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Defensive Chemistry of *Senecio miser*

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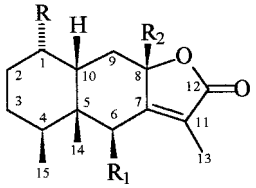
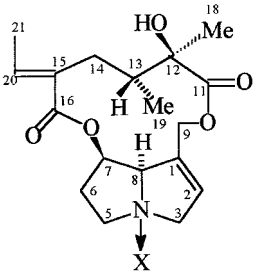
Three eremophilanolides, 1α-acetoxy-8β-methoxy-10βH-eremophil-7(11)-en-8α,12-olide (**1**); 1α-angeloyloxy-6β-hydroxy-8β-methoxy-10βH-eremophil-7(11)-en-8α,12-olide (**2**); and 1α-angeloyloxy-8βH,10βH-eremophil-7(11)-en-8α,12-olide (**3**), and two pyrrolizidine alkaloids, integerrimine (**4**) and its *N*-oxide (**5**), were isolated from bioactive fractions of *Senecio miser*. The structures of the new compounds **1** and **2** were established by NMR spectroscopic analysis and chemical transformation. The X-ray analysis of compound **1** was also performed. Eremophilanolides **1** and **2** and alkaloids **4** and **5** were found to be strong insect antifeedants, further supporting a proposed defensive role for these classes of compounds.

The genus *Senecio* (Asteraceae), widely distributed throughout the world, is known to be a source of pyrrolizidine alkaloids, eremophilanolides, and furanoeremophilanes.[1−3] There are about 210 *Senecio* species in Chile, and a large number of endemic members of this genus are present in Chilean Patagonia,[4] where they have had a negative impact on the local economy because of their toxic effects on cattle.[5−7] The toxicity exhibited by these plants has been attributed to their content of pyrrolizidine alkaloids (PAs).[8]

As part of an ongoing collaborative study on bioactive metabolites of *Senecio* species endemic to Chilean Patagonia, we have studied the defensive chemistry of the herbaceous species *S. miser* Hook f.[4] In the present work we report on the isolation, structure determination, and biological activity of two new eremophilanolides (**1** and **2**), the known compound **3**, the acetylated derivative of **2** (**6**), and the pyrrolizidine alkaloid, integerrimine (**4**), and its *N*-oxide (**5**). The antifeedant, insecticidal, and fungicidal effects of the active fractions and the pure compounds were evaluated against several herbivorous insects [the aphid *Myzus persicae*; the Colorado potato beetle (CPB), *Leptinotarsa decemlineata*; and the lepidopteran *Spodoptera littoralis*] and the plant pathogen *Fusarium moniliforme*.



1 R = OAc; R_1 = H; R_2 = OMe
2 R = OAng; R_1 = OH; R_2 = OMe
3 R = OAng; R_1 = R_2 = H
6 R = OAng; R_1 = OAc; R_2 = OMe



4 X = ··
5 X = O

Results and Discussion

The methanolic extract of the aerial parts of *S. miser* strongly inhibited the feeding activity of *M. persicae* and *L. decemlineata* adults, but was innocuous to *S. littoralis* larvae. Bioassay-guided fractionation of the extract showed that fractions Fr-1 and Fr-2 (EtOAc in *n*-hexane) and Fr-5 (MeOH in EtOAc) had the strongest antifeedant effects. Fr-1 had a strong antifungal effect on *F. moniliforme*. Therefore, the bioactive constituents of *S. miser* could be grouped into two according to their polarity (Fr-1−Fr-2 and Fr-5).

The chromatographic purification of the active fractions of *S. miser* afforded five compounds (**1**−**5**). Compounds **1**−**3** (isolated from Fr-1 and Fr-2) proved to be sesquiterpene lactones of the eremophilanolide type, while **4** and **5** (isolated from Fr-5) are PAs. The IR and UV spectra of compound **1** showed bands at 1765 and 1731 cm^{-1} and λ_{max} 231 (log ϵ 3.46) nm, respectively, characteristic of an α,β-unsaturated γ-lactone and a saturated ester. The HREIMS showed a molecular ion peak at *m/z* 322.1789 corresponding to the molecular formula $C_{18}H_{26}O_5$. The ^{13}C NMR spectrum (DEPT experiment) showed 18 carbon atoms, five methyls, four methylenes, three methines, and six quaternary carbon atoms. Moreover, the ^1H and ^{13}C NMR spectra suggested the presence of an acetate group [δ_H 2.04 (3H, s); δ_C 170.3 (s) and 21.2 (q)], the characteristic signals of a methyl substituted α,β-unsaturated lactone with an *endo*-double bond [δ_H 1.86 (3H, s); δ_C 8,3 (q), 171.6 (s), 125.1 (s), and 156.5 (s)], and a methoxy group [3.11 (3H, s); 105.9

