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ARTICLES

Insect Attractants: Volatiles of Hydrolyzed Protein Insect Baits

Ron G. Buttery,* Louisa C. Ling, Roy Teranishi, and T. R. Mon

The volatile components of a widely used commerical hydrolyzed protein insect bait have been isolated both by Tenax trapping and by vacuum steam distillation continuous extraction. The volatile concentrates obtained were analyzed by capillary gas–liquid chromatography–mass spectrometry and 43 components identified. Major components include phenylacetaldehyde, acetic acid, furfuryl alcohol, 2-acetylfuran, benzaldehyde, methional, 2-acetylpyrrole, and furfural. Unusual components include some aldol condensation products such as 5-methyl-2-phenyl-2-hexenal and 5-methyl-2-[(methylthio)methyl]-2-hexenal.

A number of investigators [e.g., Hagen et al., (1976), van Emden and Hagen (1976), and Miller and Haarer (1981)] have explored the use of hydrolyzed protein preparations as baits for certain insects such as the green lace wing (*Chrysopa carnea*) and the Mediteranean fruit fly (*Ceratitis capitata*, Wiedemann). These insects seem to be attracted to the hydrolyzed protein by the volatile compounds associated with it. The hydrolyzed protein mixtures are assumed to be related in composition to the "honeydew" extruded by aphids which, in nature, can apparently supply a suitable diet for both the adult and larva of certain insects (Hagen et al., 1976).

Some of these baits have been used in large-scale programs to combat insect pests such as in the 1981 program in California to eradicate the Mediteranean fruit fly. The main bait used in California for this purpose was the commercial hydrolyzed protein bait "Staley Protein Bait No. 7" (abbreviated PIB-7).

There seems to be very little published information on the identity of the volatile compounds associated with these baits which must attract the insects initially to the hydrolyzed protein. Some recent studies have been reported by Morton and Bateman (1981), who identified 39 compounds in two different hydrolyzed protein preparations, one of which was PIB-7.

The present study was aimed at further identification of the major volatiles associated with the commerical PIB-7 hydrolyzed protein bait.

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

EXPERIMENTAL SECTION

Materials. The main sample of hydrolyzed protein was "Staley Protein Bait No. 7" (corn gluten hydrolyzed) manufactured by A. E. Staley Manufacturing Co., Protein Division, Decatur, IL (PIB-7). Two different lots of this material were examined. This material is an aqueous (44–50% solids) viscous liquid, pH 3.5–4.5, described as containing 18–25% protein (as amino acids) and 6–12% carbohydrate (mostly sugars) and 6–13% salt. An enzyme-hydrolyzed protein for comparison was "Ardemine Autolysate enzymatic protein hydrosylate of Brewers yeast" manufactured by Unilab Research Corp., Berkeley, CA.

Isolation Using Tenax Traps. The traps as described previously (Buttery et al., 1982) were made from Pyrex glass and contained a 1.3 cm diameter × 7 cm long (1.7 g) column of Tenax GC adsorbent. One liter of the hydrolyzed protein preparation was placed in a 12-L flask and stirred with a magnetic stirrer. Purified air (500 mL/min) was passed into the flask (via a Teflon tube) and out through the trap by applying suction to the outlet of the trap. The isolation was continued for 24 h at room temperature. The trap was then removed and the trapped material eluted with freshly distilled diethyl ether. The ether extract was concentrated by using a warm water bath and low hold up distillation columns.

Isolation by Vacuum Steam Distillation Continuous Extraction. This was carried out in essentially the same way as described previously by the authors for other materials [e.g., Buttery and Kamm (1980)] at 100 mmHg pressure. In this case methyl *tert*-butyl ether, purified by distillation through a 20-plate column, was used as the

extracting solvent. The isolation was carried out by using 6 L of PIB-7 in a 12-L flask. A dry ice reflux condenser was used above the modified Likens/Nickerson steam distillation continuous extraction head. The isolation was continued for 3 h. After the isolation the extract was dried over anhydrous sodium sulfate and concentrated under reduced pressure.

