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NATURALLY OCCURRING INSECTICIDES

Myristicin, an Insecticide and Synergist Occurring Naturally in the Edible Parts of Parsnips

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A chemical of insecticidal and strong synergistic nature was found in the edible parts of parsnips (*Pastinaca sativa* L.), which have been consumed for centuries by humans without causing any obvious harm. The insecticidal constituent, present at about 200 p.p.m., was isolated and identified as 5-allyl-1-methoxy-2,3-methylenedioxybenzene or myristicin. Its toxicity to various insects was established and compared with pyrethrum and aldrin. The knockdown effect, although definite, was not as great as that of pyrethrum. In tests with *Drosophila melanogaster* Meig., it acted as a repellent and also killed through fumigant action, characteristics not evident with pyrethrum. Comparison of the synergistic activity of myristicin and piperonyl butoxide with *Musca domestica* L., showed that piperonyl butoxide was better with the pyrethroids, and myristicin with the carbamates tested. With *Drosophila,* the synergistic activity of myristicin was superior with Sevin, similar with *p,p'*-DDT and allethrin, and inferior with pyrethrum as compared with piperonyl butoxide.

One of the most difficult aspects of the search for useful insect control chemicals is the attempt to evaluate the potential hazard of the compounds on prolonged ingestion by man. It is therefore intriguing to seek insecticidal components in the edible parts of plants long consumed by man and animals without causing any obvious harm. Such chemicals might be of further importance in naturally protecting the edible portions of certain crops from insect attack, thus reducing the necessity for applied methods of insect control.

In tests conducted at the University of Wisconsin, a chemical of insecticidal and also of strong synergistic properties was found in the edible parts of parsnips.

Experimental

Evidence of Insecticidal Activity. Parsnips (All American variety), *Pastinaca sativa* L. (*Umbelliferae*), were grown in a Carrington silt loam soil free from any insecticidal residues. After harvest, the edible part of the crops was washed with water and ground in a food grinder (Hobart, Model T-215 Food Cutter). Three grams of this material were placed on wet filter paper within each of six small bioassay jars (5.0 cm. in diameter and 6.3 cm. deep). Fifty vinegar flies (*Drosophila melanogaster* Meig.) were then introduced into each of the six jars and also into six control jars containing only wet filter paper (*6*). After an exposure time of 24 hours, 40% of the flies exposed to the parsnip material were dead, while no control mortality occurred. Apparently, parsnips contained some insecticidal substance. The ground parsnip material was then extracted and purified by a method described for other plant materials (*14*). Aliquots of the extract were then pipetted into bioassay jars, the solvent was evaporated at the opening of a fume hood, and 50 vinegar flies were introduced into each of the jars. All of the flies were dead after having been exposed overnight to residues representing 2.6 grams of parsnip material. In another test, flies were exposed to the residue of an extract representing 4.4 grams of parsnip material. After an exposure period of 100 minutes, 90% of the flies were knocked down or dead. Since some flies stuck to the plant residue deposited on the glass bottom of the bioassay jar, a fine metal screen was placed in front of this residue to prevent the insects from coming into direct contact with the insecticidal deposit. New flies were then introduced into the jars. After a 1-hour exposure time, 32% of the flies were motionless, and all had died after an additional 14-hour exposure period. No control mortality was observed. These preliminary experiments indicated that the substance derived from parsnips also killed by fumigant action.

Extraction and Purification. The following procedure was found to be the most efficient of several tried for extraction, purification, and isolation of the insecticidal principle.

After harvest, the edible parts of the parsnips were washed with water and then macerated in a food grinder. Four-hundred grams of the macerated crop material were then placed into a 2-quart, wide-mouthed Mason jar, and 900 ml. of redistilled acetone were added. After 1 hour of head-to-end tumbling, the supernatant liquid was decanted through filter paper, and the recovery volume was recorded. The acetone was then evaporated on a steam bath using a Vigreux reflux funnel. After the amount of the remaining water had been determined for correction of

the previously established recovery volume, the water was transferred quantitatively with 6% ether in pentane (commercial grade *n*-pentane, purified by passing through Florex and redistilling) into a 1000-ml. separatory funnel. Two-hundred milliliters of 6% ether in pentane and 20 grams of sodium chloride were added. After shaking and separation of the phases, the water layer was extracted two additional times with 200 ml. of this organic solvent mixture. The combined ether-pentane fractions were then dried over anhydrous sodium sulfate, combined in a 1000-ml. Erlenmeyer flask equipped with a Vigreux funnel, and concentrated in a 50° C. water bath to approximately 100 ml. The solvents were then transferred quantitatively into a 250-ml. Erlenmeyer flask and further concentrated to approximately 15 ml.

