JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Pest-Managing Efficacy of *trans*-Asarone Isolated from *Daucus carota* L. Seeds

RAFIKALI A. MOMIN AND MURALEEDDHARAN G. NAIR*

Bioactive Natural Products and Phytoceuticals, Department of Horticulture and National Food Safety and Toxicology Center, Michigan State University, East Lansing, Michigan 48824

The bioactive hexane extract of *Daucus carota* seed yielded 2,4,5-trimethoxybenzaldehyde (**1**), oleic acid (**2**), *trans*-asarone (**3**), and geraniol (**4**). Compounds **1**−**4** were evaluated for their mosquitocidal, nematicidal, antifeedant, and antimicrobial activities. Only *trans*-asarone was active in the assays performed, causing 100% mortality to fourth-instar mosquito larvae, *Aedes aegyptii*, at 200 μg mL^{-1} and the nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* at 100 μg mL^{-1}. In feeding trials, *trans*-asarone also caused significant weight reductions of the caterpillars *Helicovarpa zea*, *Heliothis virescens*, and *Manduca sexta* when incorporated into artificial diet at a concentration of 100 μg mL^{-1}. Also, it exhibited slight activity at 100 μg mL^{-1} against the yeasts *Candida albicans*, *Candida parapsilasis*, and *Candida kruseii*.

KEYWORDS: ***Daucus carota*; Umbelliferae; *trans*-asarone; insecticidal; mosquitocidal; nematicidal; antifeedant; antimicrobial**

INTRODUCTION

Daucus carota L., carrot, a herb belonging to the family Umbelliferae, is grown throughout the temperate regions of the world for its roots, which are consumed raw and cooked as well as used in canned, frozen, and dehydrated products. Carrot seeds have a pungent taste and are used as a folk medicine in Asia (*1*), and oil from carrot seed has been studied for its hypotensive, diuretic, cardiac, anticonvulsant, antifertility, and carminative effects as well as for treatment of digestive and skin disorders (*2*−*5*). Carrot seed oil from selected varieties has also demonstrated antibacterial and antifungal activities (*6*−*8*).

Implementation of the Food Quality Protection Act (FQPA) in the United States has encouraged scientists to seek alternatives to synthetic commercial pest-managing compounds. Recently, efforts are made to accelerate the search for more toxicologically and environmentally safe pest-managing agents. It is evident that plants have their own defense systems to protect them from pests. Several plant secondary products have been associated with activities against plant pests. In an attempt to add value to agricultural products, we have investigated carrot seeds for potential pest-managing compounds. The preliminary bioassay of carrot seed extracts showed that the hexane extract was active against mosquito larvae, *Aedes aegyptii*. Therefore, the hexane extract was subjected to mosquitocidal assay-guided purification, and the resulting compounds were tested for nematicidal, mosquitocidal, antifeedant, and antimicrobial activities. In this paper, we report results of assays of these compounds from the hexane extract of carrot seeds against a broad spectrum of pests including insects, nematodes, and yeast species.

* Author to whom correspondence should be addressed [telephone (517) 353-2915; fax (517) 432−2310; e-mail nairm@msu.edu].

MATERIALS AND METHODS

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on Varian INOVA 300 and 500 MHz spectrometers. ^{13}C NMR spectra were recorded at 75 or 126 MHz. Chemical shifts were recorded in $CDCl_3$, and the values are reported in δ (parts per million) based on δ residuals of 7.24 for ^{1}H NMR and 77 for ^{13}C NMR. Coupling constants, *J*, are in hertz. The silica gel used for MPLC was Merk silica gel 60 (30−70 μm particle size). TLC plates (Analtech, silica gel GF, coated on PE sheets, 200 μm) and preparative TLC plates (Analtech, silica gel, 20 × 20 cm; 250, 500, and 1000 μm) were viewed under UV light (254 and 366 nm) after developing. All organic solvents used were of ACS reagent grade (Aldrich Chemical Co., Milwaukee, WI).

Plant Material. Carrot seeds were provided by Asgrow Seed Co., Kalamazoo, MI, and stored at −20 °C until extraction.

