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Effect of pH on the Volatiles of Hydrolyzed Protein Insect Baits

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Four volatile concentrates were prepared from acidic corn protein hydrolysate and from basified hydrolysate, under atmospheric and vacuum conditions. Examination by capillary gas chromatography/mass spectrometry revealed that both the atmospheric and vacuum concentrates prepared from acidic hydrolysate were qualitatively very similar, with phenylacetaldehyde and several other aromatic oxygenated compounds predominating. In contrast, nitrogenous compounds were the major components of the two basic concentrates. These were primarily alkyl-substituted pyrazines in the atmospheric concentrate, but in the vacuum concentrate a group of 3-methylbutylamine-derived imines predominated. Some attraction was shown for all four concentrates in field bioassays with *Dacus dorsalis*, *Ceratitis capitata*, and *Dacus cucurbitae*, but with one exception (basified atmospheric concentrate vs *D. dorsalis*), none were as attractive as basified protein hydrolysate itself. No attractancy could be demonstrated for the four major imines.

Hydrolyzed protein products from various protein sources have been used as baits for certain insects (Steiner, 1952; Hagen et al., 1976; van Emden and Hagen, 1976; Miller and Haarer, 1981). Such insects, which include the green lace wing (*Chrysopa carnea*), the onion fly (*Hylemya antigua* Meigen), the seedcorn fly (*Hylemya platura* Meigen), and several fruit flies, including the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann), the oriental fruit fly (*Dacus dorsalis* Hendel), and several *Anastrepha* species, are thought to be attracted to these baits by the volatile compounds associated with the baits. Hagen et al. (1976) have proposed that these protein preparations are related in composition to the "honeydew" produced by aphids, which in nature can apparently supply a suitable diet for both the adult and larval stages of certain insects.

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The usefulness of hydrolysate baits prepared from corn gluten protein in large-scale programs to combat insect pests has been demonstrated in several Mediterranean fruit fly eradication projects. Suppression efforts in the 1980–1982 California Mediterranean fruit fly infestation included both aerial and ground spraying of commercial hydrolyzed corn protein bait Staley Protein Bait No. 7 (PIB-7) combined with malathion (Jackson and Lee, 1985).

Several papers reporting the identities of volatile compounds associated with PIB-7 have recently appeared. Morton and Bateman (1981) in Australia have identified 39 compounds in two different hydrolyzed protein preparations, a yeast hydrolysate (NBS) and PIB-7. Buttery et al. (1983) in this laboratory have reported the identities of some additional components. A second paper from this laboratory (Matsumoto et al., 1985) presented some preliminary results from the present study. Reports on the identification of volatiles from other hydrolyzed protein sources include those by Manley and Fagerson (1970a,b), Markh and Vinnikova (1973), and Withycombe et al. (1978). Bateman and Morton (1981) have also reported that raising the pH of their standard yeast protein hydrolysate mixture (NBS) significantly increased the attractiveness of their bait for the Queensland fruit fly

Table I. Synthesized Imines: Kovats Indices and Mass Spectra

imine	DB-1	DB-WAX	mass spectrum [m/z (relative intensity)]
N-ethylidene-2-methylbutylamine	790	957	56 (100.0), 98 (60.9), 57 (27.2), 41 (11.9), 43 (9.6), 42 (6.4), 55 (5.6), 44 (5.2), 39 (5.0), 99 (4.3), 70 (3.4), 84 (3.4), 112 (2.7), 54 (2.6), 71 (1.8)
N-ethylidene-3-methylbutylamine	792	974	98 (100.0), 56 (85.3), 57 (61.1), 43 (25.9), 41 (17.5), 42 (12.5), 70 (11.2), 55 (9.5), 44 (7.6), 71 (7.4), 99 (7.0), 39 (6.5), 112 (5.7), 58 (4.2), 54 (3.6)
N-(2-methylpropylidene)-3-methylbutylamine	927	1040	98 (100.0), 43 (43.2), 70 (30.0), 71 (29.6), 84 (26.3), 41 (14.6), 55 (14.2), 56 (11.0), 85 (10.5), 99 (7.7), 126 (6.4), 42 (5.7), 39 (5.3), 72 (3.0), 57 (2.5)
N-(2-methylbutylidene)-2-methylbutylamine	1026	1130	98 (100.0), 70 (32.1), 71 (27.1), 43 (25.9), 41 (19.6), 127 (16.0), 69 (15.4), 42 (13.8), 84 (12.1), 140 (9.5), 99 (8.3), 56 (7.3), 113 (6.5), 55 (5.1), 39 (5.0)
N-(2-methylbutylidene)-3-methylbutylamine	1025	1139	98 (100.0), 71 (38.1), 43 (37.2), 70 (28.5), 84 (25.0), 41 (19.1), 140 (16.3), 99 (10.7), 127 (9.0), 69 (8.7), 42 (8.6), 57 (7.4), 56 (6.7), 55 (6.3), 39 (4.7)
N-(3-methylbutylidene)-2-methylbutylamine	1033	1158	98 (100.0), 42 (50.0), 56 (38.7), 43 (25.9), 140 (22.3), 113 (20.2), 84 (19.8), 41 (19.1), 57 (16.1), 44 (14.9), 71 (8.4), 70 (7.8), 99 (7.7), 55 (6.6), 112 (6.0)
N-(3-methylbutylidene)-3-methylbutylamine	1032	1167	98 (100.0), 57 (51.9), 56 (33.8), 43 (33.6), 84 (29.9), 140 (28.2), 42 (20.7), 41 (18.0), 113 (15.0), 99 (9.5), 71 (9.5), 70 (9.1), 55 (7.1), 112 (5.9), 69 (4.4)