One gram of Nuchar activated carbon (C-190 N, pH 6) was added. The mixture was swirled gently for 1 minute and then filtered through a 1.3-cm. layer of asbestos with some glass wool on top, held in a glass tube (2 × 18 cm.). After several washings with a total of 140 ml. of 6% ether in pentane, the filtrate was concentrated in a 50° C. water bath to about 15 ml. and then added to a 10-gram Florisil (60/100 mesh) column (2.0 cm. diameter). To elute, 200 ml. of 6% ether in pentane were used. The extract was then concentrated in a 50° C. water bath to about 8 ml. and transferred quantitatively into a 12-ml. conical centrifuge tube. After all the solvents had evaporated, a yellow, viscous liquid remained in the tube. This material was next subjected to thin layer chromatography. It was spotted as a 17.8-cm. long band across silica gel (silica gel G, containing calcium sulphate as a binder, DESAGA-Heidelberg) on a glass plate (20 × 20 cm.), 2.5 cm. away from the lower edge. The chromatogram was then developed with 2,2,4-trimethylpentane so that the solvent front moved 15 cm. within 90 minutes. After it dried, three bands were clearly visible under a short wave ultraviolet lamp: a lower one (R_f 0.00–0.05) close to the spotting area; a wide medium one (R_f 0.05–0.64) of bluish appearance; and a few narrow bands (R_f 0.64–0.69) of a yellowish to white appearance. The various bands were scraped off the glass plate and tested individually by exposing vinegar flies to them. Only the medium band (R_f 0.05–0.64) of bluish appearance in ultraviolet light was insecticidal. This band was then used for further purification by placing the silica gel into a beaker containing 100 ml. of redistilled acetonitrile. After stirring this mixture for 15 minutes by means of an electric stirrer, the acetonitrile was filtered off under suction and transferred with the solvent washings of the silica gel into a 250-ml. separatory funnel containing 50 ml. of purified pentane. This acetonitrile-pentane mixture was then shaken to remove a pentane-soluble impurity. After the phases had separated, the acetonitrile was dried over anhydrous sodium sulfate and evaporated within a beaker, which was immersed in a 60° C. water bath, with a gentle stream of purified nitrogen. When a volume of about 8 ml. had been obtained, the contents of the beaker were transferred quantitatively into a 12-ml. conical centrifuge tube. The rest of the acetonitrile was then blown out with purified nitrogen. The yellowish, viscous material remaining in the tube was then centrifuged for 5 minutes at 2000 r.p.m. and the supernatant liquid spotted for a second time on silica gel as described above. The bands obtained were very similar to those previously mentioned, although this time the medium band appeared completely homogeneous under ultraviolet light. This band was removed, placed into 100 ml. of redistilled acetone, and stirred for 15 minutes. The acetone was then filtered off under suction, dried over anhydrous sodium sulfate, and evaporated with nitrogen as previously described. A clear, golden liquid of very pleasant odor which did not solidify at −18° C. was obtained. As will be discussed, this material was found to consist almost entirely of a single component. Its concentration within the edible part of parsnips was approximated as 200 p.p.m.

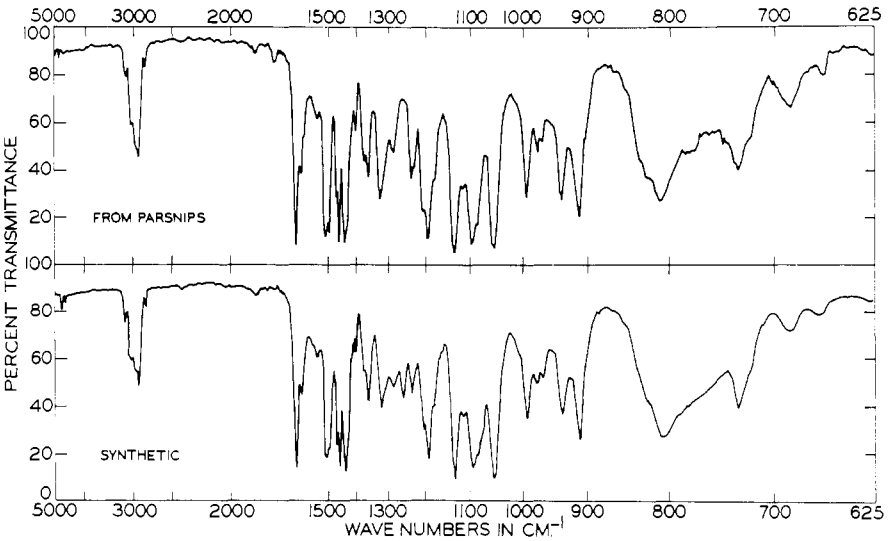


Figure 1. Infrared absorption spectra of myristicin from parsnips and synthetic

Spectra prepared from 5% carbon tetrachloride solutions with Baird B infrared spectrometer

