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Chemistry

Structure, stereochemistry, and thermal isomerization of the male sex pheromone of the longhorn beetle *Anaglyptus subfasciatus*

(3-hydroxy-2-hexanone/3-hydroxy-2-octanone/cerambycidae)

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ABSTRACT **Male-released sex pheromone constituents of the longhorn beetle *Anaglyptus subfasciatus* (Coleoptera: Cerambycidae) are identified by GC–MS and GC–Fourier transform infrared as a 7:1 molar mixture of 3-hydroxy-2-hexanone and 3-hydroxy-2-octanone. These two compounds undergo thermal isomerization during GC analyses to give the corresponding 2-hydroxy-3-alkanones. Comparison of GC retention times of the natural products with those of synthesized enantiomerically pure compounds revealed that both semiochemicals have (*R*)-stereochemistry. These absolute configurations were confirmed by comparisons of the (*R*)-methoxy(trifluoromethyl)phenylacetic acid esters of insect-derived and synthetic samples.**

Since the milestone identification of bombykol more than three decades ago, increasingly sophisticated analytical techniques combined with a great deal of research effort has led to the identification of hundreds of insect sex pheromones (1). While our knowledge of pheromonal communication in some groups, such as the Lepidoptera, is substantial, very little is known about chemical communication in many other groups of insects, including many of economic interest, such the longhorn beetles (Cerambycidae). Only recently has a female-released long-range sex pheromone for this group been identified (2) as an interesting and unusual semiochemical; this finding encouraged us to extend our study of longhorn beetle chemical communication.

The cryptomeria twig borer *Anaglyptus subfasciatus* PIC is one of the most harmful insect pests of the Japanese cedar *Cryptomeria japonica* and the Japanese cypress *Chamaecyparis obtusa*, the most abundant tree species of forest plantations in Japan. Larvae of the beetle bore from dead twigs into the trunk and feed on sapwood, inducing discoloration of the heartwood and decreasing its commercial value. Since forest plantations are usually located near riverheads, spraying insecticides for the control of beetles must be avoided to prevent contamination of groundwater; alternative methods of control are, therefore, badly needed.

It has been shown that *A. subfasciatus* females are attracted to males in a wind tunnel and that a male-specific cuticular structure in the pronotum seems to be involved in pheromone secretion (3). We have confirmed that reproductive behavior in *A. subfasciatus* utilizes male-released sex pheromones, which are now fully characterized as 3-(*R*)-hydroxy-2-hexanone and 3-(*R*)-hydroxy-2-octanone.

MATERIALS AND METHODS

Analytical Procedures. GC was carried out on Hewlett–Packard 5890 II instruments equipped with split/splitless and cold on-column injectors, flame ionization detectors, and Hewlett–Packard 3396A integrators. High-resolution GC analyses were performed with polar and nonpolar capillary columns: DB-Wax (30 m × 0.25 mm; 0.25 μm; J & W Scientific; Folsom, CA) and CP-Sil 5 CB (25 m × 0.32 mm; 0.12 μm; Chrompack; Raritan, NJ), respectively. Unless otherwise mentioned, the DB-Wax column was operated at 50°C for 1 min, programmed at 4°C/min to 180°C and then at 10°C/min to 230°C, and held at this temperature for 10 min [hereafter expressed as 50(1)–180(1)/4–230(10)/10] while the CP-Sil 5 CB column was operated at 40(4)–180(1)/4–270(15)/10. Chiral resolutions of the 3-hydroxy-2-alkanones were achieved on a trifluoroacetylated γ-cyclodextrin-based capillary column, Chiraldex GTA (20 m × 0.25 mm; 0.125 μm; Astec; Whippany, NJ), operated either at 60(1)–90(20)/0.5 or at 55(1)–90(20)/0.5, with helium as a carrier gas at a head pressure of 2 kg/cm^2 (flow rate, 1.6 ml/min). Low-resolution MS was carried out either on a Hewlett–Packard 5891 mass selective detector or on a Finnigan–MAT (San Jose, CA) ion-trap detector 800. GC separations were performed on a DB-Wax column under the above described conditions and on a DB-1 column (30 m × 0.25 mm; 0.25 μm; J & W Scientific) operated at 40(4)–180(1)/4–250(20)/10, respectively. IR spectra of the natural products were acquired on a Tracer FTS-60 A (Bio-Rad) instrument and those of the synthetic compounds were recorded on a Jasco (Easton, MD) IR-810 instrument. Optical rotations were measured on a Perkin–Elmer 241 polarimeter and ^{1}H NMR spectra were obtained in C^2HCl_3 solutions on a Varian XL-400 instrument.

