

Insecticidal and Synergistic Components Isolated from Dill Plants

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Dill plants (*Anethum graveolus* L.), whose seeds have been used for centuries for flavoring of various human foods, were found to contain insecticidal components which also were synergistic for carbamate and organophosphorus insecticides with some insect species. Four major compounds were isolated and identified: *d*-carvone was present in the aerial parts of dill, including seeds ("dill greens") at 3625 ppm, myristicin was found in both dill "greens" (24 ppm) and roots (41 ppm), while apiol and its isomer, dill-apiol, were present only in roots at 271 and 227 ppm, respectively. Crude dill "green" extracts were more active as insecticides and synergists than were crude root extracts. The relatively high biological activity of the greens was due to the high concentration of *d*-carvone. This compound, utilized at

sublethal dosages, increased considerably the toxicity of carbaryl, carbofuran, and parathion to insects. However, apiol, dill-apiol, and myristicin were much more toxic to insects than *d*-carvone and also synergized the insecticidal activity of the synthetic insecticides at much smaller dosages. Apiol, having two methoxy groups, appeared to be more toxic to fruit flies than myristicin, which has one methoxy group. Apiol was also more effective as a synergist with parathion, but not with carbaryl. These findings might point to a potential problem in that food plants may contain substances which by themselves exhibit negligible biological activities, yet which could interact with residues of synthetic insecticides or other synthetic chemicals in the animal body.

The study of biologically active substances in man's environment has become increasingly important in recent years. This coincided with our awareness of some potential undesirable side effects exhibited by agricultural chemicals, in particular those with excessive residual properties. Problems that require investigation pertain to the potential effects of man-produced chemicals and/or their metabolites on biological systems and to their potential interaction in living cells with naturally occurring compounds that also affect biological systems. Chemicals of the latter category include plant-derived insecticidal substances.

Surveys of plants that have been tested for insecticidal properties have been published at different times during this century; the major ones include those by Roark (1919), McIndoo and Sievers (1924), Roark and Keenan (1931), Worsley (1934), Holman (1940), McIndoo (1941), Heal *et al.* (1950), Jacobson (1958), and Feuill (1965). Jacobson and Crosby (1971) published a recent survey pertaining to naturally occurring insecticides. All tests mentioned in the various publications were primarily designed for the measuring of toxicity as indicated by relatively rapid insect mortalities.

Research efforts in our laboratory were directed toward the isolation and investigation of biologically active substances in food plants that have been consumed for centuries by humans. As a result the insecticide, 2-phenyl ethylisothiocyanate was isolated from the edible parts of turnips (Lichtenstein *et al.*, 1962) and myristicin, a substance of insecticidal and synergistic properties, was isolated from the edible parts of parsnips (Lichtenstein and Casida, 1963). A potential problem exists in that food plants may contain substances which by themselves exhibit negligible insecticidal activities, yet they could interact with residues of synthetic chemicals in our body and produce physiological effects. It was for this reason that we grew a variety of food plants on an insecticide-free silt loam soil in Madison, Wis., and tested them initially for their potential insecticidal activities.

This study reports on the presence, isolation, and structure of biologically active substances in the dill plant, on

their insecticidal activity, and on their joint action with synthetic insecticides on some insects.

EXPERIMENTAL SECTION

Evidence of Insecticidal Activity. *With Macerated Plant Tissue.* Dill, *Anethum graveolus* L. (Umbelliferae), was grown on an insecticide-free soil. When mature seeds were present, the plants were harvested and roots were removed and washed with water. Roots and greens (all aerial plant parts, including seeds) were then separately macerated in a food grinder (Hobart Model 215 Food Cutter) and used for experimental purposes or were frozen (-5°) for future testing. Initially, the insecticidal activity of the roots or greens was investigated by placing 3 g of each of the freshly macerated materials on wet filter paper in each of five bioassay jars (5 cm diameter and 6.3 cm deep). Fifty fruit flies (*Drosophila melanogaster* Meig.) were then introduced into each jar. As a control, flies were exposed to wet filter paper only. In addition, the same experiment was repeated except that cylindrical screen inserts were placed into the bioassay jars. Flies were then introduced into the inserted screen containers which prevented the insects from direct contact with the plant material. In this way fumigant toxicity could be tested. One day later 47% of the flies had died after direct exposure to the macerated dill greens, but none were killed when kept in the screen inserts. With root material 11% of the flies died in 48 hr by direct exposure and none died by fumigation. After this initial indication of some insecticidal activity, macerated dill roots and greens were extracted for further testing.

With Plant Extracts. In the early stages of this investigation, various extraction procedures were utilized. Ground roots or greens were primarily extracted with a 1:1 mixture of redistilled pentane-acetone or redistilled hexane-acetone as previously described (Lichtenstein and Casida, 1963). In addition, plant material was extracted with water at a ratio of 2 ml/g of plant material.

