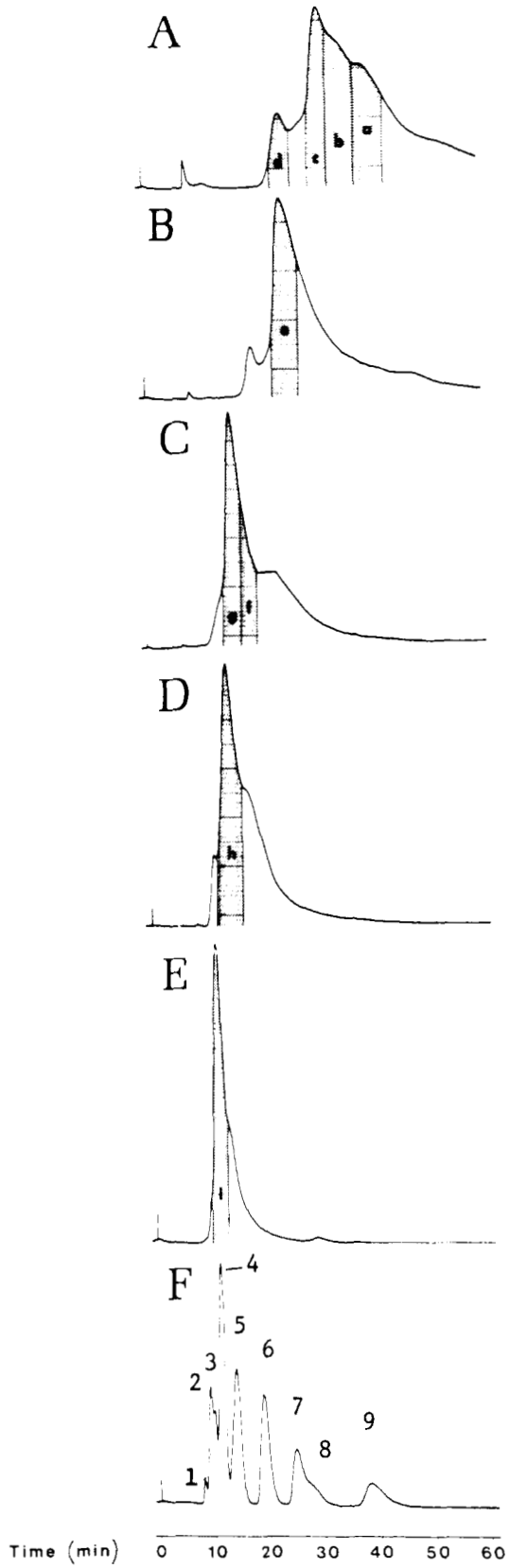


Figure 4. High-pressure liquid chromatograms of fractions resulting from Sephadex LH-20 chromatography of marijuana-smoke condensate

Chromatograms *A*, *B*, *C*, *D*, and *E* correspond to fractions VII, VI, V, IV, and III in Table I, respectively. Chromatogram *F* represents chromatography of standard compounds. Key: (1) benzene; (2) biphenyl; (3) fluorene; (4) anthracene; (5) benzo[*a*]fluorene; (6) triphenylene; (7) benzo[*a*]pyrene; (8) perylene; (9) dibenz[*a*,*c*]anthracene

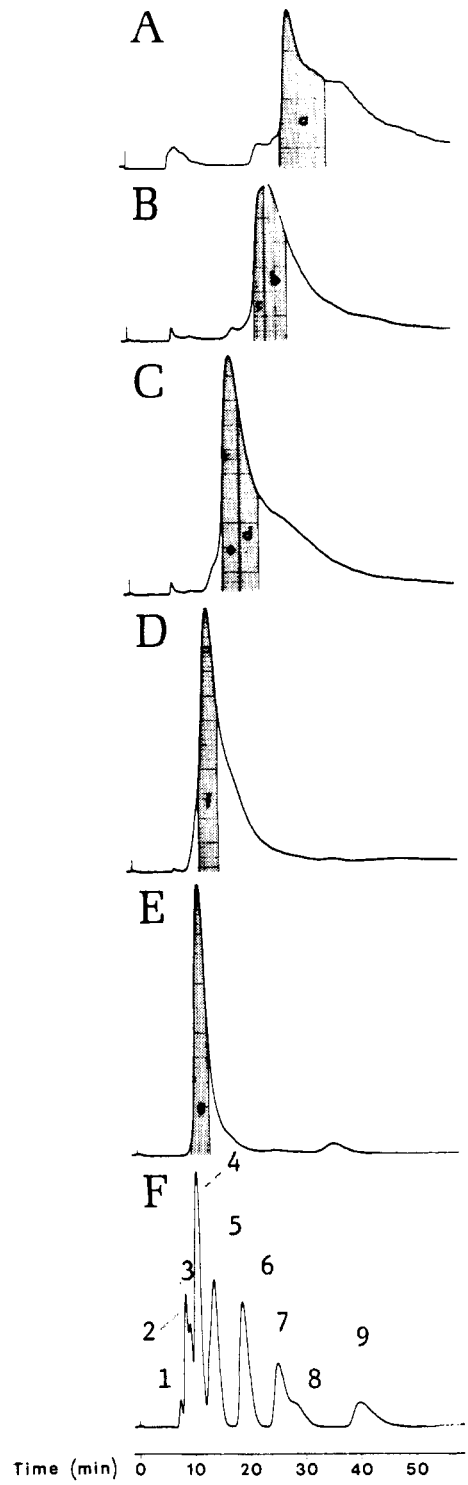


Figure 5. High-pressure liquid chromatograms of fractions resulting from Sephadex LH-20 chromatography of tobacco-smoke condensate.

Chromatograms *A, B, C, D,* and *E* correspond to fractions VII, VI, V, IV, and III in Table I, respectively. Chromatogram *F* represents chromatography of standard compounds. Key: Same as in Figure 4

size, from Waters Associates, Inc., Milford, Mass.) was dry-packed into a series of 2.0-mm i.d. columns of total length 4.25 meters. Semi-preparative separation of the fractions obtained from Sephadex LH-20 chromatography was accomplished by adding 500 μl of methylene chloride to each fraction and making a series of 30-μl injections on the column with *n*-hexane as the mobile phase. Appropriate fractions were successively collected for further investigations. A Varian Series 4100 Liquid Chromatograph with UV detector was used in this study. Flow rates of 2 ml/min were obtained at a pressure of 1500 psi.

Capillary Column Gas Chromatography. Glass capillary columns coated with SE-52 methylphenylsilicone stationary phase were used to monitor complexity, degree of cleanup, and fractionation throughout the whole analysis scheme. All gas-chromatographic work was carried out with a modified Varian 1400 gas chromatograph. With the injection port at 250°C and the analyt-ear temperature programmer. Helium was used as carrier gas. Sample solutions to be analyzed were introduced onto the capillary column via a precolumn (*24*) consisting of 2 mg of a specially treated solid support packed into a 1-mm i.d., glass tube. The solvent was then flushed off at room temperature with helium and the glass tube was placed in the modified injection port of the gas chromatograph. With the injection port at 250 °C and the analytical column at room temperature, the carrier gas was passed through the capillary for 30 min. The samples were thermally released and trapped in the first part of the column prior to temperature programming.

