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Transformation of Presumptive Precursors to Frontalin and *exo*-Brevicomin by Bark Beetles and the West Indian Sugarcane Weevil (Coleoptera)

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Abstract—(*Z*)-6-Nonen-2-one (**1**) has recently been shown to be the biosynthetic precursor for the aggregation pheromone *exo*-brevicomin (**2**) in mountain pine beetle (MPB) males, *Dendroctonus ponderosae* (Hopkins). We tested the hypotheses that (1) 6-methyl-6-hepten-2-one (**3**) is the biosynthetic precursor for the aggregation pheromone frontalin (**4**) in the spruce beetle (SB), *Dendroctonus rufipennis* (Kirby), and (2) that frontalin and *exo*-brevicomin are produced from **3** and **1**, respectively, only by beetles that utilize them as aggregation pheromones. Exposure of scolytids MPB, SB, pine engraver (PE), *Ips pini* (Say) and *Ips tridens* (Mannerheim) and West Indian sugar cane weevil (WISW), *Metamasius hemipterus sericeus* (Olivier) to deuterio- or protio-**3** invariably resulted in the production of deuterio- or protio-**4**. Similarly, exposure of SB, WISW and *I. tridens* to **1** resulted in the production of **2**. We were unable to demonstrate the presence of **3** in SB volatiles, nor were we able to demonstrate the conversion of 6-methyl-5-hepten-2-one to **3** by SB. Production of enantiomerically enriched frontalin and *exo*-brevicomin by all the beetles exposed to respective precursors reveals widespread occurrence of nonspecific polysubstrate monooxidases in the Coleoptera. Copyright © 1996 Elsevier Science Ltd

Introduction

Pheromone biosynthesis in the Coleoptera is thought to proceed via transformation of host plant volatiles or de novo.[1] Evidence for the conversion of plant-derived terpenes to pheromones is widespread. Exposure of *Dendroctonus* beetles to α-pinene results in the production of *trans*-verbenol,[2] whereas *Ips* beetles convert oleoresin and myrcene to, respectively, verbenols, and ipsdienol and ipsenol.[2a,b,3] In a recent study, however, the California fivespined ips, *Ips paraconfusus* (Lanier), produced large amounts of ipsdienol when feeding on phloem tissue with insignificant myrcene content, suggesting an alternative pathway for ipsdienol biosynthesis.[4]

De novo biosynthesis has been demonstrated for the cotton boll weevil (CBW), *Anthonomus grandis* (Boheman). CBW was originally hypothesized to produce its pheromone from host terpenes,[5] but Mitlin and Hedin[6] revealed CBW pheromone production also from radiolabeled acetate, mevalonate and glucose.

Although bicyclic acetals represent an important group of Coleopteran aggregation pheromones, detailed biosynthesis studies have only been conducted for *exo*- and *endo*-brevicomin. Vanderwel and co-workers[7] demonstrated that mountain pine beetle (MPB) males, *D. ponderosae* (Hopkins), converted (*Z*)-6-nonen-2-one (**1**) to (+)-*exo*-brevicomin [(+)-**2**] by nonstereospecific oxidation of the olefin followed by enantioselective cyclization.

6-Methyl-6-hepten-2-one (**3**) has been suggested as a possible biosyntheic precursor for 1,5-dimethyl-6,8-dioxabicyclo[3.2.1]octane (frontalin, **4**; Brand et al.;[8] Vanderwel and Oehlschlager, 1987;[1c] Francke et al., 1995[a]). Frontalin is an important aggregation pheromone of many *Dendroctonus* beetles, including southern pine beetle, *Dendroctonus frontalis* (Zimmerman)[10] and spruce beetle (SB), *Dendroctonus rufipennis* (Kirby).[11] 6-Methyl-6-hepten-2-one, possibly deriving from 6-methyl-5-hepten-2-one (sulcatone, **5**) or the corresponding alcohol sulcatol (**6**), may undergo epoxidation and cyclization to frontalin.

In this study, we tested the hypotheses (1) that 6-methyl-6-hepten-2-one is a precursor for frontalin biosynthesis in SB, and (2) that biosynthesis of frontalin and *exo*-brevicomin from, respectively, 6-methyl-6-hepten-2-one and (*Z*)-6-nonen-2-one are specific to beetles that utilize them as aggregation pheromones (Fig. 1).

Results and Discussion

Exposure of male and female SB to (4,4-$^2H_2$)-6-methyl-6-hepten-2-one (**3**-$^2H_2$) (Exp. 1, Table 1), invariably resulted in the production of $^2H_2$-frontalin (**4**) (Fig. 2). The EIMS of beetle-produced **4** clearly indicated enrichment of two deuteriums (*m/z* 144, 114, 102) relative to nondeuterated **4** (*m/z* 142, 112, 100; Fig. 3). Male or female PE exposed to **3** (Exp. 2, Table 1) also produced **4**, as indicated by GC–MS and GC–MS–SIM analyses of beetle-produced volatiles. Further exposure of female MPB to **3**-$^2H_2$ (Exp. 3, Table 1),

and exposure of male and female *I. tridens* and WISW to **3** (Exps 4–5, Table 1), resulted in the production of deuterio- and protio-**4**, respectively.

