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Characterization of Meat and Bone Meal Flavor Volatiles

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The vacuum distillate of meat and bone meal (MBM), a pet food ingredient, has been analyzed by WCOT glass capillary gas-liquid chromatography-mass spectrometry. A total of 64 components were identified in this volatile oil. The major volatile identified was 1-hexanal (43% relative concentration). Other major volatiles identified in decreasing order of relative concentration are 1-heptanal, 1-octanal, 3-octen-2-one, 1-nonanal, 1-pentanal, 3,5-octadien-2-one (two isomers), 1-octen-3-ol, and 2-(*n*-pentyl)furan. Other important flavor compounds identified include *trans*-2-alkenals, *trans,trans*-2,4-alkadienals, and several pyrazines. These compounds contributed to the notes characteristic of MBM which may play a role in the acceptance of MBM-containing pet foods by pet and pet owner.

Meat and bone meal (MBM) is a relatively inexpensive protein source that is used extensively in animal feed (meals for poultry and pigs) and pet foods. The rendering industry prepares MBM from viscera, bone scraps, and fallen and unsound animals from the livestock dressing processing. The processing and utilization of MBM have been described by Herbert et al. (1974). The application of meat and bone meal as a food protein concentrate for human use has been described by Nash and Mathews (1971).

The composition of MBM has been studied by Doty (1969) and Nash and Mathews (1971). These authors found MBM to contain 54-59% protein, 10-12% crude fat, 28-29% ash, and 3-6% moisture. Amino acid composition studies indicated that glutamic acid and glycine were the most abundant amino acids. The least abundant amino acids were the sulfur-containing amino acids cystine and methionine.

Few studies exist in the literature that describe the flavor chemistry of meat byproducts which are utilized in pet foods. A recent study by Greenberg (1981) described the flavor volatiles found in poultry byproduct meal (PBPM), a meat meal used in dog and cat foods. The lack of literature reports on meat byproduct volatiles is in contrast to the enormous volume of literature on the flavor chemistry of prime meats used primarily in human consumption. The flavor chemistry of prime meats has been

reviewed recently by Wasserman (1979) and Shibamoto (1980).

It is the objective of this study to identify key flavor components in meat and bone meal (MBM). A discussion of how these MBM volatiles may be formed and how they differ from volatiles of prime meats and meat byproducts such as PBPM will also be presented.

EXPERIMENTAL SECTION

Materials. Meat and bone meal was obtained from a major supplier, Badger By-Products Co., Milwaukee, WI. The meal was refrigerated and, within 1 week after purchase, subjected to flavor isolation. The MBM was used "as is" during the isolation procedures.

Authentic chemical reference compounds were obtained from commercial sources (e.g., Aldrich Chemical Co. and Alpha Chemical Co.).

Isolation of the Volatile Flavor: Vacuum Degassing. The volatile flavor components were isolated from a total of 28 lb of meat and bone meal by using a vacuum degassing technique.

Each isolation involved degassing 1600 g of meat and bone meal at room temperature with a pressure of 0.02 mmHg and collecting the volatiles in a series of coiled traps such as those described by Chang et al. (1977) which were immersed in dry ice-2-propanol. At the end of 6 h the volatiles were taken up in redistilled diethyl ether. The ether was dried over anhydrous sodium sulfate, concentrated to 0.5 mL by using a Kuderna-Danish concentrator, and concentrated to a final volume of 0.3 mL under a nitrogen stream.

Capillary GLC-Mass Spectral (GLC-MS) Analysis.

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The two GLC columns used were 50 m × 0.50 mm i.d. Pyrex WCOT glass capillaries coated with SE-30 or OV-225. A number of different GLC-MS runs were made with the two columns using a Hewlett-Packard 5840A gas chromatograph.

The main temperature programming conditions (those used for the chromatogram in Figure 1 with the SE-30 column) were to hold the capillary at 40 °C for 3 min after injection and then to program linearly from 40 to 170 °C at 3 °C/min and hold at the upper limit. The carrier gas (helium) had a linear velocity of 29 cm/s with a head pressure of 8.0 psi. The capillary inlet system was in the split mode with a split ratio of 1.5:1.

The glass capillary column effluent was split in half by a platinum-iridium splitter to a flame ionization detector and a Model 5982 Hewlett-Packard quadrupole mass spectrometer via a platinum-iridium transfer line. A mass spectrometer source temperature of 180 °C and an ionizing potential of 70 eV were used. Mass spectral identifications were confirmed by computer-assisted spectra matching of authentic standards or literature spectra. The calculation of Kovats retention indexes was compared to those of standards to further confirm mass spectral data.