Extraction and Isolation. Ground carrot seeds (1 kg) were sequentially extracted with hexane, EtOAc, and MeOH (1.5 L × 4, 24 h each) and yielded 121.1, 57.8, and 25.1 g of residue, respectively, upon removal of solvent. A portion of the hexane extract (32 g) was stirred with MeOH and filtered to yield MeOH soluble (4.84 g) and insoluble (26 g) fractions. The bioactive MeOH soluble fraction (3.09 g) was fractionated by MPLC on silica gel (Sanki Engineering Ltd., model LBP-V pump operating at 1−15 psi, Chemco MPLC tayperling type glass column, 35 × 4 cm) using hexane with an increasing amount of acetone and finally with MeOH as eluting solvent. Fractions collected were A (720 mL, 220 mg), eluted with 100% hexane and hexane/acetone (8:1); B (155 mL, 1.84 g), eluted with hexane/acetone (8:1); C (75 mL, 145 mg), D (75 mL, 74 mg), and E (75 mL, 15 mg), eluted with hexane/acetone (4:1); F (75 mL, 17 mg), eluted with hexane/acetone (4:1 and 1:1); and G (195 mL, 165 mg) and H (165 mL, 300 mg), eluted with 100% acetone followed by 100% MeOH.

10.1021/jf020209r CCC: $22.00
Published on Web 06/27/2002

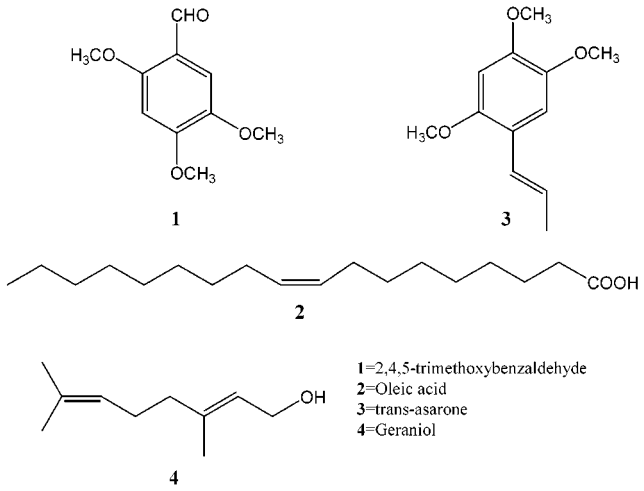

Figure 1. Molecular structures of bioactive compounds from *D. carota* seeds.

Fraction H (40 mg) was purified by preparative TLC using hexane/acetone (4:1 × 3) as the mobile phase and yielded compound **1** (7.8 mg). Similarly, purification of fraction G by preparative TLC using hexane/EtOAc, 3:1, as the mobile phase afforded compound **2**. Fraction B was further subjected to MPLC on silica gel using hexane with an increasing amount of acetone and yielded fractions I (565 mL, 60 mg), eluted with 100% hexane and hexane/acetone (15:1); II (30 mL, 92 mg), III (90 mL, 942.4 mg), IV (60 mL, 73 mg), and V (120 mL, 215.1 mg), eluted with hexane/acetone (15:1); and VI (660 mL, 135.2 mg), eluted with 100% acetone. The bioactive fraction IV was further purified by preparative TLC using hexane/chloroform/toluene/MeOH (3:2:2:0.1) as the solvent system and afforded compound **3** (5.9 mg). The purification of fraction A (40 mg) by preparative TLC (100% hexane) yielded compound **4** (20.3 mg) (**Figure 1**).

Nematicidal Assay. Cultures of the nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* were maintained in the Bioactive Natural Products and Phytoceuticals Laboratory, Michigan State University. *C. elegans* was cultured on NG agar medium containing a strain of *Escherichia coli* in disposable Petri dishes with 2−4 mL of physiological saline solution, and *P. redivivus* was grown in axenic, liquid basal heme medium (5 mL) in scintillation vials. The cultures were stored at room temperature and subcultured prior to the assay. A nematode suspension diluted in physiological saline (15−20 nematodes in a 48 μL aliquot) was delivered to each of two wells per treatment in Corning polystyrene 96-well plates. Two microliters of DMSO (50%) or test compound in 50% DMSO was added to each corresponding well. The plate was covered, wrapped with Parafilm, and kept in a humid chamber. The number of dead nematodes was recorded 2, 4, 6, 8, and 24 h after treatment by microscopic observation (*9*, *10*).

