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Identification and synthesis of vesperal, the female sex pheromone of the longhorn beetle *Vesperus xatarti*

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Summary — 10-Oxoisopiperitenone and 10-hydroxyisopiperitenone (for which the trivial names vesperal and vesperol are proposed) were identified using various EIMS (or MS-MS), CIMS data and GC-Fourier transform infrared obtained from the female-specific semiochemicals of the longhorn beetle *Vesperus xatarti*. Their structure was confirmed by synthesis via a Diels-Alder reaction and appropriate oxidation steps. The role of the female sex pheromone for vesperal was demonstrated. Comparison of the HPLC retention time of natural vesperal with those of synthesized pure enantiomers revealed that the natural compound has (*S*)-stereochemistry.

10-oxoisopiperitenone / 10-hydroxyisopiperitenone / mass spectrometry / pheromone / Cerambycidae / *Vesperus xatarti*

Résumé — **Identification et synthèse du vesperal, la phéromone sexuelle femelle du longicorne *Vesperus xatarti*.** Deux nouveaux monoterpènes, la 10-oxoisopipériténone et la 10-hydroxyisopipériténone (pour lesquels les noms triviaux de vesperal et vesperol ont été attribués), ont été identifiés par différentes techniques de spectrométrie de masse, microhydrogénation et infrarouge à transformée de Fourrier dans les collectes d'effluves des femelles du longicorne *Vesperus xatarti*. Leur structure a été confirmée par synthèse. Celle-ci utilise une réaction de Diels-Alder et des aménagements fonctionnels adéquats. Seule l'activité phéromonale du vesperal a été mise en évidence. La configuration absolue (*S*) du vesperal a été établie par synthèse des deux antipodes optiques et comparaison par HPLC des temps de rétention avec le produit naturel.

10-oxoisopipériténone / 10-hydroxyisopipériténone / spectrométrie de masse / phéromone / Cerambycidae / *Vesperus xatarti*

Introduction

Vesperus xatarti Dalman (Coleoptera, Cerambycidae) is a major pest of vineyards in French and Spanish Catalonia. Its biology, known since the 19th century [1], differs considerably from that of other cerambycid beetles. The larvae are not endophytic, but develop as grubs on vine roots. The adults appear in winter. While the males are winged and may be attracted by light, the females are totally apterous and live in the soil. When the night temperature reaches 10 °C, the females climb on the vine plants, mate and start laying eggs.

The weak mobility of females suggested a mating system mediated by a female-emitted pheromone attracting males over a distance. The present paper reports the identification and synthesis of the two female-specific compounds, the precise role of the major compound in the chemical communication of the species is demonstrated.

Identification

Airborne volatile collections from males and females were done using Supelpak™-2 as adsorbent. After elution with dichloromethane, the volatiles were analysed by GC-MS. Only two female-specific compounds were detected under the analytical conditions utilized by using a gas chromatograph linked to an electroantennographic apparatus [2]. The response of the male antenna to the column effluents showed unambiguously that these two female-specific compounds were highly active [Zagatti et al (in preparation)]. Their peaks appeared respectively on BPX-5 column at 8.21 min and 8.99 min. In relation with the structure described below, we proposed the trivial name 'vesperal' and 'vesperol' for these two compounds. Because of the low number of available insects, identification was conducted directly on a crude extract of airborne volatiles containing less than 2 μg of a mixture of vesperal and vesperol (90:10).

Vesperal

CI(NH_4)GC-MS of vesperal gave ions in the upper part of the spectrum at *m/z* (%) 182 (5) and 165 (100) which could correspond to $[M + NH_4]^+$ and MH^+ respectively. Under CI(ND_4)MS, ions at *m/z* 186 eg $[M + ND_4]^+$ and 166 eg $[MD]^+$ were obtained confirming the molecular weight as 164 and further indicating (no supplementary mass shift through H-D exchange) the absence of any mobile hydrogen (ie, hydroxyl group).

* Correspondence and reprints

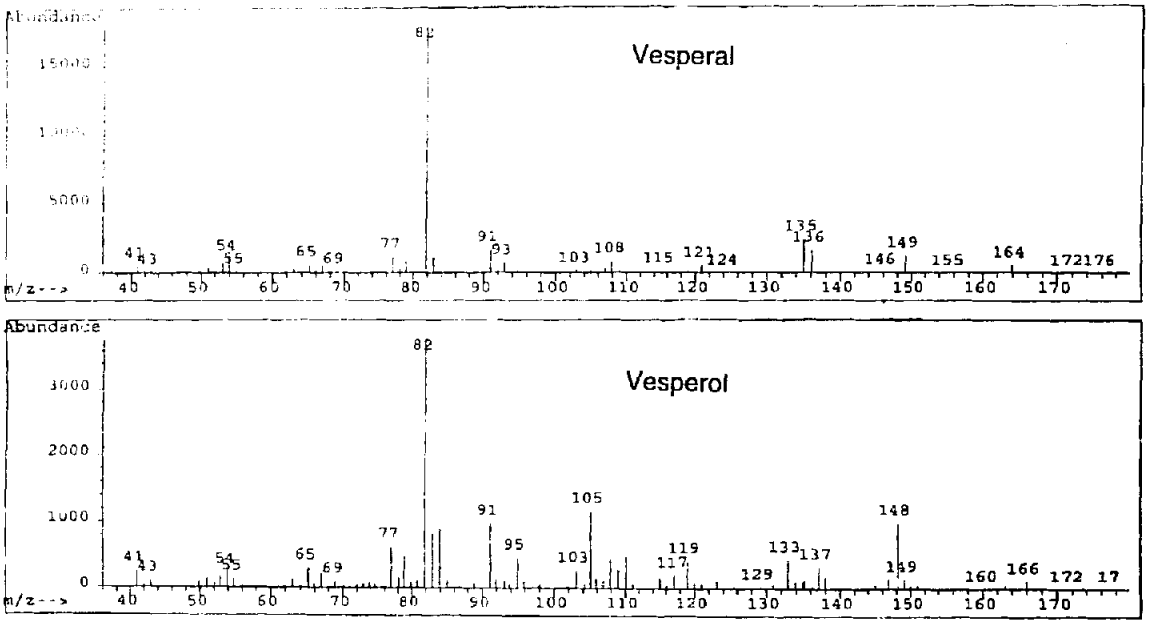
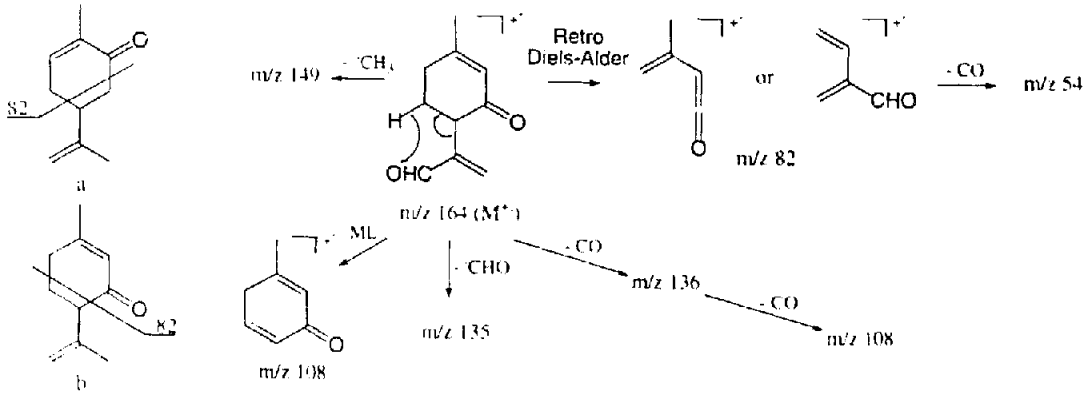


