Volatile Methoxybenzene Compounds in Grains with Off-Odors

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More than 20 volatile methoxybenzene compounds were found in a set of 745 corn, sorghum, soybean, and wheat samples obtained from official grain inspectors. Most samples containing methoxybenzenes were off-odor. By using an autosampler, volatiles were purged from whole grain at 80 °C, collected on Tenax, and then thermally desorbed and transferred to a gas chromatograph−mass spectrometer for separation and identification. Use of an infrared detector aided identification of some compounds, especially isomers with similar mass spectra. Samples with insect odor had 1,4-dimethoxybenzene and its 2-methyl, 2-ethyl, and 2-methoxy derivatives that appeared to be derived from 1,4-quinones, which are known (except for 2-methoxy) defensive secretions of *Tribolium* insects. Samples with mostly musty, sour, and/or smoke odors commonly contained methoxybenzene and 1,2-dimethoxybenzene along with their 4-ethyl and 4-ethenyl derivatives, 4-chloro-1-methoxybenzene, and/or 2-methoxyphenol and its 4-ethyl derivative. Other methoxybenzenes were also found, including methoxy derivatives of other phenols and N-heterocyclic compounds. Co-occurrences and correlations of levels of some compounds were also reported to indicate relationships with odors and inter-relationships among compounds.

Keywords: *Grain; volatile methoxybenzenes; odor*

INTRODUCTION

Odor is an important factor in grain grading in the United States. All samples submitted for grading are smelled according to guidelines in the *Grain Inspection Handbook* (U.S. Department of Agriculture, 1997). Development of objective off-odor detection and classification methodology for possible use in grain grading has stimulated research on volatile components of grains (Seitz and Sauer, 1992). In previous studies, many volatile compounds from grains were identified and possible associations with off-odors were described (Seitz and Sauer, 1994, 1996; Seitz, 1995; Seitz et al., 1999). To further establish compound−odor associations, the set of samples described herein was collected. Because the set contained a large number of samples and had good representation of odors that occur in commercial grain samples, it provided the opportunity to identify additional compounds as well as some information on origins of compounds.

In our previous investigations of volatiles in grains mentioned above, the presence of several methoxybenzene compounds along with various alcohols, aldehydes, acids, esters, enals, terpenoids, phenolics, alkylbenzenes, benzofurans, geosmin, and insect pheromones was described. It was known that beetles in the genus *Tribolium* produced quinones (Howard, 1987; Howard et al., 1986), but those compounds were not detected in inoculated grain samples or in commercial samples from inspectors (Seitz and Sauer, 1996). In the current set of samples, we found >20 volatile methoxybenzene compounds, along with evidence that some of them were derived from conversion of quinones produced by *Tribolium* species. The objective of this paper was to focus on the methoxybenzene group of compounds, especially in regard to (1) identification of isomeric compounds, (2) possible associations of compounds with various off-odors, and (3) evidence that certain compounds came from the genus *Tribolium*. Methoxybenzenes and other volatile compounds associated with musty odors (Chambers et al., 1998a; Maga, 1987, Wasowicz et al., 1988), smoke odors (Baltes et al., 1981; Chambers et al., 1998b; Guillen and Ibargoitia, 1998; Maga, 1988; Wittskowski et al., 1992), and insects (Howard, 1987; Howard et al., 1986; Seitz and Sauer, 1996; Bartelt and Wicklow, 1999) in grains and food products have been described previously.

EXPERIMENTAL PROCEDURES

Grain Samples and Odor Assessments. A set of commercial grain samples was collected by the Grain Inspection Packers and Stockyards Administration/Federal Grain Inspection Service. The sample set consisted of 745 samples total, including 202 soybean, 210 corn, 96 sorghum, and 237 wheat samples. The samples were kept in cold storage (~4 °C, or frozen if the moisture content of the sample was >15%) until analyses were conducted.

The odor of each sample was assessed by official grain inspectors and by a panel at our laboratory. Odors were classified on an intensity scale of 0−3 as okay (normal), musty, sour, insect, smoke, and COFO (commercially objectionable foreign odor, which includes insecticides, solvents, weed, and other miscellaneous off-odors). In addition, most of the samples were evaluated by the Sensory Analysis Center at Kansas State University using an intensity scale of 0−10 and a group of descriptors including those mentioned above. Odors listed

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in the tables were deduced from intensity and description (odor type) information from all panels that evaluated each sample.

Analysis of Samples. The primary analytical system used to analyze all of the samples in the set has been described in detail (Ram et al., 1999). Handling the large number of samples was aided by an Archon autosampler (model 5100A, Varian Associates, Walnut Creek, CA). Each sample placed in the autosampler was 18 g of whole grain. The autosampler automatically added an internal standard (1 μL of ethylbenzene-d_{10} in methanol and 1 mL of water), preheated the sample to 80 °C, and then maintained it at 80 °C while the sample was purged with helium at 40 mL/min for 8 min. The volatiles were transferred to the trap (glass lined, Tenax-TA, Tekmar Co., Cincinnati, OH) in the purge and trap system via a glass-coated (Silcosteel, Restek Corp., Bellefonte, PA) transfer line heated to 130 °C. A Hewlett-Packard Co. (Palo Alto, CA) purge and trap instrument (model G1901A-60500) equipped with a capillary interface module (model G1908-60500) was used with described modifications (Ram et al., 1999). After volatiles were collected, a 10 min dry purge was performed to remove excess moisture from the trap. The trap was preheated at 220 °C, and the volatiles were desorbed at 225 °C for 6 min. With the capillary interface module, the desorbed volatiles were cryofocused at −140 °C at the top of the GC column. The cryofocused zone was heated at 200 °C for 0.85 min before initiation of the analytical run. Volatiles were analyzed with a GC [model 5890, Series II, Hewlett Packard (HP)] coupled with an MS detector (model 5971, HP). A BPX5 column (50 m × 0.32 mm i.d. × 0.25 μm film thickness) from Scientific Glass Engineering Inc. (Austin, TX) was used for separation. Carrier gas was helium at a constant flow rate of ∼1.0 mL/min. Oven temperature was held at 50 °C initially for 2 min, increased to 140 °C at a rate of 7 °C/min, and then increased to 260 °C at a rate of 17.5 °C/min. Effluent from the GC column was delivered directly to the MS detector with the transfer line temperature set at 280 °C. Electron impact energy of the ion source was 70 eV, and masses were scanned over the range of 33−250 amu (m/z). The mass spectrometer was tuned using the high sensitivity tune procedure provided by the HP Chemstation software, which enhanced the sensitivity severalfold over that provided by the standard tune procedure.

Compounds were quantified by using target analysis procedures in the Environmental Data Analysis (EDA) software from Hewlett-Packard. Ethylbenzene-d_{10} was used as the internal standard (ISTD). Anisole, 4-chloro-1-methoxybenzene, 4-ethenyl-1-methoxybenzene, and 1,2-dimethoxybenzene were quantitated from plots of the ratio of concentration of the target compound to that of ISTD using four to eight data points for each compound and a linear fit with zero intercept in the EDA software as described previously (Ram et al., 1999). Other compounds were quantitated by arbitrarily assigning an amount for a particular compound found in a particular sample, so that the amounts of these compounds were relative to the amount found in that particular sample. For all of the compounds, the target amounts were converted into areas of total ion chromatograms by using a conversion factor for the compound from the TIC of the sample containing the highest amount of that compound. Therefore, this method indicates the amounts of compounds proportionate to the size of the peaks in the chromatogram.

To obtain infrared spectra on volatiles from selected samples, a second analysis system equipped with an infrared detector was used. Samples (whole grain) were placed in U-shaped sparge tubes (without glass frit) and attached to a HP purge and trap instrument equipped with a capillary interface module the same as described for the primary system except that samples were heated with a pocket heater (model 14-5737-020). Purge and trap parameters were similar to those used with the primary system. A model 5890 series II gas chromatograph (GC), coupled with a model 5965B FTIR detector (IRD), and a model 5970 mass selective detector (MSD), all from Hewlett-Packard Co. were used to analyze the volatiles. A BPX35 column (50 m × 0.32 mm i.d. × 0.25 μm film thickness) from Scientific Glass Engineering Inc. was used for separation. Column head pressure was 124 kPa (18 psi) at 50 °C. Carrier gas was helium at a constant flow rate of ∼1.7 mL/min. Oven temperature was identical to that used with the primary system. Effluent from the column first passed through the IR detector and then into the MS detector. The temperature and the flow cell temperatures of the IRD were maintained at 250 °C. MSD conditions were as follows: direct transfer line temperature, 280 °C; ion source temperature, 280 °C; ionization voltage, 70 eV; mass range, 33−300 amu; scan rate, 1.91 scans/s.

