New Components with Potential Antioxidant and Organoleptic Properties, Detected for the First Time in Liquid Smoke Flavoring Preparations

María D. Guillén* and María L. Ibargoitia

Tecnología de Alimentos, Facultad de Farmacia, Universidad del País Vasco,
Paseo de la Universidad 7, 01006 Vitoria, Spain

A commercial aqueous smoke preparation was exhaustively extracted, using dichloromethane as solvent, until the carrier had totally lost its smoky odor. Qualitative and quantitative characterizations of the extract were performed by means of gas chromatography/mass spectrometry and gas chromatography with flame ionization detector, respectively. Carbonyl derivatives including aldehydes and ketones as well as acids and esters are almost absent; however, the high proportion of phenol, guaiacol, and syringol derivatives is noticeable. The presence of di-*tert*-butylhydroxytoluene, several hopanes, and a number of lignin dimers must be pointed out; these latter components had apparently not been detected before either in smoke flavorings or in wood smoke. The mass spectral data of the compounds considered as lignin dimers and of the unidentified components are given. The presence of lignin dimers is very interesting from the point of view of health and food technology for their therapeutic, organoleptic, and antioxidant properties.

Keywords: *Liquid smoke preparations; extraction; gas chromatography; gas chromatography/mass spectrometry; phenol, guaiacol, and syringol derivatives; di-tert-butylhydroxytoluene; hopanes; lignin dimers*

INTRODUCTION

The use of smoke flavoring preparations for food smoking instead of the traditional techniques is increasing. This is due to the numerous advantages that the use of smoke flavorings has over the traditional smoking process (Pszczola, 1995).

Smoke flavoring preparations can show different physical states, colors, and odors. These different properties are due to the carrier used to support the smoke components and to the nature and concentration of the smoke components in the corresponding carrier.

Commercial smoke flavorings previously studied have been shown to belong to two different types of preparations with regard to the proportions of carbonylic and phenolic derivatives in the blend.

One type of commercial smoke flavoring is characterized by a higher proportion of carbonylic derivatives than of phenolic derivatives, similar to the proportions of these compounds in smoke (Guillén and Ibargoitia, 1996a,b). The main differences found between preparations belonging to this group are the absolute concentrations of the components in the mixture, the acidity degree, and the carrier's nature (Guillén and Manzanos, 1996a,b).

Another type of commercial smoke preparation is characterized by an overall higher proportion of phenolic derivatives (phenol, guaiacol, syringol, and their derivatives) than of carbonylic derivatives in different proportions from those found in smoke (Guillén and Ibargoitia, 1996a,b). Differences between preparations of this group are also due to the absolute concentrations of the compounds, the acidity degree, the carrier's nature, and in some cases the presence of fats, salt, or aromatic compounds coming from plants (Guillén et al., 1995; Guillén and Manzanos, 1997).

In this paper the composition of a commercial smoke flavoring is reported, comprising the extraction of the supposed aromatic compounds, by liquid−liquid extraction with an organic solvent, study of the nature of the extracted compounds and identification by GC/MS, and their quantification by GC with FID detector. The composition found allows us to classify this flavoring in a new group of smoke flavorings; in addition, new components have been found, some of them with potential antioxidant and organoleptic properties. These compounds have not been previously detected either in smoke flavorings or in wood smoke.

MATERIALS AND METHODS

Sample, Solvents, and Standard Compounds. The sample studied was a water-based smoke flavoring of a light brown color and an intense and pungent odor, used in the Spanish food industry. The extraction of the volatile compounds was carried out with CH_2Cl_2. This organic solvent was selected for its high effectiveness in extracting polycyclic aromatic compounds and aromatic compounds in general (Guillén et al., 1991, 1995; Guillén, 1994) and for its high volatility. Standard compounds, available from Aldrich, Fluka, and Sigma, were used for identification of some components and for the gas chromatographic quantification; these are asterisked in Table 1.

Extraction, GC/MS, and GC. A sample of 5 mL of liquid smoke was exhaustively extracted until the carrier totally lost its smoky odor. The solvent was partially evaporated, under vacuum in a rotary apparatus using mild and careful conditions to avoid the loss of very volatile compounds, until a solution volume of 1 mL was reached, and this was kept in a refrigerator.

---

* Author to whom correspondence should be addressed (fax 34-45-130756; e-mail knpgulod@vf.ehu.es).

S0021-8561(97)00952-7 CCC: $15.00

The GC/MS employed a Hewlett-Packard chromatograph, model 6890 series II, equipped with a mass spectrometer selective detector 5973 (MS), and a Hewlett-Packard Vectra XM Pentium computer. A fused-silica capillary column (30 m long, 0.25 mm diameter, 0.25 μm film thickness), coated with a nonpolar stationary phase (HP-5 cross-linked 5% phenyl methyl silicone), was used. The temperature program began at 50 °C (0.5 min) and increased at 5 °C/min until 280 °C was reached (10 min). Helium was used as carrier gas. Injector and detector temperatures were 250 and 280 °C, respectively. The injection technique used was splitless. The volume of sample injected was close to 1 μL. Mass spectra were recorded at an ionization energy of 70 eV. Components were identified by their retention times, by their mass spectra, by comparing their mass spectra with those in a commercial library (Wiley 138K, Mass Spectral Database, Wiley 1990), and in some cases by using standards, as in previous studies (Guillén et al., 1995).

A Hewlett-Packard gas chromatograph model 5890 series II, equipped with a flame ionization detector (FID) and a Vectra VL2 4/66 computer, was used for the quantitative study. A fused-silica capillary column (30 m long, 0.32 mm internal diameter, 0.25 μm film thickness), coated with a nonpolar stationary phase (HP-5, cross-linked 5% phenyl methyl silicone), was used. The temperature program began at 50 °C (0.5 min) with an increase of 5 °C/min until 290 °C (10 min), and nitrogen was used as carrier gas. Injector and detector temperatures were 250 and 300 °C, respectively. The injection technique used was splitless, and the volume of the sample injected was 1 μL. All compounds asterisked in Table 1 were used as external standards for quantification, and response factors of compounds of a similar nature were used for the quantification of those compounds not commercially available.

Each stage of this experiment was performed several times to obtain accurate results.

RESULTS AND DISCUSSION

The liquid smoke has a clear brown color and a very intense and pungent odor and its acidity is 6 mequiv/L. A sample of 5 mL was exhaustively extracted by liquid−liquid extraction using CH_2Cl_2 as solvent, and the yield, determined by gas chromatography, was 1451 mg/L.

Figure 1 shows the total ion chromatogram of the obtained extract. It can be observed that the compounds in appreciable concentrations in the sample elute between 7 and 55 min, and compounds that elute down to 7 min are scarce and are in very small concentrations.

Table 1 shows most of the identified compounds by groups. One can see the scarce number (29 compounds) of carbonyl, acid, and alcohol derivatives in the sample as well as their very small concentrations. The total concentration of these types of compounds in the flavoring is 38 mg/L. Compounds such as propanal, 1-acetoxypropan-2-one, 3-methylcyclopentane-1,2-dione (cyclotene), 2-furancarboxaldehyde, 2(5*H*)-furanone, and 3-hydroxy-5-methyl-2(5*H*)-furanone among others, which are generated in the thermal degradation of cellulose and hemicellulose and which are present in high proportions in the smoke (Maga, 1988; Guillén and Ibargoitia, 1996a,b) and in other commercial smoke flavorings (Guillén et al., 1995; Guillén and Manzanos, 1996a,b, 1997), are absent in this liquid smoke. This shows that this flavoring does not contain all of the typical smoke components, nor are they in the proportions in which they are present in the smoke; that is, this preparation is made of only a fraction of the smoke components due to the small proportion of acid, alcohol, ester, carbonyl, and furan derivatives.

