Isolation and Identification of an Antifungal Agent from Seeds of American Elm

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ABSTRACT

Capric acid, a 10-carbon straight-chain fatty acid isolated from American elm, *Ulmus americana,* seeds, was identified as the antifungal agent active against the Dutch elm disease fungus *Ceratocystis ulmi* and several other fungi.

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Resistance of American elm, *Ulmus americana* L., seedlings less than 3 years old to Dutch elm disease has been noted by several workers (1, 3, 8). The nature of this resistance is not known. Schreiber (7) reported the presence of a fungitoxicant to *Ceratocystis ulmi* (Buism.) C. Moreau in water extracts from stems and leaves of American elm seedlings 1-5 months old. Similar extracts from American elm seeds also were toxic to the Dutch elm disease fungus (Schreiber, *unpublished*). This research was undertaken to identify the fungitoxicant in American elm seeds.

Three-hundred-eighty grams of American elm seed were ground in 5.5 liters of water in a Waring Blendor, and filtered, and the marc was reextracted with 2 liters of water and filtered again. Extraction of the combined aqueous extract four times with 1 liter of chloroform yielded 15.4 g of chloroform solubles with antifungal activity. The fraction was chromatographed on a 400 g silicic acid column, with benzene as a solvent, and dry weight of 20-ml effluent fractions was analyzed. Two peaks emerged early, of which the second contained 2.5 g of inhibitory material. Rechromatography of this fraction on a column of 100 g of silicic acid and later crystallization from petroleum ether (60-68 C bp) produced 2 g of crystals with 30.5 - 32.0 C mp.

The nuclear magnetic resonance (nmr) spectrum of the antifungal substance showed a pattern typical of long-chain fatty acids (4), the mass spectrum exhibited a molecular ion peak at m/e 172 corresponding to the formula $C_{10}H_{20}O_2$, and the infra red (IR) spectrum showed a peak at ν_{max} 1705 cm^{-1} for carbonyl absorption. A comparison of the isolated material with authentic capric (decanoic) acid by use of nmr and IR spectra, thin-layer chromatography, and melting point showed them to be identical. In addition, the amide derivative with 96.5 - 98.0 C mp was prepared by a standard method (2) with the acid chloride and ammonia. The derivative and authentic capramide gave identical nmr and IR spectra, and an undepressed mixture melting point.

The crude seed extract and the fractions obtained from it during the process of purification and identification of the fungitoxic agent were tested for fungitoxic activity. We used a spore germination-filter paper pad method described by Schreiber (7) and a standard paper-disk plate method (5). In both, *C. ulmi* was the test organism.

We extracted capric acid from American elm seeds and bioassayed it at 5, 10, 50, and 100 mg/ml to determine its fungitoxicity to *C. ulmi.* The capric acid was solubilized in $CHCl_3$. A crude water extract from seeds at 10 and 100 mg/ml concentration was used as a standard. The solutions were tested by our placing 0.1 ml of the solutions on 12.7-mm diameter filter paper disks and drying them. The dried disks were placed in the center of a petri plate seeded with *C. ulmi* conidia. After 48 hours, the diameters of the zones of inhibited growth were measured.

The fungitoxic effects of capric acid on *C. ulmi, C. minor* (Hedgc.) Hunt, *C. fagacearum* (Bretz) Hunt, *Nectria cinnabarina* Tode ex Fr., and *Fusarium solani* (Mart.) Appel & Wr. were determined by the spore germination-filter paper bioassay (7). A 0.1-ml drop of 10-mg/ml concentration of capric acid in $CHCl_3$ was applied to each pad. The pads were air-dried and remoistened with 0.15 ml H_2O. Spores of the test fungi were applied and counted after 19 hours. The results of this, and the preceding, bioassay represent the averages of nine replications for each treatment.

The average sizes of zones of inhibition around filter paper pads with 5, 10, 50, and 100 mg/ml of capric acid were 23, 26, 27, and 26 mm, respectively. The crude extract at 10 and 100 mg/ml was less active, producing zones of inhibition of 13 and 20 mm, respectively. The lower activity of crude extract was probably caused by the diluting effect of stimulatory or neutral compounds.

The fungitoxic effects of capric acid were not specific to *C. ulmi;* capric acid reduced spore germination of all other fungi tested. The percentages of spore germination of *C. ulmi, C. minor, C. fagacearum, N. cinnabarina,* and *F. solani* on filter paper pads treated with 0.1 ml of a 20 ml/liter solution of capric acid in chloroform were 3, 2, 3, 0, and 6, respectively, and on untreated checks were 94, 24, 94, 77, and 98, respectively.

The seed oil of several members of the family Ulmaceae contains substantial amounts of capric acid. Oil from American elm seed contains 55.5% (by weight) capric acid (6). The relationship between capric acid and the factors contributing to Dutch elm disease resistance in juvenile American elms is yet to be determined. However, we have shown capric acid is a naturally occurring plant product toxic to *C. ulmi.* This or similar chemicals may prove to be useful chemotherapeutants by combining fungitoxicity in the absence of phytotoxicity. Their use would, in addition, minimize environmental contamination often associated with synthetic fungicides.

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