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Note

Synthesis and nematocidal activity of ascaridole derivatives against *Meloidogyne incognita* and *Aphelenchoides besseyi*

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A series of ascaridole derivatives **1**–**10** were successfully synthesized and their structures were characterized by ^{1}H, ^{13}C NMR and high-resolution mass spectroscopy. The nematocidal activities of these compounds were evaluated and the results indicated that these derivatives have better nematocidal activities against *Meloidogyne incognita* than *Aphelenchoides besseyi*. Interestingly, *cis*-isomers of *Z*-3,6-epidioxy-1-methyl-4-isopropylcyclohexene (**2**) and *Z*-3,6-epidioxy-1-methyl-4-isopropenylcyclohexene (**4**) are more active than their corresponding *trans*-isomers. Among them, 1,4-epidioxy-4-isopropyl-2-cyclohexenoic acid (**8**) and 1,4-epidioxy-4-isopropyl-2-cyclohexenecarboxylic acid methyl ester (**10**) exhibited significant higher nematocidal activities than those of other analogs; therefore the carboxylic acid or carboxyl ester substituent on the ring might be essential for high nematocidal activity. Compared with the toxicity of a commercial nematocide, Oxamyl, these derivatives can be considered effective and promising. © Pesticide Science Society of Japan

Keywords: ascaridole, nematocidal activity.

Introduction

Plant diseases can be caused by parasitic nematodes.[1] The most destructive species are *Meloidogyne incognita* and *Aphelenchoides besseyi*, which can cause serious problems for a number of economically important agricultural crops including grape, papaya, guava, watermelon, rice and strawberry.[1] The current management of *M. incognita* and *A. besseyi* primarily relies on toxic nematocide application[2]; however, due to these chemicals creating a potential hazard to the environment and human health, alternatives to new environmentally friendly nematocides are desirable.

Chenopodium ambrosioides is a natural product which has shown to be effective in controlling nematodes.[3] It is used to produce Chenopodium oil, which has been used as an anthelmintic for the treatment of intestinal worms for many years.[4] The anthelmintic property of Chenopodium oil is due to its major component, ascaridole.[4] Recently, ascaridole was reported to have nematocidal activity to kill *Caenorhabditis elegans*[5] Pollack *et al.* observed that ascaridole could inhibit the growth of *Plasmodium falciparum in vitro*, and a similar compound, cineol, lacking the internal 1,4-endoperoxide, was found to be inactive. Artemisinin, on the other hand, which contains a 1,4-endoperoxide, was active against *Plasmodium falciparum*. These observations prompted Pollack *et al.* to conclude that the endoperoxide in ascaridole was essential for its anthelmintic activity.[6] Kiuchi *et al.* reported that the natural product, (−)-(1*S*,4*S*)-*p*-mentha-2,8-dien-1-hydroperoxide, which has an isopropenyl group appended to the cyclohexenyl ring, showed stronger trypanocidal activity than ascaridole.[3] Mao *et al.* also reported that derivatives of Tebufenozide containing a carboxylic acid or ester substituent resulted in increasing their insecticidal activities.[7] Encouraged by these reports and with the aim of further exploring new potential nematocides, ascaridole derivatives bearing some functional groups such as carboxylic acid or ester were considered. In this paper we describe the synthesis of a series of ascaridole derivatives and the comparison of their nematocidal activities against *M. incognita* and *A. besseyi*.

Materials and Methods

1. *Chemicals*

α-Terpinene, rose bengal, α-phellandrene, carvone, and isopropenyl acetate were obtained from Aldrich. The precursor compounds, 1-methyl-4-isopropenyl-2,6-cyclohexadiene (**11**), 1-methyl-2-acetoxy-4-isopropenyl-2,6-cyclohexadiene (**12**), 4-isopropyl-1,3-cyclohexadiene-carboxylic acid (**13**), 4-isopropyl-1,3-cyclohexadiene-1-methanol (**14**), and 4-isopropyl-1,3- cyclohexadienecarboxylic acid methyl ester (**15**) were prepared following published procedures.[8–11]