In addition to the compounds mentioned above, a high number of phenol derivatives (27 compounds) have been found, and those in higher concentrations are phenol and methyl- and some dimethyl- or ethylphenols; the total concentration of this group in the flavoring is 146 mg/L. The presence of di-*tert*-butylhydroxytoluene (BHT), in small concentration, must be pointed out; this well-known antioxidant has not been found before in other smoke flavorings, and only Baltes et al. (1982) have detected it in the Curiepoint pyrolysis of tars from concentrated smoke aroma condensates. This compound could have been generated in the wood pyrolysis and detected for the first time because of the high sensitivity of the selective mass detector used, or it might have been added to the smoke flavoring.

Guaiacol and derivatives constitute the second main group of phenolic derivatives by concentration (334 mg/L); in this group guaiacol, 4-methyl-, 4-ethyl-, and 4-propylguaiacol and eugenol are those in highest concentrations. Syringol and derivatives have been found to be the most concentrated phenolic derivatives in the sample (385 mg/L), and also in this group 4-methyl-, 4-ethyl-, 4-propyl-, and 4-propenylsyringol derivatives are the main components, as might be expected. Taking into account the components cited above, the flavor of this liquid smoke will be basically due to phenol derivatives, which have aromas judged as pungent, cresolic, burnt, and smoky (Kim et al., 1974; Baltes and Söchtig, 1979), but incomplete and different from the global smoke aroma (Olsen, 1976).

A small number of other well-known terpenic compounds (in total 9 mg/L) have been found in this preparation; compounds of this nature have been detected before in commercial liquid smoke flavorings (Guillén and Manzanos, 1997). Likewise, a considerable number of alkyl aryl ethers in small concentrations have been detected (in total 85 mg/L) as have a very high number of lineal hydrocarbons (in total 126 mg/L); these latter types of compounds are generated in the wood pyrolysis and are present in smoke (Maga, 1988).

Hopanes are another group of compounds found in small proportions (44 mg/L) and not detected before either in smoke or in smoke flavorings. These compounds are present in higher plants, and some of them have been considered as biomarkers for the study of petroleum and sediments (Rohmer et al., 1992; Philp, 1985). These compounds could have been generated in the wood pyrolysis and detected for the first time because of the high sensitivity of the selective mass detector used, or it could arise from a possible external contamination by petroleum derivatives.

The phenol derivatives basically result from the thermal degradation of lignin. Lignin is a high molecular mass randomly cross-linked polymer, consisting of an irregular array of differently bonded hydroxy- and methoxy-substituted phenylpropane units (Fengel and Wegener, 1989; Sjöström, 1993; Alén et al., 1996). During pyrolysis, competing thermal degradation reactions take place that generate different bond cleavages according to their bond energies.

Due to the high structural diversity of lignin, its pyrolysis should provide a high number of products. In smoke flavorings and smoke studied previously (Maga, 1988; Guillén et al., 1995; Guillén and Ibargoitia, 1996a,b; Guillén and Manzanos, 1996a,b, 1997) only lignin monomers with a phenolic functional group have been detected; however, the smoke flavoring studied here is constituted by a high number of lignin dimers in small concentration. Table 2 shows the mass frag-

Table 1. Identified Compounds in the Dichloromethane Extract of the Liquid Smoke, Their Retention Times (RT), and Their Concentrations

no.	RT (min)	compound[a]	concn (mg/L)
		Carbonylic, Acid, and Alcohol Derivatives	37.7
1	1.56	acetaldehyde*	0.6
2	1.62	2-propanone*	8.9
3	1.95	2-butanone*	0.3
4	1.98	acetic acid*	0.6
5	4.41	furanmethanol (furfuryl alcohol)*	2.2
6	5.47	1-(2-furanyl)ethanone (acetylfuran)*	0.7
7	6.60	benzaldehyde*	0.2
8	6.80	3-methyl-2-cyclopenten-1-one*	0.3
9	6.85	2,4,5-trimethyl-2-cyclopenten-1-one	nd[b]
10	7.55	4,5-dimethyl-2-cyclopenten-1-one	0.3
11	7.64	1-cyclopentylethanone	nd
12	7.77	1-(2-furanyl)propanone	nd
13	8.00	*trans*-4,5-dimethyl-2-cyclopenten-1-one	nd
14	8.19	benzyl alcohol*	0.4
15	8.34	3,4,5-trimethyl-2-cyclopenten-1-one	0.6
16	8.40	2,3-dimethyl-2-cyclopenten-1-one	5.9
17	9.12	2,3,4-trimethyl-2-cyclopenten-1-one	0.6
18	9.22	1-phenylethanone (acetophenone)*	0.7
19	10.09	trimethyl-2-cyclopenten-1-one	1.8
20	10.40	2-phenylethanol	3.2
21	10.72	*trans*-4-methyl-5-isopropylcyclopent-2-en-1-one	0.2
22	10.88	3,4,4-trimethyl-2-cyclopenten-1-one	1.5
23	12.05	1-(2-methyphenyl)ethanone	tr[c]
24	12.38	2-ethyl-2,5-dimethylcyclopenten-2-one	2.9
25	13.32	4-methylbenzaldehyde	1.7
26	16.35	ethylbenzaldehyde	1.5
27	17.63	2-methyl-1,4-benzenedicarboxaldehyde	1.3
28	18.35	2,4,5-trimethylbenzaldehyde	1.3
29	19.73	2,6-bis(1,1-dimethylethyl)-2,5-cyclohexadiene-1,4-dione*	nd
		Phenol Derivatives	145.8
30	7.07	phenol*	23.7
31	8.89	2-methylphenol*	16.5
32	9.47	3-methylphenol* and 4-methyphenol*	46.5
33	10.20	2,6-dimethylphenol*	1.5
34	11.15	2-ethylphenol*	2.3
35	11.46	3,5-dimethylphenol*	6.1
36	11.78	2,5-dimethylphenol* and 2,4-dimethylphenol*	16.2
37	11.99	3-ethylphenol*	11.7
38	12.01	2,3-dimethylphenol*	3.8
39	12.25	3-ethyl-5-methylphenol	tr
40	12.88	2,4,6-trimethylphenol*	0.8
41	13.35	hydroxybenzaldehyde (isomer)	0.3
42	13.40	4-propylphenol	0.7
43	13.59	4-ethyl-3-methylphenol	1.9
44	13.63	2,3,6-trimethylphenol*	1.5
45	13.86	2,4,5-trimethylphenol	tr
46	14.08	3-(1-propenyl)phenol	1.3
47	14.23	2-propylphenol	0.2
48	14.43	2,3,4-trimethylphenol	3.8
49	14.59	2,3,5-trimethylphenol*	2.0
50	14.74	trimethylphenol	1.1
51	15.51	2,5,6-trimethylphenol	0.7
52	15.98	4-propenylphenol	0.5
53	18.45	2-(1,1-dimethylethyl)phenol	2.3
54	20.14	3-(1,1-dimethylethyl)phenol	nd
55	20.75	di-*tert*-butyl-hydroxytoluene (BHT)*	nd
56	26.34	4-hydroxy-3,5-di-*tert*-butylbenzaldehyde	0.4
		Methoxy Phenols and Derivatives	334.2
57	9.75	2-methoxyphenol (guaiacol)*	119.5
58	12.57	4-methyl-2-methoxyphenol (4-methylguaiacol)*	109.3
59	14.88	4-ethyl-2-methoxyphenol (4-ethylguaiacol)*	53.2
60	15.83	2-methoxy-4-vinylphenol (4-vinylguaiacol)*	3.1
61	16.04	1-(3-hydroxy-2-methoxyphenyl)ethanone	1.6
62	16.96	4-ethyl-6-methyl-2-methoxyphenol	tr
63	16.98	4-(2-propenyl)-2-methoxyphenol (eugenol)*	28.5
64	17.19	4-propyl-2-methoxyphenol (4-propylguaiacol)*	12.8
65	17.90	4-hydroxy-3-methoxybenzaldehyde (vanillin)*	1.9
66	18.24	4-(1-propenyl)-2-methoxyphenol (*cis*-isoeugenol)*	1.9
67	19.28	4-(1-propenyl)-2-methoxyphenol (*trans*-isoeugenol)*	nd
68	19.45	1-(2-hydroxy-5-methoxyphenyl)ethanone	nd
69	19.71	1-(4-hydroxy-3-methoxyphenyl)-2-propenyl alcohol (coniferyl alcohol)	1.5
70	20.05	1-(4-hydroxy-3-methoxyphenyl)ethanone (acetovanillone)*	0.7
71	21.22	1-(4-hydroxy-3-methoxyphenyl)-2-propanone (2-propiovanillone)	0.2
72	23.80	4-hydroxy-3-methoxybenzeneacetic acid (vanillic acid)*	nd

