Pyrolytic Formation of Polynuclear Aromatic Hydrocarbons from Petroleum Ether Extractable Constituents of Flue-Cured Tobacco Leaf

William S. Schlotzhauer,* Ray F. Severson, Orestes T. Chortyk, Richard F. Arrendale, and Howard C. Higman

A petroleum ether extract of flue-cured tobacco leaf was separated by silicic acid column chromatography into eight major fractions which were then pyrolyzed. Polynuclear aromatic hydrocarbons (PAH) were isolated from the pyrolyzates by silicic acid column chromatography and gel filtration. Major components of fractions that produced highest amounts of PAH were characterized by various techniques including gas chromatography, column chromatography, ultraviolet (uv) spectrometry, and mass spectrometry. Fractions containing sterols and steryl esters, and terpenes, especially the C_{45}-isoprenoid alcohol, solanesol, produced the greatest amounts of PAH per gram pyrolyzed. The paraffin fraction and the fraction containing the most polar constituents produced relatively low levels of PAH. Because of its relatively high concentration in tobacco leaf, solanesol may contribute more than 30% of the total PAH in the pyrolyzate of the total extract. Thus, solanesol may be a major precursor of cigarette smoke PAH.

Bioassays (Wynder and Wright, 1957; Seelkopf et al., 1963; Bock et al., 1970) of tobacco smoke condensates have indicated that condensate fractions containing the polynuclear aromatic hydrocarbons (PAH) possess significant biological activity. The low levels of PAH present in or on tobacco leaf (Bentley and Burgan, 1960) suggest that these components are pyrosynthesized during the smoking process. Pyrosynthesis of PAH from paraffins (Wynder et al., 1958; Grimmer et al., 1966) and sterols (Badger et al., 1965) led to the examination of tobacco leaf lipids (generally extractable with petroleum ether) as likely precursors of smoke PAH. Wynder et al. (1958) demonstrated the carcinogenicity of tars derived from pyrolysis of a hexane extract of tobacco at temperatures exceeding 640 °C. Schlotzhauer and Schmeltz (1968) suggested that as much as two-thirds of the amounts of the carcinogen benzo[*a*]pyrene, formed during tobacco pyrolysis (860 °C), arises from the hexane-extractable components of leaf. Such extractable material constitutes from 5 to 10% of dry leaf weight and consists of a mixture of paraffinic and polyunsaturated hydrocarbons, glycerides, solanesol, tocopherols, aliphatic esters, phytosterols and steryl esters, neophytadiene, phthalates, resins, high molecular weight fatty acids, other acids, and bases (Swain et al., 1961; Cook et al., 1969). It was apparent that any meaningful evaluations of the contribution of hexane-extractable leaf constituents to smoke PAH should entail a separation of these constituents prior to pyrolytic studies. In the present study, we chromatographed a petroleum ether extract of flue-cured tobacco leaf, pyrolyzed the separated fractions under conditions which yielded PAH patterns similar to those observed in cigarette smoke condensate, and identified the major constituents of the principal PAH-producing fractions. These data allowed correlations of the contributions of individual leaf constituents to the PAH content of cigarette smoke.

EXPERIMENTAL SECTION

Preparation of Extract Fractions. Flue-cured tobacco (3.8 kg) (Old Belt variety) was ground to 16 mesh (Wiley Mill) and extracted (Soxhlet) with 20 l. of nanograde petroleum ether (PE) (bp 30–60 °C) for 72 h, after which time no further material was extractable under these conditions. The extract was taken to dryness in vacuo (30–35 °C) to yield a 290-g residue (7.63% of dry leaf weight). Silicic acid (Mallinkrodt, 100 mesh) was washed with nanograde methanol and activated 17 h at 150 °C for the preparation of columns (Swain et al., 1969). Extract (in PE) was applied to a silicic acid column (4400 g) at a 15:1 loading ratio and eluted with a series of solvents of increasing polarity, ranging from petroleum ether to methanol (Table I). The 42 eluted fractions were characterized by thin-layer chromatography and combined on the basis of similar physical and chemical characteristics into eight major fractions (F1–F8) for pyrolytic studies and compositional analyses.

Pyrolyses of Extract Fractions. The eight extract fractions were pyrolyzed according to Severson et al. (1976) under conditions that result in PAH patterns similar to those isolated from cigarette smoke condensate. The pyrolysis apparatus consisted of a 2 in. i.d. × 48 in. long Vycor chamber positioned in a Lindberg "Hevi-Duty" tube furnace. One end of the chamber was adapted with a Vycor joint inlet tube that allowed the introduction of dry nitrogen gas at 150 ml/min. The other end consisted of a similar joint with both an exit tube for product effluent, which was directed into the trapping system, and a 2-mm Vycor tube fitted with a silicon septum, through which a Chromel/Alumel 22-gauge wire was inserted. The furnace was preheated to 700 ± 5 °C, and a Vycor boat containing the sample (generally 5.00 g) was pulled by the wire into the furnace hot zone from the inlet end at a rate of 13.37 in./min. The rate of sample travel was established by a rotating drum kymograph around which the wire was wound. The pyrolyzate effluent was condensed in a train of traps that included a 5-l. flask at room temperature, a series of coldfingers immersed in ice/water and dry ice/acetone, and, finally, a gas scrubbing bottle containing 250 ml each of benzene/ethyl ether (1:1, v/v), and 0.5 N aqueous sodium hydroxide.

