

# Antitermite Activity of $\beta$ -Caryophyllene Epoxide and Episulfide

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Caryophyllene-6,7-epoxide and caryophyllene-6,7-episulfide can be easily synthesized from  $\beta$ -caryophyllene by autoxidation or episulfidation. The bioactivities of  $\beta$ -caryophyllene and its derivatives were investigated against the subterranean termite *Reticulitermes speratus* Kolbe. The antifeedant, feeding, and termiticidal activities of each compound were tested using no-choice, dual-choice, and non-contact methods. Antitermitic activities were not shown by  $\beta$ -caryophyllene, but were observed for the oxide and sulfide derivatives. Caryophyllene-6,7-episulfide showed especially high antifeedant and termiticidal activities. Thus, naturally abundant, non-bioactive  $\beta$ -caryophyllene can be easily converted into an antitermite reagent via a non-biological process.

**Key words:** <sup>13</sup>C NMR, Bioactivity, Autoxidation

## Introduction

$\beta$ -Caryophyllene, a sesquiterpene hydrocarbon, has a unique structure consisting of a medium-ring olefin and is found widely in plant extracts (Collado *et al.*, 1998).  $\beta$ -Caryophyllene-6,7-epoxide is easily formed from  $\beta$ -caryophyllene by autoxidation (Ashitani and Nagahama, 1999; Sköld *et al.*, 2006). The bioactivities of  $\beta$ -caryophyllene and/or caryophyllene-6,7-epoxide have been studied for mites (Furuno *et al.*, 1994; Macchioni *et al.*, 2002; Kim *et al.*, 2003), ticks (Ashitani *et al.*, unpublished data), termites (Park and Shin, 2005), fungi (Yang *et al.*, 1999), mice (Sköld *et al.*, 2006), and rats (Chavan *et al.*, 2010).

Caryophyllene-6,7-episulfide is easily obtained through the reaction between caryophyllene and elemental sulfur (Ashitani and Nagahama, 1999; Ashitani *et al.*, 2008), however, the bioactivity of caryophyllene-6,7-episulfide has not been reported. It is of great interest to develop reagents, such as  $\beta$ -caryophyllene, for synthesis various bioactive compounds against harmful insects from natural and abundant resources. The structures of the above caryophyllene derivatives are shown in Fig. 1. The absolute configuration of caryophyl-

lene-6,7-episulfide has been previously reported (Ashitani and Nagahama, 1999). Caryophyllene-6,7-episulfide and caryophyllene-6,7-epoxide have the same configuration at C-6 and C-7. A comparison of the bioactivities of caryophyllene-6,7-episulfide and caryophyllene-6,7-epoxide is therefore interesting in the field of natural products chemistry. In this study, bioactivities of  $\beta$ -caryophyllene epoxide and  $\beta$ -caryophyllene episulfide were investigated against a subterranean termite, *Reticulitermes speratus* Kolbe, which is known as a major harmful insect to the Japanese wood industry.

## Material and Methods

### Preparation of samples

$\beta$ -Caryophyllene was purified by silica gel column chromatography from the commercially available reagent (Tokyo Chemical Industry Co. Ltd., Tokyo, Japan). Caryophyllene-6,7-epoxide was separated by silica gel column chromatography from autoxidized  $\beta$ -caryophyllene samples kept at room temperature. Caryophyllene-6,7-episulfide was synthesized by direct episulfidation of  $\beta$ -caryophyllene as described by Ashitani and Nagahama (1999).

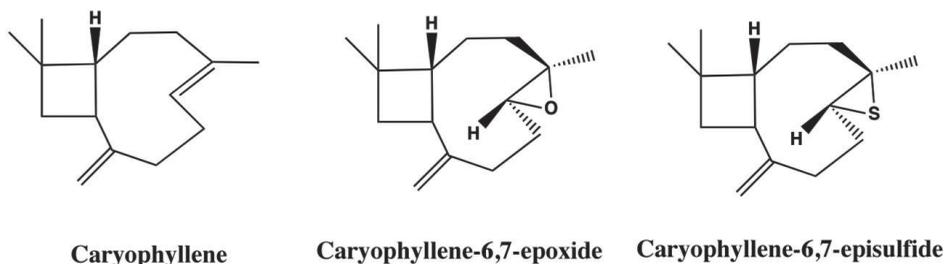


Fig. 1. Chemical structures of the compounds studied.