Compounds were identified by comparing, with the aid of a computer and careful visual examination, experimental infrared (IR) spectra and mass spectra (MS) of compounds with standard spectra in two IR vapor phase libraries (HP 59963A EPA and HP 59964A flavors and fragrances) and in two MS databases (HP 59943B Wiley PBM and Wiley 6), respectively. A mass spectral database from The National Institute of Standards and Technology (NIST/EPA/NIH), PC version 4.5, U.S. Department of Commerce) also was used when necessary. GC retention times (GC t_R) were used in the compound identifications, and authentic standards were utilized when possible. In addition, compound identifications were based on information (GC t_R and MS and IR of authentic standards) from many previous studies conducted in this laboratory on volatiles in grains and breads (Seitz, 1995; Seitz et al., 1998, 1999; Ram et al., 1999).

RESULTS AND DISCUSSION

The methoxybenzene compounds found in the set of 745 grain samples are listed in Table 1. Table 2 gives the number of samples containing each compound for the entire set and for each grain type, along with corresponding means for total ion chromatogram (TIC) areas normalized to the ISTD response. For each compound that was calibrated with an authentic standard, nanogram amounts shown in Table 2 represent 18 g of sample. Note that, generally, each compound appears predominantly in a particular grain type.

It is evident from Table 3 that methoxybenzene compounds were usually found in samples with off-odors. With each compound, incidence increased as odor intensity increased, especially when intensity exceeded 0.99. On average, the amount of compound was lowest when the odor intensity level was 0−0.49.

To show inter-relationships among compounds and compound−odor relationships, coincidence (co-occurrence) and correlation of amounts of some compounds were included in the discussion below. Coincidence of compounds for the entire set of samples is given in Table 4, whereas coincidence numbers given in Tables 6−10 are for the samples included in each respective table. For each compound listed in the left column of Table 4, the corresponding row going from left to right first shows the number of samples with that compound and then the percent of samples also containing each respective compound listed along the top of the table. Some of the percentages listed in Table 4 are dependent on (or limited by) the number of samples with a particular compound. For example, in the row for compound **I** it is shown that 65% of the samples also contain compound **II**, whereas in the row for compound **II** it is shown that only 31% of the samples also contain compound **I**. The percentage in the second row is lower because only 111 samples contained **I** as compared to 232 samples containing **II**. The ratio of samples is nearly the sample as the ratio of percentages, that is, 111/232 versus 31/65. Correlation of compound amounts was generally not expected, but significant correlation of amounts along with high coincidence of compounds seemed to give evidence for inter-relationships among certain compounds as indicated below.

Table 1. Names and Identification Information for Methoxybenzenes Found in Grain Samples with Off-Odors

compd name	compd no.	t_R (min)	IR[a] (cm^{-1})	mass spectrum[b] [m/z (intensity)]
methoxybenzene (anisole)	**I**	9.1		108 (100), 78 (69), 65 (75)
4-ethyl-1-methoxybenzene	**II**	13.6	1248	121 (100), 122 (8), 136 (28), 91 (8), 77 (10), 78
4-ethenyl-1-methoxybenzene	**III**	14.6		134 (100), 119 (55), 91 (56), 65 (31)
4-(1-propenyl)-1-methoxybenzene (anethole)	**IV**	16.5		148 (100), 147 (57), 133 (21), 115, 116, 117 (27), 118, 121, 103, 105 (19), 91 (19), 77 (20), 78, 79 (15)
4-(2-propenyl)-1-methoxybenzene (estragole)	**V**	16.9		148 (100), 147 (63), 133 (25), 115, 116, 117 (24), 121 (16), 91 (25), 105 (18), 77, 78, 79
4-chloro-1-methoxybenzene	**VI**	13.6		142 (100), 144 (35), 127 (61), 129, (20), 99 (59)
1,2-dimethoxybenzene (veratrole)	**VII**	14.2	1261	138 (100), 123 (37), 95 (41), 77 (37)
1,3-dimethoxybenzene	**VIII**	14.8		138 (100), 107, 108, 109 (42), 91, 92, 93, 94, 95 (28), 77, 78 (36), 79, 80, 81, 62, 63, 64, 65 (29)
1,4-dimethoxybenzene	**IX**	14.7	1236	123 (100), 138 (74), 95 (26)
2-methyl-1,4-dimethoxybenzene	**X**	16.3	1223	137 (100), 152 (65), 109 (13), 91 (5), 94 (9), 77 (15), 78, 79
4-ethyl-2-methoxyphenol (4-ethylguaiacol)	**XI**	16.6		137 (100), 152 (33), 122 (11), 109 (4), 94 (7), 91 (9), 77, 79
4-ethyl-1,2-dimethoxybenzene	**XII**	17.3	1272	151 (100), 166 (56), 135 (7), 95 (15), 91 (13), 77, 78, 79
2-ethyl-1,4-dimethoxybenzene	**XIII**	17.4	1222	151 (100), 166 (93), 136 (15), 119, 121 (15), 123, 91 (30)77 (20), 78, 79
4-ethenyl-1,2-dimethoxybenzene	**XIV**	18.0	1273	164 (100), 149 (38), 135 (3), 121 (15), 103 (22), 91 (39), 77 (34), 78
1,2,3-trimethoxybenzene	**XV**	17.2	1250	168 (100), 153 (67), 125 (30), 110 (39), 95 (28), 93 (24)
1,2,4-trimethoxybenzene	**XVI**	18.0	1215	168 (100), 153 (90), 125 (53), 110 (21), 95 (10), 93 (10)
4-ethenyl-1,2,3-trimethoxybenzene	**XVII**	18.5		179 (100), 194 (52), 164 (26), 149 (5), 91 (14), 77 (9)
2-methoxyphenol (guaiacol)	**XVIII**	13.1		109 (100), 124 (75), 81 (68), 53 (22)
3,4-dimethoxyphenol	**XIX**	14.0		139 (100), 154 (80), 126 (14), 111 (55), 96 (15), 95 (7), 79 (27), 81, 83
methoxyresorcinol	**XX**	16.0		140 (100), 125 (90), 111 (56)
2,3,5-trichloro-6-methoxypyridine	**XXI**	17.9		182 (100), 184 (85), 186 (31), 211 (61), 213 (56), 215 (20), 170 (31), 168 (31), 146 (35), 148 (29), 133 (5), 135 (8), 107 (40), 109 (33), 98 (42), 100 (17)

[a] IR, C−O stretching frequency. [b] When there was a multiplet of adjacent ions, the ion listed had the highest intensity.

Table 2. Distribution of Methoxybenzene Compounds among Four Grain Types

		corn		sorghum		soybeans		wheat		total				max
compd name	compd no.	no. of samples	mean TIC[a]	no. of samples	mean TIC	no. of samples	mean TIC	no. of samples	mean TIC	no. of samples	mean TIC	min TIC	max TIC	amt (ng)
methoxybenzene	**I**	42	40	45	294	8	367	16	125	111	179	0.55	4180	175
4-ethyl-1-methoxybenzene	**II**	168	42	41	49	15	6	8	11	232	40	4.00	1080	
4-ethenyl-1-methoxybenzene	**III**	143	86	55	130	34	38	10	146	242	92	16.00	847	26.1
4-(1-propenyl)-1-methoxybenzene	**IV**	47	16	2	8	11	149	16	16	76	15	0.85	90	
4-(2-propenyl)-1-methoxybenzene	**V**	13	28	1	40	1	19	20	36	35	33	2.71	123	
4-chloro-1-methoxybenzene	**VI**	50	9	21	41	21	7	23	23	115	17	0.46	117	2.54
1,2-dimethoxybenzene	**VII**	114	49	52	118	50	37	46	26	262	57	5.00	624	62.6
1,3-dimethoxybenzene	**VIII**	14	7	11	19	29	25	10	16	64	19	8.55	284	
1,4-dimethoxybenzene	**IX**	92	29	34	51	34	7	90	55	250	38	0.40	1563	
2-methyl-1,4-dimethoxybenzene	**X**	5	10	4	12	1	44	23	28	33	23	1.20	194	
4-ethyl-2-methoxyphenol	**XI**[b]	51	21	11	47	8	19	3	19	73	25	3.83	383	
4-ethyl-1,2-dimethoxybenzene	**XII**	137	20	43	12	22	5	20	6	222	16	0.42	301	
2-ethyl-1,4-dimethoxybenzene	**XIII**	0	-[c]	2	13	0	-[c]	10	40	12	35	6.25	173	
4-ethenyl-1,2-dimethoxybenzene	**XIV**	84	10	35	14	5	8	13	13	137	11	0.62	47	
1,2,3-trimethoxybenzene	**XV**	7	9	4	4	17	24	2	6	30	16	1.06	66	
1,2,4-trimethoxybenzene	**XVI**	1	11	0	-[c]	1	10	17	52	19	48	3.34	560	
4-ethenyl-1,2,3-trimethoxybenzene	**XVII**	2	1	4	4	2	10	11	197	19	116	0.57	990	
2-methoxyphenol	**XVIII**	35	92	22	170	61	183	13	56	131	144	4.05	1920	
3,4-dimethoxyphenol	**XIX**	14	37	2	10	5	22	7	17	28	28	0.68	400	
methoxyresorcinol	**XX**	12	3	5	12	34	11	12	10	63	9	1.13	67	
2,3,5-trichloro-6-methoxypyridine	**XXI**	4	1	4	0.4	1	1	37	5	46	4	0.11	30	