GC–Electroantennographic Detector (EAD). The responses of *A. subfasciatus* female antennae were recorded with an amplifier and a passive high-pass filter, according to a reported method (4). The antenna was set on an acrylic stage (5) and placed inside the transfer tube 2 cm from the point of introduction of the GC effluent.

GC–Behavioral Bioassay. Coupled chromatographic separations and behavioral observations were performed as described (6), using the same system described for GC–EAD experiments.

Wind Tunnel Experiments. Females were placed at the downwind end of a wind tunnel (2 m long and 30 cm i.d.) and observed with lights on for 15 min at an airflow of 30 cm/s and 25°C.

Insects. *Cryptomeria* logs infested by *A. subfasciatus* were collected in the field in winter and kept in a field cage until the next spring, when the adults emerged from the logs. Beetles were then kept separately in plastic cages (12 cm in diameter and 5 cm high) under 16 h light/8 h dark conditions and fed on a 5% (wt/vol) sucrose solution.

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Abbreviations: ee, enantiomeric excess; MTPA, methoxy(trifluoromethyl)phenylacetic acid; EAD, electroantennographic detector.

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Aeration. Beetles were placed in a collection system as described (5); the airborne volatiles were trapped on Super Q (Alltech Associates) and extracted with hexane.

Syntheses. *(±)-3-Hydroxy-2-octanone (4).* To a stirred suspension of HgO (0.5 g, 2.3 mmol) in dry methanol (60 ml) was added $BF_3 \bullet Et_2O$ (5 ml); stirring was continued until the HgO had completely dissolved. After cooling the mixture to −60°C, (±)-1-octyn-3-ol (**5b**) (24 g, 190 mmol) was added dropwise, and the mixture was stirred at −10°C for 1.5 h. The reaction mixture was then acidified (HCl, 1 M; 30 ml), stirred for 1 h, extracted with ether, washed with brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent and distillation at 71–72°C/6 mmHg (1 mmHg = 133 Pa) gave (±)-**4** (9 g, 63 mmol; 33% yield); ^{1}H NMR δ 0.87 (t, J = 6.4 Hz, 3H), 1.24–1.36 (m, 5H), 1.40–1.58 (m, 2H), 1.74–1.86 (m, 1H), 2.19 (s, 3H), 3.46 (br, 1H, OH), and 4.16 (dd, J = 7.2, 4.0 Hz, 1H); IR ν_{max} 3500 (vs, broad), 2970 (vs), 2950 (vs), 2880 (s), 1722 (vs), 1370 (s), 1140 (s), and 1100 (s) cm^{-1}.

(±)-3-Hydroxy-2-hexanone (2). In a manner similar to that described above, (±)-1-hexyn-3-ol (**5a**) (9.8 g, 0.1 mol) afforded crude (±)-**2**. Purification on a silica gel column (eluent: hexane/ether, 40:60) gave (±)-**2** (4.8 g, 40 mmol; 41%); ^{1}H NMR δ 0.96 (t, J = 6.8 Hz, 3H), 1.30–1.42 (m, 1H), 1.44–1.55 (m, 2H), 1.74–1.85 (m, 1H), 2.20 (s, 3H), 3.45 (d, J = 4.4 Hz, 1H, OH), and 4.18 (ddd, J = 8.0, 4.4, and 3.6 Hz, 1H); IR ν_{max} 3480 (s, broad), 2970 (vs), 2950 (vs), 2880 (s), 1722 (vs), 1370 (s), 1140 (s), and 1085 (s) cm^{-1}.