2D-NMR data for caryophyllene-6,7-episulfide are reported in Ashitani and Nagahama (1999). Optical rotation of caryophyllene-6,7-episulfide was analysed in this study.

*Caryophyllene-6,7-episulfide*:  $[\alpha]_D -77.9^\circ$  ( $c$  0.70, 16.9 °C,  $\text{CHCl}_3$ ). – MS:  $m/z = 236$  [ $\text{M}^+$ ], 221, 203, 189, 161, 147, 133, 121, 105, 93, 77, 69, 55. – IR (KBr):  $\nu = 2954$  (s), 1636 (w), 1454 (m), 1379 (w), 1050 (w), 890 (m), 629 (m)  $\text{cm}^{-1}$ . –  $^1\text{H}$  NMR (270 MHz,  $\text{CDCl}_3$ ):  $\delta = 0.98$  (3H, s), 1.00 (3H, s), 1.08–1.20 (1H, m), 1.44–1.59 (2H, m), 1.52 (3H, s), 1.66–1.70 (8H, m), 2.00–2.09 (1H, m), 2.41–2.62 (4H, m), 2.99 (1H, dd,  $J = 11.4, 3.5$  Hz), 4.89 (1H, d,  $J = 0.99$  Hz), 5.00 (1H, dd,  $J = 0.66, 0.66$  Hz). –  $^{13}\text{C}$  NMR (67.8 MHz,  $\text{CDCl}_3$ ):  $\delta = 20.5$  ( $\text{CH}_3$ ), 21.6 ( $\text{CH}_3$ ), 28.1 ( $\text{CH}_2$ ), 29.8 ( $\text{CH}_3$ ), 33.3 ( $\text{CH}_2$ ), 33.5 ( $\text{CH}_2$ ), 33.9 (C), 38.7 ( $\text{CH}_2$ ), 45.2 ( $\text{CH}_2$ ), 48.6 (CH), 48.9 (CH), 50.2 (C), 51.9 (CH), 112.0 ( $\text{CH}_2$ ), 151.5 (C).

#### Antitermite tests

For antitermite tests, each test compound was purified by silica gel column chromatography until a purity of 95% was reached, as measured by gas chromatography (GC).

Antitermite activities of the samples were measured according to methods described in previous reports (Kusumoto *et al.*, 2009; Sekine *et al.*, 2009). A colony of *R. speratus* Kolbe was collected from Tsuruoka city, Japan. Each test compound was dissolved in acetone and applied to paper discs (thickness, 1.5 mm;  $\phi$ , 8 mm; Advantec, Tokyo, Japan). Discs with increasing contents (sample weight/paper disc weight  $\cdot$  100) of each sample were prepared (0.25, 0.50, 1.00, and 2.00%). The paper discs were vacuum-dried and then used in the antitermite tests.

No-choice tests were used to investigate the termiticidal activity and antifeedant effect of the samples. Dual-choice and non-contact tests were

