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Note

Determination of the Absolute Configuration of Quercivorol, (1*S*,4*R*)-*p*-Menth-2-en-1-ol, an Aggregation Pheromone of the Ambrosia Beetle *Platypus quercivorus* (Coleoptera: Platypodidae)

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A pair of enantiomers of *trans*-*p*-menth-2-en-1-ol, an aggregation pheromone of *Platypus quercivorus*, was synthesized from (*S*)- and (*R*)-limonene. The retention time of the aggregation pheromone from the insect coincided with that of (1*S*,4*R*)-*p*-menth-2-en-1-ol synthesized from (*S*)-limonene from GC analyses with a chiral column, enabling the absolute configuration of the aggregation pheromone to be determined as (1*S*,4*R*).

Key words: (1*S*,4*R*)-*p*-menth-2-en-1-ol; quercivorol; aggregation pheromone; *Platypus quercivorus*

Mass attack of by the ambrosia beetle, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae), on oak trees, especially *Quercus crispula* Blume, has resulted in the widely spread and continuous heavy mortality of oak in deciduous broadleaf forests in Japan since the late 1980s.[1)] A phytopathogenic fungus, hypothesized by Hijii *et al.*,[1)] was isolated from the gallery and mycangia of the ambrosia beetle and named as *Raffaelea quercivora*.[2)] Dysfunction of the water flow in attacked tree trunks was caused by long gallery digging in sapwood by *P. quercivorus* and by the invasion of *R. quercivora* through the gallery walls.[3)] This is the first case of the ambrosia beetle being demonstrated to kill a living host tree with a vectoring pathogen.

Although sound communication is important as a cue for the mating behaviour of the beetles,[4)] field experiments have indicated that chemical communication by using an aggregation pheromone was responsible for the mass attack on oaks by the beetles.[5)] The results of GC–EAD and GC–MS analyses of the volatiles from boring dust and field trap experiments using a synthetic racemic mixture identified the aggregation pheromone of the beetle as *trans*-*p*-menth-2-en-1-ol, although its the stereochemistry has not yet been clarified.[6)] To clarify the absolute configuration of the aggregation pheromone of the beetle, (1*S*,4*R*)- and (1*R*,4*S*)-*p*-menth-2-en-1-ol were synthesized (shown in the Scheme 1), and the retention times of these synthetic products and that of the aggregation pheromone from crushed male volatiles were compared.

(*S*)-limonene (**1**) was converted into (*S*)-carvomenthene (**2**) by Raney nickel W4-catalyzed reduction, and the carbon-carbon double bond at C1 of **2** was oxidized by *m*-CPBA to give *cis*- and *trans*-epoxy-(*S*)-carvomenthane (**3**). The epoxide ring of **3** was opened to form a new carbon-carbon double bond at C2 by sodium 2-heptanoxide to be converted into (1*S*,4*R*)- and (1*R*,4*R*)-*p*-menth-2-en-1-ol (**4** and **5**). Each isomer was identified by its retention time from a GC analysis with the same column[7–9)] and by comparing its GC–MS data with that in the literature.[9)] The total yields of **4** and **5** were respectively 11.2% and 10.2% based on (*S*)-limonene. (1*S*,4*S*)- and (1*R*,4*S*)-*p*-menth-2-en-1-ol (**6** and **7**) were synthesized from (*R*)-limonene in the same way.

The retention times of the four diastereoisomers of *p*-menth-2-en-1-ol synthesized from (*R*)- and (*S*)-limonene and the aggregation pheromone of *P. quercivorus* from GC analyses with a chiral column are shown in Table 1. The retention time for the aggregation pheromone of *P. quercivorus* was 16.72 min, almost the same as that of **4** synthesized from (*S*)-limonene. On the other hand, the retention time of **7** synthesized from (*R*)-limonene was 16.96 min. We concluded from these results that the aggregation pheromone of *P. quercivorus* was (1*S*,4*R*)-*p*-menth-2-en-1-ol, for which the name quercivorol is