Semi-quantitative determination of individual PAH was accomplished by collecting smoke condensate from four batches of 100 g each of marijuana and tobacco and subjecting the condensates to the previously described analysis scheme except that the total PAH fraction was collected during Sephadex LH-20 chromatography and analyzed directly by capillary GC. Solutions containing 0.5 μg each of a series of standard PAH (phenanthrene, pyrene, triphenylene, dibenz[*a,c*]anthracene, benzo[*e*]pyrene, and coronene) were subjected to the same analysis scheme to monitor any losses or occurrence of contamination.

Peak heights in the total profiles were compared to a calibration graph constructed for phenanthrene to give the semi-quantitative values contained in Table II.

Spectral Measurements. Proton NMR spectra were recorded for solutions in $CDCl_3$ at 90 MHz on a Bruker HX-90 spectrometer with Fourier transform facility. The number of scans varied between 4 000 and 22 000 for pulse widths of 4 μs. Chemical shifts were measured relative to internal tetramethylsilane.

Mass-spectral data were obtained by direct coupling of the glass capillary column to the ion source of a Hewlett-Packard Model 5980A combined gas chromatograph/dodecapole mass spectrometer. Electron-impact ionization spectra were obtained with an electron energy of 70 eV. Chromatographic peaks were scanned at the rate of 100 amu/s and mass spectra were recorded on oscillographic paper.

RESULTS AND DISCUSSION

Literally hundreds of papers dealing with the analysis of PAH isolated from tobacco smoke have been published during the past 20 years, but two problems have always seemed to plague the analyst. First, until the advent of thermally stable high-resolution capillary columns, the resolution obtained by conventional packed GLC columns, TLC, or column chromatography was far from satisfactory for separating parent compound PAH, let alone their alkylated derivatives. Second, the isolation of "clean" PAH fractions free from interfering non-PAH compounds from such an incredibly complex organic mixture has previously been difficult. These problems have severely limited the use of GC-MS as a tool for structural elucidation and have led almost universally to detection by UV or fluorescence spectroscopy (*25*).

Total GLC profiles of the PAH fraction isolated from marijuana and tobacco smoke condensates on short, but highly efficient glass capillary columns coated with SE-52 methylphenylsilicone stationary phase (Figures 1 and 2) demonstrates the most superior resolution of these mixture components achieved to date. Many hitherto inseparable trace isomers have been resolved, thus increasing greatly the analytical information necessary to complement the more recent interest in alkylated PAH (*9, 10, 26, 27*). Table II lists those compounds identified by combined GC-MS, proton NMR, and standard compound retention data.

As mentioned earlier, mass spectrometry is limited in the information it can provide concerning the position of alkyl substitution on the ring. For this reason, larger amounts of marijuana and tobacco condensates were subjected to the

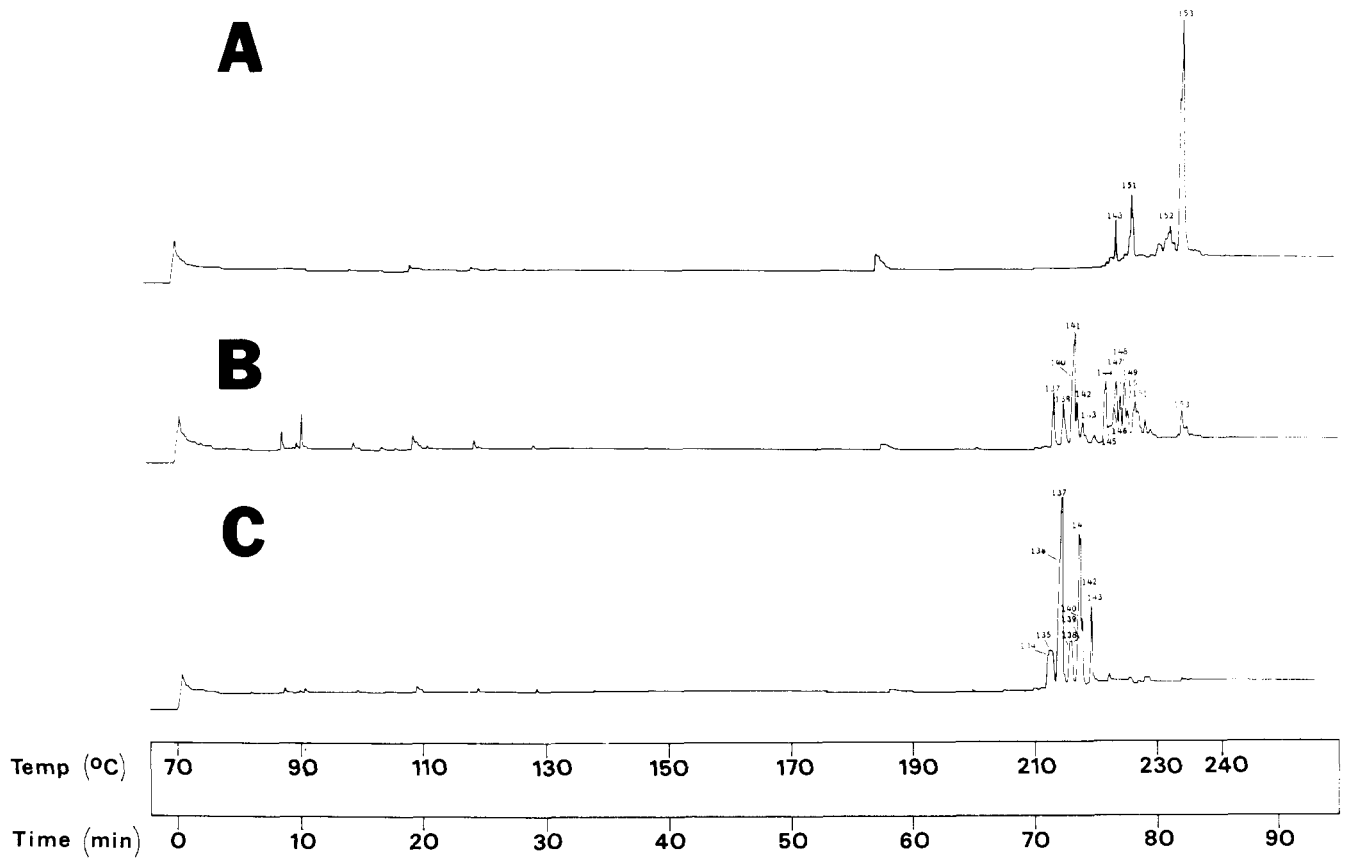


Figure 6. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure of LC of marijuana-smoke condensate

