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BIOACTIVE SATURATED PYRROLIZIDINE ALKALOIDS FROM *HELIOTROPIUM FLORIDUM*

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Key Word Index—*Heliotropium floridum*; Boraginaceae; pyrrolizidine alkaloids; 3′-acetyltrachelanthamine; floridine; floridinine; floridimine; heliovicine; biological activity.

Abstract—Here we describe the isolation and structural determination of the new saturated pyrrolizidine monoester alkaloids, 3′-acetyltrachelanthamine, floridine, floridinine and floridimine, along with the known one, heliovicine, from *Heliotropium floridum*. Their structures were established by high resolution NMR (including 2D NMR experiments), mass spectrometry, chemical reactions and by correlation with published data of known compounds. Bioassays of the alkaloidal extract and its major components against several insect pests and plant pathogens showed that 3′-acetyltrachelanthamine is a strong anti-feedant, with low toxicity against *Leptinotarsa decemlineata* and a moderate anti-fungal agent against *Fusarium monoliforme*; floridinine only showed the anti-fungal effect. © 1997 Elsevier Science Ltd

INTRODUCTION

There are numerous plant species containing pyrrolizidine alkaloids (PAs) and they mainly belong to the Boraginaceae, Compositae and Leguminosae [1–3]. Toxicologically, these compounds are known to be the cause of liver damage and have been identified as both carcinogenic and mutagenic agents [4–6]. Furthermore, they are implicated in the defensive strategy of several insect species, among them *Danaid*, *Arctiid* and some *Itomiine Lepidopterans* and Oreina Chrysomelids [5, 7].

The genus *Heliotropium*, a known source of PAs, is comprised of *ca* 250 species distributed throughout both hemispheres with 24 species endemic to Chile [8]. As part of our ongoing study of bioactive PAs from Chilean *Heliotropium* species, we have studied *H. floridum* var. *latifolium*, a shrub localized in the north Chilean desert (Atacama). Herein, we describe the isolation and characterization of the alkaloids **1**–**5** and the biological activity of the major ones (**1** and **3**), against several species of insect pests and plant pathogens.

RESULTS AND DISCUSSION

The crude alkaloidal extract (0.25%) obtained from the plant as described in the experimental was subjected to successive chromatographies and preparative TLC on silica gel to give five alkaloids. The structures of the alkaloids were established by spectroscopic methods (mass spectrometry, ^{1}H and ^{13}C NMR and 2D NMR experiments). The least polar alkaloid (**1**) was isolated as an oil (0.003%). The ^{13}C NMR spectrum (DEPT experiment) showed 17 carbon atoms (four methyls, six methylenes, four methines and three quaternary carbon atoms) and its molecular formula $C_{17}H_{29}NO_5$ was established by high resolution mass analysis. The remaining fragmentation ions are consistent with the presence of a trachelanthamidine, rather than an (+)-*iso*-retronecanol, esterified with a necic acid [9, 10].

The ^{1}H and ^{13}C NMR spectra exhibited ring proton and carbon positions similar to those published for a trachelanthamidine ester [11, 12]. Furthermore, the ROESY [13] spectrum showed a positive NOE between the protons at δ_H 2.09 (*m*, H-1β) and 1.63 (*m*, H-7β) and no NOE between the H-1β and 3.38 (*dd*, H-8) protons, indicating a *trans*-configuration for the H-1 and H-8 protons, and that (−)-trachelanthamidine was the necine of alkaloid **1**.

The ^{1}H NMR ($CDCl_3$) spectrum of **1** showed the esterified necic acid signals at δ_H 0.90 and 0.96 (*d*, J = 7.0 Hz) corresponding to two isopropyl methyl groups, the methyl signals H-4′ at 1.23 (*d*, J = 6, 4 Hz), two methine signals at 2.02 (*sept*, overlapped, H-5′) and 5.22 (*q*, J = 6.4 Hz, H-3′) and 1.97 (*s*, OCOC$\underline{H}_3$). The HMQC [14] experiment showed that

