Composition of an Ether-Soluble Fraction of a Liquid Smoke Solution

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An ether-soluble fraction of a commercial liquid smoke solution was separated by gas–liquid chromatography. Thirty-four components were identified by comparison of their GLC retention times and infrared spectra with those of authentic samples, and fourteen were tentatively identified by their retention times on two different columns: 23 phenols, six acids, four furan carbonyl derivatives, four 1,2-cyclopentanediones, three lactones, two cyclopentenones, two hydroxyketones, one furanone, one ester, maltol, and 2,5-hexanedione. Several were not previously reported in wood smoke. Certain compounds found in smoke are also found in other food flavors and their possible formation is discussed briefly.

In previous studies at this laboratory the composition of wood smoke used in the preparation of smoked food products was investigated, utilizing whole smoke and smoke condensates as sources of components (Doerr *et al.*, 1966; Fiddler *et al.*, 1966). Whole smoke is difficult to handle and subject to changes in composition because of the condensation and precipitation of components prior to collection. Smoke condensates, which are easier to use, are the readily condensable components of smoke but may not represent all of the important constituents of whole smoke. Our knowledge of the composition of smoke, however, has been so limited (until recently) that a study of the condensate has provided valuable information on smoke constituents.

Another form of liquid smoke solutions that is being used commercially is prepared by passing smoke through water. These solutions can be prepared by a fairly reproducible procedure providing a large scale, constant source of material for study. The method of preparing one of these commercial solutions has been described in a patent (Hollenbeck, 1963) and some of the components have been identified. Hollenbeck and Marinelli (1963), using paper chromatography, reported the presence of formic, acetic, propionic, vanillic, and syringic acids, acetaldehyde, glyoxal, methyl glyoxal, furfural, acetone, ethanol, guaiacol, and syringol (2,6-dimethoxyphenol) in this preparation. Paper chromatography, however, does not permit adequate separation and identification of the trace components in smoke and smoke solutions (Wasserman *et al.*, 1967).

We continued our study of the constituents of smoke, utilizing the smoke solution described above as a more convenient source of material. The smoke solution, however, was too dilute for analysis of trace components. This paper describes a procedure for concentrating the constituents of the solution by ether extraction and the identification of a number of components.

EXPERIMENTAL

Preparation of Smoke Flavor Concentrate. Charsol, a liquid smoke solution, was obtained from the Red Arrow Products Corp., Milwaukee, Wis. A smoke concentrate was prepared from the smoke solution by continuous extraction with diethyl ether for 30 hours in a liquid–liquid Palkin Automatic Extraction Apparatus, then evaporation of the ether with a stream of nitrogen at room temperature.

Gas–Liquid Chromatography (GLC). The components of the smoke concentrate were separated with a Perkin-Elmer Model 800 dual-column gas chromatograph with flame ionization detectors equipped with a 4 to 1 effluent splitter to permit collection. The ether extract was first chromatographed with a 6-foot by $^1/_8$-inch o.d. column packed with 15% Carbowax 20M-TPA on 60- to 80-mesh Gas Chrom P. The operating conditions were: temperature programming from 70° to 180° C. at 5° per minute with a helium carrier gas flow of 125 cc. per minute. The trapped peak material was then rechromatographed on a 6-foot by $^1/_8$-inch o.d. column packed with 15% SE-30 on 60- to 70-mesh Gas Chrom Z in the same instrument. The temperature was programmed from 70° to 180° C. at 2.5° per minute. The helium carrier flow was 85 cc. per minute. Injection port and detector temperatures were 210° and 200° C., respectively.

For compounds not completely separated by use of the Carbowax 20M-TPA and SE-30 columns, a 6-foot by $^1/_8$-inch o.d. 15% DEGS on 60- to 80-mesh Gas Chrom Z column was used in an F and M Model 810 gas chromatograph with a carrier flow of 85 cc. per minute.

Infrared Spectra. The individual components were trapped in melting point capillary tubes. Each tube was rinsed with approximately 5 μl. of carbon tetrachloride into a 0.1-mm. path length KBr type D microcavity cell. The spectra were recorded on a Perkin-Elmer Model 421 infrared spectrophotometer fitted with a 4× beam condenser.

