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Identification of Sheep Liver Volatiles

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The volatile constitutents of sheep liver were investigated to find new coyote (*Canis latrans*) attractants that could be useful for reducing predation of sheep. Steam disstillation–solvent extraction of the liver yielded a concentrate which showed good coyote attractancy. In order to identify the most active substances from the many compounds present, silica gel and acid/base fractionations were carried out. Capillary column GLC/MS of these fractions showed over 200 peaks of which 108 compounds were identified. They included a series of aldehydes, hydrocarbons, esters, and aromatic compounds, 15 thiazoles, 9 pyrazines, and 5 pyridines.

Coyotes (*Canis latrans*) cause considerable damage to livestock, mainly sheep. Efforts at using lures for coyote trapping suffer from attractance of nontarget animals; therefore, development of new lures is aimed at improving specificity, as well as attractancy, solely for coyotes.

A cooperative project between the University of California, Davis, and the Western Regional Research Center, U.S. Department of Agriculture, Albany, CA has resulted in the identification of a number of substances that are promising as specific coyote lures (Teranishi et al., 1980; Fagre et al., 1981). Such lures must satisfy several requirements. They must be at least as effective as the empirically developed commercial lures used so far. They should of known composition and consist of a minimum number of components for simple and inexpensive production. From the ecological point of view, lures should be specific for coyotes.

Sheep liver volatiles were investigated as a possible source for attractants (Fagre, 1982). A study of cooked pork liver volatiles was published by Mussinan and Walradt (1974); however, there are no publications on sheep liver volatiles. Testing of cooked sheep, pork, and beef liver volatiles on penned coyotes has been carried out by cooperating scientists from the University of California, Davis. This paper presents the analytical portion of the project: the identification of volatiles from cooked sheep liver.

EXPERIMENTAL SECTION

Extraction of Volatiles. A total of 35 kg of lamb liver provided by the Animal Science Department, University of California, Davis, was extracted in 2–3-kg batches. The liver was blended with an equal amount of water (2–3 L) in a Waring Blendor, transferred to a 12-L round-bottomed flask, and atmospherically steam distilled and extracted with diethyl ether (125 mL) by using a Likens-Nickerson head, as described by Schultz et al. (1977). A dry ice–2-propanol reflux condenser was attached at the extraction head exit port. The ether extracts were combined, dried and concentrated by using a Vigreux column. The extraction yielded 1.27 g of sheep liver volatiles. Material which codistilled with ether during initial concentration was recovered by distilling off the ether with more efficient glass helix distillation system. This low-boiling fraction totalled 10 mg, which is 0.8% of the total extract.

Silica Gel Fractionation. A total of 400 mg of the sheep liver extract was placed on a silica gel column (45 cm × 1.5 cm i.d.; silica gel 100–200 mesh, deactivated with 10% H_2O) and eluted successively with hexane, hexane/diethyl ether (5%), hexane/ether (50%), ether, and methanol. The eluents were divided into nine fractions, as determined by TLC analysis, by using phosphomolybdic acid for visualization. Each fraction was concentrated and weighed. Total recovery was 32%.

Acid/Base Fractionation. A 240-mg portion of the sheep liver extract was dissolved in 50 mL of ether and extracted with 3 N HCl as described by Buttery et al. (1977) to yield the basic fraction. The remaining ether solution was treated successively with 5% $NaHCO_3$ and 1 N NaOH to obtain acidic fractions I and II. Yields were basics 12.5%, acidic I 5%, and Acidic II 38.8%. A portion of the acidic fractions was esterified with dimethylformamide dimethyl acetal (Pierce No. 49355) in order to identify the methyl esters.