Isolation of PAH from Pyrolyzates. The traps were repeatedly washed with benzene/ethyl ether and aqueous sodium hydroxide. The washings were partitioned (separatory funnel) and cross-extracted, and the organic layer removed for analysis. An aliquot (10% by volume) was evaporated in vacuo for determination of residue weight, and the remainder of the sample taken to dryness and redissolved in 200 ml of benzene/isooctane (1:1, v/v). Activated silicic acid (40 g) was added to the benzene/isooctane solution and the sample was concentrated on a

Tobacco Laboratory, Agricultural Research Service, United States Department of Agriculture, Athens, Georgia 30604.

Table I. Fractionation of Petroleum Ether Extract of Flue-Cured Tobacco (Silicic Acid Chromatography)

Extract fraction	Eluting solvent(s)	Eluate no.	Wt, g	% total extract
F-1	Petroleum ether (PE)	1-3	15.30	5.27
F-2	5% benzene/PE-20% benzene/PE	4-12	12.62	4.35
F-3	30% benzene/PE-40% benzene/PE	13-16	25.93	8.93
F-4	60% benzene/PE-80% benzene/PE	17-20	84.96	29.25
F-5	Benzene	21-24	19.13	6.58
F-6	20% diethyl ether/benzene-60% diethyl ether/benzene	25-29	58.85	20.26
F-7	Diethyl ether	30-36	45.83	15.78
F-8	Acetone-methanol	37-42	27.82	9.58

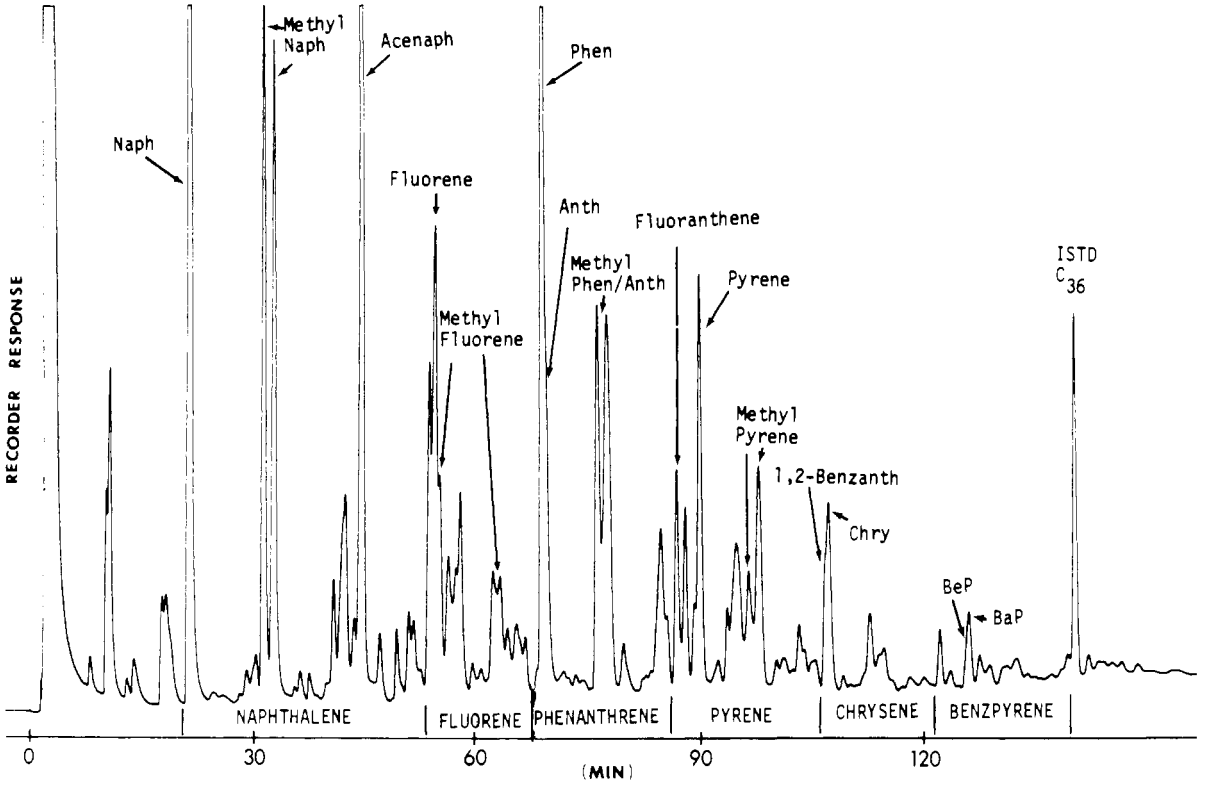

Figure 1. Typical gas chromatogram of pyrolyzate PAH.