Reference Compounds. Samples of the unsubstituted, 3-ethyl- and 3,4-dimethyl-1,2-cyclopentanediones were prepared from their corresponding 3,5-dicarbethoxy derivatives. The latter compounds and 2,3-dimethyl-2-cyclopentenone were obtained through the courtesy of M. A. Gianturco, The Coca-Cola Co., Linden, N. J. The 2,5-dimethyl-4-hydroxy-3(2H)furanone was obtained from J. A. Losekoot, Unilever Research Laboratory, Duiven, The Netherlands. The remaining authentic standard samples of chemicals were obtained from commercial sources and purified by GLC before use in the identification of smoke components.

Identification of a component was considered positive when the GLC retention times on two different columns and the infrared spectrum agree with those of an authentic sample. Tentative identification of several compounds were made based on their retention times on two columns.

RESULTS AND DISCUSSION

The chromatogram of the smoke solution concentrate, separated on the Carbowax 20M-TPA column, is given in Figure 1. The identities of the various peaks, together with their retention times on the several columns, are listed in Table I. Thirty-four components have been positively identified and 14 additional compounds were tentatively identified. One of the pitfalls of trying to identify com-

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pounds on the basis of GLC retention times alone was exemplified in this study. Many of the peaks eluting as separate entities from the Carbowax 20M-TPA column could be further separated into two or more components by rechromatography on the SE-30 column.

Separation of peak 7 on the SE-30 column showed it to consist principally of furfural with a small quantity of acetol acetate. This was the only ester identified under the gas chromatographic conditions used. More volatile methyl esters may be present, as we reported previously (Doerr *et al.*, 1966), but they would appear within the first minute of the separation and would be difficult to collect.

Although peak 11 was recovered as a single entity after chromatography on both the Carbowax 20M and SE-30 columns, the infrared spectrum indicated it was a mixture. A carbonyl absorption at 1780 and 1710 cm.$^{-1}$ suggested the presence of a 5-membered ring lactone and acid, respectively. The retention characteristics of several lactones and acids, together with the strong characteristic odor of the trapped material, assisted in narrowing the choice of possible compounds. Satisfactory identification of peak 11 as a mixture of γ-butyrolactone and butyric acid was made by resorting to separation on a third column (DEGS).

Peaks 13 and 17 were tentatively identified as the α,β-unsaturated 5-membered β-angelica lactone and γ-crotonolactone, respectively, by comparing the location and relative intensities of the doublet carbonyl absorptions in the infrared to those found by Jones *et al.* (1959). The identity of peak 13 was confirmed as β-angelica lactone. This compound may be derived from levulinic acid (peak 32). Peak 17 was only tentatively identified as γ-crotonolactone based on the similarity of its infrared spectrum to that published by Angell *et al.* (1960) and its relative retention time on Carbowax 20M reported by Tatum *et al.* (1969). An authentic sample was not available to confirm the identification.

Peak 18 is 3-methyl-1,2-cyclopentanedione (cyclotene) which occurs as the enol 3-methyl-2-cyclopenten-2-ol-1-one (Bredenberg, 1959). This compound has been identified in maple syrup (Filipic *et al.*, 1965), stored orange powder (Tatum *et al.*, 1967), and from the thermal decomposition of carbohydrates and closely related substances (Johnson *et al.*, 1969; Shaw *et al.*, 1968; Tatum *et al.*, 1969; Walter and Fagerson, 1968). A minor component of peak 18 was tentatively identified as 3,4-dimethyl-1,2-cyclopentanedione. Comparison of the IR spectra of peaks 15 and 20 with that of cyclotene indicated strong similarities. All three had free and bonded O—H stretch vibrations (enol form of the diketone), double carbonyl absorptions at approximately 1710 and 1660 cm.$^{-1}$, and a strong doublet centered at 1400 cm.$^{-1}$ as well as a strong band in the skeletal region at 1100 cm.$^{-1}$. The unsubstituted- (peak 15) and 3-ethyl-1,2-cyclopentanedione (peak 20) were subsequently identified by comparison with authentic samples prepared by the hydrolysis, then decarboxylation of their corresponding 3,5-dicarbethoxy derivatives (Gianturco and Friedel, 1963). The three alkyl cyclic diketones identified have also been found by Gianturco *et al.* (1963) in the aroma complex of roasted coffee and from the base-catalyzed degradation of fructose (Shaw *et al.*, 1968). The 3-methyl-1,2-cyclopentanedione has been prepared by refluxing an aqueous alkaline mixture of glyceraldehyde with acetone (Fray, 1961). Glyceraldehyde with butanone forms 3-ethyl- and 3,4-dimethyl-1,2-cyclopentanedione in a similar manner (Gianturco, 1967).

