Novel Labdane Diterpenes from the Insecticidal Plant *Hyptis spicigera*[1]

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Seven new labdane diterpenes with insecticidal properties were isolated from the aerial parts of *Hyptis spicigera*. Their structures were established on the basis of spectral (MS, ^{1}H NMR, and ^{13}C NMR) and chemical evidences as: 19-acetoxy-2α,7α,15-trihydroxylabda-8(17),(13*Z*)-diene (**1**); 15,19-diacetoxy-2α,7α-dihydroxylabda-8(17),(13*Z*)-diene (**2**); 7α,15,19-triacetoxy-2α-hydroxylabda-8(17),(13*Z*)-diene (**3**); 19-acetoxy-2α,7α-dihydroxylabda-8(17),(13*Z*)-dien-15-al (**4**); 19-acetoxy-7α,15-dihydroxylabda-8(17),(13*Z*)-dien-2-one (**5**); 19-acetoxy-2α,7α-dihydroxylabda-14,15-dinorlabd-8(17)-en-13-one (**6**); and 2α,7α,15,19-tetrahydroxy-*ent*-labda-8(17),(13*Z*)-diene (**7**). Absolute configurations were established by application of Mosher's method. Compound **2** significantly inhibited larval growth of the European corn borer.

Members of the Lamiaceae (Labiatae) family have provided important resources for the Old and New Worlds, and their uses in medicine, as condiments in regional cuisine, and as pest-control agents are of central importance.[2−4] The genus *Hyptis*, in particular, has yielded a great number of medicinal and economically important species that are frequently used as remedies in the treatment of gastrointestinal disturbances and skin infections, as well as insect repellents.[5] Bioactivity-guided fractionation of selected cytotoxic crude extracts have indicated the importance of flavonoids,[6] lignans (*H. verticillata* and *H. tomentosa*),[7] and 5,6-dihydro-α-pyrones (*H. pectinata* and *H. oblongifolia*)[8] as the bioactive principles of this genus.[5]

In the course of our research directed toward the discovery of bioactive constituents from economic and medicinal Mexican plants,[9] we now describe the structure and stereochemistry of seven novel diterpenes, compounds **1**−**7**, from the aerial parts of *H. spicigera* Lam. This plant material, popularly known in Mexico as "hierba del burro", is used by farmers to control insect infestations in stored grains. The whole plant is strongly scented, and it is burned in rooms to repel mosquitoes. The aerial parts of this species are used to flavor foods and for medicinal purposes.[10] It has also been a traditional practice of farmers in many regions of tropical Africa, for example, Burkina Faso (Upper Volta) and Sierra Leona, to mix this local weed with grain, because of some antifeedant and/or repellent components against bruchid beetles (*Callosobruchus* sp.), whose larvae feed on the grain, causing extensive damage and loss.[11]

The efficacy of this traditionally used insecticidal plant was previously demonstrated. Ethanolic extracts derived from this plant material were found to reduce the oviposition and hatching of bean weevils (*Acanthescelides obtectus*) under controlled environmental conditions.[11] Only two previous phytochemical investigations of this plant have been conducted. In a preliminary study of the leaves and inflorescences, the chemical composition of their essential oils was quantified by GC−MS. The oils were characterized by a high content of β-caryophyllene, α-pinene, and sabinene.[10] Recently, the defatted aerial parts afforded a 6-substituted 5,6-dihydro-α-pyrone,[12] closely related to the cytotoxic constituents from *H. oblongifolia*.[5,8]

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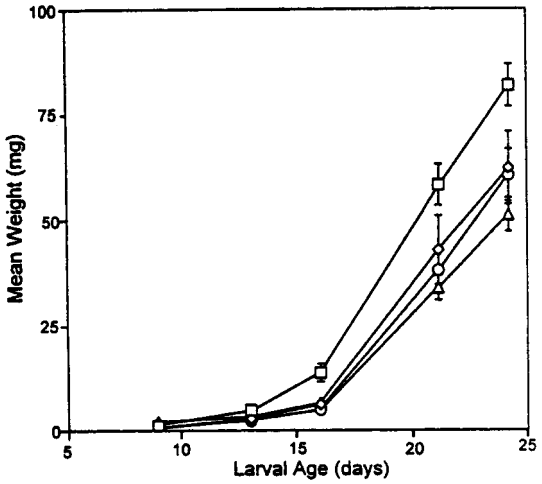

Figure 1. The growth of *Ostrinia nubilalis* reared on artificial meridic diet with test compound **2** at 0 (□), 5 (◇), 50 (○), and 100 (△) ppm. Vertical bars represent standard error deviations of the mean.

Results and Discussion

Guided by a bioassay that tested for larval toxicity of the European corn borer, *Ostrinia nubilalis* (Lepidoptera: Pyralidae), a $CHCl_3$−MeOH extract of the aerial parts of *H. spicigera* was fractionated mainly by column chromatography. The insecticidal activity was traced to a fraction rich in a mixture of diterpenes. HPLC separation furnished seven labdanes. Compound **1** was the major metabolite present in the aerial parts of the plant (4.4×10^{-2} % w/w), while **2**−**7** were minor components ($3.8-0.4 \times 10^{-3}$ % w/w). The plant was also shown to contain ursolic and *ent*-kaur-16-en-19-oic acids. Compound **2** significantly inhibited larval growth of the model insect (Figure 1). Cytotoxicity of the isolated compounds was evaluated with colon and nasopharyngeal carcinoma cell lines. Compound **1** displayed marginal cytotoxicity with HCT-15 (ED_{50} 8.7 μg/mL) and KB (ED_{50} 19.4 μg/mL) cultures. The responses displayed by diterpenes **4** and **5** were similar to each other and twofold less intense (ED_{50} 11−19 μg/mL) than **1**. All other diterpenes were inactive at concentrations below 20 μg/mL.