Chromatograms *A*, *B*, and *C* represent fractions *a*, *b*, and *c* in Figure 4, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total of 4 ml: *A*, 8.0 μl; *B*, 8.0 μl; *C*, 8.0 μl

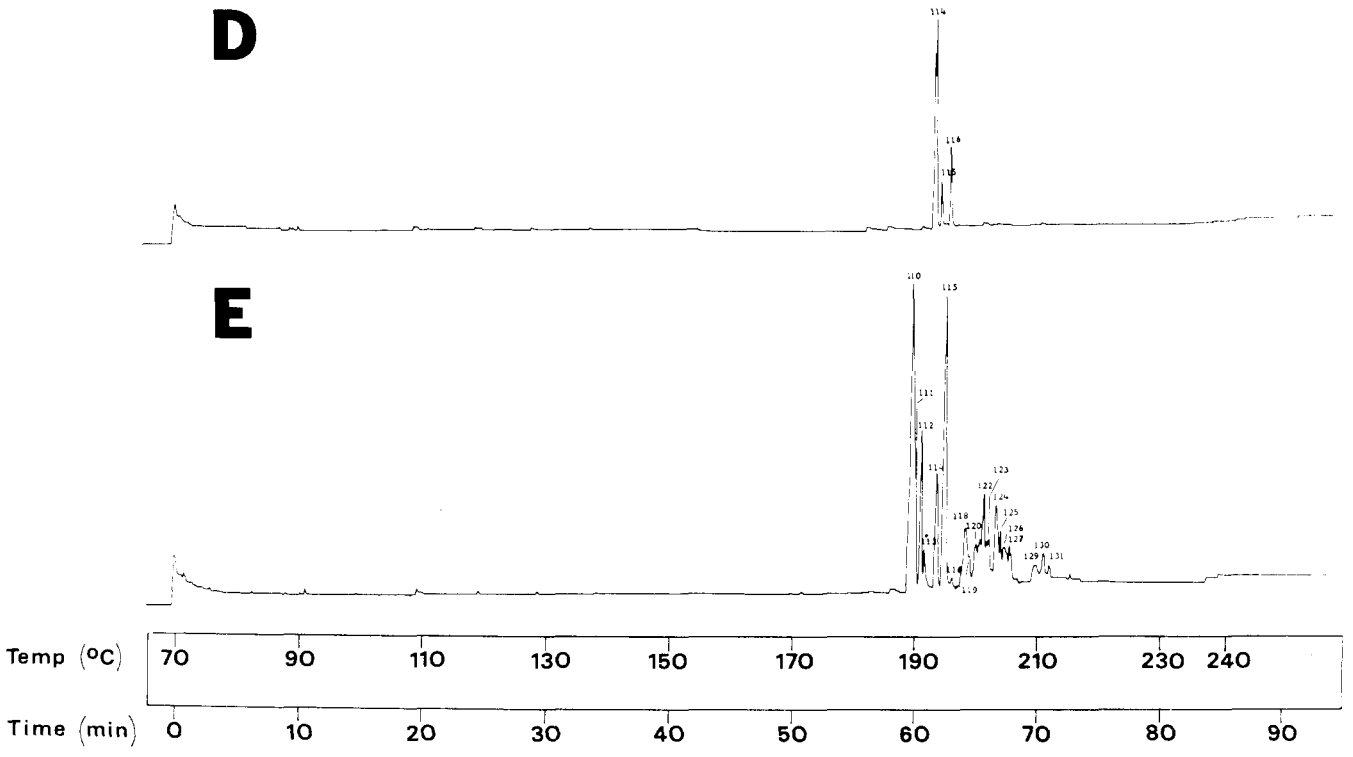


Figure 7. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure LC of marijuana-smoke condensate

Chromatograms *D* and *E* represent fractions *d* and *e* in Figure 4, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total of 4 ml: *D*, 8.0 μl; *E*, 4.0 μl

Table II. PAH Identified in Marijuana and Tobacco Smoke Condensates

Peak No.	Marijuana, μg/100 cigarettes	Tobacco, μg/100 cigarettes	Mol wt	Name
1	6.3	0.3	131	Methylindole
2	3.2		145	Ethylindole[a]
3	1.0		168	Dibenzofuran
4	1.4	0.5	166	Methylacenaphthylene
5	0.8	0.3	180	2-Methylfluorene
6	1.4	0.3	180	1-Methylfluorene
7	8.9	8.5	178	Phenanthrene
8	3.3	2.3	178	Anthracene
9	0.4	0.1	196	Ethylmethylbiphenyl[b]
10	6.5		167	Carbazole
11	0.8	0.4	192	
12	2.6	2.0	192	3-Methylphenanthrene
13	5.3	5.6	192	2-Methylphenanthrene
14	3.2	2.4	192	2-Methylanthracene
15	3.2	2.4	190	4*H*-Cyclopenta[*def*]phenanthrene
16	2.9	2.7	192	9-Methylphenanthrene
17	4.2	3.2	192	1-Methylphenanthrene
18	3.4		181	Methylcarbazole
19	3.6		181	Methylcarbazole
20	5.1		181	Methylcarbazole
21	3.1	1.6	204	Methyl-4*H*-cyclopenta[*def*]phenanthrene
22	3.0		181	Methylcarbazole
23	0.3	0.4	206	Ethylphenanthrene or ethylanthracene[a]
24	0.7	0.6	206	Ethylphenanthrene or ethylanthracene[a]
25	0.6	0.5	206	Ethylphenanthrene or ethylanthracene[a]
26	0.7	0.5	206	Ethylphenanthrene or ethylanthracene[a]
27	1.5	0.8	206	Ethylphenanthrene or ethylanthracene[a]
28	0.7	0.6	206	Ethylphenanthrene or ethylanthracene[a]
29	0.6	0.7	206	Ethylphenanthrene or ethylanthracene[a]
30	3.0	1.6	206	Ethylphenanthrene or ethylanthracene[a]
31	4.3	1.8	206	Ethylphenanthrene or ethylanthracene[a]
32	2.5	1.9	206	Ethylphenanthrene or ethylanthracene[a]
33	8.9	8.3	202	Fluoranthene
34	0.6	1.6	206	Ethylphenanthrene or ethylanthracene[a]
35	2.9	1.2	202	Benzacenaphthylene
36	4.9	3.4	206	Ethylphenanthrene or ethylanthracene[a]
37	6.6	6.8	202	Pyrene
38	1.9	0.7	218	Ethyl-4*H*-cyclopenta[*def*]phenanthrene[a]
39	2.2	0.7	218	Ethyl-4*H*-cyclopenta[*def*]phenanthrene[a]
40		X	282	*p,p'*-TDEE
41	1.3	1.4	218	Ethyl-4*H*-cyclopenta[*def*]phenanthrene[a]
42	1.9	0.8	218	Ethyl-4*H*-cyclopenta[*def*]phenanthrene[a]
43	0.6	0.5	220	Ethylmethylphenanthrene or ethylmethylanthracene[b]
44	1.4	0.7	220, 218	Ethylmethylphenanthrene or ethylmethylanthracene,[b] Ethyl-4*H*-cyclopenta[*def*]phenanthrene[a]
45	2.4	1.6	218	Ethyl-4*H*-cyclopenta[*def*]phenanthrene[a]
46	4.0	4.6	216	Methylfluoranthene
47	1.8	1.8	216	Methylfluoranthene
48	3.8	3.6	216	Methylfluoranthene
49	4.2	4.9	216	Benzo[*a*]fluorene
50	5.4	5.5	216	2-Methylpyrene and benzo[*b*]fluorene
51		X	318	*o,p'*-TDE
52	2.5	1.2	220	Ethylmethylphenanthrene or ethylmethylanthracene[b]
53	4.1	4.4	216	4-Methylpyrene
54	4.8	5.6	216	1-Methylpyrene
55	0.8	0.9	216	Methylfluoranthene
56	0.6	0.3	216	Methylfluoranthene
57		X	318	*p,p'*-TDE
58	1.1	1.5	230	Ethylfluoranthene or ethylpyrene[a]
59	0.3	0.5	230	Ethylfluoranthene or ethylpyrene[a]
60	0.5	0.9	230	Ethylfluoranthene or ethylpyrene[a]
61	1.1	1.0	230	Ethylfluoranthene or ethylpyrene[a]
62	2.1	2.4	230	Ethylfluoranthene or ethylpyrene[a]
63	2.1	2.4	230	Ethylfluoranthene or ethylpyrene[a]
64	2.5	2.7	230	Ethylfluoranthene or ethylpyrene[a]
65	1.4	1.8	230	Ethylfluoranthene or ethylpyrene[a]
66	1.7	1.6	230,226	Ethylfluoranthene or ethylpyrene,[a] acefluoranthylene
67	2.4	3.0	230	Ethylfluoranthene or ethylpyrene[a]
68	2.3	2.6	230, 226	Ethylfluoranthene or ethylpyrene,[a] acepyrylene
69	1.2	1.4	230	Ethylfluoranthene or ethylpyrene[a]
70	1.6	1.7	230	Ethylfluoranthene or ethylpyrene[a]