Insects used in Exps 2–5 do not naturally produce **4**. Although **4** is a common aggregation pheromone in scolytids, it has never been reported for the genus *Ips*. Frontalin was also not detected in pheromone analyses of the curculionids WISW (unpublished data) and pineapple weevil, *Metamasius dimidiatipennis* (unpublished data). Production of **4** by *Ips* and *Metamasius* during exposure to **3** therefore represents a nonspecific and nonselective biotransformation. Similarly, neither *I. tridens*, WISW nor female MPB naturally produce *exo*-brevicomin (**2**), but did so (Fig. 2) when exposed to (*Z*)-6-nonen-2-one (**1**) (Exps 7–9, Table 1), the proposed precursor for *exo*-brevicomin (**2**) in male MPB.

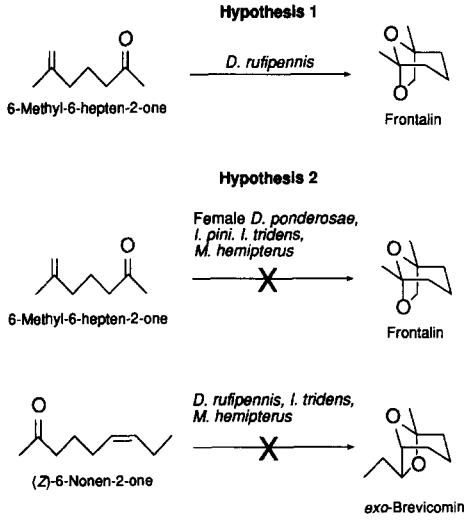

Figure 1. Presumptive precursor for frontalin biosynthesis by *D. rufipennis* (hypothesis 1) and unexpected production of frontalin and *exo*-brevicomin by *Ips* and *Metamasius* beetles (hypothesis 2).

All beetles exposed to **3**-$^2H_2$ or **3** produced **4** with slight enantiomeric excess (ee) [(−)-**4**, 22–30% ee in SB and (+)-**4**, 26–30% ee in WISW; Fig. 4]. These findings sharply contrast with the natural enantioselective production of (+)-**4** by female SB when boring into suitable spruce logs.[12] Similarly, exposed to **1** (Exps 7–9), beetles produced *exo*-brevicomin with no (WISW) or slight (SB: 56% ee, *I. tridens*: 27–30% ee) enantiomeric excess (Fig. 5). Production of enantiomerically enriched **2** from **1** or enantiomerically enriched **4** from **3** by all beetles tested (Figs 4 and 5) is likely mediated by common, nonspecific polysubstrate monooxidases.[13]

Hypothesizing that feeding in phloem tissue or exposure to tree volatiles may be required for induction of frontalin biosynthesis,[14] male and female SB were exposed to fresh spruce phloem disks together with **3**-$^2H_2$ (Exp. 10). However, even when feeding on phloem disks, beetles produced only deuterio-**4** with enantiomeric ratios comparable to those produced in the absence of phloem.

The presence of **3** in volatiles of male and female SB would have provided at least some evidence that **3** is part of the frontalin biosynthetic pathway. However, only isomeric 6-methyl-5-hepten-2-one (**5**) was detected in beetle-produced volatiles. Since **3** could conceivably derive from isomerization of **5**[8] or from oxidation and isomerization of 6-methyl-5-hepten-2-ol (**6**), beetles were exposed in separate experiments to **5** [SB (Exp. 11), PE (Exp. 12)] and **6** [SB (Exp. 13)]. Oxidation of **6** to **5** (Exp. 13) coupled with the lack of frontalin formation from **5** or **6** (Exps 11–13) indicates that neither **5** nor **6** are likely to be absorbed as direct dietary constituents during the formation of frontalin.

Table 1. Summary of exposure experiments with scolytid and curculionid beetles

Exp.	Insect	Precursor[a]	Number of exposed beetles		Number of control beetles		Number of beetles per replicate	
			Males	Females	Males	Females	Males	Females
1	*D. rufipennis*	**3**-$^2H_2$	16	16	16	6	4	4
2	*I. pini*	**3**	70	70	70	70	10	10
3	*D. ponderosae*	**3**-$^2H_2$		8		8		4
4	*I. tridens*	**3**	40	40	40	40	10	10
5	*M. hemipterus*	**3**	4	4	2	2	1	1
6	*D. rufipennis*	**3**	20	20	16	16	4	4
7	*I. tridens*	**1**	40	40	40	40	10	10
8	*M. hemipterus*	**1**	4	4	2	2	1	1
9	*D. rufipennis*	**1**	12	12	8	8	4	4
10	*D. rufipennis*[b]	**3**-$^2H_2$	16	16	4	4	4	4
11	*D. rufipennis*	**5**	24	24	24	24	4	4
12	*I. pini*	**5**	50	50	50	50	10	10
13	*D. rufipennis*	**6**	24	24	4	4	4	4

[a]**3**-$^2H_2$ = (4,4-$^2H_2$)-6-Methyl-6-hepten-2-one; **3** = 6-methyl-6-hepten-2-one; **1** = (*Z*)-6-nonen-2-one; **5** = 6-methyl-5-hepten-2-one; **6** = 6-methyl-5-hepten-5-ol.
[b]Beetles were exposed in the presence of fresh Engelmann spruce phloem.