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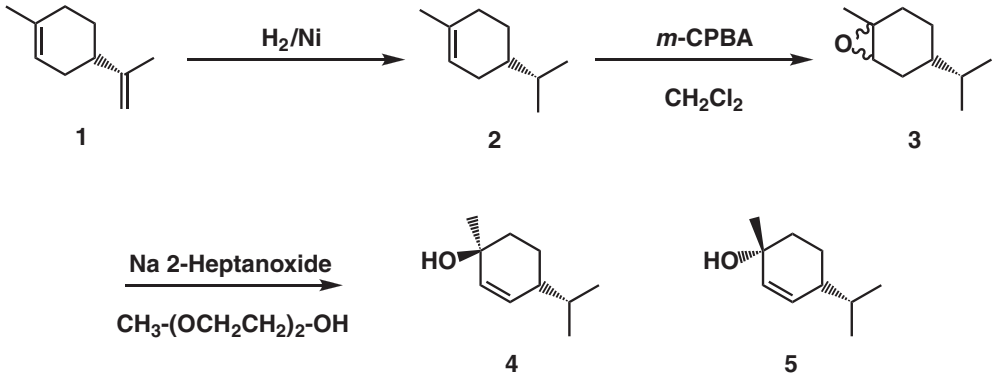

Scheme 1. Synthesis of (1*S*,4*R*)- and (1*R*,4*R*)-*p*-Menth-2-en-1-ol, Quercivorol from (*S*)-Limonene.

Table 1. Retention Times of Stereoisomers of *p*-Menth-2-en-1-ol from GC Analyses with a Chiral Capillary Column

p-Menth-2-en-1-ol	Retention time (min)
trans-*p*-Menth-2-en-1-ol from *Platypus quercivorus*	16.72
cis-*p*-Menth-2-en-1-ol from *P. quercivorus*	16.06
(1*S*,4*R*)-*p*-Menth-2-en-1-ol (**4**)	16.71
(1*R*,4*R*)-*p*-Menth-2-en-1-ol (**5**)	16.05
(1*S*,4*S*)-*p*-Menth-2-en-1-ol (**6**)	16.08
(1*R*,4*S*)-*p*-Menth-2-en-1-ol (**7**)	16.96

proposed. The precise biological assay results, in which quercivorol attracted the beetles in field-trap experiments and evoked an EAD response in antenna of the beetle, will be reported elsewhere.

Experimental

Collection of volatiles from the males. Emerged male adults of *P. quercivorus* were collected from logs of *Q. crispula* which had been killed by the *R. quercivora* and *P. quercivorus* complex and harvested from damaged forests in Kushibiki Town, Yamagata Prefecture and Mikawa Village, Niigata Prefecture. The insects were crushed in 1.5-ml vials by a tiny glass rod. The headspace volatile components containing the aggregation pheromone of the beetle were collected by SPME (Supelco, USA) from crushed males in the vials.

Instruments. GC–MS data was recorded with a JEOL JMS600 mass spectrometer with a capillary column (HP-5, crosslink 5% PH ME Siloxane, 30 m × 0.32 mm i.d., 0.25 µm thickness, He carried gas:, temperature program from 80 °C with a 1-min hold to 150 °C at 4 °C/min). To elucidate the absolute structure, a sample was analyzed in a chiral capillary column (Chirasil-Dex CB, 25 m × 0.25 mm i.d., 0.25 µm thickness, Varian, USA) installed in a Hewlett-Packard (HP) 6890 GC instrument equipped with an HP 5973 mass spectrometer, using He as the carrier gas. The oven temperature was held at 60 °C for 1 min, and then programmed to 150 °C at 5 °C/min. ^{1}H- and ^{13}C-NMR data were measured with a JEOL JNM-AL 400 (400 MHz) instrument. TMS was used as the internal standard. Letters (br.)s, d, t, q, and m represent (broad)singlet, doublet, triplet, quartet, and multiplet, respectively, and coupling constants are given in Hz. The solvent used was $CDCl_3$. Specific rotation values were recorded with a Horiba SEPA-200 high-sensitivity polarimeter.

Chemicals. All reagents were purchased from Nacalai Tesque Ltd. and Wako Chemical Ltd. in Japan.

Syntheses.