Mosquitocidal Assay. First-instar larvae of the mosquito *A. aegyptii* were provided by Drs. Alexander Raikel and Alan Hays, Department of Entomology, Michigan State University. The larvae were reared in reverse osmosis water in an incubator at 26 °C for 4 days, after which time 10−15 larvae were placed in 980 μL of degassed water in test tubes. Twenty microliters of a solution of test compound in DMSO, or DMSO as a control, was then added to each tube. Pure compounds were tested at concentrations of 200 and 100 μg mL^{-1}. Treatments and controls were replicated three times. The number of dead larvae was recorded at 2 h intervals for 24 h (*10*, *11*).

Antifeedant Assay. Eggs of the lepidoptera species *Helicovarpa zea*, *Heliothis virescens*, and *Manduca sexta* were purchased from the Department of Entomology, North Carolina State University, Raleigh, NC. For all three insects the antifeedant assay was performed according to a published procedure (*12*). The weights of larvae that had fed on diet containing test compounds were compared with those that had fed on diet containing DMSO alone. The results were analyzed by using ANOVA ($p \leq 0.01$), and the means were compared by calculating the least significant difference (LSD).

Antimicrobial Assay. Pure compounds were evaluated for antimicrobial activities according to reported procedures (*9*, *13*) against the yeasts *Candida albicans* (MSU strain), *Candida kruseii* (MSU strain), and *Candida parapsilasis* (MSU strain), which were cultured in Petri dishes containing YMG medium (20 mL); the bacteria *E. coli*, *Streptococcus aureus*, and *Staphylococcus epidermidis*, which were cultured in Emmons medium (20 mL); and the fungi *Aspergillus flavus*, *Aspergillus parasiticus*, and *Fusarium oxysporum*, which were cultured in PDA medium (20 mL). DMSO was used as a control treatment. DMSO or test compounds dissolved in DMSO (20 μL) at 100 μg mL^{-1} were spotted on the bioassay plates containing a lawn of test organisms. The plates were allowed to dry in a laminar flow hood and then incubated at 27 °C for 72 h. The zone of inhibition was measured in millimeters.

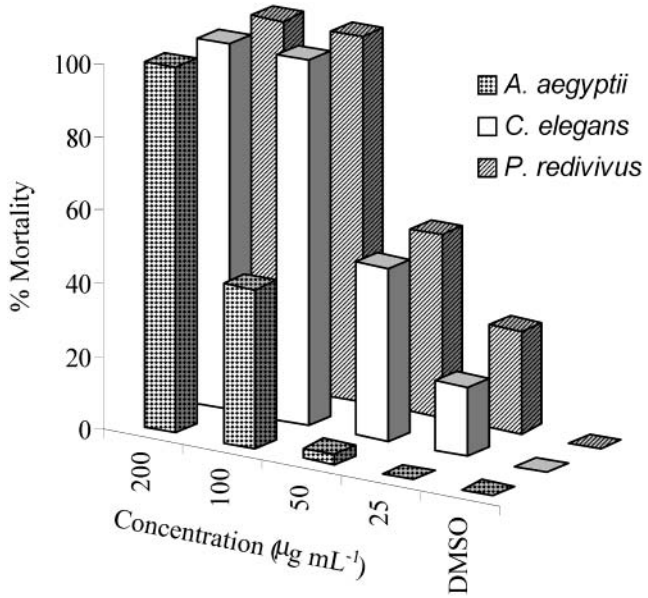

Figure 2. Effects of a range of concentrations of *trans*-asarone (**3**) on mortality of *A. aegyptii*, *C. elegans*, and *P. redivivus* after exposure for 24 h. Statistical analysis was done using ANOVA ($p \leq 0.01$), and the means were compared by calculating the LSD. The data are presented as percent mortality to the control DMSO.