Diterpene **1** was obtained as a colorless oil exhibiting a positive optical rotation ($[\alpha]_D$ +58°). It formed two derivatives on partial acetylation, which were identical to natural products **2** and **3**. Peracetylation of these compounds gave the same derivative, indistinguishable (HPLC, NMR, $[\alpha]_D$

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Table 1. ^{1}H NMR, HMQC, and HMBC Data of Compound **1**[a]

		correlated carbon		
			HMBC	
position	δ ^{1}H	δ ^{13}C HMQC	$^2J_{C-H}$	$^3J_{C-H}$
1	2.06 m	47.6 t	2	3, 20
	0.94 dd (11.5)[b]			
2	3.90 m	64.8 d	1, 3	
3	2.03 m	45.1 t	2	1,18,19
	0.99 dd (12)[b]			
4		38.6 s	3, 5, 18, 19	
5	1.27 dd (4)[b]	53.0 d	6	1, 18, 20
6	2.16 m	33.4 t	5, 7	
	1.28 m			
7	3.90 m	73.7 d	6	17
8		149.2 s	9, 17	6
9	1.53 dd (10.1)[b]	53.7 d	11	17, 20
10		40.3 s	1, 5, 9, 20	
11	1.64 m	21.6 t	9, 12	
	1.53 m			
12	2.06 m	29.9 t		14, 16
	1.23 m			
13		139.8 s	12, 16	11, 15
14	5.40 t (7)	124.9 d	15	12, 16
15	4.03 br d (7)	59.0 t	14	
16	1.71 br s	23.3 q		12, 14
17	5.26 br s	104.3 t		9
	4.76 br s			
18	1.03 s	27.7 q		3, 5, 19
19	4.14 d (11.1)	66.9 t		3, 5, 18
	3.75 d (11.1)			
20	0.70 s	16.1 q		1, 5, 9
Me-CO−	2.03 s	20.9 q		
Me-*CO*−		171.1 s		

[a] Chemical shifts (δ) are in ppm relative to TMS; coupling constants J (Hz). C-multiplicities were established by DEPT. HMBC optimized for J_{C-H} = 8 Hz. [b] Approximate triplet-like signals.

+20°) from compound **8** produced by total acetylation of **1**, thus showing the carbon skeleton, oxygenation pattern, and stereochemistry to be the same for each natural product.

Chemical ionization MS of the principal component (**1**) suggested the molecular formula $C_{22}H_{36}O_5$, allowing five degrees of unsaturation. ^{13}C NMR (DEPT) indicated 20 carbons, with 30 directly attached protons for the diterpene nucleus, in addition to signals for an acetyl group. This spectrum also indicated the presence of two double bonds, one exocyclic, and a trisubstituted olefin as part of a side chain. The ^{1}H NMR spectrum (Table 1) showed four methyl groups as singlets, of which one was part of an acetate (δ 2.03), one was attached to an unsaturated carbon (δ 1.71), and two were identified as tertiary groups (δ 1.03 and 0.70). Other functionalities that were apparent from the ^{1}H and ^{13}C NMR data of **1** (Table 1) included an allylic primary alcohol (δ_H 4.03 br d, H_2-15; δ_C 59.0 CH_2-15) and two secondary hydroxyl groups (δ_H 3.90 m, 2H; δ_C 64.8 CH-2, and 73.7 CH-7), which were initially recognized by MS fragments at *m*/*z* 363, 345, and 327, indicating consecutive losses of three H_2O molecules from the $[M + H]^+$. The olefinic proton at δ 5.40 (H-14) was coupled to the hydroxyl methylene protons (H_2-15) and displayed allylic coupling with the vinylic methyl group (H-16), thus defining the side chain substitution. There were also observed signals for two diastereotopic protons at δ_H 4.14 and 3.75 (AB, d, H_2-19, J = 11.1 Hz), whose chemical shifts were characteristic of an acetyloxy methylene group with an axial orientation (δ_C 66.9 CH_2-19).[13,14] The presence of this functionality was corroborated in the MS by loss of ketene (C_2H_2O, 42 mass units) from the dehydration fragments to produce peaks at *m*/*z* 303, 285, and 267.

The nature of the diterpene skeleton of **1** was established by means of the 2D NMR experiments ^{1}H−^{1}H COSY, NOESY, HMQC (^{13}C and ^{1}H chemical shift values are given in Table 1), and HMBC (two- and three-bond correlations between ^{13}C and ^{1}H are included in Table 1). These results allowed the construction of a *trans*-decalin system with the exocyclic methylene at C-8, attachment of the side chain at C-9, and placement of the acetyloxy methylene group at C-4 to complete a normal labdane skeleton.[14,15] Support for this assignment was obtained from the HMBC, which showed a $^3J_{C-H}$-interaction between C-18 (δ_C 27.7) and H_2-19.

