Volatile Components of Hardwood Sawdust Smoke

Components of Phenolic Fraction

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Thirty-one compounds in the phenolic fraction of mixed hardwood smoke were separated by gas chromatography on a variety of columns. They were identified by comparison of their retention times and their mass and infrared spectra with known authentic compounds except that in cases where reference materials were not available, degradative and synthetic reactions to known standard compounds were employed. The presence of 2-methoxy-4-allylphenol, 4-hydroxy-3,5-dimethoxybenzaldehyde, and all three isomeric methylphenols was confirmed. Compounds identified that have not been previously reported are: *o*-hydroxybenzaldehyde; 3-hydroxy-2-methylpyrone; 2-methoxy-4-*cis*-propenylphenol; 2-methoxy-4-*trans*-propenylphenol; 2,6-dimethoxy-4-vinylphenol; 2,6-dimethoxy-4-allylphenol; 2,6-dimethoxy-4-*cis*-propenylphenol; 2,6-dimethoxy-4-*trans*-propenylphenol; 4′-hydroxy-3′,5′-dimethoxyacetophenone; 3-(4′-hydroxy - 3′ - methoxyphenyl) - 2 - propenal; 4′-hydroxy-3′,5′-dimethoxypropiophenone; and 3 - (4′ - hydroxy - 3′,5′ - dimethoxyphenyl) 2-propenal. Condensates prepared from mixed hardwood sawdust and from hickory sawdust smoke were compared and found to contain the same compounds.

Exposure of foods to wood smoke is one of the oldest means of food preservation. In addition to partial dehydration during smoking, compounds with antimicrobial and antioxidant activity are deposited in meat and fish during smoking. In current commercial practice, this process is employed primarily for the color and flavor which are developed.

Wood smoke is a complex system consisting of vapor and particulate phases. Foster *et al.* (1961) developed a model of the smoking process based on a vapor absorption mechanism. They postulated that the particles act as a reservoir of components which are partitioned between phases depending on temperature and the removal of material from the vapor phase by the food in the smoking chamber. Variation in the extent to which smoke components are absorbed by foods may be expected, depending on the composition of the absorbing surface and the solubility of individual smoke components, in water, fat, and other food constituents. Chemical reactions between smoke components and food constituents are likely. Therefore, smoke composition is not the only consideration in determining the composition of smoked foods, but investigation of the composition of smoke is a logical starting point for study of the chemistry of the smoking process.

Acids, carbonyls, alcohols, and other neutral components have been identified in wood smoke (Fiddler *et al.*, 1967; Hamid and Saffle, 1965; Hoff and Kapsalopoulou, 1964; Jahnsen, 1961; Love and Bratzler, 1966; and Porter *et al.*, 1964). Using an approach based on volatility rather than functionality, Doerr *et al.* (1966) identified sixteen volatile components of hickory sawdust smoke. Recent capillary gas chromatographic analyses of hardwood smoke vapor phase (Kornreich and Issenberg, 1968) indicate that this fraction contains more than 100 components with boiling points lower than that of furfural (162° C). This volatile fraction is more complex than has been previously realized and deserves further investigation.

Special significance has been attributed to phenolic compounds as contributors to the flavor of smoked food products (Fiddler *et al.*, 1967; Tilgner *et al.*, 1962). Phenols have been determined in investigations of the technological aspects of the smoking process (Foster and Simpson, 1961; Foster *et al.*, 1961; Porter *et al.*, 1964; Tilgner *et al.*, 1962; Ziemba, 1963) because of their flavor significance and because most of the phenolic compounds present in smoke are not normal food components and their presence in foods and model systems can be attributed to exposure to smoke. Many of these investigations of the smoking process have employed colorimetric methods for determination of total phenols in smoke condensates and foods. Work in these laboratories (Proctor, 1959) showed that these methods, though rapid and simple, are not reliable since individual phenols yield different color intensities, and in some cases different colors with the reagents employed. Large differences in flavor thresholds of individual phenols have been demonstrated (Wasserman, 1966) and similar variation in physical properties and chemical reactivities may be expected.

Fiddler *et al.* (1966) reviewed methods employed for generation of smoke and identification of smoke components. Their investigation combined modern analytical methods with rigorous identification criteria for characterization of the phenols of hickory sawdust smoke. Eighteen components were identified by comparing gas chromatographic retention times and infrared spectra with known authentic samples. Three components were tentatively identified, based on chromatographic data.

The present study of the composition of the phenolic fraction of hardwood sawdust smoke was undertaken to characterize this fraction prior to studies of interactions between smoke components and foods. This information will be used in investigations of the partitioning of individual smoke components between smoke and food components and in studies of the chemical interactions which might occur.

