if internal organs from hens were continuously consumed over a long period of time. On a fresh weight basis (including fat and water), the highest concentration of Cd in liver of hens was 1.45 mg/kg and well within the upper range of concentrations that Kreuzer et al. (1977) found in the livers of swine produced under normal farm management conditions. At the age of 80 weeks, gizzards (lining removed) from hens on high-Cd diets contained 9.96 mg of Cd/kg on a dry weight basis, which translates to a wet weight concentration of about 3.3 mg of Cd/kg. Shellfish and crustaceans used for human foods frequently contain concentrations of Cd that are as high or higher than those found in the gizzard of hens (Ministry of Agriculture, Fisheries, and Food, 1973; Fassett, 1975), and thus the latter may present about the same potential human health hazard. But Cd contents of gizzards could be markedly reduced by switching the hens to a low-Cd diet a few weeks before they are marketed. The kidneys are removed from carcasses of spent hens before they are further processed and would not impact human food chains.

Lifetime ingestion of biologically incorporated Cd was about 3634, 21 687, and 36 949 μg/bird for chickens on low-, intermediate- and high-Cd diets, respectively. On the basis of amounts in those tissues that accumulated Cd, it was estimated that total amounts of the metal retained in 80-week-old hens were 47.6, 212.6, and 298.3 μg for those on low-, intermediate-, and high-Cd diets, respectively. This corresponds to a retention of 1.31, 0.98, and 0.81% of the total ingested Cd, respectively, by the several organs from hens on diets containing 0.095 ± 0.05, 0.57 ± 0.11, and 0.97 ± 0.14 mg of Cd/kg. The decrease in retention of Cd at higher contents in diets may indicate that either one or more of the organs was approaching saturation with the metal and more was excreted or it was translocated to other tissues whose contents were not considered in the calculation. At the highest dietary level, there was some indication that Cd was perhaps translocated to bones and lungs.

Registry No. Cd, 7440-43-9.

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Crude Oleic Acid Volatiles

Donald J. Stern,* Robert A. Flath, Thomas R. Mon, Roy Teranishi, Robert E. Lundin, and Mabry E. Benson

Commercial-grade crude oleic acid has been shown to be a coyote attractant and may be effective in predator control. Pure oleic acid was found to lack attractancy; therefore, a study of the volatiles associated with the crude oleic mixture was made in an effort to identify the most active compounds. Volatiles obtained via steam distillation–extraction were separated into basic, neutral, and acidic fractions and analyzed by GC–MS. The total number of compounds identified was 132.

Coyotes (*Canis latrans*) cause considerable damage to livestock, especially sheep. A cooperative project between the U.S. Department of Agriculture and the University of California, Davis, has resulted in the investigation of various materials that could be useful as lures in coyote trapping (Lorenz et al., 1983; Teranishi et al., 1977). Crude oleic acid volatiles but not pure oleic acid have shown promise as an attractant (Teranishi et al., 1981).

There are numerous literature references to the flavor of cooked meats (Buttery et al., 1977; Caporaso et al., 1977; Mussinan et al., 1974) but none on crude oleic acid. Wa-

Western Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture, Berkeley, California 94710.

tanabe and Sato (1971) reported on the volatiles obtained from heated beef tallow. Crude oleic acid or red oil is obtained commercially as an extract of beef tallow (Formo, 1982). We report on over 100 compounds identified from the volatiles of crude oleic acid.

EXPERIMENTAL SECTION

Extraction. Commercial-grade crude oleic acid (Armac) was extracted by simultaneous distillation and extraction at atmospheric pressure, as reported by Teranishi et al. (1977), with a yield of 0.11%.

Acid–Base Fractionation. An aliquot (10 g) of the distillate was dissolved in 250 mL of ether and fractionated by acid–base extraction. The ether solution was treated with 3 N HCl to yield the basic fraction and then was extracted successively with 5% $NaHCO_3$ and 1 N NaOH to yield acid fractions I and II. The remainder of the ether solution was the neutral fraction. Yields were basic fraction 1%, acid I 5%, acid II 54%, and neutrals 14%.