(*Dacus tyroni*). They associated this increase with the release of ammonia by the basified mixture. In a preliminary cooperative study with this laboratory, Landolt (1983) has seen similar results in Caribbean fruit fly (*Anastrepha suspensa*) bioassays with basified PIB-7, as have Gothilf (1984) and R.T.C. with the Mediterranean fruit fly. More recently, Mazor et al. (1987) examined the attractancies of ammonia solutions and of a number of protein-based baits for female Mediterranean fruit flies. They demonstrated that ammonia is indeed attractive to the female but that the increase in attractancy of protein-based baits at elevated pH is not solely attributable to corresponding increases in ammonia release. They concluded that other volatiles released on basification are also involved in the increased attractancy.

The present study was initiated to identify additional major volatiles from commerically available corn protein hydrolysate insect bait and to examine the changes in the volatiles profile that occur after basification of the protein hydrolysate.

EXPERIMENTAL SECTION

Materials. The hydrolyzed protein used was Nu-Lure Insect Bait (NLIB; Miller Chemical and Fertilizer Corp., Hanover, PA). This is identical with the PIB-7 (Staley Protein Bait No. 7) used in the previous study from this laboratory (Buttery et al., 1983). PIB-7 is manufactured by A. E. Staley Manufacturing Co., Decatur, IL, but is now marketed by Miller as Nu-Lure Insect Bait. The pH of the material as received was 4.3.

Authentic chemical compounds were obtained from reliable commercial sources or were synthesized by established methods. Among the synthesized compounds were a number of aliphatic imines, prepared by direct combination of the appropriate aldehyde and primary amine, in the presence of anhydrous sodium sulfate. They are listed in Table I with their mass spectra and Kovats index values. The experimental conditions used in determining these values are described below.

Concentrate Preparation. *Nonbasified NLIB: Vacuum Conditions.* In a typical preparation, 5.0 L (6.19 kg) of NLIB, 1.8 L of distilled water, and 50 mL of a silicone antifoam/distilled water mixture [9 g of Harwick Antifoam 60 (Harwick Chemical Co., Akron, OH) plus 400 mL of distilled water, boiled down to ca. 100 mL of mixture] were combined in a 12-L round-bottomed flask fitted with heating mantle, glass/Teflon stirrer, and modified Likens and Nickerson extraction head. Chilled antifreeze solution at 0 °C was circulated through the extraction head condensers. A Dewar-type condensor containing solid carbon dioxide/2-propanol was mounted at the vacuum port of the extraction head. Hexane (100 mL; Burdick & Jackson) was used as extracting solvent. At an operating pressure of 100 mm, the maximum sample pot temperature was 58.6 °C. After a 3-h period of distillation/extraction, the flask containing the hexane extract was placed in a freezer overnight. The colorless hexane solution was decanted from a few frozen water droplets, and then the volume was adjusted to 100 mL with hexane. To determine the extraction yield, a 5-mL portion of this solution was concentrated by distilling the hexane. The residue from 5 mL of solution weighed 2.9 mg; therefore, the total yield from 6.19 kg of NLIB was approximately 58 mg (9 ppm yield).

