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Sex pheromone of the oleander scale, *Aspidiotus nerii*: Structural characterization and absolute configuration of an unusual functionalized cyclobutane

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ABSTRACT **The sex pheromone emitted by the female oleander scale, *Aspidiotus nerii* (Homoptera, Diaspididae), has been isolated and characterized as (1*R*,2*S*)-*cis*-2-isopropenyl-1-(4′-methyl-4′-penten-1′-yl)cyclobutaneethanol acetate by using advanced MS and NMR spectroscopic methods, as well as a variety of microderivatization sequences. The structure has been confirmed by stereo- and enantioselective synthesis of the four possible stereoisomers. The absolute configuration has been determined by comparison of the activity of the *cis* (1*S*,2*R*) and (1*R*,2*S*) enantiomers with that exhibited by the natural material in greenhouse bioassays and field tests. The structure of this sesquiterpenoid pheromone is new in the coccids and in the pheromone field in general.**

The oleander scale, *Aspidiotus nerii* Bouché, formerly *A. hederae* (Homoptera, Diaspididae), is a cosmopolitan pest, mainly found in tropical and subtropical areas, particularly in the Mediterranean countries. It is polyphagous and has been reported from hosts corresponding to more than 100 plant families (1). Particularly important is the damage caused on lemon and olive trees and ornamental plants, such as oleander. The scale is sap-sucking, causing a general weakening of the tree, discoloration of leaves, and severe deterioration of the fruit (2). *A. nerii* has parthenogenetic (or uniparental) and bisexual (or biparental) populations, but it remains controversial whether both forms correspond to one or two separate species (3). Nevertheless, it seems generally accepted at the present time that *A. nerii* is at least the biparental form. The life cycle of the insect has two nymphal instars before molt to the adult female and four to the adult male. The adult female is immobile, but the male has two wings and, therefore, is able to fly. The sex pheromone of the scale presumably is produced by the female's pygidial glands and released through the rectum as in *Aonidiella aurantii* and *A. citrina* (4).

Studies were initiated independently in the United States and in France on the pheromone of this species not only because a sensitive pheromone-monitoring trap was needed for this pest, but also because pheromone structures identified in other armored scale species were found to be unusual (5–9). We report herein studies directed on the identification of the sex pheromone of the oleander scale by GC-MS, low-energy MS-MS, ^{1}H NMR, microchemical reactions, and, finally, by high-resolution two-dimensional ^{1}H-^{1}H and ^{1}H-^{13}C NMR correlations. The proposed structure, a sesquiterpenic functionalized cyclobutane acetate, has been confirmed—including its absolute configuration—by synthesis and biological activity in greenhouse bioassays and field tests.

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MATERIALS AND METHODS

Insects. The insect laboratory colonies used in this study were established from crawlers collected in Greece from olive trees and in Israel from oleander leaves. The rearing was achieved on "Bintje" potato tubers at 25°C and 70% relative humidity under 12-h light/12-h dark photoperiod. Virgin females were obtained by eliminating males at the second molt stage (0.1% triprene sprays) (10).

Pheromone Collection and Purification. The pheromone was obtained by airborne volatile trapping using Porapak Q (Waters) as adsorbent (11). In France, pheromone collections typically were made by drawing purified air (1.8 liters/min) over 20–50 scale-infested potatoes (*ca.* a few thousands of sexually mature females) contained in a 10- (or 20)-liter reaction vessel and through a 1.2 × 7-cm glass tube containing Porapak Q (*ca.* 2 g). After a 2-week period under ambient conditions of light and temperature, extraction of the trap with 25 ml of pesticide grade hexane followed by concentration (100 μl) yielded 1–3 μg of pheromone. A number of collections were combined and concentrated. The combined extracts were subjected to fractionation either directly by micropreparative GLC (France) or after a prior Florisil chromatography (USA). In the latter case, chromatography was performed on 9.2 g of Florisil (7% water) in a 50 × 1.5-cm glass column. The column was eluted with 20 ml hexane and then 50 ml hexane/ether (100:5). Fractions (10 ml each) were collected and bioassayed. Activity was located in the fourth fraction. This fraction was combined with the fourth fractions from other runs and the solvent was evaporated. The residue was taken up in 200 μl hexane and placed in a 1-ml dram vial. It was concentrated further under a stream of nitrogen and collected from an OV-101-packed GLC column (2 m × 4 mm i.d.) at 180°C. Biological activity was located on a peak eluting at 10.7 min, relative to heptadecane at 9.2 min and octadecane at 13.8 min. Multiple collections were made to obtain material for further analyses. After this procedure *ca.* 900 μg pheromone was finally purified. The direct micropreparative GLC fractionation (French group procedure) was carried out on a 2 m × 4-mm i.d. SE-52 (5% on 100–120 mesh Gas Chrom Q) stainless-steel column by using nitrogen as carrier gas and an effluent splitter. The splitter diverted 90% of the chromatographic effluent to a U-glass capillary tube immersed in liquid N_2.