The relative stereochemistry was assigned on the basis of the observed NOESY correlations for the peracetyl derivative **8**, although the coupling constants ($^3J_{H-H}$) (Table 2) for the methine signals H-2 and H-7 provided enough evidence for their spatial orientation. Thus, H-2 displayed two large diaxial couplings ($J_{1a-2} = J_{2-3a} = 11.8$ Hz) and two small axial−equatorial interactions ($J_{1e-2} = J_{2-3e} = 4.1$ Hz). The coupling pattern for the triplet-like signal of H-7 with small values ($J_{6a-7} = J_{6e-7} = 4.2$ Hz) required an equatorial orientation. NOESY experiments showed correlations between the H-20 methyl group and H-2, which in turn displayed an additional cross peak with the most shielded proton (δ_H 4.06) of the acetyloxy methylene functionality (H_2-19) on C-4, confirming their axial orientation on the β-face of the decalin system. Finally, the geometry of the side chain double bond was established as *Z* by virtue of the NOE interactions between H-14 and H-16 methyl group. These data provided conclusive evidence for formulation of **1** as 2α,7α,15,19-tetrahydroxylabda-8(17),(13*Z*)-diene,19 acetate.

^{1}H and ^{13}C NMR spectra (Tables 2 and 3) of natural products **2** and **3** were similar to those of compound **1**. After measuring the ^{1}H−^{1}H COSY and HMQC, the additional acetate group was placed at the primary carbinol group on C-15, whose methylene protons and carbon ($\delta_{(C-15)2}$ 61.1 − $\delta_{(C-15)1}$ 59.0 = 2.1) were paramagnetically shifted in **2**. Similarly, the data obtained for diterpene **3** supported the placement of the third ester substituent at C-7. The signal for H-7 was shifted 1.15 ppm downfield relative to the same resonances in compounds **1** and **2**.

The CIMS of compound **4** exhibited a peak at *m*/*z* 379 $[M + H]^+$ in accordance with the molecular formula $C_{22}H_{34}O_5$, suggesting an additional degree of unsaturation in relation to compound **1**. The presence of an α,β-unsaturated aldehyde (IR 1667 cm^{-1}) was supported by ^{1}H and ^{13}C NMR (Tables 2 and 3). Chemical shift values registered for this vinylic methyl group (δ_H 1.95 and δ_C 24.7) were in agreement with the *Z*-configuration for the 13,14 double bond.[15] Final evidence for the relative stereochemistry of compound **4** was achieved by chemical correlation with **1**. Treatment of **1** with MnO_2 in THF resulted in oxidation of the allylic position C-15. Surprisingly, the hydroxyl group on C-7 remained unreacted under room-temperature conditions. During a prolonged storage in $CDCl_3$, compound **4** suffered isomerization of the side-chain double bond to yield **9**. The main differences of this derivative in relation to **4** were observed in the chemical shifts for the vinylic methyl group (Me-16, δ_H 2.14 and δ_C 17.6), which indicated an *E* configuration for the double bond.[16]

Compound **5** yielded a formula of $C_{22}H_{34}O_5$ by FABMS. NMR data for **5** were almost identical to those of **1** except for absence of the H-2 signal, suggesting oxidation of the parent diterpene nucleus at this position. The presence of a ketone C-2 function was supported by the ^{13}C NMR signal

Table 2. ^{1}H NMR Data for Compounds **2**−**9** (500 MHz in $CDCl_3$)[a]

