Insect Growth Regulatory Effects of Linear Diterpenoids and Derivatives from *Baccharis thymifolia*

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Linear diterpenes isolated from aerial parts of *Baccharis thymifolia* (**1–3**) were tested for insect growth inhibitory activity against *Tenebrio molitor* larvae. Compounds **4–16** were prepared by various chemical transformations. The diterpenes 6,10-(*E,E*)-thymifodioic acid (**1**), the butenolide **3**, and the derivatives **4**, **11**, and **14** produced developmental deficiencies in assays using topical application on fifth instar larvae of *T. molitor*. Compound **3** is a new natural product.

During insect development, there are alternate periods of feeding and growth followed by shedding of the cuticle; this progression is known as the molting process. Molting provokes several changes, known as morphogenesis, which are regulated by hormones such as neurohormones, ecdysteroids, and juvenile hormones (JHs). The JHs include several sesquiterpenoids, particularly those possessing the fundamental structure of farnesol, that are synthesized from the mevalonate pathway. They are associated with physiological processes, such as metamorphosis and inhibition of imaginal disks, that affect the expression of the mature characters.[1]

Some synthetic compounds with skeletons quite different from the natural JHs possess JH activity.[2] This fact suggests that the hormone-like properties are induced by stereoelectronic factors not directly noticeable from simple scrutiny of the molecular structures. Juvenile hormones and antijuvenile hormones have been isolated from a variety of plants.[3–5] Genera of the Asteraceae and Labiatae families grow in the semiarid central western area of Argentina, and some of these plants contain compounds active toward insects.[6]

In previous phytochemical studies carried out with the aerial parts of *Baccharis thymifolia* Hook & Arn. (Asteraceae: Asteroideae: Baccharineae), the linear diterpenes **1** and **2** were isolated.[7,8] The novel compound **3** was recovered in this study, and the complete assignment of all NMR signals for compounds **1** and **2** is described. Synthetic derivatives **4–16** were prepared by various chemical transformations in order to evaluate their possible JH or anti-JH bioactivities against *Tenebrio molitor* L. (Coleoptera: Tenebrionidae) larvae.

Results and Discussion

The ^{1}H NMR and ^{13}C NMR spectra (see Experimental Section) of compound **3** were similar to those previously reported for compound **1**.[7] However, the ^{1}H NMR signals corresponding to the furan moiety of **1** were replaced by a set of signals centered at δ 4.80 (2H, br s, H-1 and H-1′) and at δ 7.15 (1H, br s, H-2). The last signal was in agreement with a proton on the β-carbon of an α,β-unsaturated carbonyl system. The pattern suggested an α-substituted butenolide heterocycle. Butenolide rings with β-substitution usually exhibit the olefinic proton near δ 5.90.[9,10] The ^{13}C NMR signals of **3** corresponding to C-1 (δ 70.1), C-2 (δ 145.0), and C-3 (δ 133.0) indicated α-substitution of the butenolide moiety. Two methyl groups on the C-15 olefinic carbon showed resonances at δ 1.66 and 1.57, whereas the H-14 signal appeared at δ 5.10 (t, J = 7.2 Hz). The proton on C-14 was clearly coupled with the two methyl groups and with the C-13 methylene protons (2H, δ 2.06, m). Two olefinic protons (multiplets at δ 6.70 and δ 6.80) were indicative of two α,β-unsaturated carboxyl groups. Treatment of compound **3** with diazomethane yielded the corresponding dimethyl ester. The ^{13}C NMR spectrum of compound **3** indicated *E* configuration for both the Δ^{6} and Δ^{10} double bonds.[11] Hence, compound **3** was assigned as (2*E*,6*E*)-2-(4-methylpent-3-enyl)-6-(3-(2-oxo-2,5-dihydrofuran-3-yl)propylidene)hept-2-enedioic acid.

Experiments directed to detect JH or anti-JH properties are based on observations of morphological changes in the insect development. Alterations in the reproductive system are frequently associated with a shortening of the pupal period. In the present work, fifth stage larvae of *T. molitor* were treated with compounds **1–16** by topical application using doses similar to those used for juvenile hormone antagonist studies.[12–14] The imaginal molt inhibition (Table 1) includes several abnormalities associated with premature metamorphosis.[15]

Diterpene **1** produced deformities without changing the pupal instar duration (Table 1). Its geometrical isomer (**2**) induced a reduction in length of the pupal stage, although growth abnormalities were not observed. The butenolide **3**, with the polyene chain similar to that of **1**, interfered with larvae development. But, in this case, the pupal stage duration was significantly decreased.

Compound **4** showed molt inhibition similar to its precursor **1**, but with a significant change in the duration of the pupal instar. A similar outcome was observed when the related compounds **2** and **5** were evaluated. These compounds (**1–5**) appear to have hormonal-like activity.

Considering that the majority of compounds with JH activity have a *gem*-dimethyl group or a *gem*-dimethyl-substituted oxirane ring on the terminal double bond, compounds **6–9** were prepared in order to assess the role of this functionality in the bioactive compounds **4** and **5**. Application of the Sharpless dihydroxylation reaction to diterpenes **4** and **5** afforded *vic*-diol moieties with high stereocontrol at C-14.[16]

Compound **6** was prepared by using AD-mix-α as chiral reagent. The ^{1}H NMR spectrum of **6** showed singlets at δ 1.12 (H-16) and 1.15 (H-17), as well as resonance at δ 3.25 (br d, J = 9.0 Hz, H-14), indicating the presence of two hydroxylated carbons (C-14/C-15). The ^{13}C NMR spectrum was in agreement with this substructure from the signals at δ 60.3 (C-14) and 72.6 (C-15).