Nonbasified NLIB: Atmospheric Pressure. The same apparatus as that described above was used at atmospheric pressure, with the following changes: The Dewar condenser at the exit port was removed, and the extraction head was cooled with tap water, rather than refrigerant. The same quantities of NLIB, water, antifoam, and hexane were used, and the distillation/extraction was again run for 3 h (maximum temperature of NLIB/water mixture 107 °C). The pale yellow hexane solution was placed in the freezer overnight, and then the volume was adjusted to 100 mL as above. Residue from 5 mL was 6.4 mg; the total amount of extracted material was 128 mg (21 ppm yield).

Basified NLIB: Vacuum Conditions. A 5-L quantity of NLIB (6.19 kg) was basified to pH 8.7 with concentrated aqueous potassium hydroxide solution (7.2 mol of KOH). The basified material was stored at room temperature for 3 days, and then a concentrate was prepared with the same apparatus and under the same conditions as described above for the unbasified starting material. The volume was adjusted to 100 mL, and then a 5-mL portion was stripped, leaving 2.1 mg; total extract was then approximately 42 mg (7 ppm yield).

Basified NLIB: Atmospheric Pressure. A 5-L quantity of NLIB was basified as above and then was stored at room temperature overnight. The hexane extract was prepared in a manner identical with that employed with the nonbasified NLIB at atmospheric pressure. Of the 100-mL solution, 5 mL was stripped, leaving 39.9 mg of residue. The total weight of extracted material was then 798 mg (129 ppm yield).

Component Separation, Identification, and Quantitation. Hewlett-Packard 5830A and 5840A gas chromatographs fitted with flame ionization detectors (FID) and a Finnigan MAT 4500 quadrupole gas chromatograph/mass spectrometer (GC/MS) were used to separate the volatiles concentrates. Identical cross-linked bonded methyl silicone columns (DB-1; 60 m × 0.32 mm (i.d.), 0.25-μm film; J&W Scientific, Inc., Folsom, CA) were installed in the FID units and in the GC/MS. A DB-WAX column (identical dimensions and source) was used on occasion to separate components whose GC peaks overlapped on the DB-1 phase. The columns were operated at constant head pressure (23 psi, FID; 14 psi, GC/MS) and were programmed from 50 to 250 °C (230 °C maximum for the DB-WAX) at 4 °C/min. Split injectors (25/1 split ratio) were installed on all instruments.

Components were tentatively identified in most instances by mass spectrum matching with a mass spectral library collection, using the Finnigan MAT Incos data system. The reference library is basically the NIH–EPA collection, supplemented by 1400–1500 additional spectra from other collections and from previous work in our laboratory. When no suitable reference spectra were available, samples of suspected compounds were synthesized. Tentative identifications were verified by comparison of a component's experimental Kovats index (KI) value on methyl silicone (DB-1) with that determined under identical GC conditions with an authentic sample. A homologous series of normal hydrocarbons (C_6–C_{20}) was coinjected with each of the four volatiles concentrates in separate runs; experimental KI values were based upon the resulting chromatographic data.

Area percent integration values from the H/P 5800 series GCs were used without correction (all response factors 1). GC/FID

and GC/MS chromatograms were correlated by inspection and by matching experimental KI values.

Bioassay. *NLIB Concentrates.* The concentrates were field-tested by R.T.C. in Waiakea, HI, in a series of four tests of ten replicates each. The test solutions (0.1 mL of the respective 100-mL stock solution; 0.01 mL represents the volatiles from 6.19 g (5.0 mL) of NLIB starting material) were pipetted directly onto wicks in standard Jackson sticky traps placed in a randomized complete block design. They were compared with 0.1 mL of neat NLIB (basified to pH 8.5 with concentrated aqueous NaOH) against both males and females of three fruit fly species, *D. dorsalis* (Oriental), *C. capitata* (Mediterranean), and *Dacus cucurbitae* (Melon). Each test was terminated after 2 days.

3-Methylbutylimines. Four imines (*N*-ethylidene-, *N*-(2-methylpropylidene)-, *N*-(2-methylbutylidene)-, and *N*-(3-methylbutylidene)-3-methylbutylamine) were tested (R.T.C.) in an outdoor rotating laboratory olfactometer in Honolulu, HI. Neat samples were pipetted onto wicks in Jackson traps and were compared against water blanks. The same test was run on two occasions with two traps of each sample on each run. On the second run the dose of each sample was increased 10-fold. Four fruit fly species (males and females) were employed: *D. dorsalis*, *C. capitata*, *D. cucurbitae*, and *Dacus latifrons* (Malaysian).

RESULTS AND DISCUSSION

The study results are summarized in Tables II–IV. Table II lists the compounds identified in the two concentrates prepared at atmospheric pressure. Table III is a corresponding listing of the vacuum concentrate components. Quantitative data in both tables are in parts per million. Quantitation values are not included for several short retention time components; the corresponding GC/FID peaks overlapped considerably, and area percent values could not be reliably extracted from the data. These are so indicated in the yield columns.