RESULTS AND DISCUSSION

The volatile oil obtained from the meat and bone meal (MBM) by vacuum degassing amounted to 130 parts per million (ppm) of the meat and bone meal. The essence has an odor very similar to that of the intact MBM which can be described as having green, fatty, fried fatty, hay, straw, and cardboard-like notes.

The results of the analysis of the MBM essence by capillary GC-MS are presented in Table I. Each compound in Table I is identified by a peak number pertaining to the indicated peak in Figure 1, the SE-30 capillary gas chromatogram of the volatile oil. The relative concentration of the components in the volatile oil is presented in the last column of Table I. These are based on a single measurement of peak areas of a typical essence.

Compounds presented in Table I had mass spectra (see paragraph at end of paper regarding supplementary material) and Kovats retention indexes (K_1) consistent with those of authentic standards. Compounds for which standards were not available were tentatively identified by comparing their mass spectrum with literature mass spectral data. The mass spectra of 2-(*n*-heptyl)- and 2-(*n*-octyl)furan were consistent with those published by Jennings and Shibamoto (1980). The mass spectrum of 3,5-nonadien-2-one was consistent with that published by Tressl et al. (1977). These three compounds have measured K_1 values which are consistent with their homologues. The mass spectrum of 3-nonyne was consistent with that published in "Eight Peak Index of Mass Spectra" (1974).

Peak no. 60 and 62 were tentatively identified by their mass spectra to be two isomers of 3,5-undecadien-2-one. Mass spectra or K_1 values for 3,5-undecadien-2-one have not been reported in the literature. Badings (1970) has identified the 2,4-dinitrophenylhydrazone of this compound by infrared spectroscopy. Mass spectral data (above m/e 26, intensities are in parentheses with the base peak taken as 100) were as follows: for 3,5-undecadien-2-one ($K_1 = 1344$), molecular ion 166 (13) and major ions 95 (100), 81 (58), 43 (45), 67 (21), 41 (12), 55 (12), 109 (4), and 151 (3); for 3,5-undecadien-2-one ($K_1 = 1379$), molecular ion 166 (10) and major ions 95 (100), 43 (33), 81 (30), 67 (14), 41 (12), 55 (12), 109 (3), and 151 (2).

The spectra of these two compounds are similar to the other 3,5-dien-2-ones reported. They have major ions at

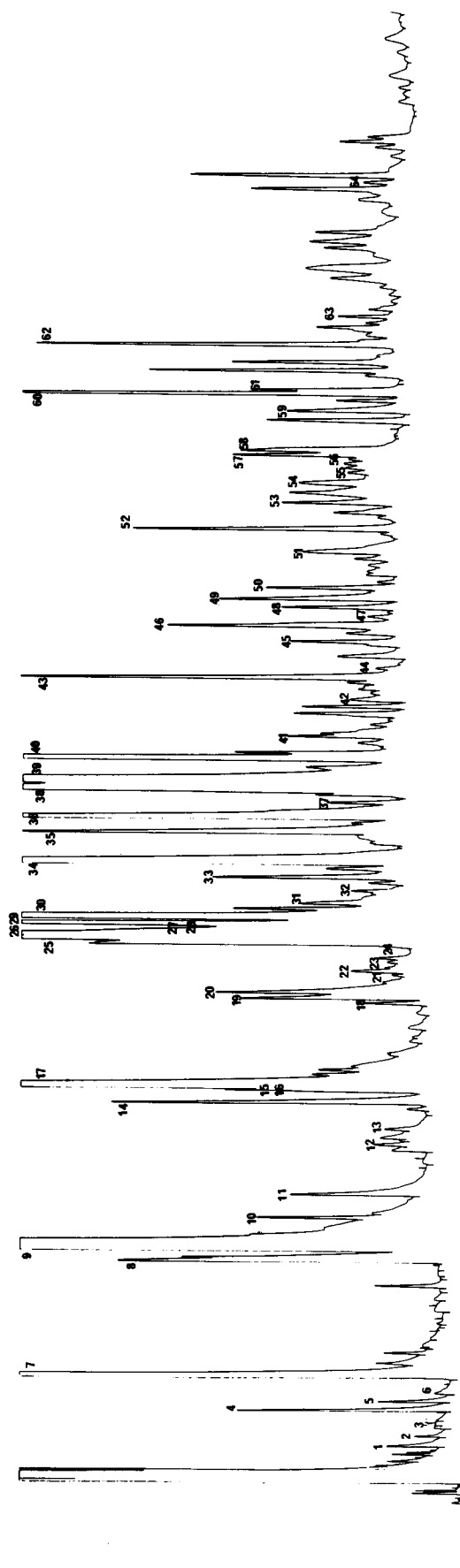


Figure 1. Capillary GLC analysis of the volatile oil of meat and bone meal (MBM). For GLC conditions, see the text. The identity of the numbered peaks is shown in Table I.

