

New Mosquito Repellent from *Eucalyptus camaldulensis*

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A new compound named eucamalol and 4-isopropylbenzyl alcohol were isolated as mosquito repellents from the essential oil of *Eucalyptus camaldulensis*. The structure of eucamalol was elucidated to be 3-formyl-6 α -isopropyl-2-cyclohexen-1 β -ol by ¹H NMR analysis and confirmed by synthesis. Both compounds exhibited potent repellent activities against *Aedes aegypti*.

INTRODUCTION

N,N-Diethyl-*m*-toluamide (DEET) has been used as an insect repellent against blood-sucking insects such as the mosquito. However, DEET has many drawbacks, such as an unpleasant odor and its skin penetration (Moody et al., 1986). Furthermore, DEET also reacts with certain plastics and synthetic rubber, resulting in considerable damage to eyeglasses and watchbands. Therefore, a search for new repellents lacking these defects has recently been initiated.

The isolation and identification of *p*-methane-3,8-diols [1 (*trans*) and 2 (*cis*)] as potent mosquito repellents from *Eucalyptus citriodora* have been already reported (Nishimura et al., 1986). It has been reported that the essential oil from *E. camaldulensis* exhibited significant repellent activity against *Aedes albopictus* (Nishimura et al., 1987). These observations prompted us to purify the mosquito-repelling principles in *E. camaldulensis* and elucidate their structures. This paper describes the structure elucidation and synthesis of a new mosquito repellent, eucamalol, and its mosquito-repelling activity.

EXPERIMENTAL PROCEDURES

¹H NMR spectra were recorded on a Bruker AMX-600 spectrometer at 600 MHz or on a Bruker AC-300P spectrometer at 300 MHz with TMS as an internal standard. IR spectra were observed on a Hitachi 270-30 spectrophotometer in chloroform solution. Mass and high-resolution mass spectra were recorded on a Hitachi M-008 spectrometer by direct inlet at 70 eV. Specific rotation was observed on a Perkin-Elmer 241 polarimeter. Analytical TLC and preparative TLC (0.5 mm) were performed on precoated silica gel 60 GF₂₅₄ (Merck).

Isolation of Compounds 3 and 4. The leaves of *E. camaldulensis* (1.5 kg) were collected in Matsudo, Chiba prefecture, Japan, in October 1989. They were cut into small pieces and steam-distilled to yield 1.6 g of the essential oil. This was purified by successive preparative TLC [0.5 mm, hexane-ethyl acetate 4:1 and 2:1 (v/v)] to give 25 mg of compound 3 and 18 mg of compound 4. The structures of compounds 3 and 4 are given in Figure 1.

4-Isopropylbenzyl Alcohol (3): MS, *m/z* 150 [M]⁺, 132 [M - H₂O]⁺ (base peak), 107 [M - C₃H₇]⁺; ¹H NMR $\delta_{\text{CDCl}_3}^{\text{TMS}}$ 7.05 (2H,

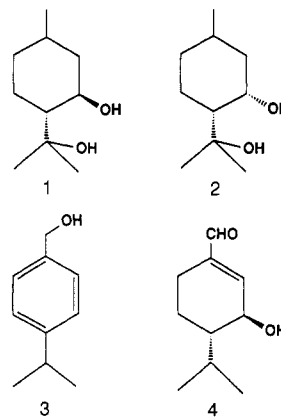


Figure 1. Structures of *p*-methane-3,8-diols 1 (*trans*) and 2 (*cis*), 4-isopropylbenzyl alcohol (3), and eucamalol (4).

d, *J* = 7.0 Hz), 6.95 (2H, *d*, *J* = 7.0 Hz), 4.00 (2H, *s*, CH₂OH), 2.85 (1H, *m*, CH), 1.00 (6H, *d*, *J* = 6.8 Hz).