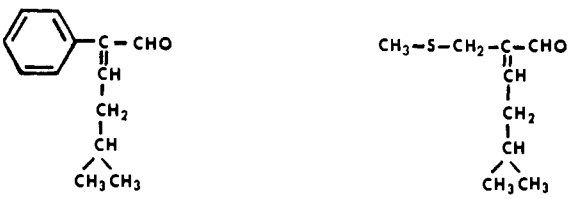
Capillary Gas–Liquid Chromatography–Mass Spectrometry (GLC–MS). The main part of the work was carried out by using a 150 m long by 0.64 mm i.d. Pyrex glass capillary column coated with Carbowax 20-M. The effluent from the capillary was introduced directly into the mass spectrometer (a modified Consolidated 21-620 cycloidal type) by using a Llewellyn-Littlejohn single-stage silicone rubber membrane molecular separator. The GLC programming conditions were to hold the column temperature at 50 °C for 30 min after injection and then to program from 50 to 170 °C at 1 °C/min, holding the temperature at 170 °C for another 60 min. For some studies, in a search for tryptophan degradation products, the GLC–MS analysis was carried out, using a 150 m long by 0.64 mm i.d. Pyrex glass capillary coated with silicone OV-3, at 170 °C constant temperature. The silicone OV-3 capillary column was also used for the GLC–MS analysis of some very volatile compounds by direct injection of 10 mL of the atmosphere above the PIB-7.

Most authentic chemical compounds, for comparison (mass spectra and GLC Kovats' index), were obtained from reliable commercial sources. Other compounds were synthesized by established methods [e.g., Buttery (1973)]. All compounds were repurified by GLC separation and their identities verified by spectral (MS and IR) means.

RESULTS AND DISCUSSION

Two main methods of isolating the volatiles were used. In the first method air was drawn over the hydrolyzed protein and the volatiles were adsorbed on a Tenax trap. The second method used vacuum steam distillation continuous extraction. Preliminary tests with the vacuum steam volatile oil from PIB-7 hydrolyzed protein showed that it was attractive to the Mediteranean fruit fly, although less so than the original PIB-7 (in 1 day 600 flies were trapped with the PIB-7 bait, 210 flies trapped with the vacuum steam volatile oil, and none with blank traps). Effectiveness to the green lace wing was indicated by the attraction of more than 50 green lace wing insects to the corridor immediately outside the authors' laboratory when briefly handling the vacuum steam volatile oil in open containers in the laboratory. The amount of total volatile concentrate obtained was of the order of 5 parts per million (ppm) of the original PIB-7 (w/w) by using the vacuum steam distillation method and 0.5 ppm by using the Tenax trapping method.

The concentrates obtained by removing the solvent in the extraction methods were analyzed by capillary GLC–MS. Components identified for the PIB-7 hydrolyzed protein volatile oils are shown in Table I. Approximate relative percentages of components based on GLC peak areas are also listed. There were some variations between samples, and these figures are only meant to give a general idea for a typical sample. Major components identified include the Strecker degradation aldehydes phenylacetaldehyde (10%) and methional (9%). Other members of this group, also identified, include the 2- and 3-methylbutanals which were only identified in the vacuum steam volatile oil (conditions used in the Tenax trapping method were not suitable for the lower boiling compounds). Other major compounds identified included acetic acid (30%) and various sugar degradation products such as furfuryl alcohol (11%), furfural (9%), and 2-acetylfuran (6%).



I II

Figure 1. Structures of 5-methyl-2-phenyl-2-hexenal (I) and 5-methyl-2-[(methylthio)methyl]-2-hexenal (II).

Most of these major compounds had also been identified by Morton and Bateman (1981), who had pointed out the importance of Strecker degradation in producing many of the volatile compounds.

A group of unusual aldehydes (cf. Figure 1) such as 2-phenyl-2-butenal and 5-methyl-2-[(methylthio)methyl]-2-hexenal, identified in the present work, apparently arise from the aldol condensation type reactions of acetaldehyde and 3-methylbutanal with phenylacetaldehyde and methional in the normal protein hydrosylate. These aldol compounds were obtained by using both methods of isolation, vacuum steam distillation, and Tenax trapping. It is difficult to completely rule out the possibility that some of this condensation might occur during the isolation. The authors, however, designed the extraction conditions to minimize this possibility (by using relatively cool temperatures throughout, handling the volatiles in dilute solutions, and storing at −20 °C). It seems more likely that these aldol compounds were mainly formed in the PIB-7 during the vigorous conditions used in its manufacture. PIB-7 is manufactured by heating corn gluten with hydrochloric acid followed by neutralization with alkali. It is supplied as an aqueous viscous liquid (50–56% water) at a pH of 3.5–4.5.