(2S,3R)-1,2-Epoxy-3-hexanol [(2S,3R)-7]. To stirred and cooled (−20°C) dry CH_2Cl_2 (200 ml) were added Ti(*i*PrO)$_4$ (2.5 ml, 8.4 mmol) and D-(−)-isopropyl tartrate (2.1 ml, 10 mmol). After stirring for 10 min, (±)-1-hexen-3-ol (**6**) (0.84 g, 8.4 mmol) and *t*-BuOOH (5–6 M in decane, 3 ml, 16.8 mmol) were added to the mixture at −20°C. The mixture was left for 12 h at this temperature. Tartaric acid (10% solution, 40 ml) was then added to the stirred and cooled mixture and stirring was continued for 1 h at −20°C and 2 h at room temperature. The organic phase was separated, washed with water, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. The residue was diluted with ether (300 ml) and extracted with 1 M NaOH (50 ml) at 0°C for 5 min. The organic phase was separated and analyzed by GC. This washing procedure was repeated (three times) until the diisopropyl tartrate was completely removed. The ethereal solution was washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Purification on a silica gel column (eluent: hexane/ether, 85:15) afforded (2S,3R)-**7** [0.31 g, 2.7 mmol, 32%, 88% enantiomeric excess (ee)]; [(*R*)-methoxy(trifluoromethyl)phenylacetic acid (MTPA) ester 16.67; (*S*)-MTPA ester, 16.98 min]; ^{1}H NMR δ 0.91 (t, J = 7.3 Hz, 3H), 1.26 (dd, J = 7.0, 2.8 Hz, 1H), 1.49 (m, 3H), 2.23 (br, 1H, OH), 2.73 (dd, J = 5.2, 4.0 Hz, 1H), 2.82 (dd, J = 5.1, 2.8 Hz, 1H), 3.02 (ddd, J = 6.1, 4.0, 3.0 Hz, 1H), and 3.85–3.87 (m, 1H); MS m/z (relative intensity) 43 (67), 45 (37), 55 (100), 57 (33), 73 (61), and 98 (1).

(2S,3R)-1,2-Epoxy-3-[2'-(tetrahydropyranyl)oxy]hexane [(2S,3R)-8]. Tetrahydropyranylation of (2S,3R)-**7** (30 mg, 0.26 mmol) according to ref. 7 gave the protected epoxide (2S,3R)-**8** (50 mg, 0.25 mmol, 96%); ^{1}H NMR δ 0.95 (t, J = 6.8 Hz, 3H), 1.38–1.90 (m, 10H), 2.75 (dd, J = 2.0, 1.2 Hz, 1H), [2.80 (dd, J = 4.0, 4.0 Hz) and 2.71 (dd, J = 2.8, 2.4 Hz), diastereotopic, 1H], 3.25 (dd, J = 6.4, 6.0 Hz), 3.63 (dd, J = 6.0, 5.2 Hz) (diastereotopic, 1H), 3.47 (m, 1H), 3.65–3.90 (m, 1H), and [4.61 (dd, J = 4.2, 3.0 Hz) and 4.76 (dd, J = 4.0, 2.8 Hz), diastereotopic 1H]; MS m/z (relative intensity) 85 (100%), 43 (54), 55 (43), 67 (26), 81 (28), 101 (18), and 157 (11).

(2S,3R)-3-[2'-(Tetrahydropyranyl)oxy]-hexan-2-ol [(2S,3R)-9]. The protected epoxide (2S,3R)-**8** (46 mg, 0.23 mmol) was reduced ($LiAlH_4$), according to a reported method (8) to give (2S,3R)-**9** (39 mg, 0.19 mmol, 82%); ^{1}H NMR δ 0.91 (m, 3H), [1.12 (d, J = 6.4 Hz) and 1.3 (d, J = 6.4 Hz), diastereotopic, 3H], 1.20–1.61 (m, 10H), 1.83 (m, 1H), 3.51 (m, 1H), 3.62 (m, 1H), 3.9–4.1 (m, 2H), 4.4 (m, 1H), and [4.65 (dd, J = 5.6, 5.2 Hz) and 4.76 (dd, J = 5.2, 4.2 Hz), diastereotopic, 1H]; MS m/z (relative intensity) 43 (22), 55 (21), 67 (12), 85 (100), 101 (8), 129 (3), and 201 (1).

(R)-3-[2'-(Tetrahydropyranyl)oxy]-2-hexanone [(R)-10]. To a solution of (2S,3R)-**9** (34 mg, 0.17 mmol) in CH_2Cl_2 (2 ml) were added 4-Å molecular sieves (three pellets), tetra-*n*-propyl ammonium perruthenate (30 mg, 0.09 mmol), and *N*-methylmorpholine *N*-oxide (30 mg, 0.26 mmol). The mixture was stirred for 40 min and subjected to column chromatography over silica gel (eluent: hexane/ether, 9:1) to yield (*R*)-**10** (33 mg, 0.16 mmol, 94%); ^{1}H NMR δ [0.92 (t, J = 7.2 Hz), 0.95 (t, J = 7.2 Hz), diastereotopic, 3H], 1.30–1.90 (m, 10H), [2.14 (s, 2H) and 2.22 (s, 3H), diastereotopic, 3H], 3.85 (m, 1H), [3.85 (m) and 4.20 (dd, J = 8.0, 5.2 Hz), diastereotopic, 1H], and [4.49 (dd, J = 7.0, 2.4 Hz) and 4.53 (dd, J = 3.6 and 3.6 Hz), diastereotopic, 1H]; MS m/z 43 (46), 55 (17), 67 (14), 85 (100), 99 (2), and 157 (4).