Comments about yields are only valid relative to the 3-h distillation period used in each of the concentrate preparation sequences. The NLIB/water mixture is a dynamic system, and additional volatile material appears to be generated during the distillation process, especially at elevated temperatures. In earlier efforts by K.E.M. and by Teranishi (1984) to exhaustively deplete volatile components from protein hydrolysate samples under various experimental conditions, they found that additional volatile material could be obtained indefinitely, as the distillation time period was extended. In addition, under the experimental conditions employed in the present study, transfer of organic volatiles from the sample pot to distillate is slower under vacuum than at atmospheric pressure. The lower yields of volatiles under vacuum conditions (9 and 7 ppm vs 21 and 129 ppm at 1 atm) are attributed to this and to the lower sample pot temperature (bp = 58–59 °C) under vacuum.

Several entries in each table are only tentative identifications; mass spectral evidence was not supported by comparison of experimental and reference Kovats index values, either because authentic samples were not available or because difficulties in calculating accurate experimental KI values near the front of the gas chromatographic runs were encountered. Such tentative identifications are enclosed in parentheses.

Components previously reported by other workers are indicated in the tables with superscript letters.

Atmospheric Pressure Concentrates. When the two concentrates prepared at atmospheric pressure and elevated temperature were compared, alkylpyrazines were found to predominate under basic conditions. The 2,5- and 2,6-dimethyl compounds were especially prominent. Mass spectral evidence from the basic concentrate indicates the presence of several additional pyrazines in significant concentrations, including methylvinyl- and diethylmethylpyrazines. Assignment of substituent positions and verification of tentative identifications were not possible because authentic samples were not available. Quantities of several imines, or Schiff bases, were also found. These are readily formed by addition of primary amines to aliphatic aldehydes. A number of minor components remain unidentified; the seven largest of these collectively total ca. 22 ppm of the total concentrate.

In marked contrast with the basic concentrate, the preparation at pH 4.3 afforded relatively little nitrogen-containing volatile material. Only traces of several pyrazines were detected, and no imines were found. Nearly all components present at significant levels were identified. The major components were phenylacetaldehyde, followed by 4-vinylguaiacol, furfural, 5-methylfurfural, benz-

Table II. Atmospheric Pressure Steam Distillates

component	basic ref KI	basic exptl KI	basic yield, ppm	acidic exptl KI	acidic yield, ppm
(methanethiol)[a,d]		nd[b]	nr[c]	nd	nr
(dimethyl sulfide)[d,e]	508	nd	nr	nd	nr
(2-methylpropanal)[d,e]	531	nd	nr	nd	nr
(3-methylbutanal)[d,e]	627	nd	nr	nd	nr
(2-methylbutanal)[d,e]	637	nd	nr	nd	nr
dimethyl disulfide[d]	722	724	0.87	725	0.08
toluene	748	753	0.13		
2-methyl-3-oxotetrahydrofuran[e]	770	774	0.08	771	0.16
N-ethylidene-3-methylbutylamine	792	797	0.35		
methylpyrazine	795	802	4.46	798	tr[f]
furfural[e]	799			800	1.08
2,4,5-trimethyloxazole	822	827	0.03		
furfuryl alcohol[e]	826	829	tr	825	tr
5-methylhexan-2-one	832	835	tr		
3-(methylthio)propanal (methional)[d,e]	861			862	0.36
2-acetylfuran[d,e]	876	878	0.15	878	0.41
2,5-dimethylpyrazine[e,g]	882	884	22.03	888	0.07
2,6-dimethylpyrazine[e,g]	882	884	28.47	888	0.09
ethylpyrazine	886	889	1.34		
2,3-dimethylpyrazine	889	892	1.80		
vinylpyrazine	901	900	0.07		
5-methylfurfural[e,g]	926			926	0.75
benzaldehyde[d,e,g]	925	925	0.83	927	0.58
dimethyl trisulfide[d]	940	941	0.08	941	0.06
2-ethyl-6-methylpyrazine[e]	969	971	3.78	971	0.04
2-ethyl-5-methylpyrazine[g]	973	974	1.75	975	0.02
trimethylpyrazine[g]	974	975	6.44		
2-ethyl-3-methylpyrazine[e]	977	978	0.70		
2-pentylfuran[e]	977			978	0.03
2-acetylpyridine	998	998	0.08		
phenylacetaldehyde[d,e]	1006			1006	10.39
2-acetylpyrrole[d,e]	1023	1022	0.04	1024	0.28
N-(2-methylbutylidene)-3-methylbutylamine	1025	1026	0.20		
N-(3-methylbutylidene)-3-methylbutylamine	1032	1033	1.42		
2,5-dimethyl-3-ethylpyrazine	1053	1055	2.48		
2-methoxyphenol (guaiacol)[d,e]	1057	1059	0.47	1058	0.17
2,5-diethylpyrazine	1063	1062	0.49		
1-phenylpropan-2-one	1090	1093	0.30	1092	0.11
(3-phenylfuran)[e]		1193	0.08	1193	0.15
2-phenylbut-2-enal[e]	1233	1233	0.08	1233	0.39
4-vinylguaiacol[e]	1280	1280	1.06	1280	1.34
5-methyl-2-phenylhex-2-enal[e]	1456			1456	0.19