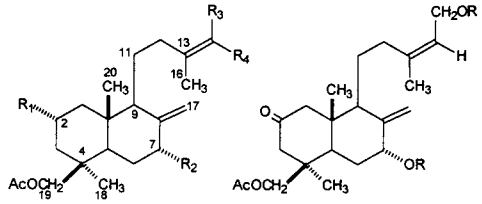
proton(s)	**2**	**3**	**4**	**4**[b]	**5**	**6**	**7**[c]	**8**	**9**
H-1	0.97 dd (9.5, 9.5)	0.97 dd (9, 9)	0.93 m	0.74 dd (11, 11)	2.14 d (13.5)	1.04 m	0.89 dd (11,11)	1.04 dd (11.5, 11.5)	0.93 m
	2.10 m	2.12 m	2.06 m	1.99 m	2.44 d (13.5)	2.06 m	2.16 m	2.05 m	2.06 m
H-2	3.90 m	3.91 m	3.88 m	3.57 m		3.88 m	3.79 dddd (4, 11.5)	4.95 dddd (4.1, 11.8)	3.88 m
H-3	1.02 dd (10.5, 10.5)	1.02 dd (9.5, 9.5)	0.98 m	0.84 dd (10.5, 10.5)	2.19 d (14)	1.00 m	0.93 dd (11.5, 11.5)	1.11 dd (12.4, 12.4)	0.98 m
	2.07 m	2.06 m	2.04 m	1.95 m	2.56 d (14)	2.04 m	2.03 ddd (2, 4, 12)	2.07 m	2.04 m
H-5	1.29 m	1.36 m	1.22 m	1.14 m	1.83 dd (3, 13.5)	1.26 m	1.28 m	1.4 dd (11.1, 13.4)	1.22 m
H-6	1.31 m	1.38 m	1.26 m	1.40 m	1.39 m	1.26 m	1.24 m	1.35 m	1.26 m
	2.21 m	2.22 m	2.17 m	2.03 m	2.27 m	2.17 m	2.10 m	2.15 m	2.17 m
H-7	3.90 m	5.05 dd (4.5, 4.5)	3.88 m	3.57 m	3.99 dd (5, 11)	3.88 m	3.84 m	5.01 dd (4.2, 4.2)	3.88 m
H-9	1.56 dd (10, 10)	1.64 dd (9.5, 9.5)	1.55 dd (10.2, 10.2)	1.29 dd (10.5, 10.5)	1.77 dd (1, 7)	1.58 dd (10.4, 10.4)	1.58 dd (10.5, 10.5)	1.64 dd (10, 10)	1.55 dd (10.2, 10.2)
H-11	1.60 m	1.50 m	1.63 m	1.31 m	1.48 m	1.62 m	1.55 m	1.49 m	1.63 m
	1.67 m	1.56 m	1.75 m	1.39 m	1.60 m	1.88 m	1.66 m	1.56 m	1.75 m
H-12	1.26 m	1.27 m	2.48 m	2.33 m	1.18 m	2.30 m	2.07 m	2.04 m	2.48 m
	2.09 m	2.10 m	2.65 m	2.41 m	2.10 m	2.55 m		2.14 m	2.65 m
H-14	5.36 dd (6.9, 7.0)	5.37 dd (7.4, 7.4)	5.88 d (8.1)	5.85 d (8.1)	5.37 dd (7.5, 7.5)		5.36 dd (7, 7)	5.34 dd (7.3, 7.3)	5.83 d (8)
H-15	4.47 dd (7.2, 12.3)	4.47 dd (7.2, 10)	9.78 d (8.1)	9.90 d (8.1)	4.45 dd (7, 12.5)		3.95 dd (7, 12.5)	4.50 dd (6.6, 12.3)	9.96 d (8)
	4.54 dd (7.5, 12.6)	4.53 dd (7.2, 10)			4.55 dd (7, 12.5)		4.02 dd (7, 12.5)	4.44 dd (6.6, 12.2)	
H-16	1.75 br s	1.75 br s	1.95 br s	1.44 br s	1.74 br s	2.08 br s	1.73 br s	1.73 br s	2.14 br s
H-17	4.78 br s	4.76 br s	4.76 br s	4.58 br s	4.83 br s	5.21 br s	4.77 br s	4.73 br s	4.70 br s
	5.28 br s	5.12 br s	5.32 br s	5.45 br s	5.34 br s	4.65 br s	5.27 br s	5.10 br s	5.26 br s
H-18	1.06 s	1.05 s	1.03 s	0.89 s	1.15 s	1.03 s	1.02 s	1.02 s	1.04 s
H-19	3.77 d (11.1)	3.79 d (11.1)	3.74 d (11.2)	3.72 d (11.1)	3.88 d (11.5)	3.77 d (11.1)	3.27 d (11.2)	3.87 d (11.3)	3.76 d (11.2)
	4.18 d (11.1)	4.13 d (11.1)	4.15 d (11.2)	4.19 d (11.1)	3.92 d (11.5)	4.12 d (11.1)	3.59 d (11.2)	4.06 d (11.3)	4.14 d (11.2)
H-20	0.73 s	0.75 s	0.72 s	0.55 s	0.74 s	0.71 s	0.89 s	0.77 s	0.71 s
19-OAc	2.04 s	2.14 s	2.03 s	1.69 s	2.05 s	2.03 s		2.02	2.02 s
15-OAc	2.05 s	2.05 s						2.03	
7-OAc		2.06 s						2.01	
2-OAc								2.12	

[a] Chemical shifts (δ) are in ppm relative to TMS. Observed splittings J (Hz). [b] In C_6D_6. [c] In CD_3OD.

at δ 209.9 (Table 3).[14] Oxidation of compound **3** with Jones's reagent produced **10**, which was identical to the peracetylated derivative of compound **5**.

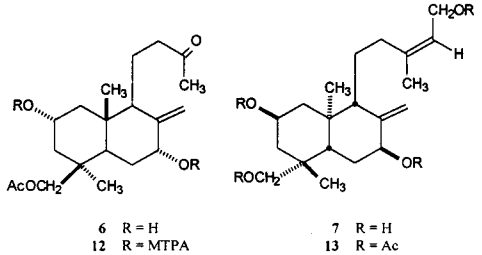
The FABMS of **6** supported a molecular formula of $C_{20}H_{32}O_5$. Examination of the NMR spectra of **1** and **6** confirmed a common bicyclic *trans*-decalin moiety, and the suggestion for a bisnorlabdane skeleton was made evident from the H-16 methyl group signal, which shifted significantly downfield (δ 2.03). This observation indicated the presence of a methyl ketone functionality (δ_C 208.7 C-14, 30.0 CH_3-16) in **6** reminiscent of the partially degraded side chain of its biogenetic precursor, diterpene **1**.

Compound **7** was found to have a molecular formula of $C_{20}H_{34}O_4$ as determined by FABMS. This polar diterpene displayed a negative specific rotation ($[\alpha]_D$ −70°), in contrast with the positive values recorded for the rest of the novel compounds isolated from *H. spicigera*. The major difference from **1** in the NMR spectra was the absence of the acetyl moiety. Accordingly, it was assumed that **7** was the deacetylated form of **1** and, as a consequence, both natural products would share the same relative configuration. On acetylation, both compounds afforded peracetates that were identical by NMR and HPLC experiments, but exhibited opposite optical rotations. The observed rotation was positive for peracetate **8** and negative for derivative **13** prepared from natural product **7**. These optical activity measurements implied that the two enantiomeric labdane skeletons could be present in the plant material, that is, in the 10-Meα and 10-Meβ series.[17]



	R_1	R_2	R_3	R_4
1	OH	OH	CH_2OH	H
2	OH	OH	CH_2OAc	H
3	OH	OAc	CH_2OAc	H
4	OH	OH	CHO	H
8	OAc	OAc	CH_2OAc	H
9	OH	OH	H	CHO
11	OMTPA	OMTPA	CH_2OAc	H