After organic solvent extractions, the acetone was removed with water and the dried pentane or hexane was concentrated and adjusted to volume. Aliquots of these fractions were then pipetted into bioassay jars, and the solvent was evaporated in a fume hood. Contact and fumigant toxicity of residues to fruit flies was then determined as described. Dry residues from extracts representing 2 g of dill greens caused a 100% mortality of the insects within 10 min of direct exposure to these residues,

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Table I. Increase in the Toxicity of Synthetic Insecticides with Crude Extracts from Dill Greens and Dill Roots (Results Are Means plus Standard Deviation of Three Replicates)

Insecticide	% mortality ^b with hexane or water extracts from					
	None (control)	Dill greens		None (control)	Dill roots	
		Hexane ^a	Water ^b		Hexane ^a	Water ^b
None	0	0	0	0	0	0
Carbaryl, 100 μg^c (DR)	5 \pm 3	93 \pm 3 ^e	0	5 \pm 2	80 \pm 11 ^e	0
0.6 ppm ^d (AE)	13 \pm 6		97 \pm 3 ^e	7 \pm 3		83 \pm 8 ^e
Carbofuran, 0.7 μg (DR)	21 \pm 8	91 \pm 6 ^e		5 \pm 1	79 \pm 13 ^e	
0.18 ppm (AE)	17 \pm 3		67 \pm 13 ^f	15 \pm 10		43 \pm 8 ^g
Parathion, 0.35 μg (DR)	10 \pm 3	93 \pm 8 ^e		7 \pm 3	73 \pm 10 ^e	
0.01 ppm (AE)	13 \pm 3		90 \pm 5 ^e	15 \pm 5		27 \pm 10
DDT, 2.0 μg (DR)	17 \pm 4	9 \pm 6		11 \pm 5	15 \pm 3	

^a Dry residue of a hexane extract representing 0.56 g of plant material. *Drosophila* bioassay. ^b Water extract representing 0.25 g of plant material, added to 20 ml of H₂O. *Aedes* larvae bioassay. ^c Deposit of microgram of insecticide in bioassay jars for *Drosophila* bioassay. ^d Concentration of insecticide in 20 ml of water for *Aedes* larvae bioassay. ^{e-g} Differences observed in mortalities obtained with and without plant material (controls) are significant at the e (0.1%), f (1%), and g (5%) levels. ^h Per cent mortality in 24 hr of mosquito larvae (AE) or fruit flies (DR).

or within 2 hr exposure to fumes that emanated from this deposit. With root material (dry residues representing 2 g) all insects were dead after 7 hr of a direct exposure to the deposits and after 24 hr of exposure to fumes.

Water extracts from greens and roots were tested for insecticidal components by introducing 20 third instar mosquito larvae (*Aedes aegypti* L.) into triplicate 20-ml aliquots representing 4.5 g of plant material. With extracts from greens, all the insects were dead within 3 hr. This water was further extracted with methylene chloride. A subsequent direct exposure of fruit flies to dry residues representing 6 g of greens or to fumes thereof resulted in a 100% insect mortality within 20 min or 24 hr, respectively. With water extracts from roots, all the mosquito larvae were dead within 19 hr. Exposure of fruit flies to dry residues from methylene chloride extracts of this water resulted in a 100% insect mortality within 24 hr exposure to the dry residue directly or to fumes that emanated from it. These data indicated that the insecticidal chemical or chemicals in dill greens were either more toxic or were present in greater concentrations than in roots.

To determine potential synergistic effects of dill components with synthetic insecticides, sublethal dosages of organic solvent or water extracts from greens or roots were added to the analytical grade insecticides carbaryl, carbofuran, parathion, or *p,p'*-DDT. Residues of these insecticides were deposited into bioassay jars at dosages that resulted in relatively low mortalities of fruit flies during a 24-hr exposure, or acetone solutions of the insecticides were added to water at concentrations that caused low mortalities of mosquito larvae. The addition of small amounts of plant extracts—selected at dosages that by themselves were not insecticidal—increased the toxicity of the insecticides significantly (Table I). Thus, the mortalities of fruit flies due to carbaryl were increased from 5% (carbaryl only) to 93 and 80%, respectively, after equivalents of 0.56 g of dill greens or dill roots had been added. Similar increases in mortality were registered with mosquito larvae. Corresponding results were obtained with both carbofuran and parathion. However, the toxicity of DDT to fruit flies was not affected. The dill plant, therefore, contains materials which are insecticidal and also exhibit synergistic properties with the carbamate and organophosphorus insecticides tested. This case might be an example where plant constituents could affect the biological activity of conventional insecticides in living systems.

Extraction and Isolation Procedures. For quantitation of the insecticidal components in dill, the plant material was extracted as described (Lichtenstein and Casida, 1963) with a mixture of hexane-acetone (1:1).

When water extracts of dill plants were concentrated in a vacuum evaporator, the distillate was found to be toxic

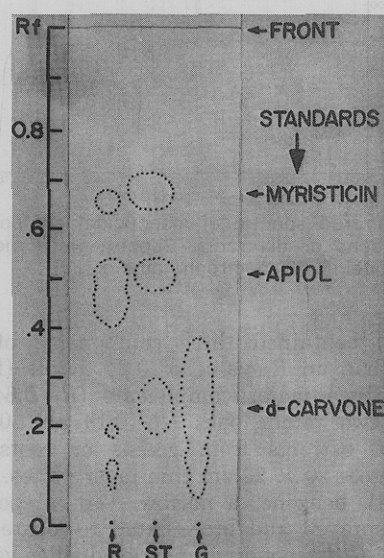


Figure 1. Thin-layer chromatogram of extracts from dill roots (R) and dill greens (G). St = synthetic reference materials (standards).

to mosquito larvae (100% mortalities in 4 hr with dill greens and in 20 hr with dill roots), indicating that the insecticidal principle was rather volatile. It was also demonstrated that steam distillates from both macerated roots and greens were highly toxic to mosquito larvae. Therefore, steam distillation was subsequently utilized for the extraction and isolation of larger amounts of these compounds. This method also eliminated a clean-up procedure. Generally, 300 g of dill greens or dill roots and 300 ml of water were placed into a 2-l. two-necked distillation flask. While steam was passed through this material for 3–4 hr, the distillate was collected in a round-bottomed flask which was immersed in Dry Ice and contained 50 ml of hexane. After 800–900 ml of the distillate had been collected, it was extracted twice with 150-ml portions of hexane, followed by concentration of the solvent and adjustment to a volume of 10 ml.