[a] TIC area × 10^{-4}. [b] One corn sample with very high TIC value (1280) was excluded. [c] No samples: No TIC areas available.

Identification of Compounds. Several sets of isomeric methoxybenzenes were found in the sample set, that is, **VII**, **VIII**, and **IX**; **XII** and **XIII**; **XV** and **XVI**; **X** and **XI**; and **IV** and **V**. In many cases, MS and IR data, along with GC t_R, had to be considered carefully to identify the different isomers in the samples. Table 1 provides identification information such as GC t_R, MS, and selected IR data. When isomeric compounds had similar MS and similar GC t_R, the C−O stretching frequency became especially useful in identifying 1,2- and 1,4-dimethoxybenzenes, that is, values >1250 cm^{-1} were typical for the former and <1250 cm^{-1} for the latter, and this difference was greatest with the substituted dimethoxybenzenes (Table 1). As an example, IR data aided identification of **XII** and **XIII**. Neither compound was represented in the IR databases, but the IR spectrum of **XIII** was similar to that of **X** and the C−O stretches for **XII** and **XIII** were 1273 and 1222 cm^{-1}, respectively. Observed and database MS of **XIII** matched well; however, none of the databases had an entry for **XII**.

Identification of compounds was also aided by knowing the type of sample and the odor associated with it. As discussed below, we found that 1,4-dimethoxybenzene and its derivatives (such as **XIII**) occurred mainly in samples with insect odor, in contrast to 1,2-dimethoxybenzene and its derivatives (such as **XII**) that occurred mostly in samples with musty, sour, smoke, or other noninsect odors. Interestingly, **XII** was recently observed among volatiles from a fungus, *Fusarium verticillioides*, growing on autoclaved corn (Bartelt and Wicklow, 1999). Compound **X** was predominantly found

Table 3. Comparison of Sample Odor Intensity to Incidence and Amount of Each Methoxybenzene Compound

group no.	odor intensity range	no. of samples	methoxybenzenes																			
			XVI	**IX**	**X**	**XIII**	**IV**	**V**	**I**	**II**	**III**	**VI**	**VII**	**VIII**	**XI**	**XII**	**XIV**	**XV**	**XVII**	**XVIII**	**XIX**	**XX**
			Percent Incidence[a]																			
1	0−0.49	118	0	1	0	0	7	0	3	6	2	1	2	8	0	5	0	0	0	4	0	6
2	0.50−0.99	54	0	4	3	0	5	17	3	4	4	2	3	8	1	3	2	0	5	2	4	3
3	1.00−1.49	70	0	10	0	0	11	6	8	13	11	8	11	6	15	14	12	7	0	9	4	13
4	1.50−1.99	139	5	16	3	0	18	23	18	26	26	14	25	17	18	27	24	3	5	14	25	24
5	2.00−2.49	181	11	33	6	8	29	23	28	27	31	35	30	30	18	21	29	23	48	32	29	30
6	2.50−3.00	183	84	36	88	92	30	31	41	23	25	41	30	31	48	30	33	67	43	39	39	24
			Amount[b]																			
1			0	0.7	0	0	1.1	0	2	4	7	0.8	1	0.7	0	0.9	0	0	0	5	0	0.6
2			0	5.0	0.02	0	2.7	3.9	4	4	11	0.5	6	6.2	0.4	1.5	0.6	0	0.2	2	0.5	0.2
3			0	3.5	0	0	1.3	0.7	22	14	38	2.2	23	0.7	6.1	9.4	2.0	1.0	0	17	0.1	1.4
4			0.10	1.8	0.03	0	1.9	1.1	11	12	37	1.3	23	1.5	1.2	5.7	2.2	0.1	0	8	0.6	1.0
5			0.19	7.2	0.06	0.05	1.5	1.6	11	8	35	3.6	17	1.4	0.7	2.6	2.5	0.4	11.4	11	0.6	0.9
6			4.71	41.0	4.05	2.27	1.3	2.5	79	25	43	5.3	37	1.5	12.7	7.3	3.4	1.8	0.7	79	3.1	0.6

[a] Percent incidence = (no. samples in a group with the compound/total no. of samples in the set with the compound) × 100. [b] Average amount: TIC values ($\times 10^{-4}$).

Table 4. Coincidence Table: For Each Compound Listed in the Left Column, the Table Shows the Number of Samples Containing That Compound and the Percent of Samples Also Containing Each Compound Listed in the Column Head (Compounds IX, X, XIII, and XVI Represent the Insect Group)

compd no.	no. of samples with	% of samples also containing																			
		I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**XI**	**XII**	**XIV**	**XV**	**XVII**	**XVIII**	**XIX**	**XX**	**IX**	**X**	**XIII**	**XVI**
I	111		65	81	17	5	40	85	19	16	63	49	12	3	32	10	5	66	14	5	6
II	232	31		81	20	6	29	69	8	23	70	44	4	3	18	8	12	50	3	0	1
III	242	37	75		17	5	35	81	15	21	65	51	6	3	21	8	10	57	6	2	4
IV	76	25	62	61		22	28	71	4	24	62	39	13	9	29	9	9	55	8	0	0
V	35	17	40	51	49		11	46	3	14	49	40	9	26	14	3	11	40	6	0	3
VI	115	38	62	86	18	3		85	19	15	48	46	9	1	32	13	9	75	14	5	9
VII	262	36	61	82	19	5	35		14	20	58	43	11	3	28	10	8	61	7	3	5
VIII	64	33	31	64	5	2	34	62		9	36	25	17	2	30	19	6	56	9	2	3
XI	73	26	73	73	25	7	24	73	8		84	53	12	7	41	8	7	52	3	0	1
XII	222	32	74	77	21	8	25	73	10	27		48	9	4	23	8	10	49	6	0	1
XIV	137	39	78	93	22	10	39	87	12	28	78		8	4	26	9	9	65	9	3	8
XV	30	45	38	59	34	10	34	97	38	31	69	38		7	69	14	10	69	21	3	0
XVII	19	16	32	42	32	37	5	37	0	26	37	21	0		26	5	16	32	0	0	5
XVIII	131	28	34	50	17	4	28	57	15	23	40	27	15	5		13	8	41	3	0	1
XIX	28	39	64	82	25	4	54	93	43	21	64	46	14	7	61		7	82	7	4	4
XX	63	8	49	51	11	6	16	32	6	8	37	21	5	6	17	3		27	0	0	2
IX	250	30	50	71	17	6	34	68	14	15	44	36	8	3	22	9	7		12	4	7
X	33	45	27	55	18	6	48	58	18	6	39	39	18	0	12	6	0	94		36	33
XIII	12	50	25	58	0	0	50	67	8	0	0	33	8	0	0	8	0	92	100		58
XVI	19	35	15	50	0	5	50	65	10	5	15	55	0	5	5	5	5	90	55	35	

in samples (mostly wheat) with insect odor, whereas **XI** was found in corn and wheat samples with musty, sour, and/or smoke odors. Care was taken to avoid confusing **X** and **XI** with the commonly observed compounds β-cyclocitral and isopiperitone (methylisopropylcyclohex-2-enone), which had MS and GC t_R similar to those of **X** and **XI**. Although both isomers **XV** and **XVI** have been associated with musty odor (Chambers et al., 1998a), we found that **XV** occurred primarily in samples with musty odors, whereas **XVI** occurred mainly in samples with insect odors. Compounds **XVII** and **XX** were identified only tentatively because they were generally present in relatively few samples at low concentrations.