[a] Components in parentheses only tentatively identified, by mass spectrum matching. [b] nd = not determined; fronts of GC/FID and GC/MS runs too compressed for reliable KI determination. [c] nr = not resolved; multiple GC/FID peak overlap at front precluded area measurements. [d] Previously found by Morton and Bateman (1981) in corn protein hydrolysate. [e] Previously found by Buttery et al. (1983) in corn protein hydrolysate. [f] tr = 0.005 ppm or less. [g] Relative amounts of coeluting compounds determined on a DB-Wax column.

Table III. Vacuum Steam Distillates

component	basic			acidic	
	ref KI	exptl KI	yield, ppm	exptl KI	yield, ppm
(dimethyl sulfide)[a,d,e]	508	nd[b]	nr[c]	nd	nr
(2-methylpropanal)[d,e]	531	nd	nr	nd	nr
(3-methylbutanal)[d,e]	627	nd	nr	nd	nr
dimethyl disulfide	722	722	0.02	723	0.01
toluene	748	748	tr[f]	749	tr
1-methylpiperidine	750	749	tr		
N-ethylidene-3-methylbutylamine	792	790	0.14		
methylpyrazine	795	803	tr	803	tr
furfural[e]	799			804	tr
2,4,5-trimethyloxazole	822			823	0.01
furfuryl alcohol[e]	826	825	tr	825	0.01
5-methylhexan-2-one	832	832	0.01	832	0.01
2,6-dimethylpyridine	857	857	0.01		
3-(methylthio)propanal (methional)[d,e]	861			862	0.06
heptan-2-one	864	865	tr	865	0.01
2-acetylfuran[d,e]	876	877	0.13	876	0.44
2,5-dimethylpyrazine[e,g]	882	883	0.12	885	0.03
2,6-dimethylpyrazine[e,g]	882	883	0.10	885	0.07
ethylpyrazine	886	890	0.01	889	0.01
2,3-dimethylpyrazine	889	893	0.01	892	0.01
5-methylfurfural[g]	926			926	0.15
benzaldehyde[d,e,g]	925	925	0.03	926	0.15
N-(2-methylpropylidene)-3-methylbutylamine	927	927	0.46		
dimethyl trisulfide[d]	940	940	tr	941	0.02
methyl 4-oxopentanoate (levulinate)[e]	946			948	0.04
hexanoic acid[d,e]	970			961	0.10
2-furfuryl acetate	962	962	0.02	964	0.04
2-ethyl-6-methylpyrazine[e]	969	970	0.08	971	0.17
2-ethyl-5-methylpyrazine[g]	973	974	0.04	975	0.03
trimethylpyrazine[g]	974	975	0.09	976	0.03
2-furanylpropan-1-one[e,g]	976	978	0.01	976	0.08
2-ethyl-3-methylpyrazine[e,g]	977	981	tr	977	tr
2-pentylfuran[e,g]	977			977	tr
ethyl 2-amino-3-methylbutyrate (valine)	992	992	0.01		
phenylacetaldehyde[d,e]	1006			1007	2.05
2-acetylpyrrole[d,e]	1023	1021	0.02	1023	0.16
ethyl 4-oxopentanoate (levulinate)[e]	1023			1024	0.04
N-(2-methylbutylidene)-2-methylbutylamine	1025	1025	0.83		
acetophenone	1030	1031	0.01	1031	0.09
N-(3-methylbutylidene)-3-methylbutylamine	1032	1033	1.82		
acetylthiophene	1049	1043	0.01	1041	0.01
2,5-dimethyl-3-ethylpyrazine	1053	1055	0.04	1054	0.01
linalool oxide A (*trans*-tetrahydrofuranyl)	1056	1056	0.02		
2-methoxyphenol (guaiacol)[d,e]	1057	1058	0.01	1057	0.44
2-phenylethanol[e]	1080	1079	0.01	1081	0.01
3,5,5-trimethylcyclohex-2-enone	1088	1088	0.02	1088	0.04
ethyl 2-amino-4-methylpentanoate (leucine)	1090	1090	0.02		
1-phenylpropan-2-one	1090	1091	0.38	1091	0.46
(2-methyl-5-propionylfuran)[e]		1098	0.04	1098	0.13
methyl phenylacetate	1144	1144	tr		
(3-phenylfuran)[e]		1194	0.01	1193	0.05
quinoline	1200	1199	tr		
4-phenylbutan-2-one	1205	1206	0.01		
2-phenylbut-2-enal[e]	1233	1234	0.02	1232	0.05
4-vinylguaiacol[e]	1280			1280	0.34
(2-methyl-4*H*-1-benzopyran-4-one)		1318	0.01		
4-methyl-2-phenylpent-2-enal[e]	1341			1341	0.06
(3-phenylthiophene)[e]		1377	0.01	1377	0.03
4-methyl-2-phenylhex-2-enal[e]	1433	1434	tr	1433	0.07
5-methyl-2-phenylhex-2-enal[e]	14556	1456	0.02	1456	0.12