EXPERIMENTAL

Generation of Smoke and Isolation of Aqueous Smoke Condensates. The smoke used in composition studies was generated and collected in the system shown in Figure 1. With the exception of the generating chamber (*B*) and specially constructed connectors, the system was assembled from commercially available glassware. From generation until collection and during subsequent fractionation, the smoke

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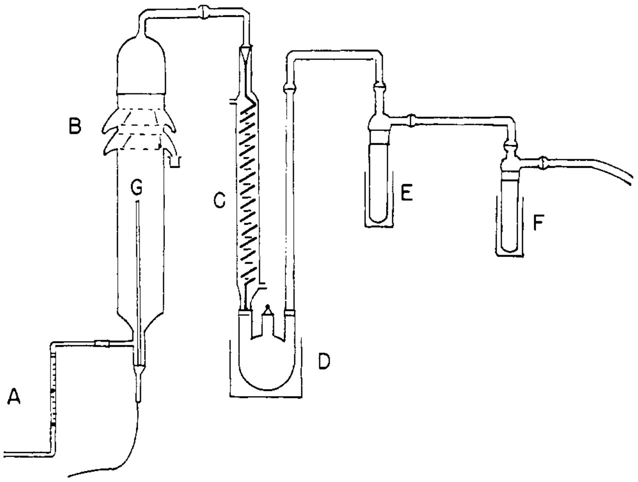

Figure 1. Smoke generator and collection system

A, Flowmeter; *B*, 64 × 330 mm. combustion chamber; *C* tap-water cooled condenser; *D*, ice-water cooled trap; *E*, solid carbon dioxide cooled condenser; *F*, liquid nitrogen cooled condenser; *G*, iron-constantan thermocouple

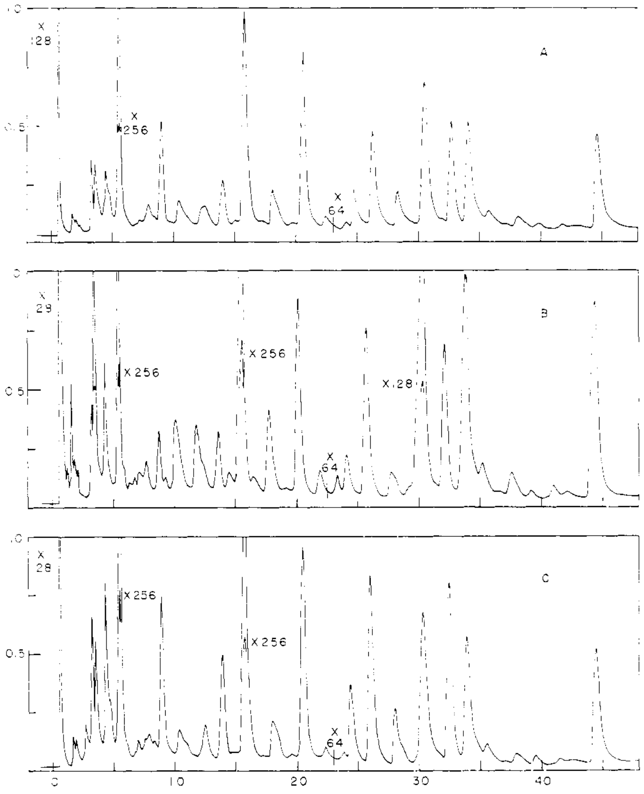

Figure 2. Analytical gas chromatograms of phenolic fractions from hardwood sawdust smoke

A and *B*, mixed hardwood smoke. *C*, hickory smoke. Column A. Temperature programmed from 80° to 200° C. at 2° C./min. Sample volume: 0.1 μl.

and smoke condensates contacted only glass surfaces to minimize contamination and decomposition. An iron-constantan thermocouple (*G*) was positioned at the central axis of the cylindrical glass generator. Its position can be adjusted to locate it at the point of maximum temperature within the generator. This highest measurable temperature was assumed to be the combustion temperature of the sawdust.

Table I. GC Columns Employed

	Column[a]	Flow Rate Used
A.	1.52 m. × 2.3 mm. I.D. 3% SE-30 on 100/120 mesh Aeropak 30	20 ml./min.
B.	1.83 m. × 2.3 mm. I.D. 5% Carbowax 20M-TPA on 100/120 mesh Chromosorb W	20 ml./min.
C.	1.83 m. × 5.3 mm. I.D. 20% SE-30 on 60/80 mesh Chromosorb W	60 ml./min.
D.	3 m. × 5.3 mm. I.D. 5% FFAP on 100/120 mesh Chromosorb G	100 ml./min.

[a] Stainless steel tubing was used for all columns.

Batches of 200 to 300 grams of mixed hardwood sawdust, prepared for commercial smokehouse use (J. F. Herne Co., Everett, Mass.) or of hickory sawdust (Koch Supplies, Inc., Kansas City, Mo.) were placed in the glass combustion tube and ignited. Air flow, from a compressed air cylinder, was controlled at 500–600 cc. per minute by a needle valve and measured with the flowmeter (*A*). Combustion temperature was maintained between 350° and 450° C. by control of air flow rate. In this temperature range, the sawdust charred to a dark brown residue, but did not exhibit a visible glow. Sawdust moisture content was approximately 10%.

The smoke passed through a tap-water cooled condenser (*C*) into an ice-water cooled trap (*D*). The barely visible smoke emerging from the ice-water cooled trap passed through a condenser cooled by solid carbon dioxide in ethanol (*E*) and a liquid nitrogen cooled condenser (*F*) in series. Noncondensed gases were exhausted into a laboratory fume hood.

