High-Performance Liquid Chromatographic Determination of Sesquiterpene Dialdehydes and Antifungal Activity from *Polygonum hydropiper*

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The antifungal sesquiterpene dialdehydes polygodial and warburganal from *Polygonum hydropiper* were determined by high-performance liquid chromatography. Polygodial and warburganal were clearly separated on an ODS column using methanol–H_2O as a mobile phase. These sesquiterpene dialdehydes accumulated in young leaves and shoots and possessed antifungal activity. The antifungal activity of *P. hydropiper* was attributed to these sesquiterpene dialdehydes.

INTRODUCTION

A series of unique sesquiterpene dialdehydes, polygodial (1), ugandensidial (4), warburganal (2), and muzigadial (3), were originally isolated as insect antifeedants from the East African medicinal plants *Warburgia stuhlmannii* and *Warburgia ugandensis* (Kubo et al., 1976, 1977; Nakanishi and Kubo, 1977; Meinwald et al., 1978). The ground bark of these plants is used not only in folk medicine but also as a spice for food. Among the oxidation products of the drimane skeleton, polygodial, warburganal, and muzigadial were found to have potent antifungal activity (Taniguchi et al., 1983, 1984). Further research revealed that the antifungal activity of these sesquiterpene dialdehydes was due to cell membrane damage in susceptible fungi (Taniguchi et al., 1988a; Yano et al., 1991). The membrane permeability of other drugs was enhanced by the dialdehydes, and synergistic effects were observed (Taniguchi et al., 1988b; Yano et al., 1989).

Polygodial and warburganal occur in *Polygonum hydropiper* L. (Fukuyama et al., 1982, 1985). *P. hydropiper* is used as a medicinal herb against cancer (Hartwell, 1970) and hemostatics (Steinberg, 1928). The young shoot is eaten and used as a spice with raw fish in Japan.

The occurrence of these sesquiterpene dialdehydes as metabolites in some plants is of interest from the viewpoint of biogenesis, pharmacology, and chemotaxonomy. This paper describes a method for the determination of polygodial and warburganal in plant materials, and the relationship between accumulation of these sesquiterpenes and antifungal activity in plant extracts was examined. Their structures are shown in Figure 1.

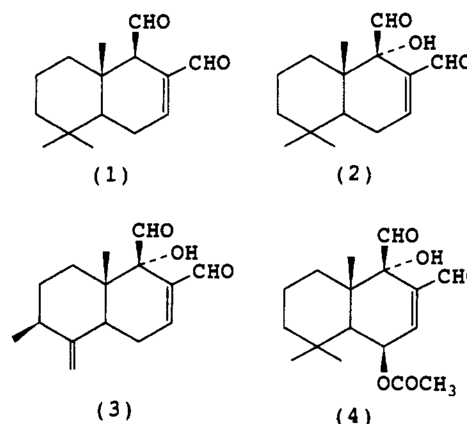

Figure 1. Structures of antifungal sesquiterpene dialdehydes. (1) Polygodial; (2) warburganal; (3) muzigadial; (4) ugandensidial.

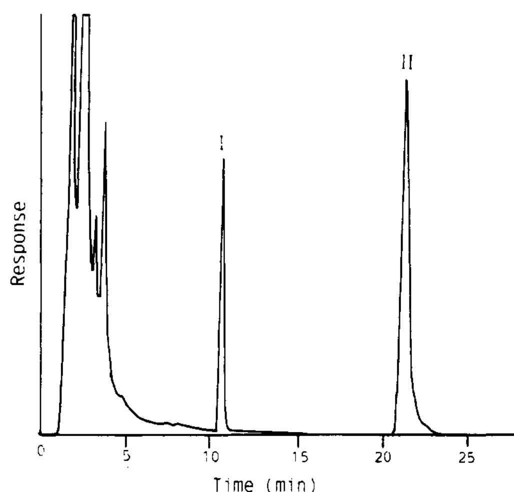

Figure 2. HPLC of antifungal sesquiterpene dialdehydes from the extract of *P. hydropiper*. (I) Warburganal; (II) polygodial.

