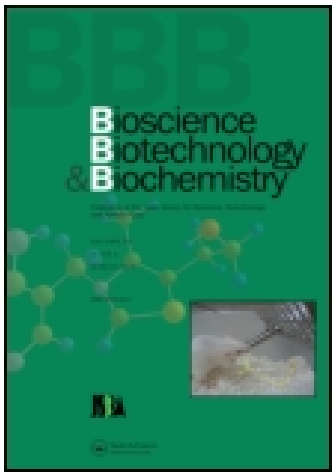

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Absolute Configuration of a New Mosquito Repellent, (+)-Eucamalol and the Repellent Activity of Its Epimer

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Note

Absolute Configuration of a New Mosquito Repellent, (+)-Eucamalol and the Repellent Activity of Its Epimer

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(+)-Eucamalol (1) and (−)-1-*epi*-eucamalol (2) were synthesized from (*S*)-(−)-perillaldehyde to determine the absolute configuration of 1, the structure of natural (+)-eucamalol being determined to be (1*R*,6*R*)-(+)-3-formyl-6-isopropyl-2-cyclohexen-1-ol. (+)-Eucamalol (1) and its 1-epimer (2) exhibited significant repellent activity against *Aedes albopictus*, and inhibited its feeding as well as DEET.

N,*N*-Diethyl-*m*-toluamide (DEET) has been used as a repellent against bloodsucking insects. However, DEET has many disadvantages, such as an unpleasant odor, suspected of carcinogenicity and skin penetration.[1] In recent years, some terpenoids have been isolated as repellents against bloodsucking insects, *e.g.*, *p*-menthane-3,8-diols (**3** and **4**)[2] and 1,8-cineole (**5**) in Fig. 1.[3] In previous studies on mosquito repellents, we have reported (+)-eucamalol (**1**) from the essential oil of *Eucalyptus camaldulensis*[4] (Fig. 1). The chemical structure of (+)-eucamalol (**1**) has been determined, except for its absolute configuration. This paper deals with the synthesis of (+)-eucamalol (**1**) and its 1-epimer (**2**) from (*S*)-(−)-perillaldehyde (**6**) to determine the absolute configuration, and their repellent activities against *Aedes albopictus*. (+)-Eucamalol and its 1-epimer were synthesized from (*S*)-(−)-perillaldehyde as shown in Fig. 2. (*S*)-(−)-Perillaldehyde (**6**) was converted to 8,9-dihydroperillaldehyde (**7**) by homogenous hydrogenation with tris(triphenylphosphine)rhodium chloride as a catalyst in a 73% yield. Conversion of **7** to 3-bromo-8,9-dihydroperillaldehyde (**9**) was performed by the procedure of Ishihara *et al.*[5] Enol acetylation of **7** with isopropenyl acetate gave an enol acetate (**8**) in a 38% yield. This enol acetate (**8**) was brominated by *N*-bromosuccinimide. Since 3-bromo-8,9-dihydroperillaldehyde (**9**) was unstable, nucleophilic substitution of bromide **9** was subsequently carried out by treating with potassium hydroxide to give two alcohols, (+)-eucamalol (**1**) and (−)-1-*epi*-eucamalol (**2**) in yields of 7.7 and 8.4%, respectively.

The $J_{1,6}$ value (9.2 Hz) of synthetic (+)-eucamalol (**1**) shows axial–axial coupling, while the smaller $J_{1,6}$ value (<2.0 Hz) of synthetic (−)-1-*epi*-eucamalol (**2**) shows axial–equatorial coupling. Thus the $J_{1,6}$ value of synthetic (+)-eucamalol (**1**) indicates that the relative configuration at C-1 and C-6 was, like that of natural (+)-eucamalol, of *trans*-form. The specific rotation of synthetic (+)-eucamalol was +14.1° in methanol, this being very close to the specific rotation of natural eucamalol, $[\alpha]_D = +13.5°$ ($c = 0.80$, MeOH).[4] Consequently, the absolute configuration of (+)-eucamalol was determined to be (1*R*,6*R*)-(+)-3-formyl-6-isopropyl-2-cyclohexen-1-ol.

The repellent activities of the synthetic eucamalol and its epimer were evaluated by using *Aedes albopictus* as the test mosquito strain (Table).