Aqueous condensate accumulated in the condensing chambers at the top of the generator during the two- to four-hour period of smoke generation and collection. It was drained from these chambers and combined with the contents of the ice-water cooled trap for analysis of smoke constituents of moderate volatility. The volume of pooled aqueous condensate ranged between 20 and 25 ml.

Isolation of Phenol Fraction from Smoke Condensates. A phenolic fraction was prepared from the aqueous smoke condensate by fractionation based on acidity (Braus and Middleton, 1952). The condensate was diluted to a volume of 100 cc. and brought to pH 12 with 15 ml. of 40% sodium hydroxide. This solution was extracted consecutively with 200-ml., 100-ml., and two 50-ml. volumes of diethyl ether. The extract contained neutral compounds. Phenols were removed from the alkaline aqueous layer by saturation with carbon dioxide at 0° C., to pH 6.6 and extraction with 200-ml., 100-ml., and two 50-ml. portions of ether. Free acids remained in the aqueous phase. The ether solution of phenols was dried with anhydrous soldium sulfate and concentrated in a rotary evaporator at 30° C. to a volume of approximately 15 ml. Final concentration to a volume of 0.25 ml. was accomplished by careful evaporation of solvent in a stream of prepurified nitrogen.

The terms "neutral fraction," "phenolic fraction," and "acidic fraction" describe the general nature of these fractions. The procedures employed did not provide quantitative separation into specific compound classes, but were enrichment techniques employed to simplify subsequent separations. Many components were expected to be present in more than one fraction.

Separation of Phenolic Fraction. Comparison of the composition of the phenolic fractions from smoke produced from mixed hardwood and hickory sawdust (Figure 2) was carried out by separations on Column A. Chromatographic columns are described in Table I. Temperature was programmed

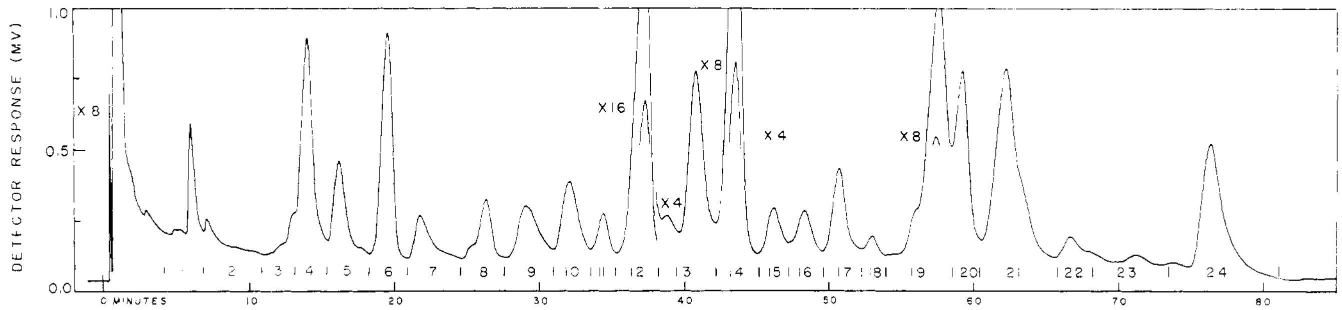

Figure 3. Preparative gas chromatogram of phenolic fraction of smoke from mixed hardwood sawdust

Column C. Sample volume: 20 μl. Fraction numbers correspond with those in Table II

from 80° to 200° C. at 2° C. per minute and sample volumes were approximately 0.1 μl.

Isolation of Components. The concentrated phenolic fraction was separated by gas chromatography on high resolution 0.5-mm. × 160-m. capillary columns coated with SF96 (50), OV-101, and OV-17 and on support-coated-open-tubular-columns (Perkin-Elmer Corp., Norwalk, Conn.) coated with SE-30, Carbowax 20M, and OV-17 liquid phases. However, no single column provided a separation adequate for unambiguous identification of all components.

Preparative chromatographic separations were, therefore, carried out on Column C and individual fractions were collected for further investigation. Samples (20 μl.) were injected and column temperature was programmed from 77° to 250° C. at 2° C. per minute. An Aerograph model 200 gas chromatograph (Varian Aerograph, Walnut Creek, Calif.) equipped with a thermal conductivity detector was used.

Individual fractions were collected in U-shaped capillary tubes measuring approximately 7 cm. on each leg and 2 cm. at the base. Tubes in which early fractions were collected were cooled with solid carbon dioxide. Cooling below ambient temperature was not necessary for the later fractions. A typical preparative gas chromatogram in which 24 collected fractions are indicated is given in Figure 3. Each fraction was rechromatographed on higher efficiency columns not only to determine purity but also to obtain retention data for identification. Columns A and B were used at temperatures in the range of 90° to 150° C. and 90° to 250° C., respectively. Temperatures were selected to provide optimum resolution and convenient retention times for individual components in each fraction. Detector and injector temperatures were 250° C. An Aerograph model 1200 gas chromatograph equipped with a flame ionization detector was used.

Purification of Phenol Components for Infrared Analysis. The major components collected as shown in Figure 3 were purified further by chromatography on Column D. Injection port and detector temperatures were 250° C. Column temperature in the range of 200° to 225° C. was selected to obtain maximum resolution of components. An Aerograph Model 200 gas chromatograph equipped with a thermal conductivity detector was used.