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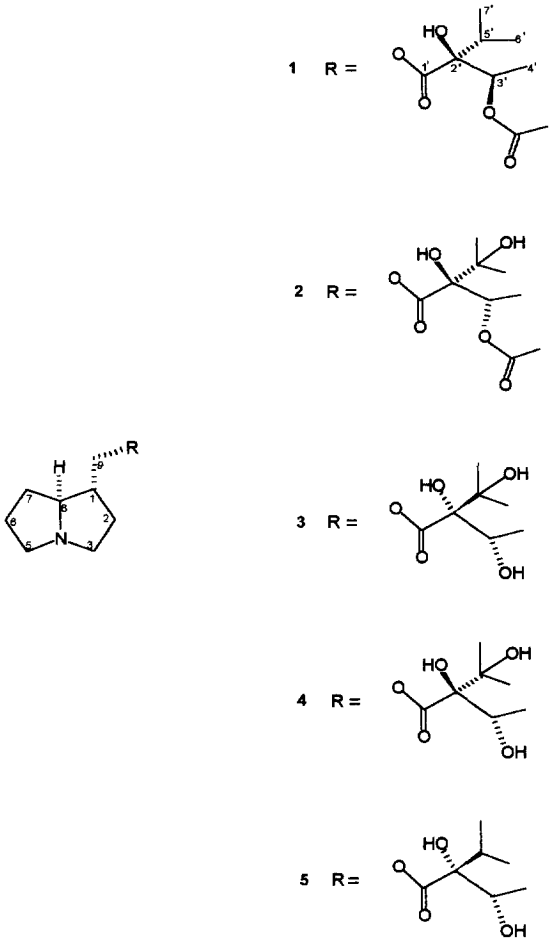

these protons were correlated with the carbon signals at δ_c 16.7 (*q*), 17.2 (*q*), 13.9 (*q*), 32.8 (*d*), 72.2 (*d*) and 31.1 (*q*), allowing us to describe the said carbon signals to C-6′, C-7′, C-4′, C-5′, C-3′ and ($OCOCH_3$), respectively. An HMBC [15] experiment confirmed those assignments.

By a HMBC experiment, the position of ester attachment at C-7 or C-9 was determined. The proton δ_H 4.16 (*d*) gave connectivities with carbonyl carbon at 174.3 (C-1′), the methine carbon at 44.7 (C-1), the methylene carbon 30.5 (C-2) and the methine carbon 67.8 (C-8), indicating the attachment at C-9 of the 3′-acetylated necic acid (Table 1).

The new alkaloid **2** was an oil and the ^{13}C NMR spectrum showed 17 carbon atoms present in the molecule. A DEPT experiment revealed four quaternary carbons, three methines, six methylenes and four methyl groups. HR-mass spectrometry indicated a $[M]^+$ at *m/z* 343.2044, corresponding to the molecular formula $C_{17}H_{29}NO_6$. The ROESY experiment gave no NOE between H-1β and H-8 but a positive NOE between H-7β and H-1β, confirming trachelanthamidine as the necine of alkaloid **2**. Its ^1H NMR spectrum was similar to that of the above-mentioned alkaloid **1**, with the exception of the chemical shifts corresponding to necic acid, δ_H 1.25 and 1.40 (3H each, *s*), 1.36 (3H, *d*, J = 6.4 Hz), 1.98 (*s*, $OCOCH_3$) and 5.45 (1H, *q*, J = 6.3 Hz), which correlate with the carbon signals at δ_c 26.1 (*q*, C-6′), 25.0 (*q*, C-7′), 15.1 (*q*, C-4′), 21.2 (*q*, $OCOCH_3$) and 72.7 (*q*, C-3′) in the HMQC experiment. The remaining carbon atom signals resonated at 173.4 (*s*, C-1′), 169.6 ($OCOCH_3$), 83.4 (*s*, C-2′) and 73.5 (*s*, C-5′) (Table 2).

Alkaloid **3** was an oil and its ^{13}C NMR spectrum exhibited 15 carbon atoms. A DEPT experiment revealed three quaternary carbons, three methines, six methylenes and three methyl groups. The molecular formula was determined by HR-mass spectrometry to be $C_{15}H_{27}NO_5$. The remaining fragments ions were similar to those observed for the alkaloid **2**. The NMR data of alkaloid **3** differed from those of **2** only in the disappearance of the acetyl group signals and in the appearance of the new signals at δ_H 4.19 (*q*, J = 6.8 Hz, H-3′) and 69.8 (*d*, C-3′) (Table 3).

Alkaloid **4** was isolated as a resin. Its ^{13}C NMR spectra (DEPT experiment) displayed 15 carbon atoms (three methyls, six methylenes, three methines and three quaternary carbon atoms). The ^1H and ^{13}C NMR spectra (Table 4) were very similar to the above-mentioned alkaloid **3**, except for the chemical shift of the H_2-9 protons ($\Delta\delta H_2$-9 = 0.10). Likewise, mild acetylation of **4** with Ac_2O–pyridine gave alkaloid **2** (NMR data identical) and its structure was thus established.