Thin-layer chromatography (tlc) was then used for the separation and isolation of various compounds and subsequent testing of their insecticidal activities. A small amount of the hexane concentrate from roots or greens was spotted on 5 × 20 cm MN-Brand precoated silica gel plates, followed by development with benzene. After spraying of the developed plates with 0.05% uranine in water and their exposure to bromine vapors, yellow spots on a reddish background became visible (Figure 1).

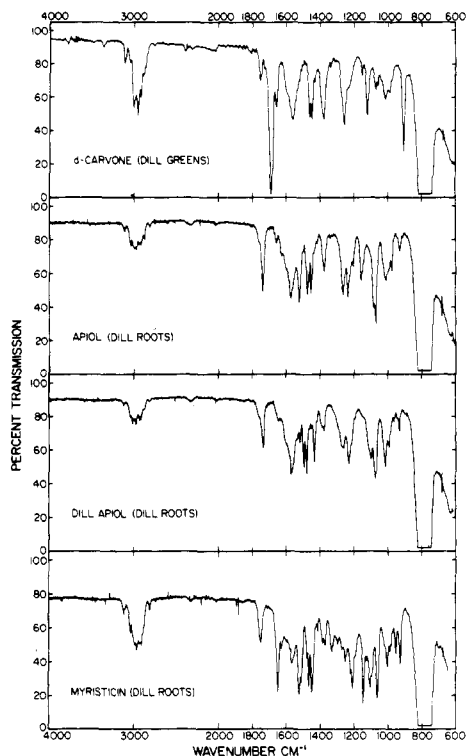


Figure 2. Infrared spectra of insecticidal compounds isolated from dill greens or dill roots. Spectra were measured on a Beckman Model IR-33 spectrophotometer.

Greens (G) contained three components of which the major one had an R_f value of 0.24. Roots (R), however, contained three major components (R_f 0.64, 0.51, and 0.44) and two minor ones (R_f 0.19 and 0.12). Larger amounts of extracts from greens or roots were then streaked across 20×20 cm thin-layer plates. After development with benzene, a narrow, vertical portion of the plate was sprayed and brominated to locate the various compounds. Corresponding unsprayed silica gel areas were then scraped off the plates, extracted with acetone, and tested for insecticidal activity. It was found that the isolate from greens (R_f 0.38–0.18) and the three isolates from roots (R_f 0.68–0.60, 0.55–0.48, and 0.46–0.40) contained insecticidal substances. These isolates were then subjected to instrumental analyses.

Identification and Quantitation of the Isolated Insecticidal Components in Dill. *d-Carvone.* The major insecticidal principle obtained from dill greens, including seeds (R_f 0.38–0.18, Figure 1), was characterized as *d-carvone* by the following evidence. Infrared absorption at 1690 cm^{-1} (Figure 2) and the band at 237 nm (ϵ 8970) in the ultraviolet spectrum (Figure 3) indicated a conjugated ketone function, and nmr signals (Figure 3) at δ 1.77 (6 H), 4.80 (2 H), and 6.80 (1 H) could be assigned to two olefinic methyl groups, a terminal methylene and an olefinic proton of the α,β -unsaturated carbonyl system. These data, together with the mass spectrum, exhibiting a molecular ion at m/e 150 and peaks at m/e 108 ($M - 42$), 107, and 82 (base peak) suggested a monoterpene skeleton. The compound was optically active ($\alpha^{20D} +59^\circ$), and direct comparison of the rotation and infrared, ultraviolet, nuclear magnetic resonance, and mass spectral data (Figure 3) of material isolated from dill with the corresponding data obtained for synthetic *d-carvone* established its identity. Isolated and synthetic *carvone* also gave identical glc retention times and tlc R_f values. Gladstone (1872) reported that dill oil, prepared from seeds of dill plants, contained up to 60% of *d-carvone*.

Myristicin. For the compound of R_f 0.68–0.60 (Figure 1) isolated from dill roots, the mass spectrum established a

molecular weight of 192. Ultraviolet absorption at 277 nm and prominent peaks at m/e 177 ($M - 15$), 161 ($M - 31$), and 147 ($M - 15 - 30$) in the mass spectrum (Figure 3) suggested a simple aromatic ether. The nmr spectrum (Figure 3) established the presence of a methyl ether (δ 3.90), a methylene dioxy group (δ 5.94), two aromatic protons (δ 6.40), and a terminal vinyl function (δ 5.00, 5.20, 5.93) attached to a benzylic methylene (δ 3.30). These results suggested the structure of myristicin for the isolated material, and direct comparison of its proton resonance, mass, infrared, and ultraviolet spectra with those of synthetic myristicin confirmed its identity. Isolated and synthetic myristicin also were indistinguishable in their glc and tlc chromatographic behavior. Schimmel (1927) reported that oil, prepared from dill greens, contained myristicin.