Table II (*Continued*)

Peak No.	Marijuana, μg/100 cigarettes	Tobacco, μg/100 cigarettes	Mol wt	Name
71	1.4	1.3	230	Ethylfluoranthene or ethylpyrene[a]
72	0.4	0.4	226, 230	Benzo[*ghi*]fluoranthene, ethylfluoranthene, or ethylpyrene[a]
73	3.3	2.6	228	Benz[*a*]anthracene
74	5.5	5.1	228	Chrysene
75	0.9	0.8	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
76	0.7	0.6	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
77	0.9	0.6	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
78	1.0	0.7	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
79	0.8	0.6	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
80	1.0	0.7	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
81	0.7	0.7	244	Ethylmethylfluoranthene or ethylmethylpyrene[b]
82	1.0	0.6	242	Methylchrysene or methylbenz[*a*]anthracene
83	1.0	0.5	242	Methylchrysene or methylbenz[*a*]anthracene
84	2.7	2.2	242	Methylchrysene or methylbenz[*a*]anthracene
85	2.1	2.2	242	Methylchrysene or methylbenz[*a*]anthracene
86	1.0	1.1	242	Methylchrysene or methylbenz[*a*]anthracene
87	0.9	0.7	242	Methylchrysene or methylbenz[*a*]anthracene
88	2.2	1.9	242	Methylchrysene or methylbenz[*a*]anthracene
89	2.7	2.9	242	Methylchrysene or methylbenz[*a*]anthracene
90	X			
91	0.5	0.5	254	Binaphthyl
92	0.5	0.3	254	Binaphthyl
93	0.8	0.7	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
94	0.6	0.6	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
95	1.0	0.7	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
96	0.5	0.6	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
97	1.5	0.7	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
98	0.7	0.7	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
99	0.4	0.3	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
100	0.7	0.7	256	Ethylchrysene or ethylbenz[*a*]anthracene[a]
101	0.6	0.6	268	Methylbinaphthyl
102	0.4	0.4	268	Methylbinaphthyl
103	0.4	0.3	268	Methylbinaphthyl
104	0.6	0.3	268	Methylbinaphthyl
105	0.3	0.3	268	Methylbinaphthyl
106	0.3	0.6	270	Ethylmethylchrysene or ethylmethylbenz[*a*]anthracene[b]
107	0.3	0.4	270	Ethylmethylchrysene or ethylmethylbenz[*a*]anthracene[b]
108	0.4	0.4	282	Ethylbinaphthyl[a]
109	0.3	0.3	282	Ethylbinaphthyl[a]
110	3.0	2.1	252	Benzo[*j*]fluoranthene
111	1.1	1.2	252	Benzo[*k*]fluoranthene
112	1.1	0.7	252	Benzofluoranthene
113	0.7	0.5	252	Benzofluoranthene
114	1.8	1.3	252	Benzo[*e*]pyrene
115	2.9	1.7	252	Benzo[*a*]pyrene
116	0.9		252	Perylene
117	0.3	0.2	266	Methylbenzopyrene or methylbenzofluoranthene
118	0.8	0.6	266	Methylbenzopyrene or methylbenzofluoranthene
119	0.5	0.5	266	Methylbenzopyrene or methylbenzofluoranthene
120	0.6	0.6	266	Methylbenzopyrene or methylbenzofluoranthene
121	0.6	0.6	266	Methylbenzopyrene or methylbenzofluoranthene
122	1.2	0.6	266	Methylbenzopyrene or methylbenzofluoranthene
123	0.9	0.7	266	Methylbenzopyrene or methylbenzofluoranthene
124		0.6	266	Methylbenzopyrene or methylbenzofluoranthene
125	0.7	0.5	266	Methylbenzopyrene or methylbenzofluoranthene
126	0.5	0.5	266	Methylbenzopyrene or methylbenzofluoranthene
127	0.5	0.3	266	Methylbenzopyrene or methylbenzofluoranthene
128		0.2	266	Methylbenzopyrene or methylbenzofluoranthene
129	0.3	0.4	266, 280	Methylbenzopyrene, ethylbenzopyrene, or ethylbenzofluoranthene[a]
130	0.4	0.5	280	Ethylbenzopyrene or ethylbenzofluoranthene[a]
131	0.3	0.3	280	Ethylbenzopyrene or ethylbenzofluoranthene[a]
132		0.3	280	Ethylbenzopyrene or ethylbenzofluoranthene[a]
133		0.3	280	Ethylbenzopyrene or ethylbenzofluoranthene[a]
134	0.3		276	*c*
135	0.3		276, 278	*c*, dibenz[*a,i*]anthracene
136	0.6		276	*c*
137	1.0	0.3	276	*c*
138	0.3		276	*c*
139	0.3	0.6	278	Dibenz[*a,h*]anthracene or dibenz[*a,c*]anthracene
140	0.4	0.2	276	*c*
141	0.7	0.3	276	Benzo[*ghi*]perylene
142	0.4		276	*c*
143	0.5		276	Anthanthrene