(+)-Eucamalol (4): [α]_D²⁵ +13.5° (*c* 0.80, CH₃OH); HRMS *m/z* 168.1151 (C₁₀H₁₆O₂, calcd 168.1150); MS, *m/z* 168 [M]⁺, 139 [M - CHO]⁺, 125 [M - C₃H₇]⁺, 69 (base peak); IR $\nu_{\text{max}}^{\text{KBr}}$ 3400 (OH group), 2980, 1680 (conjugated aldehyde) cm⁻¹. ¹H NMR data are described in Table I.

(±)-1-(1,3-Dithian-2-yl)-4-isopropyl-2-cyclohexen-1-ol (6). To a suspension of 1.2 g (10 mM) of 1,3-dithiane in 10 mL of dry tetrahydrofuran was added a solution of *n*-butyllithium (1.6 M, 6 mL, 9.6 mM) at -78 °C, and the mixture was stirred for 2 h at -78 °C. A solution of 1.38 g (10 mM) of (±)-cryptone in 2 mL of dry tetrahydrofuran was added dropwise to the above mixture at -78 °C over 1 h. The resulting mixture was stirred at -78 °C for an additional 30 min and then set aside for a further 18 h at 0 °C. The reaction mixture was concentrated to one-fifth volume under reduced pressure, and 20 mL of water was added to the mixture. The organic layer was extracted with diethyl ether (20 mL × 2). The ether layers were combined and then dried over anhydrous sodium sulfate. Evaporation of the solvent gave a yellow oil which was chromatographed on silica gel (33% ethyl acetate in hexane) to give 710 mg (28%) of (±)-1-(1,3-dithian-2-yl)-4-isopropyl-2-cyclohexene-1-ol (6): MS, *m/z* 258 [M]⁺, 240 [M - H₂O]⁺, 215 [M - C₃H₇]⁺, 119 (base peak); IR $\nu_{\text{max}}^{\text{KBr}}$ 3400 (OH group), 2980, 1680 cm⁻¹; ¹H NMR $\delta_{\text{CDCl}_3}^{\text{TMS}}$ 5.92 (1H, *dd*, *J* = 6.8 and 2.2 Hz), 5.75 (1H, *d*, *J* = 6.8 Hz), 4.26 (1H, *s*), 2.85, 2.95 (4H, *m*), 2.10 (1H, *m*), 1.80, 1.90 (4H, *m*), 1.60 (2H, *m*), 1.55 (1H, *m*), 0.96 (3H, *d*, *J* = 7.0 Hz), 0.90 (3H, *d*, *J* = 7.0 Hz).

(±)-Eucamalol (4) and (±)-*epi*-Eucamalol (5). To a mechanically stirred suspension of 0.5 g (2.3 mM) of red mercuric oxide and 0.33 g (2.3 mM) of boron trifluoride etherate in 5 mL of 15% aqueous tetrahydrofuran was added a solution of compound 6 (300 mg, 1.16 mM) in 1.2 mL of tetrahydrofuran under refluxing condition. After the addition was completed,

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Table I. ^1H NMR Data of Eucamalol (4) and *epi*-Eucamalol (5)^a

H	4	5	H	4	5
1	4.28 dd	4.44 br t	6	1.39 m	1.13 m
2	6.63 d	6.80 d	7	9.45 s	9.53 s
4 α	2.04 m	1.97 m	8	2.08 dq	1.73 dq
4 β	2.37 m	2.53 m	9 ^b	0.98 d	1.07 d
5 α	1.24 m	1.27 m	10 ^b	0.84 d	0.96 d
5 β	1.78 m	1.88 m			

^a 600 MHz in CDCl_3 ; TMS was used as internal standard.^b Assignments were interchangeable. Coupling constants (Hz) of 4: 1,2 = 2.2; 1,6 = 9.3; 4 α ,4 β = 18.2; 4 α ,5 α = 10; 4 α ,5 β = 10; 4 β ,5 α = 2.6; 4 β ,5 β = 3; 5 α ,5 β = 14.7; 5 α ,6 = 12.7; 5 β ,6 = 3.3; 6,8 = 3.3; 8,9 = 8.10 = 6.8. Coupling constants of 5: 1,2 = 5.3; 1,6 = 2.9; 2,4 α = 2.0; 2,4 β = 1.2; 4 α ,4 β = 18.2; 4 α ,5 α = 5.9; 4 α ,5 β = 12.1; 4 β ,5 α = 1; 4 β ,5 β = 5.0; 5 α ,5 β = 14.7; 5 α ,6 = 12.7; 5 β ,6 = 3.3; 6,8 = 3.3; 8,9 = 8.10 = 6.8.

