Identification of α-Santalenoic and *endo*-β-Bergamotenoic Acids as Moth Oviposition Stimulants from Wild Tomato Leaves

Robert M. Coates* and Jon F. Denissen[1]

Department of Chemistry, University of Illinois, 1209 W. California St., Urbana, Illinois 61801

John A. Juvik* and Barbara A. Babka

Department of Horticulture, University of Illinois, 1103 W. Dorner Dr., Urbana, Illinois 61801

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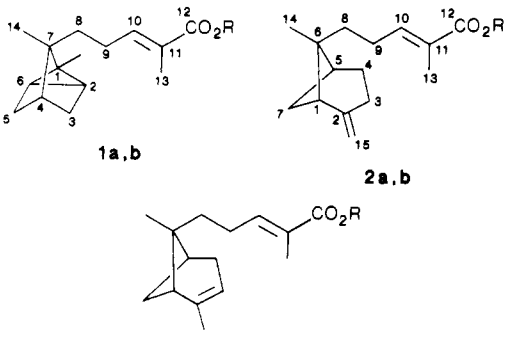
The presence of oviposition-stimulating phytochemicals in hexane extracts of whole leaves of wild tomato (*Lycopersicon hirsutum*) accession LA 1777 was indicated by oviposition preference assays with gravid female *Heliothis zea* (Boddie) moths. Three sesquiterpenes isolated from these extracts were identified as (+)-(*E*)-α-santalen-12-oic acid (**1a**), (+)-(*E*)-*endo*-β-bergamoten-12-oic acid (**2a**), and (−)-(*E*)-*endo*-α-bergamoten-12-oic acid (**3a**). Structure assignments based primarily on ^1H and ^{13}C NMR spectral interpretations were confirmed by conversion to the parent sesquiterpene hydrocarbons and subsequent comparisons with authentic *endo*-β-bergamotene and/or literature data. The identity of **1a** was verified by comparison of its methyl ester (**1b**) with a sample synthesized from (+)-α-santalol (**9**). Quantitative assays demonstrated that the two major sesquiterpene acids, **1a** and **2a**, are the principal oviposition stimulants in the tomato leaf extracts and that the activity of **2a** is about twice that of **1a**.

The influence of leaf exudates from wild tomato plants of the species *Lycopersicon hirsutum* on the oviposition behavior of *Heliothis zea* (Boddie) has been investigated recently.[2] The larvae of *H. zea* are major agricultural pests of tomatoes, corn, and cotton.[3] The oviposition preference of these moths for filter paper disks treated with leaf extracts from 12 different *L. hirsutum* accessions seemed to correlate with the presence of three constituents in the extract presumed to be isomeric sesquiterpenes on the basis of a common molecular formula $C_{15}H_{22}O_2$. The objectives of this collaboration were to isolate and identify these phytochemicals and to determine their activity as oviposition stimulants for the female moths.

A literature search revealed few chemical analyses of tomato leaf volatiles. The presence of unknown sesquiterpenes in tomato leaves has been noted.[4,5] Several mono- and sesquiterpenes in tomato leaf essential oils have been identified by GC/MS analysis.[6] Significant amounts of the sesquiterpenes α-santalene and zingeberene have been found in extracts of two wild tomato species.[7]

In this paper we report the identification of three sesquiterpene carboxylic acids present in hexane extracts of leaves of *L. hirsutum* accession LA 1777 as (+)-(*E*)-α-santalen-12-oic acid (**1a**), (+)-(*E*)-*endo*-β-bergamoten-12-oic acid (**2a**),[8] and (−)-(*E*)-*endo*-α-bergamoten-12-oic acid (**3a**).[8] The results of oviposition preference assays with *H. zae* moths implicating **1a** and **2a** as the principal oviposition stimulants in the leaves of this wild tomato species are also presented.



Isolation and Spectral Identification

Hexane extracts of whole *L. hirsutum* leaves or from cotton swabs of leaf surfaces were concentrated and separated into nonpolar and polar fractions (~0.5% and 1.1% of fresh leaf weight, respectively) by chromatography. Oviposition preference assays indicated that all of the activity was associated with the polar fraction. Capillary GC analysis showed the presence of three principal components: A (11%), B (46%) and C (43%), in order of increasing retention time.

Esterification with diazomethane provided a mixture of methyl esters which was separated into an A + B fraction (16–26:84–74) and a C fraction (>95% purity) by argentic chromatography. Saponification of the two fractions followed by crystallization of the recovered acids afforded an A/B mixture enriched in A (30% A), an A/B mixture enriched in B (84% B), and analytically pure C as a crystalline solid, mp 66–67 °C.

Tentative structures were deduced from IR, ^1H NMR, and ^{13}C NMR spectra and GC/MS analyses of the A/B/C ester mixture and/or the A/B and C ester fractions. Although the least abundant isomer (A) was not obtained in pure form, its principal ^1H NMR absorptions were ascertained by inspection of spectra of the A/B ester mixture and the A-rich A/B acid mixture. The ^1H and ^{13}C NMR line assignments for esters B and C are secured by two-

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(8) The endo/exo stereochemical descriptors are used to designate the orientation of the side chain of the bergamotene isomers instead of the less appropriate cis/trans or syn/anti prefixes commonly found in the literature.

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Table I. ^{1}H NMR Spectral Data and Assignments for α-Santalen-12-oic, *endo*-β-Bergamoten-12-oic, and *endo*-α-Bergamoten-12-oic Acids (1a, 2a, and 3a, Respectively)[a]

C no.	1a	2a	3a[b]
1		2.30 (m, 1 H)	*c*
2	0.85 (s, 1 H)		
3	1.09 (d, J = 11, 1 H)[d] 1.62 (d, J = 11, 1 H)[e]	2.55 (t, J = 5.5, 2 H)	5.22 (br s, 1 H)
4	1.59 (s, 1 H)	1.83 (m, 2 H)	*c*
5	1.06 (d, J = 11, 1 H)[d] 1.57 (d, J = 11, 1 H)[e]	2.05 (m, 1 H)	*c*
6	0.85 (s, 1 H)		
7-endo		1.44 (d, J = 10, 1 H)	1.17 (d, J = 8.3, 1 H)
7-exo		2.30 (dt, J = 5.7, 8.5, 1 H)	2.35 (dt, J = 5.7, 8.4, 1 H)
8	1.27 (m, 2 H)	1.23 (m, 2 H)	*c*
9	2.13 (m, 2 H)	2.05 (m, 2 H)	*c*
10	6.92 (t, J = 7, 1 H)	6.87 (t, J = 7.4, 1 H)	6.88 (tq, J = 1, 7.5, 1 H)
13	1.84 (s, 3 H)	1.81 (s, 3 H)	1.81 (s, 3 H)
14	0.85 (s, 3 H)	1.25 (s, 3 H)	1.28 (s, 3 H)
15	1.00 (s, 3 H)	4.60, 4.67 (2 s, 2 H)	1.70 (d, J = 1.3, 3 H)

[a] Spectra recorded in $CDCl_3$ at 300 MHz. Coupling constants are given in hertz. Assignments were confirmed by correlation analysis (COSY). [b] Absorptions for **3a** taken from a spectrum of a 70:30 mixture of **1a** and **3a**. [c] Absorption was obscured by those of **1a**. [d] These assignments may be reversed. [e] These assignments may be reversed.

dimensional correlation spectra (COSY, ^{1}H/^{13}C CSCM, and DEPT).[9] The ^{1}H NMR spectral data for the acids are presented in Table I. Elemental compositions of $C_{15}H_{22}O_2$ for the acids were confirmed by high resolution electron impact GC/MS analyses of the esters.