2. *General synthetic procedure for ascaridole (1) and its derivatives (2–10)*

To each precursor compound such as α-terpine and *etc.* (73.6 mmol) in methanol (800 ml) was added 60 mg of rose bengal. The reaction mixture was stirred at 0°C. Oxygen was bubbled through the mixture (flow rate 6.3 ml/s) and irradiated with a 500 W tungsten lamp. After the starting material disappeared according to TLC, the reaction mixture was then concentrated *in vacuo*. The residue was purified by column chromatography to give products **1**–**10**. The spectra data of ascaridol (**1**), *Z*-3,6-epidioxy-1-methyl-4-isopropylcyclohexene (**2**), and *E*-3,6-epidioxy-1-methyl-4-isopropylcyclohexene (**3**) were consistent with those

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in the literature.[3,12)]

2.1. Z-3,6-Epidioxy-1-methyl-4-isopropenylcyclohexene (4)

Yield, 29%; bp 136–138°C (36 mm Hg); $[\alpha]_D$ +17.9° (*c* 1.4, $CHCl_3$); ^1H NMR δ_H ($CDCl_3$): 1.71–1.79 (m, 1H), 1.88 (s, 3H), 1.96 (s, 3H), 2.09–2.15 (m, 1H), 2.20–2.25 (m, 1H), 4.43–4.46 (m, 1H), 4.50–4.52 (m, 1H), 4.94 (s, 1H), 5.00 (s, 1H), 6.37–6.39 (m, 1H); ^{13}C NMR δ_C ($CDCl_3$): 18.6, 21.6, 26.2, 40.8, 74.8, 75.8, 111.9, 126.0, 141.0, 145.5; HR-EIMS *m/z* (M^+): Found 166.0989, Calcd. for $C_{10}H_{14}O_2$: 166.0994.

2.2. E-3,6-Epidioxy-1-methyl-4-isopropenylcyclohexene (5)

Yield, 26%; bp 132–137°C (34 mm Hg); $[\alpha]_D$ +23.1° (*c* 1.7, $CHCl_3$); ^1H NMR δ_H ($CDCl_3$): 1.37–1.41 (m, 1H), 1.70 (s, 3H), 1.96 (s, 3H), 2.42–2.48 (m, 1H), 2.93–2.98 (m, 1H), 4.50–4.51 (m, 1H), 4.56 (s, 1H), 4.61–4.64 (m, 1H), 4.78 (s, 1H), 6.17–6.20 (m, 1H); ^{13}C NMR δ_C ($CDCl_3$): 18.4, 21.9, 28.6, 40.9, 73.8, 75.7, 111.0, 123.4, 141.4, 145.2; HR-EIMS *m/z* (M): Found 166.0985, Calcd. for $C_{10}H_{14}O_2$: 166.0994.

2.3. Z-3,6-Epidioxy-1-methyl-2-acetoxy-4-isopropenylcyclohexene (6)

Yield, 37%; bp 188–194°C (30 mm Hg); $[\alpha]_D$ +14.9° (*c* 0.8, $CHCl_3$); ^1H NMR δ_H ($CDCl_3$): 1.84 (s, 3H), 1.85 (s, 3H), 2.08–2.16 (m, 2H), 2.23 (s, 3H), 2.82–2.86 (m, 1H), 4.55 (s, 1H), 4.58–4.59 (m, 1H), 4.96 (s, 1H), 5.03 (s, 1H); ^{13}C NMR δ_C ($CDCl_3$): 12.2, 20.8, 21.6, 28.7, 40.7, 75.4, 76.3, 111.3, 124.0, 142.0, 143.9, 167.3; HR-FABMS *m/z* ($M+H^+$): Found 225.1126, Calcd. for $C_{12}H_{16}O_4$: 225.1127.

