to 0.06 percent (*8*) and is similar both structurally and pharmacologically to the furanochromones khellin and khellol (*9*). Visnagin may be used for the treatment of coronary thrombosis and angina pectoris, and it will increase coronary blood flow (*9*).

Aseptically germinated seeds of *A. visnaga* formed callus tissue on a modified Murashige and Skoog's medium (MT) (*10*) containing 1.0 part of 2,4-dichlorophenoxyacetic acid (2, 4-D) per million and 0.7 percent agar. The callus was subcultured and maintained on the agar medium for at least 6 months before being transferred to liquid MT medium containing 0.1 ppm of 2,4-D. Suspension cells were grown in 500-ml erlenmeyer flasks, containing 100 ml of the medium, while being shaken on a reciprocal shaker (80 strokes per minute, 5 cm in length) at room temperature. A daily 8-hour light period was provided (1375 lu/m$^2$ from 40-watt, cool-white, fluorescent tubes, Amplex Corp.), and the suspension cultures acquired a greenish-yellow color. The cultures were transferred every 3 weeks into new liquid medium. The cultures were approximately 2 years old at the time of collection. After each 3-week growth cycle the cells were filtered, and the filtrate was evaporated in a rotary evaporator to about one-fourth the original volume. On an average, 0.165 g (dry weight) of inoculum produced 0.612 g (dry weight) of the cells. The cells and the concentrated medium were frozen and accumulated until enough was obtained for extraction.

The accumulated frozen cells from suspension cultures were thawed and then dried in an oven at 80°C for 16 hours. The dried cells (150.5 g) were extracted with ethylene dichloride for 48 hours in a Soxhlet apparatus. Similarly, the frozen liquid medium from 13 liters of the original filtrate was thawed and diluted with an equal volume of water. Approximately 900 g of a carbohydrate-like gel that precipitated was removed by filtration. The medium was then extracted in separatory funnels with ethylene dichloride until a portion of the extract, as tested by thin-layer silica-gel chromatography, contained no visnagin.

Both the cell and liquid medium extracts were then combined and concentrated to 40 ml for subsequent application and purification on thick-layer plates (approximately 0.4 to 0.5 mm). The adsorbant used was purified silica-gel (Adsorbosil-2; Applied Science Labs. Inc., State College, Pa.); it was activated by heating the plates at 100°C for 30 minutes. A portion (1 or 2 ml) of the combined extract was applied as a streak to each plate (application-I) and the plates were developed in a mixture of chloroform and ethanol (98.5 : 1.5). Material from the yellow-green ultraviolet fluorescent zone corresponding to reference visnagin (*11*, $R_F$ 0.46) was eluted with ethylene dichloride (reagent grade). The eluate was applied again (application-II) to the thick-layer plates and developed in a mixture of chloroform and methanol (95 : 5). Material from the fluorescent zone again corresponding to reference visnagin ($R_F$ 0.38) was recovered. Small quantities of the eluate from the application-II when rechromatographed contained only a single zone in both of the above solvent systems, and in a mixture of butanol, water, and formic acid (10 : 10 : 1) as well. A single zone was also present in the three solvent systems when the plates were sprayed with either 20 percent antimony trichloride in chloroform or anisaldehyde reagent (1 ml of anisaldehyde in 2 ml of concentrated sulfuric acid and diluted to 100 ml with glacial acetic acid) and viewed under ultraviolet light.

The chromatographically pure visnagin eluate was then evaporated, and an amorphous material was obtained. A portion of this material and an authentic sample of visnagin were separately dissolved in small amounts of methylene chloride (spectral grade). Infrared spectra of both the solutions were similar (Fig. 1). The ultraviolet spectrum for the isolated compound in a methanol solution was the same as that for reference visnagin, namely a maximum absorption at 242 m$\mu$. The remainder of the eluate from application-II was dissolved in a minimal amount of methanol and water mixture (approximate ratio 90 : 10) and crystallization was initiated by scratching with a glass rod. The total yield of visnagin crystals obtained was 6.4 mg.

The melting point of the isolated visnagin crystals was 144° to 145°C, the same as that reported for standard visnagin (*8*).

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## Food Safety Program: Endrin Monitoring in the Mississippi River

Abstract. *Twelve successive monthly samplings and analyses of representative fish and shellfish and of mud and water from the lower Mississippi River showed neither a high concentration of endrin nor a time-ordered change in the concentration. The general absence of endrin from the samples indicated no significant contamination.*

The mass death of fish in the lower Mississippi River in 1963 led to speculation concerning the accumulation of insecticide residues in the environment and rumors of increasing concentration of endrin in the fauna of the "food chain"; buildup of endrin in soil and water was postulated. We investigated these rumors by monthly sampling and analysis of mud and water, top-water fish (bream), bottom-water fish (catfish), shrimp, and oysters from the lower river for one year beginning in July 1964. Fish and mud and water were sampled at Baton Rouge, Louisiana, from the east bank opposite Port Allen Lock; shrimp and oysters were obtained from the area of shellfish production below New Orleans.

Replicated determinations for endrin were made on 8 to 15 randomly selected fish. Samples of mud and water, containing about 50 percent mud by volume, were collected from the same general area as the fish; shrimp and oysters were randomly sampled. All

samples were protected from change in composition by sealing in airtight containers and by freezing between the times of collection and analysis.

