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Received for review March 12, 1973. Accepted July 30, 1973.

Isolation and Identification of the Components of the Tar of Hickory Wood Smoke

Denis E. Hruza, Sr.,* Michel van Praag,[1] and Howard Heinsohn, Jr.

The volatile components of hickory wood tar were fractionated by preparative gas chromatography. Individual fractions were analyzed on a Carbowax 20M 50 ft × 0.02 in. support coated open tubular column (SCOT) coupled to a mass spectrometer. The following compounds are among those being reported for the first time from hickory wood tar: 2-methyl-2-butenoic acid; benzaldehyde; 2-acetylfuran; 2-cyclohexenone; 4-propylguaiacol; 4-methylveratrol; 3-methyl-2-cyclopenten-1-one; 2,3-pentanedione; acetophenone; resorcinol; vinylphenol; 2,6-dimethylphenol; and 2-ethylphenol. A total of six aldehydes, eight ketones, four esters, six furans, 12 aromatic hydrocarbons, 32 phenols, and 13 acids were identified.

The smoking of foods as a method of preservation is one of the oldest methods known. Smoking not only partially dehydrates food during the process but also deposits compounds possessing antimicrobial antioxidant activities in the meat. With today's modern technology of food preservation, the smoking of foods is done for color and added flavor.

Most of the previous work in the literature on smoke flavor has been carried out on wood smoke itself. The identification of acids, carbonyls, alcohols, and other neutral components has been published (Fiddler *et al.*, 1967; Hamid and Saffle, 1965; Hoff and Kapsalopoulou, 1964; Jahnsen, 1961; Love and Bratzler, 1966; Porter *et al.*, 1964). Recently, work was carried out on the constituents of a liquid smoke solution (Fiddler *et al.*, 1970a,b). Much work has been done on the phenolic compounds and their role as contributors to the flavor of smoke foods (Fiddler *et al.*, 1967; Kornreich and Issenberg, 1972; Lustre and Issenberg, 1969; Tilgner *et al.*, 1962). The technological aspects of the smoking process have revealed the presence of phenols in smoked foods. Since phenolic compounds identified in foods are not normal components, they were attributed to the smoking process (Fiddler *et al.*, 1966; Foster and Simpson, 1961; Foster *et al.*, 1961; Porter *et al.*, 1964; Tilgner *et al.*, 1962; Ziemba, 1963).

We decided to look at a different aspect of smoke flavor by examining the tar of hickory wood smoke with a possibility of preparing a synthetic substitute.

ISOLATION

The material examined was a commerical sample obtained from Old Hickory Products Co., Atlanta, Ga. The material is a natural flavor product made from 100% hickory wood. As the hickory wood tar is a black viscous material, it was first dissolved in acetone (Matheson, Coleman and Bell, Spectroquality) and filtered to remove any carbon particles, and most of the solvent was removed by distillation using a Kuderna–Danish concentrator (Kontes Glass Co., Vineland, N. J.). The clarified sample (*ca.* 8 ml) was then used for our analytical work.

The acids present in the material were isolated and identified in the following manner. One kilogram of the hickory smoke concentrate was steam distilled and 2 l. of distillate were collected. A liter portion was made basic to pH 7.8 with sodium bicarbonate and the nonacidic components were removed by extracting with 3 × 150 ml of diethyl ether (Matheson, Coleman and Bell, ACS reagent grade). The aqueous mixture was then made acidic with concentrated hydrochloric acid to pH 1, saturated with sodium chloride, and extracted with 3 × 150 ml of diethyl ether. The ethereal extracts were combined, dried over anhydrous sodium sulfate, and filtered, and the solvent was removed using a Kuderna–Danish concentrator. The residue was then methylated using a diazomethane generator utilizing *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide (Aldrich Chemical Co.). The methylated residue was then analyzed in the same manner as the clarified sample described below.