Fig 1. MS (EI) spectra of vesperal **1** major constituent and vesperol **2** minor constituent obtained from the airborne volatiles of *Vespertilio xatartii* females.



Scheme 1

Low-resolution GC-EIMS (fig 1) showed ions at m/z: 164 (1), 149 (3), 136 (5), 135 (6), 121 (1), 108 (3), 91 (4), 82 (100), 79 (3), 67 (4), 54 (19) and 41 (6). Some of these ions might be interpreted as arising from $M^{+\cdot}$ after the loss of CO, $^{\cdot}CHO$ or 2CO (see scheme 1) thus indicating the possible presence of 2 carbonyls (including an aldehyde) and the occurrence of a prominent peak at m/z 82 was reminiscent of terpenes of carvone (a) or isopiperitenone (b) type (MW 150) [3]. The ion at m/z 108 could, however, likely result from a (b)-like structure after a McLafferty rearrangement induced by the aldehyde and/or neighbouring ketone.

High-resolution GC-EIMS measurements assigned as molecular formula $C_{10}H_{12}O_2$ (found m/z 164.0824, calculated for $C_{10}H_{12}O_2$ m/z 164.0837) in agreement with the above assumptions. Furthermore, GC-FTIR showed intense absorptions at 1 689 and 1 657 cm^{-1}, indicating that the two oxygens were involved in (distinct) conjugated carbonyl groups.

Catalytic (Pd/C in pentane) micro-hydrogenation (conditions preserving the carbonyls) led to at least three GC separable diastereoisomers exhibiting: (i) the same EI spectrum with ions at m/z 150 (2), 140 (58), 125 (27), 112 (35), 111 (48), 97 (23), 83 (28), 69 (78), 55 (100), 43 (63) and 41 (73) and (ii) identical Cl(NH_3)MS spectra (ions at m/z 186 ($[M + NH_4]^+$, 7), 169 (MH^+, 100), 151 ($[MH - H_2O]^+$, 3) and 140 (6)). These data thus assigned to vesperal two carbon-carbon double bonds (cf CIMS) and finally the structure of 10-oxoisopiperitenone if taking into account the EIMS ions of the hydrogenated derivatives at m/z 111/69 (α-cleavage on either side of the ketone) and 112 (McLafferty (ML) rearrangement also generated from the cyclic ketone).

This conclusion was confirmed by the low-collision energy CAD spectrum of ion m/z 82 formed under EIMS source conditions. This spectrum (daughter ions at m/z 67 (1.5), 54 (100), 42 (9.5), 39 (23) and 28 (1.5)) was found to be identical to that of the same ion generated from isopiperitenone but differed significantly in ion ratio from that issuing from carvone.

Vesperol

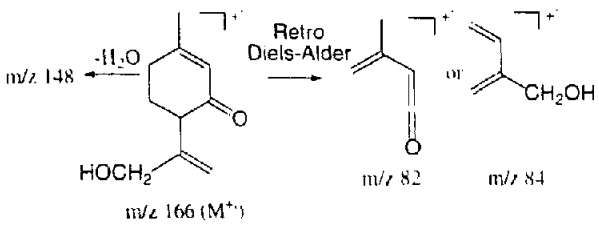
CI(NH_3)GC-MS of vesperol gave ions in the upper part of the spectrum at m/z (%) 184 (20), 167 (100) and 149 (25) which could correspond to $[M + NH_4]^+$, MH^+ and $[MH^+ - H_2O]$ respectively. Under CI(ND_3)MS, ions at m/z 189 eg $[M + ND_4]^+$ and 169 eg $[MD]^+$ were obtained confirming the molecular weight as 166 and further indicating (one supplementary mass shift through H–D exchange) the presence of one mobile hydrogen (ie. hydroxyl group).

Low-resolution GC-EIMS (fig 1) showed ions at m/z: 166 (1), 148 (12), 138 (6), 137 (10), 108 (12), 84 (24), 82 (100), 54 (20). Some of these ions might be interpreted as arising from $M^{+\cdot}$ after the loss of H_2O and retro Diels–Alder reaction as for vesperal (see scheme 2). Finally these data thus assigned to vesperol the structure of 10-hydroxyisopiperitenone ($C_{12}H_{12}O_2$, MW = 166).