Capillary GC/Mass Spectral Analyses. The nine fractions obtained from the silica column were analyzed by gas chromatography/mass spectrometry using a 42 m × 0.6 mm OV-101 glass capillary column which was temperature programmed from 60 to 200 °C at 3 °C/min and

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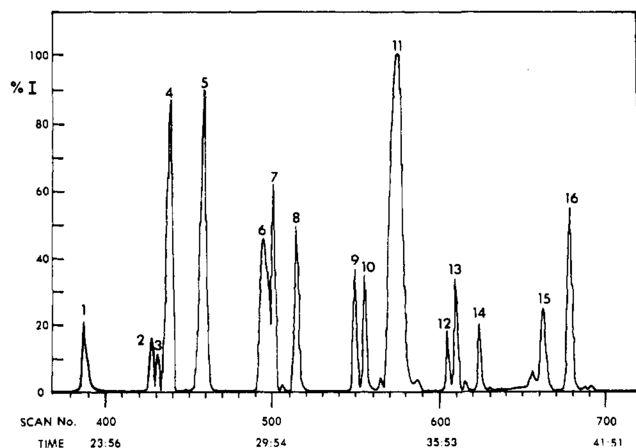

Figure 1. Reconstructed ion chromatogram (RIC) of fraction 4 obtained by silica gel chromatography of sheep liver extract and analyzed by combined GC/MS (for experimental details, see the text). Peak numbers refer to numbers of aldehydes in Table I.

held at 200 °C for 30 min. The outlet of the column was coupled to a double-focusing magnetic sector mass spectrometer (VG Organic MM 70/70F, resolution of 1000) equipped with a VG Organic DS 2035 data system. In addition to electron impact ionization which was applied to all fractions, isobutane chemical ionization was employed in the GC/MS examination of the fourth silica gel fraction.

The basic, acidic, and low-boiling fractions, as well as the esterified acidic fractions, were analyzed by combined GC/MS using a Finnigan Model 4500 gas chromatograph/mass spectrometer equipped with an INCOS data system. The gas chromatographic separation was carried out on the Finnigan system by using a fused silica column (50 m × 0.32 mm; OV-101 methyl silicone). The temperature program for the low boilers was 45 °C for 5 min, programmed to 185 °C at 2 °C/min, and then isothermal at 185 °C for 5 min. For the acidic fractions, as well as the esterified samples, the column was temperature programmed from 80 to 225 °C at 4 °C/min and held at 225 °C for 20 min. The basics were run under the following temperature conditions: 50 to 200 °C at 4 °C/min and held at 200 °C for 15 min. The identifications of all compounds were based on the comparison of the unknown spectra with known mass spectra. These assignments were confirmed when possible by relative retention times with a Hewlett-Packard 5840a gas chromatograph with a flame ionization detector. This equipment was also used for quantitative analysis.

RESULTS AND DISCUSSION

Preliminary gas chromatographic analyses of the sheep liver extract on glass capillary columns had indicated the presence of more than 200 peaks. Therefore, adsorption chromatography and acid/base fractionation seemed advisable. The fractionation by silica gel column chromatography was partially successful but considerable material losses occurred; only 32% of the applied material was recovered from the column even though the adsorbent had been deactivated. Results from GC/MS examination of the fractions indicated that highly polar compounds had not been recovered from the silica gel column. The first three fractions contained mainly straight chain hydrocarbons. Fractions 4, 5, and 6 contained a series of aliphatic aldehydes and also detectable amounts of some methyl and ethyl esters of fatty acids. The last three fractions showed mainly traces of components found in earlier fractions. The identities of the compounds are listed in Table I. Fraction 4 contained almost exclusively a series of aliphatic aldehydes some of which could be only partially characterized. The RIC (reconstructed ion chromatogram) of fraction 4 is given in Figure 1. Application of chemical ionization mass spectral analysis supported the electron impact ionization derived identifications. The peak numbers in Figure 1 correspond to the peak numbers of the aldehydes in Table I. As shown in Table I, some of the compounds have been reported before