Table II (*Continued*)

Peak No.	Marijuana, μg/100 cigarettes	Tobacco, μg/100 cigarettes	Mol wt	Name
144	0.5		290	*d*
145	0.2		290	*d*
146	0.4		290	*d*
147	0.5		290	*d*
148	0.4		290	*d*
149	0.5		290, 302	*d*, dibenzopyrene
150	0.3		290, 302	*d*, dibenzopyrene
151	0.4		290	*d*
152	0.3		304, 306	Diphenylacenaphthylene, Quaterphenyl
153	1.2		306	Quaterphenyl

[a] Could also be dimethyl-. [b] Could also be trimethyl- or propyl. [c] Compounds with molecular weight 276 can be any of the following: Indeno[1,2,3-*cd*]pyrene, Indeno[1,2,3-*cd*]fluoranthene, Aceperylene, Phenanthro[10,1,2,3-*cdef*]fluorene, Acenaphth[1,2-*a*]acenaphthylene, Dibenzo[*b*,*mno*]fluoranthene. Further possibilities are the benzo derivatives of acepyrylene and acefluoranthylene. [d] Compounds with molecular weight 290 are methyl derivatives of those with molecular weight 276.

analysis scheme and separated into fractions consisting of the parent compound and its alkylated derivatives. The comparison of fraction weights resulting from Sephadex LH-20 chromatography according to Table I can be seen in Figure 3. Fractions I and II were not analyzed further because they were found to contain in addition to mono- and bicyclic aromatics many other interfering compounds that were not completely removed by previous steps. It was found that the cannabinoids were some of the major contributors to these two fractions in marijuana smoke and that Δ^9-tetrahydrocannabinol had almost the same retention as naphthalene. High-resolution GLC of fractions III through VII of both marijuana and tobacco showed some overlapping of fractions which suggested that further frac-

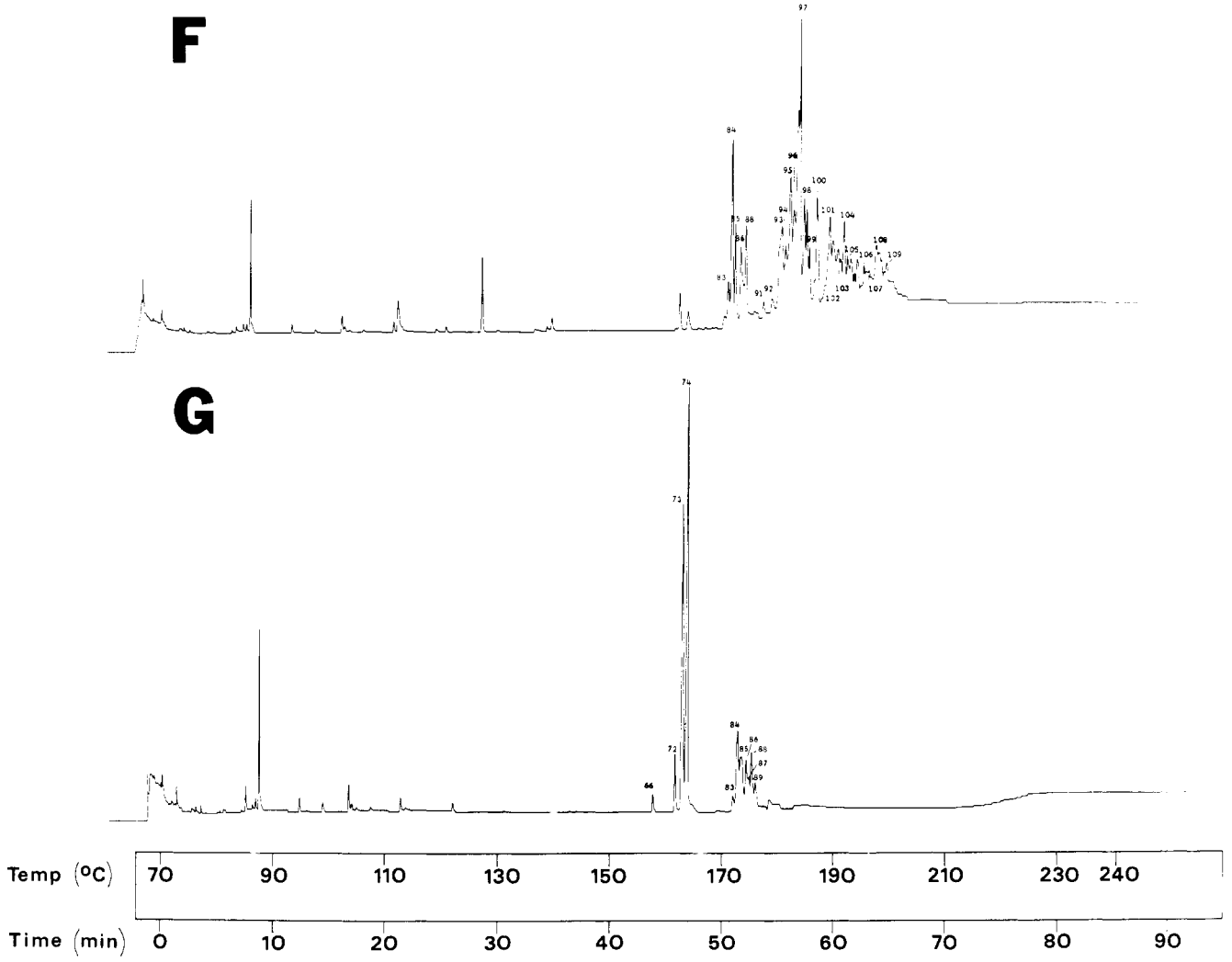


Figure 8. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure LC of marijuana-smoke condensate

Chromatograms *F* and *G* represent fractions *f* and *g* in Figure 4, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total of 4 ml: *F*, 1.5 μl; *G*, 1.5 μl

tionation was necessary, but at the same time demonstrated the power of the combination of silicic acid and Sephadex LH-20 chromatographies for cleaning of the PAH fractions obtained from complex organic mixtures.

High-pressure liquid chromatograms of the Sephadex LH-20 fractions are shown in Figures 4 and 5. The shaded portions represent the fractions collected for identification purposes. Capillary GC of these final, more refined, fractions are shown in Figures 6 through 9 for marijuana and Figures 10 through 12 for tobacco. It is readily noticeable that the concentrations of PAH of molecular weights greater than that of chrysene are significantly increased in marijuana compared with tobacco. This observation corresponds well with the comparison of fraction weights obtained by Sephadex chromatography (Figure 3). The semiquantitative data are listed in Table II. This increase in concentration of higher PAH could greatly increase the carcinogenic activity of marijuana condensates. The concentration of the potent carcinogen, benzo[*a*]pyrene, in marijuana is almost twice (170%) that determined in tobacco, and the occurrence of higher PAH may have even greater toxicological significance.