*(S)-Carvomenthene (**2**).* A 13.6 g amount of (*S*)-limonene (100 mmol) and 1.4 g of Raney nickel W4 were stirred, and a hydrogen gas stream was then fed into the solution under atmospheric pressure at room temperature until 3.4 liters of the hydrogen gas had been absorbed. After purging with N_2 gas, the mixture was filtered and then distilled under atmospheric pressure to produce 12.3 g of pure (*S*)-carovomenthene as a colorless oil, bp 168–172 °C at 760 mmHg in a 89.4% yield. $[\alpha]_D^{22}$ +78.5° (c = 2.0, $CHCl_3$). EIMS m/z (%): 138 (11), 96 (19), 95 (99), 94 (10), 82 (22), 81 (38), 79 (17), 77 (13), 69 (30), 68 (100), 67 (22), 55 (61), 53 (34), 51 (10). ^{1}H-NMR ($CDCl_3$) δ: 5.36 (1H, br.s, H-2), 2.00 (1H, m, H-6b), 1.96 (1H, m, H-3b), 1.93 (1H, m, H-6a), 1.75 (1H, m, H-5b), 1.72 (1H, m, H-3a), 1.63 (3H, br.s, H-7), 1.46 (1H, octet, H-8), 1.25 (1H, m, H-4), 1.20 (1H, m H-5a), 0.89 (3H, d, J = 6.4, H-9), 0.87 (3H, d, J = 6.4, H10). ^{13}C-NMR ($CDCl_3$) δ: 133.7 (s, C-1), 121.0 (d, C-2), 40.1 (d, C-4), 32.4 (d, C-8), 30.9 (t, C-6), 29.0 (t, C-3), 26.6 (t, C-5), 23.5 (q, C-7), 20.1 (q, C-9), 19.7 (q, C-10).

(R)-Carvomenthene. $[\alpha]_D^{22}$ −78.2° (c = 2.0, $CHCl_3$).

*(1S,4R)- and (1R,4R)-p-menth-2-en-1-ol (**4** and **5**).* (*S*)-Carvomenthene (8.28 g, 60 mmol) was dissolved in 300 ml of CH_2Cl_2 with 180 ml of 0.5 *N*-aqueous sodium bicarbonate. *m*-CPBA (7.74 mg, 60 mmol in 200 ml of CH_2Cl_2) was slowly added over 1 h into the solution, and the mixture stirred at room temperature under N_2 gas for 9 h. The CH_2Cl_2 layer was successively washed twice with 1 *N*-aqueous NaOH and saturated-aqueous NaCl. After drying over anhydrous Na_2SO_4, the solvent was removed to give 7.96 g of mixture of the two

diastereoisomers, *cis*- and *trans*-epoxy-(*S*)-carvomenthane, as a colorless oil.

After displacing the air with N_2 gas, small pieces of sodium (1.15 g, 50 mmol) were slowly added over 1 h to 2-heptanol (13.0 g, 100 mmol) in the flask, and the mixture stirred at 90 °C until the sodium had completely reacted. After 100 ml of diethylenglycol monomethyl ether had been mixed with the solution, the mixture was added dropwise to the epoxides (7.70 g in 100 ml of diethylenglycol monomethyl ether) at 90 °C under N_2 gas. The reaction mixture was then stirred and heated at 140 °C for 14 h. After cooling to room temperature, the reactant was diluted with 100 ml of water and extracted with hexane (110 ml × 3). The hexane layer was successively washed with water, 1 *N*-HCl and saturated-aqueous NaCl, and dried over anhydrous Na_2SO_4. After removing the solvent under reduced pressure, the product was distilled under reduced pressure to give 2.32 g of mixture of (1*S*,4*R*)- and (1*R*,4*R*)-*p*-menth-2-en-1-ol as a colorless oil, bp 120–137 °C/23 mmHg. The mixture (1.5 g) was chromatographed in a silica gel (Wako C300) column eluted with 3% and 5% diethyl ether in hexane to obtain 723 mg of (1*S*,4*R*)-*p*-menth-2-en-1-ol and 663 mg of (1*R*,4*R*)-*p*-menth-2-en-1-ol as colorless oils in respective yields of 12.1% and 11.4%.

*(1S,4R)-p-menth-2-en-1-ol (**4**).* $[\alpha]_D^{22}$ −25.2° (*c* = 1.0, $CHCl_3$). EIMS *m/z* (%): 154 (3), 139 (21), 136 (59), 134 (15), 121 (41), 119 (54), 111 (11), 105 (11), 94 (31), 93 (100), 92 (43), 91 (85), 84 (11), 83 (11), 81 (13), 80 (14), 79 (37), 78 (13), 77 (74), 71 (12), 69 (19), 67 (10), 65 (16), 55 (10), 53 (10). ^{1}H-NMR ($CDCl_3$) δ: 5.60 (2H, s, H-2 and 3), 1.93 (1H, dt, J = 9.2 and 5.6, H-4), 1.84 (1H, ddd, J = 12.4, 6.0 and 2.8, H-6a), 1.72 (3H, ddd, J = 13.2, 5.6 and 2.8, H-5a), 1.60 (1H, td, J = 12.4 and 3.2, H-6b), 1.57 (1H, m, H-8), 1.36 (1H, dddd, J = 13.2, 12.4, 9.2 and 3.2, H-5b), 1.21 (3H, s, H-7), 0.89 (3H, d, J = 6.8, H-9) 0.87 (3H, d, J = 6.8, H-10). ^{13}C-NMR ($CDCl_3$) δ: 134.5 (d, C-2), 131.2 (d, C-3), 69.6 (s, C-1), 41.7 (d, C-4), 38.0 (t, C-6), 31.6 (d, C-8), 28.5 (q, C-7), 23.6 (t, C-5), 19.8 (q, C-9), 19.4 (q, C-10).