[a] Components in parentheses only tentatively identified, by mass spectrum matching. [b] nd = not determined; fronts of GC/FID and GC/MS runs too compressed for reliable KI determination. [c] nr = not resolved; multiple GC/FID peak overlap at front precluded area measurements. [d] Previously found by Morton and Bateman (1981) in corn protein hydrolysate. [e] Previously found by Buttery et al. (1983) in corn protein hydrolysate. [f] tr = 0.005 ppm or less. [g] Relative amounts of coeluting compounds determined on a DB-Wax column.

aldehyde, and acetylfuran. Such compounds were also found in methylene chloride extracts of similar commercial hydrolysates by Morton and Bateman (1981) and in vacuum steam distillate extracts by Buttery et al. (1983).

Vacuum Concentrates. The concentrate prepared under vacuum at pH 8.7 contained essentially the same series of alkylpyrazines found in the atmospheric basic concentrate. Again, evidence for methylvinyl- and diethylmethylpyrazines were found. In addition, several isopropylmethylpyrazines appeared to be present. However, a group of imines predominated. All appear to be reaction products of 3-methylbutylamine and various short-chain aldehydes. The 3-methylbutanal imine was the major component, followed by the 2-methylbutanal, 2-methylpropanal, and acetaldehyde adducts. The major oxygenated components of the basic vacuum concentrate were 1-phenylpropan-2-one and acetylfuran, both of which were common to all four concentrates. Nearly all com-

Table IV. Concentrate Bioassay[a]

sample	D. dorsalis	C. capitata	D. cucurbitae
basified Nu-Lure (pH 8.5)	1290	847	1034
atmospheric concentrate, acidic Nu-Lure (13 μg/0.1 mL)	340	166	293
atmospheric concentrate, basified Nu-Lure (80 μg/0.1 mL)	1633	286	451
vacuum concentrate, acidic Nu-Lure (6 μg/0.1 mL)	492	224	248
vacuum concentrate, basified Nu-Lure (4 μg/0.1 mL)	586	167	268

[a] Total fly catches, male and female; four field tests of ten replicates each; Jackson sticky traps with No. 2 wicks; 0.1 mL of each sample.

ponents present at significant concentrations were identified, either fully or tentatively.

The vacuum concentrate prepared at pH 4.3 was similar to the pH 4.3 atmospheric concentrate. Phenylacetaldehyde was again the major volatile, followed by 1-phenylpropan-2-one, guaiacol, 4-vinylguaiacol, and acetylfuran. Very little furfural appeared in the vacuum sample, in contrast with the atmospheric concentrate.

Total concentrate yields were greater from preparations run at atmospheric pressure than at 100 mm; this difference was much more pronounced under basic conditions than at pH 4.3 (ca. 19×; ca. 2× at pH 4.3). Notably, the yields of individual alkylpyrazines at pH 8.7 were much higher at 760 mm/107 °C than at 100 mm/58 °C, by factors ranging from ca. 170 to 3900×. This presumably indicates that formation of volatile alkylpyrazines, the major components of the atmospheric basic concentrate, is favored at the higher temperature.