ANALYTICAL METHODS

Preparative gas chromatography was carried out on an F&M 770 preparative gas chromatograph equipped with a thermal conductivity detector utilizing an 8 ft × ¾ in. stainless steel column packed with 25% SE-30 on 60–80 mesh Chromosorb W A/W. The column temperature was programmed from 75° to 225° at 2°/min, with a helium carrier gas at a flow rate of 300 ml/min. The injection sizes for this separation were 2 ml. Two milliliters of the clarified sample was injected and the effluent collected as 12 distinct peaks which were trapped in capillary glass tubes cooled with crushed Dry Ice and sealed (Figure 1). The fractions were further resolved using an F&M 700 glc equipped with a thermal conductivity detector utilizing an 8 ft × ¼ in. stainless steel column packed with 20% Carbowax 20M on 60–80 mesh Chromosorb W A/W. The column temperature was programmed from 75° to 225° at 2°/min, with helium carrier gas at a flow rate of 80 ml/min. This column was used to trap out distinct peaks for nmr and ir analyses.

Nmr spectra of the samples in deuteriochloroform solution were recorded using a Varian HA-100 spectrometer. Tetramethylsilane was the internal reference compound. Sweep width was 1000 Hz, with a 50-Hz sweep offset.

Glc–ms analyses were carried out using an Aerograph 1520 gas chromatograph coupled to a Hitachi RMU-6E mass spectrometer. A 50 ft × 0.02 in. support coated open tubular (SCOT) stainless steel column coated with Carbowax 20M was used. The column temperature was programmed from 30° to 175° at 2°/min, with a helium carrier gas at a flow rate of 6 ml/min. The column was used with the column effluent split so that 5 ml was directed

International Flavors and Fragrances, Inc., Union Beach, New Jersey 07735.
[1] International Flavors and Fragrances, Tilburg, Holland.