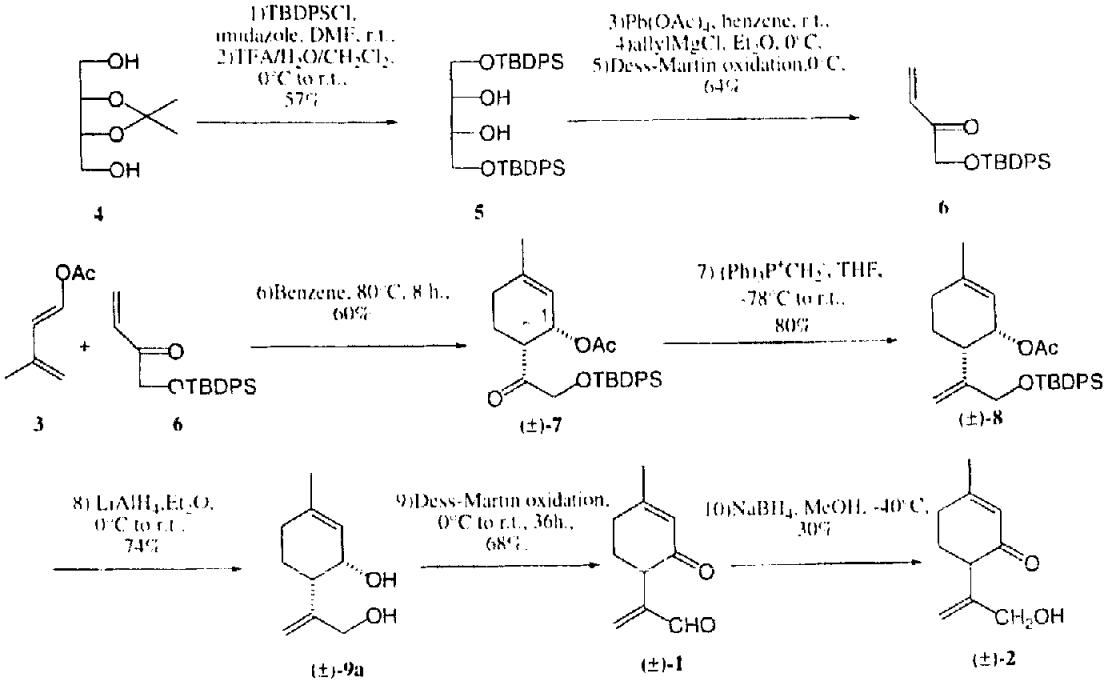
Synthesis of (±)-10-oxoisopiperitenone 1 and (±)-10-hydroxyisopiperitenone 2

Diels–Alder reaction of 3-methyl-1-acetoxybutadiene **3** [4] with enone **6** prepared from diol **4** [5] by standard procedures gave, in one step, compound **7** as a single diastereoisomer (*cis* stereochemistry, $J_{1,6}$ = 2 Hz) with the correct carbon skeleton and appropiate functionalities for conversion to (±)-**1** and (±)-**2** (scheme 3).

Wittig reaction on **7** with the ylide of methyltriphenylphosphonium bromide furnished **8**. Deprotection of the acetate and *tert*-butyldiphenylsilyl groups were performed at the same time with $LiAlH_4$ in ether at room temperature. Careful Dess–Martin [6] oxidation of diol **9a** yielded (±)-10-oxoisopiperitenone **1**. The primary hydroxy group was first fastly oxidized by the triacetoxyperiodinane. The oxidation of the secondary allylic alcohol was more difficult due to the intermediary hemiketal system. Reduction of **1** with $NaBH_4$ at low temperature (− 40 °C) followed by hydrolysis at the same temperature with acetone gave (±)-10-hydroxyisopiperitenone **2** in moderate yield (30%) accompanied by degradation products.



Scheme 2



Scheme 3

The spectroscopic data and GC retention times (of the 'native' and hydrogenated compounds) obtained from both sides (natural and synthetic) being identical led us to finally assign to vesperal the structure of 10-oxoisopiperitenone **1** and to vesperol the structure of 10-hydroxyisopiperitenone **2**.

Biological activity

The biological activity of (±)-**1** and (±)-**2** was measured by recording the electroantennographic (EAG) responses of male antennae to increasing doses of racemic vesperal and vesperol **7**. The results showed the high sensitivity of males to these compounds, with responses not different from those obtained with a natural extract of female volatiles. Field-trapping experiments were conducted in Banyuls (France) from december 1996 to february 1997 using racemic vesperal and vesperol as bait. The results showed that vesperal at the dose of 1 mg caught 126 male beetles in 3 traps whereas vesperol at the same dose caught only 7 male beetles. This result definitely established the role of vesperal as the female sex pheromone of *Vesperus xatarti*. We did not find any biological role for vesperol, assuming it could be only an intermediate in the biosynthesis of vesperal. It will be published with other biological data, in a subsequent paper. Further work is in progress to study the attractiveness of synthetic enantiomers of vesperal.

Absolute configuration assignment of vesperal

One way to expeditiously determine the stereochemistry of pheromones is to obtain enantiomeric resolution on a chiral chromatographic column. The assignments of the peaks can be carried out with samples of known stereochemistry and the absolute configuration of the natural product is determined by comparison of its retention time under the same conditions. Separation of racemic 10-oxoisopiperitenone was achieved by HPLC on a cyclobond I^{TM} column (β-cyclodextrin, H_2O/MeOH 85:15, 1 mL/min, λ = 256 nm, retention time 28.5 and 30.1 min). The natural sample gave only one peak corresponding to the peak of shorter retention time, demonstrating that *Vesperus xatarti* utilizes enantiomeric pure sex pheromone. (*R*)- and (*S*)-10-oxoisopiperitenone were prepared from diol **9a** in five steps. Formation of the diastereoisomeric mixture of (*S*)-(+)-*O*-methylmandelic ester followed by Dess Martin oxidation of the mixture of allylic alcohol **10** and **11** furnished **12** and **13** which were separated by preparative HPLC (μBondapak^{TM} C18, H_2O/CH_3CN (75:25), or Zorbax^{TM} Sil, dichloromethane/isopropanol/water (700:5:0.3)). Products **12** and **13** were separatly hydrogenated in MeOH with Pd/C (10%) with subsequent saturation of the double bonds and abstraction of the ester moiety to yield optically active menthone [3] (scheme 4). By correlation of optically active menthone from **12** with known (2*S*,5*R*)-(−)-menthone obtained by hydrogenation of commercialy available (5*R*)-(+)-pulegone (GC on Lipodex^{TM} capillary column), it is possible to assign the stereochemistry as (2*S*) for **12** and (2*R*) for **13**.

With (*S*)-**12** and (2*R*)-**13** in our hands, we transformed separately these compounds via a reduction oxidation sequence in (*S*)-(+)-10-oxoisopiperitenone ($[\alpha]_D$ = +31.9) and (*R*)-(−)-10-oxoisopiperitenone ($[\alpha]_D$ = −30.3) (scheme 5). We noticed that reduction of **12** or **13** gave a diastereoisomeric mixture (66:33) of diol **9a** and **9b**. Synthetic (*S*)-10-oxoisopiperitenone had the same retention time as the natural sample of vesperal on a chiral HPLC column. It follows that the stereochemistry of the sex pheromone has the (*S*) configuration.