rotary evaporator to a thick slurry. This slurry was then mixed with a quantity of petroleum ether sufficient for transferring to the head of a column of activated silicic acid (100 g). Elution with 500 ml of petroleum ether yielded a fraction (F-PE) containing paraffins and alkenes. Subsequent elution with 1500 ml of benzene/petroleum ether (25:75, v/v) yielded the PAH-containing eluate (F-BPE). The residual material on the column was eluted in 1000 ml each of benzene, ethyl ether, and methanol (eluates F-B, F-E, and F-M, respectively). Residue weights of F-PE to F-M from each of the eight fractions pyrolyzed are presented in Table II. Eluate F-BPE was concentrated to 10 ml, a 2.0-ml aliquot was taken to dryness for determination of residue weight, and a 1.0-ml aliquot was subjected to gel filtration (GF) on Bio-Beads SX-12 in a four-column system (Snook et al., 1975). Eluates containing the PAH were collected in two portions, the elution volumes of which were defined by those of trimethylnaphthalene (GF-B, containing GF eluates 36–39) and fluoranthene (GF-C, containing GF eluates 40–60).

Gas Chromatographic Analyses of PAH. GF-B and GF-C were analyzed by gas chromatography (GC) after the addition of a measured quantity of hexatriacontane as internal standard. The Hewlett-Packard Model 5830 gas chromatograph used was equipped with dual flame-ionization detectors and $^1/_8$ in. × 15 ft steel columns containing 5% Dexsil 300 on 100/120 mesh Chromosorb W/AW. The column temperature was held at 90 °C for 10 min, then programmed at 2 °C/min to 325 °C, at which it was held for 45 min. Carrier gas flow was 40 ml/min helium, determined at 90 °C. Detector responses and retention times were obtained for the standards: purified naphthalene, fluorene, phenanthrene, pyrene, chrysene, and benzo[*a*]pyrene (BaP). Gas chromatograms were divided into PAH groups according to relative retention times (t_{RR}) and named for major PAH present. The following PAH groups were defined: naphthalene, t_{RR} 0.238–0.568; fluorene, t_{RR} 0.581–0.731; phenanthrene, t_{RR} 0.754–0.951; pyrene, t_{RR} 0.964–1.180; chrysene, t_{RR}

Table II. Comparison of Chromatographic Yields of Neutrals in Extract Fraction Pyrolyzates

Eluates	Yields of chromatographic eluates, %,[a] for neutral pyrolyzates							
	F1	F2	F3	F4	F5	F6	F7	F8
F-PE	51.6	4.8	4.2	3.5	5.0	2.3	1.6	1.9
F-BPE	39.1	74.3	65.9	74.0	60.4	67.3	61.9	52.3
F-B	12.3	6.1	15.3	10.6	7.5	12.3	7.8	8.8
F-E	13.5	10.9	12.0	10.1	17.6	10.2	19.5	22.4
F-M	3.9	2.1	2.5	0.8	1.9	2.0	3.2	7.3
Recovery	120.4	98.2	99.9	99.0	92.4	94.1	94.0	92.7

[a] Based on weight of material placed on silicic acid column.

1.203–1.359; and benzopyrene, t_{RR} 1.384–1.594 (Figure 1). The peak areas of each group in the chromatograms of samples GF-B and GF-C were totaled. Assuming detector responses identical with those of the major parent PAH, GC volatile weight data were calculated for each group.

Identification of Constituents in Extract Fractions. *Determination of Hydrocarbon Waxes.* Extract fractions were analyzed for waxes according to Chortyk et al. (1975) by GC with Dexsil 300 GC columns. Detector responses of 23 normal paraffins (C_9–C_{36}) were plotted vs. retention times (relative to C_{36}). Neophytadiene, which co-eluted with paraffin waxes during silicic acid chromatography of the petroleum ether extract (fraction F1, Table I), was determined by the above method.

Determination of Free Acids, Free Sterols, and Steryl Esters. Portions (0.5 g each) of residues from the eight extract fractions were partitioned (separatory funnel) between equal volumes of ethyl ether and 0.1 N sodium hydroxide (aqueous). The aqueous layer, containing sodium salts of the free acids, was acidified with 8 M sulfuric acid and the acids were extracted with ethyl ether. The extracted acids were reacted with diazomethane, and the methyl esters formed were analyzed by GC on $^1/_8$ in. i.d. × 10 ft long glass columns containing 10% Apolar 10C on Chromosorb W/AW. The ethyl ether layer from the original partition step, which contained free sterols and steryl esters, was divided into equal portions. One portion was concentrated to 1.0 ml, a measured amount of cholestane was added as internal standard for quantitative analyses, and the free sterols were determined by GC on 1/8 in. i.d. × 6 ft long glass columns containing 10% SP-2250 on Supelcoport. Effluents corresponding to unknown peaks were collected and analyzed directly in a high-resolution DuPont Model 21-492 mass spectrometer. The other portion of the ethyl ether layer was taken to dryness in vacuo, and the residue redissolved in 10% KOH in 95% ethanol and heated under reflux for 1 h. An equal amount of water was added to the saponification reactants; the liberated sterols were extracted with ethyl ether and analyzed as described above for free sterols. The water–alcohol layer was acidified and treated as above for analysis of the acids as methyl esters.