Table I. Identities of the Constituents Found in Sheep Liver Extract by Combined GC/MS Analysis after Fractionation on Silica Gel

compound[a]	peak no. (Figure 1)	Kovats index[b] un-known	Kovats index[b] known	references[c]
aldehydes				
dodecanal		1388	1391	e, f
tridecanal	1	1490		e, g
1-tetradecanal	5	1592	1595	e, g
tetradecanal isomer	4	1556		
tetradecanal isomer	2			
tetradecanal isomer	3			
1-pentadecanal	8	1694		e, g, h
pentadecanal isomer	6	1658		
pentadecanal isomer	7	1665		
1-hexadecanal	11	1796		e, h
hexadecanal isomer	9	1760		
hexadecenal	10	1770		
heptadecanal	14	~1900		e, g
heptadecanal isomer	12	1862		
heptadecanal isomer	13	1870		
octadecanal	15	1976		
octadecanal	16	~2000		e
esters				
methyl hexadecanoate		1913	1911	e
methyl octadecenoate		2082	2082	
methyl octadecanoate		2111	2112	
ethyl dodecanoate		1579	1578	e
ethyl tetradecanoate		1779	1775	e
ethyl pentadecanoate				
ethyl hexadecanoate		1979	1980	
ethyl octadecenoate		>2000		
ethyl octadecanoate				
hydrocarbons				
tridecane		1300	1300	g, h
tetradecane		1400	1400	f, h, i
pentadecane		1500	1500	e, f, h, i
hexadecane		1600	1600	e, f, h, i
heptadecane		1700	1700	e, f, h, i
octadecane		1800	1800	f, h
nonadeccane		1900	1900	
eicosane		2000	2000	
heneicosane		2100	2100	
docosane		2200	2200	
tricosane		2300	2300	
tetracosane		2400	2400	
pentacosane		2500	2500	
hexacosane		2600	2600	
heptacosane		2700	2700	
octacosane		2800	2800	
2,6,10,14-tetramethyl-2-hexadecene[d]		1789		
2,6,10,14-tetramethyl-hexadecane[d]		1813		

[a] All identifications not confirmed by retention indices are to be considered tentative. [b] Kovats indices were determined by using the series of normal hydrocarbons and a 25 m × 0.2 mm methyl silicone fused silica column. [c] References are to studies on other meat volatiles in which the compound has been reported. [d] Suggested assignment among several possibilities. [e] Mussinan and Walradt (1974). [f] Dwivedi (1975). [g] Caporaso et al. (1977). [h] Chang and Peterson (1977). [i] Maga and Sizer (1973).

Table II. Identities of the High-Volatility Constituents of Sheep Liver Extract Analyzed by Combined GC/MS

compound[a]	concn, % of the fraction	Kovat index[b] unknown	Kovat index[b] known	odor threshold for humans, ppb in water	references[b]
aldehydes					
2-methylpropanal	2.0	534	<600	0.9	*d*
3-methylbutanal	36.0	632	635	0.15	*d–f*
2-methylbutanal	9.0	642	643	1.25	*d, e, g*
pentanal	0.6	677	677	12.0	*d, e, g, h*
miscellaneous					
ethanol	17.0	<500	<600	800 000	*d, e, g*
2-butanone	0.7	575	577		*d, e, g*
ethyl acetate	4.8		600	5 000	*d, e, g*
1,1-diethoxyethane	0.2		715	40	*e*
dimethyl disulfide	0.06		732	1.2	*d, f*

[a] All identifications not confirmed by retention indices are to be considered tentative. [b] Kovat indices were determined by using the series of normal hydrocarbons and a 213 m × 0.7 mm SF 96(50) plus 5% IGEPAL stainless steel column, temperature program, 45 °C/30 min to 200 °C at 2 °C/min. [c] References are to studies on other meat volatiles in which the compound has been reported. [d] Dwivedi (1975). [e] Chang and Peterson (1977). [f] MacLeod and Coppock (1976). [g] Mussinan and Walradt (1974). [h] Caporaso et al. (1977).

in studies of other meat volatiles. It is of interest that most of the compounds were also found in pork liver extract (Mussinan and Walradt, 1974) and some of the aldehydes in the neutral fraction of cooked ovine fat (Caporaso et al., 1977), whereas the other occurrences were in beef (Chang and Peterson, 1977; Dwivedi, 1975; MacLeod and Coppock, 1976). The quantities of each compound class identified in sheep liver extract were hydrocarbons 2%, esters 2%, and aldehydes 26% of the original. However, none of these compounds is regarded as very important for meat flavor (Maga, 1975).

