Unistat, and Polystat formed in the chicken tissues all give positive tests with the method for the determination of 3-amino-5-nitro-*o*-toluamide described by Thiegs, Smith, and Bevirt (*7*). These residues cannot, therefore, be distinguished by this method. If the derivatives of Zoamix and Unistat are converted back to their corresponding dinitro compounds by treatment with peracetic acid, they can then be distinguished by the 1,3-diaminopropane test.

When the derivative of Polystat is treated with peracetic acid and tested with 1,3-diaminopropane, a negative test is obtained. This will, therefore, distinguish it from the derivatives of Zoamix and Unistat.

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NATURALLY OCCURRING INSECTICIDES

Identification of 2-Phenylethylisothiocyanate as an Insecticide Occurring Naturally in the Edible Part of Turnips

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A chemical having insecticidal properties was found in the edible part of turnips and was identified as 2-phenylethylisothiocyanate. In the turnip variety investigated (Purple Top Strap Leaf), the concentration amounted to 63 p.p.m., as determined by bioassay. The toxic action of 2-phenylethylisothiocyanate against various insects has been established. It has a definite knock-down effect which is not as great as that of Lethane 384; however, its killing effect is superior to that of Lethane 384. 2-Phenylethylisothiocyanate is an insecticide which occurs naturally in plant tissue that has been consumed for centuries by humans without causing any obvious harm.

CHEMICALS having pesticidal activity are synthesized yearly and marketed in relatively large amounts for agricultural purposes. These chemicals, which are applied to either crops or soils, are in many cases of residual nature. If proper precaution is not exercised, these residues may contaminate animal and human food supplies. Therefore, all pesticides, unless they are of low persistence, necessarily have to have a low mammalian toxicity as determined by extensive animal tests. From such toxicity data, conclusions are then drawn relative to humans.

In tests conducted at the University of Wisconsin, a chemical of insecticidal action was found in the edible part of turnips. Since this crop has been consumed for centuries by humans and no harmful effects have been attributed to it, the toxicity of the insecticidal compound to humans cannot be high.

Preliminary Experiments with Turnips

Turnips (Purple Top Strap Leaf) were grown in a soil (Carrington silt loam), which did not contain any insecticidal residues. After harvest, the edible part of the crops was washed with water and then macerated in a food grinder to a puree-like consistency. Three grams of this material were placed on filter paper in each of eight small test jars (bioassay jars—$2^3/_4$ inches in diameter and 3 inches deep). Fifty vinegar flies (*Drosophila melanogastor* Meig.) were then introduced into each of the test jars (*2*). Shortly after exposure, the flies were affected by paralysis; 50% of the test insects were dead within 2 to $2\ ^1/_2$ hours. Total mortality was registered 3 hours after exposure. It was evident in these early tests that the action of the insecticidal principle resembled a relatively fast knock-down effect.

During the next step, the edible part of the turnips was extracted and purified. For this purpose, 350 grams of the macerated crop material was mixed with 750 grams of anhydrous sodium sulfate; the mixture was kept overnight in a refrigerator and then placed in a 2-quart wide-mouthed Mason jar. A mixture of 900 ml. of commercial grade *n*-pentane (purified by passing through Florex and redistilling) and acetone (4 to 1 by volume) was then added. After 1 hour of head-to-end tumbling, the jars and their contents were chilled to minimize evaporation of solvents during filtering. The supernatant liquid was then decanted through glass wool, and the recovery volume was recorded.

The acetone was removed from the extracts by washing once with water and three times with a 2% solution of sodium sulfate. The pentane solution was then dried over anhydrous sodium sulfate and concentrated to about 25 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 $^1/_2$-inch layer of asbestos with some glass wool on top, held in a glass tube ($7 \times {}^3/_4$ inch). After several washings with a total of 140 ml. of pentane, the clear filtrate was concentrated in a 50° C. water bath and then added to a 10-gram Florisil (60/100 mesh) column (20 mm. diameter). To elute, 150 ml. of 6% ether in pentane was used. Finally, the extract was adjusted to volume with pentane.