Table 1 (continued)

no.	RT (min)	compound[a]	concn (mg/L)
		Dimethoxy Phenols and Derivatives	385.3
73	16.89	2,6-dimethoxyphenol (syringol)*	253.4
74	17.07	3,4-dimethoxyphenol	3.4
75	19.21	4-methyl-2,6-dimethoxyphenol (4-methylsyringol)*	46.4
76	19.99	2,6-dimethoxyphenyl acetate	1.3
77	20.87	2-(2-propenyl)-4,5-dimethoxyphenol	0.9
78	21.12	4-ethyl-2,6-dimethoxyphenol (4-ethylsyringol)	26.9
79	21.94	4-vinyl-2,6-dimethoxyphenol (4-vinylsyringol)	8.2
80	22.90	4-(2-propenyl)-2,6-dimethoxyphenol*	15.8
81	23.05	4-propyl-2,6-dimethoxyphenol (4-propylsyringol)	11.4
82	23.83	4-(1-propenyl)-2,6-dimethoxyphenol*	4.7
83	24.07	4-hydroxy-3,5-dimethoxybenzaldehyde (syringaldehyde)*	4.0
84	25.12	4-(1-propenyl)-2,6-dimethoxyphenol (isomer)*	3.7
85	25.83	1-(4-hydroxy-3,5-dimethoxyphenyl)ethanone (acetosyringone)*	4.7
86	26.85	1-(4-hydroxy-3,5-dimethoxyphenyl)butanone	0.5
		Terpenic Compounds	9.5
87	7.94	1-methyl-4-(1-methylethyl)benzene (*p*-cymene)*	0.2
88	8.15	1,8-cineole (eucaliptol)*	nd
89	12.63	1-methoxy-4-(2-propenyl)benzene (estragole)*	5.2
90	13.89	2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (carvone)*	3.2
91	15.26	2-isopropyl-5-methylphenol (thymol)*	0.6
92	15.41	5-isopropyl-2-methylphenol (carvacrol)*	0.3
		Alkyl Aryl Ethers	85.5
93	10.57	1,2-dimethoxybenzene*	0.6
94	10.96	1,3-dimethoxybenzene*	0.3
95	11.28	1,4-dimethoxybenzene*	1.2
96	13.78	3,4-dimethoxytoluene	2.4
97	15.78	1,2,3-trimethoxybenzene*	2.1
98	17.46	1,3,5-trimethoxybenzene*	3.5
99	18.08	5-methyl-1,2,3-trimethoxybenzene	2.0
100	18.31	4-(2-propenyl)-1,2-dimethoxybenzene*	nd
101	19.35	4-(1-propenyl)-1,2-dimethoxybenzene*	0.9
102	20.34	4-(1-propenyl)-1,2-dimethoxybenzene (isomer)*	0.6
103	21.79	5-(2-propenyl)-1,2,3-trimethoxybenzene	0.9
104	23.95	5-(1-propenyl)-1,2,3-trimethoxybenzene	nd
105	24.23	5-(1-propenyl)-1,2,3-trimethoxybenzene (isomer)	0.8
106	24.60	1-(3,4,5-trimethoxyphenyl)ethanone	tr
107	25.50	3,4,5-trimethoxybenzenemethanol	70.2
108	27.46	3-phenoxy-1-methoxybenzene	nd
		Aromatics and Aliphatics Hydrocarbons	126.1
109	4.87	styrene*	nd
110	12.27	naphthalene*	2.8
111	15.89	2-butenyl-3-methylbenzene	1.2
112	17.36	4-(2-butenyl)-1,2-dimethylbenzene	0.7
113	17.73	1,3,5-triethylbenzene	nd
114	17.93	tetradecane*	3.6
115	22.72	hexadecane*	0.5
116	24.95	heptadecane*	0.5
117	26.53	phenanthrene*	0.1
118	27.06	octadecane*	0.6
119	28.54	1-methylphenanthrene	0.8
120	28.93	1-nonadecene	0.4
121	29.08	nonadecane*	0.7
122	31.01	eicosane*	1.5
123	32.84	heneicosane*	0.8
124	34.12	methyleicosane	2.1
125	34.60	docosane*	1.9
126	36.30	tricosane*	2.1
127	37.93	tetracosane*	2.1
128	39.50	pentacosane*	3.7
129	41.01	hexacosane*	7.1
130	42.46	heptacosane*	6.0
131	43.85	octacosane*	6.9
132	44.29	2,6,10,15,19,23-hexamethyl-2,6,10,14,18,22-tetracosahexaene (squalene)*	5.9
133	45.24	nonacosane*	11.9
134	46.55	triacontane*	14.0
135	47.60	1-hentriacontene	nd
136	47.95	hentriacontane	15.4
137	49.63	dotriacontane	12.6
138	51.64	tritriacontane	10.4
139	53.90	1-tetratriacontene	2.2
140	54.08	tetratriacontane	7.6

Table 1 (continued)

no.	RT (min)	compound[a]	concn (mg/L)
		Hopanes	43.9
141	47.03	17*β*(*H*)-28-norhopane	tr
142	47.32	17α(*H*)-28-norhopane	13.1
143	47.40	17*β*(*H*),21α(*H*)-29-methylhopane	7.5
144	48.60	17α(*H*),21*β*(*H*)-hopane	7.1
145	50.38	homohopane	4.4
146	50.61	homohopane (isomer)	7.2
147	52.02	bishomohopane	3.0
148	54.29	trishomohopane	0.2
149	54.94	trishomohopane (isomer)	1.4

[a] Asterisks indicate compounds used as standards. [b] nd, not determined. [c] tr, traces.

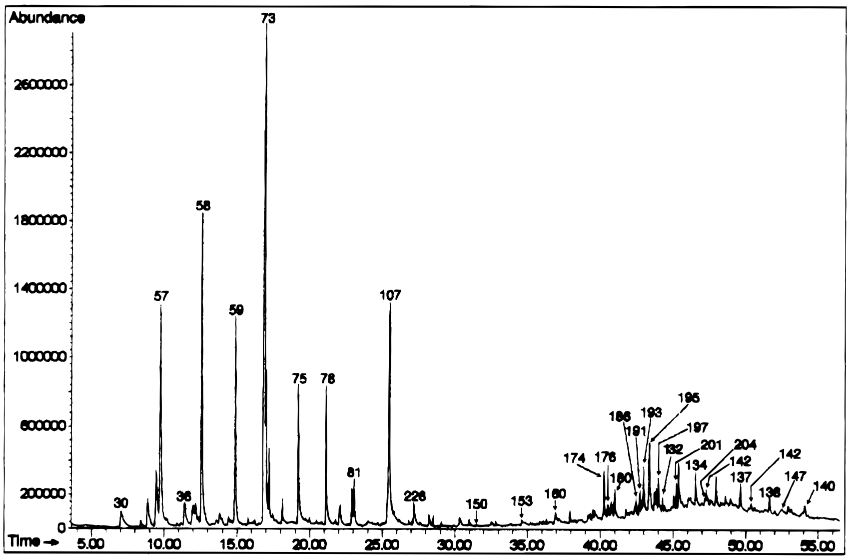

Figure 1. Total ion chromatogram of the soluble fraction in CH_2Cl_2 of the liquid smoke flavoring.

ments of the mass spectra of those compounds present in the smoke flavoring, tentatively considered as lignin dimers. The identification of each one of the compounds in Table 2 is a very difficult, if not impossible, task because many of these compounds have probably never been isolated and their spectral data are unknown.

From data in Table 2 it can be observed that different types of lignin dimers are present. From 55 compounds considered as lignin dimers in Table 2, 29 are characterized because they show base peak fragments of *m*/*z* 137, 152, 154, 164, 167, 168, 181, or 194.