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Table 1. ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR Data of Compound **1**

proton	δ (J_{H-H} in Hz)	COSY		HMQC	HMBC
1β	5.15 dt (4.8, 12.1)	H-2β, H-2α, H-10β		70.5 d	C-1′, C-9
2α	1.45 m	H-2β, H-1β		25.6 t	C-1, C-4
2β	1.75 m	H-2α, H-1β		25.6 t	C-1
3α	1.60 dq (13.2, 4.0, 3.1, 3.2)	H-3β		29.9 t	
3β	1.40 qd (13.3, 4.3)	H-3α		29.9 t	C-4
4α	1.44 m	H-15, H-3α, H-3β		29.2 d	C-14
			C-5	41.3 s	
6β	1.94 dd (1.5, 13.7)	H-6α		35.4 t	C-4, C-5, C-7, C-11, C-14
6α	2.75 d (13.6)	H-6β		35.4 t	C-4, C-5, C-7, C-8, C-10, C-11, C-14
			C-7	156.5 s	
			C-8	105.9 s	
9α	1.75 d (13.6)	H-9β, H-10β		32.5 t	C-1, C-5, C-8, C-10, C-8′
9β	2.38 dd (4.0, 13.8)	H-9α, H-10β		32.5 t	C-5, C-7, C-8
10β	2.17 dt (4.3, 13.1)	H-9α, H-9β		43.0 d	C-1, C-5, C-14
			C-11	125.1 s	
			C-12	171.6 s	
13	1.86 d (1.5)	H-6β		8.3 q	C-7, C-11, C-12
14	1.1 s			21.3 q	C-4, C-5, C-6, C-10
15	0.82 d (6.5)	H-4α		15.1 q	C-4, C-5
			C-1′	170.3 s	
2′	2.04 s			21.2 q	C-1′
OMe	3.11 s			49.9 q	C-8

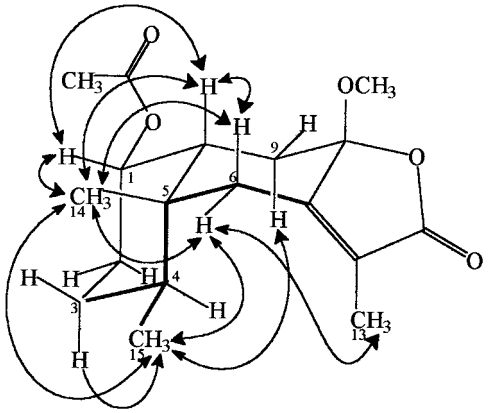

Figure 1. ROESY correlations observed for compound **1**.

(s)]. The *cis*-decalin structure of compound **1** was determined by the chemical shift of the C-5 methyl group at δ_H 1.10 (3H, s).[9,10] HMQC[11] and HMBC[12] experiments confirmed the presence of an eremophilanolide skeleton, along with the positions of the substituents and the chemical shifts of the remaining protons (Table 1).

The stereochemistry of **1** was confirmed by a ROESY experiment (Figure 1). The methyl group at C-5 gave a positive NOE, with signals corresponding to the protons at H-15, H-1β, H-3β, H-6α, H-6β, and H-10β. Similarly, the signal at δ_H 2.72 (H, d, H-6α) corresponded with protons at H-15, H-14, H-13, and H-6β, and the proton signal at δ_H 1.94 (dd, H-6b), with signals of protons H-10β, H-14, and H-6α. Therefore, the structure of **1** is consistent with a C-8α orientation of the γ-lactone.[13]

The molecular structure of **1** was confirmed by single-crystal X-ray diffraction. The structure was solved by direct methods using SHELX86.[14] Refinement was performed with SHEXL93[15] using full-matrix least squares with anisotropic thermal parameters for all non-H atoms. The H-atoms (except those of the C-13 methyl group) were located by means of difference Fourier maps and added to the refinement as a fixed isotropic contribution. The refinement converged at $R_1 = 4.40\%$ and $wR_2 = 9.82\%$, with a goodness of fit of 0.982 for 1049 reflections taken with $I > 4\alpha I$. The largest peak on the final difference map was 0.15 e/Å^3. The bond lengths and bond angles were within the usual ranges, the cyclohexane rings were cis-fused, and

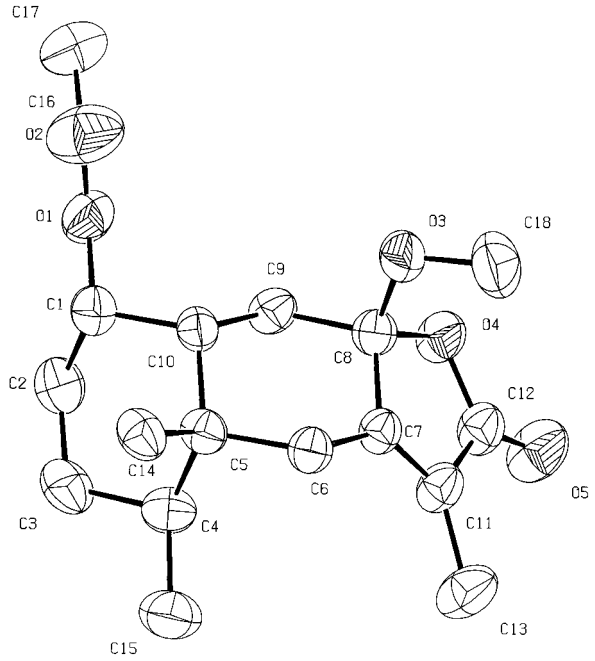

Figure 2. ORTEP drawing of compound **1**.