Most of the components identified by Buttery et al. (1983) in a vacuum steam distillate extract of acidic protein hydrolysate were found in the corresponding vacuum concentrate in this study. A number of lower molecular weight polar compounds reported by Buttery were not found by us. This is likely due to the choices of extracting solvent; Buttery et al. employed methyl *tert*-butyl ether, while we used hexane. The ether would more effectively extract very polar components from the steam distillate.

Morton and Bateman (1981) prepared a direct methylene chloride extract of Staley's No. 7 hydrolysate (PIB-7). They also examined the most volatile components of PIB-7 by a headspace trapping procedure. Most of the major compounds reported by them were also found in the present study. Again, some of the most polar constituents listed in this earlier paper were not found by us. Methylene chloride extracts hydroxy compounds and lower free acids more efficiently than does hexane. Morton and Bateman reported the presence of several γ- and δ-lactones that we were unable to detect. The high water solubility and relatively low vapor pressure of individual lactones make a steam distillation process rather inefficient for their concentration.

Bioassay Results. NLIB Concentrates. Results from the field bioassays of the four concentrates are presented in Table IV. The concentrate prepared from basified NLIB at atmospheric pressure was most attractive of the four concentrates to each of the fruit fly species tested. However, with the exception of *D. dorsalis*, all species were attracted more strongly to the control (basified NLIB) than to the concentrates. Comparing responses to the four concentrates, the basic atmospheric concentrate is most attractive of the four to all three fly species. This concentrate also represents the highest material yield of the four (acidic/atmospheric, 21 ppm; basic/atmospheric, 129 ppm; acidic/vacuum, 9 ppm; basic/vacuum, 7 ppm).

3-Methylbutylimines. Test results indicate that, when tested as neat samples against water, none of the four test imines is significantly more attractive than the water blank to the four fruit fly species.

In the bioassays of the four NLIB concentrates (Table IV), the 0.1-mL trap loading represents the volatiles collected from a 5-mL portion of the respective NLIB starting batch. However, in nearly every test comparison the reference sample (0.1 mL of basified NLIB) attracted more flies than did any of the concentrate samples. This could well reflect the absence of any ammonia in the four concentrates. The volatiles concentration process does not retain any free ammonia that might have been released by the starting NLIB (acidic or basified). Since several groups have shown that ammonia release is an important factor in the attractiveness of protein hydrolysates, the greater response to the basified NLIB reference sample might be due in part to this difference. In addition, highly volatile organic components (lower amines, aldehydes, etc.) would be lost or poorly collected during the concentration procedures employed, especially under vacuum. If any are active, they might provide additional attractancy to the basified NLIB but would likely be absent from the test samples.

Attempts to relate catches with each volatiles concentrate (Table IV) to the presence of specific components in the respective concentrate (Tables II and III) were not particularly successful. Since the imines, at least when neat, do not appear to be attractive to the fruit flies tested, the major remaining features of the two basic concentrates are the presence of an assortment of pyrazines. However, such pyrazines either are absent or are present at trace to near-trace levels inm the two concentrates from acidic NLIB. The same (non-ammonia) component(s) need not contribute to the attractancy of both acidic and basified NLIB volatiles concentrates, but the search for active components would be simplified if this were the case.

Future plans include bioassay of individual concentrate components and headspace GC/MS examination of both acidic and basified NLIB, to examine the possibility that other more volatile compounds are released by the material.