Analytical Procedures. GLC was carried out on a Girdel 300 (40 m × 0.4 mm SE-52 fused silica capillary column, helium as carrier gas and flame ionization detection). A Perkin–Elmer 3920 was used for micropreparative GLC. Mass spectrometry was carried out on a Nermag (Quad Service, Poissy, France)

Abbreviations: EI, electron impact; CI, chemical ionization.

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R-10–10 C gas chromatograph–mass spectrometer (GC-MS) or a R-30–10 triple quadrupole (MS-MS analyzer) instrument under computer control and the following electron impact (EI) source conditions: electron energy, 70 eV; filament current, 150 μA; temperature, 200°C. Chemical ionization (CI) was performed at 95 eV electron energy and 130°C source temperature by using NH_3 or NO as reagent gas at 10^{-4} torr (1 torr = 133 Pa) and 2×10^{-5} torr measured in the source housing, respectively. For MS-MS experiments, collisional activated dissociation was achieved with Ar at 3×10^{-2} torr and 20 eV collision energy. Samples were introduced via a Ros injector on a 25 m $\times$ 0.25-mm i.d. CPtm Sil 5 (Chrompack, Middelburg, The Netherlands) fused silica capillary column with helium as the carrier gas.

NMR spectra were carried out in $CDCl_3$ or C_6D_6 solutions on Bruker AM400 (operating at 400 MHz for ^1H or 100.5 MHz for ^{13}C) or AMX600 (600 MHz for ^1H) instruments. The chemical shifts are expressed in ppm relative to the residual solvent ($CHCl_3$ at δ 7.27 ppm or C_6H_6 at δ 7.15 ppm in ^1H NMR, with the central peak of the C_6D_6 ^{13}C signal at δ 128.7 ppm in ^{13}C NMR spectra).

Microderivatization. Hydrolysis of a pheromone sample typically was carried out by treating a 20-μl (*ca.* 20 ng pheromone) aliquot of GLC-collected material with 100 μl of 95% ethanol containing two drops of 10% NaOH. Hexane (0.5 ml) and water (0.5 ml) were added after 1 h at room temperature. The aqueous layer was washed with hexane, and the combined organic layers were concentrated in a 4-ml dram vial under a stream of nitrogen. Reacetylation was performed by taking an aliquot of the hydrolyzed product and adding 2 drops of pyridine and 2 drops of acetic anhydride. The reaction mixture was treated with 0.5 ml of aqueous saturated $NaHCO_3$ and 0.5 ml of hexane after 15 h at room temperature. The organic layer was decanted and the aqueous layer was washed with additional hexane. The hexane washings were combined and concentrated to *ca.* 100 μl.

Microhydrogenation was performed for 1 min on a 10- to 20-μl hexane extract (*ca.* 0.5 μg of accumulated GLC-purified material) diluted in methanol (20 μl) in the presence of a catalytic amount of PtO_2. An aliquot containing *ca.* 200 ng of hydrogenated alcohol solution was evaporated and redissolved in 50 μl of hexane. A few drops of freshly prepared triphenylphosphine bromide in methylene chloride were added, and the reaction was held at room temperature overnight. Then a few drops of water were added, the mixture was extracted with hexane several times, and the hexane extracts were combined and concentrated under nitrogen. Hydrogen skeletons were generated by two methods. In the first method, a pinch of $NaBH_4$ and a few drops of DMSO were added to OV-101 GLC-collected bromide prepared as above, the reaction was left for 2 h at room temperature, and then 0.5 ml of water was added. The reaction mixture was extracted with hexane several times, and the hexane extracts were combined and concentrated under nitrogen. The second method used lithium aluminum hydride with $Pt(Al_2O_3)$ catalyst and was performed on the alcohol (*ca.* 100 ng) according to the literature procedure (12).