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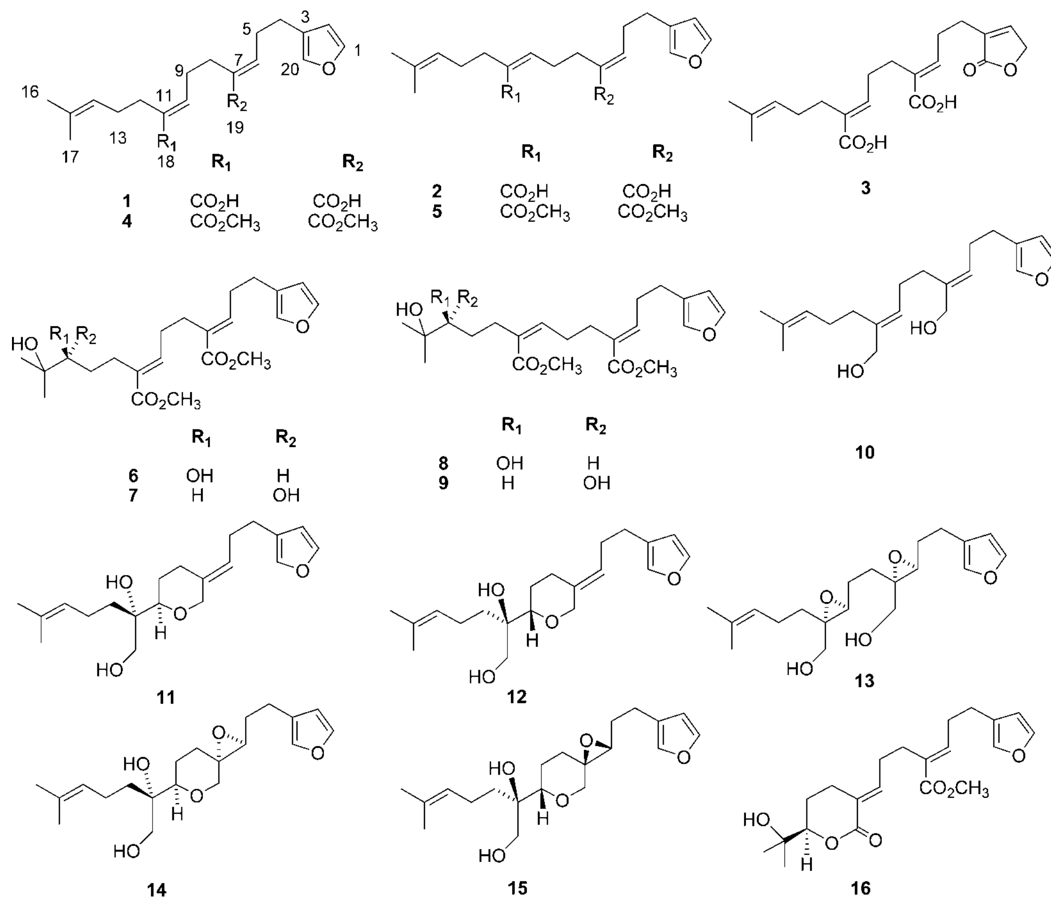

Table 1. Response of *T. molitor* Larvae to Compounds **1**−**16** in Topical Bioassays[a]

compound[b]	duration of pupal instar (days ± SEM)[c]	imaginal molt inhibition (percentage ± SEM)[d]
1	10.7 ± 0.4	33.6 ± 2.1
2	4.7 ± 0.4*	
3	5.09 ± 3.1*	24.1 ± 2.2
4	7.4 ± 1.2*	34.3 ± 2.4
5	6.0 ± 0.2*	
6	12.1 ± 0.3	
7	12.0 ± 1.4	
8	10.0 ± 1.0	
9	11.2 ± 1.9	
10	5.3 ± 2.7*	
11	16.2 ± 0.8*	14.4 ± 2.2
12	12.0 ± 0.8	
13	12.7 ± 0.5	
14	9.9 ± 1.2	30.8 ± 2.4
15	10.6 ± 0.7	
16	11.2 ± 1.9	
control	12.3 ± 1.2	

[a] $N = 25$. Experiments were carried out in three replicates. [b] Dose: 120 μg/larvae. [c] Time elapsed between the pupal ecdysis and the appearance of the first symptoms of the cuticle deposition in imaginal apolysis. Values are mean (±SEM). Data followed by an asterisk are significantly different from the control ($P < 0.05$, by Kruskal–Wallis test; multiple comparisons Dunn's test). [d] Insects showing a morphology intermediate between larvae-pupae and pupae-adultoids. Severe deficiencies in the process of the new cuticle deposition.

When AD-mix-β was used, the corresponding enantiomer (**7**) was obtained. Specific rotation values indicated that derivatives **6** and **7** were enantiomers. Compounds **8** and **9** were obtained from **5** using the appropriate chiral reagent. The ^{1}H NMR and ^{13}C NMR spectra for the new compounds were in agreement with the proposed structures.

Whereas compounds **4** and **5** were active (Table 1), derivatives **6–9** did not display any significant activity as a consequence of the stereocontroled oxidations. These results make us consider that the terminal γ,γ-dimethylallyl moiety might be a key requirement for bioactivity toward *T. molitor* larvae.