performed to investigate the repellent effect and effect of the volatile fraction of the compounds, respectively. The no-choice method consisted of a paper disc impregnated with the sample on top of sterile sea sand (2.0 g) in each Petri dish ( $\phi$ , 45 mm). Blank experiments were conducted where termites were exposed to paper discs (control paper discs) treated only with acetone. In the dual-choice method, a paper disc impregnated with the sample was put together with a control paper disc in each Petri dish. Termites could choose between the blank disc and the disc containing the sample. A further control was provided by way of blank experiments where two control paper discs, impregnated with acetone, were put in each Petri dish. The non-contact method consisted of a blank paper disc on sterile sea sand with either a disc impregnated with the sample or a control paper disc impregnated with acetone (blank experiment), stuck to the lid of the Petri dish. In this way, termites did not come into direct contact with the disc containing the compounds. Ten worker termites were introduced into each Petri dish, and the sand was moistened to supply water. Additionally, no-feed control (no paper disc was added to the Petri dishes) treatments were conducted. All Petri dishes were placed in a dark room at  $(26 \pm 1)$  °C. Each test included three replicates. The duration for no-choice, dual-choice, and non-contact tests were 32, 26, and 19 d, respectively. Survival of termites was measured each day to determine the mortality. Mass loss of each paper disc was determined by subtracting post-treatment from pre-treatment paper disc weights. Antifeedant and feeding activities of the compounds were calculated using the following equations:

$$\text{antifeedant activity (\%)} = (B - T) \cdot 100/B,$$

$$\text{feeding activity (\%)} = T/B \cdot 100,$$

where  $B$  is the mass loss of the paper disc (in mg) in the blank test and  $T$  is the mass loss of

the sample (in mg) containing the paper disc or control paper disc.

### Statistical analysis

Test samples were compared using analysis of variance (ANOVA), and means were separated using a protected Tukey-Kramer test ( $p < 0.05$ ; Statcel 2, Saitama, Japan) (Yanai, 2004).

## Results and Discussion

The no-choice test was performed to investigate the termiticidal activity of the sesquiterpenes. The hydrocarbon  $\beta$ -caryophyllene did not show termiticidal activity (Fig. 2a). Termiticidal activity was observed for caryophyllene-6,7-epoxide and the corresponding episulfide. The termites exposed to the highest content (2.00%) of the oxide and sulfide experienced close to 100% mortality at the mid-point of the test period (day 17 of 32). Caryophyllene-6,7-episulfide showed especially potent termiticidal activities, *i.e.* termite mortality was higher in caryophyllene-6,7-episulfide than in caryophyllene-6,7-epoxide treatments at the 1.00% level after 32 days [episulfide, (78  $\pm$  5.8)% mortality; epoxide, (28  $\pm$  12)% mortality;  $p < 0.05$ ]. The highest content (2.00%) of caryophyllene-6,7-epoxide and -episulfide provoked higher termite mortalities after 17 days compared to the no-feed control.

The antifeedant activities of the paper discs impregnated with  $\beta$ -caryophyllene were similar to the blank at all sample contents (Fig. 2b).  $\beta$ -Caryophyllene had no, or a very weak, anti-

feedant effect. In the case of caryophyllene-6,7-epoxide and caryophyllene-6,7-episulfide, the antifeedant activities were dependent on the sample content. The most potent antifeedant response was observed for caryophyllene-6,7-episulfide, which also exhibited strong termiticidal activity.

Dual-choice tests were performed to investigate the repellent activity of the compounds. Termiticidal activities of  $\beta$ -caryophyllene were not found, but high mortality was observed in the epoxide and episulfide dual-choice treatments (Fig. 3a). Weak repellent activity was observed in dual-choice tests with  $\beta$ -caryophyllene. On paper discs impregnated with over 0.50%  $\beta$ -caryophyllene, there was a significantly ( $p < 0.05$ ) lower feeding activity compared to the control paper (Fig. 3b). Paper discs impregnated with caryophyllene-6,7-epoxide showed repellent activity at all contents except the highest (2.00%), where the termiticidal effect was high and precluded feeding. In the case of the episulfide, significant repellent activity was observed only on the paper disc impregnated at 0.25%, which had low termiticidal activities – at higher contents mortality precluded any repellent effects. Thus, both caryophyllene-6,7-epoxide and -episulfide are toxic to and a repellent against termites. At high contents of the epoxide and episulfide (above 1.00%) in both the no-choice and dual-choice tests, it was observed that termites gathered on the edges of the Petri dishes and stopped all movements.