The presence of an (*E*)-2-methyl-2-butenoic acid grouping in all three sesquiterpenes was apparent from peaks at δ 1.80–1.83 (s, 3 H, vinyl CH_3) and 6.72–6.76 (t, 1 H, J = 7.5 Hz, vinyl H) in the ^{1}H NMR spectra of the esters.[10] The absence of absorptions for other vinyl protons or vinyl carbons in the ^{1}H and ^{13}C NMR spectra of component B indicated a tricyclic nucleus. ^{1}H NMR signals at δ 4.58 and 4.66 (2 s, 1 H each) and 5.22 (br s, 1 H) revealed the presence of an exocyclic methylene group in C, an endocyclic trisubstituted double bond in A, and therefore bicyclic skeletons for both A and C.

The preceding analysis suggested that the three tomato leaf constituents might be carboxylic acid derivatives of α-santalene,[11] β-santalene,[12,13] epi-β-santalene,[11,13] *endo*-α(or β)-bergamotene,[8,14] *exo*-α(or β)-bergamotene,[8,14b,15] sesquifenchene,[16] sesquicarene,[17] or isosesquicarene.[18] The tricyclic component B was readily identified as the known (*E*)-α-santalen-12-oic acid (**1a**).[19,20] Structures based on β-santalene, epi-β-santalene, or sesquifenchene for component C could be excluded by the large ^{1}H NMR chemical shift difference (Δδ 0.20–0.26) which characterizes the exocyclic methylene protons of these sesquiterpenes.[11b,12,13,16] The absence of high field doublets of doublets from cyclopropane ring protons[18] in the ^{1}H NMR spectra of A and C was inconsistent with the sesquicarene and isosesquicarene structures. However, the overlapping doublet of triplets at δ 2.30 (J = 5.7, 8.5 Hz, 1 H), the sharp doublet at 1.43 (J = 9.4 Hz, 1 H), and the singlet at 1.24 (3 H) in the ^{1}H NMR spectrum of component C are very similar to resonances arising from the C-7 exo proton, C-7 endo proton, and the exo quaternary methyl group, respectively, in β-pinene[21] and *endo*-β-bergamotene.[14] The same peaks can also be recognized for the minor component A in the spectra of the A/B mixtures. Consequently components A and C were tentatively assigned as the previously unknown (*E*)-*endo*-α- and (*E*)-*endo*-β-bergamoten-12-oic acids (**3a** and **2a**). The possibility of *exo*-bergamotene structures is excluded by the absence of the characteristic high field singlet for the endo methyl group.[14b,15b]

Confirmation of Structure Assignments by Chemical Correlations and Direct Comparisons

endo-β-Bergamotenoic acid (**2a**) was converted to the parent hydrocarbon (**6**) in order to confirm the structural assignment and to establish the absolute configuration. The carboxyl-to-methyl transformation was accomplished by lithium aluminum hydride reduction of **2a** to *endo*-β-bergamotenol (**4**, 81%), conversion to the chloride (**5**) with *N*-chlorosuccinimide and dimethyl sulfide[22] in the presence of 2,2,6,6-tetramethylpiperidine, and reduction to *endo*-β-bergamotene (**6**, 47% from **4**) with lithium triethylborohydride.[23] When the same hydroxyl-to-chloride conversion was attempted in the absence of the hindered amine buffer, a 23:77 mixture of exo- and endocyclic chloride isomers (**5** and **7**) was obtained in 76% yield. Evidently some hydrogen chloride liberated during the Corey–Kim procedure[22] catalyzed partial isomerization of

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(18) Uyehara, T.; Yamada, J.-I.; Kato, T.; Bohlmann, F. *Bull. Chem. Soc. Jpn.* **1985**, *58*, 861–867.
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(20) Synthesis: Kamat, S. Y.; Chakravarti, K. K.; Bhattacharyya, S. C. *Tetrahedron* **1967**, *23*, 4487–4491. The α-santalene derivatives shown as *Z* isomers must actually have the *E* stereochemistry. See: Heathcock, C. H. In *Total Synthesis of Natural Products*; ApSimon, J., Ed.; Wiley: New York, 1973; Vol. 2, p 486.
(21) (a) Bates, R. B.; Thalacker, V. P. *J. Org. Chem.* **1968**, *33*, 1730–1732. (b) Kaplan, F.; Schulz, C. O.; Weisleder, D.; Klopfenstein, C. *Ibid.* **1968**, *33*, 1728–1730. (c) Teisseire, P.; Galfre, A.; Plattier, M.; Corbier, B. *Recherches* **1966**, *15*, 52.
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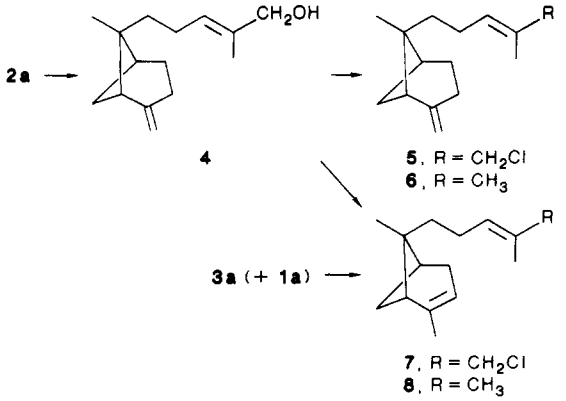
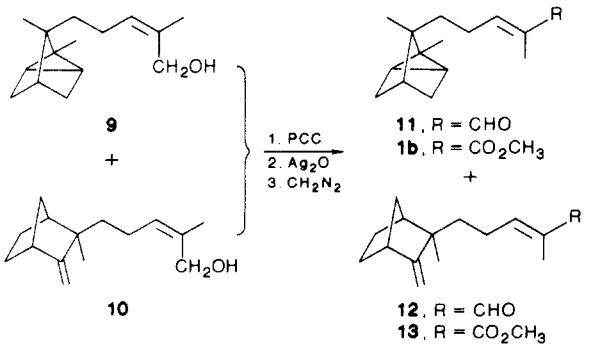
the double bond into the more stable endocyclic position. The apparent absence of byproducts in this reaction is noteworthy since acid-catalyzed reactions of the pinenes usually cause skeletal rearrangements to bornyl, camphanyl, and fenchyl derivatives.[24] Chromatographic enrichment of the chloride mixture to 10:90 (β:α) followed by reduction provided a sample of *endo*-α-bergamotene (**8**, 90% purity). An enriched 70:30 mixture of acids **1a** and **3a** was converted to a mixture of α-santalene and *endo*-α-bergamotene (**8**) by the same three-step reaction sequence.



The ^1H and ^{13}C NMR spectra, GC retention times (by coinjection), and mass spectra of *endo*-β-bergamotene (**6**) are identical with those of a synthetic sample of the racemic sesquiterpene.[25] The identity of the minor component of the above α-bergamotene + α-santalene mixture with *endo*-α-bergamotene (**8**) derived from **4** was established by comparisons of ^1H NMR spectra, mass spectra, and GC retention times.