Peaks 3 and 5 were identified as 1-hydroxy-2-propanone (acetol) and 1-hydroxy-2-butanone: two of the four C_3 and

Table I. Liquid Smoke Solution Components

Peak No.	Compounds	Retention Time, Min. CW20M-TPA	SE-30	Other
3	1-Hydroxy-2-propanone (acetol)	4.1	0.8	
	2-Methyl-2-cyclopentenone		2.8	
5	1-Hydroxy-2-butanone	5.3	4.0	
6	Acetic acid	6.0	<1.0	
7	Furfural	6.9	2.0	
	Acetol acetate		2.4	
8	2-Furyl methyl ketone	7.8	3.0	8.3[a]
	2,5-Hexanedione		3.0	9.2
9	Propionic acid	8.1	1.5	
	2,3-Dimethyl-2-cyclopentenone	8.6	5.5	
10	5-Methylfurfural	9.2	3.3	
11	Butyric acid	10.4	3.0	11.3[b]
	γ-Butyrolactone		3.0	6.5
13	β-Angelica lactone	11.5	3.4	
14	Valeric acid[c]	12.3	5.5	
15	1,2-Cyclopentanedione	13.1	3.0	
17	γ-Crotonolactone[c]	13.6	2.9	
18	3-Methyl-1,2-cyclopentanedione (cyclotene)	14.3	5.5	
	3,4-Dimethyl-1,2-cyclopentanedione[c]		6.9	
19	Guaiacol	14.9	7.4	
	Tiglic acid[c]		5.5	
20	3-Ethyl-1,2-cyclopentanedione	15.6	8.3	
22	4-Methylguaiacol	16.8	11.4	
23	Phenol	17.7	4.0	
	o-Cresol[c]		6.0	
24	Maltol	18.2	7.9	
	4-Ethylguaiacol		15.0	
25	2,5-Dimethyl-4-hydroxy-3(2*H*)-furanone	18.8	7.0	
26	*m*-Cresol	19.6	6.8	
	p-Cresol		6.8	
	2,4-xylenol[c] and/or		9.3	
	2,5-xylenol[c]		9.3	
28	4-Propylguaiacol[c]	20.1	19.0	
29	*p*-Ethylphenol	21.4	10.0	
	Eugenol		18.6	
30	3,4-Xylenol[c]	21.8	16.4	
	4-Vinylguaiacol[c]		19.0	
31	Syringol	22.7	18.4	
	cis-Isoeugenol[c]		20.6	
32	Levulinic acid	23.8	8.4	
33	4-Methylsyringol	24.4	22.0	
	trans-Isoeugenol[c]		20.6	
35	4-Ethylsyringol	25.7	26.6	
36	4-Propylsyringol[c]	28.3	30.5	
37	3-Methoxycatechol	28.6	14.2	
	5-Hydroxymethyl-2-furfural[c]		14.0	
38	Vanillin	30.5	19.7	
39	Acetovanillone	33.7	10.8	
42	"a catechol"	37.3		

Retention time in minutes on DEGS column:
[a] Isothermal at 80° C.
[b] Temperature program, 70°–180° C. at 6° per minute.
[c] Tentative identification (see Experimental).