The authors carried out test experiments with 3-methylbutanal and 2-phenylacetaldehyde (10 μL each) added to 8 L of water and otherwise exactly duplicating the conditions used during the vacuum steam distillation continuous extraction of PIB-7. A trace of aldol condensation product was found but it was less than 1% of the original aldehydes. The authors found no detectable condensation when these compounds were mixed in pure hexane or methyl *tert*-butyl ether and left at room temperature for 3 h. Aldol condensations, however, are known to be readily catalyzed by alkaline or acid conditions. Such conditions did not occur during the isolation procedures.

The compound 2-phenyl-2-butenal and other aldol condensation products of phenylacetaldehyde with aliphatic aldehydes show some slight resemblance to some known fruit fly attractants such as "Cue-Lure", 4-(*p*-acetoxyphenyl)butan-2-one (melon fly), and related compounds [cf. Beroza et al. (1960)].

The compounds 3-phenylfuran and 3-phenylthiophene probably also arise from aldol-type condensations through an intermediate something like 2-phenyl-2-butenal.

Levulenic acid is a known product of acid hydrolysis of sucrose.

The nitrogen-containing compounds 2-acetylpyrrole and the alkylpyrazines are commonly found in systems involving the heating of amino acids and sugars [cf. Hodge et al. (1972)].

Many of the compounds in Table I have been reported as volatile constituents of soy sauce (Nunomura et al., 1976a,b, 1978, 1980) and also of some cooked foods such as roasted cocoa beans (van Praag et al., 1968) and fried

Table I. Volatile Compounds Identified in PIB-7 Hydrolyzed Protein Insect Bait

compound[a]	characteristic mass spectral ions,[b] m/z	Kovats' GLC index[c]	relative % Tenax trap	relative % vac stm distill.
Aliphatic Aldehydes and Alcohols				
acetaldehyde[f]	29, *44*, 43, 42	410[e]		
2-methylpropanal[f]	43, 41, *72*, 71, 57, 55	567[e]		
2-methylbutanal[f]	57, 51, 58, *86*, 39, 71	904		2
3-methylbutanal[f]	44, 41, 43, 58, 57, *86*	904		
2-methylbutanol	57, 56, 41, 31, 70, 39	1180		3
3-methylbutanol	55, 42, 43, 70, 31, 57	1180		
Aliphatic Acids				
acetic acid[f]	43, 45, 60, 29, 42, 31	1430	2	30
butyric acid	60, 73, 41, 43, 45, *88*	1620	0.2	0.9
3-methylbutyric acid	60, 41, 43, 45, 74, 87	1650	1.0	
pentanoic acid	60, 73, 41, 45, 43, 87	1720	1.0	
hexanoic acid[f]	60, 73, 57, 45, 55, 87	1830	2.6	1.7
levulinic acid[f]	43, 56, 45, 73, 101, *116*	2340	3.2	
Aliphatic Esters and Lactones				
methyl levulinate	43, 55, 99, 115, 59, 71	1560	1.2	
ethyl levulinate	43, 99, 74, 129, 102, *144*	1600	4.6	
γ-pentalactone[f]	56, 41, 85, 43, 45, *100*	1600	0.2	
γ-hexalactone	*85*, 56, 42, 57, 70, *114*	1680	0.2	
Sulfur Compounds				
dimethyl sulfide[f]	47, *62*, 45, 35, 46, 61	528[e]		
methional[f] [3-(methylthio)propanal]	48, 47, *104*, 61, 45, 76	1440	5	9
5-methyl-2-[(methylthio)methyl]-2-hexenal	109, 81, 43, 53, 124, *172*	1820		1.0
3-phenylthiophene[d]	*160*, 115, 45, 63, 89, 128	2090	0.2	0.2
Aromatic Compounds				
benzaldehyde[f]	77, 105, *106*, 51, 50, 39	1520	9	6
phenylacetaldehyde[f]	91, 92, *120*, 65, 39, 51	1650	11	10
guaiacol[f]	109, *124*, 81, 53, 39, 51	1840	3	1.5
2-phenylethanol	91, 92, *122*, 65, 39, 51	1890	0.8	
2-phenyl-2-butenal	*146*, 117, 115, 91, 51, 78	1910	0.8	0.3
4-methyl-2-phenyl-2-pentenal	*174*, 103, 91, 131, 77, 145	1920	0.6	0.6
4-methyl-2-phenyl-2-hexenal	91, 103, 77, *188*, 131, 115	2000		0.6
5-methyl-2-phenyl-2-hexenal	117, *188*, 104, 91, 132, 145	2060	2	2
4-vinylguaiacol	*150*, 135, 77, 51, 107, 53	2160		1
Furans				
2-pentylfuran	81, 53, *138*, 39, 95, 68	1240		3
2-methyl-3-oxotetrahydrofuran	43, 72, *100*, 44, 45, 55	1250		0.8
furfural	*96*, 39, 95, 38, 37, 67	1450	1	9
5-methylfurfural	*110*, 109, 53, 39, 51, 81	1560	0.4	0.9
2-propionylfuran	95, 39, *124*, 38, 67, 53	1560	0.3	
2-acetylfuran[f]	95, *110*, 39, 43, 68, 67	1490	13	6
furfuryl alcohol	41, *98*, 42, 81, 53, 97	1650	13	11
2-methyl-5-propionylfuran	109, *138*, 53, 110, 43, 51	1700	0.9	7
3-phenylfuran[d]	*144*, 115, 89, 63, 72, 51	1830	0.3	0.4
Nitrogen Heterocyclics				
2,5-dimethylpyrazine	*108*, 42, 39, 81, 52, 65	1325	0.3	
2,6-dimethylpyrazine	*108*, 42, 39, 67, 81, 66	1330	0.9	
2-ethyl-6-methylpyrazine	121, *122*, 39, 94, 56, 66	1390	1	
2-ethyl-3-methylpyrazine	121, *122*, 67, 94, 81, 52	1410	0.3	
2-acetylpyrrole[f]	94, *109*, 66, 39, 43, 53	1950	10	2
N-methyl-2-formylpyrrole[d]	*109*, 108, 53, 39, 80, 41	2090	0.2	