RESULTS AND DISCUSSION

The structures of compounds **1**−**4** (**Figure 1**), yielded from mosquitocidal assay-guided purification of the hexane extract of carrot seeds, were deduced by ^{1}H and ^{13}C NMR spectral experiments. These compounds were identified as 2,4,5-trimethoxybenzaldehyde (**1**), oleic acid (**2**), *trans*-asarone (**3**), and geraniol (**4**). Both ^{1}H and ^{13}C NMR spectral data of compounds **1**−**4** were identical to their respective published spectral values (*14*−*17*).

Nematicidal assay of compounds **1**−**4** using *C. elegans* and *P. redivivus* indicated that only compound **3**, *trans*-asarone, was active against these species, showing 100% mortality at 100 μg mL^{-1} within 24 h (**Figure 2**). Compounds **1**, **2**, and **4** caused no mortality to *C. elegans* and *P. redivivus* at this concentration. Among the compounds **1**−**4** tested against fourth-instar *A. aegyptii* larvae, only compound **3** showed mortality after 24 h (**Figure 2**). Therefore, a dose−response study of the larval mortality was determined for compound **3** and indicated 100 and 43% mortalities at 200 and 100 μg mL^{-1}, respectively.

In the caterpillar antifeedant assays, compounds **1**, **3**, and **4** were tested against *H. zea*, *H. virescens*, and *M. sexta* neonate larvae at 100 μg mL^{-1} concentration. Only *trans*-asarone, compound **3**, demonstrated significant activity against all three species as indicated by 97, 96, and 79% weight reductions of *H. zea*, *H. virescens*, and *M. sexta*, respectively, after 6 days (**Figure 3**). Compounds **1** and **4** had no effect on larval weight

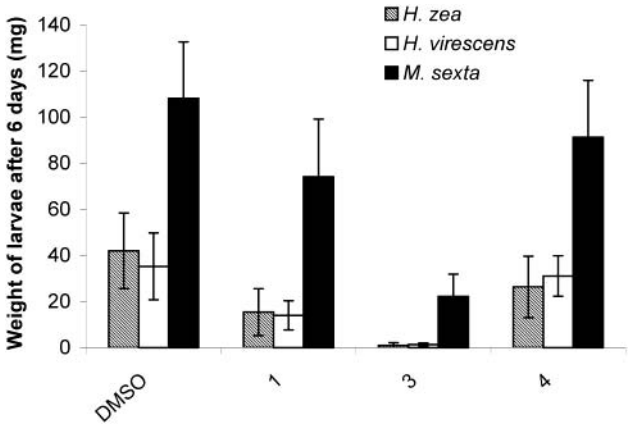

Figure 3. Effects of compounds 2,4,5-trimethoxybenzaldehyde (**1**), *trans*-asarone (**3**), and geraniol (**4**) from carrot seeds on growth of *H. zea*, *H. virescens*, and *M. sexta* neonate larvae at 100 μg mL^{-1} after 6 days. Data represent the mean of 12 replications. Statistical analysis was done using ANOVA ($p \leq 0.01$), and the means were compared by calculating the LSD.

at the concentration tested (**Figure 3**). Compound **2**, oleic acid, was not tested in the antifeedant assays.

Only *trans*-asarone (**3**) exhibited activity against the yeasts at 100 μg mL^{-1}, generating zones of inhibition of 6, 7, and 6 mm for *C. albicans*, *C. kruseii*, and *C. parapsilasis* after 72 h of incubation, respectively. However, this compound did not inhibit the growth of the bacteria and fungi when tested at 100 μg mL^{-1}. Compounds **1**, **2**, and **4** were not inhibitory to any of the microorganisms tested in our laboratory at 100 μg mL^{-1} concentration.

Other studies have also shown *trans*-asarone to have biological activity. *trans*-Asarone has been reported as an antifeedant against the cutworm, *Peridroma saucia*, when incorporated into artificial diet (*18*), and Poplawski et al. (*19*) reported that it exhibited deterrent activity against stored grain pests *Sitophilus granarius* (adult), *Tribolium confusum* (adult and larvae), and *Trogoderma granarium* (larvae) (*19*). *trans*-Asarone has also been shown to repel the red-winged blackbird, *Agelaius phoeniceus*. The mobility of the second-stage *Toxocara canis* nematode was rapidly inhibited when incubated with *trans*-asarone (*21*), and the nematode died after prolonged exposure.