(R)-3(−)-Hydroxy-2-hexanone [(R)-2]. Method A. A solution of (*R*)-**10** (28 mg, 0.14 mmol) and *p*-toluenesulfonic acid monohydrate (1.9 mg, 0.01 mmol) in methanol (2 ml) was stirred for 1 h at 25°C. The solvent was evaporated and the residue was chromatographed on a silica gel column to afford (*R*)-**2** (14 mg, 0.12 mmol, 86%). NMR data were the same as those obtained for the racemic compound. MS data were identical to those of the natural product. $[\alpha]_D^{22}$ −43° (*c* 0.07, hexane); 86% ee; [(*R*)-MTPA ester, 16.30 min; (*S*)-MTPA ester, 16.15 min]; ^{1}H NMR [(*R*)-MTPA] δ 0.94 (t, J = 7.6 Hz, 3H), 1.20–1.50 (m, 2H), 1.70–1.90 (m, 2H), 2.13 (s, 3H), 3.57 (d, J = 1.6 Hz, 3H), 5.18 (dd, J = 6.8, 6.0 Hz, 1H), 7.30 (m, 3H), and 7.57 (m, 2H).

(S)-3-(+)-Hydroxy-2-henaxone [(S)-2]. This compound was obtained via Sharpless epoxidation (9), as described above, but using L-(+)-isopropyl tartrate. $[\alpha]_D^{22}$ +47° (*c* 0.05, hexane); 82% ee.

(R)-3-(−)-Hydroxy-2-octanone [(R)-4]. (±)-1-Octyn-3-yl phthalate (45.5 g, 0.167 mol) was prepared from phthalic anhydride (29.6 g, 0.2 mol) and (±)-1-octyn-3-ol (**5b**) (25.2 g, 0.2 mol), according to a described procedure (10, 11), in 83% yield. Resolution of the monoester with (*R*)-(+)-α-methylbenzylamine (20.3 g, 0.167 mol) (11), followed by saponification, yielded (*R*)-(−)-1-octyn-3-ol [(*R*)-**5b**] (1.6 g, 12.7 mmol); hydration of [(*R*)-**5b**] gave (*R*)-**4** (1.14 g, 7.9 mmol; 62%); $[\alpha]_D^{22}$ −72° (*c* 1.3, hexane); 99.1% ee; [(*R*)-MTPA ester, 18.24 min; (*S*)-MTPA ester, 18.04 min]; ^{1}H NMR [(*R*)-MTPA ester] δ 0.88 (t, J = 6.8 Hz, 3H), 1.17–1.40 (m, 6H), 1.72–1.89 (m, 2H), 2.13 (s, 3H), 3.56 (d, J = 1.2 Hz, 3H), 5.17 (d, J = 7.2, 5.6 Hz, 1H), 7.40–7.42 (m, 3H), and 7.63–7.65 (m, 2H); MS m/z (relative intensity) 43 (29), 77 (4), 105 (10), 119 (7), 189 (100), 290 (3), and 360 (1).

(S)-3-(+)-Hydroxy-2-octanone [(S)-4]. Hydration of (*S*)-(+)-1-octyn-3-ol [(*S*)-**5b**] (6 g, 47 mmol) yielded (*S*)-**4** (3.7 g, 26 mmol; 55%); $[\alpha]_D^{22}$ +68° (*c* 1.1, hexane); 95.8% ee; [(*R*)-MTPA ester, 18.04; (*S*)-MTPA ester, 18.24 min]; ^{1}H NMR [(*R*)-MTPA] δ 0.83 (t, J = 6.8 Hz, 3H), 1.17–1.40 (m, 6H), 1.72–1.89 (m, 2H), 2.20 (s, 3H), 3.63 (d, J = 0.9 Hz, 3H), 5.17 (dd, J = 7.2, 5.6 Hz, 1H), 7.40–7.43 (m, 3H), and 7.63–7.65 (m, 2H).

(R)-2. Method B. Optically active yneol (*R*)-**5a** (1.3 g, 13 mmol), obtained by chiral resolution (9, 10), was hydrated to yield (*R*)-**2** (0.5 g, 4.3 mmol; 33%); $[\alpha]_D^{22}$ −75° (*c* 1, hexane); 97% ee.