Compounds of lower molecular weight and correspondingly higher vlatility were found in the fraction called the low boilers. They are listed in Table II. The major group of compounds consists of aldehydes. 3-Methylbutanal is the major constitutent (36% equivalent to 0.28% of total extract); it also appears to give the fraction its characteristic odor. There were a few minor constituents listed under miscellaneous whose mass spectra were of intensity sufficient for identification; these assignments were confirmed by retention time indices. All of these low-boiling compounds were found previously in other meat flavor studies.

Preliminary field studies showed that the whole sheep liver steam volatile extract was highly attractive to coyotes (Fagre, 1982). The criterion used to evaluate the large number of compounds reported for attractancy was to consider each compound's odor threshold for humans, as reported in the literature (Teranishi et al., 1974, 1975), or by personal communication (Gaudagni, 1982). Some of the low-boiling compounds shown in Table II have very low odor thresholds and are also generally found in meat volatiles. They have been submitted for study as coyote attractants.

Coyote attractancy tests are conducted at the Hopland Experiment Station, a division of the University of California, Davis. An observer, stationed in a blind, records individual coyote reaction to materials placed at a number of scent stations (Timm et al., 1977; Murphy et al., 1978). Responses are measured by total time spent at a station.

It is known that heterocyclic compounds containing an N, S, and/or O atom in their structure, such as pyrazines, thiazoles, and oxazoles, contribute significantly to meat flavors (Shibamoto, 1980). These compounds have also been reviewed separately by Maga and Sizer (1973) and Maga (1975, 1978). Although such heterocyclics are of mostly minor concentration in meat volatiles, they are attracting increasing attention because of their distinctive aroma notes and their presence in many kinds of heat-

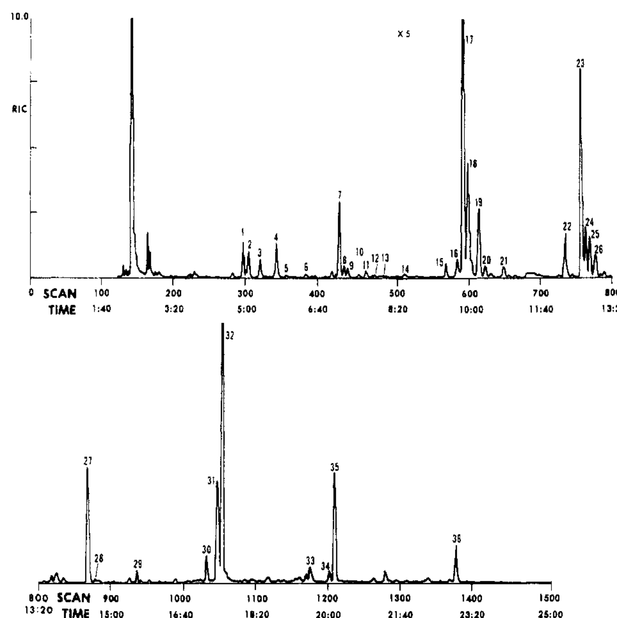

Figure 2. Reconstructed ion chromatogram (RIC) of the basic fraction of sheep liver extract analyzed by combined GC/MS. Peak numbers refer to the numbers of the compounds given in Table III.

