

CHROM. 14,580

FRACTIONATION AND CAPILLARY GAS CHROMATOGRAPHIC-MASS SPECTROMETRIC CHARACTERIZATION OF THE NEUTRAL COMPONENTS IN MARIJUANA AND TOBACCO SMOKE CONDENSATES

MILOS NOVOTNÝ*, FRANCO MERLI*, DONALD WIESLER, MARY FENCL and TALAT SAEED

Department of Chemistry, Indiana University, Bloomington, IN 47405 (U.S.A.)

(Received November 20th, 1981)

SUMMARY

Polar and non-polar "neutral" constituents of the smoke condensates of Mexican marijuana and standard tobacco were resolved by capillary gas chromatography and structurally characterized through mass spectrometry. Comparisons of the two materials reveal substantial qualitative and quantitative differences. The cannabinoid-like substances partition into both the non-polar and polar fraction. In total, over 130 "neutral" smoke components have been characterized.

INTRODUCTION

An increasing use of marijuana in our society necessitates detailed evaluation of its health hazards. Objective pharmacological and toxicological studies of this drug are handicapped by an insufficient understanding of its chemical composition. While already the plant extracts contain a great number of constituents, the problem is further complicated by the complex processes that occur during the burning of marijuana materials.

Since marijuana is usually administered through smoking, the smoke condensate must primarily be analyzed with respect to both biological effects and chemical composition. Whereas our knowledge of the chemical aspects has been steadily increasing, the obvious complexity of marijuana smoke challenges even the best separation and identification techniques. High-resolution (capillary) gas chromatography (GC), high-performance liquid chromatography (HPLC), and GC-mass spectrometry (MS) have been extensively used in the field of tobacco smoke analysis where a similar degree of complexity is encountered.

In spite of the great resolving power of glass capillary columns, a direct analysis of the smoke extracts does not yield sufficiently detailed information. An effective search for the minor smoke components requires some form of sample enrichment and

* Present address: Istituto Superiore di Sanità, Laboratorio di Igiene del Lavoro, Rome, Italy.

fractionation. Fractionation of smoke condensates is furthermore desirable to assess various biological activities.

In recent studies¹⁻⁷, marijuana smoke condensate was fractionated to yield different classes of compounds for compound identification¹⁻⁴ and toxicological evaluation⁵⁻⁷. Much interest in this approach stems from the fact that marijuana may not always be toxicologically and pharmacologically synonymous with its major components, cannabinoids.

In the solvent partition scheme⁸ used in our study, it is possible to obtain fractions according to their pH characteristics and polarities. While the previous work dealt with the analysis of polycyclic aromatics¹, acids and phenols^{2,3}, and basic substances⁴ in marijuana smoke condensate, the present study concentrates on polar and non-polar neutral components. Tobacco smoke condensate has been used here as a "baseline material" to distinguish certain constituents from the usual products of combustion.

EXPERIMENTAL

Smoke condensates were obtained by means of a standard smoking machine⁹ from either Mexican marijuana cigarettes (National Institute on Drug Abuse, Rockville, MD, U.S.A.) or standard tobacco cigarettes (Tobacco-Health Research Institute, University of Kentucky, Lexington, KY, U.S.A.). The content of major cannabinoids in marijuana, as determined by gas chromatography, was: Δ^9 -tetrahydrocannabinol, 1.18%; cannabinol, 0.18%; cannabidiol plus cannabicyclol, 0.16%. The total weight of both materials was determined prior to the smoke-collection experiments. Three separate smoke collections were carried out, each involving approximately 1000 cigarettes of the starting materials, over the total period of 15 months.

Puffs of a 2-sec duration in 1-min intervals were drawn while the smoke was trapped in pure acetone using a cryogenic trap held at approximately -60°C . After acetone was evaporated to dryness, the residual condensate weights were determined.

A previously described¹⁻⁶ partition scheme was utilized to yield different fractions of smoke condensates; the fractionation is shown schematically in Fig. 1. Thus, both types of smoke condensate were divided into acidic, basic, polar neutral, non-polar neutral, and polynuclear aromatic fractions.