(+)-Eucamalol and its epimer showed repellent and feeding-

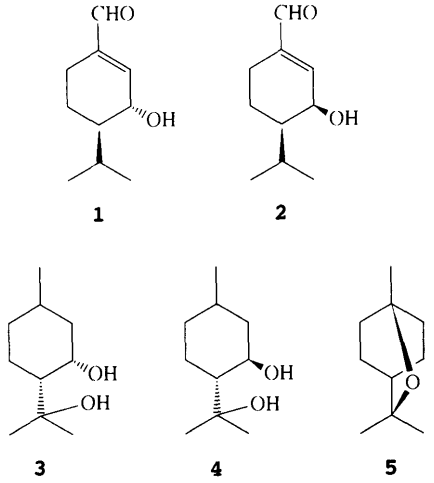

Fig. 1.

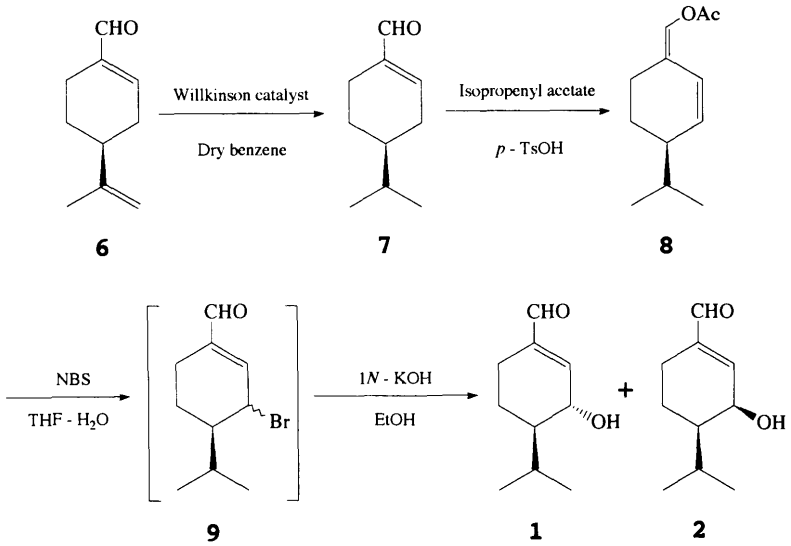

Fig. 2.

Table Repellent and Feeding Inhibition Activities of (+)-Eucamalol and Its (−)-1-Epimer against *Aedes albopictus*

Repellent activity (RA)

	500	250	50	mg/m²
(+)-Eucamalol	100	100	84.2	%
(−)-epi-Eucamalol	100	100	75.0	
DEET	100	100	80.0	

$$RA = \frac{\text{Total mosquitoes} - \text{Attracted mosquitoes}}{\text{Total mosquitoes}} \times 100\%.$$

Feeding inhibition activity (FIA)

	500	250	50	mg/m²
(+)-Eucamalol	100	100	74.5	%
(−)-epi-Eucamalol	100	100	65.0	
DEET	100	100	85.0	

$$FIA = \frac{\text{Total mosquitoes} - \text{Bloodsucking mosquitoes}}{\text{Total mosquitoes}} \times 100\%.$$

inhibition activities against *A. albopictus* to the same degree as DEET. In addition, both the repellent and feeding inhibition activities of (+)-eucamalol were the same as that of its epimer. In previous work, we have isolated 8,9-dihydroperillaldehyde (**7**) from *Eucalyptus camaldulensis* as a mosquito repellent, although the repellent activity of 8,9-dihydroperillaldehyde (44% at 167 mg/m²) was much less than that of (+)-eucamalol (96%) at the same concentration (K. Watanabe *et al.*, unpublished data). Wright[6] has demonstrated the action of a mosquito repellent as follows: (A) The carbon dioxide sensor in the antenna of the mosquito is made active by the repellent, but if exposure to the repellent continues, adaptation occurs and the mosquito cannot perceive a rise in the level of carbon dioxide. (B) The moisture sensor in the antenna of the mosquito seems to be shut off from the outset, and this could be explained by the molecules of the repellent blocking the pores in the cuticle of the sensory hairs by adsorption force. Since the configuration of the hydroxy group at the 3-position did not affect the repellent activity, we suggest that the hydroxy group enhanced the repellent activity by increasing the adsorption force.

Experimental

Instrumentation. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker AM-400 spectrometer at 400 MHz (^{1}H) and 100 MHz (^{13}C). Tetramethylsilane was used as an internal standard. All NMR spectral assignments were performed by ^{1}H-^{1}H and ^{13}C-^{1}H COSY spectra. Mass spectra (MS) were measured with a JEOL JMS-DX303 HF spectrometer at a 70 eV ionization voltage. IR spectra were recorded on a JASCO IR-810 spectrophotometer, while specific rotation values were taken with a JASCO DIP-370 digital polarimeter in a cylindrical cell (3.5 mm i.d. × 50 mm), using methanol as a solvent.