treated food. Some of these compounds were found as expected in the basic fraction of sheep liver extract. This fraction contained primarily a series of thiazoles, pyrazines, and pyridines. Figure 2 shows the RIC of the basic fraction. The peak numbers refer to the peak identities given in Table III. Compounds for which reference substances were not available for comparing Kovats indices are listed as tentatively identified. Compounds detected were 15 thiazoles, 2 thiazolines, 9 pyrazines, 5 pyridines, and 4 oxazoles, Figure 3 shows their skeletal structures. The pyrazines and pyridines which were verified by retention data have been reported previously in the volatiles from various animal sources. Most of the sulfur-containing compounds, however, have not been found before in animal-derived volatiles. In the basic fraction of the sheep liver extract they were the main constituents. The five positively identified thiazoles were 28.5% of this fraction 3.6% of the original extract), mostly 2-isobutyl-4,5-dimethylthiazole, trimethylthiazole, and 2,4-dimethyl-5-ethylthiazole.

Since these heterocyclic compounds are regarded as important constituents of meat flavors, their odor threshold

Table III. Identities of the Constituents of the Basic Fraction of Sheep Liver Extract, Analyzed by Combined GC/MS Using a Methyl Silicone Fused Silica Column

compound[a]	peak no. (in RIC, Figure 2)	concn, % of fraction	Kovats index[b]		odor threshold for humans, ppb in water	references[c]
			unknown	known		
thiazoles						
trimethylthiazole	17	8.8	969	969	9	d, e
2,5-dimethyl-4-ethylthiazole	22	1.0	1037	1038	1	
2,4-dimethyl-5-ethylthiazole	23	5.0	1047	1048	0.1	d
2-isopropyl-4,5-dimethylthiazole	27	3.4	1097	1102		
2-isobutyl-4,5-dimethylthiazole	32	10.3	1185	1183	10	
tentatively identified						
2-acetylthiazole	19	1.3	1000			e, f
2,4-diethylthiazole	25	1.2	1052			
2,5-diethyl-4-methylthiazole (or 2,4-diethyl-5-methylthiazole)	29	0.8	1131			
C_2-alkyl-5-propylthiazole		0.2	1135			
C_6-alkylthiazole	30	0.9	1176			
diisopropylthiazole	31	2.7	1182			
C_3-alkyl-2-isobutylthiazole	33	1.0	1240			
C_4-alkyl-4-propylthiazole	34	0.6	1253			
C_2-alkyl-2-pentylthiazole	36	1.4	1337			
2-isobutylthiazole						
thiazolines						
tentatively identified						
2-methyl-4-ethylthiazoline	16	0.3	966			
C_3-alkylthiazoline	15					
pyrazines						
2-methylpyrazine	2	0.6	800	802	60000	d, f, h
2,6-dimethylpyrazine	7	2.3	886	887	1500	d, f, h–j
2,3-dimethylpyrazine	9	0.3	895	895	2500	d, f, i, j
2,5-dimethyl-3-ethylpyrazine	24	2.4	1051	1054		h, j
2-ethyl-3,5-dimethylpyrazine	26	1.4	1056	1059	ca. 1	d, f, i
tentatively identified						
ethylpyrazine	8	0.3	891			f, h–j
trimethylpyrazine	18	4.0	974			d, f, i, j
2-methyl-6-vinylpyrazine	20		1008			i
5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine	28	0.4	~1100			f, i
pyridines						
2-acetylpyridine	21	0.4	1010	1011	19	d
3-isobutylpyridine		0.4	1075	1077		
tentatively identified						
3-methylpyridine	5		832			d
3,5-dimethylpyridine	13	0.1	917			d
3-ethylpyridine	14	0.2	937			d
oxazoles						
tentatively identified						
trimethyloxazole	4	0.7	824			d, f
2,5-dimethyl-4-ethyloxazole	10	0.1	900			
2,4-diethyloxazole	11	0.5	908			
2,5-diethyloxazole	12	0.2	909			
miscellaneous						
2-methyl-2-pentenal	3	1.3	813	813		
tentatively identified						
ethyl acetate						f, g, k
3-penten-2-one						f
2-hexanol	1					
dimethylbenzene (probably *p*-xylene)	6					f, h, k
benzaldehyde						f–h, k
2-acetylpyrrole						f, k