5 R = H
10 R = Ac



6 R = H
12 R = MTPA

7 R = H
13 R = Ac

Table 3. ^{13}C NMR Spectral Data of Compounds **2**,**4**, and **5**−**9** (125.7 MHz in $CDCl_3$)[a]

position	2	4	4[b]	5	6	7[c]	8	9
1	47.6	47.5	47.6	53.7	47.7	41.3	43.5	47.7
2	64.8	64.7	64.3	209.9	64.7	65.5	68.1	64.7
3	45.1	45.1	45.3	50.3	45.0	45.1	41.0	45.1
4	38.6	38.9	38.6	41.3	38.6	41.2	38.5	38.6
5	53.0	52.9	53.0	52.6	52.9	54.4	52.8	52.9
6	33.2	33.3	33.6	33.6	33.5	34.2	30.0	33.4
7	73.7	73.4	73.4	73.4	73.7	74.7	74.5	73.6
8	145.7	148.7	149.3	148.6	148.8	151.2	143.9	148.6
9	53.7	53.6	53.4	53.3	54.0	55.2	54.2	54.1
10	40.3	40.4	40.2	43.4	40.5	41.3	40.2	40.5
11	21.5	21.9	21.9	21.5	17.4	22.7	21.8	21.2
12	30.1	30.6	30.4	29.7	42.1	31.1	30.1	30.6
13	142.2	163.8	162.9	141.7	208.7	139.8	142.3	163.8
14	119.9	129.3	129.6	120.3		126.3	119.9	127.4
15	61.1	190.8	190.2	61.1		59.3	60.9	191.2
16	23.3	24.8	24.2	23.2	29.7	23.5	23.4	17.6
17	104.3	104.8	104.8	104.9	104.3	104.6	105.3	104.5
18	27.7	27.7	27.7	27.7	27.7	28.2	27.7	27.7
19	66.9	66.9	67.0	67.1	66.9	65.5	66.5	66.9
20	16.1	16.2	16.1	15.9	15.9	16.7	15.8	16.1
Me-CO-2							21.0	
Me-CO-7							20.8	
Me-CO-15	20.9						21.1	
Me-CO-19	20.9	20.9	20.4	21.5	20.9		21.4	20.9
Me-*C*O-2							169.9	
Me-*C*O-7							170.4	
Me-*C*O-15	170.9						171.0	
Me-*C*O-19	171.1	171.1	170.5	170.7	171.1		171.0	171.1

[a] Chemical shifts (d) are in ppm relative to TMS. All assignments are based on HMQC and HMBC experiments. [b] In C_6D_6. [c] In CD_3OD.

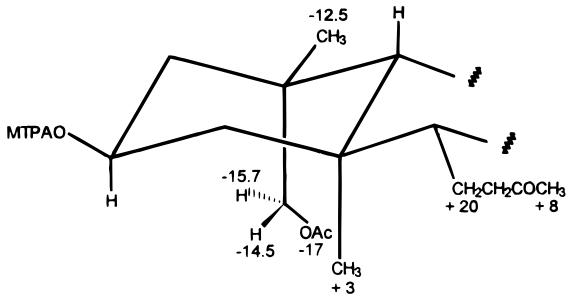


Figure 2. Stereochemical model for the MTPA ester derivatives at carbinol center C-2 of compound 6 showing $\Delta\delta$ values for diagnostic ^1H NMR signals on the normal labdane skeleton.

Determination of the absolute configuration at the asymmetric center C-2 in compounds **2** and **6** by the use of high-field NMR application of Mosher's methodology[18,19] allowed correlation of the carbon framework of the dextrorotatory natural products **1**−**6** with the normal labdane 10-Meβ-stereotype. For example, construction of the stereochemical projection for the MTPA ester derivatives of compound **6** according to the Kakisawa−Mosher model[18] is shown in Figure 2, where the arrangement of the $\Delta\delta$ ($\delta S - \delta R$) (ppm) values for diagnostic protons surrounding the C-2 carbinol center confirmed the *R* configuration. The negative curvature of the optical rotatory dispersion displayed by derivative **13** was used as the argument in favor of the enantiomeric absolute configuration for natural product **7** (i.e., 10-Meα-labdane skeleton), in contrast to the positive plain ORD curve showed by peracetate **8**.

Experimental Section

General Experimental Procedures. Melting points were obtained on a Büchi 530 apparatus and are uncorrected. The IR spectra were obtained on Perkin−Elmer model 1600. Positive ion FABMS was recorded using a glycerol matrix in a JEOL SX102A mass spectrometer. CIMS was recorded on a Hewlett−Packard 5985-B spectrometer. The optical rotations were measured on a Perkin−Elmer 241 polarimeter. ^1H (500 MHz), ^{13}C (125.7 MHz), DEPT, COSY, NOESY, HMQC, and HMBC NMR spectra were recorded either on a Brucker AMX-500 or a Varian XL-500 instrument. Chemical shifts are in δ (ppm) with TMS as internal reference. Preparative column chromatography was performed on Si gel flash (40 μm, J. T. Baker). Analytical and preparative TLC were performed on Si gel 60 F_{254} Merck plates, and the spots were visualized by spraying with 10% $Ce(SO_4)_2$ in 2N H_2SO_4, followed by heating at 110°. The instrumentation used for HPLC analysis consisted of a Waters (Millipore Corp., Waters Chromatography Division, Milford, MA) 600E Multisolvent Delivery System equipped with a Waters 410 differential refractometer detector and a computer (OptiPlex 466/Le, Dell). Control of the equipment, data acquisition, processing, and management of chromatographic information were performed by the Millennium 2000 software program (Waters). HPLC separations were performed on ISCO Si gel (μPorasil, 10 μm) columns: semipreparative (250 mm × 10 mm) and preparative (250 × 21.2 mm) scales.