The insecticidal activity of the purified extract was tested by pipetting aliquots representing 34.88 grams of turnip material into bioassay jars. After the solvent had been evaporated at room temperature at the opening of a fume hood, the jars were covered with a fine screen. Fifty vinegar flies were intro-

duced through a hole in the screen (*2*) and were thus exposed to the residue from the turnip extract. When houseflies (*Musca domestica* Linn.) or confused flour beetles (*Tribolium confusum* Duvae) were used, the insects were anesthetized with carbon dioxide, and then 25 houseflies or 50 beetles were placed into three times replicated test jars. Mortality counts were made 0.5, 0.75, 1, 2, and 18 hours after the insects had been exposed to the insecticidal residue. Approximately 50% of the flies were dead within 30 minutes, and nearly 100% mortality was registered after an additional 15 minutes. The effect on flour beetles, however, was slower, since 2 hours of exposure were necessary to cause 90 to 100% mortality.

To test the fumigant action of the insecticidal principle in turnip extracts, aliquots representing 18.48 grams of turnip material were pipetted either into bioassay jars or onto watch glasses (3-inch diameter). After evaporation of the solvent from the glass surfaces, a watch glass was placed on the bottom of each of three galvanized iron containers (*3*), 3 1/4 inches in diameter and 6 3/4 inches deep. A screened cage containing 50 vinegar flies was placed on glass rods within each of the three containers, at a distance of 2 inches 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 and compared with the contact action within the three times replicated bioassay jars. Flies were killed by the insecticidal vapors; 90% of the insects were killed after 3 hours of constant exposure within the closed container. However, when the flies were also able to contact the insecticidal residue, only 1.5 hours were required. In view of these results, efforts were next made to isolate and identify the insecticidal principle of turnip roots.

Identification of the Insecticide

A portion of the pentane-ether fraction prepared from turnip roots as described above was placed in an all-glass, short-path distillation apparatus, and the solvents were removed. The material, which sublimed across a path length of about 2 cm. at 170° C. under approximately 15 mm. of mercury gage pressure, was subjected to biological and gas chromatographic analyses. The bioassays, carried out on vinegar flies as described above, showed that all the insecticidal activity was in the volatile fraction, and that none was left in the trace of nonvolatile residue.

The pentane-ether fraction was then examined directly by gas-liquid chromatography. The components of this fraction could be separated on a column in which the liquid phase was a silicone oil. The column packing was prepared by the evaporation of 5 parts of M and B silicone oil (May and Baker Co., Dagenham, England) on 100 parts of 30/60 mesh Chromosorb W (Fischer Scientific Co., Chicago, Ill.) with brisk stirring and moderate heat. The packing was dried 48 hours at 155° C. before it was used to fill a glass column, 1.8 meters long by 7.5 mm. inside diameter. The chromatograms were run on a Barber-Coleman Model 10 gas chromatograph with a 20-mc. Sr^{90} argon ionization detector operated at 500 volts. A pressure of 11 p.s.i.g. of argon at the column entrance gave a flow rate of 100 ml. per minute. The flash heater was held at 197° C., the column at 154° C., the detector cell at 174° C., and the collection exit at 190° C. during the run. Full-scale recorder response of 50 mv. corresponded to 10^{-9} ampere in the detector cell.

Gas chromatograms carried out in this way on aliquots of the pentane-ether solution from turnip roots showed three distinct components. Figure 1, *A*, shows a typical tracing. The three components represented by peaks 2, 3, and 4 accounted for practically all of the material in the fraction, although concentrated samples prepared by evaporation of the solvent prior to chromatography revealed several other minor peaks with longer retention times.

From general considerations of the volatility and odor of the active fractions, the presence of a mustard oil was suspected. The occurrence of a compound of this type—namely, 2-phenylethylisothiocyanate—in turnip roots has been demonstrated by Stahmann *et al.* (*9*). A sample of this compound was therefore synthesized by the method of Slotta *et al.* (*8*) as modified by Stahmann *et al.* (*9*), except that magnetic stirring of the reaction mixture was employed. A 34% yield of doubly distilled product, b.p. 102–103° C. (1.4-1.5 mm.) was obtained.