The weights of the individual fractions from the three smoke collections were determined. The same fractionation scheme has also been applied to yield fractions for pharmacological screening experiments as reported elsewhere^{5,6}.

An aliquot of the non-polar neutral fraction residue was redissolved in an appropriate small volume of methylene chloride solution and injected onto a 50 m \times 0.25 mm I.D., glass capillary column coated with OV-101 methylsilicone fluid; the column was programmed from 50 to 270 $^{\circ}\text{C}$ at 1 $^{\circ}\text{C}/\text{min}$.

An appropriate aliquot of the polar neutral residue was silylated with N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) at 70 $^{\circ}\text{C}$ for 30 min, and a 0.15- μl amount of the resulting solution was injected into the same column as described above; the column was programmed from 50 to 270 $^{\circ}\text{C}$ at 1 $^{\circ}\text{C}/\text{min}$.

A Varian Model 3700 gas chromatograph was used for all GC experiments. In order to identify the individual chromatographic peaks as separated, the methylsilicone-coated glass capillary column was attached to the ion source of a Hewlett-

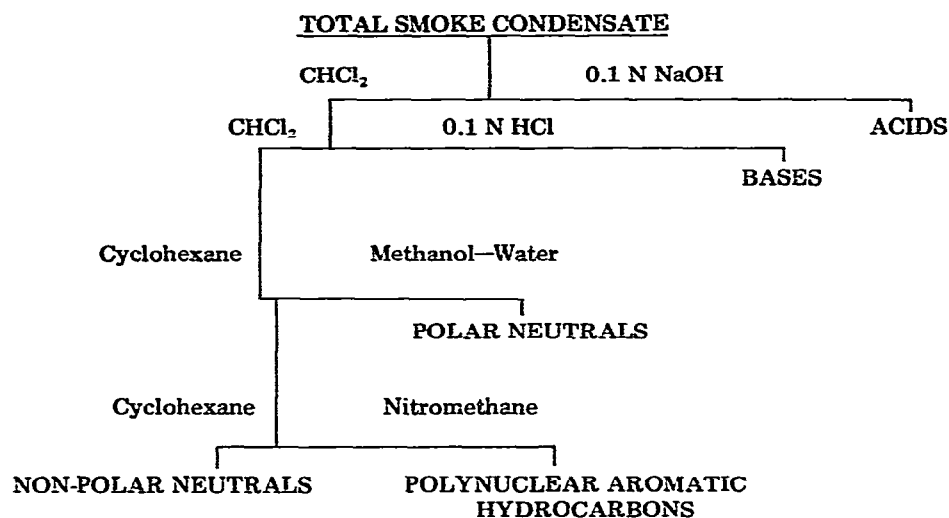


Fig. 1. The fractionation scheme used for tobacco and marijuana smoke condensates.

Packard Model 5982 combined gas chromatograph-mass spectrometer operating in the electron impact mode. The spectra were repetitively taken at appropriately selected time intervals throughout the entire chromatogram.

RESULTS AND DISCUSSION

Average yields of the individual fractions obtained through the described fractionation scheme (Fig. 1) from three smoke condensates are listed in Table I. Both the uncontrolled variations during smoke collection and losses due to evaporations of solvents cause most probably the deviations from the average weights. While in such

TABLE I

SMOKE FRACTION WEIGHT EXPRESSED AS PERCENTAGE OF THE DRY PLANT MATERIAL WEIGHT

Fraction type	Weight (%)	
	Tobacco	Marijuana
Total smoke condensate	4.97 ± 0.91	8.27 ± 1.24
Acidic	0.31 ± 0.15	0.38 ± 0.09
Basic	0.26 ± 0.06	0.23 ± 0.16
Total neutral residue	1.87 ± 0.38	3.87 ± 0.15
Polar neutral	0.87 ± 0.23	1.73 ± 0.58
Non-polar neutral	0.57 ± 0.15	0.83 ± 0.15
Polynuclear aromatic	0.20 ± 0.10	0.47 ± 0.12

