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ALLELOPATHIC EFFECTS OF *p*-MENTHANE-3,8-DIOLS IN *EUCALYPTUS CITRIODORA*

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Key Word Index—*Eucalyptus citriodora*; Myrtaceae; allelopathy; menthanediols; germination inhibitor; growth inhibitor.

Abstract—The relationship between the allelopathic *p*-menthane-3,8-diols and the ontogenetic age in *Eucalyptus citriodora* was elucidated. The diols in the soil from a *Eucalyptus* grove were analysed by mass chromatography. Germination and growth inhibitory activities of the *cis*-diol against several higher plants were examined.

INTRODUCTION

Since Molisch defined the term allelopathy in 1937 [1], many scientists have been concerned with the exploration and exploitation of allelopathic chemicals [2–15]. It is well known that some *Eucalyptus* species (gum trees) are surrounded by bare (grass-free) ground. In *E. camaldulensis* [4] this allelopathic effect is due to 1,8-cineole and α-pinene. In the course of our research on *Eucalyptus* metabolites which exhibit biological activity, we have studied the allelopathic substances from *E. citriodora* (lemon-scented gum) leaves. In the previous paper [16], (±)-*p*-menthane-3,8-*cis*-diol (**1**) (4.6 mg/g fr. wt leaves) and its *trans* isomer (**2**) (2.2 mg/g fr. wt leaves) were isolated as germination and growth inhibitors against lettuce (*Lactuca sativa* L.). Interestingly, the diols occurred in the *Eucalyptus* plants as a racemic mixture, and were absent from ontogenetically juvenile tissue of *E. citriodora*.

This paper deals with the relationships between allelopathic substances and ontogenetic age of *E. citriodora*, the presence of *p*-menthane-3,8-diols in the soil from an *E. citriodora* grove, and the germination and growth inhibitory activity of *p*-menthane-3,8-*cis*-diol against several higher plants.

RESULTS AND DISCUSSION

Allelopathic substances and ontogenetic age

The full details of the isolation and subsequent characterization of the *cis* and *trans* isomers of *p*-menthane-3,8-diol from adult leaves of *E. citriodora* are given in the Experimental.

The relationships between the amounts of constituents determined by GC and GC/MS and ontogenetic age in *E. citriodora* seedling are shown in Fig. 1. The diols were absent from ontogenetically juvenile tissue until 13 months or approximately 50 nodes. (±)-Citronellal, which is the major component of *E. citriodora* essential oils, gradually increased and after 13 months decreased dramatically. In contrast, the *cis* and *trans* *p*-menthane-3,8-diols increased with a ratio of 2 to 1 after 13 months. This suggested that the *cis* and *trans* diols are formed by cyclization of citronellal by an obvious biogenetic pathway. However, it is not clear whether or not the diols are produced enzymatically from (±)-citronellal. The diols were not artifacts since the pH of the homogenized tissues was neutral.

p-Menthane-3,8-diols in soil

Soil samples were collected at an *E. citriodora* grove in Queensland state, Australia and extracted with methanol (see Experimental). The methanol extract was concentrated and re-extracted with ether. *p*-Menthane-3,8-diols in the ether soluble fraction were detected by GC/MS. The GC trace given by the total ion current showed very complicated peaks. However, the ions monitored at *m/z* 59, 81 and 96 gave two big peaks on the mass chromatogram which were identified as the *cis* and *trans* forms of *p*-menthane-3,8-diols by comparisons of MS and GC data with those of synthetic standard compounds prepared by acid-catalysed cyclization of citronellal [17].

Furthermore, the concentrations of the diols were quantitatively determined by GC/MS. The sum of concentrations of the *cis* and *trans* diols was approximately 15 ppm although the concentration of the *cis* diol in the fresh leaves was *ca* 4600 ppm. The low concentration of diols indicates that methanol extraction of these compounds from soil is inefficient and/or that they are partially transformed by microorganisms in the soil.

Germination and growth inhibitory activity

In the previous paper [16], it was indicated that the germination inhibitory activity of the *cis* isomer was much higher than that of the *trans* isomer, and that the (+) synthetic *cis* isomer had a higher activity than the optical antipode. In this paper, the (+) synthetic *cis* isomer was used for germination and growth tests against several higher plants. Bioassays were made on filter papers or 0.35% agar solutions ranging in concentration from 10 to 300 ppm. The inhibitory activities against seeds and seedlings of several plants are shown in Figs 2 and 3, respectively. Concentrations as high as 100–300 ppm (5.8×10^{-4}–1.7×10^{-3} M) were inhibitory to seed germination and hypocotyl growth in lettuce (*Lactuca sativa* L.