Figure 1, *B*, shows a gas chromatogram of synthetic 2-phenylethylisothiocyanate run under the same conditions as the natural turnip extract. The retention times of peak 3 of the extract (Figure 1, *A*) and of the synthetic material were in excellent agreement in the numerous gas chromatograms which have been run. Under the conditions used, the retention time for this component was 5.35 ± 0.05 minutes.

About 0.3 mg. of the material from peak 3 (Figure 1, *A*) was collected in a trap cooled in dry ice–chloroform, and its infrared spectrum in chloroform solution was determined using a special microcell (Cell 24496 for I.R.5 spectrophotometer, Beckman Instruments, Inc., Fullerton, Calif.). The spectrum was essentially identical to that obtained with synthetic 2-phenylethylisothiocyanate. Like other mustard oils, 2-phenylethylisothiocyanate has a sharp, acrid odor and is a potent lachrymator. The isolated

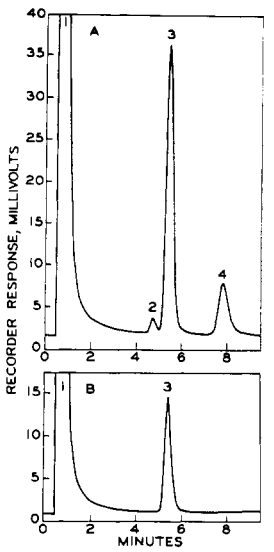

Figure 1. A. Turnip extract

B. Synthetic 2-phenylethylisothiocyanate

Peak identification
1. Solvent
2. Unknown
3. 2-Phenylethylisothiocyanate
4. Unknown

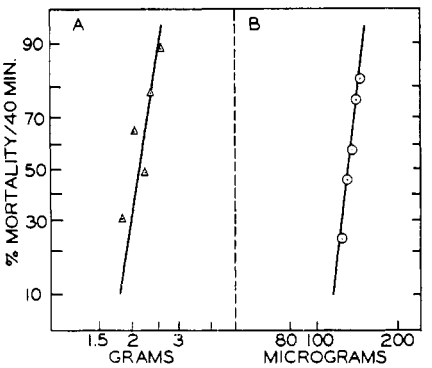

Figure 2. Dosage mortality curves obtained after 40-minute exposure of *Drosophila melanogastor* Meig. at 52% relative humidity and 72° ± 2° F. to serial dilutions of turnip extract (curve *A*) or 2-phenylethylisothiocyanate (curve *B*). For curve *A* the dosages are expressed as grams of fresh turnip tissue equivalent to the volume of extract used

and synthetic samples seemed to be identical in this regard.

Numerous isothiocyanates (mustard oils) have been encountered in various plants, in which they appear to exist in the form of glucosides (*4*). 2-Phenylethylisothiocyanate has been identified in turnips, cabbage, and horseradish (*9*), nasturtiums (*1*), and other plant species (*4*). The pronounced bacteriostatic and fungistatic activity of many naturally occurring mustard oils and of related synthetic products also has long been

Table I. Mortalities of *Drosophila melanogastor* Meig.

Grams[a]	Exposure to Insecticides, Minutes	Mortalities, % 2 hours	24 hours
SHORT EXPOSURE TO PURIFIED TURNIP EXTRACT			
2.45	10	20	26
2.45	20	16	30
3.43	10	50	88
3.43	20	63	96
SHORT EXPOSURE TO 2-PHENYLETHYLISOTHIOCYANATE			
Micrograms[b]			
150	10	6	20
150	20	10	16
170	10	21	59
170	20	33	79

[a] Weight, in grams, of fresh turnip tissue corresponding to amounts of extract tested.
[b] Weight, in micrograms, of synthetic 2-phenylethylisothiocyanate tested.