Apiol. The isolate of R_f 0.55–0.48 (Figure 1) from dill roots showed typically aromatic ultraviolet absorption (λ_{max} 279 nm (ϵ 1070)) and its mass spectrum, exhibiting a molecular ion at m/e 222 and a fragmentation pattern very similar to that of myristicin (Figure 3), characterized the substance as a methoxy analog of myristicin. The nmr spectrum (Figure 3) fully supported this assignment. The spectrum showed two methoxy groups (δ 3.84, 3.97), a methylene dioxy function (δ 5.94), one aromatic proton (δ 6.30), and the resonances of the allyl side chain (δ 5.09, 5.23, 5.95, and 3.31). The compound was identified as *apiol* by direct comparison of all spectral data (Figure 3) as well as glc (Figure 4) and tlc (Figure 1) behavior with synthetic material, obtained by purification of a commercially available sample.

Synthetic myristicin (R_f 0.67) had been kindly provided by H. Jenkins, A. H. Robins Co., Richmond, Va. Authentic *apiol* (R_f 0.51) was purchased from Pfaltz and Bauer, Inc., Flushing, N. Y., and authentic *d-carvone* (R_f 0.23) was from Aldrich Chemical Co., Milwaukee, Wis. Although their exact origin is unknown, they are referred to in this paper as synthetic.

For quantitative and qualitative analyses of the various dill components, a Tracor Model 550 gas chromatograph, equipped with a Melpar flame ionization detector, was used. The $1.83 \text{ m} \times 3 \text{ mm}$ glass column, containing 5% Carbowax 20M coated on 80–100 mesh Gas Chrom Q, was programmed from 70 to 200° at a rate of $10^\circ/\text{min}$. Nitrogen flow through the column was held at $45 \text{ ml}/\text{min}$. The injection port temperature was maintained at 250° and the detector at 170° . Under these conditions synthetic *d-carvone* had a retention time of 2.4 min, while myristicin and *apiol* had times of 6.06 and 7.56 min, respectively. When $2 \mu\text{l}$ of a hexane extract from dill roots, representing 0.02 g of plant material, was analyzed by gas-liquid chromatography (glc), three distinctive peaks were observed (Figure 4). One peak had the same retention time as myristicin (B), another had the same retention time as *apiol* (C), and a third peak (D) did not correspond to any of the available reference standards. For the identification of this compound as indicated by peak D, additional tlc was performed. Extraction of the silica gel area with an R_f 0.46–0.40 resulted in the isolation of dill-*apiol*.

Dill-Apiol. Spectral data for the component of R_f 0.46–0.40 (Figure 1) extracted from dill roots characterized this compound as an isomer of *apiol*, referred to by Karrer (1958) as dill-*apiol*. The mass spectrum of the substance indicated a molecular weight of 222 and a pattern very similar to that of *apiol* (Figure 3). Likewise its proton resonance spectrum (Figure 3) very closely matches that of *apiol*, except for the distinctive chemical shifts of the methoxy resonances. The data are entirely consistent with the structure shown in Figure 3 (dill-*apiol*). Synthetic dill-*apiol* was not available to us for direct spectral comparison, and since other isomers satisfying the spectral evidence are possible, our structural assignment must be regarded as tentative, though highly probable. The presence

NAME, MOLECULAR WEIGHT & FORMULA	STRUCTURE	MASS SPECTRUM ^a m/e (rel. intensity)	NMR SPECTRUM ^b δ (no. H's, mult, J, assign.)	UV SPECTRUM ^c λ max. (e)
d-CARVONE M. Wt. = 150 $C_{10}H_{14}O$		150(M ⁺ , 12), 108(22) 107(17), 93(27) 82(100), 67(10) 54(20), 53(20) 41(27)	1.77 (6H, br. s, H ₅ , 10) 2.2-2.8 (5H, m, H ₂ , 5, 6) 4.80 (2H, br. m, H ₃) 6.80 (1H, br. m, H ₆) 4.1(27)	237 (6970)
APIOL M. Wt. = 222 $C_{12}H_{14}O_4$		223(14), 222(M ⁺ , 100) 207(22), 195(10) 177(15), 149(15) 44(22), 43(11)	3.31 (2H, br. d, 6 Hz, -CH ₂ -) 3.84 (3H, s, -OCH ₃) 3.87 (3H, s, -OCH ₃) 5.09 (1H, br. m, CH ₂ =) 5.23 (1H, br. m, CH ₂ =) 5.94 (2H, s, -OCH ₂ O-) 5.95 (1H, br. m, -CH=) 6.30 (1H, s, H ₆)	279 (1070)
DILL APIOL M. Wt. = 222 $C_{12}H_{14}O_4$		223(14), 222(M ⁺ , 100) 207(20), 177(32) 149(16), 121(11) 101(13), 83(11) 59(27), 58(22), 55(11) 44(38), 43(94)	3.30 (2H, br. d, 6 Hz, -CH ₂ -) 3.75 (3H, s, -OCH ₃) 4.01 (3H, s, -OCH ₃) 5.09 (1H, br. m, CH ₂ =) 5.23 (1H, br. m, CH ₂ =) 5.88 (2H, s, -OCH ₂ O-) 5.89 (1H, br. m, -CH=) 6.35 (1H, s, H ₆)	283 (1010)
MYRISTICIN M. Wt. = 192 $C_{11}H_{12}O_3$		193(13), 192(M ⁺ , 100) 191(13), 165(22) 161(15), 147(10) 133(10), 131(14) 119(13), 91(24) 77(12), 65(13) 57(12), 55(17) 43(110)	3.30 (2H, br. d, 6 Hz, -CH ₂ -) 3.90 (3H, s, -OCH ₃) 4.98 (1H, br. m, CH ₂ =) 5.18 (1H, br. m, CH ₂ =) 5.93 (1H, br. m, -CH=) 5.94 (2H, s, -OCH ₂ O-) 6.40 (2H, br. s, H ₄ , 6)	277 (1090)