Base peak *m*/*z* 137 can be assigned either to the 4-hydroxy-3-methoxybenzyl group ($C_8H_9O_2$) or to the ion formed by benzylic cleavage of the 1,2-biphenylethane containing one hydroxyl and one methoxyl group in both aromatic rings or in one of them. This base peak is observed in lignin monomers such as 4-ethylguaiacol (Figure 2b), in lignin dimers such as compounds **160** (Figure 2a), **162**, and **164** in Table 2, and in several lignan dimers extracted from plants such as 3′,4-dihydroxy-3,5′-dimethoxydihydrostilbene (gigantol) (Tezuka et al., 1991) and 3,4′-dihydroxy-5,5′-dimethoxydihydrostilbene (Shimizu et al., 1988), which have mass fragmentation similar to those of the compounds **160** and **162**; taking into account the above-mentioned, it can be assumed that these latter compounds are probably two 1,2-bis(methoxyhydroxyphenyl)ethane (or 1,2-diguaiacylethane) isomer derivatives.

In the same way, base peak *m*/*z* 167, in compounds **176**, **178**, **190**, and **197**, belongs either to the 4-hydroxy-3,5-dimethoxybenzyl group ($C_9H_{11}O_3$) or to the ion formed by benzylic cleavage of the 1,2-biphenylethane containing one hydroxyl and two methoxyl groups in both aromatic rings or in one of them; this peak is also a base peak in some lignin monomers such as 4-ethyl- or 4-propylsyringol (see Figure 2g,h) and in some lignan dimers such as 4,4′-dihydroxy-3,3′,5-trimethoxybibenzyl (moscatilin or 1-guaiacyl-2-syringylethane), a compound isolated from the orchid *Dendrobium moscatum* (Majumder and Sen, 1987), which has a mass spectral fragmentation in agreement with compound **176**; taking into account the above-mentioned, it can be assumed that compound **197** in Table 2 is probably a 1,2-bis-(dimethoxyhydroxyphenyl)ethane (or 1,2-disyringylethane) isomer derivative.

Base peaks *m*/*z* 164 and 194 are observed in some compounds in Table 2; these fragments belong in some cases to propenylguaiacol and propenylsyringol, respectively, as can be observed from mass spectra in Figure 2e,f,i,j. Mass spectra of some lignan dimers extracted from plants such as dihydroconiferyl dihydro-*p*-coumarate (Tezuka et al., 1993; Grabber et al., 1996) and 4-hydroxy-5-methoxy-3′,4′-methylenedioxy-2,7′-cyclolignan-7-one (Martinez and Torres, 1997) also show base peak *m*/*z* 164 and fragmentation patterns similar to some compounds in Table 2; in the same way, the mass spectra of other lignan dimers such as sinapyl *p*-coumarate (Grabber et al., 1996) and 4-hydroxy-3,3′,5,5′-tetramethoxy-8,4′-oxyneolignan and others, extracted from *Virola pavonis* leaves (Ferri and Barata, 1992), have base peak *m*/*z* 194 and fragmentation pattern similar to some compounds in Table 2.

Table 2. Mass Spectral Data of the Compounds Considered as Lignin Dimers, Their Retention Times (RT), and Their Concentrations in the Smoke Flavoring Preparation

no.	RT (min)	mass spectral data, *m*/*z* (%)	concn (mg/L)
			150.8
150	31.49	256 (100), 239 (49), 213 (20), 181 (19), 167 (40), 129 (27), 102 (77), 97 (29), 83 (19), 71 (26), 60 (45), 57 (36), 43 (52)	0.4
151	34.45	246 (100), 231 (8), 213 (4), 203 (5), 199 (12), 185 (4), 171 (19), 131 (7), 115 (8) [bis(methoxyhydroxyphenyl) (isomer)]	0.7
152	34.48	260 (45), 227 (18), 167 (100), 137 (10), 125 (15), 111 (26), 103 (18), 92 (20), 85 (23)	0.5
153	34.72	246 (100), 231 (11), 213 (6), 203 (7), 199 (18), 185 (8), 171 (21), 167 (6), 131 (11), 115 (19) [3,3′-dimethoxy-4,4′-dihydroxy-1,1′-biphenyl]	0.7
154	34.82	260 (100), 231 (11), 213 (15), 185 (23), 169 (5), 146 (7), 130 (5), 109 (7)	0.6
155	34.98	262 (100), 234 (15), 225 (13), 192 (16), 154 (31), 138 (10), 102 (13)	0.6
156	35.36	272 (79), 256 (5), 244 (6), 230 (10), 197 (30), 164 (100), 149 (22), 135 (26), 121 (17), 103 (14), 91 (12)	0.3
157	35.92	260 (100), 243 (7), 229 (17), 213 (14), 185 (17), 137 (11), 115 (9), 103 (8) [4,4′-bis(2-methoxyphenol)-methylene]	0.7
158	36.43	290 (30), 260 (88), 242 (5), 213 (10), 185 (11), 152 (100), 137 (7), 123 (5), 115 (7)	0.3
159	36.68	286 (100), 211 (14), 208 (11), 164 (63), 149 (34), 135 (10), 115 (5), 103 (7), 91 (8)	1.3
160	36.93	274 (23), 151 (9), 137 (100), 122 (9), 94 (6) [1,2-bis(methoxyhydroxyphenyl)ethane (isomer)]	4.1
161	37.13	286 (66), 243 (9), 211 (25), 164 (100), 149 (38), 135 (13), 103 (9), 85 (19)	0.9
162	37.31	274 (20), 151 (8), 137 (100), 122 (9), 94 (6) [1,2-bis(methoxyhydroxyphenyl)ethane (isomer)]	0.7
163	38.08	306 (100), 274 (15), 263 (5), 167 (50), 165 (27), 154 (27), 123 (5), 107 (6) [3,3′,5,5′-tetramethoxy-4,4′-dihydroxy-1,1′-biphenyl]	0.4
164	38.28	288 (28), 274 (7), 246 (20), 151 (29), 137 (100), 122 (7), 91 (5)	1.0
165	38.64	300 (41), 164 (100), 149 (26), 137 (67)	0.4
166	38.73	302 (100), 194 (76), 179 (23), 135 (11)	1.2
167	38.84	272 (100), 257 (31), 229 (15), 164 (7), 137 (25), 107 (12), 79 (11) (dimethoxydihydroxydihydrophenanthrene)	nd
168	39.18	302 (72), 272 (26), 227 (12), 194 (100), 179 (12), 135 (16), 121 (9), 91 (10)	1.9
169	39.31	302 (34), 259 (9), 227 (14), 164 (100), 151 (19), 131 (7), 103 (8)	1.5
170	39.63	290 (100), 259 (23), 215 (7), 183 (5),167 (5), 137 (10)	2.6
171	39.74	304 (38), 289 (100), 194 (5), 164 (11), 137 (19), 123 (5)	0.9
172	39.84	316 (60), 300 (19), 276 (12), 194 (100), 179 (10), 163 (31), 149 (21), 135 (16)	1.4
173	39.93	290 (66), 154 (100), 139 (8)	1.4
174	40.18	316 (36), 273 (7), 241 (8), 194 (63), 181 (100), 164 (11), 149 (8), 135 (10), 97 (11), 77 (5)	7.0
175	40.36	316 (59), 286 (100), 271 (28), 194 (89), 151 (68), 137 (10), 119 (10)	1.6
176	40.52	304 (31), 167 (100), 151 (5), 137 (35), 122 (7) [4,4′-dihydroxy-3,3′,5-trimethoxybibenzyl (moscatilin)]	3.7
177	40.76	316 (61), 273 (10), 241 (11), 194 (100), 179 (12), 167 (18), 149 (16), 135 (11), 91 (9)	nd
178	40.84	304 (28), 290 (92), 243 (19), 167 (100), 151 (11), 137 (7), 122 (7), 115 (10), 107 (12)	nd
179	40.86	304 (74), 154 (100), 111 (19), 97 (17)	nd
180	40.96	290 (100), 243 (13), 215 (13), 167 (24), 145 (5), 115 (5)	5.0
181	41.04	302 (100), 287 (23), 259 (6), 166 (11), 149 (6)	1.6
182	41.11	334 (100), 297 (11), 194 (21), 180 (10)	2.4
183	41.78	330 (42), 318 (23), 194 (100), 179 (8), 167 (52), 163 (23), 149 (13), 137 (5), 91 (7)	4.1
184	41.94	304 (100), 289 (13), 272 (7), 219 (7), 194 (7), 164 (34), 151 (45)	2.0
185	42.09	330 (34), 194 (100), 179 (8), 163 (9), 149 (6)	2.0
186	42.17	332 (100), 318 (43), 257 (22), 194 (52), 165 (88), 151 (24), 131 (39)	2.3
187	42.24	330 (10), 298 (100), 283 (6), 255 (10), 194 (28), 163 (9), 149 (10)	nd
188	42.32	318 (9), 194 (15), 168 (100), 153 (16), 137 (15), 125 (11), 111 (13), 97 (20)	nd
189	42.56	334 (100), 181 (38), 165 (19), 154 (9), 137 (18)	0.8
190	42.64	332 (28), 314 (5), 298 (7), 167 (100)	nd
191	42.70	332 (45), 302 (5), 289 (7), 257 (9), 194 (100), 179 (10), 167 (14), 151 (19), 137 (5)	5.1
192	42.82	334 (100), 319 (11), 180 (39), 167 (30), 137 (10), 123 (5)	3.3
193	42.97	320 (100), 303 (4), 289 (27), 273 (5), 259 (4), 245 (5), 229 (3), 213 (5), 194 (4), 167 (17), 153 (4) 137 (5), 123 (5) [4,4′-bis(2,6-dimethoxyphenol)methylene]	9.4
194	43.21	346 (32), 330 (8), 316 (23), 301 (11), 292 (22), 271 (8), 194 (100), 179 (26) 167 (19), 151 (9), 137 (9), 91 (10)	2.2
195	43.31	348 (21), 181 (100), 167 (6)	10.7
196	43.37	306 (100), 291 (8), 263 (17), 248 (6), 194 (13), 182 (9), 167 (7), 153 (8)	2.4
197	43.75	334 (20), 167 (100), 123 (6) [1,2-bis(dimethoxyhydroxyphenyl)ethane (isomer)]	12.7
198	44.01	346 (38), 271 (7), 194 (100), 179 (15), 165 (15), 109 (7), 91 (5)	7.7
199	44.42	360 (35), 332 (62), 317 (24), 285 (12), 194 (100), 179 (21), 165 (10), 151 (6), 133 (6), 91 (7), 77 (7)	4.2
200	45.07	360 (29), 285 (8), 194 (100), 179 (21), 167 (11), 123 (11), 111 (10), 97 (14)	6.1
201	45.39	386 (100), 371 (11), 355 (6), 339 (7), 311 (9), 232 (72), 217 (36), 194 (5), 181 (17), 167 (15), 154 (5), 139 (7)	10.5
202	45.64	328 (100), 310 (6), 285 (9), 194 (7), 164 (9) [dehydrodihydrodiisoeugenol (isomer)]	5.7
203	45.84	358 (38), 315 (5), 301 (7), 283 (6), 194 (100), 179 (8), 177 (21), 167 (6), 137 (5), 95 (8)	3.4
204	47.20	386 (100), 371 (11), 339 (7), 311 (9), 232 (73), 217 (37), 181 (17), 167 (14), 152 (10), 137 (12)	16.1