the reaction mixture was refluxed for an additional 2 h. An additional 0.25 g (1.15 mM) of red mercuric oxide was added to the mixture, and the resulting mixture was refluxed for 1 h. The mixture was cooled to room temperature, and 10 mL of ethyl ether and 4 mL of brine were added to the mixture. The organic layer was separated and washed with 10 mL of brine and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 120 mg of yellow oil which was purified on silica gel (33% ethyl acetate in hexane) to give 10 mg (5.1%) of ($\pm$)-*epi*-eucamalol (5, R_f 0.70) and 15 mg (7.7%) of ($\pm$)-eucamalol (4, R_f 0.65). The ^1H NMR, IR, and MS data of synthetic ($\pm$)-eucamalol (4) were identical with those of the natural specimen. Compound 5: MS, m/z 168 [M^+], 139 [$\text{M} - \text{CHO}^+$], 125 [$\text{M} - \text{C}_3\text{H}_7^+$], 69 (base peak); IR, $\nu_{\text{max}}^{\text{KBr}}$ 3400 (OH group), 2980, 1680 (conjugated aldehyde) cm^{-1} . ^1H NMR data are described in Table I.

Bioassay. A chick whose abdominal feathers were removed with a haircutter was fixed on a wood board (7 $\times$ 15 cm). The shaved abdominal skin of this chick (2.5 $\times$ 4 cm) was exposed, and an ethanol solution of a test compound was applied to the skin (1.5 g/m^2). About 500 adult mosquitoes which were 6–8 days old after emergence (*Aedes aegypti*; approximately equal number of females and males) were released in a cage (21 $\times$ 21 $\times$ 30 cm) made of stainless steel and nylon gauze. Two chicks (one was treated and the other untreated) were put in the cage for 2 min. Then, the total number of landed mosquitoes on each chick was counted. Repellency (percent) was calculated according to

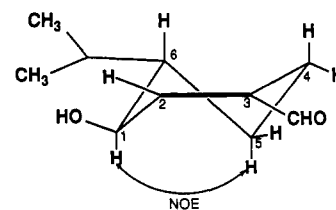
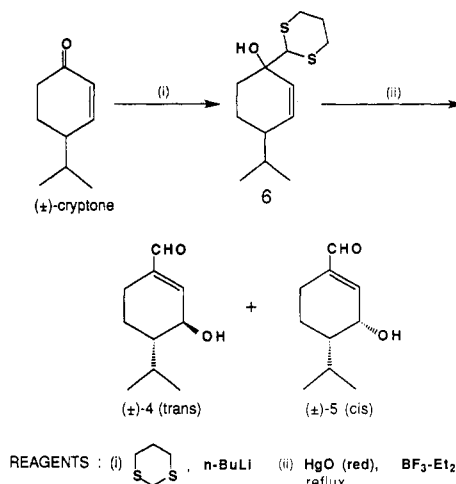
$$\text{repellency (\%)} =$$

$$\left(1 - \frac{\text{no. of landed mosquitoes on the treated chicks}}{\text{no. of landed mosquitoes on the untreated chicks}}\right) \times 100$$

The repellency was evaluated every hour after treatment, until the repellency was reduced to less than 80%.

