ORIGINAL ARTICLE

Chemical structures of *p*-menthane monoterpenes with special reference to their effect on seed germination and termite mortality

Nobuhiro Sekine · Sakae Shibutani

Received: 5 September 2012/Accepted: 21 January 2013/Published online: 22 February 2013 © The Japan Wood Research Society 2013

Abstract A series of *p*-menthane monoterpenes was investigated to confirm any correlation between their bioactivity (effect on seed germination and termite mortality) and chemical structure. The germination percentages of Brassica rapa seeds at a concentration of 0.1 mg/Petri dish of (+)-pulegone, isopulegol, piperitone, (-)-dihydrocarveol, terpinen-4-ol and (-)-menthol were found to be 21.6, 27.3, 27.3, 29.1, 42.9 and 43.4, respectively. The lethal concentration 50 values of carvacrol, (+)-pulegone, thymol, (-)-menthol and (-)-terpinen-4-ol for termites (Reticulitermes speratus) were 0.34, 0.50, 0.65, 0.92 and 1.26 (mg/Petri dish), respectively. Of all the compounds tested, phenols produced the highest levels of termite mortality, with ketones and alcohols also showing bioactivity. An assessment of the bioactivity revealed that the presence of a phenol group was effective for termite mortality, with a carbonyl group also showing strong bioactivity. The presence of an alcohol or isopropyl group in a ring also contributed to the bioactivity, whereas the presence of an isopropenyl group at the same position, however, exhibited an inhibitory effect on seed germination. In conclusion, the bioactivity of the *p*-menthane monoterpenoids was dependent upon the presence and position of certain functional

N. Sekine (🖂)

Faculty of Agriculture, Yamagata University, 1-23 Wakaba-machi, Tsuruoka, Yamagata 997-8555, Japan e-mail: kineno128@yahoo.co.jp

N. Sekine · S. Shibutani Institute of Wood Technology, Akita Prefectural University, 11-1 Kaieizaka, Noshiro, Akita 016-0876, Japan groups and the degree of saturation in the functional group of the side chain.

Keywords Monoterpenoid $\cdot p$ -Menthane \cdot Termite \cdot Seed

Introduction

Monoterpenes are a class of organic compounds composed of two isoprene units and can be further classified as either acyclic (e.g., myrcene), cyclic (e.g., *p*-menthanes) or bicyclic (e.g., bornanes, fenchanes, caranes, pinanes and thujanes) depending upon their molecular connectivities. In addition to their basic hydrocarbon forms, they can exist as the corresponding oxygenated compounds, containing aldehyde, alcohol, ketone, ester and ether functionalities [1, 2]. Monoterpenes have been isolated from the essential oils of many higher plants. Essential oils are very complex natural mixtures and, in the majority of cases, contain somewhere in the region 20–60 components at quite different concentrations. Monoterpenes are an important class of compounds in perfumery and flavor industries [3].

Thyme oil contains thymol, carvacrol, *p*-cymene and γ -terpinene as its major components [3, 4], and is used as a flavoring in the processed food industry, as well as in soaps and detergents [3]. Thyme oil has been shown to exhibit a range of biological activities, including antimicrobial [5, 6], antifungal [7], trypanocidal [8], and antioxidant activities [9].

Peppermint oil contains about 40 % menthol and 20 % menthone, and is used in various medicinal, pharmaceutical, cosmetic and perfumery products as well as in foodstuffs [3, 10]. (–)-Menthol is a particularly important flavoring compound and is used extensively in toothpastes and chewing gums [2, 3, 11]. Peppermint oil is known to possess bioactivities such as antimicrobial [10], and genotoxic activities [12].

Part of these results was presented at the 61st Annual Meeting of the Japan Wood Research Society held in Kyoto, Japan, March 18–20, 2011.

p-Menthane monoterpenes are characteristic of thyme and peppermint oils [2] and the bioactivities of both of these oils are possibly related to the functions of *p*-menthane monoterpenes.

