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 (1R,6R)(+)-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.11 In recent years, some terpenoids have been isolated as repellents against bloodsucking insects, e.g., p-methane-3,8-diols (3 and 4)21 and 1,8-cineole (5) in Fig. 1.21 In previous studies on mosquito repellents, we have reported (+)-eucamalol (1) from the essential oil of Eucalyptus camaldulensis4 (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 homogeneous 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.23 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 +141° in methanol, this being very close to the specific rotation of natural eucamalol, \( [\alpha]_D^{20} = +13.5^\circ \) (c = 0.80, MeOH).21 Consequently, the absolute configuration of (+)-eucamalol was determined to be (1R,6R)(+)-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-
Repellent and Feeding Inhibition Activities of (+)-Eucamolal and Its (−)-1-Epimer against *Aedes albopictus*

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<tr>
<th></th>
<th>500</th>
<th>250</th>
<th>50</th>
<th>mg/m²</th>
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<td>100</td>
<td>100</td>
<td>84.2</td>
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<td>100</td>
<td>100</td>
<td>75.0</td>
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</tr>
<tr>
<td>DEET</td>
<td>100</td>
<td>100</td>
<td>80.0</td>
<td></td>
</tr>
</tbody>
</table>

RA = \( \frac{\text{Total mosquitoes – Attracted mosquitoes}}{\text{Total mosquitoes}} \times 100\% \)

Feeding inhibition activity (FIA)

<table>
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<tr>
<th></th>
<th>500</th>
<th>250</th>
<th>50</th>
<th>mg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Eucamalol</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>74.5</td>
</tr>
<tr>
<td>(−)-epi-Eucamalol</td>
<td>100</td>
<td>100</td>
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<tr>
<td>DEET</td>
<td>100</td>
<td>100</td>
<td>80.0</td>
<td></td>
</tr>
</tbody>
</table>

FIA = \( \frac{\text{Total mosquitoes – Bloodsucking mosquitoes}}{\text{Total mosquitoes}} \times 100\% \)

8,9-Dihydropyridinealdehyde (7). \( \delta \)-Perillylaldehyde (6, 5g) was dissolved in 50ml of benzene, and 510mg of tris(tribenzylyphosphine)-rhodium dichloride was then added to the benzene solution. Hydrogen gas was bubbled into the mixture for 3.5h 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 Nujol, bp 136–137°C (57mmHg), giving 3.7g of 8,9-dihydropyridinealdehyde (7, 73%) as a colorless oil; \( \delta x_{20}^{\text{IR}} = 105.7^\circ \) (c= 0.52, MeOH); EI-MS: 152 (M⁺, 45), 151 (M⁺ – H), 137 (M⁺ – CH₂, 16), 124 (19), 109 (M⁺ – CH₃, 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 C₁₅H₁₀O₂; \( \delta x_{20}^{\text{IR}} \) \( \delta \) film), 3050 (olef凸显的 C-H), 3050 (olef凸显的 C-C), 1172, 780, 695; 1H-NMR \( \delta \) (CDCl₃): 9.43 (1H, s, H-7), 6.62 (1H, d, J=5.1, 1.8 Hz, H-2), 2.48-2.57 (2H, both, H-3, H-4), 1.87 (1H, m, H-5), 1.54 (1H, sept, J=2.4Hz, H-10), 1.39 (1H, m, H-4), 1.18 (1H, ddt, J=5.5, 12.6, 11.5 Hz, H-3), 0.94 (3H, d, J=2.4Hz, H-9), 0.93 (3H, d, J=2.4Hz, H-10); 13C-NMR \( \delta \) (CDCl₃): 194.1 (C), 151.6 (C), 142.1 (C), 39.8 (C), 59.1 (C), 49.8 (C), 59.1 (C), 19.7 (C), 19.4 (C). The assignments of the 9-10 positions were inter-changeable.

7-Acetoxy-p-mentha-1(7),2-diene (8). A mixture of 7 (3g) and p-toluenesulfonic acid (0.5g) in isopropyl acetate (100ml) was refluxed for 6h 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; 2mm i.d. x 20cm), using hexane (150ml) as the eluent. The hexane eluate was concentrated under slightly reduced pressure, the residue being fractionally distilled, bp 104–110°C (4mmHg), giving 1.45g of 7-acetoxy-p-mentha-1(7),2-diene (8, 38%) as a slightly yellow oil; \( \delta x_{20}^{\text{IR}} = 29.8^\circ \) (c= 0.52, MeOH); EI-MS: 194 (M⁺, 17%), 152 (47), 151 (11), 148 (13), 133 (16), 109 (100), 81 (16), 79 (18), 43 (18); EI-HR-MS: 194.1290 (M⁺), 194.1307 (calcld. for C₁₅H₂₂O₂; \( \delta x_{20}^{\text{IR}} \) (film): 3080, 3020 (olefinic C–H), 2955, 2870 (aliphatic C–H), 1755 (CO=O), 1660 (C=O), 1400, 1345, 1215; 1095 (C=O), 905, 830; 1H-NMR \( \delta \) (CDCl₃): 7.06 (1H, s, H-7), 6.03 (1H, dd, J=2.5, 10Hz, H-3), 5.74 (1H, dd, J=2.6, 10Hz, H-2), 2.73 (1H, dd, J=4.3, 15.5Hz, H-6), 2.16 (3H, s, CH₃C=O), 2.04-2.15 (2H, H-4, H-6), 1.75 (1H, d, J=13.7, 4.5Hz), 1.66 (1H, sept, J=7.1Hz, H-8), 1.37 (1H, dq, J=4.1, 12.5Hz, H-5), 0.91 (3H, d, J=7.1Hz, H-9), 0.90 (3H, d, J=7.1Hz, H-10), 13C-NMR \( \delta \) (CDCl₃): 167.9 (CH=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-22), 26.7 (CH₃C=O), 19.6 (C-9), 19.5 (C-10). The assignments of the 9-10 positions were inter-changeable.

(+)-Eucamalol (1) and (−)-1-epi-eucamalol (2). 7-Acetoxy-p-mentha-1(7),2-diene (8, 1g) and 8-bromosuccimide (1g) were dissolved in 90ml of 5:1 THF-water, and the mixture was stirred for 17h at room temperature. The reaction mixture was carefully concentrated under reduced pressure, extracted with \( \delta \)-hexane (30ml x 3). The hexane extract containing 3-bromo-8,9-dihydropyridinealdehyde (9) was washed with water (50ml x 1), dried on anhydrous Na₂SO₄, and concentrated under slightly reduced pressure.
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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 x 3). The ethyl acetate extract was washed with water (50 ml x 1), dried on anhydrous Na₂SO₄, 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. x 24 cm), using an elution gradient of n-hexane to ethyl acetate (0, 1, 2, 3, 10, 20, 30, and 100% ethyl acetate/n-hexane, 100 ml). The fraction containing I 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 (+)-eucamol (1, 7.7%) and 73 mg of (−)-1-epi-eucamol (2, 8.4%). (+)-Eucamol (1): a slightly yellow oil; [α]D₂₀ = +14.1° (c = 0.82, MeOH); EI-MS: 168 (M⁺, 65%), 151 (26), 150 (M⁺ − H₂O, 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); El-HR-MS: 168.1151 (M⁺); 168.1150 calcld. for C₁₃H₁₈O₂; IR νmax 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-CH₂), 1180, 1040, 1000, 960, 920; 1H-NMR δ (CDCl₃): 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), 2.59 (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, J = 2.5, 6.7 Hz, H-8), 1.28 (1H, d, 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); 13C-NMR δ (CDCl₃): 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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References