Asarones have also shown antimicrobial activity. *trans*-Asarone isolated from *Piper sarmentosum* inhibited *E. coli* and *Bacillus subtilis* at 25 ppm (*22*). However, in our assays, it did not show any growth inhibitory activity against the bacteria *E. coli*, *S. aureus*, and *S. epidermidis* or the fungi *A. flavus*, *A. parasiticus*, and *F. oxysporum* at 100 μg mL^{-1}. However, it exhibited slight growth inhibitory activity against yeasts *C. albicans*, *C. parapsilasis*, and *C. kruseii* at 100 μg mL^{-1}.

Studies of structure−activity relationships of *trans*-asarone and related phenylpropanoids against the green alga *Selenastrum capricornutum* showed that the biological activity depends on the number and position of methoxy groups present in the ring (*23*). Also, an increase in activity was observed with an increase in the number of methoxy groups (*23*). Similarly, methoxy groups positioned ortho and para to the alkyl side chain provided the strongest biological activity. In contrast, Poplawski et al. (*19*) reported that the presence of a methoxy group in the meta position may have a considerable influence on deterrent activity.

The mosquitocidal, nematicidal, and antifeedant activities of *trans*-asarone reported in this paper are novel. These activities in conjunction with previously reported activities against several pests suggest that *trans*-asarone has the potential to be developed as an effective and alternative pest-managing natural product as a substitute for some of the synthetic pest-managing agents on the market. Further studies are required to determine the effective dose and potential of *trans*-asarone, or its related analogues, as pest-managing activity agents under field conditions.

LITERATURE CITED

(1) Volak, J.; Stodola, J.; Severa, F. *Plantes Medicinales*; Grund: Paris, France, 1984; 140 pp.
(2) Dhar, V. J. Studies on *Daucus carota* seeds. *Fitoterapia* **1990**, *61*, 255−258.
(3) Chopra, R. N.; Chopra, I. C.; Handa, K. L.; Kapur, L. D. *Chopra's Indigeneous Drugs of India*, 2nd ed.; U. N. Dhar and Sons: Calcutta, India; 1958; 504 pp.
(4) Halim, A. F.; Mashaly, M. M.; Sandra, P. Analysis of the fruit essential oil of *Daucus carota* L. var. *Boissieri Schweinf*. In *1st Anglo-Egyptian Conference of Pharmaceutical Science*, Nov 15−17, 1988, Alexandria, Egypt, 1988.
(5) Kamboj, V. P. A review of Indian medicinal plants with interceptive activity. *Indian J. Med. Res*. **1988**, *87*, 336−355.
(6) Syed, M.; Sabir, A. W.; Chaudhary, F. M.; Bhatty, M. K. Antimicrobial activity of the essential oils of Umbelliferae Part II. *Trachyspermum amni*, *Daucus carota*, *Anethum graveolens* and *Apium graveolens* oils. *Pak. J. Sci. Ind. Res.* **1986**, *29*, 189−192.
(7) Guerin, J. C.; Reveillere, H. P. Antifungal activity of plant extracts used in therapy. Part 2: Study of 40 plant extracts against 9 fungi species. *Ann. Pharm. Fr.* **1985**, *43*, 77−81.
(8) Dwivedi, S. K.; Pandey, V. N.; Dubey, N. K. Effect of essential oils of some higher plants on *Aspergillus flavus* Link. Infesting stored seeds of *Guar cyamopsis-tetragonoloba* L. *Flavour Fragrance J*. **1991**, *6*, 295−298.
(9) Momin, R. A.; Nair, M. G. Mosquitocidal, nematicidal and antifungal compounds from *Apium graveolens* L. seeds. *J. Agric. Food Chem*. **2001**, *49*, 142−145.
(10) Nair, M. G.; Putnam, A. R.; Mishra, S. K.; Mulks, M. H.; Taft, W. H.; Keller, J. E.; Miller, J. R.; Zhu, P.-P.; Meinhart, J. D.; Lynn, D. G. Faeriefungin: A new broadspectrum antibiotic from *Streptomyces griseus* var. *autotrophicus*. *J. Nat. Prod*. **1989**, *52*, 797−809.
(11) Momin, R. A.; Ramsewak, R. S.; Nair, M. G. Bioactive compounds and 1,3-di[(*cis*)-9-octadecenoyl]-2-[(*cis*,*cis*)-9,12-octadecadienoyl]glycerol from *Apium graveolens* L. seeds. *J. Agric. Food Chem*. **2000**, *48*, 3785−3788.
(12) Zhang, D.; Nair, M. G.; Murry, M.; Zhang, Z. Insecticidal activity of Indanomycin. *J. Antibiot.* **1997**, *50*, 617−620.
(13) Chang, Y.-C.; Nair, M. G.; Nitiss, J. L. Metabolites of daidzein and genisten and their biological activities. *J. Nat. Prod.* **1995**, *58*, 1901−1905.
(14) Nawamaki, K.; Kuroyanagi, M. Sesquiterpenoids from *Acorus calamus* as germination inhibitors. *Phytochemistry* **1996**, *43*, 1175−1182.
(15) Arnhold, J.; Panasenko, O. M.; Schiller, J.; Vladimirov, Yu. A.; Arnold, K. The action of hypochlorous acid on phosphatidylcholine liposomes in dependence on the content of double bonds. Stoichiometry and NMR analysis. *Chem. Phys. Lipids* **1995**, *78*, 55−64.
(16) Siergiejczyk, L.; Paplawski, J.; Lozowicka, B.; Dubis, A.; Lachowska, B. ^{1}H and ^{13}C NMR spectral analysis of (*E*)-asarone and its isomers. *Magn. Reson. Chem.* **2000**, *38*, 1037−1038.
(17) Bunton, C. A.; Hachey, D. L.; Leresche, J. P. Deamination of nerylamine and geranylamine. *J. Org. Chem.* **1972**, *37*, 4036−4039.
(18) Koul, O.; Smirle, M. J.; Isman, M. B. Asarones from *Acorus calamus* L. oil: their effect on feeding behavior and dietary utilization in *Peridroma saucia*. *J. Chem. Ecol.* **1990**, *16*, 1911−1920.