MTPA esters. To a solution of synthetic alcohol (1 mg) or natural product in dry CH_2Cl_2 (200 μl) in a 1-ml microreaction vial were added pyridine (2 μl) and 4-dimethylaminopyridine (200 μg). The vial was sealed with a Teflon septum, flushed with argon, and then either (*R*)- or (*S*)-methoxy(trifluoromethyl)phenyl-acetyl chloride (1.5 μl) was introduced via syringe. The reaction mixture was allowed to stand at room temperature for at least 2 h and was then subjected to column

chromatography on a silica gel column (eluent: hexane/ether, 95:5) to give the desired MTPA esters. Alcohol enantiomeric purities were determined by measurement of the diastereomeric purities of these MTPA esters by GC analyses on a CP-Sil 5 CB capillary column operated at 40(4)–250(5)/10. Comparisons of retention times of the (*R*)-MTPA esters of natural and synthetic samples were performed on the same column operated at 40(4)–180(1)/4–270(15)/10.

RESULTS

The GC profiles of the airborne volatiles collected from *A. subfasciatus* males and females showed the occurrence of four male-specific peaks that were also EAD-active. These peaks appeared on a DB-Wax column at 13.01, 13.19, 19.21, and 19.51 min (Fig. 1). In a wind tunnel experiment, females placed in the downwind end of the tunnel walked upwind toward a filter paper impregnated with the airborne volatiles from males. Since this insect shows positive anemotaxis (3), this behavior alone is difficult to interpret. Nevertheless, the fact that 43% of the tested females (eight individuals; three replicates) searched the filter paper after they reached the pheromone source (control: 0%; eight individuals; two replicates) convinced us that chemotaxis plays a significant part in these upwind movements. In addition, GC–behavioral bioassay demonstrated that the biologically active compounds were those that showed EAD activity. After these compounds were eluted from the GC column, females (eight individuals; three replicates) placed inside the effluent-fed arena started to extend their antennae and move them back and forth. Because the active pairs of peaks were eluted far apart (≈6 min), it was possible to observe that the same behavior was elicited by the two group of compounds. On the other hand, this separation may have prevented aggregation of female beetles at the GC outlet, as had been observed for other insects (6).

Despite the fact that the four compounds gave mass spectra in which the intensities of the fragment ion peaks were dependent on the amount of the sample injected, typically the following profiles were obtained from ≈100 ng of the natural product. Peak **1** showed a base peak at *m/z* 45 with other fragments at *m/z* 43 (75), 55 (10), 71 (58), and 116 (2). The fragments of **2**, **3**, and **4** were 55 (100%), 43 (67), and 73 (54); 43 (100%), 45 (95), 55 (20), 71 (60), 99 (82), and 114 (2); 55 (100%), 43 (60), 73 (15), 83 (80), and 101 (32), respectively. The molecular ion peaks of **1**, **2**, **3**, and **4** were determined to be 116, 116, 144, and 144 based on the fact the M+1 ion peaks on the ion-trap detector appeared at *m/z* 117, 117, 145, and 145, respectively.

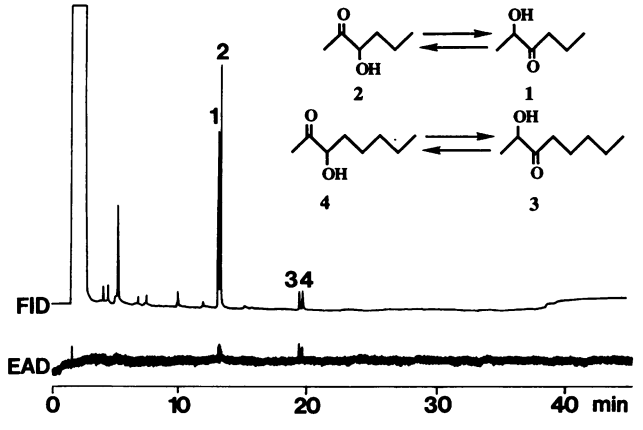


FIG. 1. Parallel flame ionization detector (FID) and EAD chromatograms obtained from the airborne volatiles of *A. subfasciatus* males separated on a DB-Wax column. A female antenna was used as the sensing element. Peaks **1** and **3** are derived from isomerization of **2** and **4** in the GC injection port.