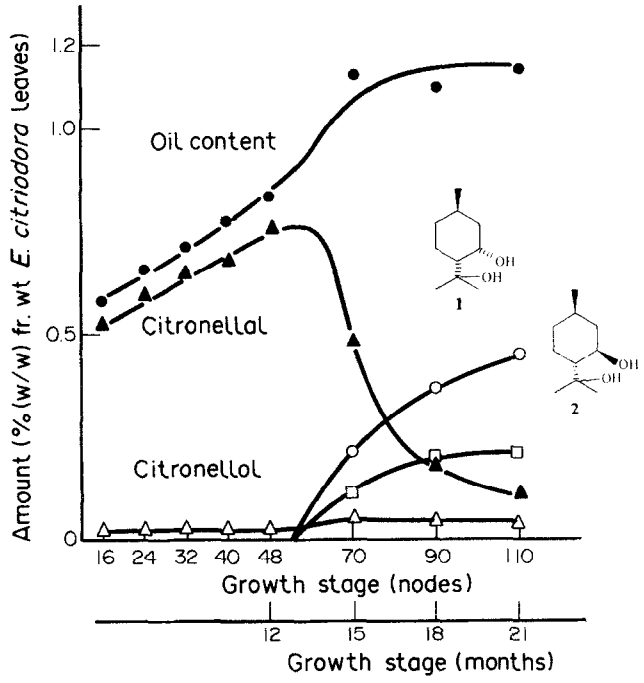

Fig. 1. Relationship between amounts of constituents and ontogenetic age of *E. citriodora*. **1**, *p*-Menthane-3,8-*cis*-diol; **2**, *p*-menthane-3,8-*trans*-diol.

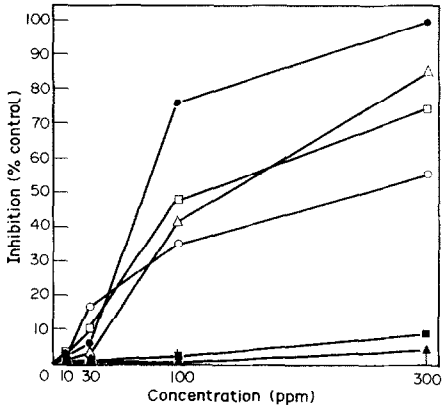

Fig. 2. Germination inhibition of *p*-menthane-3,8-*cis*-diol (**1**) against seeds of several higher plants. Experimental error was within 7%. ●, Lettuce; △, garden cress; □, green foxtail; ○, barnyard grass; ■, rice; ▲, *E. citriodora*.

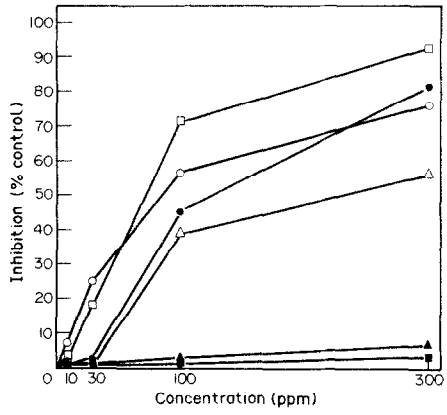

Fig. 3. Growth inhibition of *p*-menthane-3,8-*cis*-diol (**1**) against seedlings of several higher plants. Experimental error was within 7%. Symbols used are as in Fig. 2.

cv. Wayahead), garden cress (*Lepidium sativum* L.), green foxtail (*Setaria viridis* L.) and barnyard grass (*Panicum Crus-galli* L.). However, these and higher concentrations of the diol had no inhibitory effects on germination and growth of *E. citriodora* itself and rice (*Oryza sativa* L.). In terms of herbicidal properties, it is interesting that the biological activity of the *cis* diol is very selective against higher plants.

The structure–activity relationships of *p*-menthane-3,8-diols will be presented elsewhere.

EXPERIMENTAL

Plants and soil materials. Seeds and leaves of *E. citriodora* Hook. were collected near Canberra in Australia with the aid of Dr. D. M. Paton, Department of Botany, the Australia National University. Seedlings were cultivated in the greenhouse of Hokkaido University, Sapporo.

Soil samples were collected at an *E. citriodora* grove in Queensland state, Australia by Dr. I. C. MacRae, Department of Microbiology, University of Queensland. The soil (*ca* 500 g)

without leaf trash was extracted with MeOH at ambient temp. for 2 weeks.

General methods. Glass capillary GC (SCOT column, 30 m × 0.28 mm i.d. coated with PEG-HT) was used. The oven temp. was programmed from 100 to 180° at 2°/min. The injector and detector were maintained at 250°. A flow rate of 1.25 ml N_2/min was employed. GC (continuous ion monitoring)/MS was carried out by using a JEOL JMA-2000 mass data system and JMS-D300 spectrometer at an ionizing voltage of 30 or 70 eV.