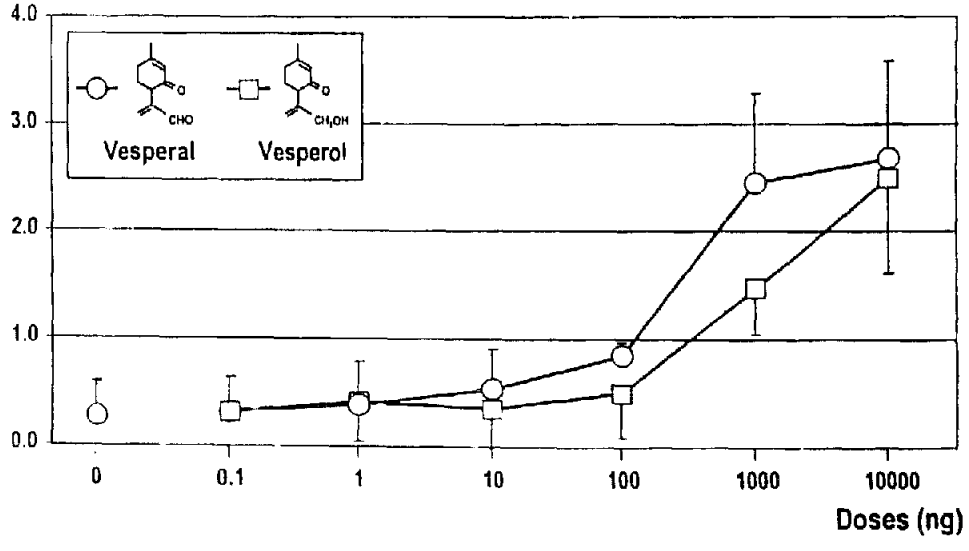
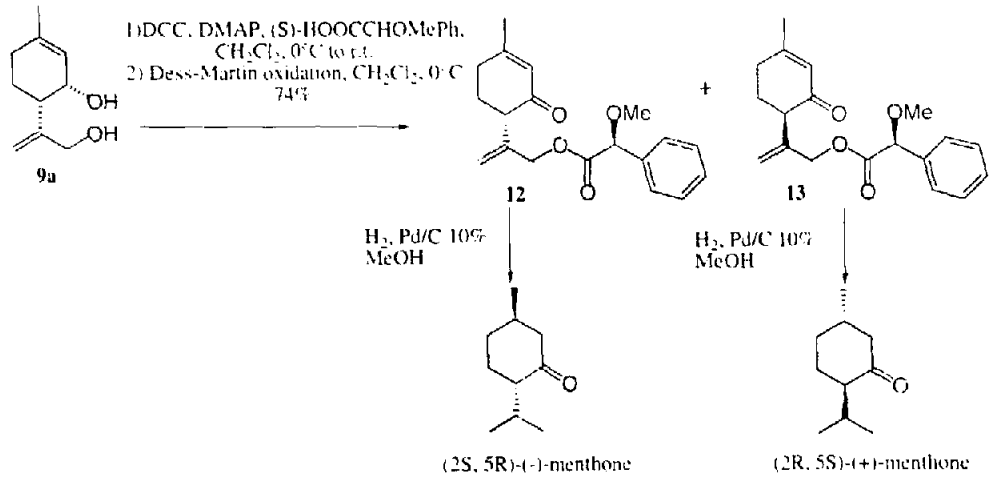
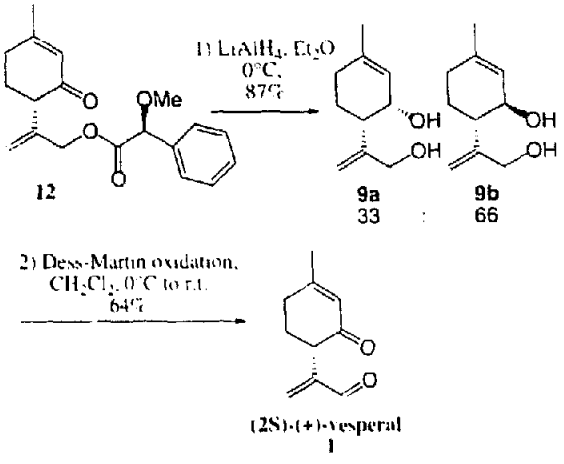


Fig 2. Corrected EAG amplitudes (± confidence intervals on the mean).



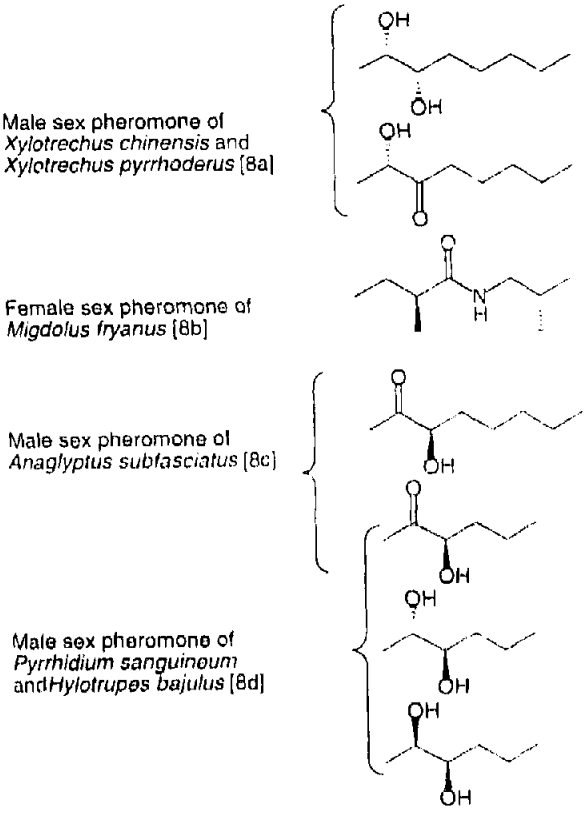
Scheme 4



Scheme 5

Conclusion

We identified two new monoterpenes ((*S*)-10-oxoisopiperitenone (vesperal) and 10-hydroxyisopiperitenone (vesperol)) as semiochemicals for *Vesperus xatarti* and demonstrated the role of pheromone for vesperal. This confirms the high diversity of structures of known male and female pheromones in the Cerambycidae [8] (scheme 6). The use of the sex pheromone of *Vesperus xatarti* may pave the way for the development of a practical and effective pest management system for this insect.