The non-contact test (Fig. 4) was performed to investigate whether the volatile fraction of the sesquiterpenes had an effect on termite behav-

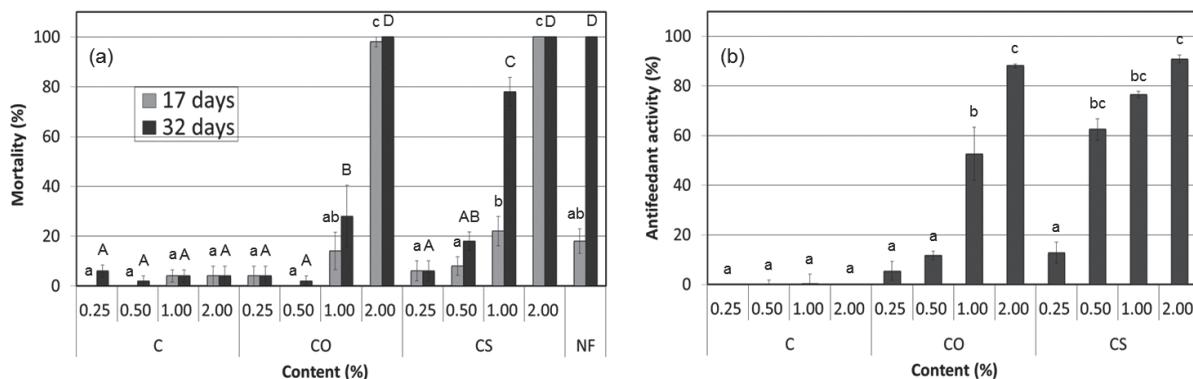


Fig. 2. (a) Termiticidal activities after 17 and 32 days and (b) antifeedant activities after 32 days of exposure to compounds in the no-choice test. C,  $\beta$ -caryophyllene; CO, caryophyllene-6,7-epoxide; CS, caryophyllene-6,7-episulfide; NF, no-feed test. Bar,  $\pm$  SE. Common letters denote no significant difference. Tukey-Kramer test,  $p \leq 0.05$ .

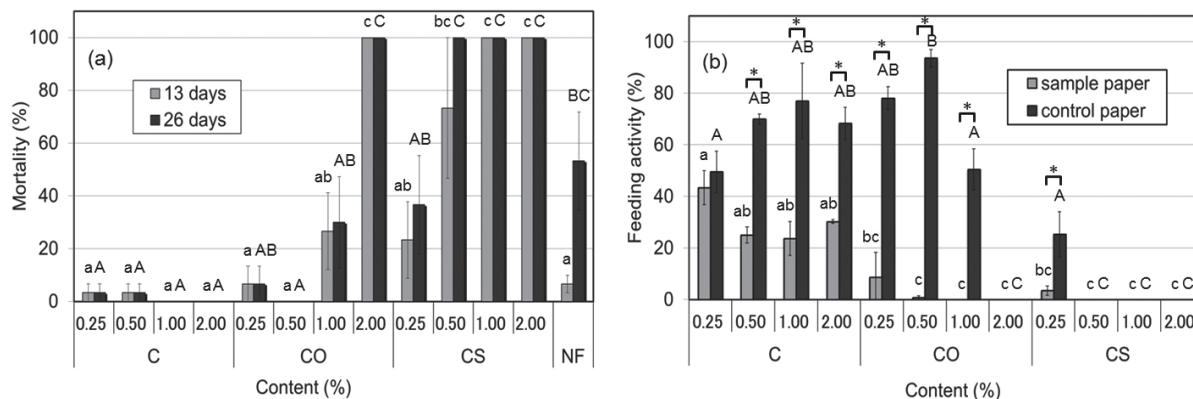


Fig. 3. (a) Termiticidal activities after 13 and 26 days and (b) feeding activities after 26 days of exposure to compounds in the dual-choice test. C,  $\beta$ -caryophyllene; CO, caryophyllene-6,7-epoxide; CS, caryophyllene-6,7-episulfide; NF, no-feed test. Bar,  $\pm$  SE. Common letters denote no significant difference. Asterisks (\*) denote a significant difference between sample and control papers. Tukey-Kramer test,  $p \leq 0.05$ .