The samples, subjected to appropriate cleaning, were analyzed for endrin by gas-liquid chromatography, with an electron-capture detector (Table 1). Homogenates of fish were subjected to hydrolysis by alcoholic potassium hydroxide and were extracted several times with normal hexane. Samples of shrimp and oyster were homogenized, extracted with a mixture of hexane and isopropanol (3 : 1), and filtered through glass wool. Samples of mud and water were extracted with a mixture of ether and isopropanol 2 : 1.

The following procedure was used with all samples. Extraction solvents were freed of alcohol (and of alkali, when present) by repeated washings with water. Drying with sodium sulfate, concentration, and liquid-phase chromatographic cleanup through a magnesium oxide–Celite No. 545 column preceded quantification. The appropriate portion of the eluate was analyzed by gas-liquid partition chromatography, with an electron-capture detector. Quantification was determined from calibration curves prepared by analysis of fortified endrin solutions in hexane. Analyses were repeatedly verified by recovery experiments; recovery averaged 85 percent.

The lower limit of confident analysis is estimated to be 0.005 parts per million; this is the lowest analytical value that is considered discernible from the analytical response of interfering substances found occurring naturally in the samples analyzed. Analyses yielding less than 0.005 ppm are reported as negative. Three readings per sample per month were usually taken. Oysters and shrimp were negative throughout. Catfish yielded 0.01, 0.02, and 0.01 ppm of endrin in July 1964; and one reading of 0.01 ppm in each of August and October 1964 and June 1965. Bream yielded one reading of 0.01 ppm in each of July and October 1964 and February 1965. Mud and water were negative throughout apart from two readings of 0.01 ppm in July 1964 and one of 0.01 ppm in each of February and June 1965.

The data show neither a high level of endrin nor a time-ordered change in the endrin level. The general absence of endrin from the samples indicates that there is no significant contamination of the environment by the pesticide, but the possibility of localized and sporadic occurrence of residues is not ruled out. We also conclude that the portion of human food supply represented by the organisms studied is not contaminated by endrin.

Table 1. Conditions for gas-chromatographic analysis for endrin with a Micro-Tek model 2000 R.

| Item | Condition |
|---|---|
| Column | |
| Substrate | 5 per cent silicone D-11 |
| Support | Chromosorb W, 60–80 mesh |
| Length, shape | 91 cm, coiled helix |
| Bore | 6 mm, Pyrex |
| Carrier gas | Methane-argon, 5:95 |
| Pressure | 2.7 atm (g) |
| Flow rate | 142 $cm^3$/min |
| Operating temp. | 200°C, column; 210°C, flash heater; 205°C, detector |
| Output polarity | Negative |
| Isothermal | Negative |
| Output attenuator | 8–16 |
| Input attenuator | 1 |
| Chart speed | 1.2 cm/min |
| Detector | Tritium<br>Electron affinity |

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## Defensive Secretion of a Caterpillar (Papilio)

Abstract. *The eversible cervical gland (osmeterium) of the caterpillar of the swallowtail butterfly,* Papilio machaon, *produces a secretion containing isobutyric and 2-methylbutyric acid. The gland is effective in defense against ants. Even when caterpillars were fed on one of three unbelliferous plants (fennel, carrot, parsnip) the secretion contained the same two acids.*

The caterpillars of butterflies of the family Papilionidae (swallowtails and parnassians) possess a remarkable defensive gland, the osmeterium, situated middorsally just behind the head. Consisting essentially of a two-pronged invagination of the neck membrane, the gland is ordinarily tucked away invisibly beneath the integument, but it is forcibly everted when the animal is disturbed. A characteristic intense odor invariably emanates from the two extruded "horns," which glisten with a coating of secretion. When the disturbance subsides, the horns are retracted. The gross morphology of the osmeterium and the cytology of the secretory cells associated with it have been described (*1, 2*). The mechanism of operation of the gland can be inferred from its structure—the horns are evaginated by blood pressure and withdrawn by special retractor muscles. We undertook this study, on the larva of the European swallowtail, *Papilio machaon,* because very little was known about the protective effect of the osmeterium, and the active principles of the secretion had never been identified.

To observe in detail the behavior of the larva when it brings the osmeterium into action, we subjected individual caterpillars to simulated attack by prodding, tapping, or pinching them. As a first response to such disturbance, a larva usually assumes a characteristic "threat posture," in which it suddenly thrusts its front end into an uplifted position. Poised in this fashion, it is prone to extrude the osmeterium (Fig. 1*B*), and it does so inevitably, if not at the very onset of the "attack," then certainly after sustained or repeated stimulation. Whether the horns are partially or completely everted depends on the intensity of the stimulus. Mild disturbance, such as poking the animal gently with a blunt probe, may elicit no more than incipient evagination. More complete (Fig. 1*B*) or even total eversion occurs only when considerable trauma is induced, as it is when the body is pinched with forceps. While everting the gland, the larva arches its front end toward the region traumatized, and may then wipe its horns against the instrument used for stimulation (Fig. 1*C*). The two horns are not necessarily equally protruded.