Table I. Compounds Identified in Volatile Oil of MBM

peak no.	compd ^a	characteristic mass spectral ions ^b	Kovats GLC index ^c (K_I)		rel concn, %
			measd	std	
Alkanals					
1	1-butanal	29, 41, 43, 44, 57, 72	586	586	0.04
4	3-methylbutanal	29, 41, 43, 44, 86	640	637	0.05
5	2-methylbutanal	39, 41, 57, 58, 86	651	651	0.01
7	1-pentanal	29, 41, 44, 57, 58, 86	675	678	3.20
9	1-hexanal	29, 41, 43, 44, 56, 82, 100	785	783	43.25
17	1-heptanal	44, 57, 70, 81, 86, 96, 114	884	883	9.07
30	1-octanal	44, 56, 57, 84, 100, 128	986	985	6.39
40	1-nonanal	44, 57, 98, 114, 142	1087	1086	3.80
48	1-decanal	41, 43, 44, 57, 112, 128, 156	1188	1187	0.23
Alkenals					
11	<i>trans</i> -2-methyl-2-penten-1-al	41, 55, 69, 83, 98	821	820	0.08
20	<i>trans</i> -2-heptenal	41, 55, 56, 70, 83, 112	941	941	0.43
35	<i>trans</i> -2-octenal	41, 55, 57, 70, 83, 97, 126	1039	1040	0.81
43	<i>trans</i> -2-nonenal	41, 55, 70, 83, 97, 140	1142	1142	0.66
52	<i>trans</i> -2-decenal	41, 55, 70, 83, 97, 110, 154	1244	1245	0.36
61	<i>trans</i> -2-undecenal	41, 55, 70, 83, 97, 168	1346	1347	0.15
Alkadienals					
31	<i>trans,trans</i> -2,4-heptadienal	39, 41, 81, 82, 110	994	994	0.30
49	<i>trans,trans</i> -2,4-nonadienal	41, 67, 81, 82, 138	1194	1193	0.30
56	<i>trans,trans</i> -2,4-decadienal	41, 67, 81, 82, 152	1296	1296	0.12
Aliphatic Ketones					
14	2-heptanone	39, 43, 58, 71, 114	875	873	0.25
27	2-octanone	39, 43, 58, 71, 113, 128	976	978	T ^d
34	3-octen-2-one	41, 43, 55, 97, 111, 126	1020	1019	6.34
36	3,5-octadien-2-one	39, 43, 53, 81, 95, 109, 124	1049	1048	1.48
39	3,5-octadien-2-one	39, 43, 53, 81, 95, 109, 124	1071	1070	2.50
44	3,5-nonadien-2-one	39, 43, 53, 81, 95, 138	1148		0.17
46	2-decanone	43, 58, 71, 85, 156	1177	1180	0.56
60	3,5-undecadien-2-one	41, 43, 81, 95, 151, 166	1344		0.52
62	3,5-undecadien-2-one	41, 43, 81, 95, 151, 166	1379		0.32
Aliphatic Alcohols					
6	1-butanol	31, 41, 43, 55, 56, 74	663	664	T
8	1-pentanol	31, 41, 42, 55, 70, 88	774	773	0.50
16	1-hexanol	31, 41, 43, 55, 56, 69, 102	881	881	0.17
25	1-heptanol	31, 41, 55, 56, 70, 98, 116	972	972	1.54
26	1-octen-3-ol	41, 43, 55, 57, 72, 128	974	974	1.50
38	1-octanol	31, 41, 56, 70, 84, 130	1067	1070	1.07
Lactones					
42	γ -heptalactone	29, 41, 57, 85, 128	1127	1128	0.17
51	γ -octalactone	29, 41, 43, 57, 85, 142	1229	1230	0.25
59	γ -nonalactone	29, 41, 43, 57, 85, 156	1333	1330	0.14
Aromatics					
2	ethylbenzene	51, 65, 77, 91, 106	850	851	0.02
13	<i>m/p</i> -xylene	51, 77, 91, 105, 106	859	861	0.06
15	<i>o</i> -xylene	51, 77, 91, 105, 106	881	881	T
19	benzaldehyde	51, 77, 105, 106	938	936	0.14
21	<i>n</i> -propylbenzene	51, 77, 91, 105, 120	944	945	T
22	<i>m/p</i> -ethyltoluene	51, 77, 91, 105, 120	952	952	T
23	1,3,5-trimethylbenzene	77, 105, 106, 119, 120	959	960	T
24	<i>o</i> -ethyltoluene	51, 77, 91, 105, 120	969	969	T
45	naphthalene	51, 102, 127, 128, 129	1160	1159	0.23
54	2-methylnaphthalene	51, 63, 87, 115, 141, 142	1277	1278	0.26
57	1-methylnaphthalene	51, 63, 115, 141, 142	1295	1295	T
Heteroaromatics					
28	2-ethyl-5-methylpyrazine	39, 40, 42, 56, 121, 122	979	978	T
29	2-(<i>n</i> -pentyl)furan	39, 53, 81, 95, 109, 138	981	982	1.03
37	3,5-dimethyl-2-ethylpyrazine	42, 56, 108, 135, 136	1059	1062	0.10
47	2-(<i>n</i> -heptyl)furan	39, 53, 81, 137, 166	1182		0.11
53	indole	63, 89, 90, 116, 117, 118	1263	1260	0.11
55	2-(<i>n</i> -octyl)furan	39, 53, 81, 95, 180	1284		0.15
Hydrocarbons and Miscellaneous					
2	<i>n</i> -hexane	41, 43, 56, 57, 86	600	600	0.01
3	ethylacetate	43, 45, 61, 70, 73, 88	611	611	0.01
10	<i>n</i> -octane	41, 43, 56, 57, 71, 114	800	800	0.42
18	α -pinene	41, 68, 80, 93, 121, 136	934	933	0.02
32	<i>n</i> -decane	41, 43, 57, 71, 85, 142	1000	1000	0.14
33	3-nonyne	41, 67, 95, 104, 124	1010		0.24
41	<i>n</i> -undecane	41, 43, 57, 71, 156	1100	1100	0.30
50	<i>n</i> -dodecane	41, 43, 57, 71, 170	1200	1200	0.22
58	<i>n</i> -tridecane	39, 41, 43, 57, 71, 85, 184	1300	1300	0.73
63	<i>n</i> -tetradecane	39, 41, 43, 57, 71, 85, 198	1400	1400	0.08
64	<i>n</i> -pentadecane	39, 41, 43, 57, 71, 85, 212	1500	1500	0.03