The other base peaks in Table 2 with *m*/*z* 152, 154, 168, and 181 are also assignable to lignin monomers or to fragments of these (see Figure 2c,d) indicating that these compounds could be lignin dimers. This is corroborated by the differences between the ion molecular peak and the base peak masses; these differences correspond in some cases with the above-mentioned fragments, in other cases with the mass of compounds such as phenol and methyl-, ethyl-, dimethyl-, trimethyl-, or propylphenol, having molecular weights of 94, 108, 122, and 136, respectively, and finally in other cases with the mass of methyl-, vinyl-, ethyl-, propenyl-, and propylguaiacol, having molecular weights of 138, 150, 152, 164, and 166, respectively.

In addition in Table 2 are given the mass spectral data of 26 other compounds, tentatively considered as lignans for which the base peak is the molecular ion peak and having as main fragments *m*/*z* 194, 181, 167,

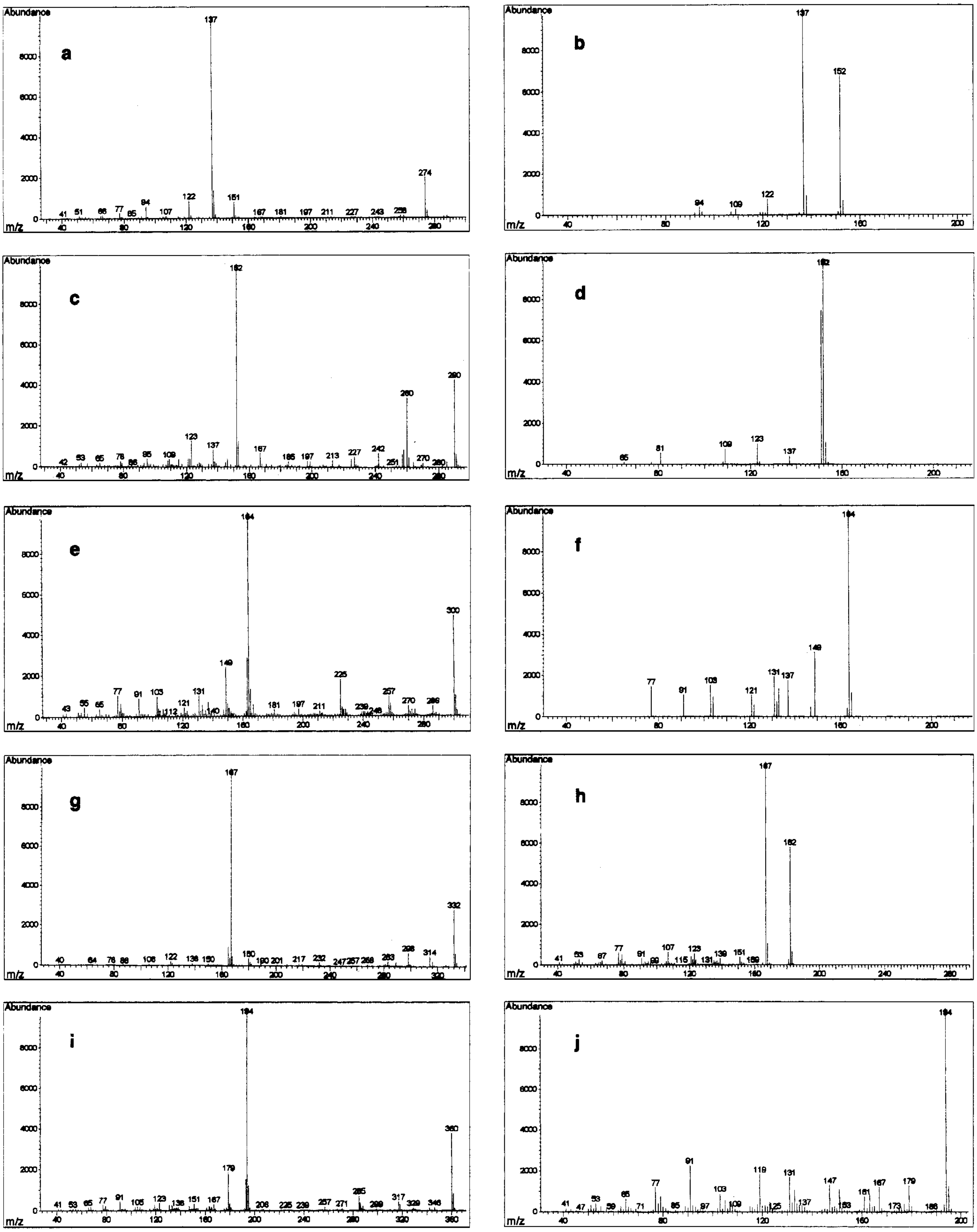

Figure 2. Examples of mass spectra of several lignin dimers and monomers: (a) mass spectrum of compound **160**; (b) mass spectrum of 4-ethylguaiacol; (c) mass spectrum of compound **158**; (d) mass spectrum of vanillin; (e) mass spectrum of compound **165**; (f) mass spectrum of 4-(2-propenyl)guaiacol; (g) mass spectrum of compound **190**; (h) mass spectrum of 4-ethylsyringol; (i) mass spectrum of compound **200**; (j) mass spectrum of 4-(2-propenyl)syringol.