[a] All identifications not confirmed by retention indices are to be considered tentative. [b] Kovats indices were determined by using the series of normal hydrocarbons and 25 m × 0.2 mm methyl silicone column. [c] References are to studies on other meat volatiles in which the compound has been reported. [d] Buttery et al. (1977). [e] Wilson et al. (1973). [f] Mussinan and Walradt (1974). [g] Chang and Peterson (1977). [h] MacLeod and Coppock (1976). [i] Mussinan et al. (1973). [j] Watanabe and Sato (1971). [k] Dwivedi (1975).

values were of special interest. The thiazoles identified should contribute significantly to the overall aroma of sheep liver extract based upon their low odor thresholds for humans.

Acidic fractions I and II were analyzed by combined GC/MS before and after esterifications. Table IV lists the compounds identified in both fractions. The free acids (2- and 3-methylbutyric) were found in acidic fraction I, as were *n*-hexadecanoic and *n*-octadecanoic acids. Dodecanoic acid could also be detected in this fraction. Major amounts of hexadecanoic, octadecanoic, and other acids larger than C_{10} listed in Table IV were present in acid fraction II. Substituted phenols were found in minor amounts in acid fraction II.

In summary, a total of 108 compounds were identified either tentatively by mass spectrum alone or by mass spectral assignment confirmed by retention indices. Tests are in progress to study the attractancy toward coyotes of

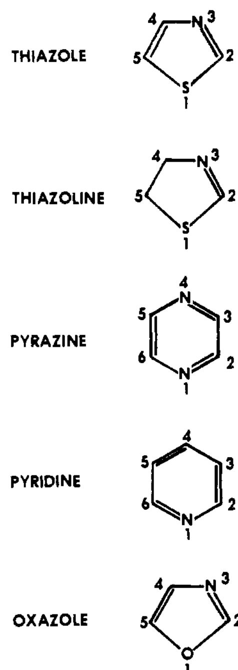

Figure 3. Skeletal structures of compounds identified in the basic fraction.

Table IV. Identities of Compounds Found in Acid Fractions I and II of Sheep Liver Extract

compound[a]	concn, % of fraction	Kovats index[b] unknown	Kovats index[b] known	references[c]
acids				
3-methylbutyric acid				
2-methylbutyric acid				
dodecanoic acid	0.3	1507	1505	
tetradecanoic acid	4.2	1708	1715	
pentadecanoic acid	0.3	1808	1813	
hexadecanoic acid	64.0	1913	1911	
heptadecanoic acid	0.8			
9-octadecenoic acid	16.0	2082		
octadecanoic acid	8.0	2111	2101	d
phenols				
tentatively identified				
phenol	0.4			d, e
methylphenol (2- or 3-)	<0.1			
4-methylphenol	0.7			
dimethylphenol	<0.1			
ethylphenol	<0.1			
ester				
ethyl acetate				d, e

[a] All identifications not confirmed by retention indices are to be considered tentative. [b] Kovat indices were determined after esterification of fractions with DMF-dimethyl acetal by using a series of normal hydrocarbons on a 25 m × 0.2 mm methyl silicone fused silica column, temperature program 100-225 °C at 4 °C/min. [c] References are to studies on other meat volatiles in which the compound has been reported. [d] Mussinan and Walradt (1974). [e] Chang and Peterson (1977).

the individual fractions and single compounds.

ACKNOWLEDGMENT

We are indebted to Dr. R. Buttery for providing mass spectral data and authentic compounds which were invaluable for many identifications, particularly in the basic fraction. T. R. Mon made a major contribution to the gas chromatographic analysis and provided many helpful suggestions. We also thank Dr. T. H. Schultz for his criticisms and suggestions regarding this manuscript.