The minor alkaloid **5** was obtained as oil and its EI mass spectrum exhibited a $[M]^+$ at *m/z* 285.15 (1%). The pyrrolizidine base was determined by ^1H and ^{13}C NMR, ^1H–^1H COSY and HMQC experiments. Furthermore, the proposed *trans*-configuration of the ring protons H-1 and H-8 was established by selective pulsed-field gradients using the so-called GOESY and 1D NMR experiment [16]. Likewise, the position of attachment of the necic component to the necine was determined by an HMBC experiment (Table 5). Thus, the H-9d proton at δ_H 4.66 gave connectivity with the methylene carbon at 29.0 (C-2), and the H-9u proton at 3.96 with the methine carbon at 68.0 (C-8) and with the carbonyl carbon at 175.6 (C-1′). Therefore, the necic acid is linked at C-9.

The stereochemistry of the component acids of alkaloids **1**–**5** was deduced from the interpretation of the values of ^1H NMR for H_2-9 and H-3′, as well as by the chemical shift for C-3′ and the shift difference between C-6′/C-7′ [12, 17], and also by comparison of these data with those published for the alkaloids, 3′-acetylintermedine [18], echimidine [19], heliovicine [12] and acetyllithosenine [20] (Table 6). Thus, (+)-3′-acetyltrachelanthic acid (2′*S*, 3′*R*) proved to be the acid component of the ester alkaloid **1**, (−)-3′-acetylviridifloric (2′*S*, 3′*S*) acid hydroxylated at position C-5′ to alkaloid **2**, the alkaloid **3** is esterified with the (−)-trachelantic (2′*R*, 3′*S*) acid hydroxylated at position C-5′, the alkaloid **4** with (−)-viridifloric (2′*S*, 3′*S*) acid hydroxylated at position C-5′, while alkaloid **5** is esterified with the (−)-trachelanthic (2′*R*, 3′*S*) acid.

Table 1. ^{1}H, ^{13}C, HMQC and HMBC NMR data of alkaloid **1**

Proton		Correlated carbon HMQC	HMBC
H-1β	2.09 *m*	44.7 *d*	C-2, C-9
H-2α	1.70 *m*	30.4 *t*	C-1
H-2β	2.02 *m*	30.4 *t*	
H-3α	3.28 *m*	54.1 *t*	C-1, C-8
H-3β	2.60 *m*	54.1 *t*	C-2, C-5,
H-5α	3.07 *dt* (10.7, 6.4, 6.4)	54.9 *t*	C-3, C-8
H-5β	2.65 *dt* (10.5, 6.5, 6.5)	54.9 *t*	C-3, C-6, C-7, C-8
H-6α	1.89 *m*	25.6 *t*	C-8
H-6β	1.84 *m*	25.6 *t*	C-5, C-7, C-8
H-7α	2.05 *m*	31.5 *t*	
H-7β	1.63 *m*	31.5 *t*	C-1, C-5
H-8	3.38 *dd* (12.8, 6.6)	67.8 *d*	C-3, C-5, C-9
H-9	4.16 *d* (6.5)	67.9 *t*	C-1, C-1′, C-2, C-8
	(C-1′)	174.3 *s*	
	(C-2′)	81.6 *s*	
H-3′	5.22 *q* (6.4)	72.2 *d*	C-1′ (174.3), C-8′ (169.7)
H-4′	1.23 *d* (6.43)	13.9 *q*	C-2′, C-3′
H-5′	2.02 overlapped	32.8 *d*	C-1′, C-2′,
H-6′	0.90 *d* (7.0)	16.7 *q*	C-2′, C-5′, C-7′
H-7′	0.96 *d* (7.0)	17.2 *q*	C-2′, C-5′, C-6′
C=O		169.7 *s*	
Me	1.97 *s*	21.1 *q*	C-8′ (169.7)

Coupling constants (Hz) are shown in parentheses.