Esterification. Fifty milligrams of acid fraction I was esterified with dimethylformamide dimethyl acetal (Pierce Methyl-8) to facilitate identification of the esters.

Capillary Gas Chromatographic–Mass Spectrometric (GC–MS) Analysis. Acid fraction I was analyzed with an EAI quadrupole mass spectrometer coupled to a 150 m by 0.6 mm i.d. Pyrex column coated with OV-3–Igepal–Silanox, 95%:5%:<1%. The temperature was programmed: starting temperature 75 °C; rate 2 °C/min; final temperature of 200 °C held for 2 h.

Acid fraction II, basic, and neutral fractions were analyzed with a Finnegan Model 4500 gas chromatograph–mass spectrometer equipped with an INCOS data system. The column was a fused capillary column coated with OV-101 (methyl silicone), 50 m by 0.32 mm i.d., with an inlet pressure of 98 kPa. The starting temperature of 50 °C was held for 0.1 min and programmed at 4 °C/min to a final temperature of 225 °C, which was held for 30 min.

Kovats indices and confirmation of retention data were verified with a Hewlett-Packard 5840 gas chromatograph equipped with a flame ionization detector, using the same column and conditions as the Finnegan GC–MS experiments. Compounds whose reference Kovats indices were not determined with authentic samples are to be considered as tentatively identified.

Preparative GC. A glass column measuring 3 m by 0.63 cm was used for isolation of 5-methyl-2,4-diisopropylphenol. Packing material was Chromosorb G (100–120 mesh) coated with SF 96(50); column was operated at 140 kPa with an isothermal temperature of 150 °C.

Preparative HPLC. Phenols were isolated from the neutral fraction by means of a Waters Associates liquid chromatograph with a differential refractometer detector using a preparative Porasil column, flow rate 2 mL/min at 3500 kPa. The solvent was hexane–acetone, 50:1.

NMR. ^1H spectra were run on a Varian EM 390 NMR spectrometer. ^{13}C spectra were run on a JEOL PFT-100 NMR spectrometer using a pulse sequence to determine carbon multiplicities (LeCocq and Lallemand, 1981). A Nicolet 200-MHz FT spectrometer was used for the nuclear Overhauser difference experiments.

Infrared. A Perkin-Elmer Model 197 IR spectrophotometer was used. The phenol sample was measured as a film on ultramicro salt plates.

Odor Thresholds. Procedures used for determining human odor responses have been described previously (Guadagni et al., 1966). Aroma responses for each fraction were measured as a percent of response to the unfractionated oleic acid mixture. Odor units are expressed in terms of concentration over threshold.

Table I

fraction	% of total weight	% of aroma contribution[a]
basic	1	56
acid I	7	1
acid II	73	18
neutral	19	25

[a] See the text for an explanation.

Table II. Acid Fraction I: Low Molecular Weight Fatty Acids of Crude Oleic Acid

	RT[a]	mol wt[b]	amount[c]
butyric	13.6	102	0.3
3-methylbutyric	15.6	116	tr
pentanoic	18.3	116	0.4
a pentanoic acid	21.3	114	tr
a hexanoic acid	22.6	130	1.0
hexanoic[d]	25.8	130	13.0
heptanic	35.1	144	12.9
benzoic	43.7	136	tr
octanoic[d]	45.7	158	32.5
phenylacetic	52.5	150	tr
an octanoic acid	54.1	158	1.8
a dimethylbenzoic acid	59.3	164	3.4
decanoic[d]	61.5	186	1.0
a tridecenoic acid	64.2	234	tr

[a] RT in minutes. [b] Mol wt of methyl ester. [c] Percent of acid fraction I. [d] Reported by Allen et al. (1969).