Laboratory Bioassays. Bioassays were conducted in Petri dish lids (95-mm diameter). Hexane solutions of the GLC-collected fractions were deposited and evaporated on squares of filter paper (1.5 $\times$ 1.5 cm, Whatman) placed in the center. Males (5–10 per set) were placed around the odorant source within a few-centimeters distance, and their behavior was analyzed during a 5-min exposure to determine the number of individuals that responded positively. A response was counted positive when a male was attracted to the source and stayed on it at least 1 min. The males were collected phototropically from potato tubers during the emergence period and used immediately for assays.

Greenhouse Bioassays. The bioassay is similar to that described previously (5, 6). An aliquot of the sample to be tested was spotted on a 2.4-cm-diameter filter paper disk that was placed on a 3.5-cm steel planchet. The planchet was set in the middle of a wooden block having two vertical split rods that held a 12.7 $\times$ 7.5-cm sticky card in a vertical position above the planchet. The holders with planchet and sticky card were positioned around the outside of a 1.8-m-diameter turntable placed in the greenhouse and turned at a 0.17 rpm rate. Trays of scale-infested potatoes were set out in the greenhouse around the turntable in the afternoon during the male flight period, and then the sticky cards collected and the number of attracted males were counted for each treatment. Synthetic compounds were assayed individually at a dose rate of 30 μg on 5 $\times$ 9-mm rubber septa (Thomas).

Field Tests. In Israel, traps consisting of white sticky cards (20 cm $\times$ 10 cm) with a rubber-septum lure (30 μg/compound) were placed randomly around a tall oleander tree (*ca.* 5 m high, 6 m diameter) at a height of 1.5 m and at a distance of 2 m from the canopy edge. Two replicates of the treatments were left in the field from November 11, 1997, to December 8, 1997, and then were collected, and the male scales were counted.

RESULTS

Mass Spectra of Purified Pheromone. Crude pheromone extracts resulting from the first airborne collections were bioassayed and the active fraction was obtained after micropreparative GLC. This fraction consisted of a unique compound that gave the following spectroscopic data. The EI-MS spectrum (Fig. 1*a*) exhibited fragment ions (relative intensities in parentheses) at *m/z* 204 (4), 189 (4), 161 (9), 121 (72), 107 (45), 93 (75), 68 (95), and 43 (100). The presence of an acetate group could be deduced from the ions at *m/z* 204, 189, and 161 that could be interpreted as $[M - AcOH]^{+\cdot}$, $[M - AcOH - \cdot CH_3]^+$, and $[M - AcOH - \cdot C_3H_7]^+$, respectively. A NH_3-CI-MS spectrum showed ions at *m/z* 282 ($[M + NH_4]^+$, 100), 265 ($[M + H]^+$, 8), and 205 ($[M + H - AcOH]^+$, 95), and a molecular mass of 264. Catalytic microhydrogenation yielded a unique derivative (Scheme 1) that produced a NH_3-CI-MS spectrum with ions at *m/z* 286 ($[M + NH_4]^+$, 100) and m/z 269 ($[MH]^+$, 4), which indicated the existence of two unsaturations in the initial compound. The same +4u shift was observed in some ions of the EI spectrum (see high mass region): *m/z* 208

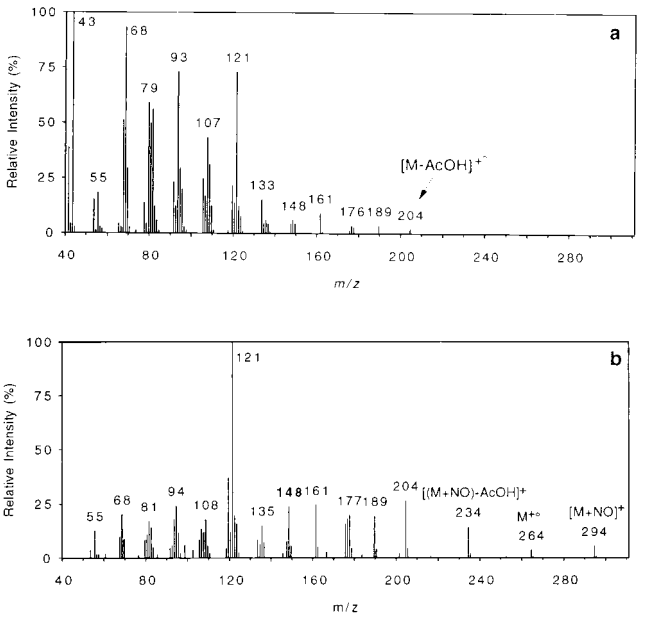
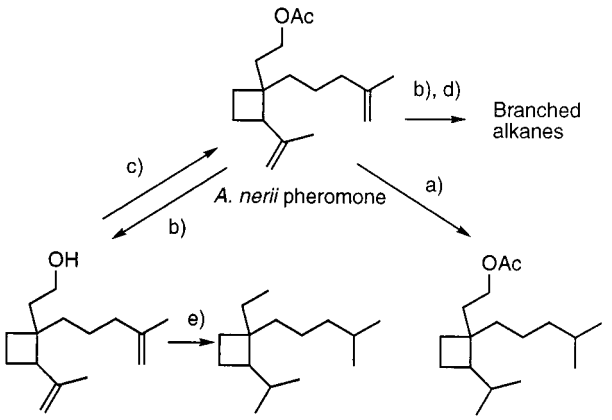