Compounds Associated with Insects. Evidence for methoxybenzenes associated with *Tribolium* insects is presented in Tables 4 and 5. The finding of compounds **XIII**, **X**, **IX**, and 1-pentadecene in samples with predominant insect-type odors (Table 5) and coincidence among compounds (Table 4) were the key to making the association with *Tribolium* infestation. Especially note in Table 5 that all of the samples that had **XIII** contained **X**, 1-pentadecene, and a sesquiterpene, and most of them also contained **IX** and **XVI**. Because 2-methyl-1,4-benzoquinone, 2-ethyl-1,4-benzoquinone, 1,4-benzoquinone, and 1-pentadecene are known *Tribolium* metabolites (Howard 1987; Howard et al., 1986), it appeared that **XIII**, **X**, and **IX** were derived from the insect 1,4-benzoquinones. The route for the benzoquinone−dimethoxybenzene conversion process is not known but may involve methyl radicals from lignins, possibly generated thermally or by photolysis (Wittkowski et al., 1992) that could react with the 1,4-benzoquinones to yield 1,4-dimethoxybenzenes. Alternatively, hydroquinone, easily generated from 1,4-benzoquinone, could be methylated biologically during storage of the grain. None of the benzoquinones were detected in any of the samples.

Although 1,2,4-trimethoxybenzene (**XVI**) was found in only 19 samples, all of the samples had strong insect odor (Table 5) and contained one or more of **IX**, **X**, **XIII**, or 1-pentadecene (Tables 4 and 5). Unlike the other substituted 1,4-dimethoxybenzenes described above, the possible precursor to **XVI**, 2-methoxy-1,4-benzoquinone, is not a known *Tribolium* secretion. Also, it is possible that methoxyhydroquinone reported in wheat flour (Koh and Hoseney, 1994) was a precursor for this compound. In this regard, it is interesting to note that 17 of the 19 samples with **XVI** were wheat.

Most of the samples listed in Table 5 contained a sesquiterpene that has not been reported as an insect

Table 5. Compounds Associated with *Tribolium* Insects

	odor		methoxybenzenes TIC area × 10^{-4}				sesquiterpene	1-pentadecene
sample	type[a]	intensity	**XIII**	**X**	**XVI**	**IX**	ng	ng
45 W	IM	2.9	173	193	560	1563	41	5
352 W	I	2.9	103	176	67	809	74	15
434 W	I	3.0	41	62	0	27	78	40
320 W	IB	3.0	23	19	0	54	79	11
483 S	IM	3.0	14	17	0	78	79	17
315 W	IMB	2.7	13	21	7	67	43	6
482 S	IM	3.0	11	12	0	62	60	10
383 W	I	2.9	11	13	12	17	37	20
218 W	I	2.7	10	7	75	412	15	3
248 W	I	3.0	10	13	6	46	22	11
240 W	IM	2.1	10	8	0	29	9	6
216 W	I	2.5	6	6	11	0	4	121
47 W	IMW	2.7	0	46	0	339	14	7
321 C	BS	3.0	0	28	0	36	69	10
57 W	IW	3.0	0	21	0	122	9	5
76 C	M	2.9	0	14	0	10	5	15
484 S	I	3.0	0	11	0	53	58	9
444 W	IMC	2.9	0	11	0	49	0	4
209 W	I	2.6	0	9	45	363	20	4
355 W	IB	3.0	0	8	0	14	60	20
637 W	KC	2.8	0	7	0	22	0	0
120 W	I	3.0	0	7	0	0	147	22
456 S	I	2.7	0	7	0	11	54	10
210 W	I	2.7	0	6	8	216	14	2
511 B	M	2.9	0	5	0	6	3	4
379 W	I	2.7	0	5	33	22	6	11
241 W	I	2.5	0	5	0	18	28	3
73 C	M	2.5	0	4	0	17	0	0
412 W	I	2.7	0	4	3	6	10	6
399 W	I	1.7	0	3	0	9	3	1
219 W	I	2.7	0	3	0	14	34	10
638 C	C	2.4	0	3	0	45	0	0
86 C	M	1.0	0	1	0	11	0	0
329 W	IM	2.2	0	0	21	11	10	0
148 W	IM	1.8	0	0	14	5	0	1
335 W	MS	2.0	0	0	14	9	0	0
106 C	MI	2.7	0	0	11	5	5	2
556 B	MK	2.5	0	0	10	6	0	0
211 W	I	2.7	0	0	7	22	1	1
447 W	I	2.9	0	0	4	26	23	7
378 W	I	3.0	0	0	3	0	7	7

[a] Odor types: I, insect; M, musty; S, fermenting sour; B, barnyard sour; K, smoke; C, COFO. When more than one odor type is indicated, the one at the left was predominant.

metabolite. This sesquiterpene was not observed in sorghum samples inoculated with red flour beetle (Seitz and Sauer, 1996). However, it has been commonly found in commercial samples (especially wheat) with insect-type odors, not only in this sample set but in other sets of samples previously analyzed (Seitz and Sauer, 1992, 1994). The MS and IR of the sesquiterpene observed in this sample set were identical to those for the compound observed in previous sample sets (data for subsequent publication).

Compound **IX** was found in 250 samples, and the coincidence data in Table 4 suggest that it was present in many more samples than could be accounted for by insect odors alone. It was quite coincident with compounds that were associated with non-insect-related odors, that is, **II**, **III**, **VII**, and others as shown in subsequent tables.

Soybeans are not usually infested with insects and, accordingly, insect volatiles were rarely detected in soybeans. The two soybean samples listed in Table 5 were of very poor quality with dark appearance, strong odor (mostly musty), and some seeds had damage that appeared to be from insects. One soybean sample was a rare sample that had several insect indicators including the lesser grain borer pheromones, dominicalures-1 and -2, and the *Tribolium* insect pheromone, 1-pentadecene.

Compounds Found in Samples with Musty, Sour, Smoke, and Other Odors. 1,2-Dimethoxybenzene (**VII**) was found in a large number of samples, mostly corn and sorghum (Table 2). Samples with **VII** commonly contained other methoxybenzenes (Table 4), especially those listed in Table 6. Compound **IX**, the 1,4-isomer of **VII**, was present in 68% of the samples with highest levels of **VII** (Table 6) and in 61% of all samples containing detectable levels of **VII** (Table 4). With some compounds, especially **I**, **XII**, **XIV**, and **XVIII**, coincidence with **VII** increased considerably when all samples with **VII** were compared with samples with highest levels of **VII** (Tables 4 and 6). Even though most of the compounds included in Table 6 were highly coincident with **VII**, only the level of **II** was significantly correlated ($r = 0.64$, $P < 0.0001$) with the level of **VII**. Some other columns in Table 6 were correlated with each other, that is, **VI** versus **IX** ($r = 0.62$, $P < 0.0001$), **XII** versus **II** ($r = 0.49$, $P < 0.001$), **III** versus **XIV** ($r = 0.63$, $P < 0.0001$), **I** versus **XVIII** ($r = 0.34$, $P < 0.05$), **I** versus **VI** ($r = 0.38$, $P < 0.01$), and **I** versus **XIV** ($r = 0.28$, $P < 0.05$).

The majority of samples with high levels of **VII** had musty odor, either as the predominant odor or mixed with other odors (Table 6). With the samples listed in Table 6, average odor intensity was high and levels of compounds **VII**, **I**, and **XVIII** were significantly correlated ($P < 0.05$) with odor intensity ($r = 0.33$, 0.31, and 0.41, respectively). The samples in Table 6 with definite smoke odor tended to have high levels of **XVIII**, especially samples 686 S, 677 S, 362 B, 364 B, and 365 B. The three soybean samples were badly damaged and discolored (nearly black).

Samples with 4-chloro-1-methoxybenzene (**VI**) had mostly musty and/or sour odors and generally high off-odor intensities (Table 7). Interestingly, a number of samples with **VI** had insect odors and contained insect-related compounds such that they were also included in Table 5. Compound **VI** was highly coincident with **VII** and **III** as well as other methoxybenzenes included in Table 7. Also, the level of **VI** was significantly correlated with the level of **VII** ($r = 0.54$, $P < 0.001$), **III** ($r = 0.37$, $P < 0.01$), **I** ($r = 0.51$, $P < 0.01$), **IX** ($r = 0.56$, $P < 0.001$), and **XVIII** ($r = 0.44$, $P < 0.01$). Compound **VI** was found mainly in sorghum, which was consistent with our previous observations on **VI** and other halogenated anisoles in musty sorghum samples (Seitz et al., 1999).