Identification of Insecticidal Material as Myristicin. Elemental analyses and molecular weight determinations on two separate batches of the insecticidal oil from parsnips as recovered directly from the thin layer chromatograms were as follows: for the first, carbon 68.97%, hydrogen 6.57%, and molecular weight 184; and for the second, carbon 69.46%, hydrogen 6.68%, and molecular weight 188. No nitrogen or sulfur was present. These analyses suggested $C_{11}H_{12}O_3$, molecular weight 192, and 68.74% carbon and 6.30% hydrogen. Interpretation of the infrared (Figure 1) and ultraviolet absorption spectra indicated that the material might be myristicin (5-allyl-1-methoxy-2,3-methylenedioxybenzene) (*4*, *9*). Bromination of the oil in cold hexane and recrystallization of the product from absolute ethanol yielded crystals, m.p. 131.5°–132° C., uncorrected [m.p. reported for dibromomyristicin dibromide, 130.5°–131.5° C. (*18*); analyses calculated for $C_{11}H_{10}O_3Br_4$, carbon 25.91%, hydrogen 1.98%, and bromine 62.69%; analyses found, carbon 25.87%, hydrogen 2.19%, and bromine 60.21%].

A sample of synthetic myristicin, prepared by the procedure of Trikojus and White (*21*), was kindly provided by H. Jenkins, A. H. Robins Co., Richmond, Va. An ultraviolet spectrum of this product was identical to that of the oil from parsnips, and an infrared spectrum (Figure 1) was similar except for additional bands in the synthetic material, particularly at 1255 cm.$^{-1}$, and a shoulder at 1075 cm.$^{-1}$. Bromine, 45 μl., in cold glacial acetic acid, was slowly added to 30 mg. of the synthetic and natural oils in cold glacial acetic acid. Following bromination, the products were crystallized and recrystallized from ethanol to yield 62 mg., m.p. 130°–131° C., from the parsnip oil, and 54 mg., m.p. 130°–131° C., from the synthetic myristicin. The mixed m.p. was 129°–131° C.

The sample of synthetic myristicin was compared with the natural product using thin layer chromatography. One hundred micrograms of each sample were spotted on a silica gel thin layer plate, and the chromatogram was developed with 2,2,4-trimethylpentane and acetone

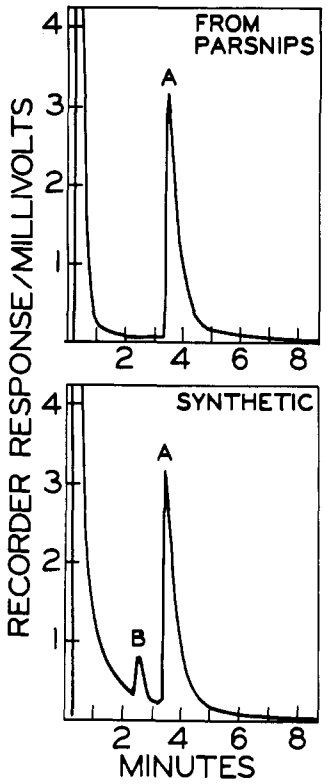


Figure 2. Gas chromatographic patterns obtained with myristicin from parsnips and synthetic

Insecticidal component designated as peak A and nontoxic as peak B

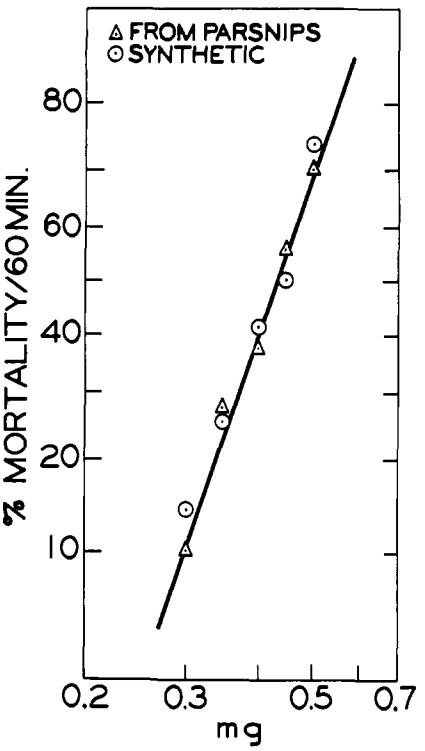


Figure 3. Dosage-mortality curves obtained after 60-minute exposure of *Drosophila melanogaster* Meig. to myristicin and synthetic