Figure 3. Data obtained by instrumental analyses for the four insecticidal constituents isolated from dill plants: (a) determined on a double-focusing mass spectrometer, Model MS902 (A.E.I.); peaks >10% of base are listed; (b) obtained with Varian T-60 or Bruker Model HX90E instruments; $CDCl_3$ solvent; chemical shifts in parts per million downfield from TMS; data in parentheses: number of protons, multiplicity (s, singlet; d, doublet; m, multiplet; br., broad; incompletely resolved, signal), coupling constant, and assignment; (c) obtained with a Beckman DB spectrophotometer, in methanol as solvent.

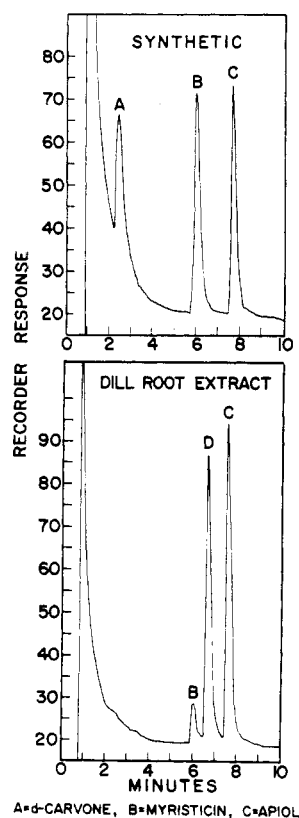


Figure 4. Gas chromatographic pattern obtained with an extract from dill roots in comparison to the chromatogram obtained with synthetic reference materials.

of dill-apiol in dill seeds was reported by Ciamician and Silber (1896).

The concentrations of the various biologically active compounds in the dill plant were determined by glc, utilizing synthetic *d*-carvone, myristicin, and apiol as reference materials. For the quantitation of dill-apiol, synthetic apiol was used as a standard. Results (Table II) indicated that *d*-carvone was the major insecticidal and synergistic component in dill greens, and its concentration amounted to 3625 ppm. Small amounts (24 ppm) of myristicin were also present. In dill roots, however, *d*-carvone was not detected. The major insecticidal and synergistic components were apiol and dill-apiol, present at concentrations of 271 and 227 ppm, respectively. Myristicin was also found in dill roots at a concentration of 41 ppm.

The biological activities of the various compounds isolated from dill plants were now investigated. Since the in-

Table II. Biologically Active Substances in Dill

Compd	Concn (ppm) in		LD ₅₀		
	Roots	Greens	<i>Drosophila</i> , ^a mg/jar	<i>Aedes</i> , ^b ppm	Mice, ^c mg/kg
<i>d</i> -Carvone	Trace	3625	2.21	107	1500
Apiol	271	ND ^e	0.29	11.5	500 ^d
Dill-apiol	227	ND	0.27	11.0	NA ^e
Myristicin	41	24	0.34	10.8	200 ^f

^a Fruit flies exposed to dry residues of each compound for 24 hr. ^b Mosquito larvae exposed to each compound in water for 24 hr. ^c Intravenous. ^d Intravenous with dogs (Merck Index, 8th Edition). ^e ND, nondetectable; NA, not available. ^f Lichtenstein and Casida, 1963.

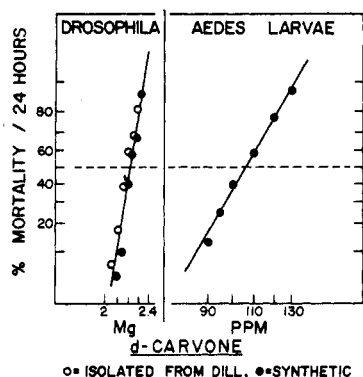


Figure 5. Dosage-mortality curves obtained after a 24-hr exposure of *Drosophila melanogaster* Meigen or *Aedes aegypti* L. larvae to naturally occurring and synthetic *d*-carvone.

secticidal and synergistic properties of myristicin had previously been reported (Lichtenstein and Casida, 1963), experiments were primarily conducted with naturally occurring *d*-carvone, apiol, and dill-apiol and also with synthetic *d*-carvone and apiol. In some cases synthetic myristicin was used for comparison purposes. In addition, data are provided relative to the mammalian toxicity of some of these plant derivatives.