the cyclopentene moiety was in a flat conformation. Figure 2 shows a computer-generated perspective of the final X-ray model of **1**.[16]

The spectroscopic data of compound **2**, $C_{21}H_{30}O_6$, were similar to those obtained for **1**, except for the absence of the acetate group signals and the presence of additional signals at δ_H 1.89 (3H, m), δ_C 20.6 (q); δ_H 2.00 (3H, dq, J = 7.2, 1.2 Hz); δ_C 16.4 (q) and δ_H 6.08 (H, q, J = 7.0 Hz); δ_C 138.4 (d), assignable to an angelate group, and δ_H 4.48 (d, J = 9.9 Hz, H-6α); δ_C 70.1 (d) which could be attributed to a geminal proton of a hydroxyl group (Table 2). This proton signal gave a spatial correlation (ROESY experiment, Figure 3) with resonances at δ_H 1.92 (s, H-13), 1.19 (s, H-14), and 0.81 (d, H-15). The proton at δ_H 2.32 (dt, H-10β) correlated with δ_H 1.19 (s, H-14). The above data are in accord with the proposed structure for compound **2**. Acetylation of **2** with Ac_2O−pyridine led to a monoacetate [δ_H 5.62 (H, s, H-6α); δ_C 69.5 (d), 2.10 (3H, s); δ_C 20.6 (q), 170.4 (s)].

Table 2. ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR Data of Compound **2**

proton	δ (J_{H-H} in Hz)	COSY		HMQC	HMBC
1β	5.20 dt (11.4, 4.7)	H-2β, H-2α, H-10β		70.8 d	C-1′, C-2, C-9, C-10
2α	1.55 m	H-2β, H-1β		25.2 t	C-1, C-3, C-4
2β	1.82 m	H-2α, H-1β		25.2 t	C-1, C-4, C-10
3α	1.58 m	H-3β		29.0 t	C-1, C-4, C-5
3β	1.58 m	H-3α		29.0 t	C-1, C-4
4α	1.40 m	H-15		28.8 d	C-3, C-5
			C-5	44.8 s	
6α	4.48 d (9.9)			70.1 t	C-4, C-5, C-7, C-8, C-10, C-11, C-14
			C-7	153.5 s	
			C-8	105.7 s	
9α	1.81 d (14.0)	H-9β, H-10β		30.9 t	C-8, C-10
9β	2.50 dd (14.2, 3.9)	H-9α, H-10β		30.9 t	C-5, C-7, C-8, C-10
10β	2.32 dt (4.3, 13.5)	H-1β, H-2β (w), H-9α, H-9β		37.7 d	C-1, C-2, C-5, C-6, C-9
			C-11	126.3 s	
			C-12	170.8 s	
13	1.92 d (1.5)			8.6 q	C-7, C-11, C-12
14	1.19 s			16.4 q	C-4, C-5, C-6, C-10
15	0.81 d (6.5)	H-4α		15.4 q	C-4, C-5
			C-1′	167.0 s	
			C-2′	127.7 s	
3′	6.08 q (7.2)	H-4′		138.4 d	C-1′, C-4′, C-5′
4′	2.0 dq (7.2, 1.2)	H-3′		15.8 q	C-2′, C-3′
5′	1.89 m			20.6 q	C-1′, C-2′, C-3′
OMe	3.31 s			51.0 q	C-8

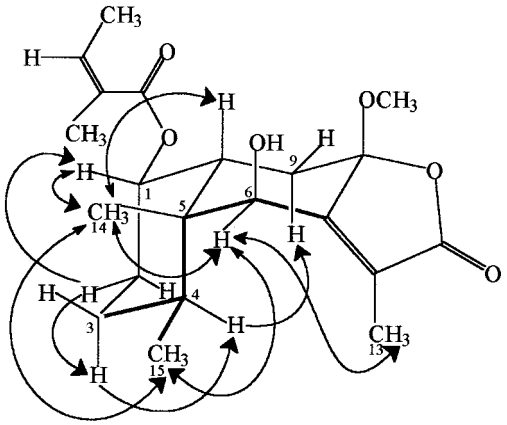

Figure 3. ROESY correlations observed for compound **2**.