Infrared spectra of eluted components were recorded on a Perkin Elmer model 257 spectrophotometer fitted with a 4X beam condenser. Carbon tetrachloride or carbon disulfide (10 μl.) was added to the capillary tube and the solution was transferred to a 0.05-mm. path length type D sodium chloride cavity cell (Barnes Engineering Co., Stamford, Conn.). Chloroform was used when compounds were insoluble in the above solvents. When quantities permitted, spectra of thin films or of KBr pellets of purified compounds were also recorded.

Mass Spectra Determinations. Columns A and B were placed in the mass spectrometer inlet chromatograph (Aerograph Model 204-C). The effluent passed through a stainless steel capillary restriction (1.9 m. × 0.50 mm. I.D.) and fritted glass enricher (Watson and Biemann, 1965) both of which were silanized to minimize adsorption of polar compounds. The effluent then passed into the ion source of a double-focusing mass spectrometer (Hitachi Perkin-Elmer, RMU-7). The glass enricher was maintained at 200° C. The capillary restriction temperature was 300° C. Injector temperature was 250° C. and the chromatograph detector oven temperature was 220° C. Ion source temperature was 250° C. Electron energy was 70 eV and ionizing current was 100 μA. Mass spectra were scanned over the range *m/e* 4–400 in 5 seconds. Source and collector slits were set at 0.5 mm., providing a resolving power of approximately 400. Chromatograms were recorded from the total ion current monitor located between the electrostatic and magnetic analyzers. Spectra of reference compounds were recorded under conditions identical to those employed for components of fractions collected from smoke concentrates.

Characterization of Components by Hydrogenation and Ozonolysis. Certain components, for which reference samples or spectra were not available, were characterized by study of their hydrogenation and ozonolysis products.

Hydrogenation of selected fractions was carried out at room temperature and atmospheric pressure in a 2-ml. test tube in which Adam's platinum oxide catalyst (0.5 mg.) had been shaken with 100 μl. of ethanol under H_2 for 40 minutes. The unknown fraction (10–50 μg.) in 5 μl. of ethanol was added and the reaction mixture was shaken under H_2 for 1 hour. The resulting mixture was filtered through a 0.45-μ Millipore filter type HAWG (Millipore Corp., Bedford, Mass.) using a Flath-Lundin Filter Kit (Hamilton Co., Whittier, Calif., Model No. 76500) into a 3-ml. centrifuge tube. The catalyst was washed with two 50-μl. portions of ethanol. The washings were filtered, combined with the initial filtrate, and the total solution was concentrated to approximately 0.25 μl. by careful purging with prepurified nitrogen. The resulting concentrate was subjected to GC-MS and IR analysis.

Ozonolysis of 10–50 μg. of selected components in ethanol was carried out in the apparatus described by Beroza and Bierl (1967). Reaction times between 10 and 50 seconds were employed.

Synthesis of Reference Compounds. 3-(4′-ACETOXY-3′-METHOXYPHENYL)-2-PROPENOIC ACID (ACETYLFERULIC ACID) was prepared by acetylation of 3-(4′-hydroxy-3′-methoxyphenyl)-2-propenoic acid (ferulic acid). A solution of 7.4 grams of ferulic acid in 50 ml. of 5% sodium hydroxide was treated with 10 ml. of acetic anhydride and the mixture was heated below its boiling point until a clear colorless solution resulted. On cooling, white crystals of acetyl ferulic acid were obtained. Recrystallization from glacial acetic acid yielded 8.3 grams (92%) m.p. 192–194° C. The melting point was identical with that reported by Fosdick and Starke (1940).

ACETYL FERUOYL CHLORIDE was prepared as described by Freudenberg (1951). The white crystalline product melted at 136° C.

3-(4′-HYDROXY-3′-METHOXYPHENYL)-2-PROPENAL (CONIFERALDEHYDE) was synthesized in a manner analogous to the synthesis of 3-(4′-hydroxy-3′-5′-dimethoxyphenyl)-2-propenal (sinapaldehyde) by Pearl (1959).

Acetylferuoyl chloride (1 gram, 4 millimoles) was dissolved in 18 ml. of dry tetrahydrofuran (Fieser and Fieser, 1967). Lithium-tri-(*tert.*-butoxy) aluminum hydride (Alfa Inorganics, Inc., Beverly, Mass.) (1 gram, 4 millimoles) in 20 ml. of dry tetrahydrofuran was added and the solution was held at 20° C. for 6 hours. It was then poured into 100 ml. of cold water. A gelatinous precipitate formed which was removed by filtration. The filtrate was found by combined GC-MS analysis to contain 95% acetyl coniferaldehyde and 5% coniferaldehyde. The acetyl group was removed by hydrolysis in aqueous 5% sodium hydroxide. The product, a yellow viscous oil, contained 80% coniferaldehyde. Attempts to crystallize it from a variety of solvents were unsuccessful.