An authentic sample of methyl α-santalen-12-oate was prepared from a commercial sample of α- and β-santalols (**9** + **10**, 63:37) which has the *Z* configuration about the side-chain double bond. Oxidation with pyridinium chlorochromate (PCC) was accompanied by *Z*–*E* isomerization and provided an impure 60:40 mixture of (*E*)-α- and (*E*)-β-santalols (**11** + **12**, 66% purity). Further oxidation with silver oxide followed by diazomethane esterification and purification by argentic chromatography afforded a sample of methyl (*E*)-α-santalenoate (**1b**, 89% purity). The identity of this sample with the ester of component B was established by comparisons of ^1H NMR, ^{13}C NMR, and mass spectra and by their coincident GC retention times.



The absolute stereochemistry assigned to the three sesquiterpenes is based on the correspondence in sign and approximate magnitude of observed specific optical rotations with various literature values[26] (see Table II). Since the optical rotation of *endo*-α-bergamotene (**8**) derived from **3a** was not determined, the configurational assignment in this case is based only on the rotation of **3a** calculated from measurements of the two **1a** + **3a** mixtures. However, the data in Table II and in the Experimental Section support the assumption that the remote carboxyl groups have at most a small effect on the magnitude of the optical rotations.

Table II. Specific Optical Rotations for (*E*)-α-Santalenoic Acid (1a), (*E*)-*endo*-β-Bergamotenoic Acid (2a), (*E*)-*endo*-α-Bergamotenoic Acid (3a) and Their Derivatives

compd	$[\alpha]_D$ ($CHCl_3$), deg obsd	lit. value
1a	+35.1[a]	+18.0[b]
1b	+27.9,[c] +25.9[d]	+18.8,[b] +18.6[e] +32.8[f]
2a	+35.0	
6	+33.5	+40.2[g]
3a	−46.3[a]	
8	−51.8[h]	−39.4[g,i]

[a] Calculated from rotations measured for two mixtures of **1a** and **3a**. [b] Reference 20. [c] Derived from **1a**; corrected for contribution of **3b** (16%). [d] Derived from **9**; 89% purity. [e] Ethyl ester, ref 20. [f] Ethyl ester, ref 26. [g] Reference 14a. [h] Derived from **2a**; corrected for contribution of **6** (7%). [i] Measured at 546 nm.

Table III. *Heliothis zea* Oviposition Preference for Hexane Extracts and Purified Sesquiterpene Acids from Leaves of the *Lycopersicon hirsutum* accession LA 1777

expt no.	filter paper disks treated with	rel oviposition preference[a,b]	least significant difference
1	hexane (control)	0.95a	0.82
	1a[c]	3.66b	0.82
	2a	4.94c	0.82
	LA 1777 extract[d]	7.94d	0.82
2	hexane (control)	1.04a	1.08
	(1a +2a) mixture[c,e]	10.09b	1.08
	LA 1777 extract[d]	9.82b	1.08

[a] Mean number of eggs on treated disks divided by mean number of eggs on adjacent, untreated disks. [b] Numbers followed by different letters are significantly different at $p = 0.05$ (protected LSD test). [c] An 84:16 mixture of **1a** and **3a** was used. [d] From 1 g of leaves. [e] 0.72 mg of 84% **1a** and 0.47 mg of **2a**.

Oviposition Preference Assays

The oviposition assays with tomato leaf (LA 1777) extracts and the purified sesquiterpene acids were conducted by using premated female *H. zea* moths according to methods described previously (see Experimental Section).[2] The oviposition preference values (Table III) are ratios of eggs laid on treated filter paper disks to those oviposited on two adjacent untreated disks. Thus, 3.7–10.1 times as many eggs were laid on disks treated with the LA 1777 extract or the purified sesquiterpenes, **1a** and **2a**.

The data from experiment 1 indicate that (+)-α-santalenoic acid (**1a**, 84% purity) and (+)-*endo*-β-bergamotenoic acid (**2a**) possess kairmonal properties that modify *H. zea* moth orientation and oviposition response.[27] This is the first report on the existence and identification of natural

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(27) We are assuming that the oviposition stimulating activity of the minor component (**3a**) is equal to or less than that of **1a**. There is a possibility that **1a** is actually inactive, in which case all, or most, of the activity would reside in **3a**.

phytochemicals from the leaves of wild and cultivated tomato species that influence the oviposition behavior of *H. zea*. The magnitude of oviposition stimulation (3.7 times that of the control for **1a** and 4.9 times the control for **2a**) indicates that these sesquiterpenes elicit significant behavioral modification of the processes of *H. zea* site selection for oviposition.

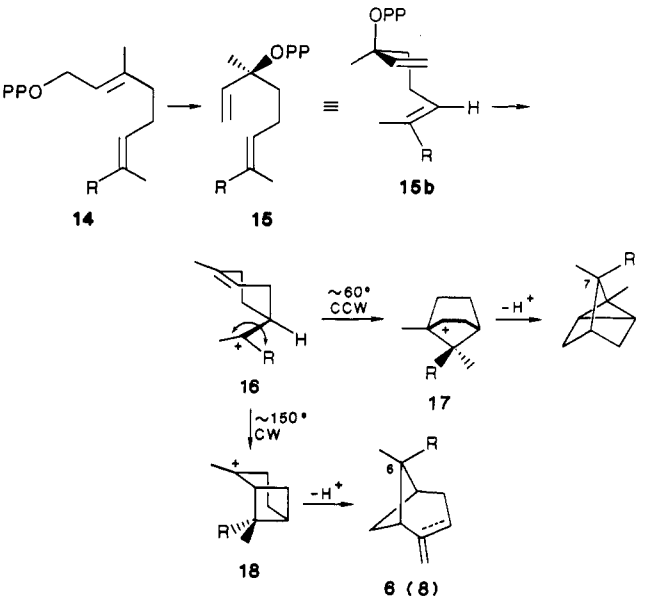
Moth response to **1a** and **2a** was also significantly different. Filter disks permeated with β-bergamotenoic acid stimulated 35% more oviposition than the α-santalenoic acid fraction (84% **1a** + 16% **3a**) even though filter disks treated with the former had 35% less sesquiterpene content. If a linear relationship exists between oviposition response and *endo*-β-bergamotenoic acid concentration, this sesquiterpene should be approximately twice (2.06 times) as active in stimulating oviposition as α-santalenoic acid. This significant difference in insect behavioral response to structurally similar phytochemicals is not unusual. For example, the spotted cucumber beetle (*Diabrotica undecimpunctata* Barber) can detect and respond to the triterpene, cucurbitacin B, at concentrations 100 times more dilute than levels that stimulate feeding with the structurally related cucurbitacin I.[28]

When **1a** (84%) and **2a** were combined on filter disks at concentrations equivalent to those found in the hexane extract of the tomato leaves, oviposition preference by *H. zea* for these disks was not significantly different from disks permeated with the LA 1777 extract (experiment 2, Table III). Consequently these sesquiterpenes apparently account for all of the observed biological activity of the leaf hexane extracts, and no synergistic effects between **1a** and **2a** are detectable.