Compound **3** was isolated as an oil. Its ^{1}H and ^{13}C NMR spectra were very similar to those of compound **1**, except for the methoxy and acetate group signals and the presence of angelate group signals. It was identified as 1α-angeloyloxy-8βH,10βH-eremophil-7(11)-en-8α,12-olide, which has been already reported from *Roldana sessilifolia*.[17]

Compound **4** was identified by comparison of its spectral data (^{1}H and ^{13}C NMR and mass spectra) with those reported for the PA integerrimine.[18] Compound **5** was isolated as an oil, and its structure was established as integerrimine *N*-oxide from the downfield ^{1}H NMR shifts of the necine protons and from the downfield ^{13}C NMR shifts of the C-3, C-5, and C-8 signals, in comparison with analogous data for integerrimine (**5**) (Table 3). Chemical evidence was obtained by oxidation of **5** with *m*-chloroperbenzoic acid in $CHCl_3$ to afford its *N*-oxide, previously isolated from *S. nebrodensis*.[19]

Table 4 shows the results of the antifeedant effects of compounds **1**−**6** on *M. persicae* and *L. decemlineata* adults. Among the eremophilanolides, **1** proved to be a strong aphid repellent, with an activity level more than 4 times that reported for the sesquiterpene farnesol, a substance structurally related to the aphid alarm pheromone farnesane.[20] This compound accounted for most of the aphid-repellent activity of Fr-2, although the possible activity of **2** and **3** cannot be ruled out.

Compound **2** was the most active deterrent to *L. decemlineata* in choice tests, with an antifeedant potency (EC_{50} value) within the range of the positive control silphinene, a strong CPB sesquiterpene antifeedant,[21] while compounds **1** and **6** had lower antifeedant effects in choice tests. None of these sesquiterpenes was active in no-choice situations. In contrast, fractions Fr-1 and Fr-2 were very active in no-choice experiments, suggesting a possible synergistic effect for the no-choice antifeedant effects of these compounds.

Among the PAs tested, integerrimine (**4**) and its *N*-oxide (**5**) exhibited similar activity (overlapping confidence limits at EC_{50} values). The PA senecionine, with activity levels similar to **4** and **5** in choice tests (overlapping confidence limits),[22] was included as a positive control substance because of its structural similarity to integerrimine (**4**). The activity of **4** and **5** accounted for that of Fr-5 in both choice and no-choice tests.

Compound **1** (31% mycelial growth inhibition) did not account for the antifungal effect of Fr-1, and, therefore, such activity could be the consequence of the individual action of compound **3** (not enough material was available for testing of this substance) or from a synergistic effect among its components. None of the test compounds proved toxic when injected in the CPB except for integerrimine (**4**). Orally injected *S. littoralis* larvae were unaffected by all of the test compounds (Table 5).

Some furanoeremophilane sesquiterpenes are effective feeding deterrents to polyphagous lepidopterans and also play a protective role against soil-dwelling herbivores[23−25] and, thus, have been proposed as defensive chemicals. Here we have demonstrated that the eremophilanolides present in the aerial parts of *S. miser* have negative effects on insect herbivores and plant pathogens, further confirming the hypothesized defensive role of these compounds.

Previously, the importance of an exocyclic methylene group at the lactone ring for the antifeedant and insecticidal activity of eremophilanes against *S. littoralis* has been indicated.[23] However, none of the eremophilanolides studied here had antifeedant or post-ingestive effects against this insect species, which may be due to the absence of an exocyclic methylene group among these substances. Such a structural requirement must, however, be species-de-

Table 3. ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR Data of Compound **5**

proton	d (J_{H-H} in Hz)	COSY		HMQC	HMBC
			C-1	130.1 s	
2	6.27 s	H-3a		131.1 s	C-8
3a	4.62 d (16.4)	H-3b		78.0 t	C-1
3b	4.51 d (16.4)	H-3a		78.0 t	
5a	3.95 m	H-5b, H-6a, H-6b		68.6 t	
5b	3.66 m	H-5a, H-6a, H-6b		68.6 t	
6a	2.95 m	H-6b		32.4 t	
6b	2.42 m	H-6a		32.4 t	
7d	5.49 m	H-6a, H-6b, H-8		73.4 d	
8	4.95 m	H-7, H-9u, H-9d		94.9 d	
9u	4.24 d (12.2)	H-9d		60.6 t	C-11
9d	5.41 d (12.2)	H-9u		60.6 t	C-2, C-8
			C-11	178.0 s	
			C-12	77.8 s	
13	1.80 m	H-19		39.4 d	
14a	2.25 m	H-14b		31.8 t	
14b	2.0 m	H-14a		31.8 t	
			C-15	133.0 s	
			C-16	167.9 s	
18	1.34 s			25.1 q	C-11, C-12, C-13
19	0.93 d (6.8)	H-13		11.8 q	C-12, C-13, C-14
20	6.65 q (7.1)	H-21		138.1 d	C-16
21	1.79 d (7.5)	H-20		14.3 q	C-15, C-20