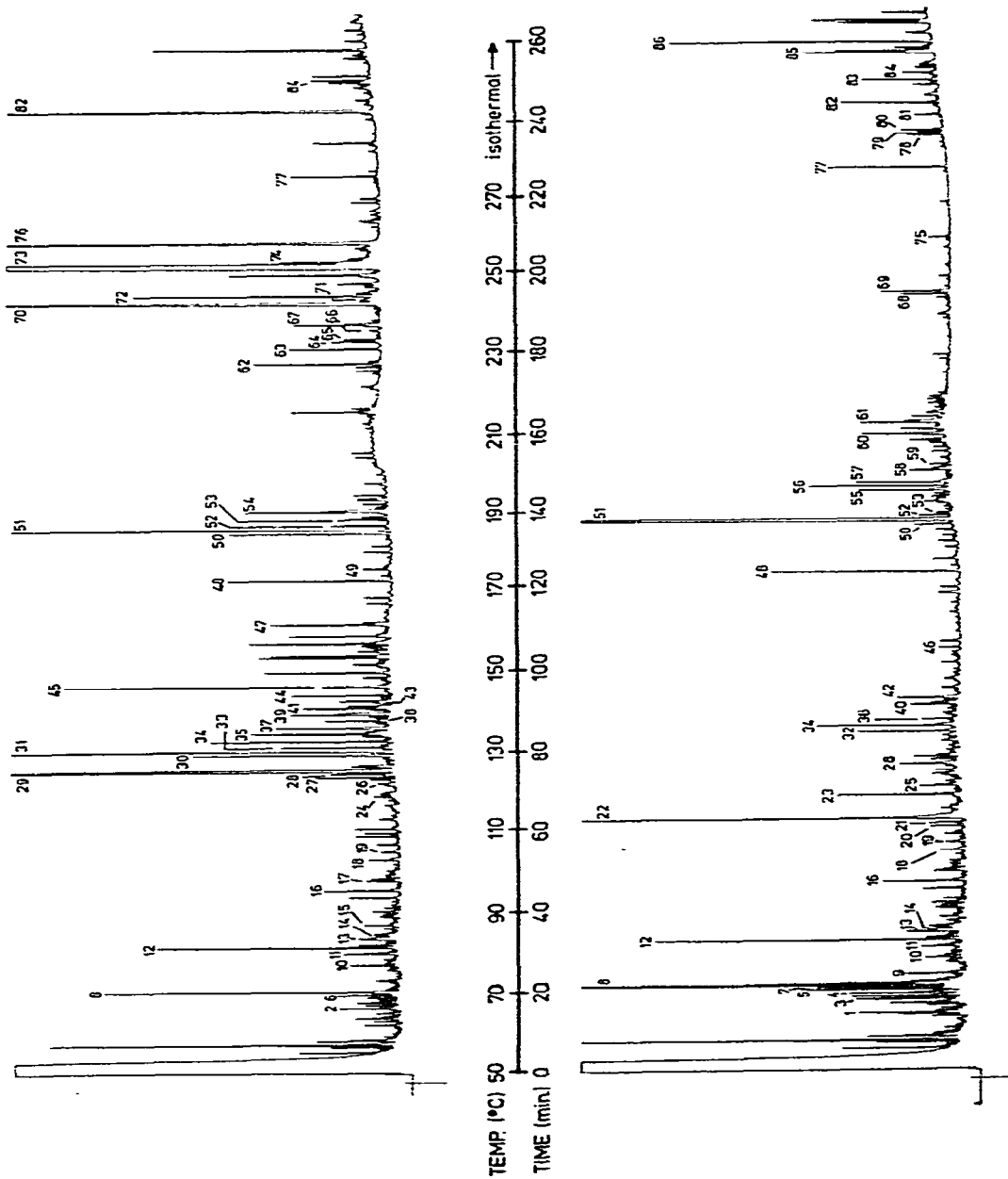


Fig. 2. Comparison of chromatograms obtained from the non-polar neutral fraction of marijuana (above) and tobacco (below) smoke condensates. For compound identifications, see Table II.

a case it is appropriate to look at the trends rather than absolute values, it is quite clear that marijuana smoke differs significantly from tobacco smoke in both the total amount of condensate and the proportion of the individual fractions.