Table 3. Mass Spectral Data of the Unidentified Compounds Present in the Liquid Smoke, Their Retention Times (RT), and Their Concentrations in the Sample

no.	RT (min)	mass spectral data, *m/z* (%)	concn (mg/L)
			131.8
205	10.89	124 (100), 122 (28), 109 (58), 95 (26), 81 (59), 67 (48)	0.3
206	14.91	146 (14), 138 (100), 131 (13), 123 (55), 110 (12), 90 (9), 83 (5)	0.4
207	15.24	136 (68), 121 (100), 107 (14), 93 (37), 79 (35), 65 (10), 45 (13)	0.4
208	15.61	146 (45), 131 (100), 125 (9), 116 (35)	0.5
209	16.21	166 (33), 151 (100), 134 (11), 121 (42), 91 (22), 77 (8)	1.1
210	17.73	164 (30), 149 (26), 136 (58), 121 (100), 109 (7), 93 (41), 65 (15)	0.7
211	18.61	178 (100), 163 (29), 146 (19), 137 (26), 111 (34), 105 (15), 94 (9)	0.7
212	18.84	180 (43), 165 (100), 147 (23), 133 (13), 102 (6), 91 (5), 77 (10)	0.7
213	19.01	180 (31), 160 (39), 145 (100), 140 (47), 121 (35), 115 (27), 95 (25), 82 (46)	0.9
214	19.82	176 (96), 162 (58), 145 (46), 140 (41), 134 (48), 119 (67), 93 (100), 70 (43), 56 (51)	0.8
215	19.95	178 (100), 160 (38), 147 (15), 131 (79), 117 (36), 103 (17), 93 (21)	0.4
216	20.10	174 (90), 162 (100), 160 (35), 145 (23), 133 (56), 104 (9)	1.1
217	20.59	194 (15), 176 (58), 166 (17), 161 (100), 150 (11), 138 (27), 135 (34), 115 (11), 103 (7)	2.1
218	21.70	194 (17), 151 (100), 119 (16), 107 (12), 91 (22)	0.6
219	22.09	196 (39), 181 (100), 165 (39), 137 (26), 122 (10), 91 (14), 77 (14)	1.6
220	22.57	190 (22), 174 (68), 151 (100), 145 (23), 111 (31), 97 (61), 83 (47), 69 (44)	0.7
221	23.75	212 (32), 208 (11), 197 (100), 179 (52), 133 (19), 91 (45)	1.0
222	24.48	212 (62), 197 (100), 176 (19), 161 (24), 155 (46), 151 (71), 141 (29), 137 (32), 129 (23), 106 (13), 98 (20), 83 (26), 67 (26)	0.5
223	24.70	226 (62), 211 (100), 184 (38), 171 (56), 167 (19), 143 (17), 123 (31), 115 (7)	0.5
224	25.30	212 (18), 184 (100), 176 (11), 155 (15), 123 (23), 109 (24), 95 (22), 81 (16)	11.4
225	25.95	220 (11), 208 (65), 190 (12), 179 (17), 151 (18), 137 (100), 119 (21), 91 (24)	0.7
226	26.16	208 (100), 193 (69), 175 (10), 165 (7), 150 (28), 147 (22)	0.4
227	26.91	206 (98), 190 (16), 188 (100), 173 (46), 145 (39), 101 (49), 88 (49), 83 (24)	0.3
228	27.20	212 (58), 183 (100), 155 (24), 140 (21), 123 (53), 95 (22), 77 (12)	7.1
229	27.61	228 (25), 200 (100), 185 (43), 157 (49), 128 (16), 102 (22)	0.5
230	27.78	222 (10), 210 (23), 206 (9), 199 (9), 181 (100), 169 (11), 153 (6)	nd
231	28.12	222 (97), 207 (100), 192 (8), 183 (15), 175 (13), 159 (16)	nd
232	28.23	204 (100), 189 (84), 157 (33), 146 (22), 118 (10), 101 (14), 89 (12)	3.0
233	28.39	238 (19), 224 (5), 200 (6), 181 (100), 138 (8), 73 (10)	nd
234	29.96	284 (60), 240 (100), 225 (79), 209 (95), 195 (15), 178 (14), 148 (13), 141 (17), 126 (15), 113 (21), 99 (10), 85 (23), 71 (44)	0.9
235	30.21	218 (100), 203 (79), 171 (30), 160 (24), 132 (15), 115 (26), 103 (8)	1.1
236	30.27	216 (100), 192 (56), 183 (22), 155 (11), 127 (10)	2.4
237	30.47	218 (100), 203 (92), 171 (26), 160 (15), 129 (12), 115 (11), 99 (10)	3.3
238	31.69	236 (100), 221 (93), 193 (45), 180 (15), 150 (12), 137 (13), 122 (8), 107 (9), 94 (9), 79 (11), 69 (12)	0.3
239	32.52	230 (100), 215 (22), 187 (27), 172 (10), 155 (5), 141 (8), 137 (6), 127 (8), 115 (17), 97 (7), 77 (7)	2.0
240	33.98	290 (8), 260 (63), 245 (11), 203 (9), 187 (11), 178 (100), 161 (44), 134 (18), 124 (13), 118 (15), 91 (8), 77 (11)	1.0
241	37.65	322 (7), 239 (9), 216 (9), 193 (13), 111 (13), 97 (17), 82 (100), 67 (12)	0.3
242	44.89	374 (44), 314 (10), 220 (100), 205 (22), 137 (7), 123 (21), 111 (11)	4.2
243	45.49	360 (97), 344 (37), 329 (23), 319 (36), 206 (100), 191 (46), 163 (12), 137 (67)	10.5
244	46.05	400 (10), 386 (100), 371 (17), 339 (14), 311 (9), 279 (6), 246 (5), 232 (17), 217 (13), 181 (17), 167 (27)	15.4
245	46.64	400 (24), 385 (40), 334 (82), 303 (17), 259 (15), 218 (100), 203 (22), 181 (61), 163 (11), 149 (32), 123 (37), 109 (27)	5.4
246	47.62	408 (13), 386 (100), 372 (19), 355 (38), 232 (13), 214 (14), 206 (27), 203 (11), 191 (18), 175 (6), 144 (11)	10.1
247	48.28	386 (100), 368 (27), 353 (37), 301 (38), 275 (43), 255 (20), 213 (30), 145 (26), 107 (18), 95 (23)	5.2
248	48.70	412 (10), 380 (10), 327 (53), 272 (20), 229 (15), 173 (15), 155 (100)	5.9
249	49.18	472 (14), 440 (20), 421 (100), 402 (20), 350 (17), 330 (19), 252 (26), 151 (25), 125 (24), 96 (26)	4.3
250	49.43	396 (3), 354 (3), 332 (100), 252 (4)	7.6
251	52.94	438 (8), 386 (6), 355 (47), 299 (74), 285 (15), 229 (15), 211 (49), 186 (17), 155 (100), 145 (30), 112 (7)	3.2
252	53.16	438 (10), 409 (10), 383 (26), 271 (88), 239 (47), 201 (16), 145 (19), 127 (100)	4.6

164, 154, or 137, which correspond to the above-mentioned groups or ions that are characteristic of lignin monomers and so are considered by other authors that study the lignin structure by pyrolysis/mass spectrometry (Hartley and Haverkamp, 1984; Haider and Schulten, 1985; Lapierre, 1993); for these compounds also the differences between the ion molecular mass and the main fragment mass, in all cases, correspond with lignin monomers or fragments of these.

