Lane, H. W.; Taylor, B. J.; Stool, E.; Servance, D.; Warren, D. C. *J. Am. Diet. Assoc.* **1983a**, *82*, 25.
Lane, H. W.; Warren, D. C.; Martin, E.; McCowan, J. *Nutr. Res.* **1983b**, *3*, 805.
Lane, H. W.; Warren, D. C.; Taylor, B. J.; Stool, E. *Proc. Exp. Biol. Med.* **1983c**, *173*, 87.
Lane, H. W.; Lotspeich, C. A.; Moore, C. E.; Ballard, J.; Dudrick, S. J.; Warren, D. C. *JPEN, J. Parenter. Enteral Nutr.* **1987**, *11*, 177.
Lawler, M. R.; Klevay, L. M. *J. Am. Diet. Assoc.* **1984**, *84*, 1028.
Levander, O. A. *J. Am. Diet. Assoc.* **1975**, *66*, 338.
Lunde, G. *J. Sci. Food Agric.* **1970**, *21*, 242.
Morris, V. C.; Levander, O. A. *J. Nutr.* **1970**, *100*, 1383.
Murphy, E. W.; Willis, B. W.; Watt, B. K. *J. Am. Diet. Assoc.* **1975**, *66*, 345.
Prasad, A. S. *Zinc in Human Nutrition*; CRC: Boca Raton, FL, 1979.
Solomons, N. W. *Am. J. Clin. Nutr.* **1982**, *35*, 1048.
Tanticharoenkiat, O.; Chastain, M. F.; Lane, H. W. *J. Food Sci.* **1988**, in press.
Thorn, J.; Robertson, J.; Buss, D. J. *Br. J. Nutr.* **1978**, *39*, 391.
Truesdell, D. D.; Whitney, E. N.; Acosta, P. B. *J. Am. Diet. Assoc.* **1984**, *84*, 28.
Varian *Analytical Methods for Flame Spectroscopy*; Varian Techtron: Springvale, Australia, 1979.
Walravens, P. A.; Hambidge, K M. *Am. J. Clin. Nutr.* **1976**, *29*, 1114.

Received for review November 18, 1986. Accepted February 2, 1988.

Investigation of the Main Components in Insect-Active Dill Seed Extract

Helen C. F. Su* and Robert Horvat

In previous studies, dill (*Anethum graveolens* L.) seed acetone extract was biologically active against several species of stored-product insects and also gave long-lasting repellency against the confused flour beetle, *Tribolium confusum* Jacquelin du Val. The extract was analyzed by HPLC monitored with a UV detector at various wavelengths. The major components were isolated, purified by TLC and HPLC techniques, and identified as 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one (*d*-carvone) and 4,5-dimethoxy-6-(2-propenyl)-1,3-benzodioxole (dillapiol).

Anethum graveolens L. (dill) of the Apiaceae family is a common herb. Its leaves and seed are used as a condiment in cooking and also in pickling. The composition and characteristics of the dill plant and its essential oil (from leaves and seeds) have been investigated extensively (Baslas and Baslas, 1972; Salzer, 1975; Stahl and Herting, 1976; Herrmann, 1978; Teuber and Herrmann, 1978; Koedam et al., 1979; Henry, 1982; Huopalahti and Linko, 1983; Porter et al., 1983). The insecticidal properties and the synergistic activities of dill plants were reported by Hartzell (1944) and Lichtenstein et al. (1974).

The effects of the lyophilized dill seed acetone extract to several species of stored-product insects were reported (Su, 1985, 1987). We now report the isolation and identification of the main components from the insect-active extract of dill seed by chromatographic and spectral methods.

MATERIALS AND METHODS

Preparation of Dill Seed Extract. The extract of dill seed was prepared by acetone extraction of the pulverized seed powder at 40–50 °C as described by Su (1985). The extract was lyophilized at 0–5 °C to obtain a light brown syrupy material.

High-Performance Liquid Chromatographic Study of Dill Seed Extract. A Waters Associates Model ALC/GPC 244 HPLC equipped with a Model 6000A pump, a U6K injector, and a Model 440 UV detector with a 300 × 7.8 mm (i.d.) μBondapak C_{18} column (octadecyltrichlorosilane covalently bonded to 10-μm μPorasil packing) was used. Methanol–water (70:30, v/v, degassed) was used as the eluting solvent. The effluent was monitored at 254, 280, 313, 340, 365, and 405 nm, and the response (1.0 AUFS) was recorded on a Waters Associates M730 Data Module recorder.