our. Sample discs were placed inside the lid of the Petri dish, not in contact with the termites. In this way, behavioural responses from the termites could be attributed to volatiles emitted from the sample discs, and not via direct ingestion. At high contents of caryophyllene-6,7-epoxide and -episulfide the termites gathered in one place and stopped all movements, as in the no-choice and dual-choice tests. Mortality was observed for contents over 1.50% of the epoxide and over 1.00% of the episulfide (Fig. 4a). The highest content (2.00%) of the episulfide caused a statistically significant ( $p < 0.05$ ) higher mortality compared to other samples. Antifeedant activities were shown clearly for caryophyllene-6,7-epoxide

and -episulfide (Fig. 4b). The antifeedant activities depended on the sample content. The results of the non-contact tests strongly suggest that caryophyllene-6,7-epoxide and -episulfide vapour can affect the termite behaviour and mortality. We speculate that the toxicities of caryophyllene-6,7-epoxide and -episulfide in the vapour state result from some action on the neuronal system of the termites, because the termites stopped all movements at high contents. The mechanism of the toxicity will be investigated as part of our future work plans.

Cheng *et al.* (2004) investigated antitermite activities of  $\beta$ -caryophyllene and caryophyllene epoxide against *Coptotermes formosanus*, a subter-

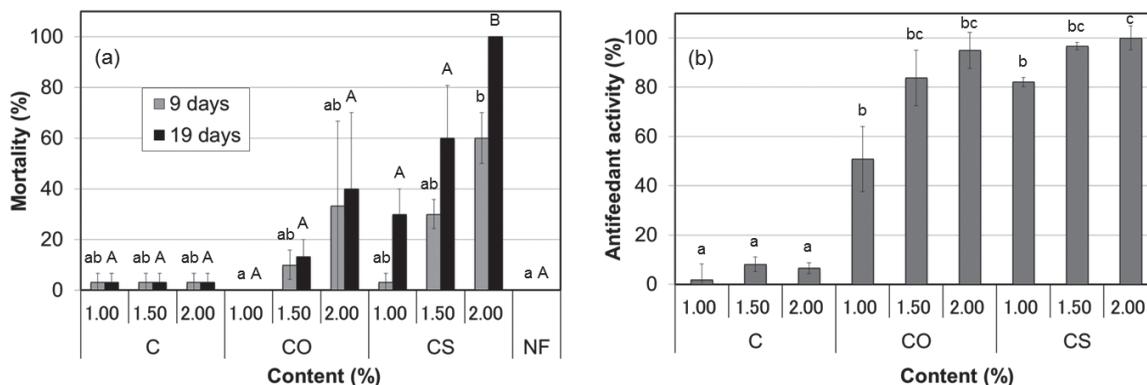


Fig. 4. (a) Termiticidal activities after 9 and 19 days and (b) antifeedant activities after 19 days of exposure to compounds in the non-contact test. C,  $\beta$ -caryophyllene; CO, caryophyllene-6,7-epoxide; CS, caryophyllene-6,7-episulfide; NF, no-feed test. Bar,  $\pm$  SE. Common letters denote no significant difference. Tukey-Kramer test,  $p \leq 0.05$ .

ranean termite similar to *R. speratus*, and reported that the antitermite activity of  $\beta$ -caryophyllene was equal to or higher than that of caryophyllene epoxide. Park and Shin (2005) reported that  $\beta$ -caryophyllene had no, or a very weak, antitermite activity against *R. speratus* – similar to our results. We showed that the antitermite activity against *R. speratus* was obviously increased by the addition of an oxygen or a sulfur atom to the C-6–C-7 end-double bond of  $\beta$ -caryophyllene. The antitermite activity of the episulfide was stronger than that of the epoxide. Thus, a minor structural change to  $\beta$ -caryophyllene caused obvious antitermite activity.

