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EFFECTS OF *p*-METHOXYCINNAMALDEHYDE FROM STAR ANISE AND RELATED CINNAMIC ACID DERIVATIVES ON VELVETLEAF GERMINATION

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In our continuing search for natural compounds that may have potential for use as herbicides, we found that extracts of star anise (*Illicium verum* Hook.) fruit inhibited velvetleaf germination. Further investigation showed the component responsible was *p*-methoxycinnamaldehyde (PMCA). Although previously unknown to occur in star anise, this compound has been found in various other plants, including sweet basil (*Ocimum basilicum* L.) (1), *Limnophila rugosa* (Roth.) Merrill (2), *Artemesia dracunculus* L. (3), *Sphaeranthus indicus* L. (4), *Agastache rugosa* (Fischer & C. Meyer) (5), and *Acorus gramineus* Ait. (6); it is also a flavor component of baked potatoes at 0.06% (7). Of those sources with reported concentrations, *Sphaeranthus indicus* contains the greatest amount of PMCA with 7.4% of above-ground plant parts.

The present study undertook to quantitate PMCA in the fruit of star anise and to determine its activity against velvetleaf germination. Additionally, six similar compounds were investigated in order to compare structure/activity relationships.

MATERIALS AND METHODS

EXTRACTION AND IDENTIFICATION OF PMCA.—Approximately 100 g of dried star anise (*I. verum*) fruit (seed+hull) were ground in a centrifugal grinding mill (Brinkmann model #192) to a fine powder and extracted in a Soxhlet apparatus with 250 ml hexane for 24 h. The extract was reduced on a rotary evaporator, yielding about 8 g residue. This was applied to a silica (Mallinckrodt, 60-100 mesh type 60 Å special) column and eluted with 250 ml Me_2CO-hexane (1:9). The resulting fraction was further separated by hplc [Partisil PAC M9 column, Whatman, with Me_2CO-hexane (1:9) using a differential refractometer as detector]. Gc/ms and nmr (8,9) elucidated the identity of PMCA. Each step was followed by bioassay (see below). Tlc plates (0.25 mm silica, Brinkmann) were developed in Me_2CO-hexane (1:1), and spots were visualized under shortwave uv light).

QUANTITATION OF PMCA.—Another hexane extraction of 64 g of fruit was conducted as above. This was followed by a 24-h Me_2CO extraction, which yielded no PMCA as detectable by tlc. The hexane extract was diluted to a known volume and analyzed by hplc (as above, except with a uv detector at 320 nm) with β-ionone (Distillation Products Industries, Rochester, NY) as an internal standard. The extract alone showed no peak at the same elution time as the internal standard. A calibration curve was prepared using seven mixtures of authentic PMCA and β-ionone. All samples were analyzed twice and the results averaged.

BIOASSAY.—Seeds were sown on treated filter paper in petri dishes, 20 per dish and 2 dishes per treatment; H_2O was added, and the dishes were wrapped in foil (10). Seeds were incubated for 4 days uniformly, after which the number of germinated seeds was recorded. For every two treatments, a blank control was added. Germination of controls varied from 85 to 100%. Bioassays of authentic compounds were conducted at least twice. Sources of chemicals were: cinnamaldehyde and cinnamyl alcohol, Aldrich, Milwaukee, WI; *o*-methoxycinnamaldehyde, ICN Pharmaceuticals, Plainview, NY; *p*-methoxycinnamic acid, Lancaster Synthesis, Ltd., Windham, NH; cinnamic acid, Eastman Kodak, Rochester, NY; and anethole, Sigma, St. Louis, MO.

RESULTS AND DISCUSSION

Mass spectra obtained of authentic *p*-methoxycinnamaldehyde and of the unknown isolated from star anise were identical. Nmr analysis matched exactly

[1]The mention of firm names or trade products does not imply that they are endorsed or recommended by the U.S. Department of Agriculture over other firms or similar products not mentioned.

that of PMCA published by Liptaj, *et al.* (11). Although the composition of star anise has been studied extensively (12-15) because of the use of its oil as a flavoring, this compound had not been previously found in the plant. This may be due to the fact that it occurs in only a trace amount, 0.01% as determined by methods discussed above. It is found in essential oils of other seeds to a much greater extent: as previously mentioned 7.4% in *S. indicus* (4); 2.5% in *A. rugosa* (5), and 1% in *A. gramineus* (6).

After 4 days exposure to a 2.5 mM dose of PMCA, essentially no velvetleaf seeds germinated (Table 1). A 1-mM dose did not affect the seeds. It appears that *o*-methoxycinnamaldehyde was slightly more active inasmuch as a 1-mM dose of it caused a reduction in germination that was significantly different from controls. However, the data from these two compounds are within statistical range of each other. Few reports on the biological activities of these natural chemicals are available. The Flavor and Extract Manufacturers' Association has determined that human consumption levels of PMCA should not exceed 2 to 5 ppm ("generally recognized as safe"), depending on the type of food involved (16). However, it is known that a 0.05 mM dose of this compound causes 50% inhibition of cyclic AMP phosphodiesterase activity (17). In this case, the other isomer was slightly less active in the same system. *o*-Methoxycinnamaldehyde has also been reported to exhibit antifungal activity at a dose as low as 0.6 mM (18). However, it was determined that the acute LD_{50} in rats of this isomer exceeded 5 g/kg (19).

Of all the compounds tested, cinnamaldehyde was the most active against velvetleaf germination (Table 1). Only about half as many seeds germinated when exposed to 1 mM of this compound as did in the controls. This suggests that the addition of the methoxy group limits antigermination activity. This hypothesis is supported by comparing *p*-methoxycinnamic acid with cinnamic acid: a 5-mM dose of the latter effectively inhibits germination whereas the same amount of the former has no effect.

TABLE 1. Effect of Cinnamic Acid Derivatives on Velvetleaf Seed Germination

Compound	Percent Germination, Relative to Controls		
	5 mM	2.5 mM	1 mM
p-methoxycinnamaldehyde	1 a*	8 a	83 cd
o-methoxycinnamaldehyde	0 a	3 a	77 bc
p-methoxycinnamic acid	83 cd	83 cd	107 e
cinnamic acid	5 a	77 bc	100 de
cinnamaldehyde	0 a	0 a	57 b
cinnamyl alcohol	0 a	0 a	73 bc
anethole	95 d	100 de	98 de
controls	100 de	100 de	100 de

*Values followed by the same letter are not statistically different at the 0.05 level by Chi-square 1-tail test.

Cinnamyl alcohol compares closely with the methoxy aldehydes and is more active than cinnamic acid. Anethole (1-methoxy-4-propenylbenzene) showed no effect on velvetleaf germination whatsoever. As the major constituent of star anise oil, it has been presumed to be an inhibiting component in several bioassays (e.g., insecticidal, bactericidal, fungicidal) (20,21,29,31), and in larger doses it may inhibit this species as well. It is also possible that anethole may act synergistically with some of the cinnamic acid derivatives present.

In reviewing the data, it appears that aldehydes and alcohols are more active

against velvetleaf germination than acids. Relatively little data are available comparing these compounds in other biological systems; however, cinnamic acid (22-25), cinnamaldehyde (26-29), and anethole (21,28-32) have all been reported to inhibit various activities, from fungal growth and toxin production to insect pupation. Interestingly, cinnamic acid also stimulated the germination of several plant seeds in very low doses (33). Cinnamaldehyde and, more prevalently, anethole are used as flavorings and perfumes in many products (12,34) and reputedly have very low toxicities (34,35).

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