Most of the samples with 2-methoxyphenol (guaiacol, **XVIII**) were soybeans, and 35 of the 50 samples listed in Table 8 were assigned smoke odor. High off-odor intensities were also common. This compound and other phenolic compounds possibly formed from the pyrolysis of lignin have been linked to smoke odor (Wittskowski et al., 1992). Two isomers of 3,5-octadien-2-one and methylacetylcyclopentene had masses *m*/*z* 81, 109, and 124 in common with **XVIII** and were eluted close to **XVIII**. However, the octadienones and the methylacetylcyclopentene had prominent masses at *m*/*z* 95 and 43, respectively, when considered along with GC t_R, and database match information helped discriminate **XVIII** from the potential interferences.

Samples with highest levels of 4-ethyl-2-methoxyphenol (4-ethylguaiacol, **XI**) were mostly corn and generally did not contain 1,2,3-trimethoxybenzene (**XV**), whereas

Table 6. Fifty Samples with Highest Amounts of 1,2-Dimethoxybenzene (VII)

	odor		methoxybenzenes TIC area × 10^{-4}								
sample	type[a]	intensity	**VII**	**IX**	**I**	**II**	**III**	**XII**	**XIV**	**XVIII**	**VI**
686 S	K	3.0	625	105	0	903	0	48	1	766	0
217 S	SM	2.7	488	203	460	188	309	12	24	0	55
737 S	SM	2.7	401	22	561	64	410	41	0	138	0
346 C	MS	1.8	363	6	20	56	54	65	5	0	0
677 S	KM	2.9	344	92	4180	0	0	27	0	430	112
92 C	S	2.8	251	0	0	53	335	25	45	152	9
357 S	MS	2.2	237	168	189	37	302	0	25	108	82
742 S	SM	1.8	237	0	212	0	339	13	21	0	0
45 W	IM	2.9	217	1563	300	25	104	0	26	0	117
89 C	SM	3.0	217	6	45	44	270	38	39	36	6
741 S	SM	1.8	215	0	137	28	240	12	19	46	0
347 C	SM	1.2	211	0	0	80	88	35	12	0	0
171 S	MSI	2.9	210	186	1007	44	282	5	20	89	84
61 S	MS	2.8	208	0	0	0	164	0	40	397	0
320 W	IB	3.0	199	54	90	0	0	0	0	0	0
76 C	M	2.9	198	10	34	20	311	20	29	0	8
163 B	KMB	1.5	196	0	0	0	16	9	0	47	0
364 B	K	3.0	192	24	889	0	32	0	0	1654	0
370 S	MSI	2.3	191	157	186	37	298	8	27	82	84
743 S	SM	1.7	185	0	119	23	189	9	18	0	0
365 B	K	3.0	182	25	995	0	0	0	0	1844	0
359 S	M	1.3	177	130	146	34	233	8	21	78	71
62 S	MS	2.5	174	8	102	0	142	0	24	273	0
254 C	SM	2.5	171	0	88	243	240	70	25	0	0
740 S	SM	2.1	169	0	113	0	237	9	14	39	0
14 C	S	2.9	168	61	17	41	219	13	28	50	0
678 S	KM	3.0	154	18	2402	0	0	1	0	168	0
293 C	MS	1.8	150	8	67	58	141	18	13	0	0
108 S	M	1.2	146	0	218	0	289	10	0	0	0
350 S	MSI	2.2	146	139	119	30	253	6	16	47	64
321 C	BS	3.0	142	36	0	23	108	33	15	48	16
371 S	MSI	2.2	140	113	138	26	187	6	22	77	56
362 B	K	3.0	137	26	991	0	0	0	0	1272	0
744 S	SM	1.8	135	0	70	0	153	7	16	0	0
250 C	MK	3.0	134	8	58	223	243	92	19	0	5
202 C	SM	1.9	124	13	0	158	234	82	22	0	5
338 C	M	1.8	119	4	25	35	40	11	3	0	0
696 S	S	1.1	109	0	123	34	170	21	0	0	0
693 S	S	1.1	104	6	193	30	211	23	0	0	0
104 C	BM	3.0	104	15	39	22	98	14	18	22	6
692 S	S	1.2	101	5	167	22	90	10	0	0	0
111 C	M	2.5	100	14	33	30	319	13	34	48	10
382 C	BS	2.1	100	6	136	34	126	14	10	0	0
694 S	S	1.1	92	4	329	33	140	17	9	0	0
149 W	M	1.7	91	15	83	13	135	0	10	58	41
420 S	MI	1.8	90	0	0	0	74	0	7	0	0
720 S	B	2.2	90	0	95	29	59	14	0	0	0
341 C	M	1.6	87	0	13	21	35	8	0	0	0
59 C	BM	2.1	86	10	40	11	68	0	0	48	5
162 B	KS	2.5	86	0	0	0	0	4	0	56	0
av odor intensity		2.24									
% coincidence with **VII**				68	82	70	86	78	68	54	38

[a] Odor types: K, smoke; M, musty; S, fermenting sour; B, barnyard sour; I, insect. When more than one odor type is indicated, the one at the left was predominant.

samples with **XV** were mostly soybeans and some of them contained relatively low levels of **XI** (Table 9). Generally high odor intensities and descriptions of smoke, sour, and/or musty were common in samples containing either **XI** or **XV**, although smoke odor was especially prevalent in samples with **XV**. These compounds are probably not solely responsible for the off-odors because of the presence of other compounds such as short-chain carboxylic acid esters in samples with sour odors and benzofurans and amyl- and hexylbenzenes in samples with smoke odors. However, **XI** has been described as having a moderately smoky odor (Baltes et al., 1981; Chambers et al., 1998b; Maga, 1988; Wittskowski et al., 1992). **XI** can be considered to be a precursor to **XII** (4-ethyl-1,2-dimethoxybenzene), which is a substituted 1,2-dimethoxybenzene associated with musty odor as described below. The presence of **XI** was quite coincident with the presence of **XII** (Table 9), but amounts of the compounds were not significantly correlated. Although **XI** is isomeric with the insect-related compound 2-methyl-1,4-dimethoxybenzene (**X**), its actual 1,2-dimethoxybenzene-related analogue (4-methyl-1,2-dimethoxybenzene) that we thought might be in samples with musty odors was not observed in any of the samples.

Most of the 25 samples with highest levels of **XV** were soybeans and corn with smoke, sour, and musty odors, and only 3 samples had some insect odor (Table 9). The lack of insect odor is in contrast to samples with **XVI**, the isomer of **XV**, which was associated with insect

Table 7. Fifty Samples with Highest Amounts of 4-Chloro-1-methoxybenzene (VI)