Table I. Compounds Identified from Tar of Hickory Wood Smoke

Compound	Reported in smoke	I_E value, gc–ms	Mol wt	Mass spectral data[b]
Acids				
Propionic acid	X[c]	2.26	88	57–29–*88*–59–89–27
Butyric acid	X	3.34	102	43–74–71–27–41–59–*102*
2-Methylbutyric acid		3.43	116	57–88–41–29–85–59–*116*
Isovaleric acid	X	3.79	116	74–43–41–59–29–57–*116*
2-Methylpropenoic acid			100	41–69–*100*–39–15–99
Valeric acid	X	4.51	116	74–29–27–57–43–41–*116*
3-Methylpentanoic acid (T)[a]			130	74–43–59–41–29–99–*130*
4-Methylpentanoic acid		4.63	130	43–74–57–55–27–87–*130*
2-Methyl-2-butenoic acid (cis)		5.59	114	55–83–27–*114*–29–39
2-Methyl-2-butenoic acid (trans)	X		114	55–83–27–*114*–29–39
Hexanoic acid	X	5.53	130	74–43–27–87–29–41–*130*
Octanoic acid	X	7.56	158	74–87–43–55–41–57–*158*
Benzoic acid		10.00	136	105–77–*136*–51–50–29
Aldehydes				
Acetaldehyde	X	0.44	44	29–*44*–43–26–42–27
Propionaldehyde	X	0.81	58	29–28–27–*58*–26–57
Tiglaldehyde (T) (2-Methyl-2-butenal)	X	4.61	84	55–29–*84*–27–39
2-Furfural	X	8.24	96	39–*96*–95–66
Benzaldehyde		9.03	106	*106*–77–105–57–50
5-Methyl-2-furaldehyde	X	9.36	110	*110*–109–53–27–51
Aromatic hydrocarbons				
Benzene	X	2.92	78	*78*–51–52–49–39–77
Toluene	X	4.14	92	91–*92*–39–65–51
Styrene (T)		6.32	104	*104*–103–78–51–77
Indene		8.61	116	*116*–115–63–39–89
Naphthalene		11.12	128	*128*–51–129–64–127–63
3-Methylindene		9.80	130	*130*–115–129–128–51–63
2-Methylnaphthalene		11.92	142	*142*–141–115–143–139
Dimethylindane (T)			146	131–*146*–91–115–129–39
Esters				
Methyl formate	X	0.63	60	31–29–32–*60*–15
Methyl acrylate		2.70	86	55–27–85–58–26–*86*
Ethyl benzoate		10.41	150	105–77–51–122–*150*
Cresyl acetate		10.9	150	108–107–43–77–51–*150*
Furans				
Furan	X	0.82	68	39–*68*–38–29–37
2-Methylfuran (T)	X	2.00	82	*82*–53–81–39–27–29
2-Ethylfuran (T)		3.00	96	81–39–*96*–41–51–65
2-Acetylfuran		8.70	110	95–*110*–39–43–96
2-Methylbenzofuran (T)			132	131–*132*–51–77–39
Dimethylbenzofuran			146	*146*–145–117–39–115
Ketones				
Acetone	X	1.00	58	43–*58*–27–26–42–29
Methyl ethyl ketone		2.21	72	43–29–27–*72*–42–57
3-Methyl-3-butene-2-one (methyl isopropenyl ketone)		2.70	84	43–41–39–*84*–69–42
2,3-Butanedione (diacetyl)	X	3.17	86	43–*86*–42–44
2-Cyclohexenone		8.15	96	68–39–*96*–40–27–42
2,3-Pentanedione		4.13	100	43–29–57–29–*100*
3-Methyl-2-cyclopenten-1-one		8.73	110	82–39–*110*–54–27–41
Acetophenone		10.26	120	105–77–*120*–51–43
Phenols				
Phenol	X	13.38	94	*94*–39–66–65–40
o-Cresol	X	13.08	108	*108*–107–79–77–39
m-Cresol	X	13.98	108	107–*108*–77–79
p-Cresol	X	14.11	108	107–*108*–79–77
1,3-Dihydroxybenzene (resorcinol)		10.50	110	*110*–82–81–69–53–55
Vinylphenol (*o,m,p*-?)		16.91	120	*120*–91–26–119–39
2,6-Dimethylphenol		12.51	122	*122*–107–121–77–39–91
2-Ethylphenol		13.99	122	107–*122*–77–39–79
2,4-Dimethylphenol	X	14.00	122	*122*–107–121–77–39
2,5-Dimethylphenol	X	14.73	122	*122*–107–121–77–39–27
2,3-Dimethylphenol		14.79	122	107–122–121–77–39
4-Ethylphenol	X	15.01	122	107–*122*–77–39–27
3-Ethylphenol		15.05	122	107–*122*–77–39–27
o-Methoxyphenol (guaiacol)	X	12.71	124	109–*124*–81–53–27–39

Table I (*Continued*)

Compound	Rnported in smoke	I_E value, gc-ms	Mol wt	Mass spectral data[b]
Phenols				
m-Methoxyphenol			124	*124*-94-81-39-53-95
Trimethylphenol (T)		15.23	136	121-*136*-135-39-91-77
3-Ethyl-5-methylphenol			136	121-*136*-91-77-39-122
2-Methoxy-4-methylphenol (4-methylguaiacol)	X	12.98	138	*138*-123-95-39-55-27
2-Methoxy-4-vinylphenol (4-vinylguaiacol)	X	15.23	150	*150*-135-107-77-51-39
tert-Butylphenol (T)		16.13	150	135-107-*150*-91-39-41
4-Ethyl-2-methoxyphenol (4-ethylguaiacol)	X	13.83	152	137-*152*-39-*91*-*122*-*94*
2,6-Dimethoxyphenol (syringol)	X	15.88	154	*154*-139-111-96-107-93
4-Allyl-2-methoxyphenol (eugenol)	X	15.09	164	*164*-77-149-39-103
2-Methoxy-4-propenylphenol (isoeugenol)	X	16.77	164	*164*-149-77-103-91-55-39
4-Isopropyl-2-methoxy-phenol (T) (4-isopropyl-guaiacol)			166	137-*166*-138-122-94-51
2-Methoxy-4-propylphenol (4-propylguaiacol)	X	14.41	166	137-*166*-138-122-94-51
2,6-Dimethoxy-4-methyl-phenol (T)	X	16.77	168	*168*-153-125-53-65
2,6-Dimethoxy-4-ethyl-phenol (T)	X	17.26	182	167-*182*-77-107-79
2,6-Dimethoxy-4-allyl-phenol (T)	X	18.99	194	*194*-91-119-77-167-39-131
2,6-Dimethoxy-4-pro-penyl phenol	X	21.18	194	*194*-91-77-119-79-39
2,6-Dimethoxy-4-isopropylphenol (T)			196	181-*196*-137-77-91-121
2,6-Dimethoxy-4-propylphenol	X		196	167-*196*-168-41-53-197
Miscellaneous				
Ethanol	X	2.46	46	31-45-*46*-27-29-43
1,2-Dimethoxybenzene (veratrol)	X	10.73	138	*138*-95-123-77-52-51
1,4-Dimethoxybenzene (T)		11.7	138	*138*-95-123-77-52-51
1,2-Dimethoxy-4-methyl-benzene (4-methylveratrol)		11.7	152	*152*-137-109-91-77-39