Table 4. Effective Antifeedant Doses (EC_{50},[a] $\mu g/cm^2$) and 95% Confidence Limits of Compounds **1**−**6** Against the Aphid *M. persicae* and the Beetle *L. decemlineata*

	M. persicae	*L. decemlineata*	
compound	EC_{50} choice	EC_{50} choice	EC_{50} no-choice
1	3.49 (1.79, 6.83)	12.24 (4.30, 34.84)	36.40 (32.40, 40.89)
2	na	3.76 (1.05, 13.41)	∼50
3	na	>50	
4		3.41 (1.28, 9.05)	6.87 (2.76, 17.14)
5		5.26 (2.85, 9.71)	6.96 (2.82, 17.15)
6		16.49 (6.16, 46.59)	>50
senecionine[b]		1.30 (0.20, 7.87)	>50

[a] EC_{50} = concentration needed to produce 50% feeding inhibition. [b] Position control compound.

Table 5. Hemolymph and Oral Injection Effects of Compounds **1**−**6** on *L. decemlineata* Adults and *S. littoralis* Larvae

	L. decemlineata	*S. littoralis*	
compound	% mortality[a] (72 h)	B gain (% control)	consumption (% control)
1	0	99.09	93.84
2	0	83.77	90.43
3	17	133.20	99.93
4	0	92.46	94.55
5	58[b]	143.37	106.83
6	0	95.86	91.91
senecionine[c]	0	91.3	88.2
p[d]		>0.05	>0.05

[a] Corrected according to Abbott.[35] [b] Significant difference from the control, $p < 0.05$, Fisher's exact test (2 × 2 contingency tables). [c] Positive control compound. [d] Treatment *p*-level ANCOVA analysis with initial larval body weight as covariate.

pendent because compounds **1** and **2** were found to be effective antifeedants against *M. persicae* and *L. decemlineata*, respectively. Similarly, several sesquiterpene lactones with a γ-butenolactone group have been reported as having moderate to high potency as *L. decemlineata* antifeedants.[26]

The PA integerrimine (**4**) was an effective antifeedant against *L. decemlineata* (not adapted to PAs). Previous data have shown that the CPB is sensitive to the macrocyclic diester senecionine, and the open diester PA echimidine and the saturated monoester PA 3′-acetyltrachelanthamine were also strong antifeedants to this insect with similar potencies, but have few structural features in common to draw any conclusions on structure−activity relationships.[23] Integerrimine *N*-oxide (**5**) was found in this investigation to be a strong CPB antifeedant. Previous reports have shown that some PA *N*-oxides, including senecionine *N*-oxide, are active antifeedants against some aphid species and *S. littoralis*, but are less potent than their tertiary bases.[27,28]

These present data suggest that the CPB putative taste receptors can interact with different PAs (and *N*-oxides) of different structural classes with high molecular selectivity.[29,30] Little is known, however, about the molecular mechanisms that modulate PA-insect taste reception. Several PAs, including *N*-oxides and senecionine, have shown significant binding activity to muscarinic and serotonin receptors, indicating that these compounds can affect several molecular targets besides long-term toxicity through DNA alkylation by PA metabolites generated in the liver.[31] Therefore, the interference of PAs with neuronal

signal transduction could mediate insect taste regulation as proposed for chrysomelid beetles.[30,31]

Here we have found that the PA **4** and senecionine were not toxic to *S. littoralis*. Tertiary PAs are deleterious for organisms with a microsomal cytochrome P450 system, but *S. littoralis* larvae can clearly tolerate PAs. These larvae prevent PA poisoning by rapid and efficient excretion of the absorbed tertiary alkaloid.[32] In contrast, integerrimine (**4**) was moderately toxic to *L. decemlineata*, but senecionine was not. A previous experiment has shown that 10 other PAs were not toxic to this insect.[22] *Oreina* (Chrysomelidae) beetles are able to take up plant alkaloid *N*-oxides and eliminate tertiary PAs.[33,34] Similarly, *L. decemlineata* beetles could eliminate tertiary PAs efficiently enough to avoid poisoning, with some exceptions (such as compound **4**).

Experimental Section

General Experimental Procedures. Optical rotations were determined in $CHCl_3$ at room temperature using a Perkin-Elmer 137 polarimeter. IR and UV spectra were obtained on a Perkin-Elmer 1600 FT and a Varian Carey 1E spectrometer, respectively. NMR spectra were measured on a Bruker AMX2 500 MHz spectrometer with pulsed field gradient, using the solvent as internal standard ($CDCl_3$, at δ_H 7.26 and δ_C 77.0). Exact mass measurements and EIMS were recorded on an Autospect instrument at 70 eV. HPLC was carried out with a Beckman System Gold apparatus, equipped with a model 168 UV−vis diode array detector. Si gel (Merck Art. 15111, 7741) and alumina (Aldrich Art. 19,944-3 and Merck 5550) were used for column chromatography and TLC. Sesquiterpenes and alkaloids were visualized on TLC with a 25% H_2SO_4 solution and Dragendorff's reagent, respectively.

