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Synthesis of (−)-callicarpenal, a potent arthropod repellent

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ABSTRACT

Callicarpenal (**1**), a natural terpenoid isolated from American beautyberry (*Callicarpa americana*), has shown significant repellent activities against mosquitoes, ticks, and imported fire ants. Here we report our efficient synthetic approach to this natural product, and preliminary results of the mosquito biting-deterrent effects of callicarpenal as well as its synthetic precursors and related C_8-epimers. The synthetic strategy allows rapid access to various epimers and analogues of the natural product that can be used to explore its structure–activity relationship and optimize its biological properties.

1. Introduction

Blood-feeding arthropods transmit many of the world's most dangerous diseases, such as malaria[1,2a] and dengue fever.[2b] An estimated 243 million malaria cases led to approximately 863,000 deaths in 2008,[2a] while nearly one third of the world's population is at risk of dengue fever.[2b] Very recently, an outbreak of infection cases caused by tick biting has been reported resulting in a fatality rate as high as 30% in some area of central China.[3] Research on the cause of this infection led to the isolation and identification of a new type of Bunyavirus. As a tick-borne virus, this new species of Bunyavirus family has been considered as a new emerging deadly virus.[3] Consequently, aside from the urgent need of study for new antiplasmodium/antivirus agents, the development of new safe and effective repellents against blood-feeding arthropods is in high demand.[4]

The best known insect repellent, due to its potency and simple synthesis, is *N,N*-diethyl-3-methylbenzamide (DEET, **4**), a compound developed by the US Army in 1946.[1] Although it is considered as safe, extensive use of this compound can lead to skin irritation.[5] Picaridin (KBR3023, **5**) is another synthetic repellent that has been reported to be as effective as DEET but not as toxic in humans.[6] On the other hand, *p*-menthane-3,8-diol (PMD, **6**), the main component in citrus/eucalyptus solutions, is the best representative of natural product-repellents.[7] The growing demand for new arthropod repellents, led to the evaluation of plants of the genus Beautyberry (*Callicarpa*).[8] Interestingly, freshly crushed American beautyberry leaves have been used in ethnomedicine as a traditional biting-insect repellent in certain southern states of the United States.[9] Inspired by this information, Cantrell et al. studied the chemical origins of the bioactivities of the beautyberry extracts against mosquitoes,[9] ticks,[10] and imported fire ants.[11] These investigations led to the isolation of three potent mosquito-repellent

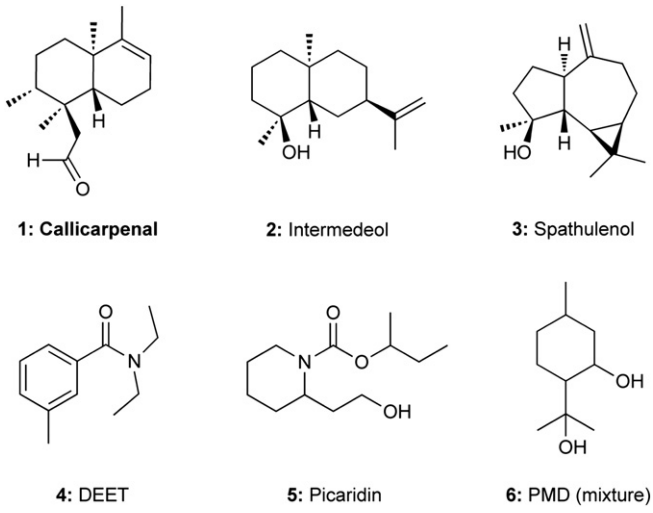

Fig. 1. Structures of various insect repellents.

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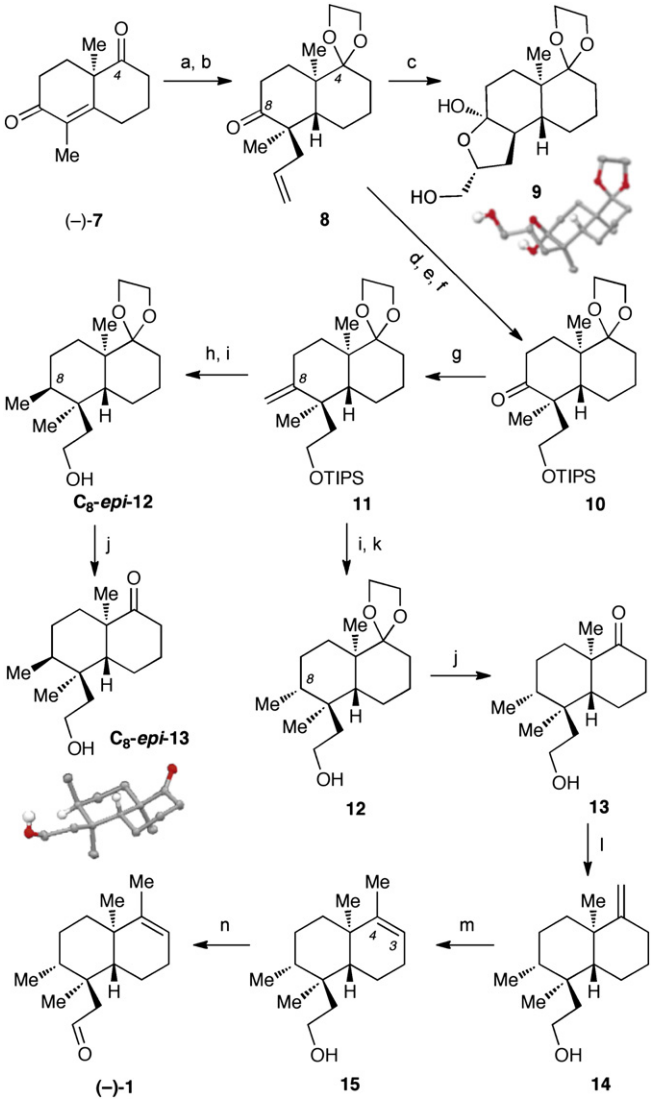
compounds: callicarpenal (**1**) and intermedeol (**2**), from the American plant, and spathulenol (**3**), from the Japanese plant (Fig. 1).