Determination of Solanesol and Solanesyl Esters. Preliminary TLC of extract fractions indicated that a substance with an R_f value identical with that of authentic solanesol was concentrated in F4. A portion of F4 (2.90 g) was chromatographed on a 45 mm diameter × 180 mm column of neutral alumina by a modified procedure of Rowland et al. (1956). The column was eluted with 1200 ml of benzene/chloroform (2:1, v/v), 900 ml of benzene/chloroform (1:2, v/v), and 300 ml of chloroform. The benzene/chloroform (1:2) eluate was taken to dryness and the residue (1.50 g) was redissolved in petroleum ether. The solution was cooled to −70 °C (dry ice/acetone bath), and the resulting precipitate separated by vacuum filtration. The precipitate was redissolved, and the recrystallization process repeated until the supernatant was colorless. For further purification, the precipitate was dissolved in benzene, and the solution passed through a micro-column of Florisil. Removal of the solvent yielded 0.93 g of a white wax which gave an infrared spectrum identical with that of an authentic sample of solanesol from tobacco leaf provided by a commerical source. The mass spectrum of the isolate indicated a molecular ion at m/e 630 and was in agreement with that for solanesol published by Reed (1963).

The 2:1 benzene/chloroform eluate, when treated as above, yielded a product (0.10 g) with ir spectrum suggestive of solanesyl palmitate (Rowland and Latimer, 1959). Saponification of this material in 10% KOH/ethanol yielded a series of fatty acids and a wax with ir spectrum identical with that of solanesol.

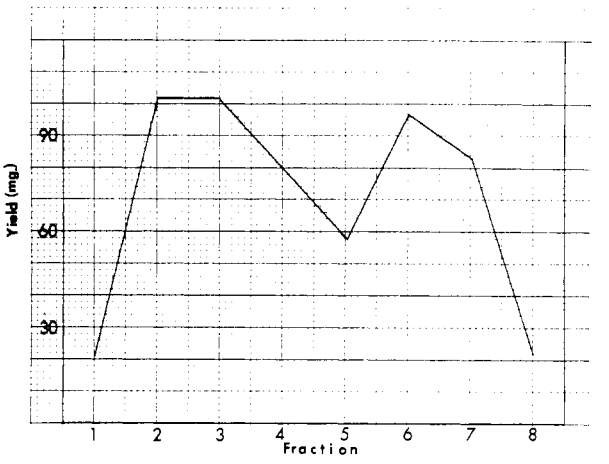

Figure 2. Yields of PAH produced per gram of extract fraction pyrolyzed (for fractions F1 to F8).

Determination of Glycerides. The method of Christopher and Glass (1969) was modified for analysis of glycerides in the extract fractions. For the determination of triglycerides, an aliquot (100 mg) of extract fraction was dissolved in 8.0 ml of benzene/methanol (1:1, v/v), and 2.0 ml of 2 N sodium methoxide in methanol was added. The reaction mixture was mechanically agitated for 3 min, and then taken to dryness in vacuo. Reaction products were redissolved in 2 N acetic acid in methanol, and the solution was extracted with petroleum ether for removal of the liberated fatty acids. Glycerol in the acidified alcohol layer was determined by GC on $^1/_8$ in. i.d. × 6 ft long glass columns containing Tenax GC; injector and detector temperature was 210 °C; column temperature was programmed from 125 to 200 °C at 4 °C/min; carrier gas flow was 32 ml/min helium, determined at 125 °C. Glycerol had a retention time of 13.2 min and an average retention window deviation of 4%. Glycerol was quantified by use of response data determined for authentic glycerol, and yields were adjusted according to those obtained from authentic tristearin under the same reaction conditions.

RESULTS AND DISCUSSION

Table II shows that the neutral pyrolyzates of extract fractions F2 through F8 contained relatively high levels (52 to 74%) of PAH-containing F-BPE eluates. Extract fraction F1, which consisted largely of paraffin waxes, yielded a pyrolyzate in which 39% of the neutrals were eluted in F-BPE, and 52% in F-PE which contains distilled paraffins. Using [^{14}C]dotriacontane in cigarette smoking studies, Jenkins et al. (1969) estimated that 95% of this typical leaf paraffin survived the smoking process unchanged. Neophytadiene, which co-elutes from silicic acid with the paraffins in F1, would be expected to form PAH during pyrolysis (Chortyk et al., 1975).