With the aim of analyzing the significance of the C-18 and C-19 functional groups, compound **4** was reduced with DIBAL-H. Surprisingly, the diol **10** only shortened the pupal phase without malformations in the molt process. In derivative **10**, carbons C-6 and C-10 do not possess the electrophilic property shown by compounds **1** and **4** due to the lack of the α,β-unsaturated carbonyl system. Electrophilic centers of this type can be attacked by nucleophiles such as the thiol group of enzymes quenching the enzyme activity, or nucleophiles from other key proteins or nucleic acids.[4] Compound **5**, which displays the aforementioned electrophilic center, but with a different configuration of the C-10−C-11 double bond, showed behavior similar to that of diol **10**. This is in accordance with complex stereoelectronic requirements involved in the recognition phenomenon.

The Katsuki−Sharpless[17] asymmetric epoxidation of allylic alcohols is a valuable reaction that exhibits high regio- and stereoselectivity. It is well-known that allylic alcohols increase the reactivity toward the epoxidation system when the electron density of the double bond increases. On the other hand, the olefinic bonds are important in the biological recognition through $\pi-\pi$ charge transfer complexes.[18,19]

Using the bis(allylic alcohol) **10**, asymmetric epoxidations were carried out by means of the two chiral ligands. By Ti(OPr-*i*)$_4$ (1.2 equiv), (*R,R*)-(+)-DET (1.4 equiv), and TBHP (1.2 equiv), compound **10** was converted into derivative **11**. The ^{1}H NMR data of **11** revealed that the furan ring was unaffected after the reaction. The signals corresponding to H-10 (1H, $\delta = 5.40$, br s) and H-19 (2H, δ 4.01, s) in compound **10** were changed to δ 3.53 (d, $J = 6.7$ Hz, H-10), and the two-proton singlet corresponding to H-19 was resolved as an AB system with resonances at δ 3.74 and 3.38. Moreover, the H-6 signal that appeared as a triplet ($J = 6.0$ Hz) at δ 5.45 in compound **10**, appeared at δ 5.29 in **11**. All of the above

data, as well as the ^{13}C NMR analysis, were in agreement with the presence of a 2-substituted-5-methylenetetrahydro-2*H*-pyran system. It is possible to reach this framework by an oxirane-ring-opening attack by the C-19 hydroxy group. From this hypothesis, the two new chiral carbons (C-10 and C-11) were obtained with high stereocontrol. The optical rotation value for compound **11** was $[\alpha]^{25}_D$ +9.3 (0.53, $CHCl_3$). When the chiral ligand was (*S,S*)-(−)-DET (1.2 equiv), the derivative **12** was recovered, and the optical rotation value was $[\alpha]^{25}_D$ −9.6 (0.99, $CHCl_3$). This data indicated that the configuration of this compound was opposite that of derivative **11**.

When 2.8 equiv of (*R,R*)-(+)-DET was used, compounds **13** and **14** were obtained. The ^{1}H NMR spectrum of **13** showed two one-proton multiplets at δ 2.90 and 3.47, corresponding to H-6 and H-10, respectively. Moreover, the hydroxymethylene proton signals (2H-18 and 2H-19) were slightly shielded, exhibiting some changes in multiplicities. These results are in agreement with the presence of two oxirane functionalities. The optical rotation value for this chiral compound was $[\alpha]^{25}_D$ −5.8 (0.15, $CHCl_3$). The presence of a 2-substituted-5-methylenetetrahydro-2*H*-pyran moiety in the byproduct **14** was determined on the basis previously discussed for compound **11**. The remaining NMR data were in agreement with the proposed structure for this compound; the optical rotation was $[\alpha]^{25}_D$ +2.5 (0.40, $CHCl_3$).

Compound **15** was obtained from **10** by means of (*S,S*)-(−)-DET. The ^{1}H NMR and ^{13}C NMR data were equivalent to those of its enantiomer (**14**). However, the optical rotation, $[\alpha]^{25}_D$ −1.8 (0.20, $CHCl_3$) was not in agreement, possibly due to the small amount available.

It is known that lactones that have a carbonyl group conjugated with an exocyclic double bond are frequently toxic toward insects. The deleterious effect has been attributed to the electrophilic β-carbon, which could act as an acceptor of nucleophiles in a Michael-type addition. In an attempt to examine this type of structure, diol **7** was subjected to an intramolecular transesterification reaction catalyzed by PPTS, yielding compound **16**. The ^{1}H NMR of **16** exhibited signals typical of a β-substituted furan ring. Resonances of the C-19 carboxymethyl protons (δ 3.75, s) and a one-proton signal at δ 6.83 (t, J = 7.3 Hz, H-6) were consistent with an α,β-unsaturated carboxymethyl group. The chemical shift for H-10 (δ 7.0, t, J = 5.5 Hz) was consistent with an α,β-unsaturated δ-lactone ring. On the other hand, the H-14 proton was shifted from δ 3.25 (br d, J = 9.0 Hz) in the starting material (compound **7**) to δ 4.01 (dd, J = 9.6, 1.9 Hz) in compound **16**. The ^{13}C NMR data were in agreement with the proposed structure.

Only compounds **11** and **14** were significantly active (Table 1); the former produced elongation of the pupal stage with some morphological abnormalities. Compound **14** did not vary the duration of the pupal stage, but the morphological abnormalities were increased. A remarkable result was that the corresponding enantiomers, **12** and **15**, did not display any activity, indicating that the stereochemistry plays a significant role in the biological activity.

In conclusion, although it is not possible to clearly describe the structure–activity relationships, it appears that the active compounds act as antagonists of JH, inducing premature metamorphosis or a range of intermediate morphogenetic responses. Finally, based on test results of compounds **11** vs **12**, and **14** vs **15**, it may be possible to design additional compounds suitable for insect pest control.