FIG. 1. EI (*a*) and NO^+-CI (*b*) mass spectra of *A. nerii* pheromone obtained under GC-MS conditions.



SCHEME 1. Microderivatizations performed on *A. nerii* pheromone and resulting structures. (*a*) H_2/PtO_2; (*b*) 5% NaOH in 95% EtOH; (*c*) Ac_2O, pyridine; (*d*) $Pt(Al_2O_3)$, $LiAlH_4$; (*e*) (*i*) H_2, PtO_2, EtOH, (*ii*) Br_2, PPh_3, hexane/CH_2Cl_2, (*iii*) $NaBH_4$, DMSO.

($[M - AcOH]^{+\bullet}$, 1), 193 ($[M - AcOH - \cdot CH_3]^+$, 3), 179 ($[M - AcOH - \cdot C_2H_5]^+$, 5), 165 ($[M - AcOH - \cdot C_3H_7]^+$, 10), 123 (45), 95 (100), 82 (65), 81(42), 69 (48), 68 (70), and 43 (65). Thus, in the absence of any further oxygen function (which was confirmed later by high-resolution EI-MS and NMR), the pheromone was proposed to be a cyclic, diunsaturated C_{15} acetate.

Microreactions of Purified Pheromone. A number of other microreactions were performed to determine the structure of the carbon skeleton (Scheme 1). At first, alkaline hydrolysis was achieved to confirm the existence of the acetate. This reaction gave an alcohol (according to the new spectroscopic data) that was inactive toward *A. nerii* males. However, biological activity was recovered through "reacetylation," and the regenerated compound was found identical (GLC, MS) to the natural pheromone. The alcohol also was derivatized according to two different sequences to obtain more structural information: hydrogenolysis by the $Pt(Al_2O_3)$/lithium aluminum hydride system (12) and bromination of the hydrogenated alcohol followed by sodium borohydride reduction in DMSO (13). The first process gave *ca.* seven compounds of apparently branched hydrocarbon type according to the MS, which indicated C_2—C_3 and/or C_5—C_6 alkyl substituents. The complexity of the reaction might result from the various opening sites of the initial ring (C_3 and C_4 rings being the most probable candidates) through the cleavage of a σ carbon–carbon bond and eventual chain shortening. In contrast, the second microreaction sequence produced a single, substituted cyclic hydrocarbon of 210 molecular mass that corresponded to a $C_{15}H_{30}$ molecular formula [EI-MS ions at m/z 210 ($M^{+\bullet}$, 6), 182 ($[M - C_2H_4]^{+\bullet}$, 6), 140 ($[M - C_5H_{10}]^{+\bullet}$, 23), 97 ($[C_7H_{13}]^+$, 38), 70 ($[C_5H_{10}]^{+\bullet}$, 100), and 69 ($[C_5H_9]^+$, 61).