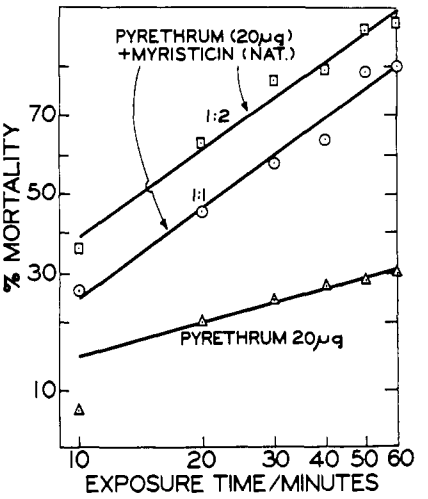


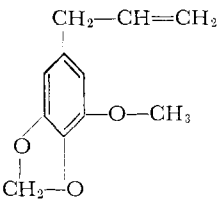
Figure 4. Time-mortality curves obtained with *Drosophila melanogaster* Meig. showing the potency of myristicin from parsnips as a pyrethrum synergist

(8:2). The spots were located by spraying with 0.05% sodium fluorescein in water, brominating, and observing the plate under short wavelength ultraviolet light. The parsnip oil yielded one purple spot, R_f 0.59, while the synthetic myristicin yielded two purple spots, R_f 0.59 and 0.70. To obtain larger amounts of these fractions, 10 mg. of synthetic myristicin were resolved into four components by thin layer chromatography with this solvent system. The synthetic myristicin yielded three components (R_f values of 0.22, 0.28, and 0.59) clearly distinguishable under ultraviolet light. These bands and the region of R_f 0.70, which had no components visible under ultraviolet light, were scraped from the glass plate and extracted with either acetone for examination by gas-liquid chromatography and bioassay, or carbon tetrachloride for determination of infrared spectra. The infrared spectrum of the R_f 0.59 component was almost identical with that of the parsnip oil, the infrared band at 1255 cm.$^{-1}$ and the shoulder at 1075 cm.$^{-1}$ having been greatly reduced on this purification. These bands appeared as major peaks in the R_f 0.70 impurity.

Gas-liquid chromatography also proved useful in comparing the synthetic myristicin with the parsnip oil. A Jarrell-Ash gas chromatograph, Model 700, equipped with a 100-mc. tritium argon diode ionization detector operated at 1200 volts was used. A 1.22-meter column (4.76-mm. i.d.) containing Chromosorb "W", 60/80 mesh, coated with 5% Dow 11 silicone grease was conditioned for 48 hours at 200° C. before use. A column pressure of 9 p.s.i. of argon gave a flow rate of 80 ml. per minute. The injector temperature was held at 250° C., the column temperature at 170° C., and the detector cell at 200° C. After injection of 0.5 μl. of acetone containing 5 μg. of the parsnip oil, one peak with a retention time of 3.41 minutes was registered (Figure 2). The injection of 5 μg. of synthetic myristicin, however, resulted in two peaks, one with a retention time identical to that of the natural product, 3.41 minutes, and the other with a 2.46-minute retention time. When the separated fractions from thin layer chromatography of the synthetic myristicin were used, the R_f 0.59 band yielded a single peak of retention time 3.41 minutes, while the R_f 0.70 band yielded the 2.46-minute retention time component as the only peak. The R_f 0.22 and 0.28 bands did not yield any peaks.

Bioassay with vinegar flies of the components from the synthetic myristicin separated by thin layer chromatography established that only the R_f 0.59 band was insecticidal. This indicated that the synthetic myristicin contained a nontoxic impurity responsible for the differences from the compound derived from parsnips, as evidenced by gas chromatography, thin layer chromatography, and infrared spectroscopy.

The structure of the insecticidal oil from parsnips was therefore concluded to be 5-allyl-1-methoxy-2,3-methylenedioxybenzene or myristicin:



To the best of the authors' knowledge, this compound has not been previously reported either as an insecticide or as a constituent of the edible part of parsnips. It is, however, known to be present in many other plants, including several Umbelliferae (*7, 10, 12, 13, 18, 20*).