F1 and F8 yielded relatively low amounts of PAH on pyrolysis (Figure 2): the former because of the ready distillation of its paraffinic constituents, and the latter, apparently, because it contained polar, oxygenated constituents. Such compounds, having a relatively low carbon content, yield low amounts of alkyl radicals essential to PAH formation. F2, F3, and F6 produced the greatest amounts of PAH on pyrolysis; therefore, compositional analyses were initially concentrated on these extract fractions. For correlation with cigarette smoke PAH, however, the yields should be expressed as percentages of

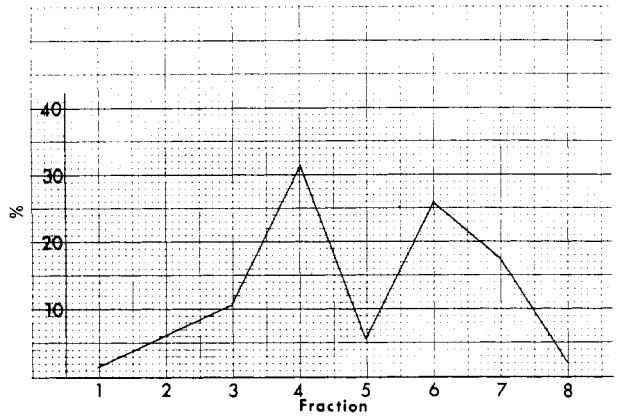

Figure 3. Relative PAH yields produced by individual extract fractions F1 to F8.

Table III. Total Sterols in Petroleum Ether Extract Fractions

	Fraction content, mg/g		
	Esterified sterols		Free sterols, F6
Components	F2	F3	
Cholesterol	28.48	10.50	5.87
Campesterol	69.44	27.15	11.56
Stigmasterol	57.31	27.90	17.01
Sitosterol	102.88	36.14	9.95
β-Amyrin	120.45	29.75	
α-Amyrin	13.30	3.11	
Total	391.86	134.55	44.39

the total PAH formed on pyrolysis of the whole PE extract (Figure 3), thus reflecting the relative abundance of each fraction in tobacco leaf. Although they produced the greatest amounts of PAH per gram pyrolyzed, F2 and F3 (4.35 and 8.93% of total extract) contributed relatively little to PAH composition of the total extract pyrolyzate. Thus, F4 (29.25% of extract weight) and F6 (20.26%) would be expected to contain a major proportion of the precursors of smoke PAH. Therefore, F4 was included in compositional analyses.

Steryl esters were isolated from F2 and F3, whereas free sterols were found in F6 (Table III). No sterols and steryl esters were found in F4. The steryl ester fractions contained the four common tobacco leaf sterols, and, in addition, two major components which gave mass spectra with molecular ion at m/e 218, characteristic of steroids. These components were identified as β- and α-amyrin and also co-chromatographed on GC with authentic standards. The total quantity of esterified sterols in the hexane extract amounted to 0.22% of leaf weight, while free sterols accounted for 0.06%. These values are consistent with those reported by Grunwald et al. (1971). The distribution of fatty acids associated with steryl esters is reported in Table IV. Unsaturated C_{18} acids, palmitic acid, and C_{20}–C_{32} fatty acids predominated. Interestingly, in the pyrolyzates of the extract fractions, only palmitic acid was present in significant amounts. This suggests that the unsaturated C_{18} acids are more liable to pyrolytic degradations than the saturated fatty acids, a behavior previously observed in experiments involving pyrolysis of stearic and linolenic acids (Schlotzhauer and Schmeltz, 1969). The steryl ester containing fractions F2 and F3 were the most potent PAH precursors (not surprising since sterols contain a phenanthrene skeleton), but, because these fractions constitute a relatively low percentage of the total extract, contribution to cigarette smoke PAH may be minimal.

Table IV. Fatty Acid Distribution in Major PAH-Producing Fractions

	Composition, %			
	Esterified acids			Free acids,
Fatty acid	F2	F3	F4	F6
n-C_{14}	1.5	2.5	2.0	2.9
n-C_{16}	9.1	15.4	26.3	19.8
n-C_{18}	3.1	3.2	5.0	3.8
n-$C_{18:1}$=	4.5	5.2	13.6	8.1
n-$C_{18:2}$=	17.3	18.8	22.1	18.6
n-$C_{18:3}$=	9.5	30.3	14.4	29.5
n-C_{20} to n-C_{32}	22.6	12.4	9.9	5.0

Table V. GC PAH Profile Groups in Pyrolyzates

	Yield, mg/5 g pyrolyzed			
PAH group	F1	F2	F4	F6
Naphthalene	48.4	167.5	189.3	196.8
Fluorene	21.1	60.4	26.1	69.9
Phenanthrene	22.9	134.0	90.9	112.4
Pyrene	9.3	72.2	44.7	63.4
Chrysene	2.9	38.2	24.5	24.6
BaP	2.3	36.7	15.6	13.4
Total	106.9	509.0	391.1	480.5
% F-BPE	35.4	46.8	41.2	52.5