Discussion

The spectra and correlations described above establish that the oviposition stimulants are side-chain *E* carboxylic acid derivatives of the known sesquiterpenes (+)-α-santalene,[11] (+)-*endo*-β-bergamotene (**6**),[14,29] and (−)-*endo*-α-bergamotene (**8**).[14,30] While (+)-(*E*)-α-santalenoic acid (**1a**) has been isolated previously from *Ayapana amygdalina*,[19,31] the (*E*)-*endo*-bergamotenoic acids (**2a** and **3a**) appear to be new natural products. However, several side-chain methyl oxidation metabolites of the more common *exo*-α-bergamotene[29b] have been reported. Although the (*Z*)-santalols and related metabolites with *Z* stereochemistry are widely encountered, the occurrence of (*E*)-α-santalenal has also been noted.[32] (*E*)-α-Santalenoic acid (**1a**)[33] and its esters[20,26] have been synthesized. While the presence of significant amounts of α-santalene in another wild tomato species has been established,[7] the occurrence of *endo*-bergamotenes in tomatoes has not been mentioned in the literature.

Scheme I. Proposed Biogenesis of (7*R*)-α-Santalene and (6*S*)-*endo*-Bergamotenes from (*E*,*E*)-Farnesyl Pyrophosphate



The cooccurrence of (+)-α-santalenoic acid with (−)-*endo*-α- and (+)-*endo*-β-bergamotenoic acids is of biogenetic interest since the two related types of sesquiterpenes have the opposite stereochemistry at the bridging carbon to which the side chain is attached (7*R* for **1a**; 6*S* for **2a** and **3a**). If it is assumed that their α-santalene and *endo*-bergamotene precursors are both biosynthesized from (*E*,*E*)-farnesyl pyrophosphate (**14**) via anti,endo S_N' cyclization[34] of (3*S*,6*E*)-nerolidyl pyrophosphate (**15**) and a common (4*S*)-bisabolyl carbocation (**16**), the second cyclization must take place with the opposite stereochemistry and rotational motions (Scheme I). Thus, a 60° counterclockwise rotation toward the endocyclic double bond would enable cyclization to the 7*S* campherenyl cation (**17**) to occur whereas a 150° clockwise rotation is required to form the (6*R*)-*endo*-bergamotenyl cation (**18**).

The maximum motion pathway to the *endo*-bergamotenes contrasts with the enantiomeric least motion pathways recently established for the enzymatic cyclizations of geranyl pyrophosphate to (+)- and (−)-α-pinene by purified cyclases from *Salvia officinalis*.[35] Alternatively the *endo*-bergamotenes might be formed by a similar cyclization of (*E*,*Z*)-farnesyl pyrophosphate with a final least motion ring closure to form the bicyclo[3.1.1]heptane skeleton.

H. zea moths have apparently evolved both the sensory apparatus to detect these sesquiterpene acids selectively and a behavioral linkage between their presence and moth oviposition. This suggests that these compounds may be serving a functional role for *H. zea* as cues for suitable host plant location and selection.

The isolation and identification of these *H. zea* oviposition stimulants may have several potential applications in agriculture. The genetic variation in their presence and amount among species of *Lycopersicon* could be utilized in a breeding program to develop tomato cultivars lacking

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(29) *endo*-β-Bergamotene has been identified in (a) fruit essential oil of *Sium latifolium L.* [Krzeminski, K.; Daniewski, M. *Herba Pol.* **1984**, *30*, 187–190] and (b) carrot seed oil (*Daucus carota L.*) [Hogg, J. W.; Terhune, S. J.; Lawrence, B. M. *Cosmet. Perfum.* **1974**, *89*, 64, 66, 69.

(30) *endo*-α-Bergamotene has been identified in the following essential oils (a) Black pepper [Russel, G. F.; Murray, W. J.; Muller, C. J.; Jennings, W. G. *J. Agric. Food Chem.* **1968**, *16*, 1047–1049], (b) opoponax [Wenninger, J. A.; Yates, R. L. *J. Assoc. Offic. Anal. Chem.* **1969**, *52*, 1155] and (c) Spanish sage and basil [Lawrence, B. M.; Hogg, J. W.; Terhune, S. J. *J. Chromatog.* **1970**, *50*, 59].

(31) Santalenoic acid without designation of α/β structure or *E*/*Z* stereochemistry has been reported as a minor component of *Heracleum candicans* seed oil: Ashraf, M.; Bhatty, M. K. *Pak. J. Sci. Ind. Res.* **1978**, *21*, 70–77.

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(35) Coates, R. M.; Denissen, J. F.; Croteau, R. B.; Wheeler, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 4399–4401.

the chemical cues used by *H. zea* or other insect pests for host plant location and recognition. This could result in improved host plant resistance to insects and reduced crop damage from larval feeding. These sesquiterpenoids might also be used to attract and trap moths for field monitoring of female *H. zea* population levels. Alternatively, the purified sesquiterpenoids might prove effective when combined with pesticide baits for actual pest control.

Experimental Section

General Aspects. Melting points were determined in open-ended capillary tubes and are uncorrected. GC analyses were carried out on either a 60 m × 0.25 mm (column A) or 30 m × 0.32 mm (column B) J&W Scientific DB-5 fused silica capillary column. A linear flow velocity of 26 cm/s and a split ratio of 190:1 were used for the 30-m column, while a linear flow velocity of 22 cm/s and a split ratio of 245:1 were used for the 60-m column.

Flash chromatography was performed as described by Still[36] on Woelm 32–63-μm silica gel or Woelm silica gel impregnated with 10% silver nitrate. Analytical thin layer chromatography (TLC) was conducted on Merck glass plates precoated with 0.25 mm of silica gel 60 F-254 or Merck precoated glass plates dipped in 10% silver nitrate in 90% ethanol/10% water solution and oven-dried overnight. TLC plates were visualized with 5% phosphomolybdic acid reagent in 95% ethanol or iodine vapor. The eluent was 20% ethyl acetate in hexane in all chromatographic purifications unless specified otherwise.

All reactions, except those performed in aqueous solvents, were carried out in a nitrogen atmosphere using glassware dried at 140 °C for at least 2 h. All reagents and solvents were reagent grade and used without further purification unless specified otherwise. Technical grade hexane and pentane used for column chromatography were distilled prior to use. Diethyl ether and dichloromethane were freshly distilled from sodium benzophenone ketyl and calcium hydride, respectively, before use as reaction solvents. Ethereal diazomethane was generated from *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, (Diazald). *N*-Chlorosuccinimide was recrystallized from benzene prior to use.

Isolation of Sesquiterpene Acids as Methyl Esters. A. Analysis of the Tomato Leaf Extract. Hexane extracts of whole, undamaged leaves of LA 1777[2] were concentrated to an average of 10 g of original fresh leaf weight per mL. GC analysis (column A, 150 °C for 3 min/20 °C increase per min/250 °C for 20 min) showed six peaks having retention times between 8.82 and 10.72 min (peak at 10.72 min = 16.1%, all others <1.8%) and three peaks at higher retention time: 13.9 min (8.5%), 14.09 min (35%), and 14.63 min (34%). TLC showed a major spot at R_f 0.87 and a second major spot that tailed between R_f 0 to 0.32.