Table II. Effect of Corn Oil on the Persistence of 2-Phenylethylisothiocyanate on a Glass Surface

Hours after Depositing Insecticide	2-Phenylethylisothiocyanate 1 Mg.			0.5 Mg.		
	Time for 90% Mortality of Vinegar Flies, Minutes					
0	9[a]	18[b]	21[c]	11[a]	29[b]	36[c]
1	15[a]	22[b]	25[c]	19[a]	30[b]	50[c]
2.5	19[a]	24[b]	31[c]	21[a]	41[b]	63[c]
4.0	37[a]	47[b]	56[c]	[d]	97[b]	122[c]
5.5	[d]	100[b]				
22.0	[d]	[d]	[d]		[d]	[d]

[a] Insecticidal deposit only.
[b] 0.5 ml. of a 0.25% corn oil solution in hexane added.
[c] 1.0 ml. of a 0.25% corn oil solution in hexane added.
[d] No deaths occurred during the following 24-hour period.

Table III. Effect of 2-Phenylethylisothiocyanate and Lethane 384 on Various Arthropods

Insecticide	Per Cent W./V.	Houseflies 0.15	0.2	0.3	24	Pea Aphids 48	Mexican Bean Beetles 48	German Cockroaches 48	Mites 120
		Per Cent Knock-Down or Mortality at Various Times after Exposure, Hours							
2-Phenylethyl-isothiocyanate	1.00			100	96	100	100		100
	0.75	75	90	100	100	100	0	3	100
	0.50	50	75	90	96	100	0	0	100
	0.10				0	0	0		32
Lethane 384	0.75	100	100	100	63	100	0	0	90
	0.50	100	100	100	28	100	0	0	87

recognized (*4*, *6*). However, relatively few reports of the insecticidal activity of mustard oils have appeared, although the simplest representative of the group, methylisothiocyanate, has been offered commercially by Schering in Germany as an insecticide and fumigant. To the best of our knowledge, no previous report of the insecticidal activity of 2-phenylethylisothiocyanate has appeared.

Biological Activity of Purified Turnip Extracts and 2-Phenylethylisothiocyonate

Standard Curves and Threshold Concentrations. To obtain dosage mortality curves, vinegar flies were exposed to serial dilutions of either purified turnip extract or 2-phenylethylisothiocyanate (Figure 2). Using an exposure time of only 40 minutes (R. H. = 52%, $T = 72° \pm 2°$ F.), the dosage required for a 50% fly mortality was an extract representing 2.1 grams of turnip material or 130 μg. of 2-phenylethylisothiocyanate. A sample equivalent to 1.8 grams of turnip material or a dosage of 110 μg. of 2-phenylethylisothiocyanate was the lower limit (threshold) for significant mortality. However, any small increase in dosage above this threshold concentration resulted in a relatively large increase in mortality. The two dosage-mortality curves were nearly parallel and unusually steep, as compared to standard curves obtained with the chlorinated hydrocarbon group of insecticides. Using the data in Figure 2, the amount of 2-phenylethylisothiocyanate in the fresh tissue of turnip roots was estimated as 63 ± 5 p.p.m. This figure was in good agreement with similar tests conducted at different times. Stahmann *et al.* (*9*) reported 85 to 245 p.p.m. of 2-phenylethylisothiocyanate in different varieties of turnips.

A quantitative chromatographic estimate of the amount of 2-phenylethylisothiocyanate contained in the pentane-ether turnip extract fraction was also made. By chromatographing various dilutions of a pentane solution of the synthetic material, we found that the average detector response was 3.6×10^4 mv. per μl. From several comparative runs with the natural and synthetic materials using solutions of such dilutions as to give very similar peak heights, we estimated that the turnip extract contained 2-phenylethylisothiocyanate in an amount corresponding to about 29 ± 2 p.p.m. in the original roots. If the detector sensitivity to the substances represented by peaks 2 and 4 in Figure 1, *A*, is assumed to be the same, the heights of these peaks would correspond to about 1.7 and 5.6 p.p.m. of these components, respectively, in the turnips used. The lower value obtained by the GLC analysis as compared to bioassay may indicate another insecticidal substance in the pentane-ether fraction. This possibility is now being investigated.

