JOURNAL OF NATURAL PRODUCTS

Volume 64, Number 9 September 2001

Full Papers

Heliotropium huascoense Resin Exudate: Chemical Constituents and Defensive Properties

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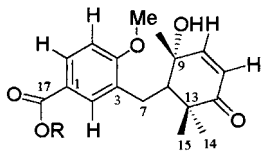
Received December 19, 2000

From the resinous exudate of *Heliotropium huascoense* a new compound, *rel*-(8*R*,9*R*)-carrizaloic acid, (**1**) {3-[*rel*-(8*R*,9*R*-9-hydroxy-9,13,13-trimethyl-12-oxo-10-cyclohexenyl)methyl]-4-methoxybenzoic acid}, and three known flavonoids, [3-methylgalangin, 3,7-dimethylgalangin, and (−)-alpinone] have been isolated. The structure of **1** was determined by spectral and chemical methods. Several plant defensive properties of **1** (insecticidal and antifungal) have been evaluated.

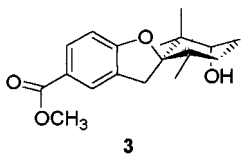
The genus *Heliotropium*, a known source of pyrrolizidine alkaloids, flavonoids, and geranyl aromatic derivatives, is comprised of about 250 species distributed throughout both hemispheres, with 24 species endemic to Chile.[1−6] Species of *Heliotropium* of the *Cochranea* section are endemic to the coastal hills of northern and central Chile and southern Peru. Like many of the plants of this geographic area, they characteristically produce a resinous exudate that covers the leaves and stem.[7] These exudates have been associated with a complex defense mechanism. There is experimental evidence indicating that the resins are part of a protective cover that prevents excessive water evaporation.[8] These resins have been proposed to play an extensive role against phytophagous organisms and UV radiation in the range 180−320 nm.[9,10] Herein we describe the isolation and structural elucidation of *rel*-(8*R*,9*R*)-carrizaloic acid (**1**) {3-[*rel*-(8*R*,9*R*-9-hydroxy-9,13,13-trimethyl-12-oxo-10-cyclohexenyl)methyl]-4-methoxybenzoic acid}, a new compound, and three known flavonoids from the resin exudates of *Heliotropium huascoense* J.M. Johnston (Boraginaceae). We have also studied the antifeedant, insecticidal, and antifungal effects of **1** and its methyl derivative **2** against several divergent phytophagous insects (the beetle *Leptinotarsa decemlineata*, the lepidopteran *Spodoptera littoralis*, and the aphid *Myzus persicae*) and plant pathogens (*Fusarium moniliforme* and *Aspergillus niger*). The related compound filifolinol (**3**), an aromatic geranyl derivative isolated from *H. filifolium*,[3] has been included in the biological tests for comparison purposes.



R

1 H

2 CH_3



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10.1021/np000608l CCC: $20.00
Published on Web 08/10/2001

Table 1. ^{1}H, ^{13}C, COSY, HMQC, and HMBC NMR Data of *rel*-(8*R*,9*R*)-Carrizaloic Acid (**1**)[a]

				correlated carbon	
proton	δ (J_{H-H} in Hz)	COSY	carbon	HMQC	HMBC
			C-1	121.7 (s)	
2	7.96 br,s		C-2	132.7 (d)	C-3, C-4, C-6, C-7, COOH
			C-3	130.5 (s)	
			C-4	161.5 (s)	
5	6.89 d (8.4)	H-6	C-5	110.3 (d)	C-1, C-6
6	7.94 d (8.8)	H-5	C-6	130.7 (d)	C-2, C-4, COOH
7a	2.94 dd (6.1, 14.5)	H-7b, H-8	C-7	28.4 (t)	C-2, C-3, C-4, C-8, C-9, C-13
7b	3.02 dd (6.5, 14.5)	H-7a, H-8			
8α	2.56 t (6.5)	H-7a, H-7b	C-8	53.8 (d)	C-3, C-7, C-9, C-16, C-13, C-14, C-15
			C-9	72.1 (s)	
10	6.67 d (10)	H-11	C-10	155.0 (d)	C-8, C-12
11	5.84 d (10)	H-10	C-11	124.9 (d)	C-9, C-13
			C-12	203.8 (s)	
			C-13	46.0 (s)	
14α	1.12 s		C-14	25.2 (q)	C-8, C-12, C-15
15*β*	1.16 s		C-15	21.1 (q)	C-8, C-12, C-13, C-14
16*β*	1.5 s		C-16	25.5 (q)	C-8, C-9, C-10
			COOH	170.2 (s)	
OMe	3.91 s			55.7 (q)	C-4

[a] Coupling constants (Hz) are shown in parentheses.