The low isolation yield of callicarpenal (0.05–0.15% from dry biomass) together with the tedious bulk isolation and purification techniques, restrict substantially the availability of this compound from natural sources. With this in mind, we report here an efficient stereoselective synthesis of (−)-callicarpenal. We also report initial mosquito deterrency studies of this natural product and various synthetic derivatives.[12]

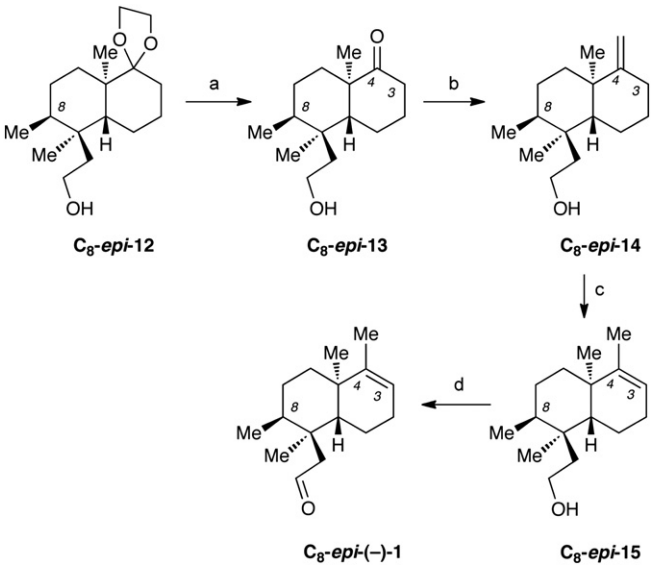
2. Results and discussion

Our synthesis of (−)-**1** departed from the readily available enantiomeric pure diketone (−)-**7** (ee >95%)[13] (Scheme 1). The more electrophilic C-4 carbonyl group of (−)-**7** was selectively protected with 1.2 equiv of ethylene glycol to form the corresponding C-4 ketal.[14] The C-8 enone was then reduced under lithium/ammonia conditions and the resulting enolate was alkylated in situ with allyl bromide to afford ketone **8** as a single isomer (70% over two steps). Conversion of **8** to silyl ether **10** was achieved in three steps: (a) ozonolysis of the terminal alkene followed by reduction of both carbonyl groups; (b) selective silylation of the primary alcohol; and (c) oxidation of the remaining C-8 hydroxyl group. The latter step was performed using catalytic amount of TPAP (3.5 mol %) and NMO as the co-oxidant (88% for three steps).[15] We also attempted to replace the ozonolysis reaction with a sequence of alkene dihydroxylation followed by oxidative cleavage of the resulting 1,2-diol. However, treatment of **8** with OsO_4/NMO gave rise to hemiacetal **9** complicating subsequent functionalization strategies. The chemical structure of **9** was unambiguously confirmed via a single crystal X-ray analysis.[16]

We then turned our attention to the installation of the C-8 methyl group. To this end, ketone **10** was converted to alkene **11** under standard Wittig conditions (91% yield). Hydrogenation of **11** under the standard Pd/C-catalyzed conditions proceeded predominantly from the sterically more accessible bottom face of the alkene forming the C_8-*epi*-**12** as the major product (94%, dr=10:1.) The stereochemistry of the newly formed C-8 center was confirmed initially by comparing the NMR data of both epimers to those reported in the literature[17] and ultimately by a single crystal X-ray analysis[16] of the undesired fully deprotected epimer C_8-*epi*-**13**. In order to produce the desired C-8 epimer, alkene **11** was initially desilylated with TBAF and subsequently reduced under Pd/C conditions. This sequence afforded the desired compound **13** as the major product (97%, dr=8:1). Importantly, treatment of **11** with Crabtree's catalyst[18] (3.5 mol %) under H_2 atmosphere (70 psi) gave even higher diastereoselectivity (dr >99:1). Obviously, the better interaction between the iridium compound and the free alcohol exclusively delivered the hydrogen from the same face of the molecule as the alcohol.[19] The acetonide was subsequently removed under acidic conditions to form ketone **13** that, after Wittig olefination, produced alkene **14** (92% for two steps). Isomerization of the exocyclic double bond of **14** was achieved with $RuCl_3$[20] under refluxing ethanolic conditions to form the desired *endo*-alkene **15**



Scheme 1. Reagents and conditions: (a) $(CH_2OH)_2$ (1.2 equiv), *p*-TsOH, PhH, 80 °C, 24 h (90%); (b) Li/NH_3, THF, −78 to −30 °C, 2 h, then CH_2=$CHCH_2Br$, 2 h (78%); (c) OsO_4 (1 mol %), NMO, acetone/H_2O (3:1), rt, 12 h (95%); (d) O_3, THF, −78 °C, then PPh_3, 2 h, then 3.0 equiv $NaBH_4$, 0 °C, 2 h; (e) 1.0 equiv TIPSOTf, 2,6-lutidine, CH_2Cl_2, −80 °C, 1 h; (f) TPAP (3.5 mol %), NMO, CH_2Cl_2, 18 h (88% for three steps); (g) CH_3PPh_3Br, NaHMDS, THF, 0 °C to rt, 16 h (91%); (h) Pd/C, H_2, rt, 12 h (99%, dr=10:1); (i) TBAF, THF, 15 h (94%); (j) 0.1 N HCl, THF, 8 h (97% for **13**, 93% for C_8-*epi*-**13**); (k) H_2, Crabtree's catalyst ([Ir(COD)(PCy_3)(Py)]PF_6, 3.5 mol %), CH_2Cl_2, 70 psi, 15 h (94%, dr >99:1); (l) CH_3PPh_3Br, NaHMDS, THF, 0 °C to rt, 18 h (95%); (m) $RuCl_3\cdot H_2O$, EtOH, reflux, 18 h (97%); (n) TPAP (20 mol %), NMO, CH_2Cl_2, 15 h (76%).



Scheme 2. Reagents and conditions: (a) 0.1 N HCl, THF, 8 h (93%); (b) CH_3PPh_3Br, NaHMDS, THF, 0 °C to rt, 18 h (91%); (c) $RuCl_3\cdot H_2O$, EtOH, reflux, 18 h (89%); (d) TPAP (20 mol %), NMO, CH_2Cl_2, 15 h (90%).