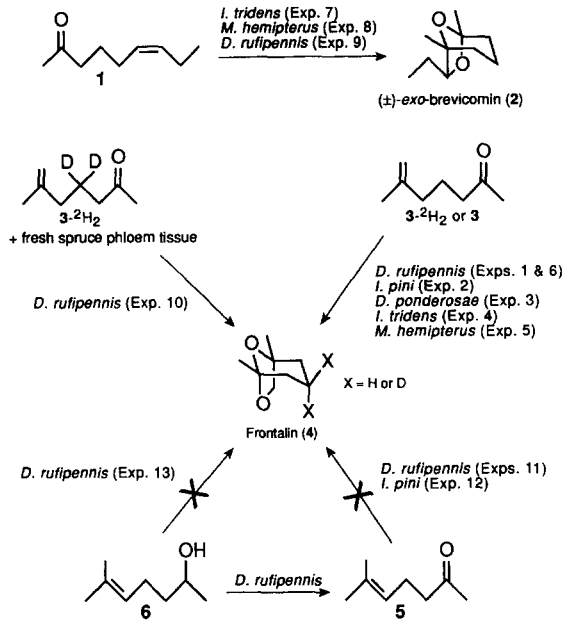

Figure 2. Summary of exposure experiments with scolytid and curculionid beetles.

Enantioselective conversion of **1** to (+)-*exo*-brevicomin by male MPB[7] suggests that **1** may be a natural precursor. Failure to demonstrate enantioselective conversion of **3** to **4** by SB in this study does not eliminate the possibility that **3** is a natural precursor of **4**. During exposure experiments, **3** may have overloaded the pheromonal biosynthetic apparatus and nonspecific oxidases may have operated to convert excess **3** to (±)-**4**. Although we attempted to avoid 'overloading' by employing the same amount of **3** (0.1 μL), as used for **1** in the MPB pheromone biosynthesis study,[7] 0.1 μL of precursor in SB may still have exceeded natural levels. It is also possible that under experimental conditions the pheromonal biosynthetic apparatus in SB was not triggered. Beetles were naturally feeding while exposed to **3**, but lacked a preceding dispersal flight which may be a prerequisite for pheromone production in SB (flight and starvation of male PE prior to myrcene exposure, however, reduced rather enhanced

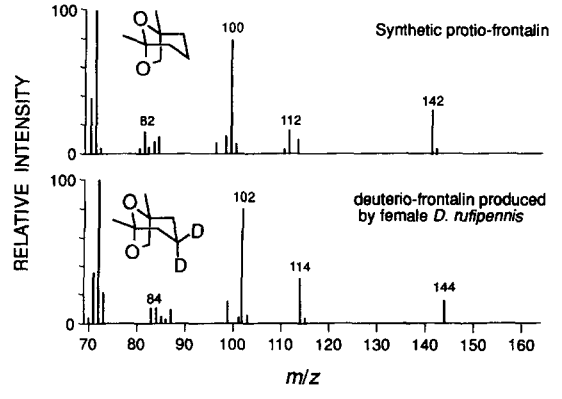

Figure 3. MS of synthetic protio-frontalin and deuterio-frontalin, produced by *D. rufipennis* upon exposure to (4,4-$^2H_2$)-6-methyl-hepten-2-one (**3**-$^2H_2$).

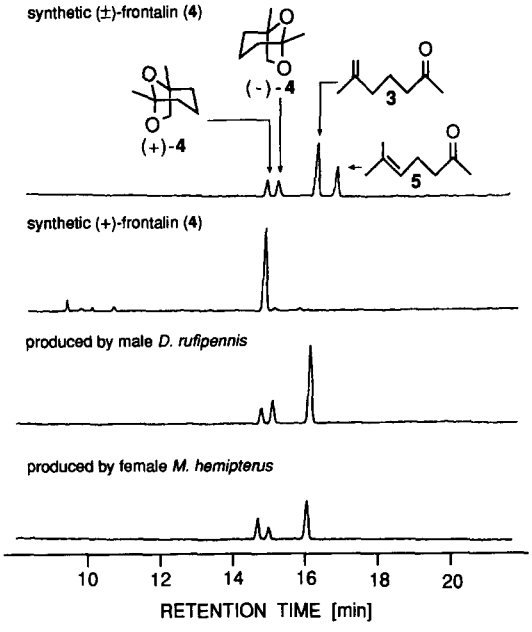

Figure 4. GC analyses of synthetic (±)- and (+)-frontalin (**4**), and **4** produced by *D. rufipennis* and *M. hemipterus* during vapor exposure to 6-methyl-6-hepten-2-one (**3**). Traces for male and female beetles were virtually identical. Exposure of female *D. ponderosae* to deuterio-**3** resulted in the production of racemic deuterio-**4**. Chromatography: Cyclodex-B column; 80 °C isothermal; split injection; linear flow velocity of carrier gas: 35 cm/s; injector temperature: 200 °C.