Table 2. ^{1}H, ^{13}C, HMQC and HMBC NMR data of alkaloid **2**

Proton		Correlated carbon HMQC	HMBC
H-1β	2.23 *m*	44.2 *d*	C-9
H-2α	2.15 *m*	31.2 *t*	C-1
H-2β	2.15 *m*	31.2 *t*	
H-3α	3.75 *m*	54.0 *t*	C-1
H-3β	2.77 *m*	54.0 *t*	C-5
H-5α	3.48 *m*	54.0 *t*	C-3, C-6, C-8
H-5β	2.65 *m*	54.0 *t*	C-3, C-6, C-8
H-6	2.04	25.4 *t*	C-8
H-7α	2.20 *m*	29.5 *t*	
H-7β	1.70 *m*	29.5 *t*	C-1, C-5, C-9
H-8	4.01 *m*	67.6 *d*	C-3, C-5, C-9
H-9u	4.16 *dd* (11.7, 5.0)	66.2 *t*	C-1, C-2, C-8
H-9d	4.27 *dd* (11.7, 5.0)	66.2 *t*	C-1, C-1′, C-2, C-8
	(C-1′)	173.4 *s*	
	(C-2′)	83.4 *s*	
H-3′	5.45 *q* (6.3)	72.7 *d*	C-8′ (169.6)
H-4′	1.36 *d* (6.4)	15.1 *q*	C-2′, C-3′
H-5′		73.5 *s*	
H-6′	1.25 *s*	26.2 *q*	C-2′, C-5′, C-7′
H-7′	1.40 *s*	24.9 *q*	C-2′, C-5′, C-6′
C=O		169.6 *s*	
Me	1.98 *s*	21.2 *q*	C-8′ (169.6)

Coupling constants (Hz) are shown in parentheses

Anti-feedant assays showed that the crude plant extract had a strong effect against *L. decemlineata* (Colorado potato beetle, CPB) in choice assays (Table 7). Furthermore, the alkaloidal fraction was similarly anti-feedant against this insect in both choice and no-choice assays, while being inactive against *S. littoralis* (% FI = 27.48 + 11.46 for 100 $\mu g\ cm^{-2}$ of alkaloidal extract in choice test). Among the major alkaloids of this fraction, compound **1** had a strong anti-feedant effect on *L. decemlineata*, while compound **3** had five

Table 3. ^{1}H, ^{13}C, HMQC and HMBC NMR data of alkaloid **3**

Proton		Correlated HMQC	HMBC
H-1β	2.36 *m*	43.3 *d*	
H-2α	2.08 *m*	28.6 *t*	C-3, C-9
H-2β	2.20 *m*	28.6 *t*	
H-3α	3.51 *m*	53.8 *t*	C-8
H-3β	2.66 *q* (10.4)	53.8 *t*	C-5
H-5α	3.24 *ddd* (12.0, 6.8, 6.8)	54.0 *t*	C-3
H-5β	2.85 *ddd* (11.6, 7.0, 6.0)	54.0 *t*	C-3, C-7, C-8
H-6α	2.0 *m*	24.6 *t*	C-5, C-8
H-6β	1.94 *m*	24.6 *t*	C-5, C-8
H-7α	2.15 *m*	30.6 *t*	C-5, C-8
H-7β	1.63 *dddd* (12.7, 8.0, 7.0, 7.0)	30.6 *t*	C-1, C-5, C-8
H-8	3.70 *m*	68.4 *d*	C-3, C-5, C-9
H-9u	4.20 *dd* (10.7, 3.9)	66.2 *t*	C-1′, C-8′
H-9d	4.53 *dd* (10.7, 3.9)	66.2 *t*	C-2, C-1′
	(C-1′)	174.6 *s*	
	(C-2′)	82.5 *s*	
H-3′	4.19 *q* (6.8)	69.8 *d*	C-1′ (174.3)
H-4′	1.27 *d* (7.0)	18.6 *q*	C-2′, C-3′
H-5′		73.9 *s*	
H-6′	1.28 *s*	25.0 *q*	C-5′, C-7′
H-7′	0.96 *d* (7.0)	26.0 *q*	C-2′, C-5′, C-6′

Coupling constants (Hz) are shown in parentheses.

Table 4. ^{1}H, ^{13}C, HMQC and HMBC NMR data of alkaloid **4**

Proton		Correlated carbon HMQC	HMBC
H-1β	2.30 *m*	44.3 *d*	C-2
H-2α	2.17 *m*	28.7 *t*	C-8
H-2β	2.28 *m*	28.7 *t*	C-1, C-3
H-3α	3.89 *m*	54.2 *t*	C-1, C-8
H-3β	2.83 *m*	54.2 *t*	C-5
H-5α	3.59 *dt* (11.6, 6.0, 6.9)	54.3 *t*	C-3, C-7
H-5β	2.89 *dt* (12.3, 6.3, 6.3)	54.3 *t*	C-3, C-7
H-6	2.11 *m*	25.3 *t*	C-8
H-7α	2.26 *m*	30.6 *t*	C-1, C-5
H-7β	1.800 *dddd* (12.3, 6.1, 6.1, 6.1)	30.6 *t*	C-1, C-5
H-8	4.30 *m*	67.6 *d*	C-9
H-9u	4.35 *dd* (11.6, 2.6)	63.2 *t*	C-8
H-9d	4.45 *dd* (11.6, 2.6)	63.2 *t*	C-2
	(C-1′)	174.7 *s*	
	(C-2′)	84.1 *s*	
H-3′	4.26 *q* (6.2)	69.9 *d*	C-1′ (174.7)
H-4′	1.32 *d* (6.3)	18.6 *q*	C-3′
H-5′		73.8 *d*	
H-6′	1.28 *s*	26.0 *q*	C-2′, C-5′, C-7′
H-7′	1.33 *s*	24.9 *q*	C-5′, C-6′