Table 1
Mosquito biting-deterrent effects of (−)-callicarpenal (**1**) and its analogues **13**, **14**, and **15** against 7–10 days old adult females of *Ae. aegypti*

Treatment (n=100)	Amount applied (nmoles/cm^2)	Mean proportion not biting (SE)
DEET	25	0.91 (0.029)a
(−)-Callicarpenal (**1**)	25	0.72 (0.045)b
13	25	0.61 (0.049)c
14	25	0.61 (0.049)c
15	25	0.64 (0.048)c
Ethanol	—	0.22 (0.041)d

n=Number of females tested against each treatment.
SE=Standard error of the mean.
Means within a column not followed by the same letter are significantly different (P<0.05, DMRT).

in 97% yield.[12e,21] Oxidation of **15** was achieved with TPAP and NMO and produced callicarpenal (−)-**1** in 76% yield. The analytical and spectroscopic data (see the Experimental section) of our synthetic (−)-**1** perfectly matched the reported data of the natural product.[22]

To evaluate the effect of the C8 stereocenter on the insect-repellent activities of callicarpenal, we synthesized C$_8$-*epi*-(−)-**1**. Scheme 2 summarizes our approach.

The aldehyde functionality of (−)-callicarpenal (**1**) could give rise to long-term stability issues, thereby limiting its applications. To overcome such potential limitations we sought to compare its bioactivity to that of its synthetic precursors. Along these lines, analogues **13**, **14**, and **15** were chosen due to their availability and structural similarity with **1**. All these compounds were evaluated for mosquito biting-deterrent effects against 7–10 days old adult females of *Aedes aegypti* (Table 1). Analogues were tested side-by-side against DEET and **1**. All of the compounds tested were more effective than ethanol control at 25 nmol/cm^2. DEET was more effective than **1** and all of its analogues **13**, **14**, and **15**. Reduction of the aldehyde functionality of **1** to the corresponding alcohol decreased considerably the activity of the natural product. On the other hand, isomerization of the C4 double bond to the C-3,4 endocyclic position appears to improve marginally the compound potency. These results are in agreement with previous observations.[23]

The C-8 epimers of callicarpenal and some callicarpenal analogues, synthesized above from the *trans*-epimer of compound **12**, were also evaluated for mosquito biting-deterrent effects against 7–10 days old adult females of *Ae. aegypti* (Table 2). Analogues **C$_8$-*epi*-13**, **C$_8$-*epi*-14**, **C$_8$-*epi*-15**, and **C$_8$-*epi*-(−)-1** were tested side-by-side against **1** and ethanol control. Again, all of the compounds tested were more effective than ethanol control at 25 nM/cm^2. Callicarpenal (**1**) was more effective than **C$_8$-*epi*-13**, **C$_8$-*epi*-14**, and **C$_8$-*epi*-15**; while **C$_8$-*epi*-(−)-1** was as effective as **1**.

Evaluation of the data shown in Tables 1 and 2 suggests that the relative stereochemistry at C8 does not play a significant role in determining the biological activity of the different epimers. Moreover, the functionalities at the C4 center do not appear to influence the bioactivity. These observations suggest that it is possible to further optimize the structure–activity of this natural product and develop exclusively synthetic analogues of callicarpenal with a simpler chemical motif, less lengthy synthesis, and optimized mosquito biting deterrency.

Table 2
Mosquito biting-deterrent effects of (−)-callicarpenal (**1**) and its analogues **C$_8$-*epi*-13**, **C$_8$-*epi*-14**, **C$_8$-*epi*-15**, and **C$_8$-*epi*-(−)-1** against 7–10 days old adult females of *Ae. aegypti*

Treatment (n=100)	Amount applied (nmoles/cm^2)	Mean proportion not biting (SE)
(−)-Callicarpenal (**1**)	25	0.78 (0.048)a
C$_8$-*epi*-13	25	0.56 (0.057)b
C$_8$-*epi*-14	25	0.56 (0.057)b
C$_8$-*epi*-15	25	0.61 (0.056)b
C$_8$-*epi*-(−)-1	25	0.76 (0.049)a
Ethanol	—	0.23 (0.049)c

n=Number of females tested against each treatment.
SE=Standard error of the mean.
Means within a column not followed by the same letter are significantly different (P<0.05, DMRT).

3. Conclusions

We have developed an efficient enantioselective synthesis of (−)-callicarpenal, a promising arthropod repellent. Key to the synthesis is the stereoselective reduction of C-8 alkene of **11** that depending on the conditions can deliver either epimer in excellent yield and diastereoselectivity. The synthesis of (−)-callicarpenal proceeds in 12 steps and 36% overall yield from diketone (−)-**7**. The synthetic strategy utilizes readily available materials and reagents making the overall approach amenable to scale-up. As such, it allows access to this natural product for further biological investigation and development. Moreover, mosquito biting-deterrent studies with callicarpenal and selected analogues suggest that the chemical structure of the parent molecule can be readily modified to improve its bioactivity.

4. Experimental section

4.1. General experimental procedures

Unless indicated, all commercial reagents were used as received without further purification. All non-aqueous reactions were carried out under argon atmosphere using dry glassware that had been flame-dried under a stream of argon unless otherwise noted. THF was dried over sodium/benzophenone, dichloromethane and MeOH over CaH_2. Et_3N was dry distilled from flame dried 4 Å powdered molecular sieves. Flash column chromatography was performed on silica gel (Merck Kieselgel 60, 230–400 mesh) using hexane/ether mixtures of increasing polarity. The progress of all the reactions was monitored by thin-layer chromatography (TLC) using glass plates precoated with silica gel-60 F_{254} to a thickness of 0.5 mm (Merck). ^{13}C NMR and ^{1}H NMR spectra were recorded on either a 400 MHz Varian instrument or a 500 MHz JEOL instrument. $CDCl_3$ was treated with flame dried K_2CO_3, chemical shifts (δ) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak ($CHCl_3$), with the abbreviations s, br s, d, t, q, and m denoting singlet, broad singlet, doublet, triplet, quartet, and multiplet, respectively. J=coupling constants given in Hertz (Hz). High resolution Mass spectra (HRMS) were recorded on a trisector WG AutoSpecQ spectrometer. Optical rotation data were collected on a Jasco P-1010 polarimeter using HPLC grade $CHCl_3$ (dried over molecular sieves).