Scheme 6. Structures of sex pheromones of Cerambycidae.

Experimental section

Extraction purification

Conditions of volatile collections: volatiles emitted from batches of 6–12 field-collected females, trapped for 3 nights at 15 °C on 200 mg of Supelpak[TM]-2 (Supelco) with an airflow of 50 mL/min and eluted with 1.5 mL CH_2Cl_2. This procedure gave two extracts of 70 and 100 female/night/equiv. The extracts were concentrated under a gentle stream of N_2 to 100 µL and kept at −20 °C until used.

Spectroscopic measurements on natural products

GC-MS conditions: Nermag R10-10C linked to a Varian 3,300 GC, 25 m × 0.32 mm id, WCOT DB5-MS, 120 to 240 °C at 10 °C/min, helium as carrier gas at 6 psi.

GC/MS/MS conditions: Nermag R30-10 triple quadrupole with the above GC conditions, 7.5 eV collision energy and Ar at 7×10^{-5} Torr.

HRMS conditions for isoprenal: Finnigan MAT-95-Q, 25 m × 0.32 mm id, WCOT BPX-5, 80 °C (2 min) to 240 °C at 10 °C/min, carrier gas helium at 8 psi. Other measurements done for ions *m/z* 136 (found *m/z* 136.0872, calc for $C_9H_{12}O$ *m/z* 136.0888) and 135 (found *m/z* 135.0813, calc for $C_9H_{11}O$ *m/z* 135.0810) were in agreement with our assignments.

GC-FTIR conditions: FTS 15A Digilab, direct deposition interface Freon.

General chemical procedures

Melting points are recorded on a Buchi 510 and are uncorrected. NMR data (^1H: 300 MHz, ^{13}C: 75.5 MHz) are recorded on a VARIAN Gemini 300 instrument. All NMR spectra are recorded in deuteriochloroform ($CDCl_3$). Chemical shifts are reported in δ ppm relative to $CHCl_3$ ($CDCl_3$) as internal reference: 7.27 ppm for ^1H (77.14 ppm for ^{13}C). Coupling constants (*J*) are given in hertz (Hz). Multiplicities are recorded as s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet) and br (broad). In the ^{13}C NMR spectra the symbols s', d', t' and q' represent 0, 1, 2 and 3 attached protons. Mass spectra (MS) were obtained on a Nermag R10-10C linked to a Varian 3300 GC. Ionization was obtained either by electronic impact (EI) or chemical ionization with ammonia (CI, NH_3). Mass spectral data are reported as *m/z*. Infrared spectra (IR) were obtained on a Perkin-Elmer FT 1600 or Perkin-Elmer 397 instrument, using either NaCl salt plates (thin film) or NaCl cells (in the specified solvent) and are reported in terms of frequency of absorption (ν, cm^{-1}). Optical rotations were measured on a Perkin-Elmer 241 polarimeter in a 1-dm cell. All reactions were monitored by thin-layer chromatography (TLC) carried out on E. Merck Ref 5554 precoated silica-gel 60F-254 plates. Visualization was accomplished with UV light, then 7–10% ethanolic phosphomolybdic acid solution or $KMnO_4$ solution followed by heating were used as developing agents. Tetrahydrofuran (THF) and diethyl ether (Et_2O) were distilled from sodium benzophenone, dichloromethane (CH_2Cl_2) and dimethylformamide (DMF) from calcium hydride. Enantiomeric resolution of synthetic isoprenal was achieved on HPLC column with chiral stationary phase (Cyclobond I[TM] β-cyclodextrin) and preparative HPLC on columns (μBondapak[TM] C18, 10 mm, 30 cm × 7.8 mm, Waters ref 84176 or Zorbax[TM] Sil, 5 mm, 250 × 4.6 mm). Enantiomeric resolution of menthone was achieved on a β-cyclodextrin capillary column (Lipodex[TM] E).

• *1-[(tert-Butyldiphenylsilyl)oxy]but-3-en-2-one* **6**

To a stirred solution of **5** [5] (6 g, 10 mmol) in benzene was added $Pb(OAc)_4$ (4.87 g, 10.4 mmol). The reaction mixture was stirred at room temperature for 30 min and filtered. The solid was washed with ether and the combined organic layers evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel (AcOEt/cyclohexane (2:98)) to give 3.2 g (87%) of adduct as a colourless oil. To a 0 °C solution of aldehyde (2 g, 6.7 mmol) in Et_2O (40 mL) under argon was added dropwise vinylmagnesium chloride (1.4 mL, 7.4 mmol, 15% solution in THF). The reaction mixture was stirred at 0 °C for 1 h, diluted with Et_2O and quenched with water. The aqueous phase was acidified with HCl (0.5 N) and extracted with Et_2O. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure. The crude product was dissolved in dichloromethane (12 mL) and Dess–Martin reagent [6] (triacetoxyperiodinane) (4.14 g, 7.3 mmol) added to this solution. The reaction mixture was stirred at room temperature for 30 min, poured into a stirred saturated aqueous solution ($Na_2S_2O_3$, 42 mL), ($NaHCO_3$, 42 mL). The aqueous layer was extracted with dichloromethane (3 × 50 mL), the combined organic layers dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/cyclohexane (5:95) to AcOEt/cyclohexane (10:90)) to give 1.63 g (74%) of **6** as a slightly yellow oil.

IR (neat, cm^{-1}): 3050, 3030, 3000, 2940, 2920, 2850, 1710, 1690, 1600.

^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.7–7.2 (m, 10H), 6.65 (dd, *J* = 10.6 Hz, *J* = 17.5 Hz, 1H), 6.27 (dd, *J* = 1.5 Hz, *J* = 17.5 Hz, 1H), 5.71 (dd, *J* = 1.5 Hz, *J* = 10.6 Hz, 1H), 4.35 (s, 2H), 1.1 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 198.0 (s'), 135.9 (d'), 132.7 (s'), 131.7 (d'), 130.0 (d'), 129.0 (t'), 127.9 (d'), 69.0 (t'), 26.8 (q'), 19.3 (s').