The product was purified for infrared analysis by gas chromatography, first on Column C and then on Column D under conditions already described. Its infrared spectrum was identical with the spectrum reported for coniferaldehyde by Pearl (1959). The molecular ion at *m/e* 178 was the base peak of the mass spectrum. The abbreviated mass spectrum (Hites and Biemann, 1968) was: *m/e* 27 (3%), 29 (3%), 38 (2%), 39 (8%), 41 (16%), 53 (10%), 63 (11%), 65 (6%), 77 (24%), 89 (12%), 90 (3%), 91 (8%), 107 (25%), 109 (12%), 118 (13%), 124 (14%), 135 (33%), 145 (10%), 146 (8%), 147 (32%), 161 (16%), 163 (9%), 177 (24%), 178 (100%).

SINAPALDEHYDE was prepared from sinapoic acid in a manner analogous to that described for the synthesis of coniferaldehyde. The product was also a yellow viscous oil that could not be recrystallized from benzene or other solvents.

The IR spectrum obtained after purification by gas chromatography on Columns C and D was identical with that reported by Pearl (1959). In the mass spectrum the molecular ion at *m/e* 208 was the base peak. The abbreviated mass spectrum was: *m/e* 28 (19%), 29 (6%), 39 (9%), 43 (8%), 51 (13%), 53 (10%), 65 (14%), 66 (8%), 77 (18%), 79 (8%), 91 (11%), 94 (8%), 105 (9%), 119 (6%), 122 (8%), 133 (10%), 137 (22%), 154 (8%), 165 (31%), 177 (18%), 180 (31%), 193 (6%), 207 (10%), 208 (100%).

Preparation of 2-Methoxy-4-methylphenol, 2-Methoxy-4-ethylphenol, and 2-Methoxy-4-vinylphenol (4-methyl, 4-ethyl, and 4-vinylguaiacol) by Thermal Decomposition of Ferulic Acid. Thermal decomposition of ferulic acid was carried out in a manner similar to that reported by Fiddler *et al.* (1967).

The pyrolysis chamber was a 50-ml. volumetric pipet with $^{1}/_{4}$-inch stainless steel Swagelok fittings (Crawford Fitting Co., Cleveland, Ohio) at the ends. The straight tubing on both sides of the pipet bulb was bent to fit it into the oven of a gas chromatograph (Chromolab model #440, Glowall Corp., Glenside, Pa.). One end was connected to the injection port, through which oxygen entered, and the other end led out of the oven into a solid carbon dioxide-ethanol trap.

The chamber bulb was charged with 1 gram of ferulic acid and pyrolysis was carried out at 300° C. for 5 hours at an oxygen flow rate of 10 ml. per minute.

The product was a reddish yellow liquid and individual components were separated on Column C. The infrared and mass spectra of the isolated compounds were consistent with those of authentic samples of 4-methyl- and 4-ethylguaiacol and with the structure of 4-vinylguaiacol.

2,6-DIMETHOXY-4-METHYLPHENOL. The compound was synthesized according to the method reported by Coscia *et al.* (1961).

Most reference compounds were obtained from commercial sources. 2-Methoxy-4-propylphenol, 2,6-dimethoxy-4-propylphenol and 4′-hydroxy-3′,5′-dimethoxypropiophenone (propiosyringone) were provided by Dr. I. A. Pearl of the Institute of Paper Chemistry, Appleton, Wis. Samples of 2-hydroxy-3-methylcyclopent-2-en-1-one and 3-hydroxy-2-methylpyrone were provided by Givaudan Corp., Clifton, N. J. Dr. W. Fiddler, Eastern Regional Laboratory, U.S.D.A. provided samples of 2,6-dimethoxy-4-ethylphenol and 2,6-dimethoxy-4-allylphenol.

RESULTS AND DISCUSSION

Smoke components that have been identified in this work are listed in Table II. Identification was considered to be established when infrared and mass spectra and chromatographic retention times on both the nonpolar Column A (3% SE-30) and polar Column B (5% Carbowax 20M TPA) coincided with data recorded for reference compounds.

The tentative identification of eugenol by Fiddler *et al.* (1966) was confirmed in this work and its presence unambiguously shown. Syringaldehyde was tentatively identified on the basis of chromatographic evidence as a major carbonyl compound in wood smoke by Simpson and Campbell (1962). Its presence was established unambiguously in this work. In addition thirteen new compounds have been unambiguously identified. Salicylaldehyde, maltol, *cis*-isoeugenol, *trans*-isoeugenol, acetovanillone, acetosyringone, coniferaldehyde, sinapaldehyde, propiosyringone, 2,6-dimethoxy-4-vinylphenol, 2,6-dimethoxy-4-allylphenol, 2,6-dimethoxy-4-*cis*-propenylphenol, and 2,6-dimethoxy-4-*trans*-propenylphenol had not been previously reported as components of hardwood smoke. Identification of the latter four compounds completed the series of alkyl- and alkenyl-2,6-dimethoxyphenols (syringols) which are analogs of the corresponding 2-methoxyphenols (guaiacols) identified in this work and by other investigators.