Whereas the chemical characterization of polynuclear aromatics¹, acids and phenols^{2,3}, as well as an extensive analysis of the basic fraction⁴ were carried out on previous occasions, the principal aim of this study was to characterize the relatively volatile portion of the neutral fraction. The polar neutral constituents are mostly non-volatile, but silylation of this sample facilitates a partial characterization of the polar neutral fraction.

In contrast to marijuana smoke, chemical data on tobacco smoke composition are relatively more abundant^{10,11}. Thus, the emphasis of this communication, in relation to tobacco smoke components, is primarily in providing comparative qualitative indications.

The chromatographic profiles of the non-polar fractions for marijuana and tobacco, respectively, are compared in Fig. 2, indicating some similarities, but also both qualitative and quantitative differences. The individual components were tentatively identified through gas chromatographic and mass spectral data for both types of materials. Table II lists most major and some minor components of the analyzed mixtures. Relatively small aromatic molecules that are represented by the earlier peaks in these chromatograms appear to be rather uncharacteristic products of common combustion processes. Various hydrocarbon substances encountered throughout the chromatographic profiles are also well-recognized non-polar plant products. Some of these were found earlier^{1,2} in marijuana plant extracts, although the burning process will undoubtedly increase the degree of mixture complexity.

Numerous terpene-like substances can be found in Table II. Because of the entirely different biosynthetic pathways that are known to occur in the cannabis plant as compared to tobacco, differences in their terpenic composition are expected. A cluster of peaks (components 55–61) that suggests C₁₉ and C₂₀ unsaturated cyclic compounds appears to be typical for tobacco smoke. On the other hand, most peaks eluting in the temperature range of 120–160°C represent fairly unique components of marijuana smoke. Terpenes of these and similar structures have previously been found in the unburned marijuana samples¹³; they are believed to be responsible for a characteristic odor of marijuana and its smoke.

Many cannabinoids are encountered in the later part of the marijuana chromatogram (peaks 62–76 of the upper chromatogram, Fig. 2). As expected these are totally absent in the corresponding tobacco profile. While some identifications presented here on such compounds are tentative and we have been unable to record recognizable mass spectra from some minor components, this group of compounds appears to be the best candidate for further studies in supplementing the lists of already reported compounds of a similar nature^{14,15}. Some of these cannabinoids possess very interesting pharmacological properties. Further separation schemes must be designed to isolate such compounds from the complex smoke condensate matrix and major cannabinoids, Δ^9 -tetrahydrocannabinol, cannabinol, and cannabidiol.

Analytical results obtained with the polar neutral fraction of the smoke condensates (Fig. 3 and Table III) reveal considerable similarity between the two materials. Just as with the "neutral" polar fraction of another product of pyrolytical