Proton NMR has the unique advantage of enabling positions of substitution to be ascertained in the PAH series either through the characteristic spin–spin coupling patterns of remaining aromatic protons, or from the chemical shifts (*17, 18, 20*) and/or benzylic couplings (*28*) of proton-bearing substituents. The uses of proton NMR in structure identification in polycyclic aromatic molecules have thus been wide-spread, and have recently been reviewed (*19*).

Until recently, the poor sensitivity of proton NMR when compared to other spectrometric techniques has severely restricted its use in the identification of trace components in mixtures such as smoke condensates. However, with the advent of rapid spectrum accumulation through Fourier transform methods, proton NMR can be applied with much more facility to the identification of small quantities of PAH. Table III lists the proton NMR chemical shifts observed in the methyl region of the first three fractions of marijuana and tobacco and compares the data with values for pure compounds obtained from the literature. Up to 22 000 scans were accumulated to obtain spectra of some of the smaller fractions, and mixture components of as little as 10 μg were identified. Several of the compounds show characteristic splittings of the methyl signals, and peaks in the aromatic proton region which confirm the position of methyl substitution. The information gained from proton NMR spectrometry of the mixtures of methylphenanthrenes and methylanthracenes agrees very well with the corresponding mass spectra and retention data. Neither of

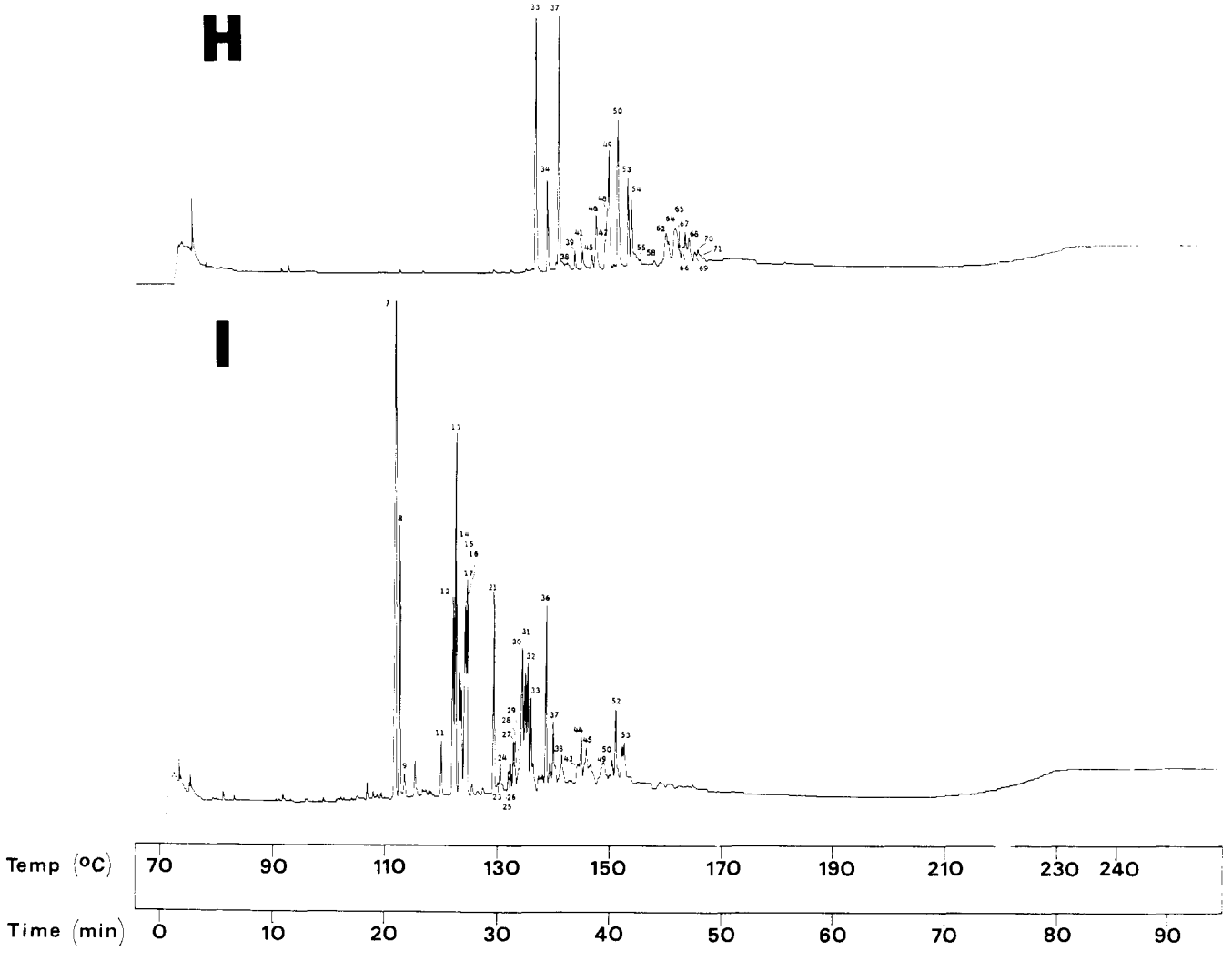


Figure 9. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure LC of marijuana-smoke condensate

Chromatograms *H* and *I* represent fractions *h* and *i* in Figure 4, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total of 4 ml: *H*, 0.5 μl; *I*, 0.5 μl