Isolation of allelopathic chemicals. Fractionation of an Me_2CO extract of fresh adult leaves was monitored by inhibitory activity against germinating seeds and seedlings of lettuce (*L. sativa*), garden cress (*L. sativum*), and green foxtail (*S. viridis*). Active fractions were obtained by steam distillation and silica gel CC using a hexane–Et_2O gradient as eluent. Rechromatography gave two inhibitors as crystals.

p-*Menthane*-3,8-cis-*diol.* Mp 81.0–82.5° (crystallized from Et_2O–hexane), $[\alpha]_D^{23}$ ±0° ($CHCl_3$; *c* 0.2). IR ν_{max}^{KBr} cm^{-1}: 3240, 2930, 2900, 1450, 1420, 1250, 1160, 930; High resolution FIMS *m/z* (rel. int.): 173.1532 $[M+H]^+$ (16), 157 $[M-Me]^+$ (37), 154 $[M-H_2O]^+$ (100), 114 (9), 96 $[M-OH-$hydroxyisopropyl$]^+$ (83), 77 (41), 59 (85); EIMS (probe) 70 eV, *m/z* (rel. int.): no M^+ peak, 157 $[M-Me]^+$ (1), 154 $[M-H_2O]^+$ (2), 139 $[M-H_2O-Me]^+$ (3), 121 (2), 111 (2), 96 (40), 81 $[M-OH-$hydroxyisopropyl$-Me]^+$ (100), 68 (16), 59 (79), 55 (21), 54 (21), 43 (42), 41 (34); ^1H NMR (200 MHz, $CDCl_3$, TMS): δ0.87 (3H, *d*, *J* = 6.4 Hz, H-7), 1.22 (3H, *s*, H-9), 1.36 (3H, *s*, H-10), 4.41 (1H, *q*, *J* = 2.4 Hz, H-3); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ20.4 (*t*, C-5), 22.3 (*q*, C-7), 25.7 (*d*, C-1), 28.8 (*q*, C-9), 29.0 (*q*, C-10), 35.0 (*t*, C-6), 42.6 (*t*, C-2), 48.4 (*d*, C-4), 68.1 (*d*, C-3), 73.3 (*s*, C-8).

p-*Menthane*-3,8-trans-*diol.* Mp 77.3–78.3° (from Et_2O–hexane), $[\alpha]_D^{23}$ ±0° ($CHCl_3$; *c* 0.1). IR ν_{max}^{KBr} cm^{-1}: 3250, 2960, 2920, 1450, 1420, 1220, 1180, 1000, 910, 870; High resolution FIMS *m/z* (rel. int.): 173.1546 $[M+H]^+$ (47), 157 $[M-Me]^+$ (11), 154 $[M-H_2O]^+$ (30), 114 (10), 113 (15), 96 $[M-OH-$hydroxyisopropyl$]^+$ (54), 77 (87), 59 (100); EIMS (probe) 70 eV, (rel. int.): no M^+ peak, 157 $[M-Me]^+$ (1), 154 $[M-H_2O]^+$ (1), 139 $[M-H_2O-Me]^+$ (3), 121 (2), 111 (1), 96 (38), 81 (100), 68 (10), 59 (90), 55 (13), 54 (20), 43 (34), 41 (19); ^1H NMR (200 MHz, $CDCl_3$, TMS): δ0.92 (3H, *d*, *J* = 6.4 Hz, H-7), 1.22 (6H, *s*, H-9 and H-10), 3.72 (1H, *dt*, *J* = 10.4, 4.3 Hz, H-3); ^{13}C NMR (50 MHz, $CDCl_3$, TMS): δ22.0 (*q*, C-9), 23.8 (*q*, C-10), 27.1 (*t*, C-5), 30.1 (*q*, C-7), 31.4 (*d*, C-1), 34.6 (*t*, C-6), 44.7 (*t*, C-2) 53.5 (C-4), 72.9 (*d*, C-3), 75.0 (*s*, C-8).

Determination of the diols. E. citriodora seedlings were grown at Hokkaido University, Sapporo. Fresh leaves (*ca* 30 g) were harvested at each growth stage (5–21 months), and Et_2O extracts of the fresh leaves analysed by glass capillary GC. α-Terpineol and lauryl alcohol were used as internal standards for the determination of citronellal, citronellol and *p*-menthane-3,8-diols. Quantitative error was within 2%.

Germination test. Fractions (300 μg/ml, 2 ml) or pure compounds (10–300 μg/ml 12 ml) dissolved in Me_2CO, were allowed to soak into filter papers in Petri dishes (dia. 6 cm). After the papers had been dried, Tween 80 soln (100 μg/ml, 2 ml) was poured into each dish which was then left overnight. Next day each dish was sown with 50 seeds of lettuce, garden cress, green foxtail, barnyard grass or rice and incubated at 22° for 1–5 days in the dark. The bioassay was repeated twice under the same conditions. Germinated seeds were counted and compared with control.

Growth test. The sample (20–600 mg) was dissolved in 10 ml Me_2CO and the resultant soln (0.1 ml) added to 20 ml 0.35% agar soln. After the agar soln had solidified, 20 seedlings were put on the agar in a deep Petri dish (60 mm × 60 mm dia.) and incubated at 22° for 7 days in 14 hr light and 10 hr dark. Hypocotyl length was measured and compared with control.

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