^a Mass spectrum (complete spectrum) and Kovats GLC index of all compounds listed are consistent with those of authentic samples. ^b Not necessarily the most intense ions but five of those considered the most unique for that compound and molecular weight shown in italic type. Ions and abundances listed in supplementary material. ^c Using the SE-30-coated Pyrex capillary column described under Experimental Section. ^d T, trace.

m/e values of 95, 81, and 43 which pertain to the methylpyrilium, pyrilium and acetyl ions, respectively. These compounds also have K_1 values consistent with those of the other 3,5-dien-2-one homologues.

The class of flavor compounds found in the greatest concentrations was the aliphatic aldehydes. 1-Hexanal was found to be the most concentrated volatile (43% relative concentration). Other major volatiles found in order of decreasing concentration were 1-heptanal, 1-octanal, 3-octen-2-one, 1-nonanal, 1-pentanal, 3,5-octadien-2-one, 1-octen-3-ol, and 2-(*n*-pentyl)furan. Other volatile compounds in Table I that may contribute to MBM flavor include *trans*-2-alkenals (*trans*-2-octenal is the most concentrated), *trans,trans*-2,4-alkadienals (*trans,trans*-2,4-hepta- and -nonadienals are the most concentrated), 3,5-undecadien-2-one, γ -lactones (γ -octalactone is the most concentrated), alkylpyrazines (3,5-dimethyl-2-ethylpyrazine is the most concentrated), and aromatic hydrocarbons (2-methylnaphthalene is the most concentrated).

The majority of the MBM volatiles in Table I have been found in lipid systems and can arise through autoxidative degradation as reviewed by Forss (1972). The major volatiles, 1-hexanal, 1-heptanal, 1-octanal, 3-octen-2-one, 1-pentanal, and 1-octen-3-ol can be formed by autoxidative degradation of fatty acids such as arachidonic, linoleic, or linolenic acids as reviewed by Eriksson (1975), Grosch et al. (1974), and Ohloff (1973).