EXPERIMENTAL PROCEDURES

Sesquiterpene Dialdehyde Standards. Polygodial and warburganal were gifts from Dr. I. Kubo (University of California), which were identified metabolites isolated from *W. ugandensis* and *W. stuhlmannii*, respectively (Kubo et al., 1976, 1977).

Equipment. Reversed-phase chromatography was performed using a Tosho HPLC system including a CCCP dual pump, a fixed-wavelength absorbance detector UV 8000, and a Sic Chromatocorder 11 electronic integrator. Separations were achieved with a TSK-gel ODS-120T (particle size, 5 μm) column (25 cm × 4.6 mm i.d.) at an elution rate of 1.0 mL/min. A TSK guardgel ODS-120T column (1.5 cm × 3.2 mm i.d.) was connected just before the separation column. Polygodial and warburganal were detected at 231 nm, the only λ_{max} these compounds possess.

Extraction of Sesquiterpene Dialdehydes. Young seedlings (3-cm shoot length) of *P. hydropiper* were collected near the Ashida River (Fukuyama, Japan) in May 1990 and were further grown hydroponically (Kyowa Hyponica system) in a greenhouse. At various growth stages (8-, 15-, 25-, 35-, 45-, and 55-cm shoot length, which correspond to 6, 13, 19, 24, 30, and 39 days further growth, respectively), each plant was harvested and divided into leaves, stems, and roots. Each sample was extracted in a screw-capped test tube with 20 mL of 95% methanol/g of fresh tissue for 24 h at room temperature. The methanol extract was evaporated and the dried extract partitioned between ethyl

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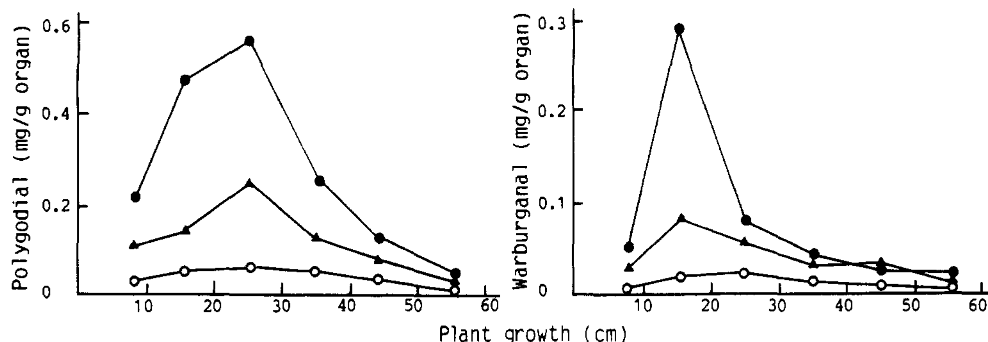

Figure 3. Changes in the sesquiterpene dialdehydes contents in various organs during plant growth of *P. hydropiper*. Each plot shows the mean of three to five analyses of independent extracts. Plant materials subjected to the analysis were obtained from one hydroponic system throughout the experiment. (●) Leaf; (▲) stem; (○) root.

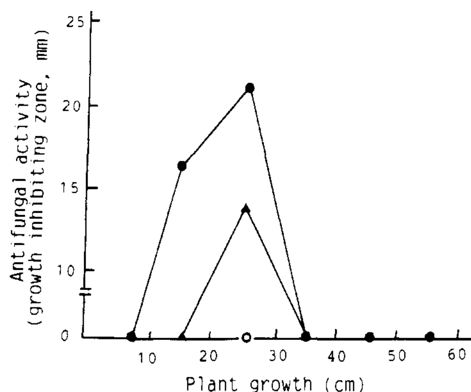

Figure 4. Changes in the antifungal activity of organ extracts during plant growth of *P. hydropiper*. (●) Leaf; (▲) stem; (○) root.