Table III. Acid Fraction II: Higher Molecular Weight Fatty Acids of Crude Oleic Acid

	mol wt[a]	scan no.[b]	amount[c]
n-octanoic[d]	144	1070	1.5
n-nonanoic[d]	158	1268	tr
n-decanoic[d]	172	1483	3.7
n-dodecanoic[d]	200	1856	1.9
n-tetradecanoic[d]	228	2220	19.9
n-pentadecanoic[d]	242	2314	tr
a pentadecanoic acid	242	2326	tr
9-hexadecenoic[d]	254	2505	18.7
n-hexadecanoic[d]	256	2534	5.4
(*E*)-9-octadecenoic[d]	282	2644	tr
(*Z*)-9-octadecenoic[d]	282	2836	59.2

[a] Mol wt of free acid. [b] Scan no. = RT in seconds. [c] Percent of acid fraction II. [d] Reported by Allen et al. (1969).

Table IV. Basic Compounds of Crude Oleic Acid

	mol wt	scan no.[a]	amount[b]
3-butylpyridine	135	964	tr
2-butylpyridine	135	1066	46
quinoline	129	1128	1.0
4-pentylpyridine	149	1194	7.1
2-pentylpyridine	149	1292	3.1
a C_4 alkyl pyridine	135	1310	0.8
a C_6 alkyl pyridine	163	1419	tr

[a] Scan no. = RT in seconds. [b] Expressed as percent of total basic fraction.

RESULTS AND DISCUSSION

Fatty acids comprise about 80% of the crude oleic material investigated. The major component is, of course, (*Z*)-9-octadecenoic acid, comprising about 43% of total crude material. Tables I and II show the composition of the fatty acids identified; the amount of each acid is expressed as a percent of the fraction in which it was found. Previous investigators (Allen et al., 1969) have shown that stearic acid is the major constituent of whole tallow, followed by oleic. In manufacture, stearic acid is separated from the crude oleic preparation (Formo, 1982) and was therefore not detected.