*4.1.1. (R)-5,8a-Dimethyl-3,4,8,8a-tetrahydronaphthalene-1,6(2H,7H)-dione ((−)-**7**).* To a solution of 2-methyl-1,3-cyclohexadione (0.90 g, 7.17 mmol) in ethyl acetate (50 ml) were added triethylamine (1.30 ml, 9.32 mmol) and ethyl vinyl ketone (0.78 ml, 7.89 mmol). The reaction mixture was heated at 70 °C for 10 h and then cooled to 25 °C. The solvent was removed under reduced pressure and the resulting crude material was chromatographed (silica, 10% ether in hexanes) to yield the corresponding triketone (1.45 g, 6.81 mmol, 95%). A solution of the triketone (1.45 g, 6.81 mmol), L-phenylalanine (1.13 g, 6.81 mmol), and D-camphorsulfonic acid (0.79 g, 3.40 mmol) in DMF (100 ml) was stirred at rt under argon atmosphere overnight. Then the mixture was heated at 30 °C for 24 h, and the temperature was raised in 10 °C intervals every 24 h during 4 days. After the mixture was stirred at 70 °C for 24 h, the oil bath was removed in order to let the reaction mixture cool down to rt. The resulting solution was then poured into cold aqueous $NaHCO_3$, extracted with ether (2×50 ml), then dried over $MgSO_4$. Evaporation of ether followed by column

chromatography (silica, 0–20% ether in hexanes) of the residue afforded the crude enone **7** as a viscous oil, which crystallized on standing at −30 °C. Recrystallization from *n*-hexane in an ice bath gave (−)-**7** (1.05 g, 5.38 mmol, 79%) as a colorless long needles; mp: 46–48 °C; R_f=0.46 (35% ether in hexanes); $[\alpha]_{D^{25}}$ +124.0 (*c* 1.0, benzene); IR (film): ν 2952, 1709, 1665, 1610, 1352, 1008; ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.92–2.81 (m, 1H), 2.73–2.64 (m, 1H), 2.55–2.37 (m, 4H), 2.18–2.02 (m, 4H), 1.81 (d, *J*=1.2 Hz, 3H), 1.42 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 212.0, 197.5, 158.1, 130.7, 50.6, 37.3, 33.3, 29.5, 27.2, 23.3, 21.5, 11.2; HRMS (ESI): calcd for $C_{12}H_{17}O_2$ $[M+H]^+$ 193.1228, found 193.1224.

*4.1.2. (4a′R,5′R,8a'R)-5′-Allyl-5′,8a′-dimethylhexahydro-2′H-spiro [[1,3]dioxolane-2,1′-naphthalen]-6′(7′H)-one (**8**).* A solution of enone (−)-**7** (1.05 g, 5.38 mmol) and *p*-toluenesulfonic acid (0.20 g, 1.08 mmol) in benzene (100 ml) was treated with ethylene glycol (0.36 ml, 6.46 mmol), then heated at 80 °C for 24 h under an atmosphere of argon. After cooling in an ice bath, the resulting solution was poured into aqueous $NaHCO_3$ and the mixture was extracted with ether (2×50 ml). The combined extracts were washed with water (20 ml) and brine (20 ml) and dried over $MgSO_4$. Evaporation of the solvent followed by flash column chromatography (silica, 0–10% ether in hexanes) yielded the corresponding ketal (1.1 g, 4.84 mmol, 90%) as a colorless liquid. To a solution of lithium (0.15 g, 21.15 mmol) in liquid ammonia (100 ml) at −78 °C was added dropwise a solution of the ketal obtained above (0.98 g, 4.23 mmol) in THF (2 ml). After reflux for 1 h at −30 °C, water (76.2 μl, 4.23 mmol) was added dropwise. The solution was refluxed for another hour, then allyl bromide (1.83 ml, 21.15 mmol) was added as rapidly as possible, and the reaction mixture was heated at reflux for 2 h. The ammonia was evaporated and the reaction was quenched with saturated NH_4Cl solution (20 ml). The resulting mixture was then extracted with ether (3×30 ml) and the organic solutions were combined, dried over $MgSO_4$, concentrated, and subjected to column chromatography (silica, 0–10% ether in hexanes) to afford ketone **8** (0.91 g, 3.3 mmol, 78%) as a colorless liquid; R_f=0.63 (50% ether in hexanes); $[\alpha]_{D^{25}}$ −64.4 (*c* 1.0, CH_2Cl_2); IR (film): ν 2946, 2872, 1703, 1441, 1186, 1045, 910; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.66 (m, 1H), 5.03–4.96 (m, 2H), 3.96–3.83 (m, 4H), 2.57–2.50 (m, 2H), 2.33 (m, 1H), 2.13 (m, 1H), 2.01–1.90 (m, 2H), 1.70–1.31 (m, 7H), 1.20 (s, 3H), 1.03 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): 215.8, 135.2, 117.3, 112.8, 65.3, 64.8, 50.9, 44.1, 42.6, 42.5, 35.0, 30.3, 28.8, 22.6, 21.7, 21.3, 16.3; HRMS (ESI): calcd for $C_{17}H_{26}O_3Na$ $[M+Na]^+$ 301.3814, found 301.3827.