(19) Poplawski, J.; Lozowicka, B.; Dublis, A. T.; Lachowska, B.; Winiecki, Z.; Nawrot, J. Feeding-deterrent activity of α-asarone isomers against some stored Coleoptera. *Pest Manag. Sci*. **2000**, *56*, 560−564.

(20) Schafer, E. W., Jr.; Jacobson, M. Repellency and toxicity of 55 insect repellents to red-winged blackbirds (*Agelaius phoeniceus*). *J. Environ. Sci. Health, Part A* **1983**, *A18* (4), 493−502.

(21) Sugimoto, N.; Goto, Y.; Akao, N.; Kiuchi, F.; Kondo, K.; Tsuda, Y. Mobility inhibition and nematocidal activity of asarone and related phenylpropanoids on second stage larvae of *Toxocara canis*. *Biol. Pharm. Bull*. **1995**, *18*, 605−609.

(22) Masuda, T.; Inazumi, A.; Yamada, Y.; Padolina, W. G.; Kikuzaki, H.; Nakatani, N. Antimicrobial phenylpropanoids from *Piper sarmentosum*. *Phytochemistry* **1991**, *30*, 3227−3227.

(23) Greca, M. D.; Monaco, P.; Pollio, A.; Previtera, L. Structure−activity relationship of phenylpropanoids as growth inhibitors of the green alga *Selenastrum capricornutum*. *Phytochemistry* **1992**, *31*, 4119−4123.

Received for review February 15, 2002. Revised manuscript received May 28, 2002. Accepted May 29, 2002. Partial funding for this project was provided by the Center for Plant Products and Technology, Michigan State University. NMR data were obtained on instrumentation that was purchased, in part, with funds from NIH Grant 1-S10-RR04750, NSF Grant CHE-88-00770, and NSF Grant CHE-92-13241.

JF020209R