Plant Material. The aerial parts of *H. spicigera* were collected in November 1989, Km-18 Xalapa-Puerto de Veracruz Road, state of Veracruz, Mexico. A voucher specimen (M-21851) is deposited at the National Herbarium, Instituto de Biología, Universidad Nacional Autónoma de México.

Extraction and Bioassay-guided Isolation Procedures. Dried aerial parts (788 g) were extracted by maceration with hexane followed by $CHCl_3$−MeOH (1:1) at room temperature. After filtration, the solvent was removed under vacuum to yield 37.5 g (hexane) and 54.5 g ($CHCl_3$−MeOH) of dark-green residues. The hexane extract afforded 75 mg of *ent*-kaur-16-en-19-oic acid.[20] The $CHCl_3$−MeOH extract was chromatographed over Si gel (450 g) in a gravity column using a gradient of Me_2CO−MeOH in $CHCl_3$. A total of 70 fractions (200 mL each) was collected. Fractions 9−16 contained 100 mg of ursolic acid, which was identified by comparing its physical and spectral data with those of authentic material in our files.[13] Combined fractions 23−37 (6 g), eluted from the original column with $CHCl_3$−Me_2CO (2:3), were found to concentrate the insecticidal activity. These active eluates were further rechromatographed over Si gel (200 g) using the same solvent system to yield the combined subfractions 83−103 (1 g) abundant in six compounds. The crude mixture was submitted to preparative HPLC. The elution was isocratic with *n*-hexane−EtOAc (1:4) and a flow rate of 6 mL/min. These chromatographic conditions were effective in isolating 350 mg of component **1** (t_R 26.2 min), 6 mg of **5** (t_R 26.8 min), 20 mg of **7** (t_R 20.6 min), 30 mg of **4** (t_R 19.1 min), 15 mg of **2** (t_R 15.3 min), and 4 mg of **3** (t_R 14.6 min). The subfractions 104−109 yielded 15 mg of the compound **6** (t_R 22 min) by preparative HPLC. The mobile phase was EtOAc−MeOH, (95:5; flow rate = 6 mL/min).

Compound 1: oil; $[\alpha]_D$ +58° (*c* 0.1, MeOH); IR (Nujol) ν_{max} 3368, 1724, 1658, 1456, 1374, 1246 and 1034 cm^{-1}; ^1H and ^{13}C NMR, Table 1; CIMS *m/z* (rel int) $[M + H]^+$ 381 (1.3), $[M + H - H_2O]^+$ 363 (29.9), $[M + H - 2H_2O]^+$ 345 (48.7), $[M + H - 3H_2O]^+$ 327 (58.6), $[M + H - 2H_2O - C_2H_2O]^+$ 303 (27.8), $[M + H - 3H_2O - C_2H_2O]^+$ 285 (89.4), $[M + H - 4H_2O - C_2H_2O]^+$ 267 (100); HRFABMS $[M + H]^+$ 381.2641 (calcd for $C_{22}H_{37}O_5$, 381.2640).

Compound 2: oil; $[\alpha]_D$ +40° (*c* 0.1, MeOH); IR (Nujol) ν_{max} 3380, 1730, 1664, 1374, 1240, 1112, and 1058 cm^{-1}; FAB-MS *m/z* $[M + H]^+$ 423; ^1H and ^{13}C NMR, Tables 2 and 3.

Compound 3: oil; $[\alpha]_D$ +25° (*c* 0.1, MeOH); IR (Nujol) ν_{max} 3378, 1730, 1660, 1456, 1372, 1240, and 1034 cm^{-1}; FABMS *m/z* $[M + H]^+$ 465; ^1H NMR, Table 2.

Compound 4: oil; $[\alpha]_D$ +10° (*c* 0.1, MeOH); IR (Nujol) ν_{max} 3382, 1732, 1666, 1374, 1246, 1128, and 1058 cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* (rel int) $[M + H]^+$ 379 (16.8), $[M + H - H_2O]^+$ 361 (47.6), $[M + H - 2H_2O]^+$ 343 (61.0), $[M + H - 2H_2O - C_2H_2O]^+$ 301 (76.5), $[M + H - 3H_2O -$

$C_2H_2O]^+$ 283 (100), $[M + H - 4H_2O - C_2H_2O]^+$ 265 (57.8); HRFABMS $[M + H]^+$ 379.2479 (calcd for $C_{22}H_{35}O_5$, 379.2484).

Compound 5: oil; $[\alpha]_D$ +66° (*c* 0.1, MeOH); ^1H and ^{13}C NMR, Tables 2 and 3; HRFABMS *m/z* $[M + Na]^+$ 401.2301 (calcd for $C_{22}H_{34}O_5Na$, 401.2303).

Compound 6: oil; $[\alpha]_D$ +23° (*c* 0.1, MeOH); IR (Nujol) ν_{max} 3380, 1732, 1658, 1372, 1244, and 1034 cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; CIMS *m/z* $[M + H]^+$ 353 (1.4), $[M + H - H_2O]^+$ 335 (11.7), $[M + H - 2H_2O]^+$ 317 (13.8), $[M + H - C_2H_40]^+$ 309 (8.1), $[M + H - 2H_2O - C_2H_2O]^+$ 275 (15.7), $[M + H - 3H_2O - C_2H_2O]^+$ 257 (50.2); HRFABMS *m/z* $[M + Na]^+$ 375.2146 (calcd for $C_{20}H_{32}O_5Na$, 375.2147).