*4.1.3. (4a′R,5′R,8a′R)-5′,8a′-Dimethyl-5′-(2-((triisopropylsilyl) oxy) ethyl)hexahydro-2′H spiro[[1,3]dioxolane-2,1′-naphthalen]-6′(7′H)-one (**10**).* A solution of ketone **8** (12.0 g, 0.043 mol) in THF (50 ml) was exposed to ozone at −78 °C until the starting material was consumed. The resulting mixture was flushed with argon, treated with PPh_3 (22.5 g, 0.086 mol) and allowed to warm up to 25 °C. After stirring for 2 h, $NaBH_4$ (4.88 g, 0.129 mol) was added to the above reaction mixture at 0 °C, followed by MeOH (5 ml), then the reaction mixture was allowed to slowly warm up to 25 °C and stirred at this temperature for 2 h, then quenched with saturated brine (100 ml). The organic layer was extracted with ether (3×100 ml), combined and dried over $MgSO_4$, concentrated, and subjected to flash chromatography (silica, 50% ether in hexanes) to afford the corresponding diol (11.2 g, 0.039 mol, 92%). A solution of the above diol in CH_2Cl_2 (150 ml) was treated with 2,6-lutidine (6.81 ml, 0.058 mol) and triisopropylsilyl trifluoromethanesulfonate (12.6 ml, 0.047 mmol) at −78 °C. The reaction mixture was stirred for 1 h and then diluted with saturated aqueous $NaHCO_3$ (100 ml) and extracted with ether (3×100 ml). The combined organic extracts were dried over $MgSO_4$, concentrated, and subjected to flash chromatography (silica, 0–20% ether in hexanes) to afford the corresponding secondary alcohol (16.9 g, 0.038 mol, 98%). A solution of above alcohol (22 g, 0.0499 mol) in CH_2Cl_2 (200 ml) was added 4-methylmorpholine-*N*-oxide (17.5 g, 0.149 mol) followed by tetrapropylammonium perruthenate (0.50 g, 1.42 mmol). The reaction mixture was stirred at 25 °C for 18 h, then directly concentrated and subjected to flash chromatography (silica, 0–5% ether in hexanes) to afford ketone **10** (21.5 g, 0.049 mol, 98%) as a colorless liquid; R_f=0.58 (25% ether in hexanes); $[\alpha]_{D^{25}}$ −62.3 (*c* 0.7, CH_2Cl_2); IR (film): ν 2939, 2865, 1703, 1461, 1098, 884, 682; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.96–3.92 (m, 2H), 3.90–3.84 (m, 2H), 3.64 (m, 1H), 3.57 (m, 1H), 2.48 (m, 1H), 2.39 (m, 1H), 2.16 (dd, *J*=12.0, 3.0 Hz, 1H), 2.07 (m, 1H), 1.99 (m, 1H), 1.71–1.40 (m, 8H, overlapped with water peak), 1.16 (s, 3H), 1.04 (m, 21H), 1.02 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ (C_8 peak is too weak to observe) 112.8, 65.0, 64.8, 59.9, 49.4, 44.6, 42.3, 41.0, 34.7, 30.1, 28.7, 22.6, 21.8, 21.6, 17.9 (6C), 16.3, 11.8 (3C); HRMS (ESI): calcd for $C_{25}H_{46}O_4SiNa$ $[M+Na]^+$ 461.3066, found 461.3068.

*4.1.4. (2-((4a′R,5′R,8a′R)-5′,8a′-Dimethyl-6′-methyleneoctahydro-2′H-spiro[[1,3]dioxolane-2,1′-naphthalen]-5′-yl)ethoxy) triisopropylsilane (**11**).* A solution of methyltriphenylphosphonium bromide (37.0 g, 0.068 mol) in THF (400 ml) was treated dropwise with sodium bis(trimethylsilyl)amide (90.0 ml of a 1.0 M solution in THF, 0.09 mol) at 0 °C and stirred at this temperature for 30 min. The resulting orange solution was treated dropwise with a solution of ketone **10** (30.0 g, 0.068 mmol) in THF (50 ml) at 0 °C. After stirring at 65 °C for 15 h, the mixture was quenched with saturated aqueous $NaHCO_3$ (500 ml), diluted with ether (500 ml), washed with brine (3×300 ml). The organic solution was dried over $MgSO_4$, concentrated, then subjected to flash chromatography (silica, 0–5% ether in hexanes) to afford alkene **11** (27.0 g, 0.062 mol, 91%) as a colorless liquid; R_f=0.74 (25% ether in hexanes); $[\alpha]_{D^{25}}$ +75.1 (*c* 1.0, CH_2Cl_2); IR (film): ν 2939, 2865, 1461, 1085, 1072, 884; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.74 (s, 1H), 4.69 (s, 1H), 3.92–3.88 (m, 3H), 3.82 (m, 1H), 3.72 (m, 1H), 3.58 (m, 1H), 2.33 (m, 1H), 2.14 (m, 1H), 1.83–1.71 (m, 2H), 1.67–1.31 (m, 11H), 1.24 (s, 1H), 1.11 (s, 3H), 1.04 (m, 18H), 0.96 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 154.4, 113.3, 106.3, 65.1, 64.7, 59.5, 44.9, 43.3, 41.9, 41.5, 31.4, 30.2, 29.4, 23.7, 22.8, 21.4, 18.0 (6C), 17.7, 11.9 (3C); HRMS (ESI): calcd for $C_{26}H_{48}O_3SiCs$ $[M+Cs]^+$ 569.2429, found 569.2432.

*4.1.5. 2-((4a′R,5′S,6′R,8a′R)-5′,6′,8a′-Trimethyloctahydro-2′H-spiro [[1,3]dioxolane-2,1′-naphthalen]-5′-yl)ethanol (**12**).* Olefin **11** (1.1 g, 2.52 mmol) in THF (20 ml) and was treated with TBAF (1.0 M in THF, 7.56 ml, 7.56 mmol) at 25 °C for 15 h. Then the reaction was quenched with water (50 ml) and extracted with ether (3×50 ml). The organic layers were dried over $MgSO_4$, concentrated and chromatographed (silica, 10% ether in hexanes) to afford the corresponding alcohol (0.664 g, 2.37 mmol, 94%). This alcohol (0.30 g, 1.07 mmol) and the Crabtree's catalyst ($[Ir(COD)(PCy_3)(Py)]PF_6$, 30 mg, 0.037 mmol) in 30 ml of CH_2Cl_2 was hydrogenated in a Parr apparatus (70 psi) for 15 h at 70 psi. The catalyst was then removed by a short silica pad to afford the reduced product **12** (286 mg, 1.01 mmol) as a single diastereomer at the C8 center (dr >99:1, 94%); $[\alpha]_{D^{25}}$ +52.1 (*c* 1.0, CH_2Cl_2); IR (film): ν 3382, 2939, 2872, 1710, 1448, 1387, 1139, 1051; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.93–3.88 (m, 3H), 3.83–3.79 (m, 1H), 3.69–3.58 (m, 2H), 1.68–1.29 (m, 15H), 1.02 (s, 3H), 0.83 (d, *J*=6.5 Hz, 3H), 0.70 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 113.4, 65.1, 64.6, 58.1, 44.4, 43.4, 40.5, 38.4, 37.1, 30.2, 30.0, 26.7, 22.7, 20.4, 17.6, 16.9, 16.0; HRMS (ESI): calcd for $C_{17}H_{31}O_3$ $[M+H]^+$ 283.2275, found 283.2273.