Chemicals. (*S*)-(−)-Perillaldehyde (Tokyo Chemical Inc.) was fractionally distilled, bp 134–135°C (39 mmHg), the specific rotation of purified (*S*)-(−)-perillaldehyde being −115.7° (neat). Benzene (Kanto Chemical Inc., 1 liter) was successively washed with conc. H_2SO_4 (300 ml × 2), water (500 ml × 1), 1 N NaOH aq. (300 ml × 2) and water (500 ml × 1). The benzene was then refluxed with $CaCl_2$, and distilled just before use. All other chemicals were purchased in the highest available grade and used without further purification.

Bioassay. Pupae of *Aedes albopictus* (Hatoyama race) were obtained from Laboratory of Parasitology at Teikyo University and incubated at 25°C for 3 weeks. The hatched adults were released into a cage (25 × 25 × 25 cm) made of stainless steel and nylon gauze, and the bioassay was performed in the cage.

Female Wistar mice (Nippon SLC Ltd.) were used at an age of 6–7 weeks.

Test samples were diluted with acetone at concentrations of 1, 5 or 10 mg/ml. The acetone solution of a test sample was applied to a wire-gauze bag (7 cm i.d. × 12 cm) at 50 ml/m² (50, 250, and 500 mg/m²), and the bag was air-dried at room temperature. A mouse was then put into the bag, and the bag placed in the cage of mosquitoes for 1 h. Each test was run using 20 female mosquitoes at 7 days old after emergence. The total number of mosquitoes landing on the mouse was counted. The mouse was then taken out of the cage, and the mosquitoes killed in a drying oven at 160°C. Each dead mosquito was crushed, and the bloodsucking mosquitoes were counted. Repellency (%) was calculated as

$$\frac{\text{total mosquitoes} - \text{attracted mosquitoes}}{\text{total mosquitoes}} \times 100\%$$

and feed inhibition as

$$\frac{\text{total mosquitoes} - \text{bloodsucking mosquitoes}}{\text{total mosquitoes}} \times 100\%$$

8,9-Dihydroperillaldehyde (**7**). (*S*)-(−)-perillaldehyde (**6**, 5 g) was dissolved in 50 ml of benzene, and 510 mg of tris(triphenylphosphine)-rhodium chloride was then added the benzene solution. Hydrogen gas was bubbled into the mixture for 3.5 h at 70°C. The reaction mixture was cooled to room temperature and concentrated under a slightly reduced pressure. The dark residue was fractionally distilled *in vacuo*, bp 136–137°C (57 mmHg), giving 3.7 g of 8,9-dihydroperillaldehyde (**7**, 73%) as a colorless oil; $[\alpha]_D = -105.7°$ ($c = 0.52$, MeOH); EI-MS: 152 (M^+, 45%), 151 ($M^+ - H$, 1), 137 ($M^+ - CH_3$, 16), 124 (19), 109 ($M^+ - C_3H_7$, 100), 95 (35), 83 (22), 81 (62), 79 (58), 77 (39), 67 (36), 55 (26), 53 (32); EI-HR-MS: 152.1178 (M^+), (152.1201 calcd. for $C_{10}H_{16}O$); IR ν_{max} cm^{-1} (film): 3050 (olefinic C–H), 2955, 2930, 2870 (aliphatic C–H), 2820, 2720 (aldehyde), 1685 (aldehyde C=O), 1645 (C=C), 1385, 1363 (*gem* –CH_3), 1182, 770, 695; ^{1}H-NMR δ ($CDCl_3$): 9.43 (1H, s, H-7), 6.62 (1H, dt, J=5.1, 1.8 Hz, H-2), 2.48–2.37 (2H, m, H-3, H-6), 2.10–2.01 (2H, m, H-3, H-6), 1.87 (1H, m, H-5), 1.54 (1H, sept, J=2.4 Hz, H-8), 1.39 (1H, m, H-4), 1.18 (1H, ddt, J=5.5, 12.6, 11.5 Hz, H-5), 0.94 (3H, d, J=2.4 Hz, H-9), 0.93 (3H, d, J=2.4 Hz, H-10); ^{13}C-NMR δ ($CDCl_3$): 194.1 (C-7), 151.6 (C-2), 142.1 (C-1), 39.8 (C-4), 31.9 (C-8), 30.1 (C-3), 24.8 (C-5), 21.7 (C-6), 19.7 (C-9), 19.4 (C-10). The assignments of the 9- and 10-positions were interchangeable.