ipsdienol production[15]). Finally, failure of SB to transform **3** to **4** with high enantiomeric purity may signal production of **4** by a route not involving **3** as a precursor. *Ips paraconfusus*, for example, produced

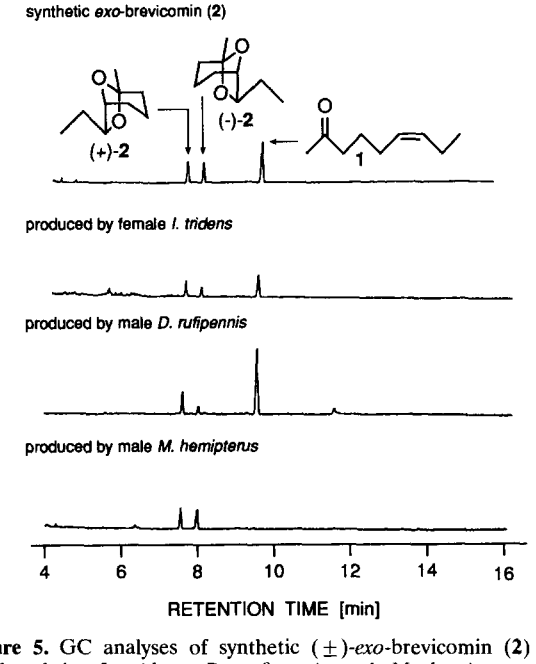

Figure 5. GC analyses of synthetic (±)-*exo*-brevicomin (**2**) and **2** produced by *I. tridens*, *D. rufipennis* and *M. hemipterus* during exposure to (*Z*)-6-nonen-2-one. Traces for male and females were virtually identical. Chromatography: Cyclodex-B column; 110 °C isothermal; split injector; linear flow velocity of carrier gas: 35 cm/s; injector temperature: 220 °C.

substantial quantities of ipsenol and ipsdienol while feeding on *Pinus sabiana* phloem with low levels of myrcene (<0.01 μg/g), suggesting that precursors other than myrcene may be utilized for ipsenol and ipsdienol biosynthesis.

In conclusion, the inability of SB to convert **5** or **6** to frontalin and the conversion of **3** to nonnatural enantiomeric mixtures rather than (+)-frontalin, suggests that these precursors may not be dietary constituents which are transformed to frontalin in SB. Production of frontalin and *exo*-brevicomin by scolytid and curculinoid beetles, not naturally producing bicyclic acetals, demonstrates widespread occurrence of nonspecific polysubstrate monooxidases in the Coleoptera.

Experimental

In vivo experiments

SB-infested Engelmann spruce logs, *Picea engelmannii* Parry), were collected near MacKenzie, Princeton, and Merritt, British Columbia. Prior to use, MacKenzie and Princeton logs were cold-stored at ca. 0 °C for several months, whereas Merrit logs were immediately placed in cages at 22–25 °C and 40–60% relative humidity. Pine engravers (PE), *I. pini* (Say) and MPB were obtained from infested lodgepole pine logs, *Pinus contorta* (var. *latifolia* Engelmann), collected near Princeton. *Ips tridens* (Mannerheim) were obtained from infested Engelmann spruce logs near Slate Creek, British Columbia. The cut ends of all logs were sealed with hot paraffin wax to prevent desiccation. Emergent beetles were collected daily, sexed and used within 48 h. West Indian sugarcane weevils (WISW), *M. hemipterus sericeus* (Olivier), were collected in the Palma Tica Oil Palm Plantation near Coto, Costa Rica.

In exposure experiments (Exps 1–9 and 11–15, Table 1), insects were placed individually in capped vials (Gries et al., 1990), containing 0 (control) or 0.1 μL of test chemical. In Exp. 10 (Table 1, Fig. 2), each vial also contained a 25–30 mm disk of fresh spruce phloem. After 24 h at 18–22 °C, beetles were crushd in dry ice-cooled pentane : ether (9 : 1, 250 mL), containing decane as internal standard. Crude extract was filtered through 1.5–2 cm of anhydrous $MgSO_4$ and pipetted onto a glass 'boat' (length, width and depth 4, 1 and 1 cm, respectively) within a glass tube (20 cm long, o.d. 22 mm). This tube placed inside a micro oven was connected upstream to a charcoal filter (coconut shell, 50–80 mesh) and downstream to a Porapak Q trap embraced by a dry ice : acetone-cooled aluminum block. While passing a gentle nitrogen stream through the system for 1 h, the oven temperature was slowly increased to 90 °C. Volatiles emanating from crude extract on the boat were captured on Porapak Q, eluted with Et_2O (1–2 mL) and concentrated for analyses. Groups of five samples were processed concurrently in a manifold of five pyrex glass tubes, each being swagelok-connected up- and downstream to charcoal filters and Porapak Q traps, respectively. A nitrogen stream was passed through the manifold for ca. 2 h and trapped volatiles were eluted from Porapak Q with 1.5 mL of pentane and concentrated.