*4.1.6. (4aR,5S,6R,8aR)-5-(2-Hydroxyethyl)-5,6,8a-trimethylocta hydronaphthalen-1(2H)-one (**13**).* To a solution of ketal **12** (10.0 g, 0.035 mol) in THF (100 ml) was added hydrochloric acid (10.0 ml, 0.1 N). The reaction was stirred at rt for 8 h, then neutralized with saturated aqueous $NaHCO_3$ (150 ml). The two layers were separated and the aqueous layer was extracted with ether (3×200 ml). The organic solutions were combined, dried over $MgSO_4$, concentrated,

and subjected to flash chromatography (silica, 10–50% ether in hexanes) to afford hydroxy ketone **13** (8.10 g, 0.034 mmol, 97%) as a white solid; R_f=0.25 (50% ether in hexanes); $[\alpha]_{D^{25}}$ +14.5 (*c* 1.0, benzene); IR (film): ν 3441, 2938, 1702, 1455, 1381, 1257, 1135, 1027, 952; ^{1}H NMR (500 MHz, $CDCl_3$): δ 3.40–3.70 (m, 2H), 2.01–2.60 (m, 3H), 1.05–1.95 (m, 12H), 1.12 (s, 3H), 0.85 (d, 3H, *J*=4.80 Hz), 0.81 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 215.8, 57.9, 49.3, 48.9, 40.6, 39.6, 37.4, 37.1, 32.9, 26.5, 26.0, 20.8, 19.1, 18.1, 16.1; HRMS (ESI): calcd for $C_{15}H_{27}O_2$ $[M+H]^+$ 239.2011, found 239.2030.

*4.1.7. 2-((1S,2R,4aR,8aR)-1,2,4a-Trimethyl-5-methylenedeca hydronaphthalen-1-yl)ethanol (**14**).* To a suspension of methyltriphenylphosphonium bromide (16.60 g, 46.6 mmol) in THF (300 ml) was added dropwise sodium bis-trimethylsilyl amide (1.0 M in THF, 38.80 ml, 38.80 mmol) and the suspension was stirred at 0 °C for 30 min. Hydroxy ketone **13** (3.70 g, 15.50 mmol) in THF (50.0 ml) was then added to the above reaction and the mixture and stirred at 25 °C for 18 h. The reaction was quenched with aqueous saturated $NaHCO_3$ (400 ml) and the mixture was extracted with ethyl ether (3×400 ml). The organic layers were collected, dried over $MgSO_4$, concentrated, and chromatographed (silica, 10–20% ether in hexane) to afford olefin **14** (3.50 g, 14.80 mmol, 95%) as a white solid; R_f=0.30 (50% ether in hexane); $[\alpha]_{D^{25}}$ +56.2 (*c* 1.0, benzene); IR (film): ν 3321, 2931, 2859, 1633, 1447, 1379, 1026, 889; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.49 (d, 2H, *J*=1.60 Hz), 3.40–3.70 (m, 2H), 1.84–2.40 (m, 3H), 1.05–1.80 (m, 13H), 1.03 (s, 3H), 0.86 (d, 3H, *J*=6.40 Hz), 0.74 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 160.2, 102.5, 58.7, 49.8, 40.9, 40.2, 39.4, 37.8, 37.3, 33.1, 28.7, 27.6, 22.1, 21.0, 18.0, 16.4; HRMS (ESI): calcd for $C_{16}H_{29}O$ $[M+H]^+$ 237.2218, found 237.2221.

*4.1.8. 2-((1S,2R,4aR,8aR)-1,2,4a,5-Tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)ethanol (**15**).* To a solution of olefin **14** (0.72 g, 3.05 mmol) in ethanol (30 ml) was added Rhodium (III) chloride hydrate (0.14 g, 0.67 mmol) and the mixture was refluxed for 18 h. The reaction mixture was cooled to 25 °C, concentrated, and chromatographed (10–20% ether in hexanes) to afford alcohol **15** (0.70 g, 2.96 mmol, 97%) as a clear oil; R_f=0.30 (50% ether in hexanes); $[\alpha]_{D^{25}}$ −39.7 (*c* 1.0, benzene); IR (film): ν 3329, 2939, 1743, 1266, 1174, 1134, 1028; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.17 (s, 1H), 3.60 (m, 2H), 1.85–2.20 (m, 2H), 1.02–1.90 (m, 14H), 0.99 (s, 3H), 0.86 (d, 3H, *J*=6.0 Hz), 0.73 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 143.9, 120.3, 58.2, 47.4, 40.8, 38.7, 38.2, 37.3, 36.7, 27.5, 26.8, 19.9, 18.6, 18.1, 17.9, 16.2; HRMS (ESI): calcd for $C_{16}H_{29}O$ $[M+H]^+$ 237.2218, found 237.2229.