Knock-Down and Killing Effects. Vinegar flies were exposed for 10 or 20 minutes to various amounts of either purified turnip extract or 2-phenylethylisothiocyanate. After this relatively short exposure period, the flies were transferred to uncontaminated bioassay jars in order to determine whether the insects were only knocked down or killed. Therefore, mortality counts were made 2 and 24 hours after removal of the insects from the insecticidal residues. All the flies which were motionless or paralyzed during or shortly (1/2 to 1 hour) after the 10- or 20-minute exposure period to the insecticidal residues did not revive during the following day (Table I). In fact, mortality counts made 24 hours after removal of the insects from the contaminated test jars resulted in larger mortality figures compared to those made 22 hours previously. Thus the insects were affected within the relatively short exposure time, and the mortalities increased during the 24 hours following exposure.

The toxicity of 2-phenylethylisothiocyanate as compared to aldrin was also studied. Two hundred microgram portions of the insecticides were pipetted in hexane solution into test jars; the solvent was evaporated at room temperature in a fume hood. Fifty vinegar flies were then exposed to the residues in each of three replicated jars. The speed of action of 2-phenylethylisothiocyanate (LD_{50} in 22 minutes, LD_{90} in 38 minutes) was higher than that of aldrin (LD_{50} in 38 minutes, LD_{90} in 42 minutes). However, during a 24-hour exposure period, a residue of 0.7 to 0.8 μg. of aldrin caused a 50% mortality of vinegar flies, whereas 2-phenylethylisothiocyanate at this concentration was inactive. Vinegar flies bounce and

spin around after being affected by aldrin, whereas exposure to 2-phenylethylisothiocyanate above the threshold level causes rapid immobilization followed by death, even after removal from the insecticidal residue.

The residual toxicity of 2-phenylethylisothiocyanate was compared with aldrin and dieldrin. Five hundred micrograms of insecticide were deposited as described above onto the glass surface (bottom) of bioassay jars; 50 vinegar flies were then introduced into each of the replicated jars. The time necessary to kill approximately 90% of the flies was 17 minutes with 2-phenylethylisothiocyanate, 39 minutes with aldrin, and 70 minutes with dieldrin. Twenty-four hours later, other flies were exposed to the same jars; no deaths occurred in those jars into which 2-phenylethylisothiocyanate had been deposited previously. With aldrin and dieldrin, however, 90% mortalities of the flies occurred after 45 and 65 minutes, respectively. After an additional 48 hours, those times for aldrin and dieldrin were 75 and 105 minutes, respectively.

These figures indicate that during the first 24 hours, the amount of 2-phenylethylisothiocyanate was reduced below the threshold level, whereas the other two insecticides persisted much longer.

In view of the relatively low residual properties of 2-phenylethylisothiocyanate, tests were conducted to find out if, and to what an extent, the residual properties of this compound could be increased. Amounts of either 0.5 or 1.0 mg. of 2-phenylethylisothiocyanate were pipetted as hexane solutions into test jars in 3 series. To one of the 3 series, 0.5 ml. of a corn oil solution (0.25% in hexane) was added and to another 1.0 ml. After evaporation of the solvent, vinegar flies were exposed to all jars initially and then 1, 2.5, 4, 5.5, and 22 hours after deposit of the insecticide. Results (Table II) indicate that the presence of small amounts of corn oil decreased the volatility of 2-phenylethylisothiocyanate or in some other manner prolonged its insecticidal activity. Moreover, corn oil apparently had some masking effect on the insecticide, since the time necessary to obtain a certain fly mortality was greater than in those tests where no corn oil was used.