3,5-Octadien-2-one and 3,5-undecadien-2-one are each present as two isomers. These ketones may be derived from linolenic and arachidonic acids, respectively, via enzymatic oxidative reactions as demonstrated by Grosch and Laskawy (1975) in model tests. It is possible that an enzymatic oxidative reaction forms a precursor compound prior to heat processing of MBM. Upon being heated, this precursor decomposes to the above unsaturated ketones.

The 2-(*n*-pentyl)furan has been identified by Smouse and Chang (1967) to be predominantly responsible for reversion flavor of soybean oil. These authors postulated that 2-(*n*-pentyl)furan originates from linoleic acid.

The *trans*-2-alkenals and *trans,trans*-2,4-alkadienals have been identified as products of autoxidized fatty acids by Badings (1970). *trans*-2-decenal and -undecenal have been isolated from autoxidized oleic acid. *trans*-2-Heptenal, -octenal, and -nonenal and *trans,trans*-2,4-nona- and -decadienals have been isolated from autoxidized linoleic acid. *trans,trans*-2,4-Heptadienal has been isolated from autoxidized linolenic acid. The *trans*-2-heptenal, octenal, and *trans,trans*-2,4-decadienal have also been isolated from autoxidized arachidonic acid.

A number of the MBM volatiles have been identified as volatile components from autoxidized triglycerides. Selke et al. (1980) have found pentanal, pentanol, hexanal, 2-heptenal, 2-octenal, 2,4-decadienal, 2-(*n*-pentyl)furan, methyl ketones, and γ -lactones, in heated trilinolein odor.

The majority of the volatiles isolated in MBM are those commonly associated with rancid foodstuff. The aliphatic aldehydes produce green, fatty, fruity, soapy, floral, and pungent odors. The green top note is the dominate odor descriptor in a freshly open container of MBM. The aliphatic ketones and alcohols impart green, floral, fruity, fatty, and soapy notes. Some of these compounds contribute mushroom-like notes. The unsaturated aldehydes and ketones help impart intense fatty, hay-straw notes which dominate the MBM essence after the top green notes have dissipated.

Badings (1970) has described the odors of 3,5-octadien-2-one and 3,5-undecadien-2-one as being fruity-fatty and fatty-fried, respectively. These compounds also have

thresholds (in paraffin oil) of under 1 ppm. *trans*-2-Octenal imparts a cardboard, woody note and has a threshold of 0.2 ppm in paraffin oil. 1-Octen-3-ol imparts a mushroom- and tin-like odor to MBM. The three furans, 2-(*n*-pentyl); 2-(*n*-heptyl); and 2-(*n*-octyl)furan impart beany-licorice-like notes.

Indole is a compound identified that is not a product of lipid autoxidation. This compound has fecal and floral notes. Indole has a flavor threshold of 0.02 ppm in deodorized butter as reported by Urbach (1972), which is below the concentration found in MBM for indole.

The volatiles identified by Greenberg (1981) in poultry byproduct meal (PBPM) and those in MBM listed in Table I have some similarities in their flavor volatile profiles. Both foods have large relative concentrations of 1-hexanal and 3-octen-2-one. Both foods have unsaturated ketones, methyl ketones, and aliphatic alcohols as well as 2-(*n*-alkyl)furans.

MBM and PBPM do have different aromas, and this is reflected in both volatile type and relative and total volatile quantity. MBM has greener top notes than PBPM. This is reflected in MBM's larger relative concentration of 1-pentanal, 1-heptanal, and 1-octanal than what is found in PBPM. MBM has a more fatty-fried base note than does PBPM. This can be attributed to the fact that MBM contains alkadienals, *trans*-2-nonenal, -decenal, and -undecenal and several alkylpyrazines which have not been detected in PBPM.

MBM and PBPM do not contain FID- or MS-detectable volatiles usually associated with pleasant cooked-meat odors. For example, they lack the presence or relative concentrations of pyrazines, thiazoles, thiazolines, tri-thiolanes, oxazoles, thiophenes, furanones, and other compounds that contribute to the savory meat-like aromas in prime meats such as beef or pork liver. This will be further verified in future studies by using flame photometric and nitrogen-phosphorus GC detectors.