Table V. Neutral Fraction of Crude Oleic Acid

	GC–MS		GC (HP 5840)		
	mol wt	scan no.[a]	Kovats	Kovats authentic sample	amount[b]
hydrocarbons					
heptane	100	222		700	
octane[d]	114	323	800	800	tr
3-ethyl-1,4-hexadiene	110	485	903	*k*	tr
n-decane[d]	142	682	1000	1000	tr
3-ethyl-2-methyl-1,3-hexadiene	124	691	1005	*k*	tr
1-methyl-4-(1-methylethynyl)cyclohexene	136	726	1020	*k*	tr
1-methyl-2-propylcyclopentane	126	738	1026	*k*	tr
butylbenzene [or (2-methylpropyl)benzene][d]	134	772	1041	*k*	tr
undecane[d]	156	904	1099	1100	tr
a C_5 alkyl bbenzene[d]	148	997	1142	*k*	tr
3,7-dimethyl-1-octene	140	1006	1145	*k*	tr
naphthalene	128	1031	1159	*k*	tr
dodecane[d]	170	1128	1199	1200	tr
dimethylisopropylbenzene	148	1264	1262	*k*	0.5
2-phenylheptane	176	1458	1354	*k*	0.3
C_{13} hydrocarbon[d]	184	1494	1371	*k*	tr
tetradecane[d]	198	1555	1399	1400	0.3
C_{14} hydrocarbon	198	1685	1464	*k*	tr
1-pentadecene	210	1723	1483	*k*	0.5
n-pentadecane[d]	212	1755	1499	1500	tr
n-hexadecane[d]	226	1943	1598	1600	0.3
1-hexadecyne	222	2060	1664	*k*	0.5
n-heptadecane[d]	240	2123	1700	1700	1.0
alcohols					
butanol[d]	74	190			
pentanol[d]	88	267	746	747	tr
hexanol[d]	102	403	849	852	tr
1,2-hexanediol	118	448	880	*k*	tr
7-octen-4-ol	128	609	963	*k*	tr
2-ethyl-1-hexanol	130	824	1064	*k*	tr
octanol[d]	130	928	1110	*k*	tr
nonanol	144	980	1134	*k*	0.7
dodecanol	186	1734	1488	*k*	1.0
esters					
butyl formate	102	228			
methyl hexanoate	130	493	905	907	tr
methyl heptanoate	144	696	1006	1006	tr
methyl octanoate	158	918	1105	1107	tr
methyl nonanoate	172	1141	1206	1207	tr
methyl decanoate	186	1361	1306	1307	*c*
methyl tetradecanoate	242	2136	1707	1708	4.4
methyl 12-methyltetradecanoate	256	2245	1771	*k*	0.3
methyl ester	256	2259	1779	*k*	0.4
methyl pentadecanoate	256	2306	1807	*k*	0.2
methyl 11-hexadecenoate	268	2432	1883	*k*	2.8
methyl hexadecanoate	270	2471	1907	1909	2.8
methyl ester	284	2584	1978		0.5
methyl oleate	296	2744	2079	2080	10.1
methyl octadecanoate	298	2787	2107	2110	0.3
aldehydes					
pentanal	86	205		*k*	
(*E*)-2-pentenal	84	245		*k*	
3-methylpentanal	100	296	773	748[e]	1.0
(*E*)-2-hexenal	98	359	823	824	tr
heptanal	114	443	876	877	0.5
benzaldehyde[d,f]	106	536	928	929	tr
(*Z*)-hept-2-enal[d,f]	112	536	928	936	tr
octanal[d]	128	641	980	980	1.4
(*E*)-2-octenal[d]	126	750	1031	*k*	tr
nonanal[d]	142	864	1082	1083	1.2
(*E*,*Z*)-2,6-nonadienal	138	958	1124	1127	tr
decanal	156	1092	1184	1184	0.3
(*E*)-2-decenal[l]	154	1208	1237	*k*	0.4
(*E*,*E*)-2,4-decadienal[d]	152	1323	1289	1289	0.6
(*E*)-2-dodecenal	182	1429	1340	*k*	0.3
tetradecanal	212	1930	1591	1592	0.2
C_{16} aldehyde	268	2287	1799	*k*	0.3
ketones					
3-pentanone	86	201			
3-pentanone	86	201			
unsaturated C_6 ketone	98	336	808	*k*	tr
cyclohexanone	98	414	858	859	tr

Table V (Continued)

	GC-MS		GC (HP 5840)		
	mol wt	scan no.[a]	Kovats	Kovats authentic sample	amount[b]
5-methyl-2-hexanone	114	425	865	*k*	tr
2-octanone	128	616	967	969	0.2
2-nonanone	142	837	1070	1072	0.8
2-(1-methylethylidene)cyclohexanone	138	897	1099	*k*	tr
2-decanone	156	1065	1171	*k*	1.1
5-butyldihydro-2(3*H*)-furanone	142	1153	1214	*k*	tr
phenyl pentyl ketone	176	1614	1431	*k*	tr
tetramethyloctadienone	180	1667	1456	*k*	tr
walogens					
chloro compound		406	854	*k*	tr
dichlorobenzene[g]	146	703	1011	*k*	tr
trichlorobenzene[g]	180	1017	1153	*k*	tr
tetrachlorobenzene[g]	214	1442	1349	*k*	tr
phenols					
phenol	94	642	969	*k*	0.16[h]
cresol	108	829	1066	*k*	0.4[h]
methylisopropylphenol[g]	150	1278	1268	*k*	2.0
thymol	150	1293	1275	*i*	5.4
carvacrol	150	1346	1300	*i*	2.0
dimethylisopropylphenol[g]	164	1361	1306	*k*	1.1[j]
methylisopropylphenol[g]	150	1379	1319	*k*	1.2
dimethylisopropylphenol[g]	164	1399	1326	*k*	0.8
diisopropylphenol[g]	178	1417	1335	*k*	0.3
dimethylisopropylphenol[g]	164	1449	1351	*k*	0.3
methyldiisopropylphenol[g]	192	1568	1407	*k*	0.7
methyldiisopropylphenol[g]	192	1584	1413	*k*	6.5
methyldiisopropylphenol[g]	192	1602	1423	*k*	0.4
5-methyl-2,4-diisopropylphenol	192	1709	1475	*k*	18.5
methyldiisopropylphenol[g]	192	1740	1491	*k*	1.6
BHT (solvent artifact)	220	1755	1499	*k*	1.4
methyldiisopropylphenol	192	1776	1506	*k*	0.7
methyldiisopropylphenol[g]	192	1862	1556	*k*	0.7[f]
dimethyldiisopropylphenol[g]	206	1862	1556	*k*	*f*
triisopropylphenol[g]	220	1895	1576	*k*	tr