Volatils from male and female SB feeding on suitable spruce logs were available in our laboratory from previous work.[11,12]

Laboratory analysis

Volatile extracts were analyzed by GC–MS (Hewlett Packard 5985 B and Varian Saturn-ion trap), employing fused silica columns (30 m × 0.25 mm i.d.; 0.25 mm film) coated with DB-1 or DB-5 (J&W Scientific, Folsom, California). Chiral determination of frontalin and *exo*-brevicomin were carried out on a Cyclodex-B-coated column (30 m × 0.25 mm i.d., J&W Scientific), which separates enantiomers with baseline resolution. Electron impact (EI, 70 eV) GC–MS analyses were conducted in both full scan and selected ion monitoring mode (SIM). A full scan MS of synthetic frontalin was obtained to select diagnostic ions. For GC–MSEI–SIM, synthetic frontalin, pentane or ether and concentrated insect extract were injected in split mode and analyzed by scanning for diagnostic ions.

Syntheses

Instruments and general procedures. NMR spectroscopy was conducted on a Bruker AMX-400 spectrometer at 400.13 and 100.62 MHz for ^1H and ^{13}C NMR spectra, respectively. ^1H chemical shifts are reported relative to TMS and ^{13}C chemical shifts are referenced to $CDCl_3$. GC analyses were performed on a Hewlett Packard 5890 instrument equipped with flame ionization detector and fused silica, DB-1 coated column (15 m × 0.25 mm i.d.; 0.25 mm film, J&W Scientific). Elemental analyses were performed using a Carbo Erba Model-1106 elemental analyzer. Diethyl ether (Et_2O) and tetrahydrofuran (THF) were freshly distilled from sodium-benzophenone-ketyl. Benzene and acetone were freshly distilled from activated 4 Å molecular sieves and anhydrous $CaSO_4$, respectively. Diisopropyl amine and cyclohexyl amine were freshly distilled from sodium under argon. Chemicals obtained from commercial sources were used without further purification unless otherwise indicated. All moisture- and air-sensitive reactions were conducted under argon. Column chromatography refers to flash chromatography using silica gel 60 (230–400 mesh, E. Merck, Darmstadt).[16] TLC was conducted on aluminum backed plates precoated with Merck Silica Gel 60F-254 as the adsorbent and visualized by treatment with an acidic solution of 1% $Ce(SO_4)_2$ and 1.5% molybdic acid followed by gentle heating.

(4,4-$^3H_2$)-6-Methyl-6-hepten-2-one (**3**-$^2H_2$) (Fig. 6) was prepared according to Pearce et al.[17] and 6-methyl-6-hepten-2-one (**3**) was prepared according to Serebryakov and Gamalevich.[18]

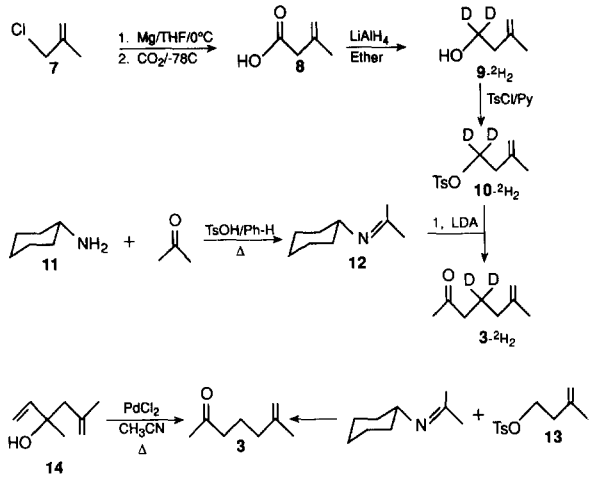

Figure 6. Scheme for synthesis of (4,4-$^2H_2$)-6-methyl-hepten-2-one (**3**-$^2H_2$) and 6-methyl-6-hepten-2-one (**3**).