Plant Material. *S. miser* Hook. f. was collected in March 1994, from the south of Chile (Sierra Baguales, XII Region) and identified by Dr. O. Dollenz from the Universidad de Magallanes. A voucher specimen (HIP no. 10730) has been deposited in the herbarium of the Instituto de Patagonia, Punta Arenas, Chile.

Insect Bioassays. *L. decemlineata*, *S. littoralis*, and *M. persicae* colonies were reared on potato foliage (cv. Desirée), artificial diet,[36] and bell pepper (*Capsicum annuum*) plants, respectively, and maintained at 22 + 1 °C, >70% relative humidity, with a photoperiod of 16:8 h (L:D) in a growth chamber.

Choice experiments were conducted with adult *L. decemlineata*, newly emerged fifth-instar *S. littoralis* larvae, and *M. persicae* apterous adults. For the chewing insects (*S. littoralis* and *L. decemlineata*), each treatment consisted of 5 to 10 plates with three insects each as described by González-Coloma et al.[21] Percent feeding reduction (%FR) was determined for each plate by the equation %FR = [1 − (treatment consumption/control consumption)] × 100. For the sucking insect (*M. persicae*), each treatment consisted of 20 boxes with 10 insects each as described by Gutiérrez et al.[20] A settling inhibition index (%SI) was calculated for each compound at an initial dose of 50 μg/cm^2 [%SI = 1 − (%T/%C) × 100, where %T = percent aphids on a treated surface, %C = percent aphids on a control surface]. Compounds with an FR/SI>50% were tested in a dose−response experiment to calculate their relative potency values (EC_{50}, the effective dose for 50% feeding reduction), which were determined by linear regression analysis (%FR or %SI on log dose).

Oral cannulation was performed with preweighed, newly emerged *S. littoralis* L6 larvae under the same environmental conditions as described. Each experiment consisted of 20 larvae orally dosed with 20 μg of the test compound in 4 μL DMSO (treatment) or solvent alone (control).[37] At the end of the experiment (72 h), larval consumption and growth were calculated on a dry-weight basis. The possible effect of variations in initial larval weight was analyzed by an analysis of covariance (ANCOVA) performed on biomass gains with initial biomass as covariate. The covariate effect was not significant ($p > 0.05$), showing that changes in insect biomass were similar among all treatments.[38,39]

With hemolymph injection, DMSO solutions of the test compounds (10 μg/insect) were injected through the metepimeron suture of the thorax of 20 adult *L. decemlineata* beetles using a Hamilton repeating dispenser fitted with a Hamilton 50-μL syringe (50-gauge pointed needle). Toxicity symptoms and mortality were recorded up to 3 days after injection by maintenance of beetles on their respective potato leaf foods. Percent mortality was analyzed with contingency tables and corrected according to Abbott.[35]

Antifungal Activity Assays. The antifungal activity of the test substances was evaluated at a single dose (0.5 mg/mL) against the plant pathogen *F. moniliforme* and estimated as mycelial growth inhibition.[40]

Extraction and Isolation. Air-dried aerial plant parts (1.6 kg) were ground and extracted with MeOH at room temperature. The extract was filtered and concentrated under a vacuum to give a dried residue (30 g, 1.8%). This crude extract was chromatographed over a Si gel (150 g) vacuum column (VLC). The elution was carried out with *n*-hexane−EtOAc and EtOAc−MeOH gradients to obtain six fractions: Fr-0 (*n*-hexane), Fr-1 (90:10) (4.0 g), Fr-2 (80:20) (3.10 g), Fr-3 (50:50) (1.12 g), Fr-4 (EtOAc) (2.10 g), and Fr-5 (EtOAc−MeOH) (50:50) (3.5 g).

Further purification of fraction Fr-1 (4.0 g) on a Sephadex LH-20 (200 × 80 mm) column, with *n*-hexane−CH_2Cl_2−MeOH (3:1:1) as eluent, resulted in eight fractions (I−VIII) being obtained. Fraction V was purified by preparative normal-phase HPLC using a 250 × 20 mm Si gel column (Gasukuro Kogyo Inertsil ODS-2, 5-μm particle size), an isocratic system of *n*-hexane−isopropyl alcohol (93:7), and a flow rate of 8 mL/min, to yield compounds **1** (200 mg) and **3** (5 mg) (peaks were detected at 256 and 290 nm). Fr-2 (3.10 g) was chromatographed on Si gel column and preparative TLC to give compounds **1** (25 mg) and **2** (14 mg). Fraction Fr-5 (3.5 g) was shaken with a mixture of CH_2Cl_2 and 0.5 M H_2SO_4 (1:1) for 12 h. The aqueous phase was removed and acidified to pH 2 with 2 N H_2SO_4, Zn dust was added and the mixture stirred for 6 h at room temperature. The solution was filtered, made alkaline with NH_4OH at pH 9, and repeatedly extracted with CH_2Cl_2. Evaporation of the solvent gave a crude alkaloidal extract (100 mg) that was chromatographed on a Si gel column. Elution was carried out with $CHCl_3$ and MeOH mixtures of increasing polarity. Further purification using preparative TLC over Si gel (20 cm × 20 cm, 0.5 mm) eluted with $CHCl_3$−MeOH−NH_3 (85:14:1) afforded integerrimine (**4**, 8 mg, 0.0005%) and its *N*-oxide (**5**, 2 mg, 0.000125%).