Experimental Section

General Experimental Procedures. NMR spectra were measured at 400 MHz (^{1}H) and 100 MHz (^{13}C); chemical shifts are reported relative to internal Me_4Si (δ = 0). NMR assignments were determined using 2D experiments (COSY, DEPT, HETCOR, HMBC, HSQC). Optical rotations were determined in $CHCl_3$. Column chromatography (CC) was performed on silica gel 60 Å (400–500 mesh), using increasing polarity *n*-hexane−EtOAc mixtures as solvent. Gel chromatography was carried out by means of Sephadex LH-20 with MeOH as solvent. TLC was carried out on silica gel 60 F_{254} (0.2 mm thick plates) using a mixture of *n*-hexane−EtOAc (1:1) as solvent. Compounds were visualized using UV light and H_2SO_4−AcOH−H_2O (4:20:1) as chromogenic reagent. HPLC chromatography was carried out using a Waters TA Instrument 515 pump and Waters 2487 dual λ absorbance and Waters 2414 refractive index detectors; the flow rate was 1.5 mL min^{-1} with *n*-hexane−EtOAc (40:60) as solvent. The column was a Macherey-Nagel SiO_2. All solvents were purified by standard techniques. Reactions requiring anhydrous conditions were performed under argon.

Plant Material. Aerial parts (2.8 kg) of *Baccharis thymifolia* were collected in February 2004 in Villavicencio, province of Mendoza, Argentina. A voucher specimen is deposited at the Herbarium of the Universidad Nacional de San Luis: L.A. Del Vitto-32491.

Isolation and Purification. The dry aerial parts were chopped and macerated (× 4) for 5-day periods with Me_2CO at rt. The solvent was evaporated under reduced pressure at low temperature, and the crude extract (480 g) was recovered. The acetone extract was subjected to flash chromatography with *n*-hexane, and the polarity of the solvent was gradually increased by addition of EtOAc. After several purifications of each fraction by flash chromatography, compounds **1** (7.5 g),[7] **2** (2.8 g),[8] and **3** (0.8 g) were recovered.

Compound 3, (2*E*,6*E*)-2-(4-Methylpent-3-enyl)-6-(3-(2-oxo-2,5-dihydrofuran-3-yl)propyli-dene)hept-2-enedioic acid: colorless oil; IR (KBr) ν_{max} 3439, 2927, 2360, 2352, 1751, 1686, 1637, 1450, 748 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) (as dimethyl ester) δ 7.15 (1H, br s, H-2), 6.80 (1H, m, H-6), 6.70 (1H, m, H-10), 5.10 (1H, t, J = 7.2 Hz, H-14), 4.80 (2H, br s, H-1), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 2.43 (6H, m, H-4, H-5, H-8), 2.26 (4H, m, H-9, H-12), 2.06 (2H, m, H-13), 1.66 (3H, s, H-16), 1.57 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) (as dimethyl ester) δ 173.8 (C, C-20), 168.3 (C, C-18), 167.6 (C, C-19), 145.0 (CH, C-2), 141.4 (CH, C-6), 141.2 (CH, C-10), 133.0 (C, C-3), 132.6 (C, C-11), 132.3 (C, C-7), 132.2 (C, C-15), 123.5 (CH, C-14), 70.1 (CH_2, C-1), 51.8 and 51.6 (CH_3, methyl esters), 29.3 (CH_2, C-5), 27.9 (CH_2, C-12), 27.7 (CH_2, C-13), 26.9 (CH_2, C-9), 26.5 (CH_2, C-8), 25.6 (CH_2, C-4), 24.6 (CH_3, C-16), 17.6 (CH_3, C-17); EIMS *m/z* 344 [$M^+ - H_2O$] (9), 326 (9), 298 (8), 229 (13), 149 (24), 121 (16), 97 (6), 91 (20), 69 (100); HRMS (FABS) *m/z* 385.1550 (calcd for $C_{20}H_{26}O_6Na$ 385.1627).