2.4. E-3,6-Epidioxy-1-methyl-2-acetoxy-4-isopropenylcyclohexene (7)

Yield, 29%; bp 192–199°C (30 mm Hg); $[\alpha]_D$ +16.0° (*c* 1.0, $CHCl_3$); ^1H NMR δ_H ($CDCl_3$): 1.46–1.51 (m, 1H), 1.74 (s, 3H), 1.83 (s, 3H), 2.20 (s, 3H), 2.39–2.46 (m, 1H), 2.96–2.98 (m, 1H), 4.60 (s, 1H), 4.66–4.67 (m, 1H), 4.68–4.69 (m, 1H), 4.85 (s, 1H). ^{13}C NMR δ_C ($CDCl_3$): 12.1, 20.3, 21.6, 27.2, 40.2, 77.2, 77.3, 112.0, 122.5, 144.7, 145.6, 169.2; HR-FABMS *m/z* ($M+H^+$): Found 225.1129, Calcd. for $C_{12}H_{16}O_4$: 225.1127.

2.5. 1,4-Epidioxy-4-isopropyl-2-cyclohexenoic acid (8)

Yield 68%. bp 228–230°C (40 mm Hg). ^1H NMR δ_H ($CDCl_3$): 0.99 (d, 3H, *J*=6.8 Hz), 1.03 (d, 3H, *J*=6.8 Hz), 1.90–2.01 (m, 3H, –OH hide in), 2.13–2.20 (m, 1H), 2.40–2.47 (m, 1H), 2.64–2.72 (m, 1H), 5.97 (d, 1H, *J*=10.4 Hz), 6.77 (d, 1H, *J*=10.4 Hz); ^{13}C NMR δ_C ($CDCl_3$): 16.4, 17.3, 30.8, 33.8, 36.7, 71.8, 76.6, 129.2, 153.1, 199.5; MS *m/z* : 154 (M^+-CO_2), 111 (100), 97 (11), 83 (56), 67 (8), 55 (41), 43 (23).

2.6. 1,4-Epidioxy-4-isopropyl-2-cyclohexene-1-methanol (9)

Yield, 56%; bp 180–182°C (40 mm Hg); ^1H NMR δ_H ($CDCl_3$): 1.02 (d, 6H, *J*=8.0 Hz), 1.33–1.43 (m, 1H), 1.56–1.66 (m, 1H), 1.77 (brs, 1H, OH), 1.92–2.14 (m, 3H), 3.75–3.83 (m, 2H), 6.57 (d, 1H, *J*=8.8 Hz), 6.65 (d, 1H, *J*=8.8 Hz); ^{13}C NMR δ_C ($CDCl_3$): 17.0, 17.1, 24.6, 24.7, 32.0, 64.3, 77.9, 80.5, 132.7, 133.6; HR-FABMS *m/z* ($M+H^+$): Found 185.1182, Calcd. for $C_{10}H_{16}O_3$: 185.1178.

2.7. 1,4-Epidioxy-4-isopropyl-2-cyclohexenecarboxylic acid methyl ester (10)

Yield 69%; bp 162–164°C (40 mmHg). ^1H NMR δ_H ($CDCl_3$): 1.00 (d, 3H, *J*=2.8 Hz), 1.02 (d, 3H, *J*=2.8 Hz), 1.52–1.59 (m, 1H), 1.72–1.79 (m, 1H), 1.93–2.00 (m, 1H), 2.06–2.13 (m, 1H), 2.40–2.46 (m, 1H), 3.89 (s, 3H, OMe), 6.60 (d, 1H, *J*=8.8 Hz), 6.78 (d, 1H, *J*=8.8 Hz); ^{13}C δ_C NMR ($CDCl_3$): 17.0, 17.1, 24.3, 27.1, 31.8, 52.8, 77.2, 80.5, 132.0, 133.9, 169.6; HR-FABMS *m/z* ($M+H^+$): Found 213.1134, Calcd. for $C_{11}H_{16}O_4$: 213.1127.

3. Nematodes

Meloidogyne incognita was isolated from the roots of a tomato plant infested by *Meloidogyne incognita*. *Aphelenchoides besseyi* was isolated from rice seed from a paddy field infested by *A. besseyi*.