Coupling constants (Hz) are shown in parentheses.

times less activity than **1** when tested at 10 $\mu g\ cm^{-2}$ (Table 7). When compared with the sesquiterpene silphenene, a strong CPB anti-feedant [21], we observed that **1** was six times less active than the silphinene in choice assays, but showed similar amounts of activity in no-choice situations (Table 7).

Adult beetles were injected with a single dose of compounds **1** to test for additional toxic effects. Seven days after the treatment the injected insects had a low mortality rate (22.5% mortality, corrected according to Abbott formula), suggesting that this compound acts mainly as an anti-feedant on this insect species. Furthermore, the strong feeding deterrency of alkaloids **1** on CPB coupled with the low effects of its

Table 5. ^{1}H, ^{13}C, HMQC and HMBC NMR data of alkaloid **5**

Proton		Correlated carbon HMQC	HMBC
H-1β	2.27 *m*	43.9 *d*	
H-2α	1.84 *m*	29.0 *t*	C-9
H-2β	2.11 *m*	29.0 *t*	C-3
H-3α	3.30 *m*	54.1 *t*	C-5
H-3β	2.73 *dt* (11.7, 63., 6.3)	54.1 *t*	C-5
H-5α	3.01 *m*	54.2 *t*	C-3
H-5β	2.56 *dist. q* (8.3)	54.2 *t*	C-3
H-6α	1.83 *m*	24.8*t*	
H-6β	1.76 *m*	24.8 *t*	
H-7α	2.00 *m*	31.1 *t*	C-5
H-7β	1.50 *m*	31.3 *t*	C-1, C-5
H-8	3.28 *m*	68.0 *d*	C-1
H-9u	3.96 *dd* (10.9, 5.9)	66.8 *t*	C-8
H-9d	4.66 *dd* (10.9, 5.9)	66.8 *t*	C-2
	(C-1′)	175.6 *s*	
	(C-2′)	83.3 *s*	
H-3′	4.03 *q* (6.1)	69.0 *d*	C-1′ (175.6)
H-4′	1.18 *d* (7.0)	17.4 *q*	C-2′, C-3′
H-5′	2.14 *sept* (6.9)	32.5 *d*	C-1′, C-2′, C-6′, C-7′
H-6′	0.92 *d* (6.9)	17.2 *q*	C-2′, C-5′, C-7′
H-7′	0.96 *d* (6.8)	16.8 *q*	C-2′, C-5′, C-6′

Coupling constants (Hz) are shown in parentheses.

Table 6. Tentative evaluation of the stereochemistry C-2′/C-3′ of alkaloids **1**–**5**. Comparison with some known PAs

Alkaloid	δ_H H-3′	δ_C C-3′	$\Delta\delta_H$ H_2-9	$\Delta\delta_C$ C-6′/C-7′	Stereochemistry C-2′/C-3′
1	5.22	72.2	0.0	0.5	2′*S*, 3′*R*
3′-Acetylintermedine	5.21	72.1	0.13	0.6	2′*S*, 3′*R*
2	5.45	72.7	0.11	1.1	2′*S*, 3′*S*
3′-Acetyllithosenine	5.28	72.1	0.14	1.1	2′*S*, 3′*S*
3	4.19	69.8	0.33	1.0	2′*R*, 3′*S*
Echimidine	4.15	69.6	0.27	1.1	2′*R*, 3′*S*
4	4.26	69.9	0.10	1.1	2′*S*, 3′*S*
Heliovicine	4.04	68.7	0.65	0.3	2′*R*, 3′*S*
5	4.03	69.0	0.70	0.4	2′*R*, 3′*S*

structurally-related compound **3** suggest a target-specific mode of action of this compound.

PAs such as senkirkine, lasiocarpine and europine have been reported as having anti-feedant effects on polyphagous and oligophagous *Lepidopterans* [22, 24], but this is the first report on this class of compounds acting as anti-feedants on CPB. On the contrary, previous studies have shown that this insect, a specialist of some Solanaceae species [25–27], showed generally low sensitivity to feeding deterrency of different classes of alkaloids (diterpenoid, indole and isoquinoline-alkaloids) [28].