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 140–300 °C, 10 °C/min), retention time 11.49 min.

EI MS *m/z* (%) 267 (15) (M-*t*Bu)$^+$, 268 (58), 247 (2), 231 (1), 211 (6), 196 (12), 189 (100), 135 (19), 91 (11), 77 (21).

• *(1R*, 6R*)-6-{2-[(tert-Butyldiphenylsilyl)oxy]-acetyl}-3-methylcyclohex-2-enyl acetate* **7**

A mixture of **6** (1.3 g, 3.9 mmol) and **3** [3] (1.0 g, 7.9 mmol) in benzene (16 mL) was heated for 8 h 30 min at reflux and overnight at room temperature under argon. The solvent was removed under reduced pressure and the crude product was purified by flash chromatography (AcOEt/cyclohexane (5:95) to AcOEt/cyclohexane (10:90)) to give 1.09 g (60%) of **7** as a colourless oil.

IR (neat, cm^{-1}): 3050, 3030, 3000, 2940, 2910, 2840, 1740, 1720, 1660, 1580.

^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.7–7.3 (m, 10H), 5.75 (m, 1H), 5.65 (m, 1H), 4.25 (AB, *J* = 18.7 Hz, 2H), 3.05 (td, *J* = 11.2 Hz, *J* = 2.0 Hz, 1H), 2.1–1.7 (m, 4H), 1.91 (s, 3H), 1.75 (s, 3H), 1.12 (s, 6H), 1.11 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 208.9 (s'), 170.6 (s'), 142.2 (s'), 135.7 (d'), 132.7 (s'), 130.0 (d'), 127.9 (d'), 118.5 (d'), 69.3 (t'), 66.7 (d'), 46.5 (d'), 29.7 (t'), 26.8 (q'), 23.6 (q'), 21.1 (q'), 19.3 (s'), 18.5 (t').

• *(1R*, 6S*)-6-{2-[(tert-Butyldiphenylsilyl)oxy]-methyl}-ethenyl-3-methylcyclohex-2-enyl acetate* **8**

To a −78 °C solution of methyltriphenylphosphonium bromide (1.1 g, 3 mmol) in THF (10 mL) under argon was added dropwise BuLi (1.5 mL, 3 mmol, 2.1 M solution in hexane). The solution of phosphonium ylide was stirred for 1 h to 0 °C then cooled to −78 °C. A solution of **7** (0.9 g, 2 mmol) in THF (20 mL) was added to the −78 °C solution of phosphonium ylide. The resultant reaction mixture was heated at room temperature overnight and quenched with water. The reaction mixture was extracted with cyclohexane. The combined organic layers were washed with brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was dissolved in pentane and stirred for 1 h at room temperature. The resultant solution was filtered and evaporated under reduced pressure. The crude product was purified by flash chromatography (AcOEt/cyclohexane (5:95)) to give 720 mg (80%) of **8** as a colourless oil.

IR (neat, cm^{-1}): 3080, 3010, 3000, 2950, 2940, 2850, 1730, 1670, 1645, 1580.

^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.7–7.3 (m, 10H), 5.57 (m, 1H), 5.24 (ds, 1H), 5.20 (m, 1H), 4.93 (ds, 1H),

4.18 (s, 2H), 2.35 (td, J = 12.6 Hz, J = 2.3 Hz, 1H), 2.1–1.7 (m, 4H), 1.90 (s, 3H), 1.73 (s, 3H), 1.07 (s, 9H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 170.6 (s'), 148.5 (s'), 141.5 (s'), 135.6 (d'), 133.7 (s'), 129.9 (d'), 127.7 (d'), 119.6 (d'), 110.7 (t'), 67.6 (t'), 66.3 (d'), 40.1 (d'), 31.1 (t'), 26.9 (q'), 23.6 (q'), 22.1 (q'), 21.2 (s'), 19.4 (t').

• *(1R*, 6S*)-6-[1-(Hydroxymethyl)ethenyl]-3-methylcyclohex-2-en-1-ol* **9a**

To a 0 °C solution of $LiAlH_4$ (0.17 g, 2.1 mmol) in Et_2O (20 mL) was added dropwise a solution of **7** (0.96 g, 2.1 mmol) in Et_2O (20 mL). The reaction mixture was stirred at room temperature for 1 h, quenched with water (0.17 mL), NaOH (15%) (0.17 mL), water (0.51 mL) and stirred for 1 h. The resultant mixture was filtered and the solvent removed under reduced pressure. The residue was purified by flash chromatography (AcOEt/cyclohexane (10:90) to AcOEt/cyclohexane (60:40)) to give 263 mg (74%) of **9a** as a white solid. Mp 41–46 °C.

IR (neat, cm^{-1}): 3350, 3010, 2960, 2930, 2950, 2900, 2860, 2820, 1670, 1645.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 5.64 (m, 1H), 5.24 (bs, 1H), 5.02 (bs, 1H), 4.11 (m, 3H), 2.28 (td, J = 12.6 Hz, J = 2.3 Hz, 1H), 2.1–1.5 (m, 4H), 1.73 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 149.7 (s'), 139.8 (s'), 122.5 (d'), 113.7 (t'), 65.4 (t'), 65.33 (d'), 43.7 (d'), 31.1 (t'), 23.5 (q'), 21.1 (t').

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 120–300 °C, 10 °C/mn), retention time 6.32 min.

EI MS m/z (%) 153 (1), 150 (2) ($M^+ - H_2O$)$^{+\cdot}$, 136 (1), 135 (7), 122 (4), 108 (1), 106 (4), 94 (5), 91 (10), 84 (100), 82 (7), 79 (12), 56 (13), 55 (12), 53 (8), 41 (17).

• *(±)-6-(1-Formylethenyl)-3-methylcyclohex-2-en-1-one* **1** *((±)-10-oxosopperatenone)*

To a 0 °C solution of **8** (210 mg, 1.2 mmol) in dichloromethane (25 mL) was carefully added Dess Martin reagent [6] (triacetoxyperiodinane) (1.2 g, 2.75 mmol) for 1 h 30 min under argon. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 36 h and poured into a stirred saturated aqueous solution ($Na_2S_2O_3$, 25 mL), ($NaHCO_3$, 25 mL). The aqueous layer was extracted with dichloromethane (3 × 25 mL), the combined organic layers dried ($MgSO_4$) and solvent evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/cyclohexane (10:90) to AcOEt/cyclohexane (60:40)) to give 135 mg (68%) of **1** as a white solid. Mp 49–50 °C.