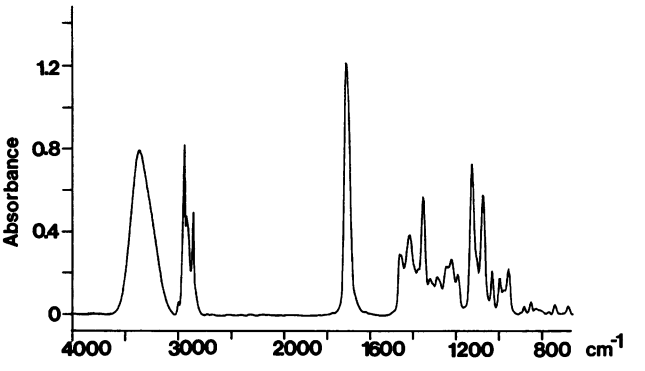


FIG. 2. GC–Fourier transform infrared spectrum of the major constituent of the male sex pheromone of *A. subfasciatus* (**2**) recorded with a Tracer instrument.

GC–Fourier transform infrared spectra of these compounds revealed that they had very similar structures; all showed carbonyl bands at 1722 cm^{-1} along with hydroxy absorption at ≈3380 cm^{-1} (Fig. 2). In addition, compounds **3** and **4** showed stronger bands at 2950 cm^{-1} due to $\nu_{as}CH_2$, which indicated that they had longer methylene chains than **1** and **2**, in agreement with the longer GC retention times observed for **3** and **4**.

Interestingly, we observed that the GC profile of the pheromone sample was dramatically influenced by the injector port temperature. When injected at 200°C, the ratios of **2**/**1** and **4**/**3** (Fig. 3*A*) were remarkably different from those observed when the sample was injected at 250°C (Fig. 1). GC analysis using a cold on-column injection system showed that the relative areas of peaks **1** and **3** decreased dramatically (Fig. 3*B*), suggesting that these two components were thermally produced artifacts.

The MS of compounds **2** and **4** showed a base peak at *m/z* 55 ($[CH_3C{=}OCHOH]^+ - H_2O$) along with a prominent peak at *m/z* 43 ($[CH_3C{=}O]^+$), indicating that the carbonyl and hydroxy groups were located in positions 2 and 3, respectively. On the other hand, the occurrence of significant peaks at *m/z* 45 ($[CH_3CHOH]^+$) for compounds **1** and **3** indicated they would have the hydroxy group in position 2. On the basis of these data, these compounds were characterized as 2-hydroxy-3-hexanone (**1**), 3-hydroxy-2-hexanone (**2**), 2-hydroxy-3-octanone (**3**), and 3-hydroxy-2-octanone (**4**). The isomerization of compounds **2** and **4** in the GC injection port into **1** and **3**, respectively (Fig. 1), simply reflects the fact that the isomeric α-hydroxyketones have a common enol through which they can easily interconvert. In fact, the mass spectral data reported for 2-hydroxy-3-octanone (**3**) (12) are very close to that found for our artifact **3**.

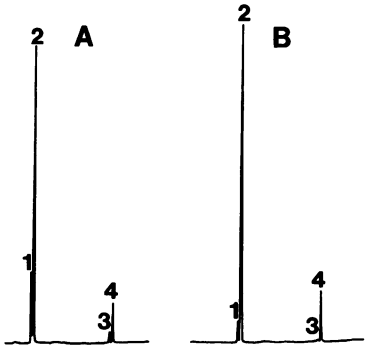
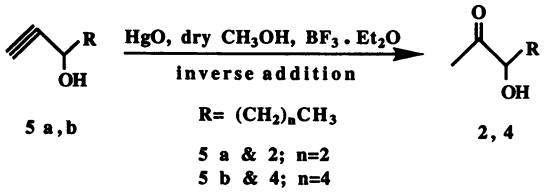


FIG. 3. Effect of the injection system on the intensity of the artifact peaks **1** and **3**. Gas chromatograms of the natural product obtained by splitless injection at 200°C (*A*) and by cold on-column injection (*B*).

To clarify this chemistry, we prepared racemic 3-hydroxy-2-hexanone (**2**) and 3-hydroxy-2-octanone (**4**) by hydration of the corresponding 1-alkyn-3-ols (Scheme I).



Scheme I

These compounds contained no traces of the corresponding 2-hydroxy isomers, as demonstrated by the absence in their ^{1}H NMR spectra of any signal for the terminal methyl (CH_3CHOH) that would appear as a doublet at δ 1.28 (8). However, during normal GC analysis on polar and nonpolar columns, 3-hydroxy-2-hexanone (**2**) gave two peaks corresponding to **1** and the insect-derived **2**, and 3-hydroxy-2-octanone (**4**) generated two GC peaks with the same retention times as **3** and the insect-derived **4**. Again, the lower the temperature of the injector port, the smaller the intensities of peaks **1** and **3**.