1α-Acetoxy-8β-methoxy-10βH-eremophil-7(11)-en-8α,12-olide (1): crystals, mp 143−145 °C; $[\alpha]^{25}_D$ +20° (*c* 0.02, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 231 (3.46) nm; IR ($CHCl_3$) ν_{max} 2940, 1765, 1731, 1460, 1258, 1029, 972, 929, 898 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m/z* $[M]^+$ 322 (15), 262 (15), 248 (10) 235 (16), 234 (100), 231 (16), 230 (37) 219 (18) 215 (19) 203 (33), 202 (12), 175 (25), 164 (14) 147 (11), 126 (15), 119 (11), 107 (26), 105 (10), 91 (12), 83 (15), 55 (13); HREIMS *m/z* $[M]^+$ 322.1797, calcd for $C_{18}H_{26}O_5$ 322.1780; $[M - C_2H_4O_2]^+$ 262.1648, calcd for $C_{16}H_{22}O_3$ 262.1569; $[M - C_3H_4O_3]^+$ 234.1668, calcd for $C_{15}H_{22}O_2$ 234.1619; $[M - C_3H_8O_3]^+$ 230.1384, calcd for $C_{15}H_{18}O_2$ 230.1306; $[M - C_4H_7O_3]^+$ 219.1370, calcd for $C_{14}H_{19}O_2$ 219.1385; $[M - C_8H_7O]^+$ 203.1300, calcd for $C_{10}H_{19}O_4$ 203.1283.

1α-Angeloyloxy-6β-hydroxy-8β-methoxy-10βH-eremophil-7(11)-en-8α,12-olide (2): oil; $[\alpha]^{25}_D$ +38.3° (*c* 0.112, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 247 (3.05) nm; IR ($CHCl_3$) ν_{max} 3500, 1771, 1717, 1691, 1235, 973 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 2; EIMS *m/z* $[M]^+$ 378 (1), 347 (1) 346 (6), 319 (1) 278 (2) 247 (13), 246 (47), 176 (5), 156 (6), 140 (8), 108 (38), 83 (100), 55 (33); HREIMS *m/z* $[M]^+$ 378.2050, calcd for $C_{21}H_{30}O_6$ 378.2042; $[M - CH_4O]^+$ 346.1790, calcd for $C_{20}H_{26}O_5$ 346.1780; $[M - C_5H_8O_2]^+$ 278.1505, calcd for $C_{16}H_{22}O_4$ 278.1518; $[M - C_6H_{12}O_3]^+$ 246.1233, calcd for $C_{15}H_{18}O_3$ 246.1255.

1α-Angeloyloxy-8βH,10βH-eremophil-7(11)-en-8α,12-olide (3): oil; $[\alpha]^{25}_D$ −17° (*c* 0.03, $CHCl_3$) {lit.$[\alpha]^{25}_D$ −44.4°

(MeOH)]};[17] HREIMS *m/z* $[M]^+$ 332.1928, calcd for $C_{20}H_{28}O_4$ 332.1987; $[M - C_5H_7O]^+$ 249.1407, calcd for $C_{15}H_{21}O_3$ 249.1490; $[M - C_5H_8O_2]^+$ 232.13.87, calcd for $C_{15}H_{20}O_2$ 232.1463; $[M - C_5H_{10}O_2]^+$ 230.1207, calcd for $C_{15}H_{18}O_2$ 230.1306; ^1H and ^{13}C NMR data identical to those reported.[17]