NMR Spectra of Purified Pheromone. ^1H NMR was recorded at this stage on various 5- to 15-μg purified samples and produced signals (Fig. 2*a* as an example; conditions: $CDCl_3$, 400 MHz) for four vinylic protons between δ 4.89 and 4.69 ppm, which correspond to two CH_3—C═CH_2 subunits (supported by resonance of two methyl groups appearing as broad singlets in the 1.6- to 1.8-ppm region). Additional resonances confirmed the presence of an acetate $CH_2CH_2OCOCH_3$ (δ 4.03, 2H, t and δ 2.04, 3H, s) and indicated a possible allylic proton (δ 2.66, t) of unusual downfield resonance. Although a cyclopropane was hypothesized at first because of the possible presence of a small signal at δ 0.88 ppm (Fig. 2*a*), a trisubstituted cyclobutane (compound **1** or **2**, Fig. 3) of grandisol **3** (or its acetate **4**) type was finally considered as a more reasonable candidate. This structure was supported by the similarity of some of the chemical shifts (especially that of the allylic proton on the C_4 ring) observed in both types of compounds when examined under the same conditions (C_6D_6 or $CDCl_3$, 400 MHz). This hypothesis also was consistent with the main odd-electron ions produced under EI-MS (pheromone or derivative obtained through sequence 2). Ions at m/z 68 (first compound) and at m/z 70 (and its complement at m/z 140, for

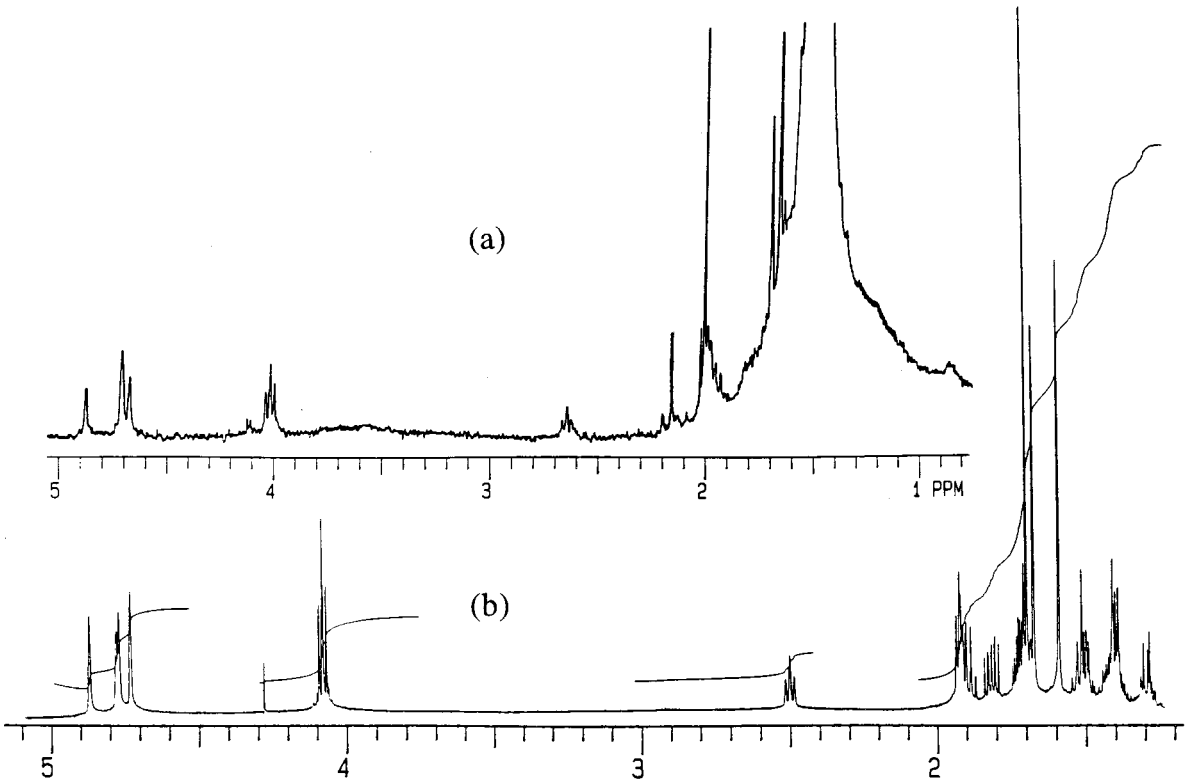


FIG. 2. ^1H NMR spectra obtained on 13 μg ($CDCl_3$, 400 MHz) (*a*) and on 900 μg (C_6D_6, 600 MHz) (*b*) of *A. nerii* pheromone.