Preparation of 4, (2*E*,6*E*)-Dimethyl 6-(3-(furan-3-yl)propylidene)-2-(4-methylpent-3-enyl)hept-2-enedioate, and 5, (2*Z*,6*E*)-Dimethyl 6-(3-(furan-3-yl)propylidene)-2-(4-methylpent-3-enyl)hept-2-enedioate. The methyl ester derivatives **4** and **5** were prepared from **1** and **2** using a solution of diazomethane in Et_2O. Compound **4**: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.33 (1H, br s, H-1), 7.21 (1H, br s, H-20), 6.81 (1H, t, J = 7.2 Hz, H-6), 6.70 (1H, t, J = 7.2 Hz, H-10), 6.25 (1H, br s, H-2), 5.09 (1H, t, J = 6.8 Hz, H-14), 3.72 (3H, s, OCH_3), 3.70 (3H, s, OCH_3), 2.55 (2H, dd, J = 7.2, 7.3 Hz, H-4), 2.41 (4H, m, H-5, H-8), 2.24 (4H, m, H-9, H-12), 2.04 (2H, dd, J =7.2, 7.3 Hz, H-13), 1.65 (3H, s, H-16), 1.56 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.2 (C, C-18), 167.8 (C, C-19), 142.9 (CH, C-1), 142.5 (CH, C-6), 141.4 (CH, C-10), 139.9 (CH, C-20), 132.5 (C, C-11), 132.1 (C, C-7), 131.5 (C, C-15), 123.8 (C, C-3), 123.6 (CH, C-14), 110.7 (CH, C-2), 51.6 and 51.5 (CH_3, methyl esters), 29.2 (CH_2, C-5), 28.0 (CH_2, C-12), 27.7 (CH_2, C-13), 26.9 (CH_2, C-9), 26.0 (CH_2, C-8), 25.6 (CH_3, C-16), 24.0 (CH_2, C-4), 14.1 (CH_3, C-17). Compound **5**: colorless oil; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.32 (1H, br s, H-1), 7.22 (1H, br s, H-20), 6.78 (1H, t, J = 7.2 Hz, H-6), 6.26 (1H, br s, H-2), 5.83 (1H, t, J =7.2 Hz, H-10), 5.05 (1H, t, J = 6.8 Hz, H-14), 3.71 (3H, s, OCH_3), 3.69 (3H, s, OCH_3), 2.54 (4H, m, H-4 and H-5), 2.46 (2H, m, H-8), 2.37 (2H, m, H-9), 2.22 (2H, m, H-12), 2.04 (2H, dd, J = 7.2, 7.3 Hz, H-13), 1.66 (3H, s, H-16), 1.56 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.2 (C, C-18), 167.9 (C, C-19), 142.8 (CH, C-1), 142.3 (CH, C-6), 140.4 (CH, C-10), 138.9 (CH, C-20), 132.2 (C, C-15), 132.1 (C, C-11), 131.7 (C, C-7), 123.9 (C, C-3), 123.3 (CH, C-14), 110.7 (CH, C-2), 51.6 and 51.0 (CH_3, methyl esters), 34.6 (CH_2, C-12), 29.0 (CH_2, C-4), 28.9 (CH_2, C-8), 27.0 (CH_2, C-13), 26.4 (CH_2, C-9), 25.5 (CH_3, C-16), 24.0 (CH_2, C-5), 17.5 (CH_3, C-17).

Preparation of 6, (2*E*,6*E*)-Dimethyl 2-((*S*)-3,4-dihydroxy-4-methylpentyl)-6-(3-(furan-3-yl)propylidene)hept-2-enedioate. Compound **4** (50 mg, 0.133 mmol) was added to a mixture of *t*-BuOH−H_2O (1:1) (1.33 mL), AD-mix-α (186 mg, 1.4 g/mmol of the starting material), and methansulfonylamide (13.1 mg, 0.132 mmol) at 0 °C. The solution

was stirred for 20 h at 0 °C. After addition of a saturated solution of $Na_2S_2O_3$ (3 mL), the mixture was extracted with EtOAc. The extracts were dried, and the solvent was removed under vacuum. Flash chromatography on silica gel yielded **6** (29 mg, 60% yield) as a colorless oil: $[\alpha]^{25}_{D}$ −9.35 (*c* 0.51, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, br s, H-1), 7.23 (1H, br s, H-20), 6.82 (1H, t, *J* = 6.8 Hz, H-6), 6.79 (1H, t, *J* = 6.8 Hz, H-10), 6.26 (1H, br s, H-2), 3.74 (3H, s, OCH_3), 3.73 (3H, s, OCH_3), 3.25 (1H, br d, *J* = 9.0 Hz, H-14), 2.58 (2H, m, H-4), 2.45 (4H, m, H-5, H-12), 2.40 (2H, m, H-8), 2.29 (2H, m, H-9), 1.25 (2H, m, H-13), 1.15 (3H, s, H-17), 1.12 (3H, s, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.4 (C, C-18), 167.9 (C, C-19), 142.7 (CH, C-1), 142.5 (CH, C-10), 142.3 (CH, C-6), 138.8 (CH, C-20), 132.0 (C, C-11), 131.3 (C, C-7), 123.7 (C, C-3), 110.7 (CH, C-2), 72.6 (CH, C-15), 60.3 (CH, C-14), 51.8 and 51.4 (CH_3, methyl esters), 31.1 (CH_2, C-13), 29.2 (CH_2, C-5), 28.1 (CH_2, C-9), 26.1 (CH_2, C-8) 26.1 (CH_3, C-16), 23.9 (CH_2, C-4), 23.3 (CH_2, C-12) 23.3 (CH_3, C-17); EIMS *m/z* 390 [M^+-H_2O] (5), 376 [M^+ − CH_3OH] (2), 299 (17), 281 (10), 149 (68), 107 (23), 81 (100).

Preparation of 7, (2*E*,6*E*)-Dimethyl 2-((*R*)-3,4-dihydroxy-4-methylpentyl)-6-(3-(furan-3-yl)propylidene)hept-2-enedioate. The procedure used above to obtain **6** was applied again to **4** (50 mg, 0.133 mmol), using AD-mix-β to yield **7** (36 mg, 72% yield) as an oil: $[\alpha]^{25}_{D}$ +9.00 (*c* 0.33, $CHCl_3$); ^1H NMR ($CDCl_3$); ^{13}C NMR ($CDCl_3$); EIMS (see compound **6**).