3-Methyl-3-butenoic acid[19] **(8)**. 2-Methyl-1-propenyl magnesium chloride [prepared by Grignard reaction between 3-chloro-2-methyl-1-propene (**7**) (6.78 g, 7.4 mL, 75 mmol) and activated magnesium powder (3.40 g, 0.14 mol) in THF at 0 °C according to Oppolzer et al.[20]] was cooled to −78 °C. Excess CO_2 gas (USP Union Carbide Canada, Vancouver) was bubbled through the reaction mixture for ~15 min. After stirring for 30 min, the cold bath was removed and the reaction allowed to warm to 0 °C. Progress of the reaction was followed by GC analysis of aliquots. Upon completion, the reaction was quenched with saturated NH_4Cl at 0 °C. The aqueous layer was extracted with Et_2O (3 × 25 mL) and the combined organic layer concentrated in vacuo. The residue was brought to pH ca. 10 with 1 M NaOH. The basic layer was extracted with Et_2O (2 × 10 mL), acidified with cold dil HCl, extracted with Et_2O (3 × 25 mL), washed with saturated NaCl and dried over anhydrous $MgSO_4$. After filtration, the solvent was removed in vacuo and the residue distilled under reduced pressure (63–65 °C at 1 mm Hg) to give **8** (5.55 g, 74% yield, 98% pure). ^1H NMR ($CDCl_3$, ppm): 1.85 (3H, s), 3.10 (2H, s), 4.90 (1H, d, J = 0.6 Hz), 4.97 (1H, d, J = 0.6 Hz), 11.92 (1H, br s). ^{13}C NMR ($CDCl_3$, ppm): 177.60, 137.91, 115.28, 43.08, 22.35.

(1,1-$^2H_2$)-3-Methyl-3-buten-1-ol (9-$^2H_2$). To a suspension of $LiAl^2H_4$ (Sigma, St Louis, Missouri, 98% atom% ^2H; 0.82 g, 19.5 mmol) in anhydrous ether at 0 °C, was added dropwise, via cannula, a solution of 3-methyl-3-butenoic acid (**8**) (2.01 g, 20 mmol) in anhydrous Et_2O. After 0.5 h, excess $LiAl^2H_4$ was destroyed at 0 °C by slow addition of water. The resulting white precipitate was filtered and rinsed with small portions of Et_2O (4 × 10 mL). The solvent was removed by fractional distillation and the residue was used without further purification. EIMS *m/z* (relative intensity): 88 (M^+, 42), 70 ($M^+ - H_2O$, 90). FT-IR (neat): 3440, 3069, 2973, 2931, 2255, 2168 cm^{-1}.

(1,1-$^2H_2$)-3-Methyl-3-butenyltosylate (10-$^2H_2$). To a solution of **9**-$^2H_2$ in 30 mL of dry pyridine was added 0.56 g (4.5 mmol) of dimethylaminopyridine (DMAP). The flask was cooled to −10 °C and *p*-toluensulfonyl chloride (4.30 g, 22.5 mmol) added in one portion. Stirring was continued for 5 h at −10 °C, monitoring aliquots by GC and TLC (9 : 1, pentane : Et_2O, R_f = 0.20). The mixture was poured into ice-cooled NaCl solution and extracted (2 × 30 mL) with Et_2O. The organic layer was washed with 3 M HCl, saturated $NaHCO_3$, NaCl and dried over anhydrous $MgSO_4$. After concentration in vacuo, the residue was purified by column chromatography (9 : 1, pentane : Et_2O) to yield **10**-$^2H_2$ (3.52 g, 72% yield based on **4**, 98% pure) as a pale yellow oil. ^1H NMR ($CDCl_3$, ppm): 1.66 (3H, s), 2.32 (2H, s), 2.44 (3H, s), 4.67 (1H, d, J = 0.8 Hz), 4.80 (1H, d, J = 0.8 Hz), 7.36 (2H, dd, J = 8, 1 Hz), 7.80 (2H, dd, J = 8, 1 Hz). ^2H NMR ($CHCl_3$, ppm): 4.12. ^{13}C NMR ($CDCl_3$, ppm): 144.63, 140.17, 133.54, 129.77, 127.89, 113.04, 67.9 (J_{C-^2H} = 16.7 Hz), 36.65, 22.29, 21.56. EIMS *m/z* (relative intensity): 70 ($M^+ - C_7H_7SO_3$, 100; 98% deuterium enriched by correlation of 68/70 peaks). FT-IR (neat): 3072, 2978, 2931, 2249, 2167, 1648, 1596, 1361, 1184, 1096, 1073, 959, 879, 816, 770 cm^{-1}. Calcd for $C_{12}H_{14}{}^2H_2SO_3$: C, 59.98; H, 6.72. Found: C, 60.10; H, 6.79.

***N*-(1-Methylethylidine)cyclohexylamine (12)**. A solution of anhydrous acetone (5.81 g, 7.34 mL, 0.1 mol), cyclohexylamine (**11**) (9.97 g, 11.4 mL, 0.1 mol) and *p*-toluensulfonic acid (100 mg) in anhydrous benzene (100 mL) was refluxed in a Dean–Stark separator until no additional water was produced (~3 days). Benzene was removed and the residue distilled at reduced pressure (35–37 °C at 1 mm Hg) to yield **8** (8.34 g, 61%, 95% pure). ^1H NMR ($CdCl_3$, ppm): 1.28 (6H, m), 1.49 (2H, m), 1.76 (2H, m), 1.84 (3H, s), 1.98 (3H, s), 3.19 (1H, m). ^{13}C NMR ($CDCl_3$, ppm): 164.01, 59.39, 36.97, 33.65, 30.82, 29.41, 25.73, 25.08, 17.92. EI–MS *m/z* (relative intensity): 139 (M^+, 20), 124 ($M^+ - 15$, 100).