3.1. Nematicidal activity

Due to the poor water solubility of ascaridole and its derivatives, the test solution was prepared by serial dilutions of each compound with 0.05 g of Ablunol AG-L. The nematocidal test against *M. incognita* and *A. besseyi* was carried out as described previously.[13)] LC_{50} values were calculated by using the computer program POLO-PC.[14)]

Results and Discussion

1. Preparations

Compounds **1**–**10** were prepared as shown in Fig. 1. Ascaridole (**1**) was synthesized by the photooxygenation of α-terpine.[15)] Analogs **2** and **3** were prepared by the reaction of α-phellandrene with singlet oxygen. Compounds **4** and **5** were prepared by the reaction of singlet oxygen with 1-methyl-4-isopropenyl-2,6-cyclohexadiene (**11**),[8)] which was synthesized by the conversion of carvone to the corresponding tosylhydrazone followed by treatment with methyl lithium. In order to introduce the acetoxy group on the 2-position of the cyclohexenyl ring of **4** and **5**, the precursor 1-methyl-2-acetoxy-4-isopropenyl-2,6-cyclohexadiene (**12**)[9)] was prepared by the reaction of carvone with isopropenyl acetate. Singlet oxygen reacted with **12** afforded two new derivatives **6** and **7**. Commercially available α-pinene was efficiently converted to 4-isopropyl-1,3-cyclohexadienecarboxylic acid (**13**)[10)] by treatment with $KMnO_4$ and H_2SO_4. Reduction of **13** with $LiAlH_4$ gave 4-isopropyl-1,3-cyclohexadiene-1-methanol (**14**).[13)] Esterification of **13** with thionyl chloride and methanol gave 4-isopropyl-1,3-cyclohexadienecarboxylic acid methyl ester (**15**).[11)] Parallel reactions of **13**–**15** with $^1O_2$ gave the corresponding derivatives **8**, **9**, and **10**, respectively. Compounds **1**–**10** were purified by column chromatography and their structures were characterized by ^1H and ^{13}C NMR and HRMS.

2. Nematocidal activity

The *in vitro* nematocidal activities of ascaridole derivatives were evaluated against *M. incognita* and *A. besseyi*. Compounds **1**–**10** exhibited 100% of mortality against *M. incognita* at the concentration of 100 μg/ml. With regard to *A. besseyi*, **8** and **10** at the concentration of 200 μg/ml showed 100% mortality, but **1**, **6**, and **9** only exhibited <50% mortality at the same concentration. These results indicate that ascaridole derivatives have better nematocidal activity against *M. incognita* than *A. besseyi*. Accordingly, their LC_{50} values were determined and are listed in Table 1.