[a] Mass spectrum (complete spectrum) and Kovats' GLC retention index are consistent with that of authentic compounds except for footnote *d*. [b] Not necessarily the most intense ions but those considered characteristic for that compound. Ions are listed in descending order of intensity with molecular ion (if found) in italic type. [c] Kovats' GLC index for the Carbowax 20-M Pyrex capillary column except for footnote *e*. [d] No authentic sample available but mass spectrum consistent with published spectra (Stenhagen et al., 1974). [e] Kovat's GLC index on a OV-3 silicone capillary GLC column. Identified only in vapor above PIB-7. No quantitative analysis made. [f] Previously identified in PIB-7 by Morton and Bateman (1981).

potato chips (Buttery, 1973).

Very volatile compounds (bp ca. less than 3-methylbutanal) were analyzed by direct injection of the vapor above PIB-7 into the GLC–MS system using the silicone OV-3 capillary column.

Tryptophan Derivatives. Baits containing only tryptophan and sucrose have been found to be very effective as attractants for green lace wing insects (Hagen et al., 1976). These authors have indicated that a volatile degradation product of tryptophan, such as indolylacetaldehyde, might be responsible. Volatile tryptophan degradation products were actively searched for in the present study but none were detected. Indole derivatives do have very long retention times on the Carbowax 20-M stationary GLC phase used for the main part of this work, but analysis was also carried out by using a Silicone OV-3 column, searching in the area where tryptophan derivatives were expected based on the known retention data of skatole and indole and estimating values for adding –CHO and –OH groups. Every GLC peak in these areas was

examined by mass spectrometry. However, no tryptophan derivatives were found with either column. Additional studies are intended to further explore this question.

Other Hydrolyzed Protein Preparations. The commercial PIB-7 hydrolyzed protein bait is produced by using an acid hydrolysis. There are indications that enzyme-hydrolyzed protein baits are more effective as attractants. Mass spectrometry–capillary GLC analysis was also carried out on the vacuum steam volatile oil from an enzymatic hydrolyzed brewers yeast protein (Autolysate). This showed many similarities to the acid-hydrolyzed protein. Phenylacetaldehyde and acetic acid were again prominent volatiles. Methional was also present. There were much lower amounts of sugar degradation products and higher concentrations of aliphatic acids, particularly isobutyric, isovaleric, hexanoic, octanoic, and decanoic acids.