B. Separation of Polar and Nonpolar Fractions. A 40-mL hexane extract was concentrated under reduced pressure to give 1.52 g (3.8% based on the weight of fresh leaves) of a green semisolid. Purification by flash chromatography with 20% ether in hexane as eluent provided two major fractions. The nonpolar fractions displayed an R_f 0.87 on TLC and GC (column A, 150 °C for 3 min/20 °C increase per min/250 °C for 20 min) retention times between 8.8 and 10.8 min, with a major peak at 10.72 min (82%). Concentration provided 0.182 g of oily yellow solid. The polar fraction displayed an R_f range between 0.0 and 0.32 on TLC. GC (column A, 220 °C) analysis showed three major peaks: A, 11.5 min (11%); B, 11.8 min (46%); and C, 12.8 min (43%). Concentration gave 0.45 g (1.1% based on fresh leaf weight) of yellow oil. Oviposition assays at this point indicated that only the polar fraction displayed significant activity.

C. Formation and Separation of Methyl Esters. A solution of 127 mg of the polar fraction in 0.5 mL of ether was treated with ethereal diazomethane until a yellow color persisted. TLC analysis showed a single spot at R_f 0.6, while GC analysis (column A, 220 °C) displayed three major peaks: component A at 10.4 min (10%), component B at 10.7 min (47%), and component C at 11.4 min (43%). Concentration and purification by flash chromatography with 20% ether in hexane as eluent gave 120 mg of clear oil. GC/MS analysis (70 eV) of the mixture exhibited a molecular ion at m/e 248 for all three major components A, B, and C.

The methyl ester mixture was applied to a 2.8 × 9.2 cm column containing 11 g of silica gel impregnated with 10% silver nitrate and eluted with hexane. The composition of the fractions was monitored by GC (column A, 220 °C). Four main fractions (1–4) were collected. Fractions 2 (5 mL) and 4 (10 mL) were mixtures containing at least three components. Fraction 1 (5 mL) consisted of a 16:84 mixture of components A and B, while fraction 3 (10 mL) consisted mainly of component C (>95%).

Concentration of fraction 1 gave 46 mg of an 84:16 mixture of component B, identified as (+)-methyl (*E*)-α-santalenoate (**1b**), and component A, identified as (−)-methyl (*E*)-*endo*-α-bergamotenoate (**3b**), as a clear oil: $[\alpha]^{24}_D$ +16.1° (*c* 2.42, $CHCl_3$); IR (CCl_4) 2924, 2874, 1713 (CO_2CH_3), 1651, 1549, 1435, 1279, 1225, 1146 cm^{-1}.

(+)-Methyl (*E*)-α-santalenoate (**1b**) displayed the following characteristics: calcd[37] $[\alpha]^{24}_D$ +27.9° ($CHCl_3$) [lit.[20] $[\alpha]^{24}_D$ +18.57 (c 2.33, $CHCl_3$)]; ^1H NMR ($CDCl_3$, 500 MHz) δ 0.84 (s, 5 H, CH_3 and $CHC(CH_3)CH$), 0.99 (s, 3 H, CH_3), 1.05 (d, J = 11 Hz, 1 H, CH_2), 1.07 (d, J = 11 Hz, 1 H, CH_2), 1.27 (m, 2 H, CH_2CH_2CH=), 1.55 (d, J = 11 Hz, 1 H, CH_2), 1.58 (s, 1 H, CH_2CHCH_2), 1.61 (d, J = 11 Hz, 1 H, CH_2), 1.83 (s, 3 H, =$C(CH_3)CO_2$), 2.08 (m, 2 H, CH_2CH=), 3.72 (s, 3 H, CO_2CH_3), 6.76 (dt, J = 1.2, 7.5 Hz, 1 H, vinyl H); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 10.6 (C15), 12.2 (C13), 17.4 (C14), 19.4 (C6), 19.5 (C2), 24.1 (C9), 27.4 (C1), 31.0 (C5), 31.5 (C3), 33.2 (C8), 38.1 (C4), 45.8 (C7), 51.6 (OCH_3), 126.9 (C11), 143.3 (C10), 168.7 (C12); GC/MS (70 eV), m/e (relative intensity) 248 (M^+, 15), 233 (7), 217 (9), 189 (10), 181 (17), 121 (54), 119 (24), 107 (43), 93 (100), 94 (49), 79 (32), 41 (26); high resolution EIMS, 248.1772 (calcd for $C_{16}H_{24}O_2$ 248.1777).

(−)-Methyl (*E*)-*endo*-α-bergamotenoate (**3b**) displayed the following partial list of spectral characteristics: ^1H NMR ($CDCl_3$, 500 MHz) δ 1.27 (s, exo CH_3), 1.69 (d, J = 1.7 Hz, CH=C-$(CH_3)CH_2$), 1.80 (s, $CH{=}C(CH_3)CO_2$), 2.36 (m, $CH{=}C(CH_3)CH_2$), 3.71 (s, CO_2CH_3), 5.22 (br s, ring vinyl H), 6.73 (t, J = 7.5 Hz, $CH{=}C(CH_3)CO_2$); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 22.9, 24.0, 29.7, 32.5, 40.3, 45.8, 46.0; GC/MS (70 eV), m/e (relative intensity) 248 (M^+, 2), 233 (1), 217 (1), 189 (4), 173 (3), 158 (5), 135 (18), 121 (17), 119 (95), 114 (41), 107 (31), 93 (100), 91 (36), 79 (30), 55 (32), 41 (25).

Concentration of the third fraction (10 mL) gave 39.6 mg (0.16 mmol) of component C, which was identified as (+)-methyl (*E*)-*endo*-β-bergamotenoate (**2b**, >95% purity), as a clear oil: $[\alpha]^{24}_D$ +30.26° (*c* 2.28, $CHCl_3$); IR (CCl_4) 2928, 1713 (CO_2CH_3), 1649, 1551, 1435, 1277, 1250, 1097, 881 ($C{=}CH_2$) cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 1.23 (m, 2 H, CH_2CH_2CH=), 1.24 (s, 3 H, CH_3), 1.43 (d, J = 9.4 Hz, 1 H, C-7 endo-H), 1.80 (s, 3 H, =C-$(CH_3)CO_2$), 1.83 (m, 2 H, $CHCH_2CH_2$), 1.98 (m, 1 H, $CHCH_2CH_2$), 2.04 (m, 2 H, CH_2CH=), 2.30 (m, 1 H, $CHC{=}CH_2$), 2.30 (dt, J = 5.7, 8.5 Hz, 1 H, C-7 exo-H), 2.54 (t, J = 5.5 Hz, 2 H, $CH_2C{=}CH_2$), 3.72 (s, 3 H, CO_2CH_3), 4.58 (s, 1 H, =CH_2), 4.66 (s, 1 H, =CH_2), 6.72 (t, J = 7.5 Hz, 1 H, vinyl H); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 12.2 (C13), 22.4 (C14), 22.8 (C4), 23.0 (C9), 23.6 (C3), 26.6 (C7), 33.7 (C8), 40.1 (C5), 42.7 (C6), 50.9 (C1), 51.6 (OCH_3), 106.8 (C15), 126.9 (C11), 143.3 (C10), 151.3 (C2), 168.8 (C12); GC/MS (70 eV), m/e (relative intensity) 248 (M^+, 5), 233 (1), 217 (1), 201 (2), 189 (5), 173 (4), 161 (12), 147 (9), 133 (54), 121 (27), 119 (82), 114 (12), 105 (17), 93 (100), 91 (34), 79 (37), 55 (30), 41 (30); high resolution EIMS, 248.1778 (calcd for $C_{16}H_{24}O_2$ 248.1777).