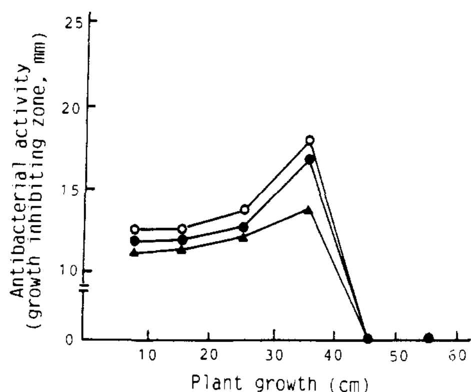

Figure 5. Changes in the antibacterial activity of organ extracts during plant growth of *P. hydropiper*. (●) Leaf; (▲) stem; (○) root.

acetate (AcOEt) and H_2O. The AcOEt layer was evaporated to dryness. Methanol was added to the residue, and 10-μL aliquots were analyzed by HPLC.

Antimicrobial Assay. The antimicrobial activity of plant extracts was examined by the paper disk method (Haraguchi et al., 1990). A paper disk (Toyo, 8 mm i.d.) was soaked in the methanol extract solution of each plant organ. After drying, disks were put onto individual agar plates seeded with *Saccharomyces cerevisiae* IFO 0203 or *Pseudomonas aeruginosa* IFO 3080. The assay plates consisted of 2.5% malt extract and 1.5% agar for fungi (*S. cerevisiae*) or 3% nutrient broth and 1.5% agar for bacteria (*P. aeruginosa*). The plates were incubated at 25 (fungi) or 37 °C (bacteria). After 2 days of incubation, the diameters of growth-inhibiting zones were measured.

RESULTS AND DISCUSSION

HPLC Analysis. With an elution solvent of 60% methanol, both warburganal and polygodial eluted as sharp peaks (Figure 2) with retention times of 11.7 and 21.5 min, respectively. Increasing the proportion of methanol to water increased the retention times. When methanol was replaced by acetonitrile, warburganal eluted too quickly. When the proportion of acetonitrile decreased, the retention time of polygodial was greater. The solvent system using methanol–H_2O (60:40) was acceptable.

The linearity of the detector response was verified using a series of methanol solutions containing sesquiterpene dialdehydes. The relationship between peak areas (detector responses) and amount of polygodial or warburganal injected was linear over 0.125–2 and 0.5–10 μg, respectively.

The results obtained from analyses of extracts are given in Figure 3 and show that the contents of polygodial continued to increase for up to 25 cm of plant growth. Warburganal was present in much younger plant. The amounts of these sesquiterpenes were larger in leaves. However, increasing plant growth decreased the contents of polygodial and warburganal.

Antimicrobial Activity. Figure 4 shows the relationship of plant growth and antifungal activity of plant extract. The extracts of younger leaves exhibited potent antifungal activity against *S. cerevisiae*. Root extract had no effect on fungal growth. Root extracts showed low sesquiterpene dialdehyde contents (Figure 3). The graphic pattern of antifungal activity corresponds with the sesquiterpene dialdehyde content. The diameter of the growth-inhibiting zone was in proportion to the amount of polygodial or warburganal (data not shown). The disk soaked in 35 μg/mL polygodial showed 22 cm of growth-inhibiting zone; this corresponds with the sesquiterpene dialdehyde content and the growth inhibitory zone of the 25-cm plant extract.

Extracts of *P. hydropiper* also possessed antibacterial activity against *P. aeruginosa*, as shown in Figure 5. This activity was observed in all organs, including roots, and continued up to 35 cm of plant growth. The antibacterial activity did not seem to be correlated with the accumulation of sesquiterpene dialdehydes.

The antifungal activity of *P. hydropiper* extract was considered to be due to the sesquiterpene dialdehyde polygodial and/or warburganal. The antibacterial activity seemed to be the effect of other components. Comparisons between hydroponically grown plants and field-collected ones and between wild species and agricultural ones are now in progress.

ACKNOWLEDGMENT

We thank Mr. N. Shiraga and Mr. K. Ikuta for their technical assistance.

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Received for review June 8, 1992. Revised manuscript received October 6, 1992. Accepted October 27, 1992.