Biological Activities of Dill Components. *d*-Carvone. The insecticidal activity of synthetic and naturally occurring *d*-carvone was measured by exposing different insects to this chemical. With fruit flies, serial dilutions of synthetic or naturally occurring *d*-carvone in hexane were pipetted in three replicates into bioassay jars. After evaporation of the solvent in a fume hood, 50 fruit flies were introduced into each jar. Insect mortalities were recorded after 24 hr. In experiments with mosquito larvae, synthetic *d*-carvone was added in acetone to water to yield a concentration of 130 ppm. From this, serial dilutions in water were prepared and pipetted into three times replicated vials, of which each finally contained 20 ml of water. Twenty third-instar larvae of *Aedes aegypti* L. were then introduced into each vial. Controls were prepared with solvents only. Mortality counts, performed after 24 hr of insect exposure, showed that increasing amounts of *d*-carvone caused increasing mortalities, resulting in typical dosage-mortality curves for the two insect species (Figure 5). With fruit flies a relatively steep curve was obtained with an LD₅₀ value of 2.21 mg of *d*-carvone per jar. With mosquito larvae, the curve was less steep with an LD₅₀ of 107 ppm. No appreciable difference in the insecticidal activity was evident between synthetic and dill-derived *d*-carvone, although the naturally occurring material appeared to be slightly more toxic. Saxena and Sharma (1972), reporting on the "Embryonic Inhibition and Oviposition Induction in *Aedes aegypti* by Certain Terpenoids," found that a deposit of 2 mg of *d*-carvone on 20 cm² of a 10-ml water surface inhibited egg hatchability by 65%.

House flies (*Musca domestica* L.) were rather unaffected when exposed to dry residues of synthetic or naturally occurring *d*-carvone in bioassay jars. With the relatively large deposit of 4 mg of synthetic *d*-carvone on glass surfaces an initial effect was generally noticed, since all flies were motionless after 1 hr of exposure. However, after an additional 23 hr, only 50% of the insects had died, thus indicating an initial knock down effect but subsequent recovery of the insects. Similar results were obtained with naturally occurring *d*-carvone.

As previously stated (Table I), crude extracts from both dill greens and roots increased the toxicity of several synthetic insecticides. These results were obtained with fruit flies utilizing equivalents of 0.56 g of dill greens and with mosquito larvae utilizing equivalents of 0.25 g of dill greens. Based on a *d*-carvone concentration of 3625 ppm,

Table III. Increase in the Toxicity of Synthetic Insecticides with *d*-Carvone^a

Insecticide, μg	% mortality of <i>Drosophila</i> /24 hr with synergist		
	None	<i>d</i> -Carvone, 2 mg	
		Synthetic	From dill
None	0	0	0
Carbofuran, 0.6	11 \pm 5	90 \pm 7 ^b	97 \pm 6 ^b
Carbaryl, 100	5 \pm 1	93 \pm 8 ^b	100 \pm 0 ^b
Parathion, 0.35	8 \pm 3	95 \pm 6 ^b	99 \pm 1 ^b
<i>p,p'</i> -DDT, 2.0	4 \pm 2	7 \pm 3	9 \pm 4

^a Test insects: *Drosophila melanogaster* Meig. Results are averages plus standard deviations of three times replicated tests. ^b Differences in mortalities observed with and without *d*-carvone (control) are significant at the 0.1% level.

the increased toxicities of the synthetic insecticides to fruit flies were probably due to the presence of 2.03 mg of *d*-carvone and to mosquito larvae due to the presence of *d*-carvone at a concentration of 45.3 ppm.

To further investigate the interaction of *d*-carvone with several synthetic insecticides, 2 mg of synthetic or dill-derived *d*-carvone was added to bioassay jars without or with analytical grade carbofuran (0.6 μg), carbaryl (100 μg), parathion (0.35 μg), or *p,p'*-DDT (2.0 μg). Fifty fruit flies were then introduced into triplicate test jars and insect mortality counts were performed after 24 hr (Table III). Although 2 mg of *d*-carvone was sublethal, a dramatic increase in insect mortality was observed with the two carbamate and the organophosphorus insecticides: insect mortalities increased from 5-11% with insecticides alone to 90%, when the insects were exposed to a combination of the synthetic insecticides plus *d*-carvone. The toxicity of DDT, however, was not affected.

To test these synergistic effects of *d*-carvone at smaller dosages, decreasing (1.8 to 0.8 mg) sublethal amounts of synthetic or dill-derived *d*-carvone were added to three times replicated bioassay jars, of which each contained a dry deposit of a standard amount of carbaryl (100 μg). Fruit flies were then exposed to these deposits for a 24-hr period. In triplicate, fruit flies were also exposed to 100 μg of carbaryl only, resulting in an 8 \pm 3% mortality of the insect population within the 24-hr exposure period. The addition of various amounts of *d*-carvone resulted, however, in typical dosage mortality curves (Figure 6). These showed that a 50% mortality of the fruit flies with 100 μg of carbaryl was achieved with 1 mg of naturally occurring *d*-carvone and with 1.4 mg of synthetic *d*-carvone. The fact that dill-derived *d*-carvone was more effective is unexplainable at this point. It is possible, however, that some impurity was present which could have been responsible for this effect.

A similar test was conducted with 0.35 μg of parathion per bioassay jar. While the insecticide alone caused only a

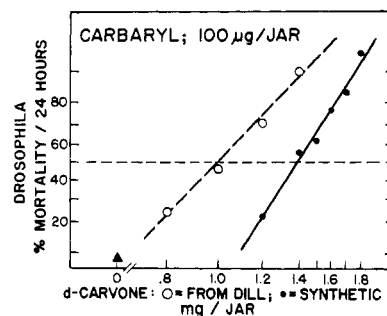


Figure 6. Effects of increasing amounts of *d*-carvone (0.8-1.8 mg/jar) on the toxicity of a constant dose of carbaryl to *Drosophila melanogaster* Meigen.