Thin-Layer Chromatographic (TLC) Separation of *d*-Carvone for Infrared (IR) Study. For TLC separation, Brinkman EM reagent, precoated silica gel F_{254}, 0.25-mm, 20 × 20 cm chromatoplates were used. About 3–4 mg of the dill extract was applied to each plate in a straight line 2.5 cm above the lower edge to a distance of 3 cm from the right edge. A spot of authentic *d*-carvone was applied at the same height, 1.5 cm from the right edge. A total of 15 plates was prepared. Each plate was developed twice in benzene–chloroform (90:10, v/v) and then examined under UV at 254 nm. The bands that corresponded to the *d*-carvone spots were collected and eluted with methanol. The methanol extract was concentrated slowly under reduced pressure, and the resulting liquid was used for the IR study.

Instrumental Analyses. A Du Pont Model 21-490B mass spectrometer was used with a direct-insertion probe at a temperature of 75–250 °C. Other conditions used were as follows: ion source temperature, 150 °C; ionizing voltage, 70 eV; ion source pressure, 4×10^{-6} Torr; scan rate, 100 s/decade from 15 to 500 amu.

GLC–MS analyses were performed with a Perkin-Elmer Model 300 GLC that was connected by an effluent splitter to a Du Pont Model 21-490B mass spectrometer. The GLC column used was a 50 m × 0.32 mm fused silica capillary column coated with SE-54 with He at 0.6 kg/cm^2 pressure as the carrier gas. Oven temperature was programmed from 50 to 215 °C at 4 °C/min. Mass spectrometer con-

Stored-Product Insects Research and Development Laboratory, U.S. Department of Agriculture—Agricultural Research Service, Savannah, Georgia 31403 (H.C.F.S.), and Richard B. Russell Agricultural Research Center, U.S. Department of Agriculture—Agricultural Research Service, Athens, Georgia 30604 (R.H.).

Table I. HPLC Analysis of Dill Seed Acetone Extract with μBondapak C_{18} Semipreparative Column Eluted by Methanol–Water (70:30, v/v) at 1.2 mL/min

	rel peak area (cm^2) at indicated wavelength					
RT, min	254 nm	280 nm	313 nm	340 nm	365 nm	405 nm
4.81	1.50[a]	1.02[a]	0.81[a]	0.64[a]	tr[b]	
5.41	14.26[a]					
5.60		8.88[a]				
5.78	7.84[a]		7.98[a]	6.37[a]	2.37	0.24
6.01		4.61[a]				
6.20	1.30[a]		4.34[a]	2.50[a]		
6.50		1.01[a]				
6.83			1.69[a]	1.54[a]	0.46	0.08
9.25	tr	tr	3.21	3.24		
10.76	10.82	7.23				
10.85			14.53	10.46	1.97	
12.61	tr		0.64	tr		
13.08		3.98	1.33	0.28		
14.61			0.80	1.07	0.80	
15.68	2.32	tr	1.45	1.74	1.06	
18.28	364.42	1.90	14.00	1.58		
25.10	69.30	183.95	4.87			
29.51	tr	7.01	tr	5.84	7.39	

[a] Fused peak. [b] Peak response too small to be calculated.

ditions were the same as those described above, except that the source pressure was 2×10^{-4} Torr.

Infrared spectra were determined as KBr pellets or films (neat) on KBr windows with an Analect FTIR Model fx6160 spectrophotometer. The UV spectra were obtained with a Varian Cary Model 210 spectrophotometer using cells with 1-cm paths, with wavelengths in nanometers.

Chemicals and Reagents. HPLC-grade methanol (Fisher Scientific Co.) was filtered through a Waters Associates solvent clarification kit with a 0.5-μm Millipore organic filtration system. All other solvents were reagent grade. *d*-Carvone was purchased from the Aldrich Chemical Co. and oil of dill from the Indiana Botanic Gardens, Inc.

RESULTS AND DISCUSSION

The HPLC analysis of 350 μg of dill seed acetone extract monitored at various UV wavelengths is summarized in Table I. The spectra showed two strong absorptions (peak areas) at retention times (RT) of (A) 18.28 and (B) 25.10 min. Other minor components gave peak areas less than 15 cm^2.

Compound A could not be obtained in dehydrated form from the HPLC eluant because it was lost when the methanol–water mixture was evaporated. Its UV absorption in MeOH–H_2O (70:30, v/v) showed very weak absorption at 313 nm and a strong absorption at 239 nm. Strong and moderate infrared absorptions obtained from MeOH elution of TLC chromatograms were observed (cm^{-1}) at 3080, 2982, 2905, 2804, 2789, 1667, 1645, 1455, 1378, 1230, 1050, and 890. A GLC–MS method was used to obtain its mass spectrum. Compound A had a RT of 33.71 min as compared to 33.73 min of an authentic sample of *d*-carvone. Its mass spectrum showed the following peaks (relative peak intensity in parentheses): *m/e* 150 (M^+, 16), 135 (20), 108 (38), 107 (30), 106 (24), 93 (52), 82 (base peak 100), 69 (32), 67 (40), 54 (21), 41 (93), 39 (82). This mass spectrum was identical with that obtained from an authentic sample of *d*-carvone, 2-methyl-5-(1-methylethenyl)-2-cyclohexen-1-one. It is a major component in oil of dill (Indiana Botanic Gardens).