The high fat content of MBM and PBPM coupled with the severe cooking (rendering) conditions such as those described by Rao and Mahadevan (1976) may promote substantial autoxidation. Thomas et al. (1971) have shown that the action of heat on lipids during cooking can accelerate autoxidation and increase the amount of carbonyl compounds. Considerable oxidative changes in PBPM during storage have been reported by Rao and Mahadevan (1976). These authors reported the fatty acid levels in lipid extracts of fresh and stored PBPM. For example, linoleic acid content decreased from 17.2 to 8.1% of the fatty acid content in the lipid extracts of fresh and 3 month stored PBPM, respectively. Thus, MBM and PBPM flavor can vary due to processing and storage conditions.

SUMMARY

Meat and bone meal (MBM), an inexpensive protein source used in pet foods and animal foods, has been subjected to volatile flavor isolation via a vacuum distillation technique. The isolated volatiles were identified by gas chromatography-mass spectrometry and further verified in most cases by Kovats retention indexes. A number of aliphatic aldehydes, ketones, and alcohols were found which contribute to the top green, soapy, fatty notes. The major volatile contributing to the top notes was 1-hexanal (43% relative concentration). Volatiles contributing to the fatty, hay, cardboard-like notes include 2-(*n*-pentyl)furan, 2-octenal, 3-octen-2-one, 3,5-octadien-2-one, and 3,5-undecadien-2-one. Volatiles contributing to the fried-fatty base notes include *trans,trans*-2,4-heptadienal, -nonadienal, and -decadienal, *trans*-2-nonenal, -decenal, and -undecenal, and several alkylpyrazines. These compounds

contribute to the flavor of MBM and pet foods containing this ingredient.

Lipids appear to be the primary source of flavor in MBM. This was also found to be the case in this laboratory's previous study of poultry byproduct meal (PBPM). The majority of compounds isolated in this study and the Greenberg (1981) PBPM study have been identified in literature reports to be products of lipid (specific fatty acid) oxidation reactions. This is further supported by the fact that MBM and PBPM have a high fat content (usually greater than 14%) which, when rendered, can accelerate autoxidation. A literature report has also demonstrated storage instability of fatty acids such as linoleic acid in PBPM.

Future work with MBM will involve attempting to identify more volatile flavor components with the aid of chemical ionization mass spectrometry. Other areas of future study will include the synthesis of authentic standards for compounds tentatively identified, such as 3,5-undecadien-2-one.

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Supplementary Material Available: A listing of the flavor volatiles in Table I and their mass spectra (m/e and relative abundance) values (4 pages). Ordering information is given on any current masthead page.

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Acid-Catalyzed Alteration of 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl (Di-*n*-butylaminosulfenyl)methylcarbamate via Nitrogen-Sulfur Bond Cleavage. 2. Separation and Identification of Polysulfide Derivatives

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2,3-Dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)methylcarbamate (DBSC) dissolved in dichloromethane-acetic acid (9:1) was converted into a mixture of polysulfide derivatives of DBSC and biscarbofuran *N,N'*-disulfide along with other alteration products. Silica gel KC₁₈ reversed-phase thin-layer chromatography provided a simple and convenient method for separating the individual components in the polysulfide mixture. The polysulfide of DBSC was separated into at least eight components, and the structures of the four major components were determined by NMR and MS analyses of purified products. The polysulfide of biscarbofuran *N,N'*-disulfide was separated into at least seven components, and the structures of the four major components were determined. Quantitative determination of the breakdown of DBSC and formation of the individual alteration products, including polysulfide derivatives, was conducted with [carbonyl-¹⁴C]DBSC. Most of the products showed good insecticidal activity against the housefly, and all of them were significantly less toxic to the white mouse than the parent methylcarbamate carbofuran.

The previous paper in this series (Umetsu et al., 1981) described the different products which were obtained when 2,3-dihydro-2,2-dimethyl-7-benzofuranyl (di-*n*-butylaminosulfenyl)methylcarbamate (DBSC or Marshal) was

allowed to stand in the 9:1 dichloromethane-acetic acid solvent at room temperature. The principal alteration products were carbofuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate), di-*n*-butylamine, and a mixture of the polysulfide derivatives of DBSC and biscarbofuran *N,N'*-disulfide. Although spectroscopic evidence was provided that demonstrated the presence of a number of these polysulfide derivatives ($n = 2$ to about 6), separation of the individual components by silica gel TLC was not accomplished. CFS_{*n*}NBu₂ ($n = 2-6$) and CFS_{*n*}CF ($n = 3-6$) gave single spots on silica gel plates after

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