	odor		methoxybenzenes TIC area × 10^{-4}								
sample	type[a]	intensity	**VI**	**VII**	**IX**	**I**	**II**	**III**	**XII**	**XIV**	**XVIII**
45 W	IM	2.9	117	217	1563	300	25	104	0	26	0
677 S	KM	2.9	112	344	92	4180	0	0	27	0	430
216 W	I	2.5	98	7	0	339	0	847	0	7	0
370 S	MSI	2.3	84	191	157	186	37	298	8	27	82
171 S	MSI	2.9	84	210	186	1007	44	282	5	20	89
357 S	MS	2.2	82	237	168	189	37	302	0	25	108
706 S	MS	2.2	73	19	27	0	0	114	0	9	0
261 C	K	3.0	71	0	1018	0	112	0	0	0	0
359 S	M	1.3	71	177	130	146	34	233	8	21	78
352 W	I	2.9	64	38	809	259	0	93	0	15	0
350 S	MSI	2.2	64	146	139	119	30	253	6	16	47
371 S	MSI	2.2	56	140	113	138	26	187	6	22	77
217 S	SM	2.7	55	488	203	460	188	309	12	24	0
70 S	S	2.0	42	78	47	0	16	79	0	5	178
149 W	M	1.7	41	91	15	83	13	135	0	10	58
209 W	I	2.6	39	42	363	37	0	46	5	21	0
132 S	MI	1.3	39	54	0	0	0	270	4	19	0
218 W	I	2.7	38	35	412	64	0	52	0	20	0
246 B	KS	1.8	37	27	0	0	0	28	0	0	68
47 W	IMW	2.7	37	21	339	96	0	33	21	0	0
475 B	MK	1.5	22	0	0	0	0	0	0	0	0
717 S	M	2.1	22	48	0	0	6	68	0	0	0
314 S	SM	2.3	22	44	29	69	11	43	0	10	0
78 C	BS	2.5	21	43	24	0	84	349	54	25	23
711 S	S	1.5	18	24	0	220	26	37	4	0	37
553 B	M	2.4	18	10	2	0	0	21	0	0	0
210 W	I	2.7	16	18	216	29	0	26	0	6	0
321 C	BS	3.0	16	142	36	0	23	108	33	15	48
41 C	MSW	2.0	15	42	17	12	24	198	7	6	0
57 W	IW	3.0	14	0	122	0	0	0	0	0	0
18 C	SM	1.6	13	60	7	0	84	149	34	20	0
387 C	M	2.5	13	12	0	11	11	21	0	0	0
65 C	M	2.1	13	13	5	0	8	146	3	16	0
86 C	M	1.0	13	30	11	0	30	114	31	13	0
12 C	MS	0.8	12	39	8	33	76	130	26	15	0
107 C	BS	2.7	12	77	10	0	58	80	19	18	53
653 C	MC	2.1	11	11	3	0	20	65	4	5	0
3 C	M	1.2	11	19	11	0	25	120	12	1	0
30 C	S	2.2	11	14	3	48	156	52	28	7	0
120 W	I	3.0	11	0	0	0	0	0	4	0	0
73 C	M	2.5	11	78	17	41	25	296	5	13	10
455 S	I	2.9	10	14	9	0	13	0	0	0	0
9 S	BM	2.4	10	65	0	183	35	16	4	4	0
82 W	IS	3.0	10	4	0	0	0	0	0	0	0
17 C	M	2.0	10	32	6	0	12	128	0	8	0
111 C	M	2.5	10	100	14	33	30	319	13	34	48
438 S	MI	2.5	9	15	0	26	9	23	0	0	14
2 C	M	1.4	9	20	10	0	23	93	13	11	0
151 B	MS	0.6	9	9	0	0	0	6	0	0	0
92 C	S	2.8	9	251	0	0	53	335	25	45	152
av odor intensity		2.23									
% coincidence with **VI**				92	74	52	70	86	56	70	36

[a] Odor types: K, smoke; M, musty; S, fermenting sour; B, barnyard sour; I, insect; W, weed; C, COFO. When more than one odor type is indicated, the one at the left was predominant.

odors as discussed above. It was also noteworthy that **VII** was generally present at higher levels in samples that had **XV** than in samples that had **XVI**. Nearly all of the samples with **XV** also contained **VII**, and it was also quite coincident with **IX**, **XVIII**, **XII**, and **III** (Tables 4 and 9). Of the 25 samples with **XV** listed in Table 9, the amount of **XV** was correlated significantly with amounts of **I** ($r = 0.67$, $P < 0.001$) and **XVIII** ($r = 0.71$, $P < 0.001$).

Like **VII** and **IX**, the 4-ethyl- and 4-ethenyl-substituted mono- and di-methoxybenzenes (**II**, **III**, **XII**, and **XIV**) were found in many samples (Tables 2 and 4), and coincidence among those compounds was common (Tables 4 and 10). Compound **XI**, a related 4-ethyl-substituted methoxyphenol, was less coincident with **II** than **III**, **XII**, and **XIV** (Table 10). With the samples listed in Table 10, the levels of the two ethyl-substituted compounds **II** and **XII** were significantly correlated ($r = 0.65$, $P < 0.001$), and the levels of the two ethenyl-substituted compounds **III** and **XIV** were significantly correlated ($r = 0.66$, $P < 0.001$). Even with all 232 samples containing **II**, coincidences of **II** with other compounds included in Table 10 remained high (Table 4) and correlations of levels of **II** to **XII** ($r = 0.71$) and **III** to **XIV** ($r = 0.75$) were significant ($P < 0.0001$). With all 137 samples that contained **XIV**, coincidences of **XIV** with **II**, **III**, **XII**, **VII**, and **IX** were 78, 93, 78, 87, and 65%, respectively, and correlation of levels of **II** to **XII** ($r = 0.73$) and **III** to **XIV** ($r = 0.53$) were significant ($P < 0.0001$).

Table 8. Fifty Samples with Highest Amounts of 2-Methoxyphenol (Guaiacol, XVIII)

	odor		methoxybenzenes TIC area × 10^{-4}									
sample	type[a]	intensity	**XVIII**	**VII**	**IX**	**I**	**II**	**III**	**XI**	**XII**	**XIV**	**XV**
193 B	KS	3.0	1919	0	0	0	0	46	75	0	0	0
365 B	K	3.0	1844	182	25	995	0	0	0	0	0	66
364 B	K	3.0	1654	192	24	889	0	32	0	0	0	48
518 B	K	2.9	1483	36	0	0	0	0	16	0	0	0
362 B	K	3.0	1272	137	26	991	0	0	7	0	0	41
686 S	K	3.0	766	625	105	0	903	0	0	48	1	2
642 C	C	1.3	744	0	0	0	0	0	207	0	0	0
730 S	K	3.0	499	0	0	0	0	0	35	0	0	0
677 S	KM	2.9	430	344	92	4180	0	0	0	27	0	0
61 S	MS	2.8	397	208	0	0	0	164	0	0	40	0
179 C	KSM	2.8	381	0	0	1	0	0	0	0	0	0
684 W	K	3.0	380	0	0	0	0	0	45	6	0	0
186 C	KM	3.0	353	0	0	472	0	0	0	0	0	0
257 C	K	1.7	341	0	0	0	0	0	0	15	0	0
486 B	K	2.7	299	0	0	0	0	0	0	0	0	0
506 B	K	2.8	285	0	0	0	0	0	25	0	0	0
62 S	MS	2.5	273	174	8	102	0	142	0	0	24	0
178 C	KM	3.0	231	0	0	0	0	0	21	0	0	0
192 B	KS	2.3	193	0	0	0	0	0	0	0	0	5
182 C	KM	3.0	193	0	0	0	0	0	8	0	0	0
70 S	S	2.0	178	78	47	0	16	79	5	0	5	0
678 S	KM	3.0	168	154	18	2402	0	0	0	1	0	0
4 B	KS	2.2	159	47	0	0	0	0	0	0	0	0
92 C	S	2.8	152	251	0	0	53	335	11	25	45	9
176 B	KS	1.6	143	0	0	0	0	0	0	0	0	0
737 S	SM	2.7	138	401	22	561	64	410	31	41	0	0
733 B	KC	2.3	124	0	0	0	0	13	0	0	0	0
138 B	KS	3.0	121	79	0	0	0	0	0	0	0	44
109 S	M	2.3	111	36	2	0	2	24	0	0	4	0
735 S	S	3.0	111	0	14	0	52	73	383	10	4	0
317 B	BMW	2.6	110	22	9	0	0	97	0	0	0	12
357 S	MS	2.2	108	237	168	189	37	302	0	0	25	0
485 B	KS	2.7	89	45	0	0	0	0	0	6	0	9
171 S	MSI	2.9	89	210	186	1007	44	282	0	5	20	0
495 B	KB	1.8	87	0	0	0	0	0	0	0	0	0
147 C	K	3.0	84	0	0	0	18	0	14	2	0	0
370 S	MSI	2.3	82	191	157	186	37	298	0	8	27	4
359 S	M	1.3	78	177	130	146	34	233	0	8	21	0
371 S	MSI	2.2	77	140	113	138	26	187	0	6	22	5
414 C	K	2.0	76	0	0	0	0	0	18	0	0	0
246 B	KS	1.8	68	27	0	0	0	28	0	0	0	0
243 B	KS	2.4	60	21	0	0	0	0	0	0	0	0
149 W	M	1.7	58	91	15	83	13	135	0	0	10	0
242 B	KB	1.8	58	0	0	0	0	0	0	0	0	0
239 B	K	1.4	57	0	0	0	0	0	0	0	0	0
637 W	KC	2.8	56	14	22	188	0	0	0	5	0	0
162 B	KS	2.5	56	86	0	0	0	0	9	4	0	16
323 B	KS	1.1	55	0	0	0	0	0	0	0	0	0
107 C	BS	2.7	53	77	10	0	58	80	17	19	18	0
731 B	K	1.2	52	0	0	0	0	0	0	0	0	0
av odor intensity		2.44										
% coincidence with **XVIII**				56	40	34	28	38	34	36	28	24

[a] Odor types: K, smoke; M, musty; S, fermenting sour; B, barnyard sour; I, insect; W, weed; C, COFO. When more than one odor type is indicated, the one at the left was predominant.