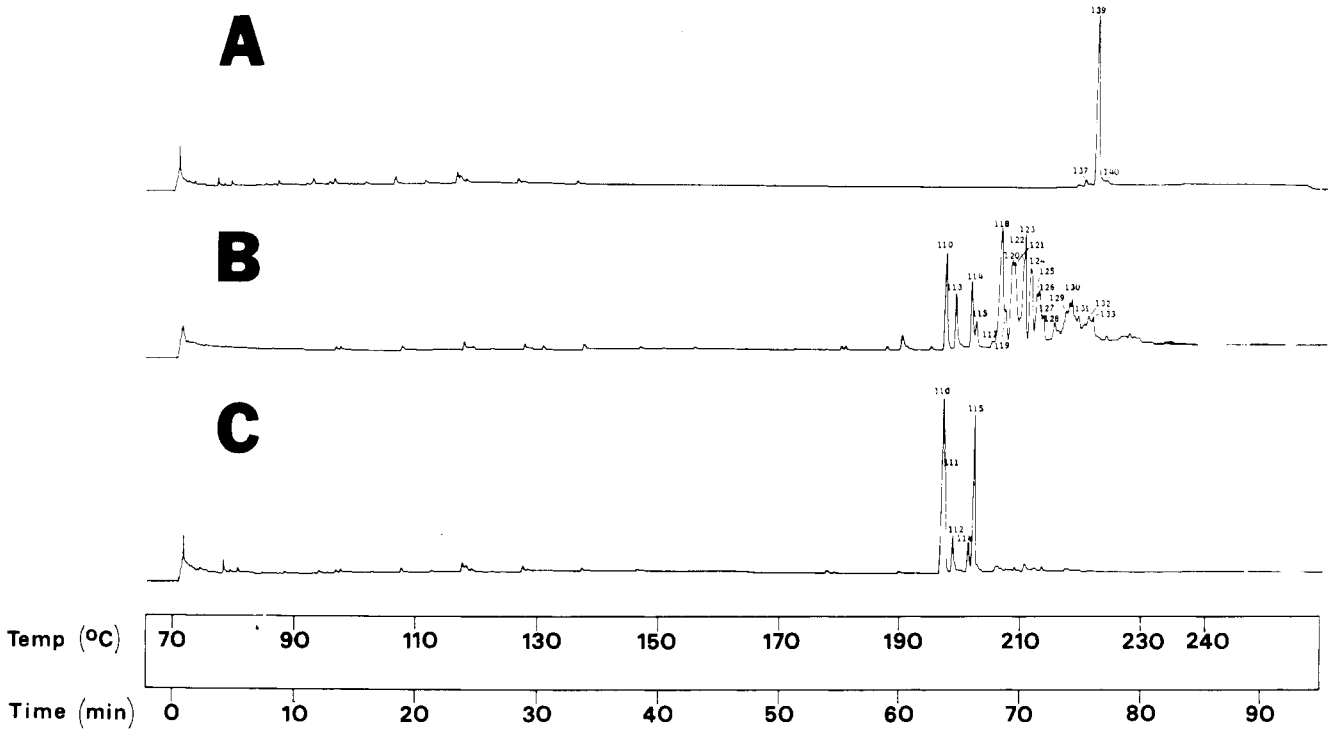


Figure 10. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure LC of tobacco-smoke condensate

Chromatograms *A*, *B*, and *C* represent fractions *a*, *b*, *c* in Figure 5, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total volume of 4 ml: *A*, 2.0 μl; *B*, 16.0 μl; *C*, 12.0 μl.

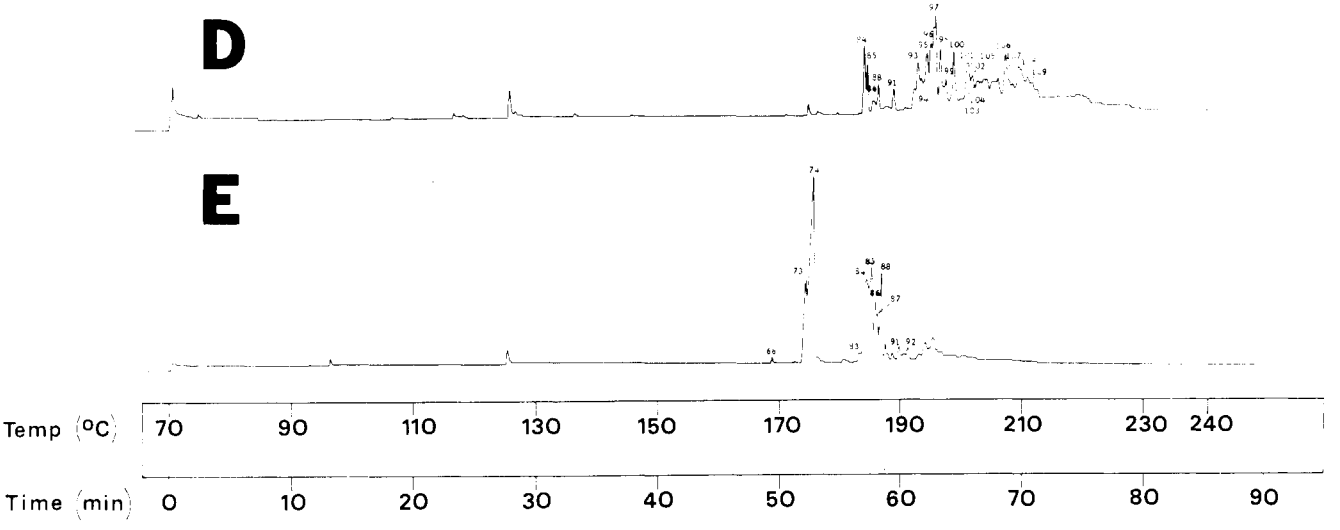


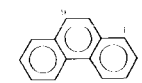
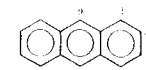
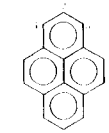
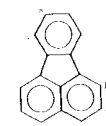
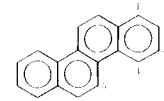
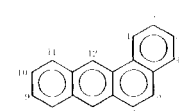
Figure 11. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure LC of tobacco-smoke condensate

Chromatograms *D* and *E* represent fractions *d* and *e* in Figure 5, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total volume of 4 ml: *D*, 16.0 μl; *E*, 8.0 μl

the sterically hindered compounds, 4-methylphenanthrene or 9-methylanthracene, appears to be present in significant amounts, although a very small peak in the NMR spectrum of tobacco fraction G (see Figure 12) can be attributed to these compounds. Likewise 1-, 7-, 10-, and 11-methylbenz[*a*]anthracenes and 4-methylchrysene are sterically hindered and not found in the later fractions. All three methylpyrenes and all five methylfluoranthenes were detected both by NMR spectroscopy and by GC-MS. For each fraction, UV and proton NMR spectrometry of the aromatic region gave further supporting information concerning the presence of the more abundant parent hydrocarbons.

The occurrence of chlorinated pesticides in the neutral fraction of tobacco condensates has previously been reported (*8, 29–31*). The possibility that pyrolysis products of these insecticides may accelerate the tumor-initiating activity of PAH (*10*) has stimulated interest in their determination. Three of these compounds (*p,p'*-TDEE, GC and GC/MS; *o,p'*-TDE, GC and GC/MS; and *p,p'*-TDE, GC, GC/MS, and NMR spectra (*32*)) were identified in the PAH fraction of tobacco condensate. Quantitation of these pesticides was not attempted because of the uncertainties of recovery during the partitioning and fractionation steps and the possible differences in FID response during gas