Solanesol, a C_{45}-isoprenoid alcohol (eluted in F4), was isolated from leaf as a free alcohol (0.71% yield) and as esters (0.10%). The subsequent development of an analytical method for solanesol, which involved high-pressure liquid chromatography and gas–liquid chromatography of derivatized solanesol, indicated that quantities of solanesol in leaf are significantly higher (up to 3% of leaf weight) than expected (Severson, 1976). The esters of solanesol were largely of palmitic and unsaturated C_{18} acids. Since F4 produced relatively high yields of PAH on pyrolysis and accounted for nearly 30% of the total petroleum ether extractables, solanesol may be a major precursor of cigarette smoke PAH. Grossman et al. (1963) examined the pyrolysis products of solanesol. At temperatures up through 550 °C, they reported monoterpenes and trace amounts of aromatic compounds. For 650 °C pyrolysis, only aromatic products were identified. Gil-Av and Shabtai (1963) established the presence of BaP in isoprene tar and proposed that solanesol, a pyrolytic precursor of isoprene, might play an important role in formation of PAH in burning tobacco. The contribution of solanesol to cigarette smoke PAH levels depends upon the thermal stabilities of solanesol and of the monoterpenes produced by solanesol upon pyrolysis at the lower temperatures present at distances removed from the burning cone of the cigarette. As solanesol is a major leaf constituent, a sufficient amount may be subjected to higher burning temperatures and, thus, produce a substantial proportion of cigarette smoke PAH.

Yields and GC profiles of PAH groups are presented in Table V (Severson et al., 1976). Paraffins in F1, as mentioned, produced the lowest yields of PAH of all groups determined, but the yields of PAH with more highly condensed ring systems were especially low. Of the fractions pyrolyzed, steryl ester fraction F2 provided highest yields of four- and five-ring PAH. The PAH group profiles of fractions F4 and F6 were similar. F6 contained free sterols and fatty acids; however, much of this fraction is as yet uncharacterized. Preliminary studies, which include monitoring of eluates from silicic acid and alumina chromatography with ir spectroscopy and use of high-pressure liquid chromatographic procedures, indicate the

Table VI. Comparative Yields of Selected PAH from Fraction Pyrolyzates

Compound	Yields, mg/5 g pyrolyzed			
	F1	F2	F4	F6
Naphthalene	10.2	38.9	21.9	30.9
Methylnaphthalene(s)	8.8	43.5	54.5	54.0
Fluorene	3.9	11.4	12.7	14.3
Phenanthrene/ anthracene	7.7	39.0	22.2	23.2
Methylphenanthrene/ methylanthracene	5.5	44.3	28.8	31.2
Pyrene	2.5	14.1	7.3	7.5
Methylpyrene	2.0	14.5	7.5	8.4
1,2-Benzanthracene/ chrysene	0.5 0.9	19.3[a]	2.6 3.3	7.3[a]
BaP/BeP	0.9	9.1	4.6	3.3
Methyl BaP/methyl BeP	0.2	5.6	0.9	2.9

[a] Not resolvable because of larger amounts of chrysene present.

presence of terpenoid alcohols with molecular weights lower than that of solanesol. These terpenoid components probably account for similarity in PAH distributions of F4 and F6 pyrolyzates. The components in F6 should be characterized as they were shown to be of major significance as PAH precursors on the basis of relative abundance in leaf. Since glycerides have been reported (Cook et al., 1969) to comprise 1.0% or more of hexane extractables of tobacco leaf, we felt that their pyrolysis may be significant in PAH formation. However, our determination indicated that the petroleum ether extract contains about 0.15% triglycerides (in F3) and 0.20% diglycerides (in F6). These data suggest that the quantitation of glycerides in leaf must be further evaluated. The data also indicate that glycerides are not present in sufficient quantities to affect smoke PAH levels.

The levels of individual PAH in the pyrolyzates (Table VI) suggest the importance of various constituents of the petroleum ether fraction. Paraffins (F1) produced low quantities of PAH, particularly alkyl-substituted ones. Neophytadiene, also in this fraction, could not have contributed significantly to PAH levels as the PAH profile was characteristic of pyrolyzed paraffins. Steryl esters (F2) yielded, in a 1:1 ratio, both parent and methyl PAH, from naphthalene through pyrene. In the F2 pyrolyzate, the level of chrysene produced was increased above those of 1,2-benzanthracene to the extent that the latter was undeterminable by GC. These compounds were produced in similar proportions in F6 which contained small amounts of free sterols. The ratio of methyl to parent PAH was highest in F4 (solanesol fraction), and is more characteristic of PAH profiles in cigarette smoke condensate (CSC) (Severson et al., 1976), than for any other fraction pyrolyzate. Thus, solanesol, and not sterol–steryl esters, appears to be of major importance in smoke PAH production. Also, the ratios of methyl to parent benzopyrenes (BaP and BeP) for CSC and F4 pyrolyzate were similar. Solanesol was subsequently pyrolyzed under conditions identical with F4, and individual PAH and PAH profile groups were similarly analyzed (Table VII). The data obtained suggest that solanesol accounts for substantial quantities of the more highly condensed PAH present in the pyrolyzate of F4. Because of its relative abundance in leaf, solanesol may contribute as much as 40% of the benzpyrenes produced on pyrolysis of the petroleum ether extract (Figure 4).