Samples with **II**, **III**, **XII**, and **XIV** were found predominantly in corn followed by sorghum and were generally off-odor with average intensities >2.0 (Tables 3 and 10). Common odors were sour (including barnyard) or musty, mixtures of sour and musty, and a few samples with strong smoke or smoke mixed with musty (Table 10). Of the 222 samples with **XII**, the isomer of the insect-related compound **XIII** discussed above, insect odor was detected in only 5 samples, 2 with dominantly strong insect odor and 3 with insect odor mixed with sour or musty odor.

Eleven of 19 samples that contained 4-ethenyl-1,2,3-trimethoxybenzene (**XVII**, tentatively identified) were wheat (Table 2). Only 2 samples were corn, which was in contrast to the preponderance of corn samples containing other 4-ethenyl-substituted methoxybenzenes such as **III** and **XIV**. Two wheat samples with strong barnyard sour odor had **XVII** at levels >10 times higher than the level in any of the other samples. Compound **III** had the highest coincidence with **XVII** (42%), and, in general, the coincidence of **IV** and **V** was higher with **XVII** than with other compounds (Table 4). None of the samples contained **XV**, the corresponding trimethoxybenzene without the ethenyl group. The average odor intensity of samples with **XVII** was high (2.4), and barnyard sour was the most common odor. Two samples (one wheat and one corn) had very strong (intensity 3.0) smoke odor, two wheat samples had strong (intensity 2.8) fumigant odor, and none of the samples had insect odor.

Table 9. Samples with Highest Amounts of 4-Ethyl-2-methoxyphenol (XI) and 1,2,3-Trimethoxybenzene (XV)

	odor		methoxybenzenes TIC area $\times 10^{-4}$										
sample	type[a]	intensity	**XI**	**XV**	**VII**	**IX**	**I**	**II**	**III**	**VIII**	**XII**	**XIV**	**XVIII**
260 C	K	3.0	1280	0	0	0	0	0	0	0	0	0	0
735 S	S	3.0	383	0	0	14	0	52	73	0	10	4	111
642 C	C	1.3	207	0	0	0	0	0	0	0	0	0	744
200 C	M	1.4	101	0	0	0	0	91	36	0	193	12	0
78 C	BS	2.5	84	0	43	24	0	84	349	0	54	25	23
193 B	KS	3.0	75	0	0	0	0	0	46	0	0	0	1919
684 W	K	3.0	45	0	0	0	0	0	0	0	6	0	380
730 S	K	3.0	35	0	0	0	0	0	0	0	0	0	499
737 S	SM	2.7	31	0	401	22	561	64	410	0	41	0	138
256 C	S	1.3	28	0	20	0	0	61	31	0	74	0	0
415 C	SB	3.0	27	0	14	5	0	30	84	0	39	0	0
202 C	SM	1.9	26	0	124	13	0	158	234	0	82	22	0
89 C	SM	3.0	25	0	217	6	45	44	270	0	38	39	36
15 C	SM	1.7	25	0	49	0	0	49	105	0	37	0	0
506 B	K	2.8	25	0	0	0	0	0	0	0	0	0	285
141 C	B	2.8	24	0	40	0	0	0	19	0	21	5	0
201 C	SM	1.8	23	0	72	14	0	113	244	0	33	11	27
113 C	S	3.0	23	0	0	0	0	166	0	0	97	6	0
639 C	S	3.0	23	0	38	0	0	8	35	0	11	5	0
654 C	S	1.1	22	0	0	0	0	0	0	0	6	5	0
86 C	M	1.0	22	0	30	11	0	30	114	0	31	13	0
16 C	SM	1.8	21	0	83	0	0	34	94	0	42	0	0
178 C	KM	3.0	21	0	0	0	0	0	0	0	0	0	231
321 C	BS	3.0	19	6	142	36	0	23	108	7	33	15	48
414 C	K	2.0	18	0	0	0	0	0	0	0	0	0	76
av odor intensity		2.36											
% coincidence with **XI**				4	52	36	8	60	64	4	72	48	52
365 B	K	3.0	0	66	182	25	995	0	0	9	0	0	1844
163 B	KMB	1.5	0	57	196	0	0	0	16	0	9	0	47
364 B	K	3.0	0	48	192	24	889	0	32	9	0	0	1654
138 B	KS	3.0	0	44	79	0	0	0	0	0	0	0	121
362 B	K	3.0	7	41	137	26	991	0	0	0	0	0	1272
28 B	KMS	2.1	0	27	25	0	22	0	0	0	0	0	51
139 B	KS	2.8	8	27	60	8	0	0	0	0	16	0	0
162 B	KS	2.5	9	16	86	0	0	0	0	0	4	0	56
76 C	M	2.9	0	14	198	10	34	20	311	0	20	29	0
346 C	MS	1.8	0	14	363	6	20	56	54	0	65	5	0
164 B	KMS	2.1	0	13	37	0	0	0	0	0	5	0	16
167 B	MK	1.2	0	13	63	0	0	0	0	0	0	0	26
317 B	BMW	2.6	0	12	22	9	0	0	97	61	0	0	110
320 W	IB	3.0	0	11	199	54	90	0	0	5	0	0	0
111 C	M	2.5	9	11	100	14	33	30	319	8	13	34	48
92 C	S	2.8	11	9	251	0	0	53	335	0	25	45	152
485 B	KS	2.7	0	9	45	0	0	0	0	0	6	0	89
459 B	KMS	2.6	4	7	12	1	10	0	10	0	6	0	20
321 C	BS	3.0	19	6	142	36	0	23	108	7	33	15	48
14 C	S	2.9	11	6	168	61	17	41	219	0	13	28	50
126 B	M	3.0	0	6	62	6	0	3	111	3	3	8	0
511 B	M	2.9	0	6	10	6	0	0	13	7	2	0	0
371 S	MSI	2.2	0	5	140	113	138	26	187	0	6	22	77
192 B	KS	2.3	0	5	0	0	0	0	0	0	0	0	193
545 B	M	2.4	0	5	23	11	0	0	42	7	0	0	0
av odor intensity		2.55											
% coincidence with **XV**			32		96	64	44	32	56	36	64	32	72

[a] Odor types: K, smoke; M, musty; S, fermenting sour; B, barnyard sour; I, insect; W, weed; C, COFO. When more than one odor type is indicated, the one at the left was predominant.

3,4-Dimethoxyphenol (**XIX**) was found in only 28 samples, and half of them were corn (Table 2). One sample of corn with a strong musty odor had 10 times more of **XIX** than the other samples. The average odor intensity was high (2.2), and most samples had musty odor or musty mixed with sour odor. One sample of sorghum had a very strong (3.0 intensity) smoke odor. Several other methoxybenzenes were coincident with **XIX**, especially **VII**, **III**, and **IX** (Table 4). It appeared that this compound was not a precursor for **XVI**.

1,3-Dimethoxybenzene (**VIII**) was found in 64 samples consisting of all four grain types, but it was most prevalent in soybean (Table 2). Considerably fewer samples contained **VIII** than the corresponding isomers **VII** and **IX** (Tables 2 and 4). Compounds **VII**, **IX**, and **III** were present in 63, 56, and 64%, respectively, of the samples that contained **VIII**. Average odor intensity for all samples containing **VIII** was 2.0, with more than half having musty odor, either alone or mixed with sour, smoke, or insect odors. Other samples had definite sour (some barnyard) or insect odors. Samples (three soybean and one sorghum) with very strong smoke odor also contained high levels of guaiacol (**XVIII**).