Recently, the essential oils of needles of *Pinus thunbergii* and Cryptomeria japonica were found to be composed predominantly of p-menthane monoterpenes at 19.8 and 27.1 %, respectively [13]. In addition, the monoterpenes α -terpineol and terpinen-4-ol, both of which possess a p-menthane skeleton, were identified as the bioactive components of the needle essential oils of P. thunbergii and C. japonica, respectively [13]. Of all of the compounds contained within the essential oils, the bioactivity levels of α -terpineol and terpinen-4-ol were found to be particularly pronounced against plant seeds (Raphanus sativus and Brassica rapa) and termites (Reticulitermes speratus) [13]. These findings suggested that the bioactivity of p-menthanes was affected by the presence of an alcoholic hydroxyl group. With this in mind, a fundamental evaluation of the correlation between the bioactivity of p-menthane compounds and their chemical structures would therefore be required to develop a better understanding of this particular series of compounds. There are, however, a few reports on the chemical structure of *p*-menthane monoterpenes with special reference to their effects on seed germination and termite mortality. Therefore, in the present study, p-menthane monoterpenes have been investigated with the aim of confirming the structure-activity relationship (SAR), in terms of their effect on seed germination and termite mortality, and their chemical structure.

Materials and methods

Test samples

p-Cymene, α-terpinene, (–)-α-phellandrene, terpinolene, thymol, carvacrol, (–)-terpinen-4-ol, *trans*-sobrerol, (–)-menthol, isopulegol, (–)-carvone, piperitone and (+)-pulegone were purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). γ-Terpinene, (+)-limonene and (–)-α-terpineol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). *p*-Menthane, *p*-cymen-8-ol, 1,8-*p*-menthadiene-4-ol and (–)-dihydrocarveol were supplied by Nippon Terpene Chemicals, Inc. (Hyogo, Japan). (–)-Carveol was purchased from Sigma-Aldrich Japan Co. LLC. (Tokyo, Japan). The chemical structures of these compounds are shown in Fig. 1.

Seeds

In the present study, *Raphanus sativus* var. *sativus* and *Brassica rapa* var. *perviridis* seeds were used, and were provided by Takii & Co. Ltd. (Kyoto, Japan).

Germination test

The germination test was carried out according to a method described previously in the literature [13]. The effects of the *p*-menthane compounds on germination were tested on the R. sativus and B. rapa seeds. A sample of each of the compounds (50, 5 and 0.5 mg) was dissolved separately in acetone (10 mL), and a portion (2.0 mL) of the resulting solutions was poured onto a filter paper (90 mm diameter, Advantec No. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) in a Petri dish. In a control Petri dish, acetone (2.0 mL) was only used. The acetone solvent was then removed by evaporation over a period of 20 min in a fume hood. Deionized water (10 mL) was then added to each of the Petri dishes together with 20 seeds of R. sativus or B. rapa. Petri dishes containing concentrations of 10.0, 1.0 and 0.1 (mg/Petri dish) of each of the compounds were prepared in this way. The screening germination test was conducted only at a concentration of 10.0 mg/Petri dish. Following on from the screening test, germination tests were conducted for the active components at concentrations of 1.0 and 0.1 mg/Petri dish. Each of the compound tests and control treatments were performed in triplicate. The Petri dishes were then covered and placed in a growth chamber (Eyelatron FLI-301NH, Tokyo Rikakikai Co. Ltd., Tokyo, Japan) at a temperature of 24 ± 2 °C for 3 days (sequential 10 h photoperiods and 14 h dark periods).

The number of germinated individuals was recorded on a daily basis throughout the experimental period to determine the germination percentage. The germination percentages were calculated according to formula (1) by considering the control as 100 (%):

Germination percent of *R*. sativus (or *B*. rapa) (%)

- = number of germinated seeds following
 - 2 days(or 1 day) of the tests for each sample/number
- of germinated seeds following 3 days of the
- control tests \times 100 (1)

Termites

Colonies of *Reticulitermes speratus* were collected from the Institute of Wood Technology at the Akita Prefectural University of Japan, in July 2009. The colonies were maintained in a room at 20 ± 2 °C for one and a half years prior to the initiation of the test.