ACKNOWLEDGMENT

The authors thank Mark W. Ivey for technical assistance in conducting these experiments.

Table VII. PAH from Pyrolysis of Solanesol

Compound	Yield, mg/5 g pyrolyzed	PAH group	Yield, mg/5 g pyrolyzed
Naphthalene	27.68	Naphthalene	108.08
Methylnaphthalene(s)	38.08		
Fluorene	7.23	Fluorene	54.40
Phenanthrene/ anthracene	22.43	Phenanthrene	96.40
Methylphenanthrene/ methylanthracene	31.90		
Pyrene	5.95	Pyrene	39.75
Methylpyrene	6.05		
1,2-Benzanthracene	2.35		
Chrysene	3.52	Chrysene	20.45
BaP/BeP	3.10	Benzopyrene	10.15
Methyl BaP/methyl BeP	1.28		
		% F-BPE	40.1

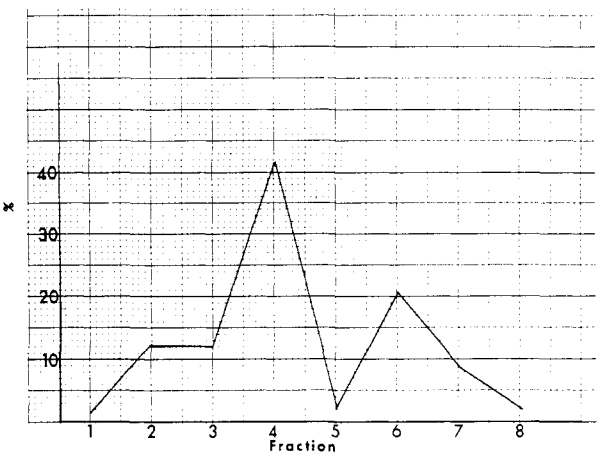

Figure 4. Relative benzo[a]pyrene yields produced by individual extract fractions F1 to F8.

LITERATURE CITED

Badger, G. M., Donnelly, J. K., Spotswood, T. M., *Aust. J. Chem.* **18**, 1249 (1965).
Bentley, H. R., Burgan, J. G., *Analyst* **85**, 723 (1960).
Bock, F. G., Swain, A. P., Stedman, R. L., *J. Natl. Cancer Inst.* **44**, 1305 (1970).
Chortyk, O. T., Severson, R. F., Higman, H. C., *Beitr. Tabakforsch.* **8**, 204 (1975).
Christopher, S. W., Glass, R. L., *J. Dairy Sci.* **52**, 1289 (1969).
Cook, C. E., Twine, M. E., Tallant, C. R., Harper, I., Hemisch, G., *Phytochemistry* **8**, 1025 (1969).
Gil-Av, E., Shabtai, J., *Nature (London)* **197**, 1065 (1963).
Grimmer, G., Glaser, A., Wilhelm, G., *Beitr. Tabakforsch.* **3**, 415 (1966).
Grossman, J. D., Ikeda, R. M., Deszyck, E. J., Bavley, A., *Nature (London)* **199**, 662 (1963).
Grunwald, C., Davis, D. L., Bush, L. P., *J. Agric. Food Chem.* **19**, 138 (1971).
Jenkins, R. S., Newman, R. H., Carpenter, R. D., Osdene, T. S., *Beitr. Tabakforsch.* **5**, 295 (1969).
Reed, R. I., in "Mass Spectrometry of Organic Ions", McLafferty, F. W., Ed., Academic Press, New York and London, 1963, p 692.
Rowland, R. L., Latimer, P. H., Giles, J. A., *J. Am. Oil Chem. Soc.* **78**, 4680 (1956).
Rowland, R. L., Latimer, P. H., *Tob. Sci.* **3**, 1 (1959).
Schlotzhauer, W. S., Schmeltz, I., *Beitr. Tabakforsch.* **4**, 176 (1968).
Schlotzhauer, W. S., Schmeltz, I., *Beitr. Tabakforsch.* **5**, 5 (1969).
Seelkopf, G., Ricken, W., Dhom, G., *Z. Krebsforsch.* **65**, 241 (1963).
Severson, R. F., private communication, 1976.
Severson, R. F., Schlotzhauer, W. S., Arrendale, R. F., Snook, M.

E., *Beitr. Tabakforsch.*, in press (1976).
Snook, M. E., Chamberlain, W. J., Severson, R. F., Chortyk, O. T., *Anal. Chem.* **47**, 1155 (1975).
Swain, A. P., Cooper, J. E., Stedman, R. L., Bock, F. G., *Beitr. Tabakforsch.* **5**, 109 (1969).
Swain, A. P., Rusaniwskyj, W., Stedman, R. L., *Chem. Ind. (London)* **14**, 435 (1961).
Wynder, E. L., Wright, G., *Cancer* **10**, 255 (1957).
Wynder, E. L., Wright, G., Lam, J., *Cancer* **11**, 1140 (1958).