*4.1.9. (−)-Callicarpenal ((−)-**1**).* A solution of alcohol **15** (40.0 mg, 0.169 mmol) in CH_2Cl_2 (5.0 ml) was added 4-methylmorpholine-*N*-oxide (39.6 mg, 0.338 mmol) followed by tetrapropylammonium perruthenate (11.90 mg, 0.034 mmol). The reaction mixture was stirred at 25 °C for 15 h, then directly concentrated and subjected to flash chromatography (silica, 0–5% ether in hexanes) to afford aldehyde (−)-**1** ((−)-callicarpenal) (30.0 mg, 0.128 mmol, 76%) as a colorless liquid; R_f=0.81 (25% ether in hexanes); $[\alpha]_{D^{25}}$ −75.0 (*c* 0.50, benzene); IR (film): ν 2938, 1710, 1450, 1385, 1055, 1010; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.83 (d, 1H, *J*=3.6 Hz), 5.17 (br s, 1H), 2.30–2.48 (m, 2H), 1.90–2.08 (m, 2H), 1.15–1.76 (m, 11H), 1.00 (s, 3H), 0.94 (d, 3H, *J*=6.8 Hz), 0.81 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 203.8, 143.6, 120.5, 51.7, 49.4, 41.7, 39.0, 38.4, 36.4, 27.3, 26.6, 19.9, 18.9, 17.9, 17.2, 16.3; HRMS (ESI): calcd for $C_{16}H_{27}O$ $[M+H]^+$ 235.2056, found 235.2059.

*4.1.10. 2-((4a′R,5′S,6′S,8a′R)-5′,6′,8a′-Trimethyloctahydro-2′H-spiro[[1,3]dioxolane-2,1′-naphthalen]-5′-yl)ethanol (C_8-epi-**12**).* Olefin **11** (110 mg, 0.25 mmol) and 10% Pd/C (11 mg) in MeOH was hydrogenated in a Parr apparatus (70 psi) for 12 h. The catalyst was then removed by a short silica pad, the corresponding reduced product was then concentrated and re-dissolved in THF (2 ml) and was treated with TBAF (1.0 M in THF, 0.75 ml, 0.75 mmol) at 25 °C for 12 h. Then the reaction was quenched with water (5 ml) and extracted with ether (3×10 ml). The organic layers were dried over $MgSO_4$, concentrated, and chromatographed (silica, 10% ether in hexanes) to afford the corresponding alcohol (67 mg, 0.24 mmol, 94%). Colorless liquid; R_f=0.33 (silica, 70% ether in hexanes); $[\alpha]_{D^{25}}$ +16.8 (*c* 2.2, CH_2Cl_2); IR (film) ν 3355, 2952, 2878, 1454, 1381, 1186, 1031, 937; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.94 (m, 3H), 3.81 (m, 1H), 3.68 (m, 2H), 1.89–1.22 (m, 15H), 1.07 (s, 3H), 0.98 (d, *J*=7.0 Hz, 3H), 0.93 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 113.8, 65.5, 65.0, 59.4, 43.9, 43.1, 42.7, 37.5, 36.1, 30.5, 25.0, 23.7, 23.2, 21.3, 20.1, 17.7, 15.0; HRMS (ESI): calcd for $C_{17}H_{31}O_3$ $[M+H]^+$ 283.2275, found 283.2271.

*4.1.11. (4aR,5S,6S,8aR)-5-(2-Hydroxyethyl)-5,6,8a-trimethylocta hydronaphthalen-1(2H)-one (C_8-epi-**13**).* See Section 4.1.6; white solid, 93% yield; mp: 95–98 °C; R_f=0.49 (silica, 80% ethyl ether in hexanes); $[\alpha]_{D^{25}}$ +44.7 (*c* 1.0, benzene); IR (film): ν 3445, 2935, 1701, 1455, 1381, 1255, 1136, 1026, 950; ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.62–3.76 (m, 2H), 2.52–2.61 (m, 1H), 2.15–2.22 (m, 1H), 2.02–2.10 (m, 1H), 1.84–1.89 (m, 2H), 1.22–1.68 (m, 10H), 1.17 (s, 3H), 1.03 (s, 3H), 0.93 (d, 3H, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 216.0, 58.4, 49.2, 47.0, 42.4, 38.3, 37.3, 35.5, 26.1, 26.1, 24.5, 21.5, 20.2, 19.6, 14.6; HRMS (ESI): calcd for $C_{15}H_{27}O_2$ $[M+H]^+$ 239.2011, found 239.2030.

*4.1.12. 2-((1S,2S,4aR,8aR)-1,2,4a-Trimethyl-5-methylenedeca hydronaphthalen-1-yl)ethanol (C_8-epi-**14**).* See Section 4.1.7; white solid, 91% yield; mp: 53–57 °C; R_f=0.62 (silica, 80% ethyl ether in hexane); $[\alpha]_{D^{25}}$ +78.0 (*c* 0.33, benzene); IR (film): ν 3323, 2932, 2856, 1632, 1445, 1372, 1026, 890; ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.53 (br s, 1H), 4.49 (br s, 1H), 3.62–3.75 (m, 2H), 1.02–2.36 (m, 15H), 1.08 (s, 3H), 0.99 (s, 3H), 0.97 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.4, 102.1, 59.5, 47.9, 42.8, 40.3, 38.1, 36.2, 33.0, 30.1, 28.8, 25.8, 21.7, 21.6, 21.5, 15.3; HRMS (ESI): calcd for $C_{16}H_{29}O$ $[M+H]^+$ 237.2218, found 237.2221.

*4.1.13. 2-((1S,2S,4aR,8aR)-1,2,4a,5-Tetramethyl-1,2,3,4,4a,7,8,8a-octahydronaphthalen-1-yl)ethanol (C_8-epi-**15**).* See Section 4.1.8; clear oil, 92% yield; R_f=0.60 (silica, 80% ethyl ether in hexane); $[\alpha]_{D^{25}}$ −28.8 (*c* 0.50, benzene); IR (film): ν 3320, 2942, 1741, 1269, 1179, 1131, 1028; ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.17 (br s, 1H), 3.68–3.79 (m, 2H), 1.25–2.09 (m, 16H), 1.03 (s, 3H), 0.97 (d, 3H, *J*=6.8 Hz), 0.96 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 144.2, 120.2, 59.5, 45.1, 42.8, 38.4, 37.6, 36.2, 30.2, 27.1, 25.9, 21.4, 20.9, 18.3, 18.2, 15.3; HRMS (ESI): calcd for $C_{16}H_{29}O$ $[M+H]^+$ 237.2218, found 237.2229.