D. Large-Scale Isolation. A 400-mL hexane extract of whole LA 1777 leaves of unknown leaf weight per mL was concentrated to 2.1 g of a green semisolid. Esterification and chromatographic fractionation as described in parts B and C provided 0.398 g of a 76:24 mixture of esters **1b** and **3b** and 0.238 g of ester **2b**.

(+)-(7*R*)-(*E*)-5-(2,3-Dimethyltricyclo[2.2.1.0^{2,6}]hept-3-yl)-2-methyl-2-pentenoic Acid ((*E*)-α-Santalen-12-oic Acid, 1a) and (−)-(1*S*,5*S*,6*S*)-(*E*)-*endo*-5-(2,6-Dimethylbicyclo-[3.1.1]hept-2-en-6-yl)-2-methyl-2-pentenoic Acid ((*E*)-*endo*-α-Bergamoten-12-oic Acid, 3a). To a solution of 0.398 g (1.6 mmol) of a 76:24 mixture of esters, **1b** and **3b**, in 12 mL of absolute ethanol was added 3.2 mL of 2 M potassium hydroxide. After 22 h at room temperature, the solution was acidified with 1 M

(36) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.

(37) The observed specific rotation of the **1a** + **3a** mixture was corrected by using an approximate specific rotation value of −46° for **3a**.

hydrochloric acid, diluted with 10 mL of water, and extracted with three portions of ether. The combined organic layers were washed with saturated sodium chloride and dried ($MgSO_4$). Removal of solvent under reduced pressure gave 0.348 g (93%) of white semisolid, which was recrystallized from pentane at −78 °C in an attempt to remove the 24% of **3a**. A white solid (0.184 g), mp 66–72 °C (lit.[20] mp 70 °C), was obtained which was shown by GC analysis (column A, 220 °C) to be enriched in **1a** (84:16 ratio of **1a** and **3a**). Evaporation of the mother liquor provided 93 mg of a semisolid slightly enriched in the minor acid **3a** (70:30 ratio of **1a** and **3a**). The following optical rotations were calculated from rotations measured for the two (**1a** + **3a**) crystalline fractions: **1a**, $[\alpha]^{23}_D$ +35.1° (*c* 1.26, $CHCl_3$); **3a**, $[\alpha]^{23}_D$ −46.3° (*c* 1.26 $CHCl_3$). The ^1H NMR spectral data for **1a** and **3a** are presented in Table I.

(+)-(1*S*,5*S*,6*S*)-(*E*)-*endo*-5-(6-Methyl-2-methylenebicyclo[3.1.1]hept-6-yl)-2-methyl-2-pentenoic Acid ((*E*)-*endo*-β-Bergamoten-12-oic Acid, 2a). Hydrolysis of 0.214 g (0.86 mmol) of ester **2b** in 7 mL of absolute ethanol with 1.7 mL of 2 M potassium hydroxide was carried out as described in the preceding procedure. Recrystallization of the crude solid (0.182 g) from pentane at −78 °C produced 0.154 g (76%) of **2a** as a white solid: mp 66–67 °C; $(\alpha]^{23}_D$ +34.97° (*c* 2.23, $CHCl_3$); IR (CCl_4) 3200–2500 (br, max at 2926), 1686 (CO_2H), 1642, 1549, 1422, 1289, 1258, 882 cm^{-1}; ^1H NMR (Table I). Anal. Calcd for $C_{15}H_{22}O_2$: C, 76.88; H, 9.46. Found: C, 76.69; H, 9.54.

(+)-(*E*)-*endo*-β-Bergamotenol (4). A solution of 26 mg (0.69 mmol) of lithium aluminum hydride in 3 mL of dry ether was stirred at room temperature while 45 mg (0.19 mmol) of acid **2a** in 1 mL of dry ether was added over 5 min. After 20 min, the mixture was quenched by careful addition of 10 mL of water. The aqueous layer was extracted with three portions of ether. The combined ether layers were washed with saturated sodium chloride, dried ($MgSO_4$), and evaporated under reduced pressure. Purification of the residue by flash chromatography with 20% ether in hexane as eluent gave 34.3 mg (0.156 mmol, 81%) of alcohol **4** as a clear oil: $[\alpha]^{23}_D$ +19.77° (*c* 3.15, $CHCl_3$); IR (CCl_4) 3619 (OH), 2924, 2363, 2336, 1553, 1252, 1217, 1005 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.15 (m, 2 H, CH_2CH_2CH=), 1.23 (s, 3 H, CH_3), 1.42 (d, *J* = 9.9 Hz, 1 H, C-7 endo-H), 1.63 (s, 3 H, =C-(CH_3)CH_2OH), 1.83 (m, 2 H, $CHCH_2CH_2$), 1.83 (m, 2 H, CH_2CH=), 2.03 (m, 1 H, $CHCH_2CH_2$), 2.29 (m, 1 H, CHC=CH_2), 2.29 (dt, *J* = 5.7, 8.5 Hz, 1 H, C-7 exo-H), 2.52 (t, *J* = 5.4 Hz, 2 H, CH_2C=CH_2), 3.97 (s, 2 H, CH_2OH), 4.57 (s, 1 H, =CH_2), 4.64 (s, 1 H, =CH_2), 5.35 (t, *J* = 7.4 Hz, 1 H, vinyl H).

(+)-*endo*-6-Methyl-2-methylene-6-(4-methyl-3-pentenyl)bicyclo[3.1.1]heptane (*endo*-β-Bergamotene, 6). A modification of the Corey–Kim procedure[22] was used. A solution of 17.7 mg (0.133 mmol) of *N*-chlorosuccinimide in 0.8 mL of dry dichloromethane was stirred at 0 °C for 10 min. Dimethyl sulfide (16 μL, 0.18 mmol) was introduced, producing a cloudy white dispersion that was stirred for 15 min at 0 °C. 2,2,6,6-Tetramethylpiperidine (14.9 μL, 12.5 mg, 0.089 mmol) and 19.5 mg (0.089 mmol) of alcohol **4** in 1.0 mL of dry dichloromethane were added. The mixture became clear after 1–2 min and was stirred at 0 °C for 1 h. GC analysis (column B, 200 °C) showed little change in the amount of chloride (t_R 5.30 min) from 0.5 h to 1 h. After 1-h total reaction time, 3 mL of saturated sodium chloride was added to the mixture, still at 0 °C. The aqueous layer was extracted with two portions of pentane. The combined pentane and dichloromethane layers were extracted with 0.1 M sodium bicarbonate, dried ($MgSO_4$), and evaporated to 0.1–0.2 mL of clear liquid. TLC analysis showed chloride (R_f 0.69) to be the major product; however, some unreacted alcohol (R_f 0.18) was also apparent.