Compound 7: white crystalline solid; mp 140−141 °C; $[\alpha]_D$ −30° (*c* 0.1, MeOH); IR (KBr) ν_{max} 3336, 1698, 1648, 1452, 1372, and 1034 cm^{-1}; ^1H and ^{13}C NMR, Tables 2 and 3; FABMS *m/z* $[M + H]^+$ 339, $[M + H - H_2O]^+$ 321, $[M + H - 2 H_2O]^+$ 303, $[M + H - 3 H_2O]^+$ 285; HRFABMS *m/z* $[M + Na]^+$ 361.2354 (calcd for $C_{20}H_{34}O_4Na$, 361.2354).

Acetylation of 1. Compound **1** (178 mg) was dissolved in 2.5 mL of Ac_2O−pyridine (2:1) and maintained at room temperature for 24 h. The reaction mixture was diluted with H_2O, extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and dried with anhydrous Na_2SO_4. Column chromatography on Si gel (*n*-hexane−EtOAc 75:25) yielded 45 mg of product **8**: oil; $[\alpha]_D$ +20° (*c* 0.1, MeOH); IR (Nujol) ν_{max} 1738, 1658, 1442, 1366, 1240, and 1030 cm^{-1}; FABMS *m/z* $[M + H]^+$ 507, $[M + H - HOAc]^+$ 447, $[M + H - 2HOAc]^+$ 387, $[M + H - 3HOAc]^+$ 327; ^1H and ^{13}C NMR, Tables 2 and 3.

Partial Acetylation of 1. Compound **1** (48 mg) was dissolved in Ac_2O (15 μL) and pyridine (11 μL). The reaction mixture was stirred at −5 °C for 10 min and treated under the same workup conditions as indicated above. The crude product was resolved by semipreparative HPLC (*n*-hexane−EtOAc, 1:1; flow rate = 1.5 mL/min) to yield 6.6 mg of diacetate **2** (t_R 20.3 min), 1.5 mg of triacetate **3** (t_R 15.6 min), and 3 mg of derivative **8** (t_R 13.5 min), besides 16 mg of unreacted starting material **1** (t_R 51.9 min).

Acetylation of 7. The procedures used for peracetylation of compound **1** were applied for derivatization of natural product **7** (4 mg) to yield 3 mg of **13**: oil; ORD (*c* 0.8, MeOH) $[\alpha]_{589}$ −2.5°, $[\alpha]_{578}$ −2.5°, $[\alpha]_{546}$ −3.7°, $[\alpha]_{436}$ −6.1°, $[\alpha]_{365}$ −8.6°; IR, ^1H and ^{13}C NMR data were identical to those of compound **8**.

Oxidation of Compound 1. To 10.8 mg of compound **1** in 2 mL of THF were added 100 mg of MnO_2. The mixture was stirred at 25° for 16 h and filtered under Celite. The solvent was removed at reduced pressure. Then the crude product was purified by semipreparative HPLC (*n*-hexane−EtOAc, 1:4; flow rate = 1.5 mL/min) to yield 5.6 mg of compound **4**.

Oxidation of Compound 3. Natural product **3** (2 mg) was dissolved in 0.5 mL Me_2CO at 0° and treated dropwise with Jones's reagent (CrO_3−HOAc) until an orange color persisted. After 5 min, the solution was diluted with H_2O and extracted with $CHCl_3$. Removal of the solvent left a residue that was analyzed by HPLC (*n*-hexane−EtOAc, 7:3; flow rate = 1 mL/min) to afford one major peak (t_R 10.2 min). This derivative was identical (GC-MS and HPLC coelution) to peracetate **10**, which was prepared by acetylation of **5**.

Preparation of C(2),C(7)-bis-MTPA Esters of 2 and 6. To a solution of compound **2** or **6** (2.8 mg in 750 μL of $CDCl_3$ in a dry NMR tube) were added 4-(dimethylamino)pyridine (1.9 mg) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (23.6 μL). The reactions were allowed to stand overnight under N_2. After 24 h, ^1H NMR spectra were recorded without further workup[21] of the mixtures and revealed complete conversion of the starting materials **2** and **6** to the MTPA esters (**11** and **12**, respectively). Isolation of derivatives prepared from compound **6** was as follows: saturated aqueous $NaHCO_3$ and Et_2O were added to the mixture and stirred vigorously for 10 min to allow efficient hydrolysis of the excess MTPA-Cl.[22] The organic phase was washed with 0.5 N HCl, dried with anhydrous Na_2SO_4, and concentrated to a yellow oil. This crude residue was purified by semipreparative HPLC (*n*-hexane−EtOAc, 7:3; flow rate = 3 mL/min) to yield 1.2 mg of the C(2),C(7)-bis-(*S*)-MTPA ester of compound **6**. Treatment with (*S*)-(+)-MTPA-Cl as describe above yielded 1.3 mg of the C(2),C(7)-bis-(*R*)-MTPA ester.

C(2),C(7)-bis-(*S*)-MTPA ester of 2: ^1H NMR ($CDCl_3$, 500 MHz) δ 7.54 (4 H, m, Ar*H*), 7.43 (6 H, m, Ar*H*), 5.43 (1 H, m, H-14), 5.29 (1 H, s, H-17), 5.28−5.25 (2 H, m, H-2 and H-7), 4.80 (1 H, s, H-17′), 4.54 (1 H, dd, *J* = 7.5, 11 Hz, H-15), 4.49 (1 H, dd, *J* = 7.5, 11 Hz, H-15′), 4.17 (1 H, d, *J* = 11 Hz, H-19), 3.79 (1 H, d, *J* = 11 Hz, H-19′), 3.56 (3 H, br s, O*Me*), 3.55 (3 H, br s, O*Me*), 2.05 (3 H, s, OCO*Me*), 2.04 (3 H, s, OCO*Me*), 1.76 (3 H, br s, Me-16), 1.06 (3 H, s, Me-18), and 0.74 (3 H, s, Me-20).