Termiticidal test

The test was conducted according to a method previously described in the literature [13]. Each of the compounds (10.0, 5.0, and 2.5 mg) was dissolved separately in acetone (10 mL) and portion (2.0 mL) of each of the resulting

Fig. 1 Chemical structures of the monoterpenoids containing

a *p*-menthane skeleton



solutions was poured onto a filter paper (90 mm diameter, Advantec No. 2, Toyo Roshi Kaisha, Ltd., Tokyo, Japan) in a Petri dish. In a control Petri dish, acetone (2 mL) was only used. The acetone solvent was then removed by evaporation over a period of 20 min in a fume hood. Deionized water (1.5 mL) was then added to each of the Petri dishes together with 20 active termites (workers). Petri dishes containing concentrations of 2.0, 1.0 and 0.5 (mg/Petri dish) of each compound were prepared in this way. Each compound test and control experiment was conducted in triplicate. The Petri dishes were then covered and placed in an incubator (ICV-450, As One Co., Osaka, Japan) at a temperature of 25 ± 1 °C (dark) for the entire test period (1 h). The numbers of surviving and dead termites were counted following the tests to determine the percentage mortality values. The percent mortality data were subjected to probit analysis to calculate the lethal concentration required to kill 50 % of the termites (LC₅₀) [14].

Statistical analysis

Test samples were compared using analysis of variance (ANOVA), and the means were separated using a protected Tukey–Kramer test (p < 0.05; Statcel 2, Saitama, Japan) [15]. The mean separation test followed transformation to arcsine square root percent of seed germination and termite mortality. The actual percentages are reported in Table 1 and Figs. 2 and 3.

Compound	Germination percent $(\text{mean} \pm \text{SD})^{II}$	
	R. sativus	B. rapa
Hydrocarbons		
<i>p</i> -Cymene	100.0 ± 11.5 C	$72.5 \pm 9.0 \mathrm{abc}$
α-Terpinene ^I	$65.3 \pm 9.6 \mathrm{BC}$	$58.8\pm5.9ab$
γ-Terpinene ^I	$82.1\pm10.9\mathrm{C}$	$75.0 \pm 10.0 \mathrm{bc}$
(-)-a-Phellandrene	76.1 ± 24.7 C	$65.4 \pm 18.5 \mathrm{abc}$
(+)-Limonene ^I	$14.7\pm9.6\mathrm{A}$	$78.8 \pm 8.8 \mathrm{bc}$
Terpinolene	$23.9\pm3.8AB$	$40.4\pm8.0a$
<i>p</i> -Menthane	$87.2\pm18.4\mathrm{C}$	$87.7 \pm 11.0c$
Control	$91.3 \pm 4.7C$	$86.4 \pm 5.1c$
Phenols		
Thymol	$0.0\pm0.0\mathrm{A}$	0.0 ± 0.0 a
Carvacrol	$2.0 \pm 3.5 \text{A}$	0.0 ± 0.0 a
Control	$91.3 \pm 4.7B$	$86.4 \pm 5.1b$
Alcohols		
p-Cymen-8-ol	$0.0\pm0.0\mathrm{A}$	0.0 ± 0.0 a
(-)-Terpinen-4-ol ^I	$4.1 \pm 5.0 A$	8.2 ± 9.4 ab
1,8-p-Menthadiene-4-ol	$2.0 \pm 3.4 \mathrm{A}$	$0.0 \pm 0.0a$
(-)-Carveol	$0.0 \pm 0.0 \mathrm{A}$	$0.0 \pm 0.0a$
(−)-α-Terpineol ^I	$3.2\pm5.3A$	$0.0 \pm 0.0a$
Trans-sobrerol	$43.5\pm10.0\mathrm{B}$	$72.2 \pm 11.1c$
(-)-Dihydrocarveol	$0.0 \pm 0.0 \mathrm{A}$	$0.0 \pm 0.0a$
(–)-Menthol	$2.2\pm3.8A$	$9.4\pm6.5b$
Isopulegol	$0.0 \pm 0.0 \mathrm{A}$	$0.0 \pm 0.0a$
Control	$91.3 \pm 4.7C$	$86.4 \pm 5.1c$
Ketones		
(-)-Carvone	$8.7\pm3.8B$	$11.3 \pm 5.7 \mathrm{b}$
Piperitone	$0.0 \pm 0.0 \mathrm{A}$	0.0 ± 0.0 a
(+)-Pulegone	$0.0 \pm 0.0 \mathrm{A}$	0.0 ± 0.0 a
Control	$91.3 \pm 4.7C$	$86.4 \pm 5.1c$