Anisole (**I**) was found in 111 samples, mostly corn and sorghum (Table 2), with a relatively high average odor intensity at 2.2. In corn and sorghum, musty and sour odors were common, with musty most prevalent in corn and sour most prevalent in sorghum. Samples of all

Table 10. Fifty Samples with Highest Amounts of 4-Ethyl-1-methoxybenzene (II)

	odor		methoxybenzenes TIC area × 10^{-4}						
sample	type[a]	intensity	**II**	**III**	**XII**	**XIV**	**XI**	**VII**	**IX**
191 C	SM	2.8	2538	83	8821	943	0	0	0
686 S	K	3.0	2120	0	1400	60	0	625	10
254 C	SM	2.5	569	240	2045	2399	52	171	0
250 C	MK	3.0	522	243	2696	1823	0	134	1
217 S	SM	2.7	442	309	361	2316	0	488	20
113 C	S	3.0	389	0	2859	625	112	0	0
202 C	SM	1.9	371	234	2390	2109	128	124	1
30 C	S	2.2	365	52	830	662	0	14	0
258 C	K	2.8	339	0	0	0	0	0	82
201 C	SM	1.8	265	244	953	1088	114	72	1
261 C	K	3.0	262	0	0	0	0	0	101
562 C	M	0.6	230	200	793	0	0	0	0
196 C	SM	2.2	222	103	637	546	33	26	0
647 C	O	0.0	217	0	259	0	0	0	0
351 C	M	3.0	215	55	266	447	0	0	0
200 C	M	1.4	214	36	5655	1183	505	0	0
714 C	M	2.9	207	46	144	400	0	3	0
624 C	S	1.1	204	0	536	0	0	0	0
18 C	SM	1.6	197	149	995	1942	55	60	1
198 C	SM	1.5	197	111	522	944	27	32	0
78 C	BS	2.5	196	349	1571	2401	419	43	2
347 C	SM	1.2	187	88	1020	1126	59	211	0
199 C	M	1.1	184	94	1300	914	52	11	1
610 C	O	0.0	183	84	536	0	0	0	0
197 C	M	1.4	179	52	1350	524	0	23	0
12 C	MS	0.8	178	130	751	1429	0	39	1
251 C	M	3.0	164	67	467	541	0	29	0
253 C	MSK	2.8	159	66	618	646	22	50	0
24 C	S	2.1	152	160	1620	0	0	74	1
737 S	SM	2.7	151	410	1216	0	156	401	2
636 C	BSM	2.0	147	121	669	806	62	38	1
256 C	S	1.3	144	31	2166	0	139	20	0
74 C	M	2.3	139	254	270	1884	0	17	0
293 C	MS	1.8	136	141	529	1231	29	150	1
107 C	BS	2.7	135	80	566	1733	83	77	1
346 C	MS	1.8	130	54	1915	506	0	363	1
92 C	S	2.8	125	335	722	4389	53	251	0
635 C	BS	2.5	125	114	805	799	47	31	1
418 C	BS	2.5	125	51	665	521	25	49	0
735 S	S	3.0	122	73	293	404	1906	0	1
84 C	MB	1.5	120	0	501	0	0	0	0
372 C	MS	1.7	118	69	198	0	0	0	0
15 C	SM	1.7	114	105	1096	0	123	49	0
650 C	C	1.6	114	0	225	0	0	0	0
252 C	MK	2.5	109	40	54	207	0	4	0
103 C	M	1.9	109	147	244	1239	0	0	0
535 C	MK	1.4	108	60	530	321	33	11	0
313 C	M	3.0	106	116	442	0	0	38	9
25 C	S	2.1	105	99	777	0	0	45	0
171 S	MSI	2.9	104	282	138	1918	0	210	18
av odor intensity		2.08							
% coincidence with **II**				84	96	70	46	70	58

[a] Odor types: K, smoke; M, musty; S, fermenting sour; B, barnyard sour; C, COFO; O, okay. When more than one odor type is indicated, the one at the left was predominant.

grains with strong smoke odor had relatively high levels of anisole. Anisole was found in only 8 of 200 soybean samples analyzed, and 3 of them that had **I** at ~40-fold higher levels than in the other 5 were severely discolored with strong smoke odor. Most of the wheat samples with **I** had insect (or insect mixed with musty or sour) odor and contained one or more of the compounds associated with insects. When all grain types were considered, the occurrence of compounds **II**, **III**, **VII**, **IX**, **XII**, and **XIV** was quite coincident with **I** (Table 4). With corn samples, the occurrence of some compounds was highly coincident with **I**, such as **II** (98%), **III** (98%), **VII** (93%), **XII** (81%), **IX** (69%), and **XIV** (62%).

In a study to be reported in a subsequent publication on samples collected from corn and sorghum piles in southwestern Kansas in 1997, the appearance of anisole and other methoxybenzenes was related to conditions that favored mold infestation. Anisole was not present initially, but it grew in fairly rapidly in parts of the piles where grain was at elevated moisture content and temperature. The amount of anisole increased more rapidly and to higher levels in sorghum than in corn piles, which was consistent with the finding reported herein that anisole was more prevalent in sorghum than in corn or the other grains. After a few weeks of storage in the piles, some corn samples that had changed to a dirty brownish white color contained compounds discussed herein as being associated with musty odor, such as **VII**, **XII**, and **XIV**. One badly molded corn sample from the piles also had 4-methylanisole, a compound that was found in only one sorghum sample (which also had a high level of anisole) in the set of commercial samples discussed herein.

Methoxyresorcinol (**XX**) was found mostly in soybeans, followed by wheat and corn (Table 2), and most of the samples were off-odor (average intensity = 1.9). Barnyard sour and musty were the most common odors in the samples. Two soybean and two sorghum samples with strong smoke odor had relatively low levels of **XX**. Levels of **XX** generally did not correlate with levels of any of the other methoxybenzenes, and it was most coincident with **II** and **III** (Table 4).

Two methoxypyrazines not listed in Table 1, 3-isopropyl- and 3-isobutyl- 2-methoxypyrazine, were found in a single corn sample with musty odor. These compounds have been described as having an earthy, musty odor (Gallois et al., 1985).

The structurally similar compounds estragole (**V**) and anethole (**IV**) were found mostly in corn (especially **IV**) and wheat (Table 2). Average off-odor intensities for samples containing these compounds were both 2.0. The compounds were not associated with any particular odor type, but musty, sour, and mixed musty−sour odors were most common. Smoke odor was much more common in samples with **IV** than in samples with **V**, that is, 12 samples with **IV** and only 1 sample with **V**. About half of the samples that contained **V** also contained **IV**, as well as **II**, **III**, **VII**, **XII**, and **IX** (Table 4). Of the samples that contained **IV**, only 22% contained **V**, but coincidence with **II**, **III**, **VII**, **XII**, and **IX** was much higher (Table 4).

2,3,5-Trichloro-6-methoxypyridine (**XXI**) was found in 46 samples, mainly wheat. It appeared to be a fragment of the insecticide Reldan. Considering that we have observed with this set (data for subsequent publication) and previous sets (Seitz and Sauer, 1994) malathion decomposition products such as *O*,*O*,*S*-trimethyldithiophosphoroate ester and diethyl *E*-2-butenoate, it was reasonable to expect that Reldan might break down similarly to **XXI** and *O*,*O*,*O*-trimethylmonothiophosphorate. We did not observe the thiophosphorate; it may have undergone further hydrolysis of the P═S bond, giving phosphate and hydrogen sulfide.

Conclusions. A number of methoxybenzenes were found in the volatiles from samples that had an off-odor. Origins of all compounds were not clear. However, it appeared to be quite likely that the methyl- and ethyl-substituted 1,4-dimethoxybenzenes (**X** and **XIII**) were derived from corresponding benzoquinones that are known defensive secretions of *Tribolium*, and these compounds were associated with insect odor. Some of **IX** probably came from insects in samples with strong

insect odor, but **IX** was commonly found in samples with noninsect odors.

Compounds found in samples with non-insect-related odors may have originated from lignin-related components in the grain. Because lignins have methoxybenzene units in their structure, it is possible that microorganisms (molds or bacteria) could break down the lignin to form mono-, di-, and 1,2,3-trimethoxybenzene compounds such as **I**−**IX**, as well as **XI**, **XII**, **XIV**, **XV**, and **XVII**. These compounds were commonly found in samples with musty and/or sour odors, and it is known that microorganisms are associated with those types of odors (Seitz and Sauer, 1992; Wasowicz et al., 1988). Bartelt and Wicklow (1999) reported on the production of **II**, **XI**, and **XII** by *Fusarium verticillioides* growing on autoclaved corn. The phenolic compounds **XI**, **XVIII**, **XIX**, and **XX** may be associated with smoke odor, and they could arise from the pyrolysis of lignins (Wittskowski et al., 1992). Accelerated respiration processes in wet grain can lead to extensive heating and possible pyrolysis of grain components (Sauer et al., 1992). The heating (possibly even leading to fire and smoke) may occur in a portion of the grain storage facility, followed by distribution of the resulting volatiles (smoke) throughout the entire grain mass.

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