*(1R,4R)-p-menth-2-en-1-ol (**5**).* $[\alpha]_D^{22}$ +28.7° (*c* = 1.0, $CHCl_3$). EIMS *m/z* (%): 154 (2),136 (16), 121 (11), 119 (14), 94 (20), 93 (100), 92 (17), 91 (32), 79 (23), 77 (36), 71 (11), 69 (13), 65 (11). ^{1}H-NMR ($CDCl_3$) δ: 5.66 (2H, s, H-2 and 3), 1.79 (1H, m, H-4), 1.76 (H, m, H-6a), 1.59 (1H, m, H-8), 1.54 (1H, m, H-5a), 1.41 (1H, m, H-6b), 1.38 (1H, m, H-5b), 1.21 (3H, s, H-7), 0.92 (3H, d, J = 6.8, H-9), 0.89 (3H, J = 6.8, H-10). ^{13}C-NMR ($CDCl_3$) δ: 133.4 (d, C-2), 133.0 (d, C-3), 67.4 (s, C-1), 42.1 (d, C-4), 37.2 (t, C-6), 31.7 (d, C-8), 29.6 (q, C-7), 21.6 (t, C-5), 19.7 (q, C-9), 19.3 (q, C-10).

*(1S,4S)-p-menth-2-en-1-ol (**6**).* $[\alpha]_D^{22}$ −28.7° (*c* = 1.0, $CHCl_3$).

*(1R,4S)-p-menth-2-en-1-ol (**7**).* $[\alpha]_D^{22}$ +25.2° (*c* = 1.0, $CHCl_3$).

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References

1) Hijii, N., Kajimura, H., Urano, T., Kinuura, H., and Itami, H., The mass mortality of oak trees induced by *Platypus quercivorus* (Murayama) and *Platypus calamus* Blandford (Coleoptera: Platypodidae)—The density and spatial distribution of attack by the beetles—. *J. Jpn. For. Soc.*, **73**, 471–476 (1991).
2) Kubono, T., and Ito, S., *Raffaelea quercivora* sp. nov. associated with mass mortality of Japanese oak, and the ambrosia beetle (*Platypus quercivorus*). *Mycoscience*, **43**, 255–260 (2002).
3) Kuroda, K., Responses of *Quercus* sapwood to infection with the pathogenic fungus of a new wilt disease vectored by the ambrosia beetle *Platypus quercivorus*. *J. Wood Sci.*, **47**, 425–429 (2001).
4) Ohya, W., and Kinuura, H., Close range sound communications of the oak platypodid beetle, *Platypus quercivorus* Murayama (Coleoptera: Platypodidae). *J. Appl. Zool.*, **36**, 317–321 (2001).
5) Ueda, A., and Kobayashi, M., Aggregation of *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae) on oak logs bored by males of the species. *J. For. Res.*, **6**, 173–179 (2001).
6) Nakashima, T., Saito, S., Kobayashi, M., Kinuura, H., Tokoro, M., Kashiwagi, T., Tebayashi, S., and Kim, C.-S., Aggregation pheromone of the oak platypodid, *Platypus quercivorus* (Murayama) (Coleoptera: Platypodidae). Proceedings of 5th Asia-Pacific Congress of Entomology, p. 89 (2005).
7) Albuquerque, M. R. J. R., Souza, E. B., Lins, M. U. D. S., Nogueura, N. A. P., Lemos, T. L. G., and Pessoa, E. R., Composition and antimicrobial activity of essential oil from aerial parts of *Baccharis trinervuis* (Lam.) Pers. *Arkivoc*, **vi**, 59–65 (2004).
8) Ciccio, J. F., and Gomez-Laurito, J., Volatile constituents of the leaves of *Siparuna thecaphora* (Siparunaceae) from Turrialba, Costa Rica. *Rev. Biol. Trop.*, **50**, 963–967 (2002).
9) Adams, R. P., "Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy," Allured Publishing Corporation, Illinois, pp. 111–117 (2001).