Table III. Proton NMR Data

Tobacco fraction	Marijuana fraction	Compound	Proton NMR Chemical Shifts (ppm) Literature	Observed tobacco	Observed marijuana	Nomenclature
G	I	1-Methylphenanthrene	2.74 *a*, *b*, *c*, *d*	2.72	2.75	
		2-Methylphenanthrene	2.54 *a*, *b*, *c*, *d*	2.53	2.55	
		3-Methylphenanthrene	2.62 *a*, *b*, *c*, *d*	2.60	2.62	
		9-Methylphenanthrene	2.72 (1.1-Hz doublet) *a*, *b*, *c*, *d*	2.71	2.74	
		1-Methylanthracene	2.82 *e*	2.82	2.82	
		2-Methylanthracene	2.54 *b*, *c*	2.53	2.55	
F	H	1-Methylpyrene	2.96 *b*, *c*	2.98	2.98	
		2-Methylpyrene	2.80 *b*, *c*	2.79	2.75	
		4-Methylpyrene	2.89 (1.1-Hz doublet) *b*, *c*	2.90	2.89	
		1-Methylfluoranthene	2.79 *f*	2.79	2.75	
		2-Methylfluoranthene	2.58 *g*	2.54	2.55	
		3-Methylfluoranthene	2.70 *f*	2.65	2.62	
		7-Methylfluoranthene	2.75 *g*	2.79	2.75	
		8-Methylfluoranthene	2.43 *f*	2.44	2.43	
		Benzo[*b*]fluorene	4.02 *h*	4.04	4.04	
		Benzo[*a*]fluorene	4.06 *h*	4.07	4.08	
		Benzo[*c*]fluorene	3.87 *h*	3.85	3.85	
E	G	1-Methylchrysene	2.73 *a*	2.70	2.70	
		2-Methylchrysene	2.54 *a*	2.60	2.55	
		3-Methylchrysene	2.59 *a*	2.60	2.55	
		5-Methylchrysene	3.20 (1-Hz doublet) *i*		3.17	
		6-Methylchrysene	2.85 (1-Hz doublet) *i*	2.82	2.85	
		2-Methylbenz[*a*]anthracene	2.65 *d*, *e*	2.60	2.62	
		3-Methylbenz[*a*]anthracene	2.56 *d*, *e*	2.60	2.55	
		4-Methylbenz[*a*]anthracene	2.75 *d*, *e*	2.70	2.77	
		5-Methylbenz[*a*]anthracene	2.71 (1-Hz doublet) *d*, *e*	2.70	2.70	
		6-Methylbenz[*a*]anthracene	2.81 (1-Hz doublet) *d*, *e*	2.82	2.85	
		8-Methylbenz[*a*]anthracene	2.83 *d*, *e*	2.82	2.85	
		9-Methylbenz[*a*]anthracene	2.59 *d*, *e*	2.60	2.55	
		10-Methylbenz[*a*]anthracene	2.59 *d*, *e*	2.60	2.55	

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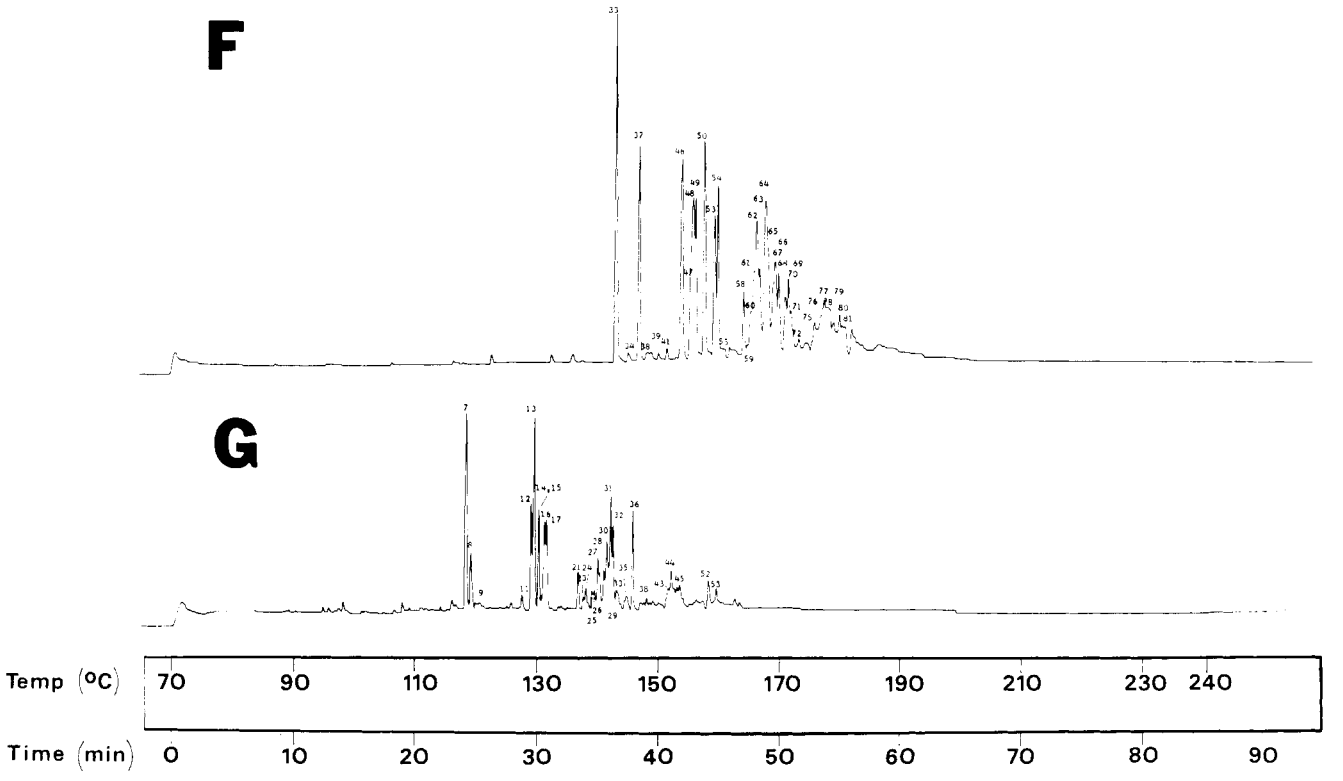

Figure 12. Capillary-column gas chromatograms of the final polynuclear aromatic hydrocarbon fractions resulting from Sephadex LH-20 chromatography and high-pressure LC of tobacco-smoke condensate

Chromatograms *F* and *G* represent fractions *f* and *g* in Figure 5, respectively. Column: Same as in Figure 1. Key: See Table II. Amount injected from a total volume of 4 ml: *F*, 2.0 μl; *G*, 1.0 μl

chromatography. No pesticide residues were found in the marijuana fraction. In addition to neutral PAH, some heterocyclic analogues have been known to occur in smoke condensates (*8*). Even though several nitrogen-containing polycyclics were found in this work, most heterocyclic PAH were undoubtedly removed during the sample preparation. This study was directed toward the identification of normal PAH.

Confirmation of the identities of many of the PAH was accomplished by comparison of GC retention times of PAH standard compounds. This approach was limited, however, because of the unavailability of a great number of standard compounds. In addition, while useful NMR data were obtained on several of the earlier fractions, there were not sufficient amounts of the later fractions to give strong signals. Published data on chemical shifts of higher molecular weight PAH compounds are also not available at this time. As a result, the exact identities of some PAH remain to be determined. With the accumulation of more standards and the resultant data obtained from proton NMR, mass spectrometry, and retention on high-resolution GLC columns of pure compounds, the identification of essentially all PAH contained in complex mixtures of the type discussed here will be possible.

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