7-Acetoxy-p-mentha-1(7), 2-diene (**8**). A mixture of **7** (3 g) and *p*-toluenesulfonic acid (0.5 g) in isopropenyl acetae (100 ml) was refluxed for 6 h under an argon atmosphere, cooled to room temperature, and then concentrated under slightly reduced pressure. The dark residue was subjected to silica gel column chromatography (Silica gel 60, 70–230 mesh, Merck; 2 cm i.d. × 20 cm), using hexane (150 ml) as the eluent. The hexane eluate was concentrated under slightly reduced pressure, the residue being fractionally distilled, bp 104–105°C (4 mmHg), give 1.45 g of 7-acetoxy-*p*-mentha-1(7),2-diene (**8**, 38%) as a slightly yellow oil; $[\alpha]_D = -29.8°$ ($c = 0.52$, MeOH); EI-MS: 194 (M^+, 17%), 152 (47), 150 (11), 148 (13), 133 (16), 109 (100), 81 (16), 79 (18), 43 (18); EI-HR-MS: 194.1290 (M^+), (194.1307 calcd. for $C_{12}H_{18}O_2$); IR ν_{max} cm^{-1} (film): 3080, 3020 (olefinic C–H),2955, 2870 (aliphatic C–H), 1755 (OC=O), 1660 (C=C), 1460, 1435, 1365, 1215, 1095 (C–O), 905, 830; ^{1}H-NMR δ ($CDCl_3$): 7.06 (1H, s, H-7), 6.03 (1H, dd, J=2.5, 10 Hz, H-3), 5.74 (1H, dd, J=2.6, 10 Hz, H-2), 2.73 (1H, dt, J=4.3, 15.5 Hz, H-6), 2.16 (3H, s, CH_3C=O), 2.04–2.15 (2H, m, H-4, H-6), 1.75 (1H, dq, J=13.7, 4.5 Hz, H-5), 1.66 (1H, sept, J=7.1 Hz, H-8), 1.37 (1H, dq, J=4.1, 12.5 Hz, H-5), 0.91 (3H, d, J=7.1 Hz, H-9), 0.90 (3H, d, J=7.1 Hz, H-10); ^{13}C-NMR δ ($CDCl_3$): 167.9 ($CH_3\underline{C}$=O), 133.4 (C-2), 133.1 (C-7), 124.9 (C-3), 122.0 (C-1), 42.0 (C-4), 31.6 (C-8), 24.3 (C-5), 22.2 (C-6), 20.7 ($\underline{C}H_3C$=O), 19.6 (C-9), 19.5 (C-10). The assignments of the 9- and 10-positions were interchangeable.

(+)-*Eucamalol* (**1**) *and* (−)-*1-epi-eucamalol* (**2**). 7-Acetoxy-*p*-mentha-1(7),2-diene (**8**, 1 g) and *N*-bromosuccimide (1 g) were dissolved in 90 ml of 5:1 THF–water, and the mixture was stirred for 17 h at room temperature. The reaction mixture was carefully concentrated *in vacuo*, and extracted with *n*-hexane (30 ml × 3). The hexane extract containing 3-bromo-8,9-dihydroperillaldehyde (**9**) was washed with water (50 ml × 1), dried on anhydrous Na_2SO_4, and concentrated under slightly reduced