The identities of 2,6-dimethoxy-4-vinylphenol, 2,6-dimethoxy-4-allylphenol, 2,6-dimethoxy-4-*cis*-propenylphenol, and 2,6-dimethoxy-4-*trans*-propenylphenol in Fractions 16, 17, 18, and 20, respectively, were initially postulated on the basis of mass spectra and GC retention data. They were confirmed by hydrogenation to the corresponding saturated products. Fraction 16, postulated to be 2,6-dimethoxy-4-vinylphenol was hydrogenated to 2,6-dimethoxy-4-ethylphenol. Fractions 17, 18, and 20 postulated to be 2,6-dimethoxy-4-allylphenol, 2,6-dimethoxy-4-*cis*-propenylphenol, and 2,6-dimethoxy-4-*trans*-propenylphenol, respectively, all yielded 2,6-dimethoxy-4-propylphenol upon hydrogenation. In all cases, GC retention times and the IR and mass spectra were identical with those of authentic samples of the expected saturated products. Fractions 18 and 20 tentatively identified as 2,6-dimethoxy-4-*cis*-propenylphenol and 2,6-dimethoxy-4-*trans*-propenylphenol, respectively, were successfully ozonized providing confirmatory evidence of structure. The GC retention time on Column A and mass spectrum of the aldehyde produced by ozonolysis were identical with those of an authentic sample of syringaldehyde.

Mass spectra of Fractions 18 and 20 are shown in Figure 4. The results of hydrogenation and ozonolysis of microgram

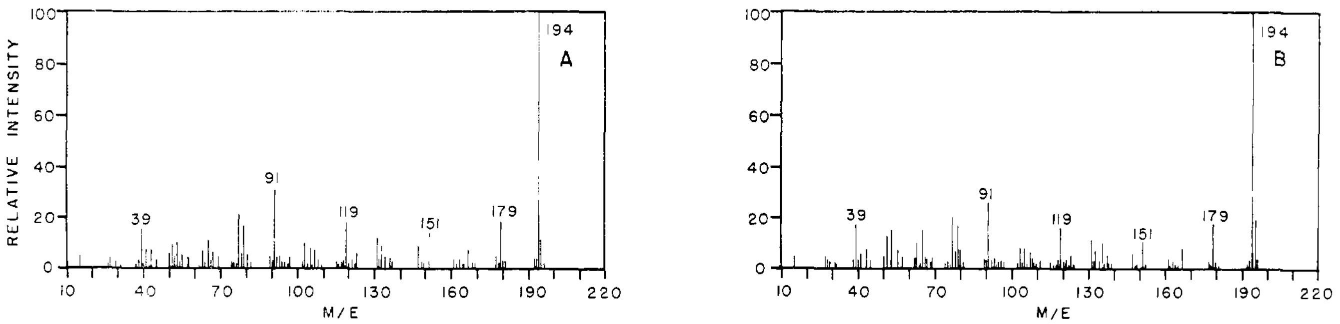

Figure 4. Mass spectra Fraction 18

A, Fraction 18. *B*, Fraction 20

Table II. Components of Phenolic Fraction

Fraction[a]	Identity	Evidence[b]
1	2-Furancarbonal (Furfural)	GC, MS
2	2-Furancarbinol (Furfurylalcohol)	GC, MS
3	5-Methyl-2-furancarbonal (5-Methylfurfural)	GC, MS
4	Phenol	GC, IR, MS
5a	*o*-Hydroxybenzaldehyde (Salicylaldehyde) (10%)[c]	GC, MS
5b	*o*-Methylphenol (*o*-Cresol) (10%)	GC, MS
5c	2-Hydroxy-3-methylcyclopent-2-en-1-one (Cyclotene) (80%)	GC, IR, MS
6a	*o*-Methoxyphenol (Guaiacol) (90%)	GC, IR, MS
6b	*m*-Methylphenol (*m*-Cresol) (5%)	GC, IR,[f] MS
6c	*p*-Methylphenol (*p*-Cresol) (5%)	GC, IR,[f] MS
7	3-Hydroxy-2-methylpyrone (Maltol)[c]	GC, IR, MS
8	2-Methoxy-4-methylphenol (4-Methylguaiacol)	GC, IR, MS
9	Unknown	
10	2-Methoxy-4-ethylphenol (4-Ethylguaiacol)	GC, IR, MS
11	2-Methoxy-4-vinylphenol (4-Vinylguaiacol)	GC, IR, MS
12a	2,6-Dimethoxyphenol (Syringol) (80%)	GC, IR, MS
12b	2-Methoxy-4-allylphenol (Eugenol) (20%)	GC, IR, MS
13a	4-Hydroxy-3-methoxybenzaldehyde (Vanillin) (60%)	GC, IR, MS
13b	2-Methoxy-4-*cis*-propenylphenol (*cis*-Isoeugenol) (10%)[c]	GC, MS
13c	2-Methoxy-4-*trans*-propenylphenol (*trans*-Isoeugenol) (30%)[c]	GC, MS
14	2,6-Dimethoxy-4-methylphenol	GC, IR, MS
15a	2,6-Dimethoxy-4-ethylpenol (60%)	GC, IR, MS
15b	4′-Hydroxy-3′-methoxyacetophenone (Acetovanillone) (40%)[c]	GC, IR, MS
16	2,6-Dimethoxy-4-vinylphenol[c]	IR, MS[d]
17	2,6-Dimethoxy-4-allylphenol[c]	IR, MS[d]
18	2,6-Dimethoxy-4-*cis*-propenylphenol[c]	IR, MS[e]
19	4-Hydroxy-3,5-dimethoxybenzaldehyde (Syringaldehyde)	GC, IR, MS
20	2,6-Dimethoxy-4-*trans*-propenylphenol[c]	IR, MS[e]
21a	4′-Hydroxy-3′,5′-dimethoxyacetophenone (Acetosyringone) (50%)[c]	GC, IR, MS
21b	3-(4′-Hydroxy-3′-methoxyphenyl)-2-propenal (Coniferaldehyde) (50%)[c]	GC, IR, MS
22	4′-Hydroxy-3′,5′-dimethoxypropiophenone (Propiosyringone) (50%)[c]	GC, IR, MS
23	Unknown	
24	3-(4′-Hydroxy-3′,5′-dimethoxyphenyl)-2-propenal (Sinapaldehyde)[c]	GC, IR, MS