Table 1 Effect of *p*-menthane monoterpenes on the germination of plant seeds^I

¹ Some of test results were cited in the literature data [13]. Sample concentration was 10.0 mg/Petri dish. Germination percent (%) = number of germinated seeds after 2 days (or 1 day) of the tests of each sample/number of germinated seeds after 3 days of the tests of control \times 100

 $^{\rm II}$ Means in the same column with the same letters are not significantly different. Tukey–Kramer, $p \le 0.05$

ns Not significant

Results

Effect of the *p*-menthane monoterpenes on seed germination

Following days 2 and 3 of the test period, the actual germination percentages of the *R. sativus* control reached 72.4 and 78.0 %, respectively. Following days 1 and 3 of the test period, the germination percentages of the *B. rapa* control reached 76.9 and 89.0 %, respectively. The germination percentages of each of the samples at a concentration of 10.0 mg/Petri dish were expressed relative to the percentages of the controls for the *R. sativus* and *B. rapa* seeds (Table 1). Of the hydrocarbon type monoterpenes, terpinolene showed inhibitory activity toward the seed germination, whereas almost all of the phenol, alcohol and ketone containing monoterpenes prevented the plant seeds from germinating.

The germination percentages of the active components at concentrations of 1.0 and 0.1 mg/Petri dish were expressed relative to the percentages of the controls for the R. sativus and B. rapa seeds (Figs. 2, 3). With the exception of terpinolene, the germination percentages of the hydrocarbon type monoterpenes for the R. sativus and B. rapa seeds at a concentration of 0.1 mg/Petri dish were almost identical to those of the control. The germination percentage of the R. sativus seeds in the presence of thymol at a concentration of 0.1 mg/Petri dish was 68.9 %, revealing that thymol exhibited a negligible inhibitory effect on the seed germination process. It was recognized that the hydrocarbons and phenols showed only weak or no inhibitory activity against both types of seeds. Of the alcohols at a concentration of 0.1 mg/Petri dish, (-)-terpinen-4-ol, (-)-carveol and (-)-menthol showed inhibitory effects on the *R. sativus* seeds, with germination percentages of 14.4, 27.0 and 37.8 %, respectively. The percentage of germination in the B. rapa seeds in the presence of isopulegol and (-)-dihydrocarveol were 27.3 and 29.1 %, respectively. The inhibitory effects of these compounds were relatively high compared to those of the other alcohols. The germination percentages of the R. sativus seeds in the compounds containing ketones at a concentration of 0.1 mg/Petri dish were 18.9, 20.0 and 31.1 % for piperitone, (+)-pulegone and (-)-carvone, respectively. The germination percentages of the B. rapa seeds in the presence of (+)-pulegone and piperitone were 21.6 and 27.3 %, respectively. Taken together, these data demonstrate that all of the ketone containing compounds exhibited significant inhibitory effects against both types of seeds.

Termiticidal activity of *p*-menthane monoterpenes

The LC₅₀ values for each of the samples are shown in Table 2. The results show that the hydrocarbons did not display any significant termiticidal activity, whereas the phenolic compounds demonstrated significantly higher levels of termite mortality than the controls, with carvacrol and thymol being the most active of the *p*-menthane compounds with LC₅₀ values of 0.34 and 0.65 mg/Petri dish, respectively. Of the alcohols, (–)-menthol and (–)-terpinen-4-ol were also quite active, providing LC₅₀ values of 0.92 and 1.26 mg/Petri dish, respectively.