(4,4-$^2H_2$)-6-Methyl-6-hepten-2-one (3-$^2H_2$). To a stirred, cold (−78 °C) solution of LDA [prepared from 10 mmol of diisopropyl amine and 10 mmol of 2.45 M BuLi in hexanes (Aldrich Chemical Co.) at 0 °C in THF] under a positive pressure of argon, was added **12** (1.38 g, 10 mmol) in 10 mL of THF. After stirring 45 min, a solution of **10**-$^2H_2$ (2.42 g, 10 mmol) in 15 mL of THF was added dropwise via cannula and the resulting mixture was stirred for 3 h at −78 °C. The mixture was quenched to slight acidity with 1 M HCl at 0 °C. The organic layer was separated and the aqueous layer extracted (2 × 10 mL) with Et_2O. The combined ether extracts were washed with saturated NaCl and dried over anhydrous $MgSO_4$. Column chromatography (9 : 1, pentane : Et_2O, R_f = 0.27) afforded **3**-$^2H_2$ (0.63 g, 57% yield, 95% pure) as a pale yellow liquid. ^1H NMR ($CDCl_3$, ppm): 1.72 (3H, s), 2.02 (2H, s), 2.16 (3H, s), 2.40 (2H, s), 4.67 (1H, d, J = 1.0 Hz), 4.72 (1H, d, J = 1.0 Hz). ^2H NMR ($CHCl_3$, ppm): 1.69. ^{13}C NMR ($CDCl_3$, ppm): 208.64, 144.98, 110.51, 42.81, 36.89, 29.84, 22.28 (J_{C-^2H} = 16.7 Hz), 22.10. EIMS *m/z* (relative intensity): 128 (M^+, 5), 110 ($M^+ - H_2O$), 65

(98% deuterium enriched by correlation of 110/108 peaks in SIM mode). FT-IR (neat): 3074, 2969, 2917, 2250, 2159, 1716, 1649, 1446, 1361, 1179, 962, 888 cm^{-1}.

3-Methyl-3-butenyltosylate (13). Commercially available 3-methyl-3-buten-1-ol (Aldrich) was tosylated using the same procedure described for the synthesis of (1,1-$^2H_2$)-3-methyl-3-butenyltosylate (**10**-$^2H_2$). ^1H NMR ($CDCl_3$, ppm): 1.65 (3H, s), 2.35 (2H, t, $J = 7.5$ Hz), 2.44 (3H, s), 4.16 (2H, t, $J = 7.5$ Hz), 4.68 (1H, d, $J = 1.4$ Hz), 4.79 (1H, s, $J = 1.4$ Hz), 7.34 (2H, dd, $J = 10$, 1 Hz), 7.78 (2H, dd, $J = 10$, 1 Hz).

3,5-Dimethyl-1,5-hexadien-3-ol (14). This compound was prepared by reaction of 2-methyl-1-propenyl magnesium chloride and 3-buten-2-one. ^1H NMR ($CDCl_3$, ppm): 1.30 (3H, s), 1.78 (3H, s), 1.86 (1H, s), 2.30 (2H, s), 4.77 (1H, d, $J = 1.3$ Hz), 4.92 (1H, d, $J = 1.3$ Hz), 5.04 (1H, dd, $J = 10.7$, 1.3), 5.23 (1H, dd, $J = 17.3$, 1.3 Hz), 5.96 (1H, dd, $J = 10.7$, 17.3 Hz). FT-IR (neat): 3445, 3075, 2933, 1710, 1643, 1107, 920, 777 cm^{-1}.

6-Methyl-6-hepten-2-one (3). This compound was prepared by the alkylation of **12** by 3-methyl-3-butenyltosylate (**13**), according to the former procedure or by palladium catalyzed [3,3] sigmatropic rearrangement of **14**.[18] In the latter process, 4.74 g of **14** and 17.5 mg of $PdCl_2$ in 35 mL of dry CH_3CN were stirred for ~72 h at room temperature. Distillation at reduced pressure (70–73 °C at 25 mHg) afforded 4.31 g of **3** (91% yield, 98% pure). ^1H NMR ($CDCl_3$, ppm): 1.72 (5H, m), 2.00 (2H, t, $J = 7.7$ Hz), 2.13 (3H, s), 2.44 (2H, t, $J = 7.7$ Hz), 4.68 (1H, d, $J = 1.2$ Hz), 4.73 (1H, d, $J = 1.2$ Hz). ^{13}C NMR ($CDCl_3$, ppm): 208.30, 145.07, 110.62, 43.04, 37.11, 29.97, 22.20, 21.58.

