

Reports

Depressant Agent from Walnut Hulls

Abstract. Crushed unripe walnut hulls (*Juglans nigra*), when extracted with ether, yield an extract which sedates or at least depresses the movements of *Daphnia magna*, leopard frogs, perch, catfish, goldfish, mice, rats, and rabbits. One purified depressant compound, 5-hydroxy-1,4-naphthoquinone (juglone), has been isolated and tested on most of these species.

Crushed unripe walnut hulls (*Juglans nigra*) have been used for generations in various types of folk medicine, including treatment of fungus infections (1). In certain regions of the United States fresh walnut hulls are employed illegally to immobilize fish, and consequently take them from small streams and impoundments. The fish gradually lose equilibrium and awareness to changes in environment, and finally can be caught by hand. When placed in fresh water, many of these animals seem to recover fully. The use of walnut hulls in folk medicine and as a depressant of fish initiated our interest in extracting the active principle from green walnut hulls, testing it on different species of fish, and assessing its effects on several other species of animals.

After using several extractive solvents—including water, acetone, ethyl alcohol, chloroform, diethyl oxide, and petroleum ether—diethyl oxide (ether, U.S.P.) and petroleum ether were judged the two best solvents for extracting the active principles from fresh, finely ground walnut hulls. After the

hulls had been macerated in ether, the solvent was allowed to evaporate at room temperature, leaving a dark, scaly residue. One kilogram of the fresh hulls extracted with 1.4 kg of ether yielded 2.13 g of residue. This residue produced the typical depression in young yellow perch (*Perca flavescens*), channel catfish (*Ictalurus punctatus*), and goldfish (*Carassius auratus*). All of these fishes could be depressed markedly by the water-soluble fraction from 7 to 15 mg of the dried ether extract per liter of tap water. Perch were the most sensitive and goldfish the most resistant of the fishes tested. The small amount of extract used in these tests produced no measurable effect on the pH or oxygen content of the water.

A study was made also to determine whether this material would be specific for fishes or would have a similar effect on more primitive organisms (*Daphnia magna*) and more highly developed animals (frogs and mammals). *Daphnia* were immobilized and, if not placed in fresh water, would die when exposed to approximately the same concentration that was lethal to perch. The water-soluble fraction was dissolved in saline (0.7 percent) and injected into frogs. The frogs became quiet and recovered in a few hours, providing the dose did not exceed 1 mg/g of body weight. Albino mice and rats also were sedated after intraperitoneal injection of this substance dissolved in corn oil.

Attempts are being made to separate the sedative principles from this ether extract. One purified compound, 5-hydroxy-1,4-naphthoquinone (juglone), has been isolated to date. This juglone was separated from the crude ether extract by sublimation and then identified by melting point, infrared spectrophotometry, and crystalline studies by comparing it with known samples of synthesized 5-hydroxy-1,4-naphthoquinone. One gram of the crude ether extract yielded 0.156 g of the 5-hydroxy-1,4-naphthoquinone. Although it has been known for years that juglone

occurs in various species of the walnut tree (2), published work on the pharmacological actions of this naphthoquinone does not include a sedative effect.

Our purified 5-hydroxy-1,4-naphthoquinone was tested on goldfish ($LD_{50} \cong 0.2$ mg/liter of water in the aquarium), mice ($LD_{50} = 0.25$ mg/100 g of body weight), and rats, and was found to have a calming or sedative effect in all of these species. Rabbits were injected intravenously with 0.07 mg of juglone (in 5-percent glucose solution) per kilogram of body weight. This resulted in tranquilization and general quietude of the animals for a period of 2 to 3 hours. Although sedation is produced by quite small amounts, the toxicity is such that profound sleep cannot be produced with less than lethal doses in these mammals.

Although the purified juglone produces sedation, there seems to be a difference between the quality of depression produced by the crude material and that produced by juglone. This suggests the presence of compounds other than juglone which may have synergistic action (3).

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References and Notes

1. Personal communications—used against "ring worm"; W. A. N. Dorland, *American Illustrated Medical Dictionary* (Saunders, Philadelphia, ed. 22, 1951), p. 772.
2. A. B. Massey, *Phytopathology* **15**, 773 (1925); E. F. Davis, *Am. J. Botany* **15**, 620 (1928).
3. This research was supported in part by National Institutes of Health grant MY-4295 (R1).

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Proof of an Adaptive Linkage Association

Abstract. Linked X-chromosome inversions of an eastern woods fly, *Drosophila robusta*, occur in two different nonrandom arrays. Since only one can be the non-random condition expected on the approach toward random equilibrium, at least one of the observed arrays must have an adaptive basis.

It is fashionable in modern evolutionary genetics to state: "The raw materials of evolution arise by mutation and recombination" and then to proceed to discuss how alleles may change frequencies under directional or nondirectional forces. Very little attention has

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].