The solution of *endo*-β-bergamotenyl chloride and unreacted alcohol **4** (in 0.1–0.2 mL of dichloromethane) was diluted with 1 mL of dry ether and added over 2 min to a stirred solution of 0.5 mmol (0.5 mL of 1 M solution in THF from Aldrich Chemical Co.) of lithium triethylborohydride[23] in 1 mL of dry ether at 0 °C. TLC and GC (column B, 200 °C) analyses showed no chloride remaining after 0.5 h, at which time 0.2 mL of water was slowly added at 0 °C. Saturated sodium chloride (2 mL) and pentane (2 mL) were added and the separated aqueous layer was extracted with two portions of pentane. The combined ether and pentane layers were extracted with two portions of saturated sodium chloride, dried ($MgSO_4$), and evaporated. Purification of the residual liquid by flash chromatography with pentane as eluent afforded 8.5 mg (47%) of *endo*-β-bergamotene as a clear oil, the purity of which was >98% by GC analysis (column B, 160 °C, t_R 5.35): $[\alpha]^{23}_D$ +33.5° (*c* 0.4, $CHCl_3$) [lit.[14a] $[\alpha]^{23}_D$ +40.2° (*c* 1.74, $CHCl_3$)]; IR (CCl_4) 3073, 2924, 1642, 1549, 1456, 1252, 1217, 1005, 980, 878 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.11 (m, 2 H, CH_2CH_2CH=), 1.23 (s, 3 H, CH_3), 1.41 (d, *J* = 9.8 Hz, 1 H, C-7 endo-H), 1.58 (s, 3 H, =CCH_3), 1.66 (s, 3 H, =CCH_3), 1.82 (m, 2 H, CH_2CH_2C=CH_2), 1.82 (m, 2 H, CH_2CH=), 2.02 (m, 1 H, $CHCH_2CH_2$), 2.29 (m, 1 H, CHC=CH_2), 2.29 (dt, *J* = 5.7, 8.5 Hz, 1 H, C-7 exo-H), 2.52 (t, *J* = 5.5 Hz, 2 H, CH_2C=CH_2), 4.57 (s, 1 H, =CH_2), 4.64 (s, 1 H, =CH_2), 5.07 (t, *J* = 7.5 Hz, 1 H, vinyl H); ^{13}C NMR ($CDCl_3$, 125.8 MHz) δ 17.5 (C13), 21.9 (C12), 22.6 (C14), 23.1 (C9), 23.7 (C4), 25.7 (C3), 26.7 (C7), 35.2 (C8), 40.1 (C5), 42.9 (C6), 51.2 (C1), 106.4 (C15), 125.3 (C11), 130.7 (C10), 151.7 (C2); GC/MS (70 eV), *m/e* (relative intensity) 204 (M^+, 10), 189 (3), 161 (28), 133 (32), 120 (27), 119 (26), 105 (23), 93 (85), 91 (39), 79 (40), 69 (100), 55 (38), 41 (55).

All spectra are identical with those of an authentic sample[25] of (±)-**6** and the ^1H NMR spectral data agree with the literature values.[14a] GC coinjection with the authentic sample displayed a single peak.

(−)-*endo*-2,6-Dimethyl-6-(4-methyl-3-pentenyl)bicyclo[3.1.1]hept-2-ene (*endo*-α-bergamotene, 8) was prepared from 39 mg (0.18 mmol) of **4** by the preceding procedure except that 2,2,6,6-tetramethylpiperidine was absent. After 2 h, two major product peaks at t_R 4.88 (40%) and t_R 5.33 (36%) were observed by GC analysis (column B, 200 °C), the latter peak corresponding to the β isomer. After allowing the reaction to warm to room temperature over 20 min, the β/α isomer ratio had decreased to 23:77. The product was isolated in the same manner and purification by flash chromatography with hexane eluent succeeded in removing more than 50% of the β isomer, providing 26 mg (0.11 mmol, 62%) of (*E*)-*endo*-α-bergamotenyl chloride (**7**) as a clear oil.

Reduction of the α chloride isomer with Super-Hydride as described for **6** above gave 19.3 mg (0.095 mmol, 87%) of **8** as a clear oil, 90% purity by GC (column B, 160 °C, t_R 4.84, ~7% β isomer): $[\alpha]^{22}_D$ −45.9° (*c* 1.9, $CHCl_3$), −51.8° (corrected for β isomer) [lit.[14a] $[\alpha]_{546}$ −39.4° (*c* 0.48, $CHCl_3$)]; IR (CCl_4) 2963, 2922, 1549, 1449, 1375, 1252, 1217, 1005, 980 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.15 (d, *J* = 8.3 Hz, 1 H, C-7 endo-H), 1.26 (s, 3 H, CH_3), 1.40 (m, 2 H, CH_2CH_2CH=), 1.58 (s, 3 H, =CCH_3), 1.67 (s, 3 H, =CCH_3), 1.70 (d, *J* = 1.5 Hz, 3 H, ring =CCH_3), 1.77 (m, 2 H, CH_2CH_2CH=), 1.97 (t, *J* = 5.6 Hz, 1 H, $CHC(CH_3)$=), 2.12 (br s, 1 H, $CHCH_2CH_2$), 2.18 (br s, 2 H, $CHCH_2CH$=), 2.33 (dt, *J* = 5.7, 8.5 Hz, 1 H, C-7 exo-H), 5.07 (t, *J* = 7 Hz, 1 H, CH=$C(CH_3)_2$), 5.20 (br q, *J* = 1.2 Hz, 1 H, ring vinyl H); GC/MS (70 eV), *m/e* (relative intensity) 204 (M^+, 3), 189 (3), 161 (7), 135 (5), 119 (84), 107 (29), 105 (26), 93 (100), 91 (37), 79 (27), 77 (22), 69 (45), 55 (38), 41 (59). All spectral data are in agreement with literature values.[14]

Reduction of Carboxylic Acids 1a and 3a to (+)-1,7-Dimethyl-7-(4-methyl-3-pentenyl)tricyclo[2.2.1.0^{2,6}]heptane (α-Santalene) and (−)-*endo*-2,6-Dimethyl-6-(4-methyl-3-pentenyl)bicyclo[3.1.1]hept-2-ene (*endo*-α-Bergamotene, 8). The conversion of **1a** and **3a** to hydrocarbons was accomplished by using the procedures described for conversion of **2a** to **8**. A 70:30 mixture (93 mg) of **1a** and **3a** obtained from the recrystallization of the mother liquor was reduced with lithium aluminum hydride to give 64 mg (74%) of the corresponding mixture of alcohols after purification by flash chromatography. Conversion to the chlorides and Super-Hydride reduction followed by flash chromatography produced 44.7 mg (0.22 mmol, 56% from alcohols) of a mixture of (+)-α-santalene and **8** as a clear oil. GC analysis showed a 34:66 ratio of **8** (t_R 4.86) and α-santalene (t_R 4.94) and coinjection with **8** synthesized via isomerization of the β analogue resulted in a conclusive enhancement of the peak at t_R 4.86. ^1H NMR and GC/MS (70 eV) spectra of **8** are also identical with those derived from **4**. The following ^1H NMR (300 MHz, $CDCl_3$) line assignments for (+)-α-satalene are in agreement with literature values:[13a,38] ^1H NMR ($CDCl_3$, 300 MHz) δ 0.83

(38) Julia, M.; Ward, P. *Bull. Soc. Chim. Fr.* **1973**, *11*, 3065–3067.