Registry No. Dodecanal, 112-54-9; tridecanal, 10486-19-8; 1-tetradecanal, 124-25-4; 1-pentadecanal, 2765-11-9; 1-hexadecanal, 629-80-1; hexadecenal, 27104-14-9; heptadecanal, 629-90-3; octadecenal, 71873-66-0; octadecanal, 638-66-4; methyl hexadecanoate, 112-39-0; methyl octadecenoate, 27234-05-5; methyl octadecanoate, 112-61-8; ethyl dodecanoate, 106-33-2; ethyl tetradecanoate, 124-06-1; ethyl pentadecanoate, 41114-00-5; ethyl hexadecanoate, 628-97-7; ethyl octadecenoate, 28555-06-8; ethyl octadecanoate, 111-61-5; tridecane, 629-50-5; tetradecane, 629-59-4; pentadecane, 629-62-9; hexadecane, 544-76-3; heptadecane, 629-78-7; octadecane, 593-45-3; nonadecane, 629-92-5; eicosane, 112-95-8; heneicosane, 629-94-7; docosane, 629-97-0; tricosane, 638-67-5; tetracosane, 646-31-1; pentacosane, 629-99-2; hexacosane, 630-01-3; heptacosane, 593-49-7; octacosane, 630-02-4; 2,6,10,14-tetramethyl-2-hexadecane, 56554-34-8; 2,6,10,14-tetramethylhexadecane, 638-36-8; 2-methylpropanal, 78-84-2; 3-methylbutanal, 590-86-3; 2-methylbutanal, 96-17-3; pentanal, 110-62-3; ethanol, 64-17-5; 2-butanone, 78-93-3; ethyl acetate, 141-78-6; 1,1-diethoxyethane, 105-57-7; dimethyl disulfide, 624-92-0; trimethylthiazole, 13623-11-5; 2,5-dimethyl-4-ethylthiazole, 32272-57-4; 2,4-dimethyl-5-ethylthiazole, 38205-61-7; 2-isopropyl-4,5-dimethylthiazole, 53498-30-9; 2-isobutyl-4,5-dimethylthiazole, 53498-32-1; 2-acetylthiazole, 24295-03-2; 2,4-diethylthiazole, 32272-49-4; diisopropylthiazole, 85924-70-5; 2-isobutylthiazole, 18640-74-9; 2-methyl-4-ethylthiazoline, 85924-71-6; 2-methylpyrazine, 109-08-0; 2,6-dimethylpyrazine, 108-50-9; 2,3-dimethylpyrazine, 5910-89-4; 2,5-dimethyl-3-ethylpyrazine, 13360-65-1; 2-ethyl-3,5-dimethylpyrazine, 13925-07-0; ethylpyrazine, 13925-00-3; trimethylpyrazine, 14667-55-1; 2-methyl-6-vinylpyrazine, 13925-09-2; 5-methyl-6,7-dihydro-5*H*-cyclopentapyrazine, 23747-48-0; 2-acetylpyridine, 1122-62-9; 3-isobutylpyridine, 14159-61-6; 3-methylpyridine, 108-99-6; 3,5-dimethylpyridine, 591-22-0; 3-ethylpyridine, 536-78-7; trimethyloxazole, 20662-84-4; 2,5-dimethyl-4-ethyloxazole, 30408-61-8; 2,4-diethyloxazole, 84027-83-8; 2,5-diethyloxazole, 40953-14-8; 2-methyl-2-pentenal, 623-36-9; 3-penten-2-one, 625-33-2; 2-hexanol, 626-93-7; benzaldehyde, 100-52-7; 2-acetylpyrrole, 1072-83-9; 3-methylbutyric acid, 503-74-2; 2-methylbutyric acid, 116-53-0; dodecanoic acid, 143-07-7; tetradecanoic acid, 544-63-8; pentadecanoic acid, 1002-84-2; hexadecanoic acid, 57-10-3; heptadecanoic acid, 506-12-7; 9-octadecanoic acid, 2027-47-6; octadecanoic acid, 57-11-4; phenol, 108-95-2; 4-methylphenol, 106-44-5; dimethylphenol, 1300-71-6.