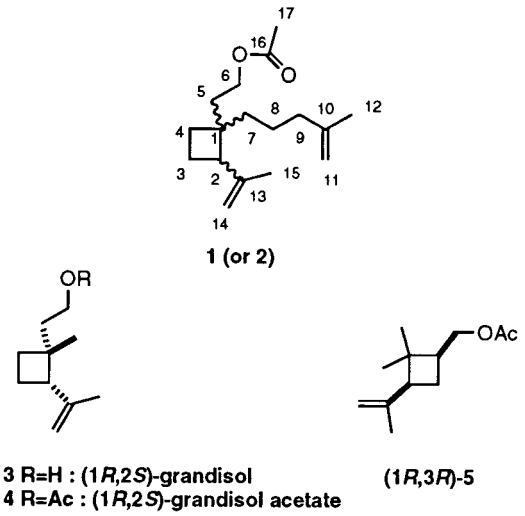


FIG. 3. Structures of *A. nerii* pheromone (**1**/**2**), grandisol (**3**), grandisol acetate (**4**), and citrus mealybug pheromone (**5**).

the derivative) therefore might result from a *retro*cycloaddition decomposition of the C_4 ring giving rise to $[H_2C{=}CH{-}C(CH_3){=}CH_2]^{+\cdot}$ and $[H_2C{=}CH{-}CH(CH_3)_2]^{+\cdot}$ (or its corresponding neutral) species, respectively. This process likely is induced directly from the molecular ion. Moreover, it appears favorable because it leads to a conjugated fragment ion in the case of the pheromone or because of the absence of any competitive pathway in the unfunctionalized cyclobutane. This decomposition does not occur with the hydrogenated pheromone molecular ion, which undergoes fast elimination of an acetic acid molecule. It is assumed that the $[M - AcOH]^{+\cdot}$ ion is rearranged immediately into a dienic-branched hydrocarbon species before any further decomposition.

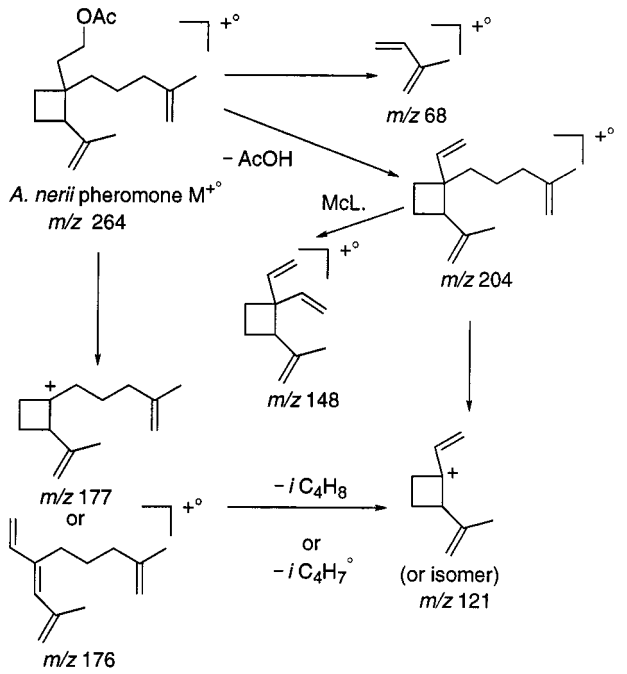
Later cumulative pheromone collections made over several years provided us with *ca.* 900 μg of purified pheromone. This unusual amount of pheromone available allowed us to record ^1H (600 MHz) (Fig. 2*b*) and ^{13}C NMR (100.5 MHz) spectra as well as two-dimensional ^1H—^1H COSY (600 MHz) and ^1H—^{13}C heteronuclear correlations (reverse mode, 400 MHz) in C_6D_6. Resonances were observed for 28 protons including 3 methyl groups and 17 carbon atoms. The ^{13}C spectrum showed resonances for three methyl groups, nine methylene carbons (two olefinic ones), four quaternary carbons (only one in the aliphatic region), and one methine carbon. Because of the extensive signal overlapping in the aliphatic region, the ^1H NMR spectrum first had to be analyzed through the ^1H—^{13}C correlations, which allowed the determination of the J^2 connectivities as shown in Table 1. The ^1H—^1H COSY spectrum then could be interpreted as containing six isolated spin systems. Extensive homonuclear decoupling experiments also were performed and allowed observation of small coupling constants (<1 Hz) between the olefinic protons of the isopropenyl moieties and either the methine proton at 2.50 ppm or the methylene protons at 1.90 ppm. Thus, the result was the determination of only three isolated spin systems: (*i*) $CH_2{-}CH_2{-}OAc$, (*ii*) $CH_2{=}C(CH_3){-}CH_2{-}CH_2{-}CH_2$, and (*iii*) $CH_2{=}C(CH_3){-}CH{-}CH_2{-}CH_2$. Because the ^{13}C NMR spectrum revealed the occurrence of only one quaternary carbon in the aliphatic region (45.4 ppm), the 1,1,2-trisubstituted cyclobutane **1**/**2** was the only structure to be compatible with these data.