Acetylation of Compound 2. Compound **2** (5.6 mg) was acetylated with Ac_2O−pyridine at room temperature for 24 h. The solvent was then evaporated under a vacuum to give a residue of 6.0 mg (96.9%). This residue was chromatographed on a Si gel column to yield 4.0 mg of an acetylated pure compound (**6**): IR ($CHCl_3$) ν_{max} 2960, 1765, 1733, 1694, 1287, 1122, 1074 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.05 (1H, dq, J = 7.0, 1.4 Hz, H-3′), 5.60 (1H, s, H-6α), 5.18 (1H, dt, J = 11.6, 4.8 Hz, H-1β), 3.20 (3H, s, OMe), 2.50 (1H, dt, J = 13.1, 4.1 Hz, H-10β), 2.42 (1H, dd, J = 13.8, 3.9 Hz, H-9β), 2.08 (3H, s, $OCOCH_3$), 1.98 (3H, s, H-13), 1.97 (3H, dq, J = 7.2, 1.5 Hz, H-4′), 1.88 (3H, m, H-5′), 1.07 (3H, s, H-14), 0.82 (3H, d, J = 6.0 Hz, H-15); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.5 (s, C-12), 170.4 (s, C-1″), 167.1 (s, C-1′), 149.9 (s, C-7), 138.4 (d, C-3′), 130.7 (s, C-2′), 127.8 (s, C-11), 106.3 (s, C-8), 71.4 (d, C-1), 69.5 (d, C-6), 50.4 (q, C-8′), 43.4 (s, C-5), 38.8 (d, C-10), 32.9 (t, C-9), 29.0 (t, C-3), 28.3 (d, C-4), 25.2 (t, C-2), 20.7 (q, C-2″), 20.6 (q, C-5′), 16.3 (q, C-14), 15.8 (q, C-4′), 15.6 (q, C-15), 8.8 (q, C-13); EIMS *m/z* $[M]^+$ 420 (6), 388 (3), 378 (3), 333 (1), 320 (4), 288 (10), 278 (17), 246 (21), 229 (11), 228 (16), 213 (11), 156 (11), 83 (100) 55 (51); HREIMS *m/z* $[M]^+$ 420.2129, calcd for $C_{23}H_{32}O_7$ 420.2148.

Integerrimine (4): amorphous; $[\alpha]^{25}_D$ −13.6° (*c* 0.12, $CHCl_3$) {lit.$[\alpha]^{25}_D$ −18.3° ($CHCl_3$)};[41] IR ($CHCl_3$) ν_{max} 3660, 3510, 1720, 1650, 1150 cm^{-1}; EIMS *m/z* $[M]^+$ 335 (6), 291 (14), 248 (11), 220 (18), 136 (100), 120 (95), 119 (85), 93 (90), 80 (31); HREIMS *m/z* 335 $[M]^+$ (9) (calcd for $C_{18}H_{25}NO_5$); ^1H and ^{13}C NMR data identical to those reported.[18]

Integerrimine *N*-Oxide (5): oil; $[\alpha]^{25}_D$ +8.5 (*c* 0.024, EtOH); HREIMS *m/z* 351 $[M]^+$ (1) (calcd for $C_{18}H_{25}NO_6$, 351.1682), 334 (1), 333 (6) (calcd for $C_{18}H_{25}NO_5$, 333.1576), 275 (1), (calcd for $C_{16}H_{21}NO_3$, 275.1521), 248 (2), 220 (2), 153 (5), 119 (100) (calcd for C_8H_9N, 119.0735), 93 (10), 81 (5), 55 (7); ^1H and ^{13}C NMR data, see Table 3.

Integerrimine *N*-Oxide (5) from Integerrimine (4). A solution of integerrimine (**4**, 5 mg) and *m*-chloroperbenzoic acid (3 mg) in $CHCl_3$ was stirred at room temperature for 3 h. After removal of the solvent under a vacuum, the residue was dissolved in H_2O (5 mL) and the aqueous solution extracted with Et_2O (6 × 5 mL). The solvent was then distilled under a vacuum to give the *N*-oxide (3 mg). Its spectroscopic properties were identical with those of the natural product.

Crystal Data for 1. $C_{18}H_{26}O_5$, mol wt = 322.4, orthorhombic, space group $P2_12_12_1$, a = 6.812(2), b = 15.945(3), c = 16271(2) Å, V = 1768.9(7) $Å^3$, Z = 4, D_c = 1.211 g·cm^{-3}, $F(000)$ = 696, μ(Mo Ka) = 0.087 mm^{-1}. A single crystal of approximate dimensions 0.5 × 0.3 × 0.3 mm was used for all X-ray measurements. The intensity data of all unique reflections within the θ range 2.5−26.3° were collected at 273 K in an Enraf-Nonius CAD4 diffractometer, using Mo Ka radiation and a graphite monochromator. Three standard reflections monitored every 2 h of X-ray exposure showed no significant intensity variation. A total of 2078 unique reflections was recorded, of which 1049 were taken into account under $I > 4\alpha I$. The intensities were corrected for Lorentz and polarization factors, but no absorption correction was made. Crystallographic data of **1**, including atomic coordinates, have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK [Fax: 44-(0)1223-336033 or E-mail: deposit@ccdc.cam.ac.uk].

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Supporting Information Available: Table showing biological effects of *S. miser* crude extract and fractions against *L. decemlineata*, *M. persicae*, and *F. moniliforme* and tables of X-ray crystallographic data for compound **1**. This material is available free of charge via the Internet at http//pubs.acs.org.

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