Fig. 2 Effect of the *p*-menthane monoterpenes on the germination of the R. sativus seeds^I. ^ISome of test results were cited in the literature data [13]. Each determination was made with three replicates of twenty seeds. Bars represent standard deviations. ^{II}Germination percent (%) = number of germination seeds of each sample after 2 days of the test/number of germination seeds after 3 days of the tests of control \times 100. ^{III}Means in the same column with the same letters are not significantly different. Tukey-Kramer, $p \le 0.05$



Fig. 3 Effect of *p*-menthane monoterpenes on the germination of *B. rapa* seeds^I. ^ISome of test results were cited in the literature data [13]. Each determination was made with three replicates of twenty seeds. Bars represent standard deviations. ^{II}Germination percent (%) = number of germination seeds of each sample after 1 day of the test/ number of germination seeds after 3 days of the tests of control × 100. ^{III}Means in the *same column with the same letters* are not significantly different. Tukey–Kramer, $p \leq 0.05$. *ns* Not significant



Table 2 LC_{50} values of the *p*-menthane monoterpenes against termites^I

Compound	LC ₅₀ (mg/Petri dish)	95 % Confidential limits
Hydrocarbons		
<i>p</i> -Cymene	n.m. ^{II}	
α-Terpinene ^I	n.m.	
γ-Terpinene ^I	n.m.	
(−)-α-Phellandrene	n.m.	
(+)-Limonene ^I	n.m.	
Terpinolene	n.m.	
<i>p</i> -Menthane	n.m.	
Control	n.m.	
Phenols		
Thymol	0.65	0.59-0.70
Carvacrol	0.34	0.19-0.46
Control	n.m.	
Alcohols		
p-Cymen-8-ol	4.49	4.09-5.00
(-)-Terpinen-4-ol ^I	1.26	1.18-1.32
1,8-p-Menthadiene-4-ol	3.96	3.64-4.32
(–)-Carveol	1.70	1.56-1.85
$(-)$ - α -Terpineol ^I	1.71	1.61-1.83
Trans-sobrerol	n.m.	
(-)-Dihydrocarveol	1.85	1.60-2.09
(–)-Menthol	0.92	0.86–0.99
Isopulegol	3.67	3.40-3.98
Control	n.m.	
Ketones		
(-)-Carvone	1.62	1.44–1.83
Piperitone	1.87	1.68-2.06
(+)-Pulegone	0.50	0.44-0.55
Control	n.m.	

^I Some of test results were cited in the literature data [13]. $LC_{50} =$ lethal concentration for 50 % mortality. Each determination was performed in triplicate for twenty worker termites

^{II} n.m. No mortality observed

In contrast, however, isopulegol, 1,8-*p*-menthadiene-4-ol and *p*-cymen-8-ol showed only weak activities with LC₅₀ values of 3.67, 3.96 and 4.49 mg/Petri dish, respectively. The LC₅₀ values of the ketones containing compounds (+)-pulegone, (-)-carvone and piperitone, were 0.50, 1.62 and 1.87 mg/Petri dish, respectively. The insecticidal activity of (+)-pulegone was greater than that of piperitone and (-)-carvone.

Discussion

A previous study on the effects of monoterpenoids on the germination of *Lactuca sativa* seeds showed that the

inhibitory effects of alcohols, phenols and ketones were larger than those of the corresponding hydrocarbons [16]. Alicyclic monoterpene compounds containing a keto group conjugated with a double bond in particular were high-lighted as showing the largest inhibitory effects against the germination of *L. sativa* seeds [17]. We have proposed that the inhibitory effects of terpinen-4-ol and α -terpineol on the germination of *R. sativus* and *B. rapa* seeds were greater than those of bornyl acetate, α -pinene and β -pinene [13].

In the present study, it was recognized that hydrocarbons and phenols showed weak to no inhibitory effects against the germination of both types of seeds, whereas alcohols and ketones, with the exception of sobrerol, exhibited stronger inhibitory effects. Although any correlation between the effects and the number of hydroxyl groups present in these compounds cannot be used as a definitive indication of their activities, these data are in good agreement with previous investigations carried out on activity [16, 17]. In particular, the inhibitory effects of alcohols and ketones against the germination of R. sativus seeds were relatively larger than those encountered against B. rapa.