pressure. The residue (1 g) was subsequently dissolved in 50 ml of 1 : 1 ethanol–1 N KOH aq, the mixture then being stirred for 1.5 h at room temperature. The reaction mixture was neutralized with 1 N HCl aq., and concentrated under reduced pressure. The residue was suspended in 50 ml of water, and extracted with ethyl acetate (30 ml × 3). The ethyl acetate extract was washed with water (50 ml × 1), dried on anhydrous Na_2SO_4, and concentrated under reduced pressure. The residue was subjected to silica gel column chromatography (Silica gel 60, 70–230 mesh, Merck; 2 cm i.d. × 24 cm), using an elution gradient of *n*-hexane to ethyl acetate (0, 1, 2, 5, 10, 20, 50, and 100% ethyl acetate/*n*-hexane, 100 ml). The fraction containing **1** and **2** (50% ethyl acetate/*n*-hexane eluate) was concentrated under slightly reduced pressure, and further purified by four repetitive PTLC steps, developing with *n*-hexane–ethyl acetate (3 : 1). Elution with methanol gave 67 mg of (+)-eucamalol (**1**, 7.7%) and 73 mg of (−)-1-*epi*-eucamalol (**2**, 8.4%). (+)-Eucamalol (**1**): a slightly yellow oil; $[\alpha]_D = +14.1°$ ($c = 0.82$, MeOH); EI-MS: 168 (M^+, 63%), 151 (26), 150 ($M^+ - H_2O$, 22), 139 (48), 133 (26), 125 (66), 124 (100), 107 (45), 98 (26), 97 (33), 96 (23), 95 (39), 79 (43), 69 (30), 68 (25), 55 (21), 43 (CHO, 24); EI-HR-MS: 168.1151 (M^+), (168.1150 calcd. for $C_{10}H_{16}O_2$); IR ν_{max} cm^{-1} (film): 3400 (OH), 2960, 2930, 2870 (aliphatic C–H), 2715 (aldehyde), 1685 (aldehyde C=O), 1650 (C=C), 1385, 1370 (*gem*-CH_3), 1160, 1050, 1030, 910, 710; ^{1}H-NMR δ ($CDCl_3$): 9.44 (1H, s, H-7), 6.62 (1H, m, H-2), 4.26 (1H, dm, $J = 9.2$ Hz, H-1), 2.34 (1H, dm, $J = 18.1$ Hz, H-4), 2.08 (1H, d sept, $J = 2.8$, 7.0 Hz, H-8), 2.05 (1H, m, H-4), 1.78 (1H, ddt, $J = 13.4$, 5.0, 2.8 Hz, H-5), 1.37 (1H, ddt, $J = 12.2$, 9.2, 2.8 Hz, H-6), 1.22 (1H, ddt, $J = 13.4$, 5.4, 12.2 Hz, H-5), 0.96 (3H, d, $J = 7.0$ Hz, H-9), 0.83 (3H, d, $J = 7.0$ Hz, H-10); ^{13}C-NMR δ ($CDCl_3$): 194.1 (C-7), 151.6 (C-2), 141.9 (C-3), 69.2 (C-1), 47.6 (C-6), 26.6 (C-8), 21.6 (C-4), 20.9 (C-9), 20.1 (C-5), 16.7 (C-10). The assignments of the 9- and 10-positions were interchangeable. (−)-1-*epi*-Eucamalol (**2**): a slightly yellow oil; $[\alpha]_D = -234.6°$ ($c = 0.97$, MeOH); EI-MS: 168 (M^+, 66%), 150 ($M^+ - H_2O$, 16), 139 (100), 125 (71), 124 (55), 121 (38), 107 (44), 99 (23), 98 (46), 97 (51), 95 (39), 81 (32), 79 (54), 77 (25), 69 (42), 43 (CHO, 31); EI-HR-MS: 168.1158 (M^+), (168.1150 calcd. for $C_{10}H_{16}O_2$); IR ν_{max} cm^{-1} (film): 3430 (OH), 3060 (olefinic C–H), 2960, 2930, 2875 (aliphatic C–H), 2840, 2725 (aldehyde), 1685 (aldehyde C=O), 1645 (C=C), 1385, 1365 (*gem*-CH_3), 1180, 1040, 1000, 960, 920; ^{1}H-NMR δ ($CDCl_3$): 9.52 (1H, s, H-7), 6.80 (1H, dd, $J = 2.1$, 5.1 Hz, H-2), 4.47 (1H, br. t, H-1), 2.50 (1H, dd, $J = 4.9$, 18.3 Hz, H-4), 1.96 (1H, m, H-4), 1.86 (1H, dd, $J = 3.5$, 21.7 Hz, H-5), 1.72 (1H, d sept, $J = 2.5$, 6.7 Hz, H-8), 1.28 (1H, dq, $J = 4.9$, 12.9 Hz, H-5), 1.12 (1H, m, H-6), 1.05 (3H, d, $J = 6.7$ Hz, H-9), 1.01 (3H, d, $J = 6.7$ Hz, H-10); ^{13}C-NMR δ ($CDCl_3$): 194.6 (C-7), 147.7 (C-2), 142.9 (C-3), 64.4 (C-1), 46.6 (C-6), 28.1 (C-8), 22.6 (C-4), 21.0 (C-9), 20.7 (C-5), 19.3 (C-10). The assignments of the 9- and 10-positions were interchangeable.

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