Some of these latter compounds have been identified; this is the case of compound **153** (molecular weight = 246) identified as 3,3′-dimethoxy-4,4′-dihydroxy-1,1′-biphenyl (1,1′-diguaiacol), of compound **157** (molecular weight = 260) identified as 4,4′-bis(2-methoxyphenol)-methylene (4,4′-diguaiacylmethylene), and of compound **163** (molecular weight = 306) identified as 3,3′,5,5′-tetramethoxy-4,4′-dihydroxy-1,1′-biphenyl (disyringol), as well as of compound **193** (molecular weight = 320) identified as 4,4′-bis(2,6-dimethoxyphenol)methylene (4,4′-disyringylmethylene) and of compound **202** (molecular weight = 328) identified as a dehydrodihydrodiisoeugenol isomer.

In addition to the compounds above-mentioned other lignan dimers with a skeleton of 9,10-dihydrophenanthrene could be present. To this group could belong compound **167** in Table 2, having a mass fragmentation pattern similar to that of the 2,5-dimethoxy-1,7-dihydroxy-9,10-dihydrophenanthrene extracted from *E. nuda* (Tuchinda et al., 1988) and the 4,7-dimethoxy-2,6-dihydroxy-9,10-dihydrophenanthrene (callosin) isolated from the orchid *Agrostophyllum callosum* (Majumder et al., 1995); the structure of compound **167** could correspond to 2,7-dimethoxy-3,6-dihydroxy-9,10-dihydrophenanthrene, as derived from the structure of related compound **160** in Table 2.

To the best of our knowledge, no reference related to the presence of lignin dimers in smoke or in smoke flavorings has ever been made. Guillén and Manzanos (1996c) have detected some compounds of this type in the polythene walls of receptacles in which smoke liquids were stored, and Edye and Richards (1991) have pointed out the presence of some compounds, possibly lignin dimers, in very small proportions in condensates from wood smoke; however, these authors do not provide more information about these compounds.

Different kinds of lignin dimers have been found and identified in extracts from plants as has been mentioned above; the current interest in the extraction and in the identification of the structure of lignan dimers present in plants is due to many of them having interesting therapeutic activity (MacRae et al., 1989; Ayres and Loike, 1990; Schröder et al., 1990; Cushman et al., 1991; Lee et al., 1995).

Lignin oligomers have also been found in wood pulp, and studies to determine the average molecular weight from several fractions have been carried out, without attempting separation and study of the components of each fraction because of the enormous difficulty that this task would involve (Ristolainen et al., 1996).

Other authors have reported the presence of the lignan lyoniresinol in oak sapwood and heartwood (Seikel et al., 1971) as well as in *Quercus robur* L. and in a brandy that has been aged for 6 years (Nabeta et al., 1987). Taking into account the above, it could be assumed that unidentified phenolic compounds, detected in oak wood extracts, the concentrations of which are important from an enological point of view because they permit the identification of wood species habitually used in cooperage, could belong to the broad family of lignin dimers, of which numerous mass spectral data are given here. These phenolic compounds of unknown structure have syringyl groups in their skeletons (Fernández de Simón et al., 1996).

Viriot et al. (1993) have found that lignin oligomers are the main polyphenols in old spirits and that these compounds are not significantly degraded or polymerized during spirit aging, unlike tannins; this shows the importance of these compounds in the organoleptic properties of old spirits. These authors have used selective methods based on chemical degradation for the quantitative determination of lignin oligomers and have studied their molecular weight distribution by gel permeation chromatography. In the same way, other authors (Galletti et al., 1995), in an attempt to study the nature of lignin oligomers and tannins responsible for, together with other substances, the organoleptic properties of wines and spirits, have studied the composition of cooperage oak and chestnut wood by means of pyrolysis/gas chromatography/mass spectrometry; these authors identified 36 compounds with molecular weights ranging from 84 to 212, coincident with the well-known smoke and smoke flavoring components, most of them lignin monomers; however, no information about lignin oligomers has been reported.

In addition to the therapeutic and organoleptic properties, lignin oligomers have been shown to have another very interesting property from the point of view of food technology: they are also potent antioxidants (Lu and Liu, 1992; Barclay et al., 1997). The latter authors have shown that the antioxidant activity decreases from tetramer lignin through lignin dimers and lignin monomers to the well-known antioxidant or BHT.

From all of the above, the importance of the presence of these compounds in smoke flavorings is clear. Furthermore, the mass spectral data collected in Table 2 may be very useful in recognizing lignin oligomers in future studies.

In addition to the numerous compounds cited, there is another group of unidentified compounds; their mass spectral data are collected in Table 3 together with their concentrations in the smoke flavoring.

In conclusion, this smoke preparation has been shown to have a composition that is very different from the known composition of smoke or other smoke flavorings previously studied; of the different groups of compounds traditionally considered as components of smoke, it contains only a fraction, the phenol derivatives, because the carbonylic derivatives, including aldehydes, ketones, and esters and acids, are very scarce and are in very small concentrations. For this reason its organoleptic properties are also different from the characteristics of the smoke. In addition, there is BHT, in a small proportion, and other components not detected before, either in smoke flavorings or in smoke, such as hopanes as well as a number of lignin dimers; the latter are very interesting from the point of view of health and food technology for their therapeutic, organoleptic, and antioxidant properties.

LITERATURE CITED

Alén, R.; Kuoppala, E.; Oesch, P. Formation of the main degradation compound groups from wood and its components during pyrolysis. *J. Anal. Appl. Pyrolysis* **1996**, *36*, 137–148.

Ayres D. C.; Loike, J. D. Lignans: Chemical biological and clinical properties. In *Chemistry and Pharmacology of Natural Products*; Cambridge University Press: Cambridge, U.K., 1990.

Baltes, W.; Söchtig, I. Low molecular weight ingredients of smoke flavor preparations. *Z. Lebensm. Unters. Forsch.* **1979**, *169*, 9–16.

Baltes, W.; Block, H.; Mevissen, L. Untersuchung höher molekularer Teerstoffe aus Raucharoma-Kondensaten (Studies on high molecular weight tars from smoke aroma condensates). *Z. Lebensm. Unters. Forsch.* **1982**, *175*, 130–136.

Barclay, L. R. C.; Xi, F.; Norris, J. Q. Antioxidant properties of phenolic lignin model compounds. *J. Wood Chem. Technol.* **1997**, *17*, 73–90.

Cushman, M.; Nagarathnam, D.; Gopal, D.; Chakraborti, A. K.; Lin, C. M.; Hamel, E. Syntesis and evaluation of stilbene and dihydrostilbene derivatives as potential anticancer agents that inhibit tubulin polymerization. *J. Med. Chem.* **1991**, *34*, 2579–2588.

Edye, L. A.; Richards, G. N. Analysis of condensates from wood smoke: Components derived from polysaccharides and lignins. *Environ. Sci. Technol.* **1991**, *25*, 1133–1137.

Fengel D.; Wegener, G. In *Wood: Chemistry, Ultrastructure, Reactions*; de Gruyter Ed: Berlin, 1989.

Fernández de Simón, B.; Cadahía, E.; Conde, E.; García-Vallejo, M. C. Low molecular weight phenolic compounds in spanish oak woods. *J. Agric. Food Chem.* **1996**, *44*, 1507–1511.

Ferri, P. H.; Barata, L. E. S. Neolignans and phenylpropanoid from *Virola pavonis* leaves. *Phytochemistry* **1992**, *31*, 1375–1377.

Galletti, G. C.; Carnacini, A.; Bocchini, P.; Antonelli, A. Chemical composition of wood casks for wine aging as determined by Pyrolysis/GC/MS. *Rapid Commun. Mass Spectrom.* **1995**, *9*, 1331–1334.

Grabber, J. H.; Quideau, S.; Ralph, J. *p*-Coumaroylated syringyl units in maize lignin: Implications for β-ether cleavage by thioacidolysis. *Phytochemistry* **1996**, *43*, 1189–1194.