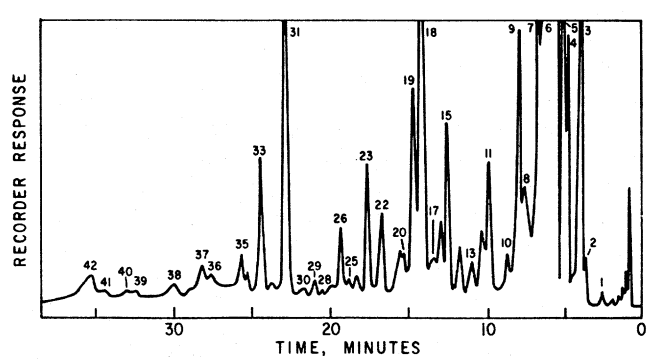

Figure 1. Gas–liquid chromatogram of ether-soluble fraction of a liquid smoke solution

C_4 hydroxyketones which Shaw *et al.* (1968) used in their proposed mechanism leading to the formation of cyclic diketones. A similar mechanism to their base-catalyzed condensation could operate as a free radical reaction during the degradation of wood when the smoke components are being formed.

Peak 24 was found to be a mixture of 4-ethylguaiacol and maltol. Maltol has been found in bread, soy sauce, heated milk, chicory, and coffee (Backe, 1910; Nakajimi and Takei, 1949; Patton, 1958; Reichstein and Beitter, 1930; Reichstein and Staudinger, 1926) and is a product of nonenzymatic browning.

The intense burnt sugar aroma of peak 25 led to its identification as 2,5-dimethyl-4-hydroxy-3(2*H*)furanone. This compound was originally identified in a pineapple flavor concentrate by Rodin *et al.* (1965) and more recently in boiled beef broth (Tonsbeek *et al.*, 1968). While Hodge *et al.* (1963) obtained this product from a Maillard-type browning reaction from rhamnose, Shaw *et al.* (1968) have obtained it by the base-catalyzed degradation of fructose. It is clear that in the case of the furanone and the cyclic diketones a carbohydrate source is necessary. In wood, cellulose, a polyglucoside, thermally degrades to form lower molecular weight products, which under the proper conditions condense and cyclize to form these compounds.

All the *ortho*-substituted alkyl phenols had an O—H stretch vibration near 3610 cm.$^{-1}$, while that of the methoxy-substituted phenols was located at 3560 cm.$^{-1}$, in agreement with published work (Rao, 1963) which indicates a $\Delta\nu$ of 52 cm.$^{-1}$. This information proved useful in the identification of several components, especially peaks 24, 26, and 29.

The small amount of material collected from peak 26 after separation on the two columns precluded further chromatography. However, the infrared spectrum was adequate for the identification of *m*- and *p*-cresol in a mixture. Peak 29 was identified as a mixture of *p*-ethylphenol and eugenol.

The major component of peak 37 is 3-methoxycatechol. The infrared spectrum indicated the same O—H stretch at 3560 cm.$^{-1}$ as the guaiacols instead of 3610 cm.$^{-1}$ as shown by the nonbonded phenols. This compound is probably a product obtained by the demethylation of syringol. There is also some infrared and mass spectral evidence that the main component of peak 42 may be another substituted catechol. These compounds, together with other polyphenols not obtained under the gas chromatographic conditions used, may be important for the antioxidative and antibacterial activity of wood smoke.

CONCLUSIONS

Forty-eight components have been reported in an ether extract of a commercial natural liquid smoke preparation. The following compounds have not been previously reported in wood smoke: 1-hydroxy-2-butanone, acetol acetate, 2,5-hexanedione, 2,3-dimethyl-2-cyclopentenone, γ-butyrolactone, β-angelica lactone, 1,2-cyclopentanedione and its 3-ethyl derivative, maltol, 2,5-dimethyl-4-hydroxy-3(2*H*)furanone, *p*-ethylphenol, eugenol, levulinic acid, 3-methoxycatechol, and acetovanillone. Also γ-crotonolactone, 3,4-dimethyl-1,2-cyclopentanedione, tiglic acid, 2,4- and/or 2,5-xylenol, *cis*- and *trans*-isoeugenol, 5-hydroxymethyl-2-furfural were tentatively identified.

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