Preparation of 8, (2*Z*,6*E*)-Dimethyl 2-((*S*)-3,4-dihydroxy-4-methylpentyl)-6-(3-(furan-3-yl)propylidene)hept-2-enedioate. The aforementioned procedure used to obtain **6** from **4** was applied to compound **5** on a 6 mg (0.016 mmol) scale, yielding **8** (4 mg, 67% yield) as an oil: $[\alpha]^{25}_{D}$ −9 (*c* 0.33, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, br s, H-1), 7.26 (1H, br s, H-20), 6.82 (1H, t, *J* = 7.2 Hz, H-6), 6.28 (1H, br s, H-2), 5.95 (1H, t, *J* = 7.4 Hz, H-10), 3.74 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 3.32 (1H, br d, *J* = 10.3 Hz, H-14), 2.54 (4H, m, H-4 and H-5), 2.46 (2H, m, H-8), 2.37 (2H, m, H-9), 2.22 (2H, m, H-12), 1.25 (2H, m, H-13), 1.18 (3H, s, H-17), 1.14 (3H, s, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.4 (C, C-18), 167.9 (C, C-19), 142.7 (CH, C-1), 142.3 (CH, C-6), 141.2 (CH, C-10), 138.8 (CH, C-20), 131.7 (C, C-11), 131.5 (C, C-7), 123.7 (C, C-3), 110.6 (CH, C-2), 72.7 (CH, C-15), 65.6 (CH, C-14), 51.7 and 51.5 (CH_3, methyl esters), 31.3 (CH_2, C-12), 29.2 (CH_2, C-5), 29.0 (CH_2, C-8), 28.9 (CH_2, C-13), 28.3 (CH_2, C-9) 26.2 (CH_3, C-16), 23.8 (CH_2, C-4), 23.3 (CH_3, C-17); EIMS *m/z* 376 [M^+ − CH_3OH] (1), 358 (5), 343 (4), 284 (14), 149 (3), 107 (25), 81 (100).

Preparation of 9, (2*Z*,6*E*)-Dimethyl 2-((*R*)-3,4-dihydroxy-4-methylpentyl)-6-(3-(furan-3-yl)propylidene)hept-2-enedioate. The same procedure to obtain **8** was applied again to compound **5** (6 mg, 0.016 mmol), using AD-mix-β to yield **9** (3.6 mg, 60% yield) as an oil: $[\alpha]^{25}_{D}$ +8.4 (*c* 0.59, $CHCl_3$); ^1H NMR, ^{13}C NMR, and EIMS (see compound **8**).

Preparation of 10, (2*E*,6*E*)-6-(3-(Furan-3-yl)propylidene)-2-(4-methylpent-3-enyl)hept-2-ene-1,7-diol. To a stirred solution of **4** (488 mg, 1.348 mmol) in dry Et_2O (13.5 mL) was added dropwise DIBAL-H (5.48 mL, 1.0 M in hexane, 5.48 mmol) at 0 °C. The reaction mixture was stirred for 60 min at 0 °C. Then, the reaction was quenched with H_2O (10 mL), allowed to warm to room temperature, and additionally stirred for 30 min. The mixture was dried over $MgSO_4$ and filtered through a pad of Celite, and the solvent was evaporated. The residue was purified by silica gel column chromatography to yield **10** (340 mg, 82% yield) as an oil: ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34 (1H, br s, H-1), 7.21 (1H, br s, H-20), 6.26 (1H, br s, H-2), 5.45 (1H, t, *J* = 6.0 Hz, H-6), 5.40 (1H, br s, H-10), 5.10 (1H, br s, H-14), 4.01 (2H, s, H-19), 3.99 (2H, s, H-18), 2.47 (2H, m, H-4), 2.31 (4H, m, H-5, H-9), 2.13 (4H, m, H-8, H-13), 2.10 (2H, m, H-12), 1.67 (3H, s, H-16), 1.59 (3H, s, H-17); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.7 (CH, C-1), 139.2 (C, C-7), 138.9 (CH, C-20), 138.9 (CH_2, C-11), 132.0 (C, C-15), 126.5 (CH, C-10), 125.3 (CH, C-6), 124.5 (C, C-3), 124.0 (CH, C-14), 110.9 (CH, C-2), 67.1 ($2CH_2$, C-18 and C-19), 28.2 (CH_2, C-9), 28.0 (CH_2, C-5), 27.1 (CH_2, C-13), 26.4 (CH_2, C-12), 25.6 (CH_2, C-8), 24.9 (CH_3, C-16), 22.6 (CH_2, C-4), 17.6 (CH_3, C-17).