C(2),C(7)-bis-(*R*)-MTPA ester of 2: ^1H NMR ($CDCl_3$, 500 MHz) δ 7.55 (4 H, m, Ar*H*), 7.42 (6 H, m, Ar*H*), 5.43 (1 H, m, H-14), 5.28 (1 H, s, H-17), 5.25 (2 H, m, H-2 and H-7), 4.79 (1 H, s, H-17′), 4.55 (1 H, dd, *J* = 7.6, 11.5 Hz, H-15), 4.49 (1 H, dd, *J* = 7.6, 11.5 Hz, H-15′), 4.19 (1 H, d, *J* = 11.1 Hz, H-19), 3.80 (1 H, d, *J* = 11.1 Hz, H-19′), 3.60 (3 H, br s, O*Me*), 3.56 (3 H, br s, O*Me*), 2.06 (3 H, s, OCO*Me*), 2.04 (3 H, s, OCO*Me*), 1.75 (3 H, br s, Me-16), 1.07 (3 H, s, Me-18), and 0.72 (3 H, s, Me-20).

C(2),C(7)-bis-(*S*)-MTPA ester of 6: oil, t_R 7.21 min; $[\alpha]_D$ +41° (*c* 0.1, MeOH);^1H NMR ($CDCl_3$, 500 MHz) δ 7.56−7.53 (4 H, m, Ar*H*), 7.43 (6 H, m, Ar*H*), 5.25−5.20 (2 H, m, H-2 and H-7), 5.09 (1 H, s, H-17), 4.65 (1 H, s, H-17′), 4.08 (1 H, d, *J* = 11.2 Hz, H-19), 3.88 (1 H, d, *J* = 11.2 Hz, H-19′), 3.56 (3 H, br s, O*Me*), 3.55 (3 H, br s, O*Me*), 2.12 (3 H, br s, Me-16), 2.03 (3 H, s, OCO*Me*), 1.05 (3 H, s, Me-18), and 0.83 (3 H, s, Me-20); FABMS $[M + H]^+$ 785, $[M + H - 234$ (MTPA-OH)$]^+$ 551, $[551 - H_2O]^+$ 533, $[533 - C_2H_2O]^+$ 491, $[M + H - 2$MTPA-OH$]^+$ 317.

C(2),C(7)-bis-(*R*)-MTPA ester of 6: oil, t_R 6.95 min; $[\alpha]_D$ −10° (*c* 0.1, MeOH);^1H NMR ($CDCl_3$, 500 MHz) δ 7.58−7.53 (4 H, m, Ar*H*), 7.42 (6 H, m, Ar*H*), 5.25−5.19 (2 H, m, H-2 and H-7), 4.86 (1 H, s, H-17), 4.56 (1 H, s, H-17′), 4.11 (1 H, d, *J* = 11.2 Hz, H-19), 3.92 (1 H, d, *J* = 11.2 Hz, H-19′), 3.60 (3 H, br s, O*Me*), 3.56 (3 H, br s, O*Me*), 2.10 (3 H, br s, Me-16), 2.06 (3 H, s, OCO*Me*), 1.07 (3 H, s, Me-18), and 0.82 (3 H, s, Me-20); FABMS $[M + H]^+$ 785, $[M + H - 234$ (MTPA-OH)$]^+$ 551, $[551 - H_2O]^+$ 533, $[533 - C_2H_2O]^+$ 491, $[M + H - 2$MTPA-OH$]^+$ 317.

Biological Screening Procedures. Larvae of *Ostrinia nubilalis* Hübner (Lepidoptera: Pyralidae) were obtained from a laboratory colony maintained at the University of Ottawa, under previously described conditions.[23] The insecticidal potential of the extract, fractions, and isolates derived from *H. spicigera* was evaluated by established procedures.[24] In brief, all test materials were dissolved in 95% EtOH and incorporated into an agar-based meridic diet at final concentrations of 5, 50, and 100 ppm. Larvae were reared at 25 °C, with a relative humidity of approximately 80%, and a 16:8 light-darkness regime. Mortality and larval weights were obtained every 4−6 days during the growth period. The results were analyzed with an ANOVA using Tukey's multiple range test.

Cytotoxicity Assay. Nasopharyngeal carcinoma (KB) and colon cancer (HCT-15) cell lines were maintained in RMPI 1640 (10×) medium supplemented with 10% fetal bovine serum. All cell lines were cultured at 37 °C in an atmosphere of 5% CO_2 in air (100% humidity). The cells at log phase of their growth cycle were treated in triplicate with various concentrations of the test samples (0.16−20 μg/mL) and incubated for 72 h at 37 °C in a humidified atmosphere of 5% CO_2. The cell concentration was determined by sulforhodamine method.[25] Results were expressed as the dose that inhibits 50% control growth after the incubation period (ED_{50}). The values were estimated from a semilog plot of the drug concentration (μg/mL) against the percent of viable cells.[26] Ellipticine was included as a positive drug control: ED_{50} 0.10 (KB); 0.17 μg/mL (HCT-15).

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