IR (CCl_4, cm^{-1}): 3088, 3035, 2977, 2947, 2870, 2825, 2701, 1699, 1677, 1647.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 9.56 (s, 1H), 6.29 (s, 1H), 6.20 (s, 1H), 5.95 (bs, 1H), 4.15 (td, J = 12.3 Hz, J = 4.8 Hz, 1H), 2.45–2.0 (m, 4H), 1.98 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 197.1 (s'), 193.1 (d'), 162.3 (s'), 148.5 (s'), 135.7 (d'), 126.6 (t'), 45.9 (d'), 30.8 (t'), 28.0 (t'), 21.1 (q').

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 120–300 °C, 10 °C/mn), retention time 5.90 mn.

MS data were identical to those of the natural product.

• *(±)-6-[1-(Hydroxymethyl)ethenyl]-3-methylcyclohex-2-en-1-one* **2** *(eupserol)*

To a −40 °C solution of (±)-**1** (13 mg, 0.07 mmol) in methanol (4 mL) was added $NaBH_4$ portionwise (3 mg, 0.07 mmol). The reaction mixture was stirred at −40 °C for 10 mn and quenched with acetone. The solution was warmed to room temperature and solvents evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/cyclohexane (60:40)) to give 1 mg (30%) of (±)-**2** as a slightly yellow oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 5.93 (bs, 1H), 5.26 (bs, 1H), 4.98 (bs, 1H), 4.15 (m, 2H), 3.11 (dd, 1H, J = 4.8 Hz, J = 11.6 Hz), 2.68 (bt, 1H, J = 5.6 Hz), 2.40–1.95 (m, 4H), 1.99 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 200.8 (s'), 163.3 (s'), 146.8 (s'), 126.6 (d'), 114.2 (t'), 66.3 (t'), 50.80 (d'), 31.1 (d'), 27.8 (t'), 24.4 (q').

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 120–300 °C, 10 °C/min), retention 8.40 mn.

MS data were identical to those of the natural product.

• *(1R, 6S)-6-{1-[((2S)-2-Methoxy-2-phenylacetoxy)-methyl]ethenyl}-3-methylcyclohex-2-en-1-ol* **10** *and (1S,6R)-6-{1-[((2S)-2-methoxy-2-phenylacetoxy)-methyl]ethenyl}-3-methylcyclohex-2-en-1-ol* **11**

To a 0 °C solution of **9a** (0.864 g, 5.1 mmol) and (*S*)-(+)-methoxyphenylacetic acid (0.847 g, 5.1 mmol) in CH_2Cl_2 (9 mL) was added dropwise under argon a solution of dicyclohexylcarbodiimide (1.045 g, 5.1 mmol) in CH_2Cl_2 (9 mL). The reaction mixture was stirred at room temperature overnight, filtered and the salts washed with CH_2Cl_2. The combined organic phases were washed with HCl (0.5 N), saturated aqueous solution of sodium bicarbonate, brine and dried on $MgSO_4$. The solvent was removed under reduced pressure and the residue purified by flash chromatography (AcOEt/cyclohexane (40:60)) to give 1.4 g (87%) of a mixture of unseparable **10** and **11** as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.3–7.5 (m, 5H), 5.50 (m, 1H), 5.4–4.4 (ms, 5H), 3.95 (m, 1H), 3.45 (s, 3H), 2.0–1.5 (m, 5H), 1.70 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 170.5 (s'), 141.4 (s'), 141.3 (s'), 139.5 (s'), 136.1 (s'), 128.93 (d'), 128.9 (d'), 128.8 (d'), 127.3 (d'), 127.2 (d'), 122.4 (d'), 114.7 (t'), 114.5 (t'), 81.6 (d'), 67.3 (t'), 67.1 (t'), 64.2 (d'), 64.1 (d'), 57.1 (q'), 42.8 (d'), 42.6 (d'), 30.9 (t'), 23.5 (q'), 20.7 (t'), 20.6 (t').

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 100–300 °C, 10 °C/min), retention time 17.06 min.

EI MS m/z (%) 208 (1), 150 (6), 132 (18), 121 (100), 105 (15), 91 (50), 77 (60), 65 (10), 55 (10), 41(15).

• *(1R, 6R)-6-{1-[((2S)-2-Methoxy-2-phenylacetoxy)-methyl]ethenyl}-3-methylcyclohex-2-en-1-one* **12** *and (1S,6S)-6-{1-[((2S)-2-methoxy-2-phenylacetoxy)-methyl]ethenyl}-3-methylcyclohex-2-en-1-one* **13**

To a 0 °C solution of **10** and **11** (1.1 g, 4.45 mmol) in dichloromethane (80 mL) was added portionwise Dess Martin reagent [6] (triacetoxyperiodinane) (2.10 g, 1.8 mmol) under argon. The reaction mixture was stirred at 0 °C for 2 h and poured into a stirred saturated aqueous solution ($Na_2S_2O_3$, 80 mL), ($NaHCO_3$, 80 mL). The aqueous layer was extracted with dichloromethane (3 × 80 mL), the combined organic layers dried ($MgSO_4$) and solvent evaporated under reduced pressure. The residue was purified by flash chromatography (AcOEt/cyclohexane (50:50)) to give 1.21 g (87%) of a mixture of **12** and **13** as a colourless oil. A part of this mixture was separated by preparative HPLC (μHondapak™ C18, 10 μm, 30 cm × 7.8 mm, Waters ref 84176, H_2O/CH_3CN (75:25), 3.5 mL/min, λ = 254 nm, retention time 72.2 min (**12**) and 76.3 min (**13**) or Zorbax™ Sil, 5 μm, 250 × 4.6 mm, dichloromethane/CH_3CN (75:25), 3.5 mL/min, λ = 254 nm, retention time 6.8 min (**13**) and 8.0 min (**12**)) to give 50 mg of **13** and 60 mg of **12** as colourless oils.

12

$[\alpha]_D$ = +106.3 (*c* 0.90 CH_2Cl_2).

