the progress of synchronized cells treated in G_1 with hydroxyurea for 5 hours (curve B) is compared with that of untreated cells (curve A). Passage of the treated cells into DNA synthesis was suppressed until the drug was removed. Almost immediately thereafter the percentage of cells labeled with tritiated thymidine rose to 100 percent. Thus even cells synthesizing DNA at the time hydroxyurea was added (and therefore destined not to form colonies) incorporated thymidine again when the drug was removed.

The data of Fig. 3 show clearly that the degree of synchrony in the cell population is markedly improved after hydroxyurea has been added and removed. Quantitatively, the labeling index of synchrony proposed by Sinclair and Morton (*8*) and indicated on curve A by L and curve B by L_H is improved from ~55 percent to ~83 percent. The spread of the S period is reduced from about 7½ hours to 6 hours.

These results indicate that hydroxyurea is likely to be an effective synchronizing agent for asynchronous populations, since the proliferative capacity of S cells can be destroyed while the remaining cells (about 40 percent in the case of hamster cells and higher percentages for cell lines such as HeLa and L, which have longer G_1 periods) merely pile up at the end of G_1. Thus, it is reasonable to expect better synchrony than is obtainable with inhibitors such as 5-fluorodeoxyuridine (FUDR) or amethopterin (*9*), which block S cells but may permit them to survive. The use of hydroxyurea may also have advantages over methods involving "suicide" with lethal amounts of tritiated thymidine (*10*). In the latter, S cells are killed but there is no inhibitory action resulting in accumulation of cells. Consequently, a relatively small portion of the population can be obtained in synchronized form. Any inhibitory drug such as hydroxyurea, however, has the inherent disadvantage that properties of surviving cells other than their proliferative capacity may be affected; the response of treated cells to other agents (such as x-rays) may not be the same as that of the untreated population. Experiments are in progress to test the possibilities of hydroxyurea as a synchronizing agent in asynchronous cells.

The lethal effect of hydroxyurea on S cells in vitro presumably has an important bearing on the behavior of the drug in vivo and may help to explain its antitumor action and the clinical complications of treatment in humans.

Furthermore, this selective action offers the prospect of damaging proliferative cells even more effectively by combining hydroxyurea with another agent [for example, in the case of hamster cells, perhaps with x-rays (*8*)] selectively effective in damaging cells at other stages of the generation cycle.

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Visnagin: Biosynthesis and Isolation from Ammi visnagi Suspension Cultures

Abstract. *During an examination of* Ammi visnaga *Lam. suspension cultures for the biosynthesis of furanochromones and related medicinal compounds, visnagin was isolated in crystalline form and identified. Thus, certain medicinally important secondary plant metabolites may be produced in appreciable amounts by plant tissue cultures.*

A number of investigators have reported the biosynthesis of medicinal compounds by plant tissue cultures, but few have reported their isolation and identification (*1*). Among the medicinally important compounds produced by plant tissue cultures are substances similar to the digitalis glycosides from *Digitalis sp.* (*2*), nicotine from *Nicotiana tabacum* L. (*3*), tropane alkaloids from *Datura sp.* (*4*), reserpine from *Alstonia constricta* F. Muell. (*5*) and vinca alkaloids from *Catharanthus roseus* L. (*6*).

We now report the isolation and identification of the furanochromone, visnagin, from suspension cultures of *Ammi visnaga* Lam. (Umbelliferae). Furanochromones have a limited distribution in plants, being reported only in the genera *Ammi* (Umbelliferae) and *Eranthus* (Ranunculaceae) (*7*). Visnagin is present in the seeds of *A. visnaga* in a concentration of 0.045

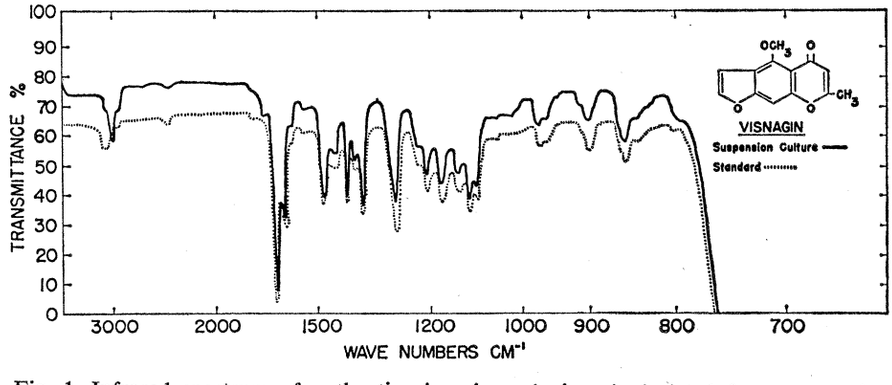

Fig. 1. Infrared spectrum of authentic visnagin and visnagin isolated from suspension cultures of *Ammi visnaga*.

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 mμ. 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