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Dynamic Heated Headspace Analyses of Volatile Organic Compounds Present in Fish Tissue Samples

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An analytical procedure was developed for the determination of selected volatile organic compounds in fish and shellfish tissue samples. The method employs a dynamic heated headspace isolation technique with solvent desorption of an activated carbon adsorbent media. Separation and identification, using capillary column gas chromatography with a flame ionization detector, allowed the determination of recovery values for all the compounds investigated in this study. The method was evaluated to quantitate the tissue burdens of estuarine organisms, which are reported herein.

Federal regulations, such as the Water Pollution Control Act Amendments of 1973 (*Fed. Regist.*, 1973), require the quantitation of organic chemicals in domestic and industrial wastewaters. The U.S. Environmental Protection Agency (EPA) has undertaken major studies to determine the concentration of organic pollutants in discharge water under the National Pollutant Discharge Elimination System (NPDES) permit program. Some states, such as New Jersey, have extended the scope of the effort to include the determination of fish and shellfish tissue burdens of volatile organic compounds. This action was initiated because of the commerical importance of the fishing industry in the State.

A survey of the literature showed that the acceptable Federal method (*Fed. Regist.*, 1979) for the determination of volatile organic compounds in water and wastewater was that developed by Bellar and Lichtenberg (1974). This procedure is based on a dynamic headspace isolation technique with adsorption of volatile components on a multiple adsorbent trap. Thermal desorption and carrier gas backflushing of the adsorbent trap is followed by gas chromatography for the separation and quantitation of the volatile organic compounds.

Another dynamic headspace procedure developed at Cook College, Rutgers University (Sabatino, 1981), replaces the thermal desorption and carrier as backflushing of the adsorbent trap by solvent desorption of an activated carbon trapping media (White et al., 1970) with carbon disulfide. The Rutgers method is the basis of the two techniques evaluated in this study. The advantages of the procedures evaluated are a lower cost purging apparatus, reduction of the attended time for the analysis, and the use of capillary column gas chromatography for high-resolution and analyte identification. In phase II of this study a self-contained tissue grinder/purging apparatus is evaluated.

The volatile organic compounds listed in Table I were investigated in this study. In general, the aromatic hydrocarbons studied exhibit greater water solubility than the halogenated aliphatic compounds, but both groups of compounds are less than 2% soluble in water (Ogata and Ogura, 1976). The compounds evaluated are commonly observed as contaminants in surface waters and discharge waters because of their widespread industrial use.

EXPERIMENTAL SECTION

Materials. All organic compounds and solvents used as standards were ACS-certified grade obtained from J. T. Baker and Fisher Chemical Co. Most of these compounds are flammable and/or cancer-suspect agents, and the appropriate handling procedures should be observed (Walters, 1979). Gas chromatography supplies were obtained from Supelco, Inc. (Bellefonte, PA). Glassware used in phase I was obtained from Wheaton Scientific, Vineland, NJ, from designs supplied by T. Sabatino. Glassware used in Phase II was fabricated by R. Shipmann from designs by T. Sabatino.

Procedures. *Phase I.* This procedure used a 100-mL glass purging vessel as shown in Figure 1. Ten grams of knife-cut tissue sample was placed into the vessel, which was secured in a water bath held at 50 °C. A National Institute of Occupational Safety and Health (NIOSH) (100/50 mg) type activated carbon tube (White et al., 1970) was attached to the glass exit arm of the apparatus with a Teflon bushing and a Viton (registered trademark of Du Pont) O ring. The helium purge gas was connected to a 227-mm Pasteur pipet by using 1 mm i.d. Teflon tubing, a cylindrical silicon through hole septum, and a 26 gauge syringe needle. The tissue was fortified with 100 μL of a

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