Unsaturated PAs exhibit mammalian toxicity plus mutagenic and carcinogenic effects after metabolic activation by microsomal enzymes [29] due to the presence of a 1,2-double bond in the pyrrolizidine ring system and esterification at C-9 or C-7 [30]. Furthermore, it has been suggested that PAs are bioactivated *via* similar pathways in mammalian liver and insect cells [31]. We did not observe any correlation between anti-feedant potency and internal toxicity of **1** on CPB, probably because of the low toxicity attributed to saturated PAs [3]. However, aqueous extracts of *H. curavassicum* containing saturated PAs significantly increased chromosomal aberrations and abnormal metaphases in a CHO cell line [32]. Further research is needed in order to assess the possible mutagenic and carcinogenic effects of **1**.

Table 8 shows the result of the anti-fungal activity tests. Juglone and Neem-azal are included as positive controls [33]. Compounds **1** and **3** showed a moderate effect (% mycelial growth inhibition >40%) on *Fus-*

Table 7. Effects on *L. decemlineata* feeding of *H. floridum* crude extracts, its alkaloidal fraction and the pure compounds **1** and **3** in choice and no-choice assays. Effective doses (EC_{50}) were calculated for the alkaloidal fraction and the pure compounds

Treatment	Choice %FI*,† AVG (SE)	Choice EC_{50} ($\mu g\ cm^{-2}$) 95% L.C‡	No choice %FI*,† AVG (SE)	No choice EC_{50} ($\mu g\ cm^{-2}$) 95% L.C‡
Plant extract	98.90 (1.04)	—	50.40 (17.43)	—
Alkaloidal extract	100 (0.0)	14.83 (8.05, 27.3)	91.90 (4.43)	45.13 (10.24, 19.85)
1	91.08 (1.86)	1.79 (0.93, 3.42)	54.20 (10.17)	2.92 (0.57, 17.78)
3	17.5 (11.24)			—
Silphinene§	—	0.27 (0.16, 0.46)	—	2.67 (1.38, 5.18)

* Concentrations of 100 and 10 $\mu g\ cm^{-2}$ were used for the extracts (plant and alkaloidal) and the pure compounds (**1** and **3**), respectively.

† %FI = $1-(T/C)\times 100$, where T = consumption of treated leaf discs and C = consumption of control leaf discs.

‡ Confidence limits (lower and upper).

§ Ref. [21].

Table 8. Mycelial growth inhibition (%) of *Fusarium* sspp. treated with *H. floridum* crude extract, its alkaloidal fraction and the pure compounds **1** and **3** inhibition growth assays.

Treatment†	% inhibition* AVG (SE) *F. oxysporum*	*F. moniliforme*	*F. avenaceum*	*F. solani*
Plant extract	15.34 (9.64)	32.5 (7.07)	11.8 (10.12)	4.49 (4.97)
Alkaloidal extract†	8.29 (9.09)	16.25 (7.44)	7.89 (7.44)	14.61 (11.91)
1	—	49.05 (7.1)	—	—
3	25.22 (7.77)	42.00 (4.47)	28.07 (7.92)	22.95 (4.80)
Juglona‡	100 (0.0)	76.8 (5.83)	54.5 (14.57)	100 (0.0)
Neem-azal-F‡	61.6 (6.92)	52.5 (6.99)	75.0 (5.48)	63.6 (6.47)

* % mycelial growth inhibition = $(1-(T/C))\times 100$, where T = mycelial grow on treatment and C = mycelial growth on control.

† Dose 0.5 mg ml^{-1}.

‡ Ref. [33].

arium moniliforme. The alkaloidal extract showed half the anti-fungal effect when compared with that of the crude plant extract on *F. moniliforme*. This extract represents a 6.45% dry weight of the crude plant extract, indicating that other anti-fungal compounds may be present in the non-alkaloid fraction of the plant.

Unsaturated PAs isolated from *Heliotropium* species, such as lasiocarpine and europine, have been shown to exert anti-microbial and anti-fungal effects. Furthermore, europine inhibited the mycelial growth of *F. moniliforme* with a similar degree of activity to that observed for alkaloids **1** and **3** (EC_{50} of 0.74 mg ml^{-1}) [23], indicating that the anti-fungal effect of this class of compounds is not related to the presence of the unsaturation.

In conclusion, we have isolated five PAs from *H. floridum*; (+)-3′-acetyltrachelanthamine, floridine, floridinine and floridimine are the names of the new structures proposed for alkaloids **1**–**4**. Heliovicine is the alkaloid **5** [12]. To the best of our knowledge, 3′-acetylviridiflorine, isolated from *Amsinckia tessellata* var. *gloriosa*, is the only acetylated derivative of a saturated pyrrolizidine monoester alkaloid reported in the literature [34]. Among the major ones, 3′-acetyltrachelanthamine is a strong anti-feedant against the alkaloid-adapted insect species, *L. decemlineata*, without showing internal toxicity. This is the first

report of an anti-feedant PA acting on this insect. Furthermore, compounds **1** and **3** showed moderate activity against *F. moniloforme*.