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Registry No. I, 21834-92-4; II, 85407-25-6; acetaldehyde, 75-07-0; 2-methylpropanol, 78-84-2; 2-methylbutanal, 96-17-3; 3-methylbutanal, 590-86-3; 2-methylbutanol, 137-32-6; 3-methylbutanol, 123-51-3; acetic acid, 64-19-7; butyric acid, 107-92-6; 3-methylbutyric acid, 503-74-2; pentanoic acid, 109-52-4; hexanoic acid, 142-62-1; levulinic acid, 123-76-2; methyl levulinate, 624-45-3; ethyl levulinate, 539-88-8; γ-pentalacetone, 108-29-2; γ-hexaloctone, 695-06-7; dimethyl sulfide, 75-18-3; methional, 3268-49-3; 3-phenylthiophene, 2404-87-7; benzaldehyde, 100-52-7; phenylacetaldehyde, 122-78-1; guaiacol, 90-05-1; 2-phenylethanol, 60-12-8; 2-phenyl-2-butenal, 4411-89-6; 4-methyl-2-phenyl-2-pentenal, 26643-91-4; 4-methyl-2-phenyl-2-hexenal, 26643-92-5; 4-vinylguaiacol, 7786-61-0; 2-pentylfuran, 3777-69-3; 2-methyl-3-oxotetrahydrofuran, 3188-00-9; furfural, 98-01-1; 5-methylfurfural, 620-02-0; 2-propionylfuran, 3194-15-8; 2-acetylfuran, 1192-62-7; furfuryl alcohol, 98-00-0; 2-methyl-5-propionylfuran, 10599-69-6; 3-phenylfuran, 13679-41-9; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-acetylpyrrole, 1072-83-9; *N*-methyl-2-formylpyrrole, 1192-58-1.

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Insecticide Inhibition of Aflatoxin Production in Corn

Frances A. Draughon,* Mohammad E. Elahi, and Dennis R. West

Inhibition of fungal growth and aflatoxin production by insecticides in culture medium and in field corn was investigated. Bux, carbofuran, carbaryl, fonofos, fensulphothion, EPN, heptachlor, and toxaphene were added to culture medium at levels of 0, 10, 50, and 100 ppm. Bux, naled, carbaryl, and fonofos at 100 ppm inhibited aflatoxin production by 97, 100, 55, and 64%, respectively. Bux, naled, and carbaryl were applied to *Asperigillus parasiticus* inoculated corn in the field at levels of 100 ppm biweekly. Corn inoculated but not treated with insecticide had a mean aflatoxin concentration of 126 ppb. Bux and carbaryl reduced aflatoxin in inoculated corn to 47 and 49 ppb, respectively. In uninoculated corn, carbaryl and Bux reduced aflatoxin from 27 ppb to less than 5 ppb. Application of naled to corn did not effectively reduce aflatoxin production.

Preharvest contamination of corn produced in the Southeastern United States is a serious agricultural problem, especially under adverse growing conditions. Contamination of preharvest corn by aflatoxins was not thought to be a problem until the 1970s (Shotwell, 1977). Aflatoxin contamiination of corn before harvest became a major agricultural problem in the southeastern states in 1977. Of the 198 million bushels of corn produced in the Southeastern United States in 1977, 56% were contaminated with aflatoxins at concentrations greater than the 20 ppb permitted by FDA in interstate commerce (CAST, 1979). These findings have resulted in much research on methods of decontamination and detoxification.

The use of antifungal agents such as propionic acid to control growth and mycotoxin production on grain during storage has shown promise (Vandegraft et al., 1975); however, little research has been performed on controlling fungal growth and toxin production on grain in the field.

Although certain pesticides are effective inhibitors of aflatoxin production in the laboratory (Hsieh, 1973; Dutton

Department of Food Technology and Science (F.A.D. and M.E.E.) and Department of Plant and Soil Science (D.R.W.), University of Tennessee, Knoxville, Tennessee 37901.

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