Insecticidal Activity of Myristicin. TOXICITY TO *Drosophila melanogaster* MEIG. To obtain standard mortality curves, serial dilutions of myristicin (synthetic or derived from parsnips) were pipetted into bioassay jars. After evaporating the solvent (acetone) at room temperature at the opening of a fume hood, the jars were covered with a fine metal screen. Fifty vinegar flies were then introduced through a hole in the screen (*6*) and exposed for a period of 1 or 24 hours (44% R.H.; 23° ± 1° C.) to the residue remaining on the bottom of the glass jars. After that time, all motionless flies and those unable to stand, walk, or fly were counted as dead. Synthetic myristicin and the myristicin derived from parsnips were equitoxic and yielded identical dosage-mortality curves (Figure

Table I. Effect of Myristicin from Parsnips and Pyrethrum on Various Arthropods

Insecticide concentration: 0.5% w./v.

	Percent Knockdown or Mortality at Various Times after Exposure, Hours							
	Mexican bean beetles	Mosquito larvae	Pea Aphids (Contact)	Pea Aphids (Systemic)	Houseflies		Southern armyworm	Mites (contact)
Insecticide	48	24	48	120	2	24	48	120
Myristicin	100	100	60	50	6	6	0	28
Pyrethrum	100	100	100	20	100	98	100	100

Table II. Revival of Vinegar Flies after Exposure to Insecticides for Periods of 1 or 24 Hours

Insecticide, μg.	% Knockdown after Exposure Time: 1 hour	24 hours	% Mortality[a] 24 Hrs. after Exposure	% Revived
Myristicin (from parsnips)				
300	17		3	83
400	41		21	49
300		82	54	34
400		100	92	8
Myristicin (synthetic)				
300	15		0	100
400	28		5	82
Pyrethrum				
90	48		19	61
100	72		46	36
50		61	34	44
100		93	76	18
Aldrin				
60	63		100	0
70	88		100	0

[a] After exposure to the insecticides for 1 or 24 hours, flies (100 per test) were transferred to noncontaminated jars and held for 24 hours (total of 25 or 48 hours after introduction of the flies), when mortality counts were made.

3). The amounts of either chemical necessary to kill 50% of the flies were 430 μg. for a 1-hour exposure period and 300 μg. for a 24-hour exposure period.

Dosage-mortality curves were also obtained with actual pyrethrum (I and II) and aldrin. The slopes of the curves after a 1-hour exposure period were 1.06 (aldrin), 1.32 (pyrethrum), and 2.94 (myristicin). Although myristicin gave the steeper curve, pyrethrum and aldrin were more toxic than myristicin, and the LD_{50} values for 1- and 24-hour exposure periods were 62 and 26 μg. with pyrethrum, and 35 and 0.9 μg. with aldrin. When flies were exposed for 24 hours to either myristicin, pyrethrum, or aldrin, 0.5 ml. of a corn oil solution (0.25% in hexane) was added to the insecticidal solution to increase the residualactio n of the toxicants.

Toxicity to Other Insects and Mites. In preliminary screening tests conducted with 0.04% and 0.2% acetone solutions of nonpurified myristicin or pyrethrum, high toxicity resulted with the Mediterranean fruit fly (*Ceratitis capitata* Wied.) and the billbug [Calandra (=*Sitophilus*) *granaria* (L.)] from both materials. These tests were conducted at the Biological Institute, Farbenfabriken Bayer, Leverkusen, Germany, during the senior author's stay at that laboratory in 1962.

Experiments were also conducted by the Wisconsin Alumni Research Foundation, Madison, Wis., comparing the effect of the naturally occurring myristicin and pyrethrum on Mexican bean beetles (*Epilachna varivestis* Muls.), mosquito larvae (*Aedes aegypti* L.), pea aphids (*Acrythosiphon pisum* Harris), houseflies (*Musca domestica* L.), southern armyworms (*Prodenia eridania* Cramer), and spider mites (*Tetranychus atlanticus* McGregor).

Insecticidal solutions were prepared by dissolving 0.35 gram of actual test sample in 2 ml. of acetone, to which 0.2 ml. of emulsifier, Triton X-100, was added prior to dispersion in distilled water to a total volume of 70 ml. This resulted in a 0.5% solution. Tests with houseflies, spider mites, pea aphids (contact bioassay), and Mexican bean beetles were performed as previously described (*14*). Tests with the southern armyworm were done in the same way as performed with the Mexican bean beetle. To test the systemic action of the toxicants on pea aphids, 25 ml. of the 0.5% solution were poured on top of the vermiculite substrate in each pot in which young pea seedlings were emerging. Three days later, 10 adult pea aphids were placed on the leaves of the pea plants and held for 48 hours. Mortality counts were made after that time. With mosquito larvae, 10 third-instar larvae were placed into a beakers containing 50 ml. of water and 0.5% of the toxicants (5000 p.p.m.).