(s, 5 H, CH_3 and $CHC(CH_3)CH$), 0.99 (s, 3 H, CH_3), 1.58 (s, 3 H, $=CCH_3$), 1.60 (s, 3 H, $=CCH_3$), 6.11 (t, J = 7.3 Hz, 1 H, vinyl H); GC/MS (70 eV), m/e (relative intensity) 204 (M^+, 13), 189 (10), 161 (13), 133 (8), 121 (30), 107 (34), 95 (46), 94 (100), 93 (93), 91 (38), 79 (38), 77 (34), 69 (34), 55 (28), 41 (57).

Oxidation of Santalol (9 and 10) to (*E*)-α- and (*E*)-β-Santalals 11 and 12. Commercial santalol (K and K Laboratories) was found to be a 63:37 mixture of (*Z*)-α-alcohol **9** (t_R 7.84) and (*Z*)-β-alcohol **10** (t_R 8.19) by GC analysis (column A, 240 °C). Careful flash chromatography using 33% ethyl acetate in hexane as eluent succeeded in removing some minor impurities and provided a sample enriched in the β isomer (53% α:47% β).

This sample (1.014 g, 4.61 mmol) was added in 10 mL of dichloromethane over 1 min to an orange solution of 2.117 g (4.66 mmol) of pyridinium chlorochromate and 0.956 g (11.3 mmol) of sodium acetate in 150 mL of dichloromethane.[39] The brown mixture was stirred for 20 h at room temperature and then filtered through Celite. The filtrate was evaporated under reduced pressure and the resulting brown oil was purified by flash chromatography. Elution with 20% ether in hexane provided 0.618 g (62%) of a clear, slightly yellow, and odorless aldehyde mixture as an oil. GC analysis (column A, 200 °C) showed a complex product mixture containing two major components, **11** (α, 40%, t_R 11.99) and **12** (β, 26%, t_R 13.07), and numerous minor impurities. The ^1H NMR spectrum (300 MHz, $CDCl_3$) displayed two singlets at 9.37 and 9.38 ppm in a ratio of ca. 2:3, indicating that the major α- and β-santalal products have trans stereochemistry about the trisubstituted double bond.[32b] Very minor singlets at 10.15 and 10.16 ppm, corresponding to the (*Z*)-α,β-unsaturated aldehyde products, were also observed. Other signals observed were in agreement with literature values.[32b]

Methyl (*E*)-α- and (*E*)-β-Santalenoates (1b and 13). A solution of 0.618 g (2.83 mmol) of the crude (*E*)-santalals **11** and **12**, 1.015 g (5.96 mmol) of silver nitrate, 8 mL of absolute ethanol, and 6 mL of water was stirred at room temperature while 0.464 g (11.6 mmol) of sodium hydroxide in 16 mL of water was added over 15 min in an adaptation of a literature procedure.[40] The mixture immediately turned black and was stirred for 2 h total. Precipitated silver oxide was removed by filtration and washed with 0.1 M sodium hydroxide. The washes and filtrate were concentrated to remove ethanol on a rotary evaporator with a warm water bath and the remaining aqueous solution was extracted with 10 mL of ether to remove trace amounts of unreacted aldehyde. The aqueous solution was acidified with 1 M hydrochloric acid and extracted with three portions of ether. The combined ether extracts were washed with saturated sodium chloride and dried ($MgSO_4$). Removal of solvent under reduced pressure provided 0.477 g (2.04 mmol, 72%) of a mixture of **1a** and (*E*)-β-santalen-12-oic acid as a clear oil containing a white solid.

A solution of the carboxylic acid mixture in 20 mL of ether was treated with ethereal diazomethane. Purification by flash chromatography using 20% ether in hexane as eluent provided a fraction that contained most of the two major esters of interest (t_R 10.72 (α) and t_R 11.53 (β), column A, 220 °C). Concentration produced 0.375 g (1.513 mmol, 53%) of the ester mixture as a clear oil, which was separated further by application to a 1.7 × 19.0 cm column of 28 g of silver nitrate impregnated silica gel and elution with 20% ether in hexane. Five 4-mL fractions were collected and analyzed by GC. Concentration of the first fraction gave 0.101 g of **1b**; estimated purity 88.5% by GC analysis (column A, 220 °C). Three unidentified impurities at t_R 6.35 (1.2%), t_R 7.02 (7.2%), and t_R 8.93 min (2.2%) were also observed. GC coinjection of this ester **1b** with that obtained from tomato leaf extracts showed them to have identical GC retention times (220 °C, t_R 10.91). GC/MS and ^1H and ^{13}C NMR spectra of ester **1b** obtained from commercial santalol are also identical with the corresponding spectra of tomato leaf extract derived ester **1b**. The specific rotation was $[\alpha]^{24}_D$ +25.94° (*c* 3.13, $CHCl_3$).

Concentration of the fifth chromatography fraction gave 43 mg of **13**, the purity of which was only 45% by GC analysis. Nevertheless, the ^1H NMR and ^{13}C NMR spectral data are consistent with the literature values[41] for methyl (*E*)-β-santalenoate.

Oviposition Preference Assays.[2] Leaf extracts from *L. hirsutum* and the purified components from these extracts were pipetted onto 4.25-cm diameter filter disks. After evaporation of the solvent, the disks were attached at even intervals to sheets of 3-mm chromatographic paper precut to cover the inner horizontal surfaces of three 30-cm diameter circular cake carriers adapted to serve as containers for the moth oviposition studies. The sheets with the treated and control disks were attached to the walls of the containers and 20 premated female *H. zea* moths were released within each container. The containers were then placed within a Percival growth chamber set at 25 °C, 75% R.H., and a 14 h/10 h light/dark cycle. At 24, 48, and 72 h after initiation the containers were removed from the incubators, the moths were anesthetized with carbon dioxide, and the sheets were removed to conduct egg counts. Oviposition preference values were calculated by dividing the number of eggs laid on treated disks by the mean number oviposited on two adjacent untreated disks. Disks permeated with hexane and then dried were also included in the experiments for controls. Values significantly less than one indicated nonpreference for *H. zea* oviposition, while those greater than one were preferred sites for oviposition. All data were tested by the protected LSD test to uncover statistically significant differences in oviposition preferences.

An enriched fraction of **1a** (84% **1a** + 16% **3a**) and pure **2a** were tested for oviposition stimulation. The concentrations of the purified sesquiterpenes were estimated by quantitative GC analysis to be 0.72 and 0.47 mg/g of fresh leaves of LA 1777, respectively. In experiment 1 333-μL aliquots of hexane solutions of **1a** (2.16 mg/mL hexane), **2a** (1.41 mg/mL hexane), the hexane extract of LA 1777 (3.00 g leaf tissue/mL extract), and pure hexane as a control were each pipetted onto nine filter disks. After drying three disks of each treatment were randomly attached to the walls of each of three containers as described above. The premated moths were then introduced and the experiment was initiated.

In experiment 2 333-μL aliquots of a mixture of **1a** (2.16 mg/mL) and **2a** (1.41 mg/mL), the hexane extract of LA 1777 (3.00 g leaf tissue/mL extract), and pure hexane were pipetted onto each of 12 filter disks. Four disks of each treatment were randomly assigned to three containers and the experiment was initiated.

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