As shown in Fig. 3, the alcohol containing compounds, isopulegol, (-)-dihydrocarveol, (-)-terpinen-4-ol, and (-)-menthol, all exhibited greater inhibitory effects against the germination of *B. rapa* seeds than any other *p*-menthane alcohols. The effect of the isopulegol was similar to that of (-)-menthol, indicating that an isopropenyl group at the C-4 position of the ring was almost equivalent to an isopropyl group at the same position in terms of its effect.

The inhibitory effect of (-)-terpinen-4-ol was also higher than that of (-)- α -terpineol, suggesting that the hydroxyl group at the C-4 position of (-)-terpinen-4-ol was contributing to the inhibition of *B. rapa* germination to a greater extent than the hydroxyl group at the C-8 position of (-)- α -terpineol. There was, however, no difference in the inhibitory effects of isopulegol and (-)-dihydrocarveol, indicating that a hydroxyl group could be well tolerated at either the C-3 or C-2 positions without any adverse impact on the observed levels of activity.

Reynolds observed that the hydrocarbons showed little inhibitory activity on the germination of *L. sativa* seeds and correlated this observation with their insolubility in water, whereas monoterpenes bearing hydroxyl groups were on the whole more active [17]. In our previous study, we proposed that the increased water solubility of oxygenated monoterpenoids corresponded well with their increased inhibitory effect on seed germination [13].

It is possible that the performance with regard to the inhibitory effect (*B. rapa*) was influenced by the position of a hydroxyl group, with hydroxyl groups attached to ring carbons providing higher levels of inhibition than those attached to an isopropyl group. Further work is needed for

p-menthane compounds containing hydroxyl groups to thoroughly determine the impact of the relationship between the stereochemistry and the inhibitory effect observed in these compounds.

In a previous report from the literature, the termite (*Coptotermes formosanus*) mortality within *Cinnamomum camphora* wood was found to depend predominantly on the camphor content in the wood meal [18]. Ohtani et al. [19] identified α -terpinyl acetate and α -terpineol as the termiticidal (*C. formosanus*) substances in *Chamaecyparis obtusa* wood. Carvacrol was also reported to possess potent insecticidal and acaricidal activities against a range of agricultural, stored-product and medical arthropod pests, and was the most active as a termiticide (*R. speratus*) [20].

In the present study, termite mortality was observed for monoterpenoids bearing particular functional groups, with the termite mortality of monoterpenes bearing phenols being greater than that of those bearing alcohols and ketones. Phenols were also more toxic toward termites than p-cymen-8-ol, and it was recognized that the presence of a phenol hydroxyl group on the benzene ring contributed more to the insecticidal activity than any alcoholic hydroxyl group attached to the isopropyl group.

As shown in Table 2, a comparison of the termite mortalities caused by (-)-terpinen-4-ol and (-)- α -terpineol indicated that a hydroxyl group at the C-4 position of the ring induced greater mortality than a hydroxyl group at C-8 position on the isopropyl group. In addition, (-)-carveol and (-)-dihydrocarveol showed higher levels of activity than 1,8-*p*-menthadien-4-ol and isopulegol, indicating that the termite mortality of the compounds was affected by the position of the hydroxyl group.

The termite mortalities of (-)-terpinen-4-ol and (-)-menthol were significantly higher than those of 1,8-*p*-menthadiene-4-ol and isopulegol, indicating that the presence of an isopropyl group at the C-4 position of the ring appeared to induce greater mortality than an isopropenyl group at the same position. The termite mortality of (-)-carveol, however, was found to be similar to that of (-)-dihydrocarveol, and the presence of a double bond at the C-1 position of the ring did not lead to decrease in the level of mortality.

The termiticidal activity of (+)-pulegone was greater than the values found for both (–)-carvone and piperitone, indicating that an external double bond at the C-4 position of the saturated six-membered conjugated with a ketone provided a more potent level of activity than the corresponding unsaturated six-membered ring with an α , β unsaturated ketone with the double portion internal to the ring.