Table 1. ^1H and ^{13}C chemical shifts (ppm) of *A. nerii* pheromone

Carbon no.	^{13}C	^1H
1	45.4 s	
2	50.0 d	2.50 (bt, *J* 9.0)
3	20.2 t	1.90, 1.69
4	28.5 t	1.51
5	39.3 t	1.80, 1.70
6	62.1 t	4.09 (t, *J* 7.5)
7	23.3 t	1.70, 1.39
8	40.6 t	1.39, 1.28
9	32.5 t	1.90
10	146.2 s	
11	111.2 t	4.80 (bs)
12	23.1 q	1.66 (bs)
13	145.6 s	
14	111.6 t	4.89 (bs), 4.79 (bs)
15	24.6 q	1.57 (bs)
16	170.8 s	
17	21.3 q	1.69 (s)

600 and 100.5 MHz, C_6D_6, coupling constants (*J*) are expressedin Hz.

High-Resolution EI-MS and MS-MS Spectra. The same sample was used for further confirmation. High-resolution EI-MS measurement of the molecular ion was consistent with a $C_{17}H_{28}O_2$ molecular formula (found, 264.2082; calculated, 264.2089). Low-energy collisional activated dissociation experiments also were performed to determine the origin and structure of ion *m/z* 121, one of the major EI fragment ions. Its daughter ion spectrum [ions at *m/z* 93 (78), 79 (46), 55 (68) 43 (42), 41 (27), and 29 (100)] was very close to that provided by grandisol acetate **4** (with an ion at *m/z* 105 of higher abundance in the latter). It was obvious, however, when examining its parent ion spectrum [ions at *m/z* 264 (<1), 204 (7), 181 (8), 177 (100), 176 (84), and 136 (100)] that the ion at *m/z* 121 had several origins and was likely a mixture of isomeric structures (Scheme 2). It was also found that when operating under low-energy charge exchange nitric oxide (NO) ionization (14), some ions were enhanced (Fig. 1*b*). Particularly interesting is the ion at *m/z* 148, which is produced exclusively through ion *m/z* 204 by a McLafferty rearrangement induced in the C_6H_{11} side chain (as shown by the parent ion spectrum under NO^+-CI). This ion could serve as a diagnostic ion in the characterization of analogues and/or precursors of the present pheromone.



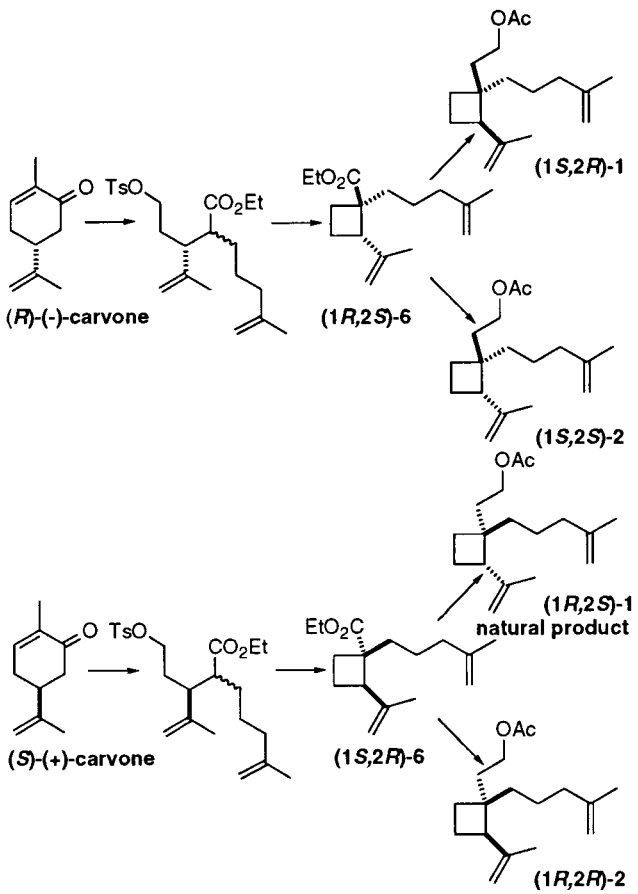
SCHEME 2. Main decomposition pathways of the *A. nerii* pheromone molecular ion $M^{+\cdot}$ according to EI-MS, EI-MS-MS, and NO^+-CI-MS data.