[a] T = tentative. [b] Mass spectral data are reported in order of decreasing intensities with the molecular weight in italics.. [c] X = previously reported in smoke condensate.

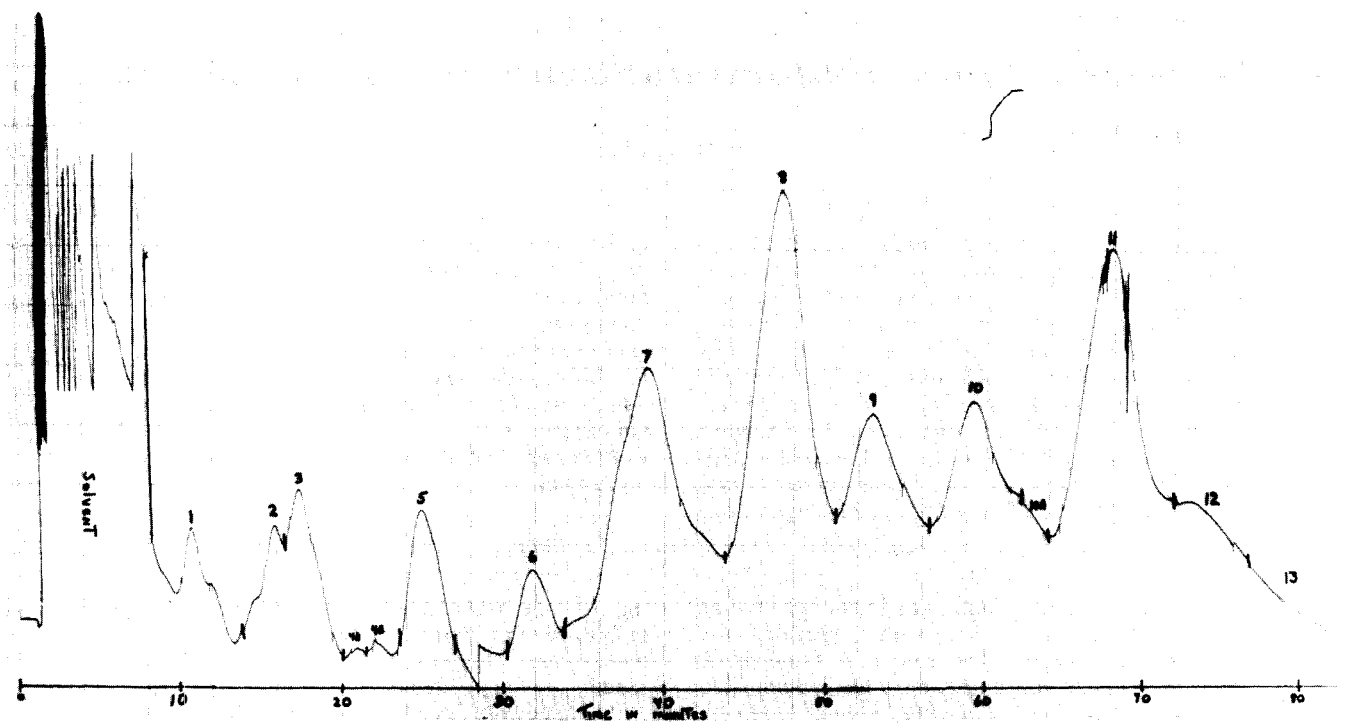

Figure 1. Separation of hickory smoke tar by preparative gas chromatography.