Received for review February 12, 1976. Accepted June 14, 1976. Reference to a company or product name does not imply approval or recommendation by the USDA.

A Gas–Liquid Chromatographic Method for the Determination of Dodine Residues on Foods

William H. Newsome

A gas–liquid chromatographic method was developed for the determination of dodine residues on fruit crops. The procedure involves extraction with methanol, partitioning with chloroform, and derivatization with hexafluoroacetyl acetone. The method yields mean recoveries of 84% or greater from 0.05 to 10.0 ppm of residue and has a lower detectable limit of 8 ppb. Confirmation of the derivative by GLC–mass spectrometry is described.

Dodine (*n*-dodecylguanidine acetate) is an agricultural fungicide introduced in 1957 and used in the control of certain diseases of fruit trees such as scab on apple or pear, leaf spot on cherry, and foliar diseases on strawberry. The presently available method for the determination of dodine residues involves a colorimetric procedure (Steller et al., 1960) and is unsuitable for regulatory purposes. Thus, it was considered desirable to develop a gas–liquid chromatographic method which would detect low levels and permit confirmation by mass spectrometry.

Because of their polar nature, guanidines require structural modification to render them sufficiently volatile for gas chromatography. Some guanido-containing compounds have been chromatographed as the corresponding trifluoroacetylated amine formed after alkaline hydrolysis (Hengstmann et al., 1974). A simpler and more sensitive method has been described which involves condensation with hexafluoroacetyl acetone to form a substituted pyrimidine (Erdtmansky and Goehl, 1975). Application of this approach to dodine provided a derivative which was amenable to gas–liquid chromatography and which could be detected at low concentration.

EXPERIMENTAL SECTION

Materials. Dodine was supplied by American Cyanamid and was labeled as assaying 100%. Solutions of dodine used for the fortification of samples were prepared in reagent grade methanol and were added to samples in volumes of 0.5 ml or less prior to initial extraction.

A reference standard of 2-dodecylamino-4,6-bis(trifluoromethyl)pyrimidine was prepared by refluxing dodine (500 mg; 1.74 mmol) for 4 h with hexafluoroacetyl acetone (2.0 g; 9.6 mmol) in benzene (10 ml). The resulting yellow solution was taken to dryness on a rotary evaporator and the amber oil taken up in warm hexane (10 ml). Upon cooling, a small quantity of dodine precipitated and was removed by filtration. The solvent was evaporated and the residual liquid permitted to crystallize at room temperature. The yield was 630 mg (91%), mp 34–35 °C. A 60-MHz NMR spectrum in deuteriochloroform gave the following resonances: δ (ppm) 7.08, 3.50, 1.30, 0.92. The ratio of their intensities was 1:2:20:3, respectively. The mass spectrum gave a molecular ion at m/e 399 as shown in Figure 1.

Analytical Procedure. The sample (5.0 g) was homogenized with methanol (50 ml) at high speed for 1 min in a Sorvall Omni-Mixer. The homogenate was filtered through Whatman No. 1 paper on a Buchner funnel using gentle vacuum. An aliquot (5.0 ml) of the filtrate was added to 0.1 M NaOH (30 ml) in a 125-ml separatory funnel. The dodecylguanidine was extracted with chloroform (10 ml) and the extract evaporated to dryness in a 15-ml centrifuge tube using a stream of nitrogen. A solution of redistilled hexafluoroacetyl acetone (10 μl) in benzene (400 μl) was added to the dried sample. A 10 × 150 mm air condenser was attached and the bottom 1 cm of the tube heated by immersion in a sand bath at 85 °C for 1 h.

After heating, the tube was cooled and the solvent removed by evaporation under a stream of nitrogen. The sample was then dissolved in hexane (1.0 ml) and applied to a small column of silicic acid. The column was prepared by placing a 5 × 25 mm bed of silicic acid (Mallinckrodt, AR, 2847, 100 mesh) on a plug of glass wool in a Pasteur pipet, slurrying it with hexane, and packing under nitrogen pressure. The sample was driven onto the column with nitrogen and the eluate discarded. The derivative was then eluted with 30% benzene in hexane (1.0 ml) and an aliquot (2 μl) analyzed by gas–liquid chromatography.

Gas–Liquid Chromatography. Analyses were carried out on a Hewlett Packard 5700 A gas chromatograph fitted with a 6 ft × 4 mm i.d. glass column and ^{63}Ni electron capture detector. The column was packed with 5% butanediol succinate on 100–120 mesh Chromosorb W, HP and preconditioned for 48 h at 200 °C under a flow of argon–methane (95:5) carrier gas. The column oven was maintained at 170 °C and detector at 300 °C. The carrier gas flow rate was 24 ml min^{-1}. Under these conditions and with a routine working attenuation, 100 pg of standard produced a peak with 40% full scale deflection. Samples

Food Research Division, Bureau of Chemical Safety, Foods Directorate, Health and Welfare Canada, Tunney's Pasture, Ottawa K1A 0L2, Ontario, Canada.