$\beta$ -Caryophyllene exists in the heartwood of various trees. In this state, the compound retains its structure because of the lack of oxygen. However, autoxidation would occur through in-

jury to the tree, if the heartwood is exposed to air. The autoxidized  $\beta$ -caryophyllene could then be a natural surface antifeedant to protect living tissues of wounded trees and timber. Considering that  $\beta$ -caryophyllene is naturally abundant and can be converted easily by direct sulfidation to its episulfide, it has the potential to be a very useful industrial material for the production of antitermite reagents.

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- Ashitani T. and Nagahama S. (1999), Direct episulfidation of caryophyllene and humulene. *Nat. Prod. Lett.* **13**, 163–167.
- Ashitani T., Borg-Karlsom A. K., Fujita K., and Nagahama S. (2008), Reaction mechanism of direct episulfidation of caryophyllene and humulene. *Nat. Prod. Res.* **22**, 495–498.
- Chavan M. J., Wakte P. S., and Shinde D. B. (2010), Analgesic and anti-inflammatory activity of caryophyllene oxide from *Annona suamosa* L. bark. *Phytomedicine* **17**, 149–151.
- Cheng S. E., Wu C. L., Chang H. T., Kao Y. T., and Chang S. T. (2004), Antitermitic and antifungal activities of essential oil of *Calocedrus formosana* leaf and its composition. *J. Chem. Ecol.* **30**, 1957–1967.
- Collado I. G., Hanson J. R., and Macías-Sánchez A. J. (1998), Recent advances in the chemistry of caryophyllene. *Nat. Prod. Rep.* **15**, 187–204.
- Furuno T., Terada Y., Yano S., Uehara T., and Jodai S. (1994), Activities of leaf oils and their components from Lauraceae trees against house-dust mites. *Mokuzai Gakkaishi* **40**, 78–87.
- Kim E. H., Kim H. K., Choi D. H., and Ahn Y. J. (2003), Acaricidal activity of clove bud oil compounds against *Tyrophagus putrescentiae* (Acari: Acaridae). *Appl. Entomol. Zool.* **38**, 261–266.
- Kusumoto N., Ashitani T., Hayasaka Y., Murayama T., Ogiyama K., and Takahashi K. (2009), Antitermitic activities of abietane-type diterpenes from *Taxodium distichum* cones. *J. Chem. Ecol.* **35**, 635–642.
- Macchioni F., Cioni P. L., Flamini G., Morelli I., Perrucci S., Franceschi A., Macchioni G., and Ceccarini L. (2002), Acaricidal activity of pine essential oils and their main components against *Tyrophagus putrescentiae*, a stored food mite. *J. Agric. Food Chem.* **50**, 4586–4588.
- Park I. K. and Shin S. C. (2005), Fumigant activity of plant essential oils and components from garlic (*Allium sativum*) and clove bud (*Eugenia caryophyllata*) oils against the Japanese termite (*Reticulitermes speratus* Kolbe). *J. Agric. Food Chem.* **53**, 4388–4392.
- Sekine N., Ashitani T., Murayama T., Shibutani S., Hattori S., and Takahashi K. (2009), Bioactivity of latifolin and its derivatives against termites and fungi. *J. Agric. Food Chem.* **57**, 5707–5712.
- Sköld M., Karlberg A. T., Matura M., and Börje A. (2006), The fragrance chemical  $\beta$ -caryophyllene – air oxidation and skin sensitization. *Food Chem. Toxicol.* **44**, 538–545.
- Yanai H. (2004), Statcel – The Useful Add-in Software Forms on Excel, 2nd ed. OMS Tokyo, Japan.
- Yang D., Michel L., Chaumont J. P., and Millet-Clerc J. (1999), Use of caryophyllene oxide as an antifungal agent in an *in vitro* experimental model of onychomycosis. *Mycopathologia* **148**, 79–82.