6-Methyl-5-hepten-2-one (**5**) and 6-methyl-5-hepten-2-ol (**6**) were purchased from Aldrich. (*Z*)-6-Nonen-2-one (**1**), *exo*-brevicomin (**2**) and frontalin (**4**) were obtained from Phero Tech Inc. (Delta, British Columbia).

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References

1. (a) White, R. A.; Agosin, M.; Franklin, R. T.; Webb, J. W. *Z. Angew. Entomol.* **1980**, *90*, 254; (b) Borden, J. H. *Aggregation Pheromones: Comprehensive Insect Physiology, Biochemistry and Pharmacology*; Kerkut, G. A.; Gilbert, L. I., Eds; Pergamon; Oxford, 1985; Vol. 9; (c) Vanderwel, D.; Oehlschlager, A. C. In *Pheromone Biochemistry*; Prestwich, D. G.; Blomquist, G. J., Eds; Academic; Orlando, 1987; (d) Bestmann, H. J.; Vostrowsky, O. In *Handbook of Natural Pesticides, Pheromones Part A*; Morgan, E. D.; Mandava, N. B., Eds; CRC; Boca Raton, 1988, Vol. IV.

2. (a) Hughes, P. R.; Renwick, J. A. A. *Physiol. Entomol.* **1977**, *2*, 117; (b) Renwick, J. A. A.; Dickens, J. C. *Physiol. Entomol.* **1979**, *4*, 377; (c) Byers, J. *Science* **1983**, *220*, 624; (d) Byers, J. A. *J. Insect Physiol.* **1983**, *29*, 5; (e) Hunt, D. W. A.; Borden, J. H.; Pierce, H. D. Jr; Slessor, K. N.; King, G. G. S.; Czyzewska, E. J. *Chem. Ecol.* **1986**, *12*, 1579.

3. (a) Renwick, J. A. A.; Hughes, P. R.; Pitman, G. B.; Vité, J. P. *J. Insect Physiol.* **1976**, *22*, 725; (b) Borden, J. H.; Nair, K. K.; Slater, C. E. *Science* **1969**, 1626; (c) Klimetzek, D.; Francke, W. *Experientia* **1980**, *36*, 1343; (d) Lindström, M; Norim, T.; Birgersson, G.; Schlyter, F. *J. Chem. Ecol.* **1989**, *15*, 541.

4. Byers, J. A.; Birgersson, G. *Naturwissenschaften* **1990**, *77*, 385.

5. Tumlinson, J. H.; Gueldner, R. C.; Hardee, D. D.; Thompson, A. C.; Hedin, P. A.; Minyard, J. P. In *Chemicals Controling Insect Behavior*; Beroza, M., Ed.; Academic; New York, 1970.

6. Mitlin, N.; Hedin, P. A. *J. Insect Physiol.* **1974**, *20*, 1825.

7. (a) Vanderwel, D.; Gries, G.; Singh, S. M.; Borden, J. H.; Oehlschlager, A. C. *J. Chem. Ecol.* **1992**, *18*, 1389; (b) Vanderwel, D.; Oehlschlager, A. C. *J. Am. Chem. Soc.* **1992**, *114*, 5081.

8. Brand, J. M.; Young, J. C.; Silverstein, R. M. *Fortsch. Chem. Org. Naturst.* **1979**, *37*, 1.

9. Francke, W.; Bartels, J.; Meyer, H.; Schröder, F.; Kohnle, U.; Baader, E.; Vité, J. P. *J. Chem. Ecol.* **1995**, *21*, 1043.

10. Kinzer, G. W.; Fentiman, A. F. Jr; Page, T. F. Jr; Foltz, R. L.; Vité, J. P.; Pitman, G. B. *Nature* **1969**, *221*, 477.

11. Gries, G.; Pierce, H. D. Jr; Lindgren, B. S.; Borden, J. H. *J. Econ. Entomol.* **1988**, *81*, 1715.

12. Gries, G. *J. Appl. Entomol.* **1992**, *114*, 240.

13. Armstrong, R. N. *CRC Crit. Rev. Biochem.* **1987**, *22*, 39.

14. Vité, J. P.; Bakke, A.; Renwick, J. A. A. *Can. Entomol.* **1972**, *104*, 1967.

15. Gries, G.; Bowers, W. W.; Gries, R.; Noble, M.; Borden, J. H. *J. Insect Physiol.* **1990**, *36*, 819.

16. Still, W. C.; Kanh, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923.

17. Pearce, G. T.; Gore, W. E.; Silverstein, R. M. *J. Org. Chem.* **1976**, *41*, 2797.

18. Serebryakov, E. P.; Gamalevich, G. D. *Bull. Acad. Sci. USSR* **1987**, *36*, 116.

19. Fujisawa, T.; Sato, T.; Gotoh, Y.; Kanashima, M.; Kanara, T. *Bull. Chem. Soc. Jpn* **1982**, *55*, 3555.

20. Oppolzer, W.; Kündig, E. P.; Bishop, P. M.; Perret, C. *Tetrahedron Lett.* **1982**, 3901.

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