Stereochemistry and Synthesis of Pheromone. The last step for the complete identification of the pheromone was to define the stereochemistry. Our unsuccessful attempts to prepare crystals (from the pheromone or via derivatives) for x-ray diffraction, as well as the limited information provided by nuclear Overhauser effect spectroscopy experiments, led us to undertake the stereoselective synthesis of the racemic material as well as the enantioselective synthesis of the four possible stereoisomers. Preparation of the racemic compound was accomplished from (*Z*)-4-hexenol as starting material, whereas the enantioselective strategy used (*R*)- and (*S*)-carvone in pure enantiomeric forms. This latter process is shown schematically in Scheme 3. The synthetic approaches will be reported elsewhere in detail.

The (1*S**,2*R**) relative stereochemistry of the natural pheromone was unambiguously determined by achiral GLC coinjection with compounds **1** and **2** prepared by the enantioselective route (9.66 min for **2** vs. 9.79 min for **1** and the pheromone on DB5-MS, 0.32 mm i.d. × 30 m, 120–300°C at 10°C/min). Furthermore, diastereomer **1** (either enantiomer) was found to exhibit the same spectroscopic data (EI and NH_3-CI-MS, ^1H and ^{13}C NMR) as those from *A. nerii* pheromone. GLC separation of the synthetic enantiomers (1*S*, 2*R*)-**1** and (1*R*,2*S*)-**1** on chiral phases was unsuccessful, so the absolute configuration of the pheromone was determined tentatively through greenhouse and field bioassays.

Field and Greenhouse Bioassays. The greenhouse bioassay was conducted for 7 days with total oleander males captured as follows: (1*R*,2*S*)-**1** = 106; (1*S*,2*R*)-**1** = 2; (1*S*,2*S*)-**2** = 10; and blank = 7.

The field test conducted in Israel gave the following total male captures: (1*R*,2*S*)-**1** = 492; (1*S*,2*R*)-**1** = 8; (1*S*,2*S*)-**2** = 5; and blank = 15.



SCHEME 3. Enantioselective synthesis of compounds **1** and **2**.

DISCUSSION

Cyclobutane derivatives are an important class of compounds because they are involved in many organic transformations (15–17), and their skeleton is common to several natural products (18–20). However, insect pheromones and/or attractants containing a cyclobutane function are relatively uncommon (21). Grandisol, (1*R*,2*S*)-(+)-*cis*-2-isopropenyl-1-methylcyclobutaneethanol (**3**), is the most important constituent of the male-produced aggregation pheromone of the cotton boll weevil, *Anthonomus grandis* (22), and has been found in other beetles, such as *Trypodendron signatum* (23), *Pityophthorus pityographus* (24), *Pityogenes bidentatus, P. quadridens*, and *P. calcaratus* (25), and *Curculio caryae* (26). Its *trans* isomer, fragranol, has been identified in extracts of the plant *Artemisia fragrans* (27). Grandisol and its oxidation product, grandisal, also have been found as aggregation pheromone components of *Pissodes strobi* and *P. nemorensis* (28, 29). A structurally related analogue, (1*R*,3*R*)-(+)-*cis*-2,2-dimethyl-3-isopropenyl-cyclobutanemethanol acetate (**5**), has been described as the sex pheromone of the citrus mealybug *Planococcus citri* (30).

The pheromone produced by the female oleander scale, *A. nerii* (1*R*,2*S*)-*cis*-2-isopropenyl-1-(4′-methyl-4′-penten-1′-yl)-cyclobutaneethanol acetate (**1**), contains an unusual trisubstituted cyclobutane structure. The sesquiterpene skeleton of the pheromone resembles that of the monoterpene grandisol, with the exception of a methyl-substituted C_5 alkenyl chain instead of the methyl group being attached to the quaternary center of the cyclobutane ring. The structure has been characterized fully by spectroscopic and chromatographic analyses, along with a variety of microreactions, and confirmed by stereoselective and enantioselective independent syntheses. The absolute configuration of the active enantiomeric form, which has been unequivocally determined by greenhouse bioassays and field tests, is similar to that of grandisol.

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