The (*R*)-MTPA esters derived from racemic 3-hydroxy-2-hexanone (**2**) gave two peaks on GC [CP-Sil 5 CB; 40(4)–180(1)/4–270(15)/10] at 28.75 and 28.98 min; the diastereomeric (*R*)-MTPA esters derived from racemic 3-hydroxy-2-hexanone (**4**) appeared at 33.31 and 33.63 min. The MS spectra of these compounds showed a distinctive peak at *m/z* 43 ($[CH_3CO]^+$). Under these conditions, the (*R*)-MTPA esters of the natural sex pheromone constituents gave only two peaks at 29.04 and 33.63 min, demonstrating that *A. subfasciatus* utilizes enantiomerically pure sex pheromone constituents. These data confirmed our conclusion that compounds **1** and **3** are not contained in the natural product.

Enantioselective synthesis of the major sex pheromone constituent (**2**) was carried out by asymmetric Sharpless epoxidation (9) as the key step (Fig. 4). Kinetic resolution of the commercially available **6** with D-(−)-isopropyl tartrate gave the epoxyalcohol **7** with the desired stereochemistry. Protection of **7** as a tetrahydropyranyl ether, followed by reduction ($LiAlH_4$) (8) afforded alcohol **9**. Oxidation of **9** (13) to give the corresponding ketone **10**, and subsequent deprotection of **10**, afforded (*R*)-**2** without loss of stereochemical integrity. Similarly, (*S*)-**2** was prepared by use of L-(+)-isopropyl tartrate.

A preliminary bioassay indicated that chirality plays a significant role in chemical signalling by *A. subfasciatus*. Since

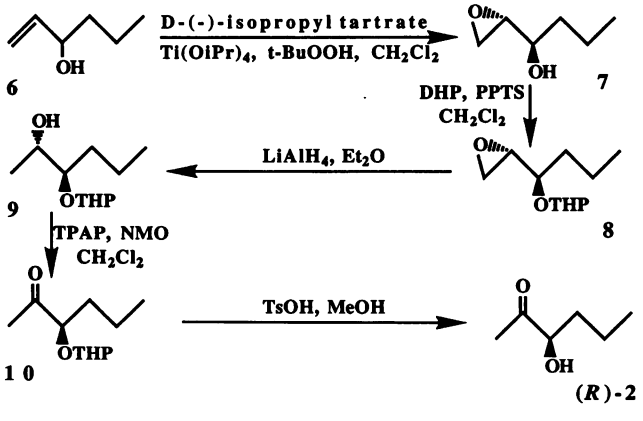


FIG. 4. Enantioselective synthesis of sex pheromone constitutient **2**. DHP, dihydropyranyl; PPTS, pyridinium *p*-toluenesulfonate; THP, tetrahydropyranyl; TPAP, tetra-*n*-propyl ammonium perruthenate; NMO, *N*-methylmorpholine *N*-oxide; TsOH, toluenesulfonic acid.

the enantiomeric purity achieved by kinetic resolution was not sufficiently high for use in field experiments, samples of much higher enantiomeric purity were prepared by resolution of racemic 1-alkyn-3-ols, followed by hydration (Scheme I).

Gas chromatographic resolution of **2** and **4** was achieved on a trifluoroacetylated γ-cyclodextrin phase, Chiraldex GTA operated at 60(1)–90(20)/0.5. (*R*)-3-Hydroxy-2-hexanone [(*R*)-**2**] appeared at 18.13 min (Fig. 5*A*), whereas its enantiomer [(*S*)-**2**] had a retention time 17.85 min. The separation of the two enantiomers (Fig. 5*B*) could be improved at lower temperature, 55(1)–90(20)/0.5, but no baseline separation was achieved [(*R*)-**2** 23.84 min; (*S*)-**2**, 23.29 min]. On the other hand, remarkable separation of the enantiomers of **4** was achieved. (*R*)-3-Hydroxy-2-octanone [(*R*)-**4**] appeared at 25.36 min (Fig. 5*C*), whereas (*S*)-**4** had a retention time of 27.60 min (Fig. 5*D*). The configurations of all four peaks were unambiguously assigned by coinjection of each pure enantiomer with its racemic mixture. The complexity of these GC resolutions is revealed by the observation that the elution order of the two enantiomers are reversed for compounds **2** and **4**; (*R*)-**2** has a longer retention time than its enantiomer, but (*R*)-**4** elutes before (*S*)-**4**.