through a Watson-Biemann separator (Watson and Biemann, 1965) to the mass spectrometer, while the remainder of the effluent went to the flame ionization detector of the gas chromatograph.

Retention indices (I_E values) were determined by the method of van den Dool and Kratz (1963) using standards of ethyl esters of *n*-aliphatic acids and programmed temperature glc. The I_E values for the unknown compounds in the glc–ms runs were obtained by interpolation between peaks of unambiguously identified compounds of known I_E values. For this reason and because of the influence of other components on retention times, the I_E values for some of the later eluting peaks could not be determined precisely as for the earlier eluting ones. The I_E values obtained on the open tubular columns were, in most cases, very close to those obtained on standard packed columns.

RESULTS AND DISCUSSION

The compounds identified from all the trappings and methylated acid fraction are listed in Table I. Identification was made by matching the mass spectra of the unknown with reference spectra and verified by comparing the retention index values of the unknown compounds in the glc–ms runs to I_E values for known compounds. Tentative identifications are indicated where the mass spectra were weak or mixed, or reference compounds were not available for comparison. Many compounds that were used for reference were commercially purchased samples. In some cases, the chemicals were synthesized by the authors. When sufficient material could be trapped out, nmr and ir spectra were obtained to aid in the identification.

Many of the major constituents of the tar of hickory wood smoke have been identified. Although there has been no previous work on the tar of hickory wood smoke, several of these compounds have been previously reported in smoke condensate and extracts. This is noted in Table I. There were also several compounds found in smoke condensate which were not found in the tar. A possible reason is that the smoke condensate was obtained in a laboratory under controlled conditions while the material for this investigation was a commercially purchased sample of which little is known.

SUMMARY

As a result of this work on the tar of hickory wood smoke, we have identified 81 of the major constituents. Although many of the identified constituents can be considered to contribute to the hickory smoke aroma, it is not possible on the basis of this study to pinpoint one or more chemicals which could be described definitely as hickory smoke.

ACKNOWLEDGMENT

The authors thank Anne Sanderson for her help in the mass spectral interpretation and Mort Jacobs for his assistance in obtaining and interpreting the nmr spectra.

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Received for review May 7, 1973. Accepted October 31, 1973.

Solubility Behavior of Soybean Globulins as a Function of pH and Ionic Strength

Wim H. van Megen

The solubility behavior of partially purified soybean globulins as a function of pH and ionic strength of the dispersing medium was investigated. It appeared that, even at the isoelectric point, the soy protein could be dissolved easily up to very high concentrations, provided that the ionic strength of the solution exceeded a critical value which, at pH 4.5, was about 0.7 for NaCl and Na_2SO_4 and 0.25 for $CaCl_2$. Below the critical ionic strength a two-phase system was formed, consisting of a protein-poor upper layer and a viscous protein-rich lower layer. At pH 7.0 no phase separation was observed at very low ionic strength but, as the salt concentration was increased, a region was passed in which the solution demixed. Outside the regions of immiscibility only homogeneous systems were obtained. The composition of the protein-poor layers in the two-phase systems was in agreement with the well known protein extractability curves for soybean meal.

The majority of the soy proteins are insoluble at their isoelectric point. Their solubility in dilute aqueous solution increases as the pH diverges from this value or, at constant pH, as the salt concentration is increased (salting-in). Therefore these proteins have been classified as globulins. The typical solubility behavior of globulins has been clearly demonstrated on β-lactoglobulin (Grönwall, 1942).

It is also well known that salting-in of proteins may be followed by salting-out when the ionic strength is in-

Unilever Research, Duiven, The Netherlands.