[a] Fraction numbers correspond to those used in Figure 3.

[b] GC, IR, MS: gas chromatographic retention time, infrared spectrum, and mass spectrum evidence, respectively. Evidence cited is consistent with data for authentic compounds.

[c] Compounds not previously reported as components of wood smoke.

[d] Identity confirmed by hydrogenation.

[e] Identity confirmed by hydrogenation and ozonolysis.

[f] IR spectrum of an equal volume mixture of standard *m*- and *p*-cresol was identical to that of the fraction, in which chromatography on a 0.5-mm. I.D. support coated open tubular Carbowax 20M column, showed that they were present in equal amounts.

quantities of Fraction 20 are illustrated in Figure 5. Mass spectra of reaction products are identical with those of the reference compounds postulated. Identical results were obtained during examination of Fraction 18. These results illustrate the applicability of the GC-MS system to analysis of products of reactions conducted on a microgram scale. Such degradative and synthetic reactions are valuable for unambiguous identification of small quantities of components isolated from natural sources when reference materials are not readily available.

Attempts to ozonize, 2,6-dimethoxy-4-vinylphenol (Fraction 16) and 2,6-dimethoxy-4-allylphenol (Fraction 17) were unsuccessful. Authentic samples of 2-methoxy-4-allylphenol and of 2,6-dimethoxy-4-allylphenol could not be ozonized in a micro-ozonolysis apparatus. This result appears to reflect a difficulty of ozonolysis of terminal double bonds under the conditions employed. Difficulty in ozonizing some compounds during the usual short reaction times employed in a micro-ozonolysis procedure has been reported by other investigators (Supelco Inc., 1969).

All the compounds found by Fiddler *et al.* (1966) in hickory smoke were identified with the exception of acetol, 2-cyclopentenone, veratrol, 2-methoxy-4-propylphenol, and 2,6-dimethoxy-4-propylphenol. The fractionation procedure used in the present work for preparation of the phenolic fraction would be expected to discriminate against the neutral compounds. Furfural, furfuryl alcohol, 5-methylfurfural, and cyclotene are not sufficiently acidic to separate with the phenols. Their presence in this fraction suggests that they are present in very high concentrations in the smoke condensate.

From retention data calculations, 2-methoxy-4-propylphenol and 2,6-dimethoxy-4-propylphenol would be expected to be present in Fractions 13 and 21, respectively. In addition, due to the lower thermal stability of a propyl side chain relative to a methyl, ethyl, or propenyl substituent, they would be expected to be present as minor components of these fractions. These compounds were found as minor components by Fiddler *et al.* (1966). Their absence in this work could be due to inadequate separation from the major components in Fractions 13 and 21. GC-MS evidence indicated the presence of 2-methoxy-4-propylphenol as a minor component in Fraction 13, but insufficient quantities of the compound were available for positive identification.

Fiddler *et al.* (1966) tentatively identified *m*-cresol, but did not find *o*- or *p*-cresol in hickory smoke phenolic concentrates separated on a Carbowax 20M-TPA column. In this investigation the three cresols were found to be minor components of the phenolic fraction and could not be separated from the major components on a single column. The *meta* and *para* isomers appeared in the same fraction as guaiacol on Column