For the valencenoid derivatives, in terms of their repellant activity against termites (C. formosanus), a reduction of the 1,10-double bond (1,10-dihydronootkatone and tetrahydronootkatone) produced compounds that were more repellent than nootkatone [21]. The isopropenyl group probably did not participate in the binding as evidenced by the absence of a significant difference in the repellent activities of nootkatone, isonootkatone and 11,12-dihydronootkatone [21]. In relation to the suppression of the furylfuramide-induced SOS response activity in the *umu* test using *Salmonella typhimurium*, the strength of suppression was related to the saturated six-membered ring, and the inhibition of the suppressive effect by (+)-menthol was stronger than that of isopulegol, indicating that the presence of an isopropenyl group led to a decrease in the strength of the inhibitory effect [22].

In the present study, the presence of a hydroxyl group in the ring of p-menthane alcohols provided a higher level of termite mortality than a hydroxyl group in the corresponding side chain. Furthermore, alcohols containing a saturated side chain showed a higher mortality level than those with an unsaturated side chain. The mortality of the p-menthane alcohols was affected by the position of the hydroxyl group and the saturation fraction of the functional group in the side chain.

In our previous work, the components, which were classified as *p*-menthane alcohols, provided an increase in water solubility that corresponded well with the associated reduction in the germination rate and LC_{50} values of the termites [13]. In this study, *p*-menthane alcohols, phenols and ketones were bioactive against plant seeds and termites. The results suggested that the bioactivities of these compounds were affected by their solubility in water, which affected their mechanism of action. Although sobrerol is one of the *p*-menthane alcohols, it exhibited a lower level of bioactivity, suggesting that the bioactivities of the compounds were not only affected by their water solubility, but also by their hydrophobicity.

In conclusion, the bioactivity of p-menthane monoterpenoids was dependent upon the presence and the position of certain functional groups and the degree of saturation fraction in the side chain functional group. A phenolic hydroxyl group was effective for termite mortality, whereas the presence of a carbonyl group provided a strong inhibitory effect on seed germination, and promoted termiticidal activity. In particular, this position was relevant for estimating the performance of p-menthane alcohols because the alcoholic hydroxyl group in the ring had been shown to have an inhibitory effect on seed germination and termite mortality. Although an isopropyl group in the ring also provided an inhibitory effect and termiticidal activity, the presence of an isopropenyl group in the ring showed a particularly marked inhibitory effect. Therefore, the selective bioactivity corresponded to the difference in isopropyl and isopropenyl groups.

More work is needed on other compounds containing the *p*-menthane skeleton to establish any further correlations between the bioactivity and chemical structure of compounds from this particular structural class.

Acknowledgments The authors would like to express their gratitude to Nippon Terpene Chemicals Inc., for providing the *p*-menthane compounds.

References

- Connolly JD, Hill RA (1991) Dictionary of terpenoids volume 1 mono- and sesquiterpenoids: monoterpenoids. Chapman & Hall, London, pp 7–152
- Mahmoud SS, Croteau RB (2002) Strategies for transgenic manipulation of monoterpene biosynthesis in plants. Trends Plant Sci 7:366–373
- Kubeczka KH, Formacek V (2002) Essential oils analysis by capillary gas chromatography and carbon-13 NMR spectroscopy second completely revised edition. Wiley, West Sussex, pp 239–254, 261–272, 355–360
- Arraiza MP, Andres MP, Arrabal C, Lopez JV (2009) Seasonal variation of essential oil yield and composition of thyme (*Thymus* vulgaris L.) Castilla-La Mancha (Central Spain). J Essent Oil Res 21:360–362
- Govaris A, Botsoglou E, Sergelidis D, Chatzopoulou PS (2011) Antibacterial activity of oregano and thyme essential oils against *Listeria monocytogenes* and *Escherichia coli* O157:H7 in feta cheese packaged under modified atmosphere. LWT-Food Sci Technol 44:1240–1244
- Hazzit M, Baaliouamer A, Verissimo AR, Faleiro ML, Miguel MG (2009) Chemical composition and biological activities of Algerian *Thymus* oils. Food Chem 116:714–721
- Klaric MS, Kosalec I, Mastelic J, Pieckova E, Pepeljnak S (2007) Antifungal activity of thyme (*Thymus vulgaris* L.) essential oil and thymol against moulds from damp dwellings. Lett Appl Microbiol 44:36–42
- Santoro GF, Cardoso MG, Guimaraes LGL, Salgado APSP, Menna-Barreto RFS, Soares MJ (2007) Effect of oregano (Origanum vulgare L.) and thyme (Thymus vulgaris L.) essential oils on Trypanosoma cruzi (protozoa: Kinetoplastida) growth and ultrastructure. Parasitol Res 100:783–790
- Safaei-Ghomi J, Ebrahimabadi AH, Djafari-Bidgoli Z, Batooli H (2009) GC/MS analysis and in vitro antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* Jaras and its main constituent carvacrol. Food Chem 115:1524–1528