TABLE II
COMPOUNDS FOUND IN THE NON-POLAR NEUTRAL FRACTION OF MARIJUANA SMOKE
CONDENSATE

Peak No.	Molecular weight	Molecular formula	Identification	Present in tobacco smoke
1	120	C ₉ H ₁₂	An ethylmethylbenzene	+
2	120	C ₉ H ₁₂	An ethylmethylbenzene	-
3	136	C ₁₀ H ₁₆	Myrcene*	+
4	138	C ₁₀ H ₁₈	An acyclic diene*	+
5	142	C ₁₀ H ₂₂	Decane*	+
6	134	C ₁₀ H ₁₄	A C ₂ ethylbenzene	-
7	138	C ₁₀ H ₁₈	A dihydrolimonene*	+
8	136	C ₁₀ H ₁₆	Limonene	+
9	134	C ₁₀ H ₁₄	A C ₂ benzene*	+
10	132	C ₁₀ H ₁₂	A C ₂ styrene	+
11	154	C ₁₁ H ₂₂	An undecene	+
12	156	C ₁₁ H ₂₄	Undecane	+
13	130	C ₁₀ H ₁₀	A methylindene or dihydronaphthalene	+
14	130	C ₁₀ H ₁₀	A methylindene or dihydronaphthalene	+
15	128	C ₁₀ H ₈	Naphthalene	-
16	170	C ₁₂ H ₂₆	Dodecane	+
17	184	C ₁₃ H ₂₈	An isomer of tridecane	-
18	142	C ₁₁ H ₁₀	2-Methylnaphthalene	+
19	142	C ₁₁ H ₁₀	1-Methylnaphthalene	+
20	182	C ₁₃ H ₂₆	A tridecene*	+
21	182	C ₁₃ H ₂₆	A tridecene*	+
22	162	C ₁₀ H ₁₄ N ₂	Nicotine*	+
23	194	C ₁₃ H ₂₂ O	Solanone*	+
24	156	C ₁₂ H ₁₂	An ethylnaphthalene	-
25	196	C ₁₄ H ₂₈	A tetradecene*	+
26	156	C ₁₂ H ₁₂	An ethylnaphthalene	-
27	204	C ₁₅ H ₂₄	A sesquiterpene	-
28	196	C ₁₄ H ₂₈	A tetradecene	+
29	204	C ₁₅ H ₂₄	beta-Caryophyllene	-
30	204	C ₁₅ H ₂₄	alpha-Bergamotene	-
31	204	C ₁₅ H ₂₄	Humulene	-
32	206	C ₁₅ H ₂₆	A dihydrosesquiterpene*	+
33	204	C ₁₅ H ₂₄	A sesquiterpene	-
34	204	C ₁₅ H ₂₄	beta-Farnesene	+
35	204	C ₁₅ H ₂₄	A sesquiterpene	-
36	212	C ₁₅ H ₃₂	An isomer of pentadecane*	+
37	204	C ₁₅ H ₂₄	A sesquiterpene	-
38	204	C ₁₅ H ₂₄	A sesquiterpene	-
39	204	C ₁₅ H ₂₄	A sesquiterpene	-
40	210	C ₁₅ H ₃₀	A pentadecene*	+
41	204	C ₁₅ H ₂₄	Bisabolene	-
42	212	C ₁₅ H ₃₂	Pentadecane	+
43	170	C ₁₃ H ₁₄	A C ₃ naphthalene	-
44	204	C ₁₅ H ₂₄	A sesquiterpene	-
45	202	C ₁₅ H ₂₂	A dehydrosesquiterpene	-
46	224	C ₁₆ H ₃₂	A hexadecene*	+

TABLE II (continued)