It was found that the *cis* configuration is preferred by two-fold

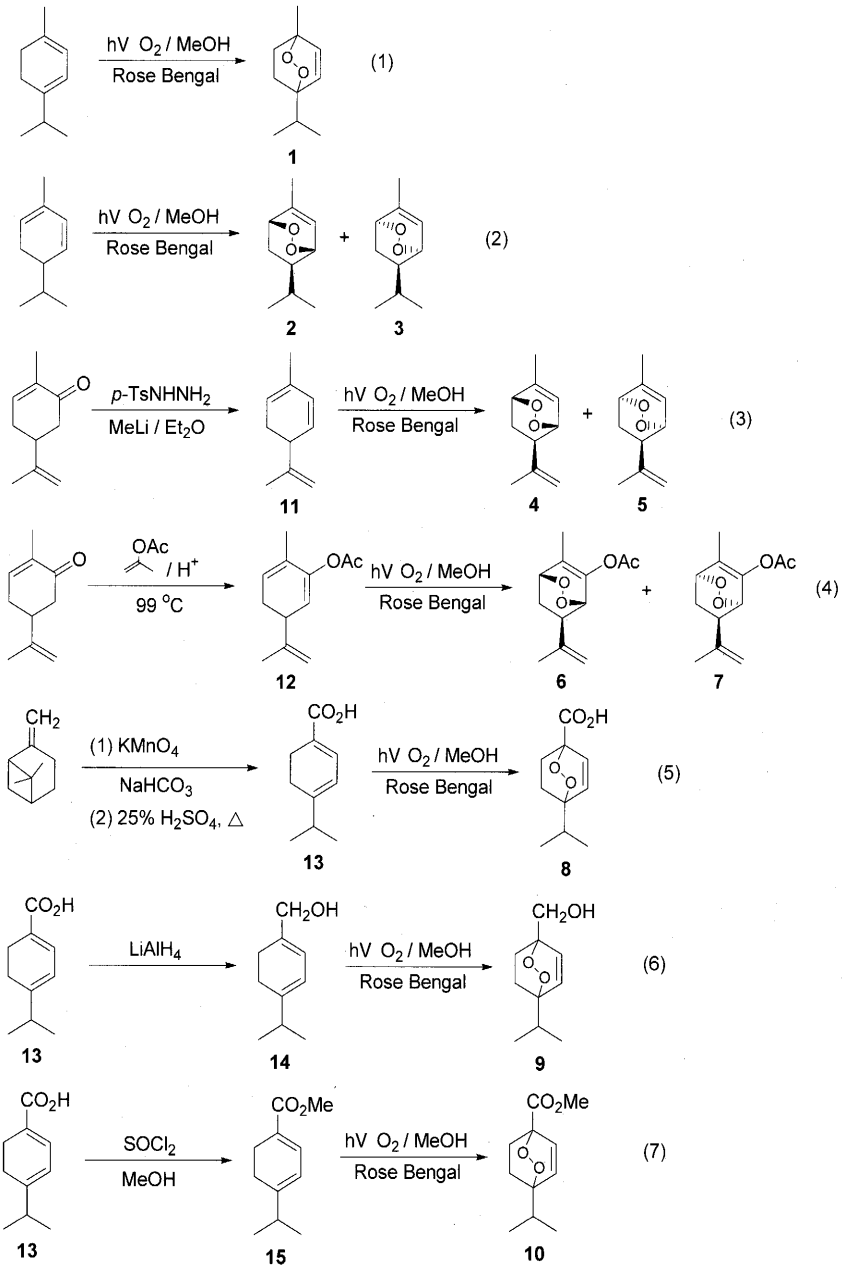

Fig. 1. Synthesis of compounds **1**–**10**.

nematocidal activity over the *trans* configuration as in the cases of **2**/**3** (25 : 51 μg/ml) and **4**/**5** (12 : 23 μg/ml). Moreover, **4** and **5** exhibited two-fold nematocidal activity as compared to **2** and **3**, respectively, indicating that an sp^2 isopropenyl group is preferred over an sp^3 isopropyl group. As regard to the cyclohexenyl ring, an acetoxy substituent at the 2-position deteriorated nematocidal activity based on the comparison of **6**/**7** with **4**/**5**. Among these ascaridole derivatives, **8** and **10** exhibited the highest nematocidal activity with LC_{50} values of 2 μg/ml. Apparently, carboxylic acid (**8**) or carboxyl ester (**10**) significantly improves nematocidal activity against both *M. incognita* and *A. besseyi*. In addition, changing the methyl group in **1** to methyl alcohol in **9** did not affect nematocidal activity. On the basis of these results, we might conclude that the carbonyl groups in **8** and **10** are essential for high nematocidal activity.

Oxamyl has been used widely to control plant parasitic nematodes, and the values of LC_{50} against *M. incognita* and *A. besseyi* were determined to be 0.07 and 0.10 μg/ml, respectively (Table 1). Oxamyl was reported to have an LC_{50} value of 3.1 mg/kg towards rats,[16] and its oral toxicity was classified as category I. These properties show that it has high mammalian toxicity and is not environmental friendly; however, ascaridole was reported to have low mammalian toxicity with LDLo of 250 mg/kg.[17] In our study, the LC_{50} values of ascaridole against *M. incognita* and *A. besseyi* were determined as 52 and 365 μg/ml, respectively; therefore, ascaridole derivatives **1**–**10** can be considered as synthetic promising nematocides with lower toxicity.

Table 1. LC_{50} values of *M. incognita* and *A. besseyi* for ten ascaridole derivatives and oxamyl[a)]

Chemicals	LC_{50} (mg/ml)	
	M. incognita	*A. besseyi*
1	52	365
2	25	69
3	51	110
4	12	21
5	23	50
6	50	302
7	51	306
8	2	4
9	55	402
10	2	2
Oxamyl	0.07	0.10

[a)] The lethal concentration expressed as μg/ml. End-point mortality is obtained at 24 hr after treatment.

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