Preparation of 11, (*S*)-2-((*R,E*)-5-(3-(Furan-3-yl)propylidene) tetrahydro-2H-pyran-2-yl)-6-methylhept-5-ene-1,2-diol. Crushed and activated 4 Å molecular sieves were added to stirred CH_2Cl_2 (1 mL), under argon. The flask was cooled to –20 °C, and Ti(OPr-*i*)$_4$(26.8 μL, 0.150 mmol), (*R,R*)-(+)-DET (27.8 μL, 0.180 mmol), and the diallylic alcohol **10** (41 mg, 0.130 mmol) dissolved in CH_2Cl_2 (0.5 mL) were added sequentially. The mixture was stirred at the same temperature for 20 min, and TBHP (25 μL, 0.150 mmol) was added slowly. The reaction was kept stirring at −20 °C for 12 h. Tartaric acid aqueous solution (15% w/v) was added at room temperature, and the stirring was continued until clear phases were reached. The phases were separated, and the aqueous phase was extracted twice with CH_2Cl_2. The combined organic phases were washed with brine, concentrated, diluted with Et_2O, and treated with precooled (0 °C) 15% (w/v) NaOH aqueous solution. The two-phase mixture was stirred vigorously for 15 min at 0 °C. The organic phase was separated, and the aqueous phase was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried, filtered, concentrated, and purified by silica gel column chromatography to yield **11** (0.025 g, 60% yield) as an oil: $[\alpha]^{25}_{D}$ +9.3 (*c* 0.53, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.34 (1H, br s, H-1), 7.21 (1H, br s, H-20), 6.26 (1H, br s, H-2), 5.29 (1H, t, *J* = 6.8 Hz, H-6), 5.08 (1H, br t, *J* = 6.8 Hz, H-14), 4.13 (1H, d, *J* = 9.8 Hz, H-18a), 3.93 (1H, d, *J* = 9.8 Hz, H-18b), 3.74 (1H, d, *J* = 8.5, H-19a), 3.38 (1H, d, *J* = 8.5, H-19b), 3.53 (1H, d, *J* = 6.7 Hz, H-10), 2.67 (2H, m, H-12), 2.47 (2H, m, H-4), 2.29 (2H, m, H-5), 1.90 (2H, m, H-13), 1.72 (3H, s, H-16), 1.72 (2H, m, H-8), 1.62 (3H, s, H-17), 1.36 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 142.7 (CH, C-1), 139.9 (C, C-7), 138.9 (CH, C-20), 131.9 (C, C-15), 124.2 (CH, C-6), 124.1 (C, C-3), 124.1 (CH, C-14), 111.0 (CH, C-2), 84.0 (CH, C-10), 74.7 (CH_2, C-18), 74.2 (C, C-11), 66.4 (CH_2, C-19), 33.7 (CH_2, C-9), 27.1 (CH_2, C-5), 25.6 (CH_2, C-8) 25.6 (CH_3, C-16), 25.4 (CH_2, C-12), 24.7 (CH_2, C-4), 21.5 (CH_2, C-13), 17.6 (CH_3, C-17); EIMS *m/z* 334 [M^+] (11), 303 (16), 191 (25), 147 (95), 81 (100), 69 (94); HRMS *m/z* 334.2155 (calcd for $C_{20}H_{30}O_4$334.2144).

Preparation of 12, (*R*)-2-((*S,E*)-5-(3-(Furan-3-yl)propylidene) tetrahydro-2H-pyran-2-yl)-6-methylhept-5-ene-1,2-diol. The procedure described to obtain **11** was applied to **10** (41 mg, 0.129 mmol), using (*S,S*)-(−)-DET instead of the (*R,R*)-(+)-DET, to yield **12** (8 mg, 20% yield) as an oil: $[\alpha]^{25}_{D}$ −9.6 (*c* 0.99, $CHCl_3$); ^1H NMR, ^{13}C NMR, and EIMS (see compound **11**).

Preparation of 13, ((2*S*,3*S*)-3-(2-(Furan-3-yl)ethyl)-2-(2-((2*S*,3*S*)-3-(hydroxymethyl)-3-(4-methylpent-3-enyl)oxiran-2-yl)ethyl)oxiran-2-yl)methanol, and 14, (*S*)-2-((2*S*,3*S*,6*R*)-2-(2-(Furan-3-yl)ethyl)-1,5-dioxaspiro[2.5]octan-6-yl)-6-methylhept-5-ene-1,2-diol. The procedure used above to obtain **11** was applied to the bis(allylic alcohol) **10** (60 mg, 0.188 mmol), using Ti(OPr-*i*)$_4$ (55.6 μL, 0.452 mmol), (*R,R*)-(+)-DET (90.0 μL, 0.528 mmol), and TBHP (108.0 μL, 0.678 mmol) to yield, after chromatographic separation, compound **13** (20 mg, 20% yield) as an oil: $[\alpha]^{25}_{D}$ −5.8 (*c* 0.15, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.36 (1H, br s, H-1), 7.26 (1H, br s, H-20), 6.26 (1H, br s, H-2), 5.08 (1H, br t, *J* = 6.8 Hz, H-14), 3.74 (2H, m, H-18), 3.61 (2H, m, H-19), 3.47 (1H, m, H-10), 2.90 (1H, m, H-6), 2.13 (2H, m, H-13), 1.89 (2H, m, H-4), 1.86 (2H, m, H-12), 1.80 (4H, m, H-5 and H-8), 1.68 (3H, s, H-16), 1.61 (3H, s, H-17), 1.45 (2H, m, H-9); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.0 (CH, C-1), 139.0 (CH, C-20), 132.0 (C, C-15), 124.0 (C, C-3), 123.0 (CH, C-14), 111.0 (CH, C-2), 66.0 (CH_2, C-19), 65.0 (CH, C-6), 63.0 (C, C-11), 62.0 (CH_2, C-18), 60.5 (CH, C-10), 60.0 (C, C-7), 31.0 (CH_2, C-13), 28.0 (CH_2, C-12), 26.0 (CH_3, C-16), 24.0 (CH_2, C-5 and C-9), 22.0 (CH_2, C-4), 20.0 (CH_2, C-8), 17.0 (CH_3, C-17); EIMS *m/z* 350 $[M]^+$ (10), 332 (1), 269 (3), 147 (15), 131 (21), 93 (25), 81 (92), 69 (100); HRMS *m/z* 350.2076 (calcd for $C_{20}H_{30}O_5$350.2093).