The natural sex pheromone mixture (Fig. 5*E*) gave two GC peaks with the same retention times as those of (*R*)-3-hydroxy-2-hexanone and (*R*)-3-hydroxy-2-octanone. Upon coinjection of the natural sample with racemic mixtures of **2** and **4**, the areas of peaks corresponding to (*R*)-**2** and (*R*)-**4** increased (Fig. 5*F*). The stereochemistry of the natural sex pheromone components can, therefore, be assigned as (*R*)-3-hydroxy-2-hexanone and (*R*)-3-hydroxy-2-octanone.

To confirm these assignments, (*R*)-MTPA esters of racemic and enantiomerically pure synthetic **2** and **4** and of the natural product were prepared. GC analyses on an achiral column [CP-Sil 5 CB; 40(4)–180(1)/4–270(15)/10] demonstrated that the (*R*)-MTPA ester of (*S*)-**2** appeared at 28.70 min, whereas the corresponding ester of (*R*)-**2** showed a retention time of 28.98 min. Similarly, (*R*)-MTPA esters of (*S*)-**4** and (*R*)-**4** appeared at 33.31 and 33.63 min, respectively. Since the (*R*)-MTPA esters prepared from the pheromonal secretion

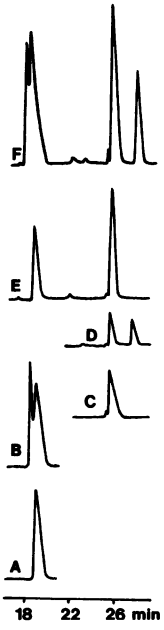


FIG. 5. Resolution of the enantiomers of compounds **2** and **4** on a chiral capillary column. (*A*) 3-(*R*)-Hydroxy-2-hexanone. (*B*) Racemic 3-hydroxy-2-hexanone. (*C*) 3-(*R*)-Hydroxy-2-octanone. (*D*) Racemic 3-hydroxy-2-octanone. (*E*) Natural product mixture. (*F*) Coinjection of the natural product mixture with racemic mixtures of the two pheromone constituents.

gave retention times of 28.98 and 33.63 min, it follows that each component has the *R* configuration.

DISCUSSION

Only after recognizing that the constituents of the sex pheromone of the longhorn beetle *A. subfasciatus* undergo isomerization under some analytical conditions were we able to identify the pheromone as a 7:1 molar mixture of 3-(*R*)-hydroxy-2-hexanone and 3-(*R*)-hydroxy-2-octanone. These ketols rearrange into the corresponding 2-hydroxy-3-alkanones at high temperatures in the injection port of a GC, making our initial GC analysis misleadingly complicated. Thermal isomerization may be in fact the cause of the confusion in the literature regarding the identification of the major sex pheromone constituent of *Xylotrechus chinensis*, reported as 3-hydroxy-2-octanone (14) and as 2-hydroxy-3-octanone (15) by two independent groups.

The first short-range male sex pheromone of longhorn beetles has been identified in the grape borer *Xylotrechus pyrrhoderus* as a mixture of (2*S*,3*S*)-octanediol and 2-(*S*)-hydroxy-3-octanone (12); however, the activity of the synthetic sample was 200 times lower than that of the natural product (16). One possible explanation is that highly volatile minor compound(s) may not have been detected under the analytical conditions utilized (12, 14, 15).

Whether the sex pheromone of *A. subfasciatus* is a short- or long-range attractant is yet to be tested in the field, but because **2** and **4** are both volatile compounds, they seem capable of serving as long-range semiochemicals.

The ratio of semiochemicals in a pheromonal bouquet often plays an important role in chemical communication. By collecting airborne volatiles from *A. subfasciatus* males of different ages, we observed that the older the insects, the smaller the ratio of C_6 to C_8 ketols. Females are, therefore, presented with information concerning the age of a potential mate. Whether this information is taken into account as a basis for sexual selection is an intriguing question.

A. subfasciatus males and females congregate on flowers for feeding, and the scents of these flowers have been demonstrated to act as an attractive kairomone; this has led to the development of a trapping system for this species (17). Use of the sex pheromone of *A. subfasciatus* in combination with these floral compounds may pave the way for the development of a practical and effective pest management system for the cryptomeria twig borer.

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