Comparison of the Effects of 2-Phenylethylisothiocyanate and Lethane 384. Experiments were conducted by the Wisconsin Alumni Research Foundation, comparing the effects of 2-phenylethylisothiocyanate and Lethane 384 (2-butoxy-2′-thiocyanodiethyl ether) on houseflies (*Musca domestica*), pea aphids (*Macrosiphum pisi*), spider mites (*Tetranychus atlanticus*), Mexican bean beetles (*Epilachna varivestis*), and cockroaches (*Blatella germanica*).

Insecticidal solutions were prepared by dissolving 1.5 grams of the test sample in 1 ml. of petroleum ether (Skellysolve B) to which 1 ml. of emulsifier, Triton x-100, was added prior to dispersion in distilled water to a total volume of 150 ml. This resulted in a 1% w./v. stock solution, from which the other test levels of 0.75, 0.50, and 0.10% solutions were prepared by further dilution with distilled water. In all experiments, the test solutions were used within 2 hours after preparation.

For testing, 50 adult houseflies (CSMA strain) or 20 adult male German cockroaches were sprayed in stainless steel cages (2 inches high and 5 inches in diameter), which were faced on top and bottom with a 14-mesh screen. The insects were sprayed for 10 seconds by means of a Waters vertical spray tower (10 p.s.i.g. pressure) which delivered approximately 30 ml. per minute. Flies were retained in these cages for 24 hours; cockroaches were retained here for 48 hours. Mortalities resulted either from residues deposited through spraying directly on the insect body or through contact with residues within the cages.

Adult pea aphids (10 per replicate) were transferred to pea seedlings and then sprayed with either 2-phenylethylisothiocyanate or Lethane 384 at various concentrations. Mortality counts were made 48 hours later.

In tests with spider mites, excised Lima beans (stems and leaves) were dipped into the test solution. After that 50 to 100 mites were exposed to each plant and held for 5 days. Mortalities of the adults were then recorded.

Mexican bean beetle larvae (late 2nd instar) were exposed (10 per replicate) to Lima bean leaves, which had been sprayed on both sides with insecticidal solutions. Mortality counts were made 48 hours later.

Results are summarized in Table III. Lethane 384 produced faster initial knockdown of houseflies than did 2-phenylethylisothiocyanate, but during a 24-hour period, the latter compound caused a total mortality, whereas Lethane killed only 28 and 63% of the flies at the concentrations tested. The mortalities of pea aphids and mites were also very high at most concentrations. However, Mexican bean beetles and cockroaches were not affected by the 0.75 and 0.5% insecticidal solutions.

The sharp threshold properties of 2-phenylethylisothiocyanate were indicated by the fact that a concentration of 1% w./v. caused a 100% mortality of Mexican bean beetle larvae, whereas none was killed with a 0.75% solution.

Screening tests with vinegar flies showed that Lethane 384 caused an extremely fast knock-down. However, mortalities observed 24 hours after a half-hour exposure period to 200 μg. of 2-phenylethylisothiocyanate were considerably higher than with 200 μg. of Lethane 384.

The toxicity of 2-phenylethylisothiocyanate to mammals has been investigated by several workers. The subcutaneous LD_{50} in mice was reported as 250 mg. per kg. by Klesse and Lukoschek (*5*) and as 840 mg. per kg. by Osswald (*7*).

Mammalian toxicity tests were also conducted by the Wisconsin Alumni Research Foundation. Adult male mice of the Swiss-Webster strain, weighing 20 to 35 grams, were used in these studies. For the determination of the acute oral toxicity, 2-phenylethylisothiocyanate was administered in a corn oil suspension as a single calculated dose orally by means of a stomach tube.

For subcutaneous and intravenous toxicity tests, a drop of Tween 80 was added to the test sample to facilitate dispersion in water. The aqueous suspension was administered as a single calculated dose either subcutaneously or intravenously via the tail vein.

All treated animals were observed for a 2-week period.

Under these test conditions, 2-phenylethylisothiocyanate had an approximate LD_{50} value of 700 mg. per kg. orally, 150 mg. per kg. subcutaneously, and 50 mg. per kg. intravenously.

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