*4.1.14. C_8-epi-(−)-Callicarpenal (C_8-epi-(−)-**1**).* See Section 4.1.9; clear oil, 71% yield; R_f=0.85 (silica, 25% ether in hexanes); $[\alpha]_{D^{25}}$ −36.4 (*c* 1.0, benzene); IR (film): ν 2933, 1710, 1460, 1381, 1060, 1000; ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.91 (d, 1H, *J*=3.6 Hz), 5.16 (br s, 1H), 2.18–2.40 (m, 2H), 1.80–2.10 (m, 3H), 1.20–1.58 (m, 10H), 1.18 (s, 3H), 1.03 (s, 3H), 1.01 (d, 3H, *J*=6.8 Hz); ^{13}C NMR (100 MHz, $CDCl_3$): δ 204.4, 144.2, 120.5, 54.0, 44.5, 39.8, 38.7, 36.7, 30.2, 26.7, 26.0, 22.6, 20.6, 18.6, 18.1, 15.8; HRMS (ESI): calcd for $C_{16}H_{27}O$ $[M+H]^+$ 235.2056, found 235.2049.

4.2. Biological experimental procedures

4.2.1. Insects. Ae. aegypti (L.) used in these studies were from a laboratory colony maintained at Mosquito and Fly Research Unit at Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, Gainesville, Florida. This colony has been maintained since 1952 using standard procedures.[24] We received the eggs and stored these in our laboratory to use as needed. Mosquitoes were reared to the adult stage by feeding the larvae on a diet of 2:1 alfalfa pellets (US Nutrition Inc. Bohemia, NY) and hog chow (Ware Milling 150 ALF Drive,

Houston, MS 38851). The diet contents were ground in a grinder and passed through sieve no. 40, 425 μm (USA Standard Sieve, Humboldt MFG. Co. Norridge, IL 60706). Eggs were hatched under vacuum (≈1 h) by placing a piece of a paper towel with eggs in a cup filled with 50 ml de-ionized water containing a small quantity of larval diet. Larvae were removed from vacuum and held overnight in the cup. These larvae were then transferred into 500 ml cups (about 50 larvae per cup) filled with de-ionized water. Larval diet was added every day until pupation and the insects were kept in an environment controlled room. Both the larvae and adults were maintained at a temperature of 27 °C±2 °C and 70±5% RH in a photoperiod regimen of 12:12 (L:D) h. The adults were fed on 10% sucrose solution. Cotton pads moistened with the sucrose solution were placed on the top of screens of 4 L cages. 5–9 days old mated females were used in these bioassays. Females were deprived of any food for 24 h prior to the test. Timings of these tests were centered around 1200 h.

4.2.2. Chemicals. CPDA-1[25] was prepared by dissolving 3.33 g sodium citrate, 0.376 g citric acid, 4.02 g dextrose, 0.28 g monobasic sodium phosphate (Fisher Scientific Chemical Co. Fairlawn, NJ), and 0.346 g of adenine (Sigma–Aldrich, St. Louis, MO) in 1026 ml of de-ionized water. ATP was added to CPDA-1 to yield 10^{-3} M ATP. CPDA-1 and ATP preparations were freshly mixed on the day of the test. CPDA-1+ATP is reported to be as attractive to mosquito females for feeding as blood.[26] DEET (*N*,*N*-diethyl-*m*-toluamide 97%) was obtained from Sigma–Aldrich (St. Louis, MO). Molecular biology grade ethanol was obtained from Fisher Scientific Chemical Co. (Fairlawn, NJ). (−)-Callicarpenal used as the positive control in bioassays was isolated in bulk as previously described.[23]

4.2.3. Mosquito biting bioassays. Experiments were conducted by using a six-celled in vitro Klun and Debboun (K and D) module bioassay system developed by Klun et al.[26] for quantitative evaluation of bite deterrent properties of candidate compounds for human use. This bioassay method determines specifically measured biting (feeding) deterrent properties of the chemicals. Briefly the assay system consists of a six well blood reservoir with each of the 3 cm×4 cm wells containing 6 ml of blood. As reported earlier,[26] female mosquitoes feed as well on the CPDA-1+ATP as they do on blood. Therefore, we used the CPDA-1+ATP instead of blood. The temperature of the solution in the reservoir was maintained at 37 °C by continuously passing the warm water through the reservoir using a circulatory bath. The reservoirs were covered with a layer of collagen membrane (Devro, Sandy Run, SC). This blood membrane unit simulated a human host for mosquito feeding. The test compounds were randomly applied to six 4 cm×5 cm areas of organdy cloth (G Street Fabrics, Rockville, MD) and positioned over the membrane-covered CPDA-1+ATP solution with a separator placed between the treated cloth and the six-celled module. A six-celled K and D module containing five females per cell was positioned over cloth treatments covering the six blood membrane wells, and trap doors were opened to expose the treatments to these females. After a 3 min exposure, the number of mosquitoes biting through cloth treatments in each cell was recorded and mosquitoes were prodded back into the cells. These mosquitoes were then squashed to determine the number, which has actually engorged the solution. A replicate consisted of six treatments: four test compounds, DEET (a standard bite deterrent compound) or callicarpenal and 95% ethanol-treated cloth as the control. The 25 nmoles DEET/cm^2 cloth dose was used as a standard, because it suppresses mosquito biting by 80% as compared to controls.[27] A set of replications were conducted on different days using new lots of mosquitoes.[28]

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Supplementary data

^{1}H NMR and ^{13}C NMR spectra for compounds **8**, **10**, **11**–**15**, (−)-**1**, **C_8-*epi*-12**, **C_8-*epi*-13**, **C_8-*epi*-14**, **C_8-*epi*-15**, **C_8-*epi*-(−)-1** as well as X-ray data of compounds **9** and **C_8-*epi*-13**. Supplementary data associated with this article can be found in online version at doi:10.1016/j.tet.2011.02.078. These data include MOL files and InChIKeys of the most important compounds described in this article.

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28. Since we are dealing with an assay on a whole organism we use statistics as a tool to explain the variation in data. These bioassays have been replicated 20 times, which is sufficient for conclusive data. For instance, increase in means from 0.6 to 0.7 is quite substantial in terms of anti-biting effects.