[a] Scan no. = RT in total seconds. [b] Expressed as percent of total neutral fraction; tr equals less than 0.1%. [c] Part of a mixture with a phenol. Total of both equals 1.1%. [d] Reported by Watanabe and Sato (1971). [e] As determined on an SF 96 (50) column. [f] Mixture. [g] Specific isomers were not determined. [h] Compounds found in acid fraction II. Amounts as percent of acid fraction II. [i] Assignment confirmed by enrichment of the original mixture with the authentic compound. [j] Part of a mixture with methyl decanoate. [k] All identifications not confirmed by retention indices are to be considered tentative.

Decanoic acid was found in both acid fractions and comprised about 2.8% of the crude material. The acid fractions elicited 1% (fraction I) and 18% (fraction II), respectively, of human aroma response to the total oleic distillate (see Table III). By contrast, decanoic acid is probably the material that causes the greatest response in coyotes to the crude oleic extract (Fagre et al., 1983; Teranishi et al., 1981). It is the basis of successful coyote lure, trimethylammonium decanoate (TMAD), which depends upon slow release of decanoic acid and trimethylamine for its activity. In addition, several phenols were identified in both acid fractions.

The basic fraction totaled a little more than 1% of the crude oleic mixture; however, its aroma response in humans was 56% of the total for crude oleic (Table III). The compounds tentatively identified (Table IV), based on MS data only, were a series of alkyl pyridines (butyl, pentyl, hexyl), as well as quinoline. The predominant isomer was 2-butylpyridine (46% of the basic fraction). Similar compounds have been reported previously in roasted lamb fat volatiles (Buttery et al., 1977). Preliminary reports show that coyotes are not attracted to these aromas (Fagre, 1982).

Neutrals comprised about 19% of the crude oleic mixture and were responsible for about 25% of the human threshold aroma response. Hydrocarbons (Table V) made up a small percentage of this fraction, and most of them were present in trace amounts. Many compounds in this class were previously reported by Watanabe and Sato (1971). Nine alcohols were also present at low concentration (Table V).

Esters (Table V) were the second largest class of compounds present in the neutral fraction. They totaled about 20% of this fraction; methyl oleate represented half of the ester total. The amounts of the individual esters present reflected the relative amounts of free acids seen in the crude material. In all probability the methyl esters are artifacts of manufacture, produced by exposure of the crude acid mixture to high concentrations of methanol.

A number of carbonyl compounds were seen, all of low abundance (Table V). Many of the aldehydes present but not the ketones have been reported in beef tallow (Watanabe and Sato, 1971).

The largest group of compounds present in the neutral fraction was phenols (Table V). In total, 19 phenolic compounds were tentatively identified or confirmed. The molecular weight range was from 150 to 220, and all had a characteristic and analogous MS fragmentation pattern, which suggested a homologous series. 5-Methyl-2,4-diisopropylphenol was the major constituent (18.5%) of the neutral fraction. Phenol, a cresol, thymol, carvacrol, and a methylisopropylphenol were identified in the neutral fraction and were also seen in acid fraction II. The presence of most of the phenols in the neutral fraction was due to the fact that exhaustive extraction with base was not employed; the phenols found only in the neutral