Table IV. Increase in the Toxicity of Synthetic Insecticides with Increasing Amounts of *d*-Carvone^a

Insecticide, ppm	% mortality of mosquito larvae/24 hr with <i>d</i> -Carvone, ppm									
	None	10		30		50		70		
		S ^b	D ^b	S	D	S	D	S	D	
None	0	0	0	0	0	0	0	5 ± 5	7 ± 3	
Carbofuran, 0.18	18 ± 3	17 ± 3	20 ± 9	23 ± 6	35 ± 10 ^c	68 ± 8 ^c	67 ± 8 ^c	83 ± 6 ^c	92 ± 6 ^c	
Carbaryl, 0.6	8 ± 3	23 ± 3 ^c	55 ± 10 ^d	42 ± 12 ^c	77 ± 3 ^c	73 ± 8 ^c	83 ± 6 ^c	98 ± 3 ^c	100 ^c	
Parathion, 0.016	13 ± 3	20 ± 5	28 ± 8 ^c	25 ± 5 ^c	37 ± 8 ^d	52 ± 8 ^d	70 ± 3 ^d	82 ± 8 ^c	90 ± 9 ^c	

^a Test insects: *Aedes aegypti* L. larvae. Results are averages plus standard deviations of three replicates. ^b S, synthetic *d*-carvone; D, derived from dill. ^{c-c} Differences in mortalities observed with and without *d*-carvone are significant at the c (0.1%), d (1%), and e (5%) levels.

9 ± 4% mortality of the fruit flies during a 24-hr exposure period, the lethal dosage to kill 50% of the insects was 0.35 µg of parathion plus 100% µg of synthetic *d*-carvone.

The synergistic effects of synthetic and naturally occurring *d*-carvone with conventional synthetic insecticides were also tested with mosquito larvae. Analytical grade carbofuran, carbaryl, and parathion were added as acetone solutions to triplicate 20-ml portions of tap water at 0.18, 0.6, and 0.016 ppm, respectively. Synthetic or naturally occurring *d*-carvone was then added to these vials at 0, 10, 30, 50, or 70 ppm, concentrations previously (Figure 5) shown to be sublethal. Twenty third-instar larvae were then introduced into each vial and mortality counts were performed after 24 hr. Again, highly significant mortality increases resulted from the presence of *d*-carvone (Table IV), particularly with carbaryl. With carbofuran alone at 0.18 ppm, 18 ± 3% of the insect larvae died within 24 hr, but 50% died when *d*-carvone was added at approximately 40 ppm. Similar results were observed with parathion.

Myristicin and Apiol. Kerr (1951), utilizing the Peet-Grady method, noted that myristicin and apiol at 2% w/v in a kerosene-based spray containing 0.1% w/v of pyrethrins increased the mortalities of houseflies and enhanced the "flight stimulating and knock-down properties of the pyrethrum-based sprays." However, he did not notice any insecticidal activity of myristicin or apiol.

The insecticidal and synergistic nature of myristicin has been described for several insect species by Lichtenstein and Casida (1963) after the chemical had been isolated from the edible parts of parsnips, a crop plant also belonging to the Umbelliferae family. In this present study, myristicin was found in both dill greens and roots, while apiol and dill-apiol were the major insecticidal components of dill roots. To test and compare the insecticidal activity of synthetic myristicin and synthetic apiol, experiments as described above were conducted with fruit flies. The insects were exposed in three replicates to serial dilutions of synthetic myristicin or synthetic apiol. Mortality counts performed after 24-hr exposure resulted in dosage-mortality curves as shown in Figure 7. Accordingly, the LD₅₀ value with myristicin was 0.34 mg per jar, but with apiol it was 0.29 mg per jar. Experiments with mosquito larvae indicated LD₅₀ values of 10.8 ppm with myristicin and 11.5 ppm with apiol.

The toxicities of naturally occurring apiol and dill-apiol were investigated with fruit flies and mosquito larvae. Both compounds were equitoxic with these two insect species, and their insecticidal activity was similar to that observed with synthetic apiol.

The synergistic activity of synthetic apiol was tested by exposing in triplicate as described 50 fruit flies to 100 µg of carbaryl, 0.6 µg of carbofuran, or 0.35 µg of parathion, to which none or 4 µg of apiol was added. After a 24-hr exposure, the per cent mortalities of the fruit flies exposed to apiol only were 0, to carbaryl only 7 ± 3%, and to carbaryl plus apiol 100 ± 0%. With carbofuran these figures were 0, 7 ± 1, and 36 ± 7%, and with parathion 0, 9 ± 1, and 62 ± 5%, respectively. This synergistic activity of apiol at 4 µg per jar was much more pronounced than the

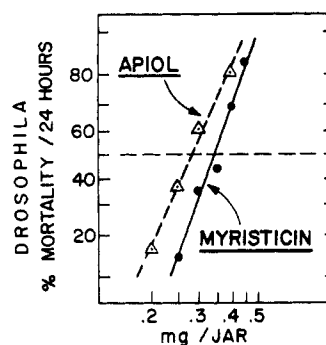


Figure 7. Dosage-mortality curves obtained after a 24-hr exposure of *Drosophila melanogaster* Meigen to synthetic apiol or synthetic myristicin.

one which previously (Table III and Figure 6) was reported with *d*-carvone at dosages of 1–2 mg per bioassay jar.