RESULTS AND DISCUSSION

The bioassay-guided chromatography of the essential oil of *E. camaldulensis* yielded two active principles. One was easily identified as 4-isopropylbenzyl alcohol (3). The other was obtained as colorless oil: $\text{C}_{10}\text{H}_{16}\text{O}_2$. The presence of the conjugated aldehyde system was shown by IR absorption at 1685 cm^{-1} and ^1H NMR signals at δ 6.63 (1H, d, $\text{C}=\text{CH}-$, $J = 2.2$ Hz) and 9.45 (1H, s, CHO). IR absorption at 3400 cm^{-1} and ^1H NMR signal at δ 4.28 (1H, dd, $J = 2.2$ and 9.3 Hz), which coupled with the above olefinic proton, demonstrated the existence of one secondary hydroxy group. The two doublets at δ 0.98 (3H, d, $J = 6.8$ Hz) and 0.84 (3H, d, $J = 6.8$ Hz) were assigned to an isopropyl group. Analysis of the ^1H - ^1H COSY spectrum of the active compound led to the assignment of the remaining signals (Table I). The $J_{1,6}$ value (9.3 Hz) as well as NOE enhancement of the multiplet at δ 1.78 (5 α -H) on irradiation at δ 4.28 (1-H) ascertained the relative configurations at C-1 and C-6, suggesting an α -orientation for 1-H and a β -orientation for 6-H (Figure

**Figure 2.** NOE enhancement of eucamalol (4).

REAGENTS: (i) 2-lithio-1,3-dithiane, $n\text{-BuLi}$; (ii) HgO (red), $\text{BF}_3 \cdot \text{Et}_2\text{O}$, reflux

Figure 3. Synthetic route to ($\pm$)-eucamalol (4) and ($\pm$)-*epi*-eucamalol (5).**Table II.** Mosquito Repellent Activities of 4-Isopropylbenzyl Alcohol (3), Eucamalol (4), and DEET against *A. aegypti*

tested compd	repellency (%)			
	immed after treatment	1 h	2 h	3 h
3	100	23		
4	100	92	87	75
DEET	100	84	55	

2). Although 3-formyl-6-isopropyl-2-cyclohexen-1-ol, with unspecified relative stereochemistry, has been prepared from 4-isopropyl-2-cyclohexen-1-one (cryptone) in a study on synthetic methodology, compound 4 with defined stereochemistry, designated eucamalol, has not previously been reported as a natural product (Ogura et al., 1978).

Rigby et al. (1988) reported a new way of converting the 1,2-adduct, derived from the lithio derivative of 1,3-dithiane and 2-cyclohexen-1-one, into 3-formyl-2-cyclohexen-1-ol. Using their method, we conducted the synthesis of ($\pm$)-eucamalol (4) from ($\pm$)-cryptone (Figure 3). A nucleophilic addition of 2-lithio-1,3-dithiane to ($\pm$)-cryptone in tetrahydrofuran gave ($\pm$)-1-(1,3-dithian-2-yl)-4-isopropyl-2-cyclohexen-1-ol (6). Treatment of 6 with red mercuric oxide and boron trifluoride etherate in aqueous tetrahydrofuran afforded a mixture of ($\pm$)-eucamalol (4) and its epimer at C-1, which was named ($\pm$)-*epi*-eucamalol (5). Both compounds were separated by preparative thin-layer chromatography. Except for the value of optical rotation, the physicochemical data (^1H NMR, MS, and IR) for the synthetic specimen of ($\pm$)-eucamalol were identical with those for the natural one. Accordingly, the structure of 4 was unequivocally confirmed. Determination of the absolute stereochemistry of 4 is now under investigation and will be reported elsewhere.

The mosquito-repelling activities of 4-isopropylbenzyl alcohol (3) and eucamalol (4) were examined against *A. aegypti* (Table II) in comparison with that of DEET. All tested compounds exhibited potent mosquito-repelling

activities against *A. aegypti* immediately after application. One hour after treatment, the effectiveness of **3** was lost. Although the duration of the effectiveness for DEET was within 2 h after treatment by our test method, eucamalol (**4**) showed 75% repellency even 3 h after treatment. This result suggests that eucamalol may be a superior repellent against *A. aegypti* compared with DEET.

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