Guillén, M. D. Polycyclic aromatic hydrocarbons: extraction and determination in food. *Food Addit. Contam.* **1994**, *11*, 669–684.

Guillén, M. D.; Ibargoitia, M. L. Relationships between the maximum temperature reached in the smoke generation processes from *Vitis vinifera* L shoot sawdust and composition of the aqueous smoke flavoring preparations obtained. *J. Agric. Food Chem.* **1996a**, *44*, 1302–1307.

Guillén, M. D.; Ibargoitia, M. L. Volatile components of aqueous liquid smokes from *Vitis vinifera* L shoots and *Fagus sylvatica* L wood. *J. Sci. Food Agric.* **1996b**, *72*, 104–110.

Guillén, M. D.; Manzanos, M. J. Study of the components of a solid smoke flavouring preparation. *Food Chem.* **1996a**, *55*, 251–257.

Guillén, M. D.; Manzanos, M. J. Study of the components of an aqueous smoke flavouring by means of Fourier transform infrared spectroscopy and gas chromatography with mass spectrometry and flame ionization detectors. *Adv. Food Sci.* **1996b**, *18*, 121–127.

Guillén, M. D.; Manzanos, M. J. Some changes in an aqueous liquid smoke flavouring during storage in polythene receptacles. *Z. Lebensm. Unters. Forsch.* **1996c**, *202*, 24–29.

Guillén, M. D.; Manzanos, M. J. Characterization of the components of a salty smoke flavouring preparation. *Food Chem.* **1997**, *58*, 97–102.

Guillén, M. D.; Blanco, J.; Canga, J. S.; Blanco, C. G. Study of the effetiveness of 27 organic solvents in the extraction of coal tar pitches. *Energy Fuels* **1991**, *5*, 188–192.

Guillén, M. D.; Manzanos, M. J.; Zabala, L. Study of a commercial liquid smoke flavouring by means of GC/MS and FTIR. *J. Agric. Food Chem.* **1995**, *43*, 463–468.

Haider, K.; Schulten, H. R. Pyrolysis field ionization mass spectrometry of lignins soil humic compounds and whole soil. *J. Anal. Appl. Pyrolysis* **1985**, *9*, 317–331.

Hartley, R. D.; Haverkamp, J. Pyrolysis-mass spectrometry of the phenolic constituents of plant cell walls. *J. Sci. Food Agric.* **1984**, *35*, 14–20.

Kim, K.; Kurata, T.; Fujimaki, M. Identification of flavor constituents in carbonyl, non-carbonyl, neutral and basic fractions of aqueous smoke condensates. *Agric. Biol. Chem.* **1974**, *38*, 53–63.

Lapierre, C. Application of new methods for the investigation of lignin structure. In *Forage Cell Wall Structure and Digestibility*; Jung, H. G., Buxton, D. R., Hatfield, R. D., Ralph, J., Eds.; American Society of Agronomy: Madison, WI, 1993; pp 133–163.

Lee, Y. H.; Park, J. D.; Baek, M. I.; Kim, S. I.; Ahn, B. Z. *In vitro* and *in vivo* antitumoral phenanthrenes from the aerial parts of *Dendrobium nobile*. *Planta Med.* **1995**, *61*, 178–180.

Lu, H.; Liu, G. T. Anti-oxidant activity of dibenzocyclooctene lignans isolated from Schisandraceae. *Planta Med.* **1992**, *58*, 311–313.

MacRae, W. D.; Hudson, J. B.; Towers, G. H. N. The antiviral action of lignans. *Planta Med.* **1989**, *55*, 531–535.

Maga, J. A. In *Smoke in Food Processing*; CRC Press: Boca Raton, FL, 1988.

Majumder, P. L.; Sen, R. C. Moscatilin, a bibenzyl derivative from the orchid *Dendrobium moscatum*. *Phytochemistry* **1987**, *26*, 2121–2124.

Majumder, P. L.; Banerjee, S.; Maiti, D. C.; Sen, S. Stilbenoids from the orchids *Agrostophyllum callosum* and *Coelogyne flaccida*. *Phytochemistry* **1995**, *39*, 649–653.

Martinez, J. C.; Torres, R. Lignans from *Virola aff pavonis* leaves. *Phytochemistry* **1997**, *44*, 1179–1182.

Nabeta, K.; Yonekubo, J.; Miyake, M. Phenolic compounds from the heartwood of europeen wood (*Quercus robur* L.) and brandy. *Mokuzai Gakkaishi* **1987,** *33*, 408–415.

Olsen, C. Z. Chemical composition and application of smoke flavor. *Proc. Eur. Meet. Meat Res. Workers* **1976**, *22*, F 7:1, F 7:8.

Philp, R. P. Fossil Fuel Biomarkers. Applications and Spectra, XXIII. In *Methods in Geochemistry and Geophysics*. Elsevier: Amsterdam, 1985; pp 26–190.

Pszczola, D. E. Tour highlights production and uses of smokes-based flavors. *Food Technol.* **1995**, *49*, 70–74.

Ristolainen, M.; Alén, R.; Knuutinen, J. Characterization of TCF effluents from kraft pulp bleaching. I. Fractionation of hardwood lignin-derived material by GPC and UF. *Holzforschung* **1996**, *50*, 91–96.

Rohmer, M.; Bisseret, P.; Neunlist, S. The Hopanoids, Prokaryotic Triterpenoids and Precursors of Ubiquitous Molecular Fossils. In *Geological Markers in Sediments and Petroleum*; Moldowan, M., Albrecht, P., Philp, P., Eds.; Elsevier: Amsterdam, 1992; pp 1–17.

Schröder, H. C.; Merz, H.; Steffen, R.; Müller, W. E. G.; Sarin, P. S.; Trumm, S.; Schulz, J.; Eich, E. Differential *in vitro* Anti-HIV activity of natural lignans. *Z. Naturforsch.* **1990**, *45C*, 1215–1221.

Seikel, M. K.; Hostettler, F. D.; Niemann, G. J. Phenolics of *Quercus rubra* wood. *Phytochemistry* **1971**, *10*, 2249–2251.

Shimizu, M.; Shogawa, H.; Hayashi, T.; Arisawa, M.; Suzuki, S.; Yoshizaki, M.; Morita, N.; Ferro, E.; Basualdo, I.; Berganza, L. H. Anti-inflammatory constituents of topically crude drugs. III. Constituents and antiinflammatory effect of paraguayan crude drug “Tamandá cuná” (*Catasetum barbatum* Lindle). *Chem. Pharm. Bull.* **1988**, *36*, 4447–4452.

Sjöström, E. In *Wood Chemistry: Fundamentals and Applications*; Academic Press: San Diego, CA, 1993.

Tezuka, Y.; Hirano, H.; Kikuchi, T.; Xu, G. J. Constituents of *Ephemerantha lonchophylla*; isolation and structure elucidation of new phenolic compounds, ephemeranthol-A, ephemeranthol-B, and ephemeranthoquinone, and of a new diterpene glucoside, ephemeranthoside. *Chem. Pharm. Bull.* **1991**, *39*, 593–598.

Tezuka, Y.; Yoshida, Y.; Kikuchi, T.; Xu, G. J. Constituents of *Ephemerantha fimbriata*. Isolation and structure elucidation of tow new phenanthrenes, fimbriol-A and fimbriol-B, and a new dihydrophenanthrene, ephemeranthol-C. *Chem. Pharm. Bull.* **1993**, *41*, 1346–1349.

Tuchinda, P.; Udchachon, J.; Khumtaveeporn, K.; Taylor, W. C.; Engelhardt, L. M.; White, A. H. Phenanthrenes of *Eulophia nuda*. *Phytochemistry* **1988**, *27*, 3267–3271.

Viriot, C.; Scalbert, A.; Lapierre, C.; Moutounet, M. Ellagitannins and lignins in aging of spirits in oak barrels. *J. Agric. Food Chem.* **1993**, *41*, 1872–1879.

Received for review November 10, 1997. Revised manuscript received January 28, 1998. Accepted January 30, 1998. This work has been supported by the Comisión Interministerial de Ciencia y Tecnología (CICYT, ALI97-1095).

JF970952X