To determine the minimum amounts of apiol or myristicin that still would increase the toxicity of some synthetic insecticides to fruit flies, various sublethal dosages of synthetic apiol (0.25–1.5 µg per jar) or myristicin (1–5 µg per jar) were deposited in test jars in combination with standard amounts of either carbaryl (100 µg per jar) or parathion (0.35 µg per jar). Typical dosage mortality curves were obtained, of which those with parathion are shown in Figure 8. While with parathion alone 5 ± 2% of the fruit flies had died after 24 hr of exposure, an LD₅₀ value was observed through the addition of 1 µg of apiol or 1.45 µg of myristicin. With carbaryl only 7 ± 2% of the fruit flies had died during a 24-hr exposure period, while 50% of the insect population died due to the addition of 0.48 µg of apiol and 0.45 µg of myristicin.

Kerr (1951) stated that "adjuvant activity was found to be related directly to the number of methoxy groups present, while a methylene dioxy group is ineffective unless one or more methoxyl groups are also present." Thus, utilizing eugenol (one methoxy group), methyl eugenol (2 methoxy groups), and elemicin (three methoxy groups) he found that the property of these compounds to increase the toxicity of pyrethrins to houseflies increased with the

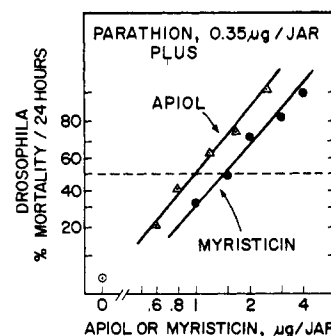


Figure 8. Effects of increasing amounts of apiol or myristicin as insecticide synergists with parathion applied at a constant rate.

number of methoxy groups present. Our tests with myristicin (one methoxy group) and apiol (two methoxy groups) seem to confirm this principle with fruit flies, since apiol was more toxic to these insects than was myristicin and also had a more pronounced synergistic activity with parathion (Figure 8) than did myristicin.

Toxicity to Mammals. Acute intravenous toxicity tests with mice and synthetic *d*-carvone were conducted by the Wisconsin Alumni Research Foundation. Adult male mice of the Swiss-Webster strain, weighing 25–35 g, were used. *d*-Carvone was diluted to a 5% concentration in distilled water and a drop of Tween 80 was added to facilitate the dispersion of the *d*-carvone in water. The test sample was administered *via* a tail vein and the treated animals were observed for a 2-week period. Under these test conditions synthetic *d*-carvone had an approximate intravenous LD₅₀ value of 1500 mg/kg.

Table II summarizes the available information pertaining to the intravenous toxicity of the insecticidal dill components. No figures for dill-apiol were obtained because of insufficient amounts of this compound at our disposal.

In summary it can be stated that of the four insecticidal and synergistic components isolated from dill plants, *d*-carvone was the least active. However, at relatively high concentrations it exhibited insecticidal properties by both contact and fumigant action. Moreover, at sublethal insecticidal dosages by itself it exhibited pronounced synergistic effects with the carbamate and organophosphorus insecticides tested. Myristicin, apiol, and dill-apiol from dill roots were considerably more toxic to insects than *d*-carvone and synergized the insecticidal activity at much smaller dosages. Based on the data reported above with standard amounts of carbaryl and parathion, the ratio of insecticide to synergist (apiol or myristicin) at the LD₅₀ value was 1:0.005 with carbaryl, 1:2.8 with parathion plus apiol, and 1:4.1 with parathion plus myristicin. Apiol, having one additional methoxy group in comparison to myristicin, appeared to be somewhat more toxic to fruit flies than myristicin, and was slightly more effective as a synergist with parathion, but not with carbaryl.

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Effect of Urea, Diethylstilbestrol, and Type of Diet on the Distribution of Aldrin and Dieldrin Residues in Finished Beef Heifers

Theron S. Rumsey and James Bond*

The effect of nutritional regimen on aldrin and dieldrin residue concentrations and distribution was studied in 16 Angus heifers. All heifers were on experiment from 14 days of age to slaughter (18 months) and were fed 1.0 mg of aldrin/kg of body weight daily from 42 days. The heifers were assigned to a factorial arrangement of nutritional regimens to compare urea *vs.* soybean meal, a concentrate *vs.* a forage diet, and diethylstilbestrol (DES) implants *vs.* no DES. The average concentration of aldrin was 7 times greater and

that of dieldrin 14 times greater in fat tissue than in organ and muscle tissues. The average tissue concentration of dieldrin was more than 100 times greater than that of aldrin. Among the fat tissues, the concentration of residues was highest in heart and kidney fats, intermediate in external rib and caul fats, and lowest in internal rib, brisket, perianal, and ruffle fats. Nutritional regimens did not affect the distribution of residues among fat tissues but did influence the average residue concentration in beef heifers.

Because of the persistence of chlorinated hydrocarbons in our environment, our food chain will be subject to low levels of residual contamination for many years after more

biologically labile substitutes are used (Fries, 1970). The select use of persistent pesticides for which there are no substitutes is also a source of low level environmental contamination. A meaningful monitoring system for persistent pesticides depends on knowledge of the distribution of these chemicals in biological systems.

Previous research has established that aldrin

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