Results are summarized in Table I and show that myristicin at this relatively high dosage was especially effective against Mexican bean beetles and mosquito larvae.

In tests conducted in the authors' laboratory, 1 mg. of myristicin derived from parsnips was added in 0.1 ml. of acetone to 50 ml. of tap water (20 p.p.m.) within each of three test tubes. Ten third-instar mosquito larvae were then added to the water in each tube. After a 24-hour exposure period, 60% of the larvae were dead. When the concentration of myristicin in water was increased to 40 p.p.m. (0.004%), complete mortality of the mosquito larvae occurred during a 24-hour exposure period.

Toxicity to Houseflies Following Topical Application. Four-day-old adult female houseflies were treated topically on the ventral portion of the abdomen with 1 μl. of acetone containing the test substance. Two fly strains were used, a DDT-susceptible one (C.S.M.A., 1948 strain) and a DDT-resistant one (Fowler housefly, a field-collected strain resistant to DDT and methoxychlor from the area of Quincy, Ill., reared and subjected to limited DDT selection pressure in the laboratories of the Wisconsin Alumni Research Foundation). The insecticidal activity of myristicin, either natural or synthetic, was relatively low (LD_{50} 700 to 750 μg. per gram, 24 hours), but did not vary appreciably between the C.S.M.A. and the DDT-resistant strain (Table IV, footnote *c*).

Knockdown and Killing Effects. In previous experiments with vinegar flies, all insects which, after a certain exposure period to the toxicant, were either motionless or unable to stand, walk, or fly were counted as dead. This procedure, however, did not indicate whether the insects were merely knocked down or if they had actually died. To obtain information on the knockdown effect of myristicin on insects, vinegar flies were exposed for either 1 hour or 24 hours to amounts of myristicin (naturally occurring and synthetic) which were close to the predetermined LD_{50} values. For comparative purposes, tests were also conducted with pyrethrum and aldrin. After the 1- or 24-hour exposure period, all "dead" flies in each test jar were counted and registered as knocked down. They were then transferred, together with the remaining flies, into a noncontaminated test jar and held for 24 additional hours therein. After that time, all motionless flies were reported as dead. Except for those exposed to aldrin, a certain percentage of flies revived during the 24-hour period following exposure to the insecticidal deposits (Table II). More insects revived after only a 1-hour exposure period. With 400 μg. of myristicin derived from parsnips, 92% of the flies were actually killed within 24 hours. Synthetic myristicin was somewhat less effective, probably due to its nontoxic impurities.

Pyrethrum resulted in the most rapid knockdown. After introduction of the flies into the test jars, they walked on the pyrethrum-contaminated glass surface and consequently were in constant contact with the toxicant. Myristicin, however, apparently had a repellent effect on

the vinegar flies. Shortly after their introduction into the test jars, they assembled on the metal screen away from the contaminated glass bottom. This indicated that myristicin affected the flies through a fumigant action also.

FUMIGANT ACTION AND RESIDUAL EFFECTIVENESS. To test the fumigant action of myristicin (synthetic and derived from parsnips) or pyrethrum, aliquots of an acetone solution of either toxicant were pipetted onto watch glasses (7.6 cm. in diameter). Amounts of 200, 300, 400, and 800 μg. of the two myristicins and 500, 1000, and 1500 μg. of actual pyrethrum were used. Each test was conducted in duplicate. After evaporation of the solvent from the glass surface, a watch glass was placed on the bottom of each of 22 galvanized iron containers, 8.3 cm. in diameter and 17.2 cm. deep (*8*). A screened cage containing 50 vinegar flies was placed on glass rods within each of the 22 containers at a distance of 5.0 cm. from the watch glass. In this way, the flies could not come into contact with the insecticidal residue itself. Therefore, its fumigant action, if any, could be tested.

No mortalities were obtained in tests with pyrethrum. However, toxic vapors emanating from deposits of myristicin from either source gave similar results. After a 24-hour exposure period to fumes from 200, 300, 400, or 800 μg. of myristicin, knockdown of 0, 34, 79, and 100% of the flies, respectively, was registered. The insects were then removed with the screened cages from the galvanized iron containers and held for an additional 24 hours in a noncontaminated environment. Counts of dead flies after that time still resulted in mortalities of 0, 19, 67, and 96%, respectively.

To test the residual action of myristicin, those iron containers holding watch glasses with deposits of 800 μg. of the toxicant were re-used, 2 days after the insecticide had been placed onto the glass surfaces. New flies were introduced as described above, and 24 hours later 52% of the flies were knocked down. All revived, however, upon removal from the iron container. Under these conditions, no insecticidal action of myristicin residues was evident 5 days after preparing the insecticidal deposit on the watch glasses.