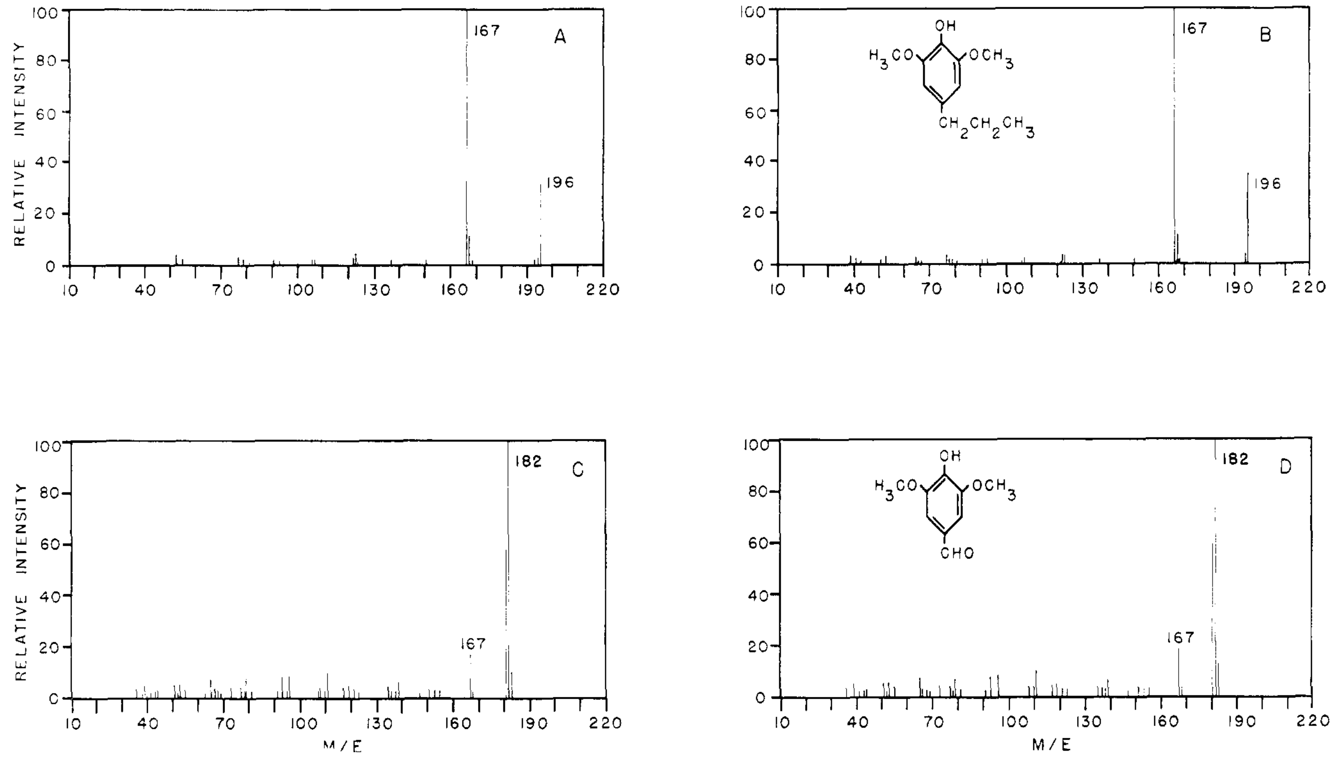

Figure 5. Mass spectra

A. Fraction 20 after hydrogenation
B. 2,6-Dimethoxy-4-propylphenol
C. Fraction 20 after ozonolysis
D. Syringaldehyde

C. On rechromatography of this fraction on Column B, they were well separated from guaiacol but could not be separated from each other. Satisfactory resolution was obtained only on a high efficiency support coated open tubular Carbowax 20-M column operated at 120° C. at a flow rate of 6 ml. per minute. Identification on the basis of mass spectra was unambiguous. Phenol and *o*-cresol have almost identical retention times on Column B but are well separated from each other on Columns A and C. Presence, of the cresols as minor components, and their similarity in structure, and, in the case of *o*-cresol, its similarity in retention behavior to phenol, has made their identification difficult. The three isomeric cresols were reported present in wood smoke by Jahnsen (1961), on the basis of paper chromatographic data. In this work the presence of all three isomeric cresols is established unambiguously.

It is evident from Table II that both monomethoxy- and dimethoxyphenols were formed as products of the oxidative thermal degradation of wood. This is not surprising since both monomethoxy- and dimethoxyphenolic monomers are present in hardwood lignins (Pearl, 1967). It is unknown whether the dimethoxyphenols contribute to the aroma of smoked food products. However, since they are present in smoke itself, the extent to which they are deposited in food and their contribution to flavor, color and bacteriostatic properties of smoked food products, should be investigated.

The comparison of chromatograms of phenolic fractions of smoke generated from mixed hardwood sawdust and from hickory sawdust (Figure 2) indicated that the same major components were present in both mixtures. Mass spectrometric analysis of column effluents confirmed this. Quantitative differences are apparent, but these are no greater than variations observed between phenolic fractions isolated from different batches of smoke condensate produced from the same kind of sawdust. No characteristic differences in odor could be detected between phenolic fractions prepared from hickory and mixed hardwood sawdust smoke condensates. All of the phenolic fractions had a mild smoky odor. Apparently the presence of a number of components is required to produce the typical pleasant character of wood smoke aroma.

ACKNOWLEDGMENT

The authors express their gratitude to I. A. Pearl, Institute of Paper Chemistry; R. E. Erickson, Givaudan Corp.; and W. Fiddler, Eastern Regional Laboratory, U.S.D.A., for samples of reference material. T. J. Mysliwy provided interesting discussions and valuable technical assistance. We are grateful to B. A. Centola, B. M. King, S. B. Udin, and C. B. Scott for technical assistance.

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Received for review May 22, 1969. Accepted August 4, 1969. Presented in part at the 156th National Meeting, American Chemical Society, Atlantic City, N. J., September 1968. Publication Number 1470 from the Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139. This investigation was supported in part by Grant No. UI-00290 from the National Center for Urban and Industrial Health, U. S. Public Health Service; and in part by a gift from Givaudan Corporation. Purchase of the mass spectrometer and associated equipment was made possible by a generous grant from the Campbell Soup Fund.