^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.42–7.33 (m, 5H), 5.51 (br s, 1H), 5.10 (s, 1H), 4.91 (s, 1H), 4.78 (s, 1H), 4.69 (AB, J = 8.0 Hz, 2H), 3.42 (s, 3H), 2.83 (t, J = 8.4 Hz, 1H), 2.22 (m, 2H), 1.93 (s, 3H), 1.87 (m, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 198.4 (s'), 170.3 (s'), 162.0 (s'), 141.5 (s'), 136.3 (s'), 128.6 (d'), 128.5 (d'), 127.4 (d'), 126.6 (d'), 115.7 (t'), 82.6 (d'), 66.9 (t'), 57.5 (q'), 50.2 (d'), 30.7 (t'), 27.6 (t'), 21.3 (q').

13

$[\alpha]_D$ = +11.7 (*c* 0.92 CH_2Cl_2).

^1H NMR (300 MHz, $CDCl_3$) δ (ppm) = 7.45–7.33 (m, 5H), 5.84 (br s, 1H), 5.10 (s, 1H), 4.91 (s, 1H), 4.78 (s, 1H), 4.69 (AB, J = 13.3 Hz, 2H), 3.41 (s, 3H), 2.83 (dd, J = 11.0 Hz, J = 5.1 Hz, 1H), 2.21 (m, 2H), 1.93 (s, 3H), 1.87 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 198.3 (s'), 170.1 (s'), 162.0 (s'), 141.5 (s'), 136.2 (s'), 128.6 (d'), 128.5 (d'), 127.2 (d'), 126.4 (d'), 115.5 (t'), 82.4 (d'), 66.7 (t'), 57.3 (q'), 49.9 (d'), 30.5 (t'), 27.5 (t'), 21.1 (q').

- *(2S, 5R)-(+)-Menthone*

A solution of **12** (9 mg, 0.028 mmol) in MeOH with Pd–C 10% (1 mg) was stirred under H_2 at room temperature for 3 h. The resultant solution was filtered on Celite and evaporated under reduced pressure to give quantitatively a mixture (60:40) of menthone, isomenthone and (*S*)-(+)-methoxyphenylacetic acid.

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 100–300 °C, 10 °C/min), retention time 3.68 min menthone, 3.79 min isomenthone and 5.41 min (*S*)-(+)-methoxyphenylacetic acid. Chiral capillary GC analysis (Lipodex^{TM} E): 33.6 min (2*S*, 5*R*)-(+)-menthone.

- *(1R, 3S)-(+)-Menthone*

This compound was obtained from **13** using the same procedure as for (2*S*, 5*R*)-(+)-menthone. Chiral capillary GC analysis (Lipodex^{TM} E): 40.7 min (2*R*, 5*S*)-(+)-menthone.

- *(1R, 6S)-6-(1-Hydroxymethylethenyl)-3-methylcyclohex-2-en-1-ol* **9a** *and (1S,6S)-6-(1-hydroxymethylethenyl)-3-methylcyclohex-2-en-1-ol* **9b**

To a 0 °C solution of $LiAlH_4$ (15 mg, 0.28 mmol) in Et_2O (2 mL) was added dropwise a solution of **12** (30 mg, 0.095 mmol) in Et_2O (2 mL). The reaction mixture was stirred at 0 °C for 1 h, quenched with water (15 mL), NaOH (1 N) (15 mL), water (15 mL) and stirred for 1 h. The resultant mixture was filtered and solvent removed under reduced pressure. The residue was purified by flash chromatography AcOEt/cyclohexane (50:50) to give 13 mg (87%) of the mixture of **9a** and **9b** (33:66) as a white solid.

9b

^1H NMR (600 MHz, $CDCl_3$) δ (ppm) = 5.93 (s, 1H), 5.46 (br s, 1H), 5.00 (s, 1H), 4.90 (m, 3H), 4.05, 4.50 (m, 2H), 2.60 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm) = 144.2 (s'), 136.0 (s'), 129.9 (d'), 112.2 (t'), 72.2 (t'), 66.3 (t'), 46.6 (d'), 30.6 (t'), 27.6 (t'), 23.1 (q').

Capillary GC analysis (BPX-5, 0.32 mm id × 25 m, 100–300 °C, 10 °C/min), retention time 7.7 min (**9b**, 66%), 7.86 (**9a**, 33%).

EI MS *m/z* (%): 168 (1, M^+), 150 (15), 135 (25), 122 (12), 94 (60), 91 (55), 84 (100), 82 (75), 79 (55), 55 (25).

- *(1S,6R)-6-(1-Hydroxymethylethenyl)-3-methylcyclohex-2-en-1-ol* **9a** *and (1R,6R)-6-(1-(hydroxymethyl)ethenyl)-3-methylcyclohex-2-en-1-ol* **9b**

These compounds were obtained using the same procedure as for **9a** (1*R*, 6*S*) and **9b** (1*S*, 6*S*) from **13** (yield = 87%).

- *(S)-(−)-6-(1-Formylethenyl)-3-methylcyclohex-2-en-1-one* **1** *(resperal)*

To a 0 °C solution of **9a** (1*R*, 6*S*) and **9b** (1*S*, 6*S*) (11 mg, 0.083 mmol) in dichloromethane (2 mL) was added carefully a solution of Dess–Martin reagent [6] (triacetoxyperiodinane) (80 mg, 0.18 mmol) in dichloromethane (1 mL) for 1 h under argon. The reaction mixture was stirred at 0 °C for 2 h and at room temperature for 24 h and then poured into a stirred saturated aqueous solution ($Na_2S_2O_3$, 25 mL), ($NaHCO_3$, 25 mL). The aqueous layer was extracted with dichloromethane (3 × 2 mL), the combined organic layers dried ($MgSO_4$) and the solvent evaporated under reduced pressure. The residue was purified by flash chromatography AcOEt/cyclohexane (10:90) to AcOEt/cyclohexane (60:40) to give 11 mg (60%) of (+)-**1** as a white solid.

$[\alpha]_D$ = +31.9 (*c* 0.77 CH_2Cl_2).

- *(R)-(+)-6-(1-Formylethenyl)-3-methylcyclohex-2-en-1-one* **1**

This compound was obtained using the same procedure as for (−)-**1** from the mixture of **9a** (1*S*, 6*R*) and **9b** (1*R*, 6*R*) (yield = 64%).

$[\alpha]_D$ = −30.3 (*c* 1.8 CH_2Cl_2).

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