When bioassay jars were used, the residual properties of 500 μg. of myristicin (synthetic and derived from parsnips) and 50 μg. of actual pyrethrum were tested. The proper acetone solutions in two series were pipetted, then 0.5 ml. of a corn oil solution (0.25% in hexane) was added to one of the series. The solvents were then evaporated, and vinegar flies were introduced into each of the test jars and held for a 24-hour period. Each day 50 new flies were introduced until such time that the deposits were no longer insecticidal. It was found that 500 μg. of myristicin (synthetic and derived from parsnips) were effective for only 1 day. However, the addition of corn oil prolonged its residual effectiveness to 2 days. Pyrethrum was effective for 2 days, and this time was doubled by adding corn oil to the original insecticidal deposit. In view of the fumigant action of myristicin and its possible volatilization, these results are not surprising.

Table III. Comparison of Synergistic Activity of Myristicin (Derived from Parsnips and Synthetic) and Piperonyl Butoxide with Several Insecticides

Test insect: *Drosophila melanogaster* Meig.

			Synergists							
			None		Myristicin				Piperonyl Butoxide	
					From Parsnips		Synthetic			
Insecticide, μg.	μg.	Ratio I:S[a]	K[b]	M[c]	K	M	K	M	K	M
Pyrethrum										
10	...	...	0	*0*	..	...	..	...	...	...
10	10	1:1			9	*0*	15	*0*	72	*13*
10	50	1:5			57	*13*	55	*7*	90	*51*
10	100	1:10			91	*92*	83	*60*	100	*95*
..	100	...			0	*0*	0	*0*	0	*0*
Allethrin										
25	...	...	4	*3*	..	...	..	...	...	..
25	25	1:1			59	*61*	15	*15*	72	*68*
25	50	1:2			69	*71*	45	*38*	89	*98*
25	100	1:4			89	*95*	63	*68*	96	*96*
..	100	...			0	*0*	0	*0*	0	*0*
Sevin										
100	...	...	0	*10*	..	...	..	...	...	..
100	20	1:0.2			61	*98*	49	*100*	8	*40*
100	50	1:0.5			93	*100*	90	*100*	20	*48*
..	50	...			0	*0*	0	*0*	0	*0*
p,p'-DDT										
25	...	...	0	*22*	..	...	..	...	...	..
25	25	1:1			3	*29*	4	*13*	10	*42*
25	50	1:2			6	*25*	9	*37*	19	*45*
25	100	1:4			16	*75*	20	*76*	16	*57*
..	100	...			0	*0*	3	*0*	0	*0*

[a] Insecticide:synergist (w./w.). [b] *K* = % knockdown after 1-hour exposure time. [c] *M* = % mortality of flies 24 hours after having been removed from contaminated jars into noncontaminated jars. Exposure time to chemicals: 1 hour.

Synergistic Activity of Myristicin. Several compounds containing the methylenedioxyphenyl group with high insecticidal activity are known (*17*, *19*), and compounds with this radical form the basis for many commercial insecticide synergists. To determine whether myristicin also acted as a synergist, experiments were conducted with *D. melanogaster* and *M. domestica*. In preliminary tests, 50 vinegar flies were exposed to deposits of either 20 μg. of actual pyrethrum (I and II), 20 μg. of pyrethrum and 20 μg. of myristicin from parsnips, or 20 μg. of pyrethrum and 40 μg. of myristicin from parsnips. Mortality counts were made at 10-minute intervals for a 1-hour exposure period. The resulting time-mortality curves (Figure 4), based on four replicates each, indicated the synergistic effect of myristicin deposited at sublethal dosages with pyrethrum. The minimum amounts of myristicin to cause any fly mortality within a 1- or 24-hour exposure period were 300 μg. and 240 μg., respectively. The addition of myristicin to pyrethrum resulted in about three times higher mortality of the flies after a 1-hour exposure period. Moreover, the slopes of the resulting dosage-mortality curves increased from 0.29 (pyrethrum) to 0.72 (pyrethrum and myristicin).

In additional tests, 50 vinegar flies were exposed in duplicated bioassay jars to the dry deposits of either pyrethrum, allethrin, Sevin, or *p,p'*-DDT. They were also exposed to deposits of these insecticides to which either myristicin derived from parsnips, synthetic myristicin, or piperonyl butoxide had been added at various ratios. After a 1-hour exposure time, all motionless flies or those unable to walk, stand, or fly were registered as knocked down (*K* in Table III). All the flies were then transferred from the contaminated jars into noncontaminated ones and held for 24 hours, when mortality counts were made (*M* in Table III).