EXPERIMENTAL

General. ^1H and ^{13}C NMR spectra were measured on a Bruker AMX 400 MHz or AMX 500 MHz spectrometer with pulsed-field gradient (only GOESY experiment) (chemical shifts reported are relative to residual $CDCl_3$, 7.26 ppm, for ^1H and 77.0 ppm for ^{13}C). Mass spectra were obtained at 70 eV. Sepns were performed by flash CC (silica gel, 63–200 mesh) and prep. TLC (silica gel GF-254, 0.2 and 1.0 mm cards).

Plant material. Heliotropium floridum var. latifolium Phil., was collected in 1991, from north of Chile (Atacama, III Region) and identified by Dr Sebastian Teillier from Museo de Historia Natural de Santiago de Chile. A voucher specimen is deposited in the Herbarium of this Museo, with the number ST 2569.

Isolation of alkaloids. Dried and pulverized aerial parts of *H. floridum* (2 kg) were first defatted with petrol and then exhaustively extracted repeatedly with EtOH at room temp. The combined extracts were evapd *in vacuo* to give 77.62 g. The syrupy residue and agitated with critic acid (7%) for 2 hr, allowed to stand for 12 hr at 4°, then filtered. The clear filtrate was washed with $CHCl_3$ (3 × 100 ml). The aq. phase was acidified to pH 2 with NH_2SO_4, Zn dust (5 g) added and the mixt. stirred for 4 hr at room temp. The soln was filtered and made alkaline with NH_4OH (pH 9) and extracted repeatedly with $CHCl_3$. Evapn of these frs gave 5.01 g (0.25%). A portion of crude alkaloidal fr. (2.5 g) was chromatographed on a silica gel column. Elution was carried out with increasing order of polarity gradients using $CHCl_3$ and MeOH. The eluate obtained with $CHCl_3$–MeOH (19:1) gave 3′-acetyltrachelanthamine (**1**) (fr. A, 77.7 mg), with $CHCl_3$–MeOH (9:1) gave a mixture of **1**–**4** (fr. B, 200 mg) and with $CHCl_3$–MeOH (17:3) gave a mixt. of **2**–**5** (fr. C, 1.0 g). Further purification by prep. TLC of frs A–C, eluted with $CHCl_3$–MeOH–NH_3 (85:14:1) gave **1** (30 mg), **2** (15 mg), **3** (20 mg), **4** (42 mg) and **5** (5 mg).

Insect bioassays. The insects species, *Leptinotarsa decemlineata* (Coleoptera: *Chrysomelidae*) and *Spodoptera littoralis* (Lepidoptera: *Noctuidae*) were used for assays based on their significance as crop pests. The *L. decemlineata* colony was reared on potato foliage and maintained at 24 + 1°, >70% r.h. with a photoperiod of 16:8 hr (light:dark) in a growth chamber. *S. littoralis* larvae were obtained from a laboratory colony reared on an artificial diet [35] and kept under the same environmental conditions.

Feeding assays. Choice and no-choice feeding assays were conducted with adult *L. decemlineata* beetles (five Petri dishes with three beetles each per treatment). Choice experiments were also conducted with sixth-instar *S. littoralis* larvae (five dishes with three larvae each per treatment) as previously described [21, 36]. Uneaten leaf disc surfaces were measured according to ref. [36] with a computer-interfaced scanner. Percent feeding inhibition (%FI) was determined for each arena by the equation %FI = [1-(treatment consumption/control consumption)] × 100 [22]. The active compounds were tested in a dose-response experiment (doses of 0.08, 0.4, 1.0, 2.0, 5.0 and 10.0 $\mu g\ cm^{-2}$) to calculate their relative potencies (EC_{50} values, the effective does for 50% feeding reduction), which were determined from log probit analysis [37].

Injected toxicity. The anti-feedant alkaloid **1** was injected abdominally into the metacoxal cavity of adult *L. decemlineata* beetles. Twenty beetles (average wt 152 mg) were injected with either 10 μg of **1** (2 μl) or solvent alone (2 μl DMSO), then kept individually in 5 cm diameter Petri dishes lined with moist filter paper and allowed to feed—*ad libitum*—on potato foliage. Mortality was checked daily for seven days and correlated according to ref. [38].