Table VI. Mass Spectral Data of Phenols Found in Neutral Fraction

scan[a] no.	molecular ion m/e	molecular ion RA, %	base peak,[b] m/e
1278	150	29	135
1293	150	27	135
1346	150	37	135
1361	164	20	149
1379	150	27	135
1399	164	22	149
1417	178	18	163
1449	164	20	149
1568	192	22	177
1584	192	29	177
1602	192	18	177
1709	192	20	177
1740	192	20	177
1764	192	25	177
1776	192	12	177
1862	206	31	191
1895	220	7	205

[a] Scan no. = RT in total seconds. [b] Relative abundance = 100% in all cases.

fraction are hindered and are weakly acidic.

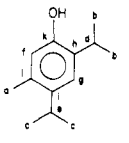
There was no attempt to determine which cresol isomer was found. Thymol and carvacrol identifications were confirmed by enrichment of an aliquot of the neutral fraction with authentic standards and observation of the increase in peak height at the assigned retention times of a GC analysis. In addition, two other phenols of mol wt 150 were observed. It is conjectured that they are also methylisopropylphenols based on their MS fragmentation patterns (Table VI).

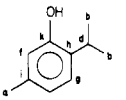
The mass spectra of all phenols reported, except phenol itself and cresol, are all quite similar in pattern. They all show a large molecular ion of 10–30% relative abundance and the base peak in all cases is $M^+ - 15$. All other fragments are of relatively low abundance. This fragmentation pattern is consistent with published spectra for methylisopropylphenols (Sendra and Cunat, 1980).

The major constituent, 5-methyl-2,4-diisopropylphenol (scan no. 1709, GC–MS), was isolated by means of preparative HPLC and preparative GC. It has been reported as a major constituent of Spanish origanum but not as an animal product (Sendra and Cunat, 1980). The mass spectrum of this compound shows a characteristic molecular ion at m/e 192 and a base ion of $M^+ - 15$ at m/e 177. There were a total of six other unisolated isomers of 5-methyl-2,4-diisopropylphenol, all with retention times within 3 min of each other. The mass spectra of the identified phenol as well as the isomers were all quite similar to each other and to published spectra (Sendra and Cunat, 1980).

The ^1H NMR of the major compound (scan no. 1709 GC–MS) confirmed that the aromatic ring structure (δ 6.53, 1 H, s; δ 7.05, 1 H, s) was substituted with a hydroxyl (δ 4.50, 1 H, s), a methyl (δ 2.25, 3 H, s), and two isopropyl groups (δ 1.20, 6 H, d, J = 7 Hz; δ 1.25, 6 H, d, J = 7 Hz; δ 3.08, 1 H, m; δ 3.13, 1 H, m). The ^{13}C spectrum (Table VII) also confirms this substitution. The ^{13}C assignments are similar to those of the analogous compound, thymol. The near-zero coupling constants of the ring protons show that they are para to each other, while nuclear Overhauser difference experiments indicated that the proton ortho to the hydroxyl is also adjacent to the methyl group. Therefore, the only possible structure for the compound at scan no. 1709 is 5-methyl-2,4-diisopropylphenol. The IR spectrum of this compound is also consistent with published spectra (Sendra and Cunat, 1980) and shows

Table VII. ^{13}C NMR of 5-Methyl-2,4-diisopropylphenol (Scan Number 1709, GC–MS)





carbon	δ	carbon	δ
a	18.7	a	20.8
b	22.7	b	22.8
c	23.5	c	
d	27.2	d	26.7
e	28.9	e	
f	117.0	f	116.4
g	122.7	g	126.3
h	131.6	h	131.8
i	133.4	i	136.5
j	139.1	j	
k	150.1	k	152.0

[a] Thymol data: Sadtler Research Laboratories (1979).

absorbances at 3600–3100, 1615, 1545, 1500, 1380, 1360, and 890 cm^{-1}.

A number of the compounds identified have been submitted for testing as coyote attractants. The results of these tests are to be published elsewhere.

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