Peak No.	Molecular weight	Molecular formula	Identification	Present in tobacco smoke
47	222	C ₁₅ H ₂₆ O	A sesquiterpene alcohol	—
48	266	C ₁₉ H ₃₈	Norphytene	+
49	252	C ₁₈ H ₃₆	An octadecene	—
50	264	C ₁₈ H ₃₂ O	A solanone-like ketone	+
51	278	C ₂₀ H ₃₈	Neophytadiene	+
52	266	C ₁₉ H ₃₈	A nonadecene	+
53	278	C ₂₀ H ₃₈	An eicosadiene	+
54	278	C ₂₀ H ₃₈	An eicosadiene	—
55	274	C ₂₀ H ₃₄	An eicosatetraene*	+
56	274	C ₂₀ H ₃₄	An eicosatetraene*	+
57	270	C ₁₉ H ₂₆ O	An androstadienone*	+
58	270	C ₁₉ H ₂₆ O	An androstadienone*	+
59	278	C ₂₀ H ₃₈	An eicosadiene*	+
60	276	C ₂₀ H ₃₆	An eicosatriene*	+
61	276	C ₂₀ H ₃₆	An eicosatriene*	+
62	314	C ₂₁ H ₃₀ O ₂	Cannabicitran	—
63	286	C ₁₉ H ₂₆ O ₂	Tetrahydrocannabidiol	—
64	314	C ₂₁ H ₃₀ O ₂	Isotetrahydrocannabinol	—
65	328	C ₂₂ H ₃₂ O ₂	Cannabidiol monomethyl ether	—
66	328	C ₂₂ H ₃₂ O ₂	Cannabichromene monomethyl ether	—
67	314	C ₂₁ H ₃₀ O ₂	Cannabicyclol	—
68	342	C ₂₅ H ₄₂	A dihydrosesterterpene*	+
69	342	C ₂₅ H ₄₂	A dihydrosesterterpene*	+
70	314	C ₂₁ H ₃₀ O ₂	Cannabidiol	—
71	332	C ₂₀ H ₂₈ O ₄	Cannabichromanone	—
72	314	C ₂₁ H ₃₀ O ₂	Cannabichromene	—
73	314	C ₂₁ H ₃₀ O ₂	Δ ⁹ -Tetrahydrocannabinol	—
74	312	C ₂₁ H ₂₈ O ₂	A dihydrocannabinol	—
75	352	C ₂₅ H ₅₂	Pentacosane*	+
76	310	C ₂₁ H ₂₆ O ₂	Cannabinol	—
77	380	C ₂₇ H ₅₆	Heptacosane	+
78	394	C ₂₈ H ₅₈	Octacosane	+
79	410	C ₃₀ H ₅₀	Squalene*	+
80	410	C ₃₀ H ₅₀	An isomer of squalene*	+
81	408	C ₂₉ H ₆₀	An isomer of nonacosane*	+
82	408	C ₂₉ H ₆₀	Nonacosane	+
83	422	C ₃₀ H ₆₂	An isomer of triacontane	+
84	422	C ₃₀ H ₆₂	Triacontane	+
85	436	C ₃₁ H ₆₄	An isomer of hentriacontane*	+
86	436	C ₃₁ H ₆₄	Hentriacontane*	+

* Present in tobacco but not marijuana

degradation, coal tar¹⁶, the substances such as phenols and certain nitrogen-containing molecules inevitably partition into the methanol-water layer under the used fractionation conditions. The only notable differences are the expected presence of nicotine and main cannabinoids in tobacco and marijuana smoke, respectively. Isoeugenol and olivetol (peaks 27 and 35, Fig. 3), tentatively identified in this work, are the expected biosynthetic correlates of cannabinoids.