With female houseflies, serial dilutions of the same insecticides plus dimetilan, or the insecticides with the synergists in a 1:1 ratio, were applied topically to the ventral portion of the abdomen. Ten insects were used in tests replicated two to four times. Twenty-four hours later, mortality counts were made to obtain dosage-mortality curves.

Myristicin, from parsnips or synthetic, was found to have relatively good synergistic activity for certain insecticides, particularly Sevin, even in a 1:0.2 in-

Table IV. Comparison of Synergistic Activity of Myristicin from Parsnips and Piperonyl Butoxide with Several Insecticides

Test insect: *Musca domestica* L.

Insecticide[a]	Fly Strain	LD_{50}, μg. per Gram Fly Within 24 Hours		
		Synergists[b]		
		None	Myristicin[c]	Piperonyl Butoxide[c]
Pyrethrum	C.S.M.A.	26.0	15.0	5.2
Allethrin	C.S.M.A.	8.6	7.1	5.6
Dimetilan	C.S.M.A.	4.1	2.2	3.7
Sevin	C.S.M.A.	>3500.0	9.0	39.0
p,p'-DDT	C.S.M.A.	4.5	4.8	4.5
p,p'-DDT	Fowler	3250.0	325.0	715.0

[a] The compounds were of greater than 98% purity with the exception of pyrethrum, where the combined pyrethrum I and II constituted 48% of the sample. The values reported for pyrethrum are for technical material. The dimetilan used was an analytically pure sample of 2-dimethylcarbamyl-3-methylpyrazolyl-(5)-dimethylcarbamate. [b] 1:1, insecticide:synergist (w./w.). [c] The LD_{50} values for myristicin (from parsnips and synthetic) alone with both the DDT-susceptible (C.S.M.A.) and DDT-resistant (Fowler) strains of flies were 700–750 μg. per gram, 24 hours. With piperonyl butoxide, LD_{50} values of 1800–2200 μg. per gram, 24 hours, for both fly strains were obtained.

secticide : synergist ratio. With *Drosophila*, the synergistic activity of myristicin was far superior to that of piperonyl butoxide using Sevin, about comparable with allethrin and *p,p'*-DDT, and inferior to pyrethrum (Table III). With *Musca* (Table IV), neither piperonyl butoxide nor myristicin synergized the toxicity of *p,p'*-DDT with the susceptible C.S.M.A. strain, but some synergistic activity was apparent with the Fowler resistant strain, particularly with myristicin. The selectivity of the synergists noted with *Drosophila* was also marked with *Musca*, where again the myristicin was superior to Sevin and possibly dimetilan; however, it was definitely inferior to pyrethrum and possibly also to allethrin. The synergistic activity of myristicin (as nutmeg oil) for pyrethrum to houseflies has been previously noted (*11*), but not the insecticidal activity.

Detoxification of the two carbamates in insects occurs by hydroxylation mechanisms (*1*, *2*). With the rat liver microsome system which degrades Sevin by hydroxylation (*5*), 100 μg. of myristicin added to the incubation mixture was highly inhibitory to these reactions. This is of further interest in respect to the results on synergism of DDT. With *Musca*, which presumably owe their resistance to an adaptive DDT-dehydrochlorinase (*15*), the synergists were less effective than with *Drosophila*, where the tolerance to this compound is associated with hydroxylation of the DDT to yield 2,2 - *bis* - (*p* - chlorophenyl) - 2 - hydroxy-1,1,1-trichloroethane (*16*, *23*).

Toxicity to Mammals. Acute toxicity tests with mice were conducted by the Wisconsin Alumni Research Foundation. Adult male mice of the Swiss-Webster strain, weighing 25 to 35 grams, were used. Myristicin derived from parsnips was diluted to a 5% concentration in distilled water, and a drop of Tween 80 was added to facilitate the dispersion of the test sample in water. For the determination of the acute oral toxicity, the aqueous suspension was administered by means of a stomach tube. For intravenous toxicity tests, the sample, prepared as above, was administered via a tail vein. All treated animals were observed for a 2-week period.

Under these test conditions, myristicin derived from parsnips had an approximate LD_{50} value of 1000 mg. per kg. orally and 200 mg. per kg. intravenously. Animals receiving 1000 mg. per kg. or more of myristicin for oral toxicity tests exhibited muscular tremors and prostration within 1 hour after treatment and until death occurred.

Several studies have been reported on the toxicity of nutmeg constituents, including myristicin, to vertebrates (*3*, *22*, *24*).

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