Compound B was obtained as an oily liquid at ambient temperature but solidified into colorless small short needles when refrigerated. Its UV absorption in methanol showed a maximum 283 nm (ε 998). Strong and moderate infrared absorption bands were observed (cm^{-1}) at 3097, 1628 (aryl CH); 2978, 2939, 2901 (CH_3 and CH_2); 1417, 1281, 998, 916 (–CH=CH–); 1500, 1479, 1464, 838, 777 (aryl multisubstitution); and 1196, 1083, 1052 (aryl aliphatic and aliphatic ethers). The mass spectrum showed the following peaks (relative peak intensity in parentheses): *m/e* 223 (13), 222 (M^+, 100), 207 (21.8), 177 (17.2), 149 (14), 121 (9), 101 (8), 59 (17), 58 (16). Compound B was identified to be 4,6-dimethoxy-6-(2-propenyl)-1,3-benzodioxole (dillapiol). Its MS and UV data were identical with those reported by Lichtenstein et al. (1974).

These two compounds were the main components apparently responsible for providing long-lasting repellency against the confused flour beetle, *Tribolium confusum* Jacquelin du Val (Su, 1987), and in serving as a protectant for wheat in storage against rice weevil, *Sitophilus oryzae* (L.) (Su, unpublished data). These biological activities are similar to the insecticidal properties reported by Lichtenstein et al. (1974) for *d*-carvone in dill greens and dillapiol in dill roots to house flies, *Musca domestica* L., and yellowfever mosquito larvae, *Aedes aegypti* (L.).

Registry No. *d*-Carvone, 2244-16-8; dillapiol, 484-31-1.

LITERATURE CITED

Baslas, B. K.; Baslas, R. K. "Biogenetic Relation between Terpenic and Nonterpenic Constituents in the Volatile Oils from *Anethum graveolens* and *A. Sowa*". *Riechst. Aromen. Koerperpflegem.* **1972**, *22*, 270–271.

Hartzell, A. "Tests on Plant Products for Insecticidal Properties". *Contrib. Boyce Thompson Inst.* **1944**, *13*, 243–252.

Henry, B. S. "Composition and Characteristics of Dill: A Review". *Perfum. Flavor.* **1982**, *7*, 39–44.

Herrmann, K. "Phenolic Components of Spices". *Nachr. Chem., Tech. Lab.* **1978**, *26*, 206, 208–209.

Huopalahti, R.; Linko, R. R. "Composition and Content of Aroma Compounds in Dill, *Anethum graveolens* L., at Three Different Growth Stages". *J. Agric. Food Chem.* **1983**, *31*, 331–333.

Koedam, A.; Scheffer, J. J. C.; Svendsen, A. B. "Comparison of Isolation Procedures for Essential Oils. I. Dills". *Chem. Mikrobiol. Technol. Lebensm.* **1979**, *6*, 1–7.

Lichtenstein, E. P.; Liang, T. T.; Schulz, K. R.; Schnoes, H. K.; Carter, G. T. "Insecticidal and Synergistic Components Isolated from Dill Plants". *J. Agric. Food Chem.* **1974**, *22*, 658–664.

Porter, N. G.; Shaw, M. L.; Shaw, G. J.; Ellingham, P. J. "Contents and Composition of Dill Herb Oil in the Whole Plant and the Different Parts during Crop Development". *N.Z. J. Agric. Res.* **1983**, *26*, 119–127.

Salzer, U. J. "Analytical Evaluation of Seasoning Extracts (Oleoresins) and Oils from Seasonings. I". *Int. Flavours Food Addit.* **1975**, *6*, 151–157.

Salzer, U. J. "The Anti-microbial Action of Certain Spice Extracts and Mixture". *Die Fleischwirtschaft.* **1982**, *62*, 885–888.

Stahl, E.; Herting, D. "The Content of Essential Oil and Coumarins in Single Fruits of Some Apiaceae". *Planta Med.* **1976**, *29*, 1–9.

Su, H. C. F. "Laboratory Study on Effects of *Anethum graveolens* Seeds on Four Species of Stored-Product Insects". *J. Econ. Entomol.* **1985**, *78*, 451–453.

Su, H. C. F. "Laboratory Study on the Long-Term Repellency of Dill Seed Extract to Confused Flour Beetles". *J. Entomol. Sci.* **1987**, *80*, 70–72.

Teuber, H.; Herrmann, K. "Flavonol Glycosides of Dill (*Anethum graveolens* L.) Leaves and Fruits. II. Phenolics of Spices". *Z. Lebensm.-Unters.-Forsch.* **1978**, *167*, 101–104.

Received for review July 6, 1987. Accepted March 14, 1988. Mention of a commercial or proprietary product does not constitute a recommendation or an endorsement by the USDA.