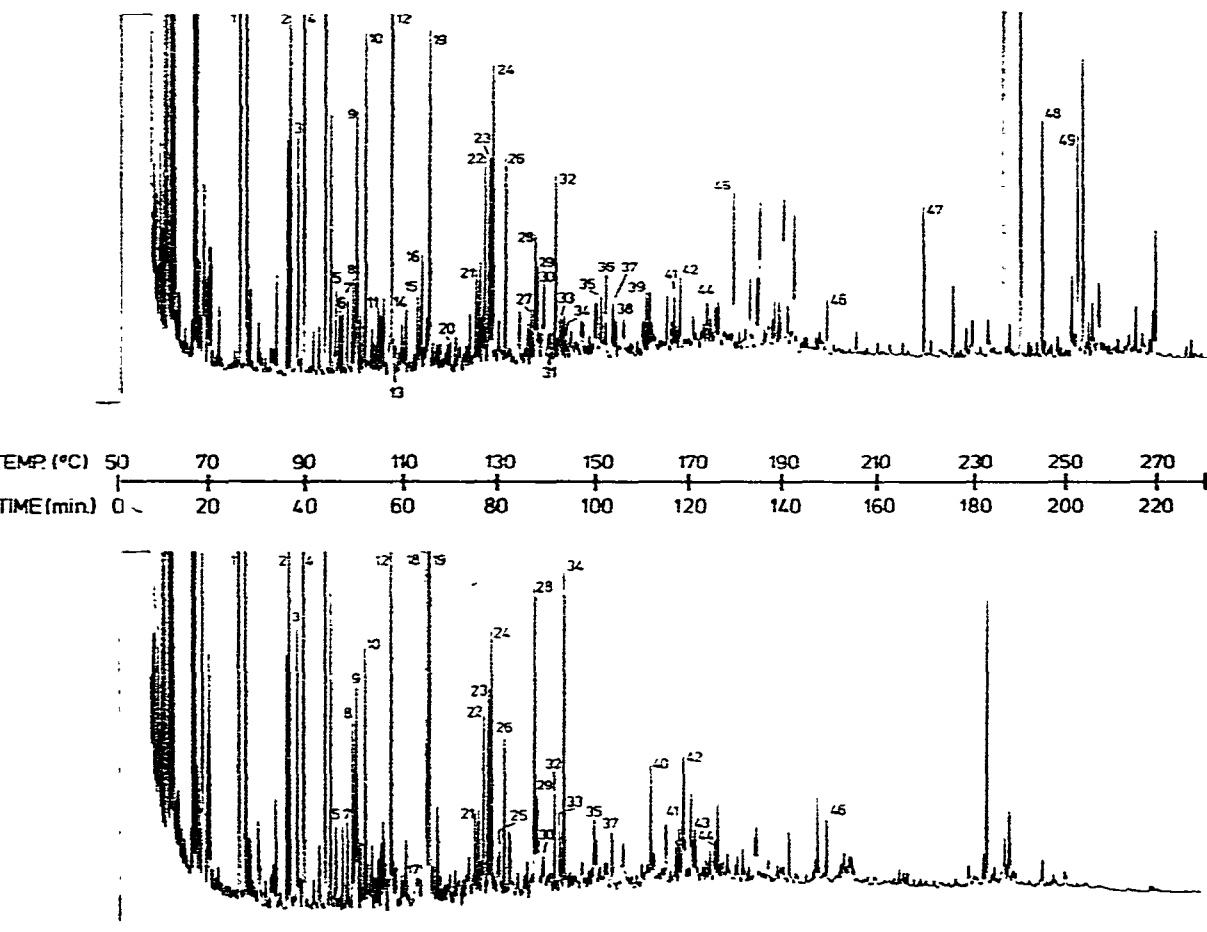


Fig. 3. Comparison of chromatograms obtained from the polar neutral fraction of marijuana (above) and tobacco (below) smoke condensates. For compound identifications, see Table III.

Inefficiency of the present partition scheme to separate completely cannabinoids into one layer was previously noted¹² with the plant materials. Again, an improved fractionation strategy is needed for isolation of minor cannabinoids from this fraction.

Various phenolic compounds were tentatively identified in both materials. Their toxicological significance resides in co-carcinogenicity, cilia toxicity and bronchial irritation; these properties have been long noted¹⁷ in connection with tobacco smoking. The profiles of phenolic substances in tobacco and marijuana, as indicated in this work, appear to be qualitatively and quantitatively similar.

It should be pointed out that the range of compounds characterized in this study represents substances that are relatively volatile, or whose volatility can be extended by a simple chemical derivatization. Characterization of the heavier smoke constituents will remain a complicated task until liquid chromatographic techniques of very high resolution become widely applicable to these sample types.