Anti-fungal assays. Test substances were bioassayed against the following species of phytopathogenic fungi: *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *F. avenaceum*. Mycelial growth inhibition was estimated according to ref. [39]. Briefly, a dose of 0.5 mg m^{-1} of test substance was dissolved in EtOH and incorporated into PDA culture media (5% final concn of solvent). For each experiment, eight replicates of 2 cm diameter media disks inoculated with the appropriate fungal sp. were incubated at 27° in darkness and colony diameters measured after 48 hr. Control experiments consisted of solvent-treated media. Juglone and Neem-azal-F were used as positive controls [33].

3′-Acetyltrachelanthamine (**1**). Oil. $[\alpha]_D$ +7° (CH_2Cl_2; *c* 0.044). IR ν^{film} cm^{-1}: 2959, 2928, 1730, 1462, 1275, 1124, 1072. EIMS *m/z* (rel. int.): 327.2049 (1) $[M]^+$ for $C_{17}H_{29}NO_5$ (calcd 327.2045), 284 (2), 240.1604 (6) for $C_{13}H_{22}NO_3$ (calcd 240.1599), 184 (6), 142 (27), 125 (38), 124.1128 (100) $C_8H_{14}N$ (calcd 124.1126), 84 (14), 83 (59), 82 (54). NMR: Table 1.

Floridine (**2**). Oil. $[\alpha]_D$ −14.2° (EtOH; *c* 0.04). IR ν^{film} cm^{-1}: 3450, 2927, 1737, 1460, 1377, 1256, 1071. EIMS *m/z* (rel. int.): 343.2044 (1) $[M]^+$ for $C_{17}H_{29}NO_6$ (calcd 343.1994), 328.1765 (2) for $C_{16}H_{26}NO_6$ (calcd 328.1760), 284.1523 (1) for $C_{14}H_{22}NO_5$ (calcd 284.1398), 240 (2), 239 (2.5), 225.1377 (10) for $C_{12}H_{19}NO_3$ (calcd 225.1364), 184 (1), 142 (8), 124.1126 (100) for $C_8H_{14}N$ (calcd 124.1126), 83.0760 (27) for C_5H_9N (calcd 83.0735). NMR: Table 2.

Foridinine (**3**). Oil. $[\alpha]_D$ −8.3 (EtOH; *c* 0.096). IR ν^{film} cm^{-1}: 3408, 2958, 2928, 1730, 1582, 1263, 1129, 1012, 955. EIMS *m/z* (rel. int.): 301.1898 (1) $[M]^+$ for $C_{15}H_{27}NO_5$ (calcd 301.1889), 242.1397 (5) for $C_{12}H_{20}O_4N$ (calcd 242.1392), 239.1509 (7) for $C_{13}H_{21}NO_3$ (calcd 239.1521), 226.1419 (5) for $C_{12}H_{29}NO_3$ (calcd 226.1443), 167 (1), 142.1230 (46) for $C_8H_{16}NO$ (calcd 142.1231), 125 (19), 124.1102 (100) for $C_8H_{14}N$ (calcd 124.1126), 122 (8), 110 (6), 96 (8), 95 (7), 83 (36), 82 (24), 70 (12), 59 (12). NMR: Table 3.

Floridimine (**4**). Oil. IR ν^{film} cm^{-1}: 3400, 2960, 2926, 2858, 1735, 1575, 1463, 1383, 1263, 1126, 1102, 958. EIMS *m/z* (rel. int.): 301 (1) $[M]^+$, 239 (8), 226 (7), 142 (36), 124 (100), 95 (12), 94 (10), 83 (55), 55 (23). NMR: Table 4.

Heliovicine (**5**). Oil. $[\alpha]_D$ −2.0° (EtOH; *c* 0.05) [Lit. [40], $[\alpha]_D$ −2.7° (EtOH; *c* 0.6)]. IR ν^{film} cm^{-1}: 3405, 2983, 2928, 1731, 1671, 1606, 1458, 1374, 1245, 1109. EIMS *m/z* (rel. int.): 285 (1) $[M]^+$, 267 (5), 252 (5), 240 (4), 226 (3), 175 (1), 142 (35), 124 (100), 96 (11), 95 (9), 83 (25), 82 (20), 55 (33). NMR: Table 5.

Acetylation of floridimine (**4**). The compound (2.4 mg), was acetylated with Ac_2O–pyridine at room temp. for 24 hr. Solvent was then distilled off *in vacuo* to give a residue of 4.6 mg. This was chromatographed over basic alumina to yield floridine (2.6 mg, 100%) ^1H and ^{13}C NMR of the acetylated product were identical to those of compound **2**.

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