Registry No. *N*-Ethylidene-2-methylbutylamine, 120144-55-0; *N*-ethylidene-3-methylbutylamine, 120144-56-1; *N*-(2-methylpropylidene)-3-methylbutylamine, 41807-57-2; *N*-(2-methylbutylidene)-2-methylbutylamine, 54518-97-7; *N*-(2-methylbutylidene)-3-methylbutylamine, 120144-57-2; *N*-(3-methylbutylidene)-2-methylbutylamine, 120144-58-3; *N*-(3-methylbutylidene)-3-methylbutylamine, 35448-31-8; methanethiol, 74-93-1; dimethyl sulfide, 75-18-3; 2-methylpropanal, 78-84-2; 3-methylbutanal, 590-86-3; 2-methylbutanal, 96-17-3; dimethyl disulfide, 624-92-0; toluene, 108-88-3; 2-methyl-3-oxotetrahydrofuran, 3188-00-9; methylpyrazine, 109-08-0; furfural, 98-01-1; 2,4,5-trimethyloxazole, 20662-84-4; furfuryl alcohol, 98-00-0; 5-methylhexan-2-one, 110-12-3; 3-(methylthio)propanal, 3268-49-3; 2-acetylfuran, 1192-62-7; 2,5-dimethylpyrazine, 123-32-0; 2,6-dimethylpyrazine, 108-50-9; ethylpyrazine, 13925-00-3; 2,3-dimethylpyrazine, 5910-89-4; vinylpyrazine, 4177-16-6; 5-methylfurfural, 620-02-0; benzaldehyde, 100-52-7; dimethyl trisulfide, 3658-80-8; 2-ethyl-6-methylpyrazine, 13925-03-6; 2-ethyl-5-methylpyrazine, 13360-64-0; trimethylpyrazine, 14667-55-1; 2-ethyl-3-methylpyrazine, 15707-23-0; 2-pentylfuran, 3777-69-3; 2-acetylpyridine, 1122-62-9; phenylacetaldehyde, 122-78-1; 2-acetylpyrrole, 1072-83-9; 2,5-dimethyl-3-ethylpyrazine, 13360-65-1; guaicol, 90-05-1; 2,5-diethylpyrazine, 13238-84-1; 1-phenylpropan-2-one, 103-79-7; 3-phenylfuran, 13679-41-9; 2-phenylbut-2-enal, 4411-89-6; 4-vinylguaiacol, 7786-61-0; 5-methyl-2-phenylhex-2-enal, 21834-92-4; 1-methylpiperidine, 626-67-5; 2,6-dimethylpyridine, 108-48-5; heptan-2-one, 110-43-0; methyl 4-

oxopentanoate, 624-45-3; hexanoic acid, 142-62-1; 2-furfuryl acetate, 623-17-6; 2-furanylpropan-1-one, 3194-15-8; ethyl 2-amino-3-methylbutyrate, 17431-03-7; ethyl 4-oxopentanoate, 539-88-8; acetophenone, 98-86-2; acetylthiophene, 39709-34-7; linalool oxide A, 34995-77-2; 2-phenylethanol, 60-12-8; 3,5,5-trimethylcyclohex-2-enone, 78-59-1; ethyl 2-amino-4-methylpentanoate, 2743-60-4; 2-methyl-5-propionylfuran, 10599-69-6; methyl phenylacetate, 101-41-7; quinoline, 91-22-5; 4-phenylbutan-2-one, 2550-26-7; 2-methyl-4*H*-1-benzopyran-4-one, 5751-48-4; 4-methyl-2-phenylpent-2-enal, 26643-91-4; 3-phenylthiophene, 2404-87-7; 4-methyl-2-phenylhex-2-enal, 26643-92-5.

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Depletion of [^{14}C]Clorsulon in Cows' Milk

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Radioactive residue levels were determined in milk and plasma of Holstein cows dosed once with an oral suspension of ^{14}C-labeled clorsulon at 7 mg/kg of body weight. Average milk residue levels decreased from 0.54 ppm at 0.9 day postdose to 0.004 ppm at 6.9 days postdose with a half-life of 0.81 day. The drug residue was isolated by a batch adsorption method on an affinity agarose gel of carbonic anhydrase–Sepharose 4B and analyzed by HPLC–reverse isotope dilution assay (RIDA). This novel method greatly simplified the extraction of drug residue from milk and provided pure drug residue isolates. RIDA results of the isolates indicated that the unchanged drug was the major residue component in milk at 0–4 days postdose accounting for 56–99% of the total radioactive residue. Depletion half-life of the parent drug was 0.82 day, in close agreement with that of the total residue in milk. About 0.7% of the dose was recovered in the milk during the 6.9-day period.

Clorsulon [MK-401, 4-amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide] (Figure 1) is a potent fasciolicide, effective against mature and immature *Fascila hepatica* in cattle and sheep (Mrozik, 1976; Mrozik et al., 1977; Ostlind et al., 1977). The drug appears to be extremely safe, since no gross toxicosis was observed in sheep after intraruminal doses as high as 400 mg/kg of body weight. The minimum effective dose for the removal of 14-week-old flukes from beef calves was ≤2 mg/kg parenterally (Wyckoff and Bradley, 1983). In vitro studies indicate that the drug acts by blocking the glycolytic pathway in the flukes, by direct inhibition of 3-phosphoglycerate kinase and phosphoglyceromutase (Schulman and Valentino, 1980). Schulman et al. (1979, 1982) performed pharmacokinetic studies in rats showing the drug is well-absorbed after oral administration. A single oral dose of clorsulon (6.25 or 12.5 mg/kg) produces peak blood concentrations about 4 h after dosing with 75% of the drug found in plasma and the rest bound to carbonic anhydrase in

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