TABLE III

COMPOUNDS FOUND IN THE POLAR NEUTRAL FRACTION OF MARIJUANA SMOKE CONDENSATE

Peak No.	Molecular weight	Molecular formula	Identification	Present in tobacco smoke
1	94	C ₆ H ₆ O	Phenol	+
2	108	C ₇ H ₈ O	<i>o</i> -Cresol	+
3	108	C ₇ H ₈ O	<i>p</i> -Cresol	+
4	108	C ₇ H ₈ O	<i>m</i> -Cresol	+
5	112	C ₅ H ₄ O ₃	Furoic acid	+
6	122	C ₈ H ₁₀ O	An ethylphenol	-
7	122	C ₈ H ₁₀ O	A dimethylphenol	+
8	124	C ₇ H ₈ O ₂	2-Methoxyphenol	+
9	122	C ₈ H ₁₀ O	A dimethylphenol	+
10	122	C ₈ H ₁₀ O	A dimethylphenol	+
11	110	C ₆ H ₁₀ N ₂	A C ₃ imidazole or pyrazole	-
12	120	C ₈ H ₈ O	A vinylphenol	+
13	124	C ₇ H ₈ O ₂	A methoxyphenol	-
14	136	C ₉ H ₁₂ O	A C ₃ phenol	-
15	136	C ₉ H ₁₂ O	A C ₃ phenol	-
16	124	C ₇ H ₁₂ N ₂	A C ₄ imidazole or pyrazole	-
17	138	C ₈ H ₁₀ O ₂	A methoxymethylphenol*	+
18	162	C ₁₀ H ₁₄ N ₂	Nicotine*	+
19	110	C ₆ H ₆ O ₂	Catechol	+
20	128	C ₅ H ₄ O ₄	A hydroxyfuroic acid	+
21	154	C ₈ H ₁₀ O ₃	A dimethoxyphenol	+
22	124	C ₇ H ₈ O ₂	A methylbenzenediol	+
23	124	C ₇ H ₈ O ₂	A methylbenzenediol	+
24	117	C ₈ H ₇ N	Indole	+
25	128	C ₅ H ₄ O ₄	A hydroxyfuroic acid	+
26	150	C ₉ H ₁₀ O ₂	A vinylmethoxyphenol	+
27	164	C ₁₀ H ₁₂ O ₂	A C ₁ vinylmethoxyphenol e.g. isoeugenol	-
28	138	C ₈ H ₁₀ O ₂	A C ₂ benzenediol	+
29	138	C ₈ H ₁₀ O ₂	A C ₂ benzenediol	+
30	142	C ₆ H ₆ O ₄	A methylhydroxyfuroic acid	+
31	138	C ₈ H ₁₀ O ₂	A C ₂ benzenediol	-
32	131	C ₉ H ₉ N	A methylindole	+
33	168	C ₁₂ H ₈ O	A hydroxyacenaphthylene	+
34	136	C ₈ H ₈ O ₂	A styrenediol	+
35	136	C ₈ H ₈ O ₂	A styrenediol	+
36	162	C ₁₁ H ₁₄ O	A pentenylphenol	-
37	180	C ₁₁ H ₁₆ O ₂	A C ₄ methoxyphenol	+
38	150	C ₉ H ₁₀ O ₂	A methylstyrenediol	+
39	154	C ₈ H ₁₀ O ₃	A methoxymethylbenzenediol	-
40	178	C ₆ H ₄ Cl ₂ O ₂	A dichlorobenzenediol*	+
41	138	C ₈ H ₁₀ O ₂	A C ₂ benzenediol	+
42	152	C ₈ H ₈ O ₃	A styrenetriol	+
43	174	C ₁₁ H ₁₀ O ₂	A methoxynaphthol*	+
44	138	C ₈ H ₁₀ O ₂	A C ₂ benzenediol	+
45	180	C ₁₁ H ₁₆ O ₂	A C ₅ benzenediol e.g. olivetol	-
46	180	C ₉ H ₈ O ₂	A methoxydihydroxybenzofuran	+

(Continued on p. 150)

TABLE III (continued)

Peak No.	Molecular weight	Molecular formula	Identification	Present in tobacco smoke
47	256	C ₁₆ H ₃₂ O ₂	Palmitic acid	—
48	314	C ₂₁ H ₃₀ O ₂	Δ ⁹ -Tetrahydrocannabinol	—
49	310	C ₂₁ H ₂₆ O ₂	Cannabinol	—

* Present in tobacco but not marijuana

ACKNOWLEDGEMENTS

This work was supported by the research grant No. DA 507 from the National Institute on Drug Abuse, U.S. Public Health Service. Generous help of Ms. Carolyn Keene, Tobacco-Health Research Institute, University of Kentucky, Lexington, KY, U.S.A., with the preparation of smoke condensates is appreciated.

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