- Jirovetz L, Buchbauer G, Bail S, Denkova Z, Slavchev A, Stoyanova A, Schmidt E, Geissler M (2009) Antimicrobial activities of essential oils of mint and peppermint as well as some of their main compounds. J Essent Oil Res 21:363–366
- 11. Serra S, Fuganti C, Brenna E (2005) Biocatalytic preparation of natural flavours and fragrances. Trends Biotechnol 23:193–198
- 12. Lazutka JR, Mierauskiene J, Slapsyte G, Dedonyte V (2001) Genotoxicity of dill (Anethum graveolens L.), peppermint (Mentha × piperita L.) and pine (Pinus sylvestris L.) essential oils in human lymphocytes and Drosophila melanogaster. Food Chem Toxicol 39:485–492
- Sekine N, Shibutani S, Yatagai M (2012) Water solubility of essential oils from coniferous needles with special reference to effect on germination and antitermite activity (in Japanese with English summary). Mokuzai Gakkaishi 58:44–53
- Yoshida M (1975) Design of experiments for animal husbandry (in Japanese). Yokendo, Tokyo, pp 348–381
- Yanai H (2004) 4-Steps excel statistics (with Statcel 2 add-in software) (in Japanese). OMS Publishing Inc., Saitama, pp 148–156
- Vokou D, Douvli P, Blionis GJ, Halley JM (2003) Effects of monoterpenoids, acting alone or in pairs, on seed germination and subsequent seedling growth. J Chem Ecol 29:2281–2301
- Reynolds T (1987) Comparative effects of alicyclic compounds and quinones on inhibition of lettuce fruit germination. Ann Bot 60:215–223
- Hashimoto K, Ohtani Y, Sameshima K (1997) The termiticidal activity and its transverse distribution in camphor (*Cinnamonum camphora*) wood (in Japanese with English summary). Mokuzai Gakkaishi 43:566–573
- 19. Ohtani Y, Hazama M, Sameshima K (1997) Crucial chemical factors of the termiticidal activity of hinoki wood (*Chamaecyparis obtusa*) III. Contribution of α -terpinyl acetate to the termiticidal activity of hinoki wood. Mokuzai Gakkaishi 43:1022–1029
- Ahn YJ, Lee SB, Lee HS, Kim GH (1998) Insecticidal and acaricidal activity of carvacrol and β-thujaplicine derived from *Thujopsis dolabrata* var. *hondai* sawdust. J Chem Ecol 24:81–90
- Zhu BCR, Henderson G, Sauer AM, Yu Y, Crowe W, Laine RA (2003) Structure-activity of valencenoid derivatives and their repellence to the Formosan subterranean termite. J Chem Ecol 29:2695–2701
- 22. Miyazawa M, Okuno Y, Nakamura S, Kosaka H (2000) Suppression of the furylfuramide-induced SOS response by monoterpenoids with a *p*-menthane skeleton using the *Salmonella typhimurium* TA1535/pSK1002 *Umu* test. J Agric Food Chem 48:5440–5443