Compound 14: (18 mg, 18% yield), oil; $[\alpha]^{25}_{D}$ +2.5 (*c* 0.40, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.38 (1H, br s, H-1), 7.27 (1H, br s, H-20), 6.31 (1H, br s, H-2), 5.09 (1H, br t, *J* = 6.8 Hz, H-14), 3.73 (1H, d, *J* = 11.0 Hz, H-19a), 3.46 (1H, d, *J* = 11.0 Hz, H-19b), 3.73 (1H, d, *J* = 11.0 Hz, H-18a), 3.38 (1H, d, *J* = 11.0 Hz, H-18b), 3.46 (1H, t, *J* =11.0 Hz, H-10), 2.90 (1H, t, *J* =5.0 Hz, H-6), 2.10 (2H, m, H-13), 1.90 (2H, m, H-4), 1.80 (6H, m, H-5, H-8 and H-9), 1.68 (3H, s, H-16), 1.62 (3H, s, H-17), 1.50 (2H, m, H-12); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 143.0 (CH, C-1), 139.0 (CH, C-20), 132.1 (C, C-15), 124.0 (CH, C-14), 123.8 (C, C-3), 110.8 (CH, C-2), 82.9 (CH, C-10), 74.3 (C, C-11), 74.0 (CH_2, C-18), 68.6 (C, C-7), 65.9 (CH_2, C-19), 64.8 (CH, C-6), 33.8 (CH_2, C-9), 26.0 (CH_2, C-8), 25.6 (CH_3, C-16), 25.4 (CH_2, C-12), 21.8 ($2CH_2$, C-4 and C-5), 21.6 (CH_2, C-13), 17.6 (CH_3, C-17); EIMS *m/z* 350 $[M]^+$ (2), 332 (5), 319 (18), 189 (8), 147 (20), 125 (17), 108 (36), 95 (26), 81 (95), 69 (100); HRMS *m/z* 350.2114 (calcd for $C_{20}H_{30}O_5$350.2093).

Preparation of 15, (*R*)-2-((2*R*,3*R*,6*S*)-2-(2-(Furan-3-yl)ethyl)-1,5-dioxaspiro[2.5]octan-6-yl)-6-methylhept-5-ene-1,2-diol. The procedure used above to obtain **13** and **14** was applied to **10** (30.0 mg,

0.0940 mmol), using (*S,S*)-(−)-DET, to yield **15** as an oil: $[\alpha]^{25}_{D}$ −1.8 (*c* 0.20, $CHCl_3$); ^{1}H NMR, ^{13}C NMR, and EIMS (see compound **14**). In the course of this procedure the corresponding enantiomer of **13** was not recovered.

Preparation of 16, (*E*)-methyl 5-(Furan-3-yl)-2-((*E*)-3-((*R*)-6-(2-hydroxypropan-2-yl)-2-oxo-2*H*-pyran-3(4*H*,5*H*,6*H*)-ylidene)propyl)pent-2-enoate. Compound **7** (12 mg, 0.294 mmol) was dissolved in dry toluene (5 mL). After that, PPTS (35 mg, 0.015 mmol) was added to the reaction. The mixture was stirred with reflux at 120 °C, for 48 h. A few drops of triethylamine and EtOAc (5 mL) were then added, and the mixture was evaporated to dryness under vacuum to yield **16** (0.007 g, 58% yield) as an oil: $[\alpha]^{25}_{D}$ −23.2 (*c* 0.4, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.35 (1H, br s, H-1), 7.25 (1H, br s, H-20), 7.00 (1H, t, J = 5.5 Hz, H-10), 6.83 (1H, t, J = 7.3 Hz, H-6), 6.27 (1H, br s, H-2), 4.01 (1H, dd, J = 9.6, 1.9 Hz, H-14), 3.75 (3H, s, OCH_3), 2.61 (2H, m, H-4), 2.43 (2H, m, H-5), 2.33 (2H, m, H-8), 2.25 (2H, m, H-9), 2.00 (2H, m, H-12), 1.66 (2H, m, H-13), 1.28 (3H, s, H-17), 1.24 (3H, s, H-16); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 167.6 (C, C-18), 166.2 (C, C-19), 144.7 (CH, C-10), 142.7 (CH, C-1 and C-6), 138.8 (CH, C-20), 131.0 (C, C-7), 125.4 (C, C-11), 123.5 (C, C-3), 110.6 (CH, C-2), 85.4 (CH, C-14), 71.1 (C, C-15), 51.6 (OCH_3), 27.6 (CH_2, C-9), 25.3 (CH_3, C-16), 25.1 (CH_2, C-5), 23.7 (CH_3, C-17), 22.5 (CH_2, C-12), 22.4 ($3CH_2$, C-4, C-8 and C-13); EIMS *m/z* 376 $[M]^+$ (4), 344 (20), 317 (8), 316 (21), 166 (12), 138 (14), 91 (10), 81 (100); HRMS *m/z* 376.1905 (calcd for $C_{21}H_{28}O_6$376.1886).

Bioassays. Recently ecdysed fifth instar larvae of *T. molitor* were randomly selected and starved for 24 h. Two acetone solutions of each compound were prepared. Test solutions were topically applied to the ventral surface of the thoracic segments with a Hamilton microsyringe (2 μL/larvae; equivalent to 120 μg/larvae of diterpene assayed). Controls were treated with the solvent alone. Three replicates of 25 larvae for each compound were tested. After treatment, insects were placed into plastic vials (diameter 10 cm, height 7 cm) containing food and held at 25 ± 1 °C with a 16:8 (L:D) photoperiod. The duration of the pupal stage (in days) was recorded every 24 h for 60 days (end-point of the experiment). The appearance of morphological alterations was documented, and the results are shown in Table 1.

Statistical Analysis. Growth assays were analyzed using the Kruskal−Wallis test and Dunn's multiple comparisons test at the $P < 0.05$ level.

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Supporting Information Available: Complete IR, ^{1}H and ^{13}C NMR, and EIMS spectral data of compounds **1** and **2**. Copies of the ^{1}H NMR spectrum of compounds **3**–**15**. Images of treated larvae. This information is available free of charge via the Internet at http://pubs.acs.org.

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