Results and Discussion

The resinous exudate obtained from the plant as described in the Experimental Section was subjected to successive column chromatography steps and preparative TLC on Si gel to give 3-methylgalangin, 3,7-dimethylgalangin, (−)-alpinone, and carrizaloic acid (**1**). The known flavonoids 5,7-dihydroxy-3-methoxyflavone (3-methylgalangin)[11,12] and 5-hydroxy-3,7-dimethoxyflavone (3,7-dimethylgalangin)[13] were identified by spectral data comparison with authentic samples. 3-Methylgalangin has been previously isolated from the resinous exudate of *Heliotropium sinuatum*.[14] (2*S*,3*S*)-3,5-Dihydroxy-7-methoxyflavanone [(−)-alpinone] showed melting point and spectral data identical to those reported for (2*R*,3*R*)-3,5-dihydroxy-7-methoxyflavanone [(+)-alpinone].[15] The only difference was the negative specific rotation, which indicated that this compound has the opposite configuration and therefore was characterized as the (2*S*,3*S*)-isomer.[16]

rel-(8*R*,9*R*)-Carrizaloic acid (**1**) was isolated as an amorphous solid. Its IR spectrum showed bands at 3435 (OH, br), 2975, 1679 (α,*β*-unsaturated ketone), and 1651 (sh) cm^{-1}. In the UV spectrum an absorption maximum was observed at 288 nm. The HREIMS gave a molecular ion peak at *m*/*z* 318.1443 corresponding to the molecular ion $C_{18}H_{22}O_5$ (calcd 318.1467). The ^{1}H NMR spectrum of **1** showed signals at δ_H 1.12 (s, H-14), 1.16 (s, H-15), 1.50 (s, H-16), 2.56 (t, J = 6.5 Hz, H-8), 2.94 (dd, J = 6.1, 14.5 Hz, H-7a), and 3.02 (dd, J = 6.5, 14.5 Hz, H-7b), which could be attributed to three methyl groups, one methine signal, and one methylene group, respectively. These protons were correlated with the ^{13}C NMR signals at δ_C 25.2 (q, C-14), 21.1 (q, C-15), 25.5 (q, C-16), 53.8 (d, C-8), and 28.4 (t, C-7) in the HMQC experiment (Table 1). Additionally, two vinylic protons were observed at δ_H 5.84 (d, J = 10 Hz, H-11) and 6.67 (d, J = 10 Hz, H-10), in agreement with the expected absorption of these protons in an α,*β*-unsaturated ketone. Furthermore, the ^{1}H NMR spectrum showed aromatic protons at δ_H 6.89 (d, J = 8.4 Hz, H-5), 7.94 (d, J = 8.8 Hz, H-6), and 7.96 (br s, H-2). These signals were shown to be coupled to each other by a COSY experiment and were correlated with the carbon signals at δ_C 110.3 (d, C-5), 130.7 (d, C-6), and 132.7 (d, C-2) in the HMQC spectrum.

The relative stereochemistry of **1** was confirmed by a ROESY experiment (Figure 1). The methyl group at C-9 gave a positive NOE with signals corresponding to H-7b and H-15. Similarly, the signal at δ_H 2.56 (1 H, t, H-8α) corresponded to the Me-14α protons and the signal at δ_H 6.67 (1H, d, H-10) with the H-11 proton. This experiment also showed NOEs between the proton methyl groups H-14α and H-15*β* and the δ_H 2.94 (dd, H-7a) and 3.02 (dd, H-7b) signals. One additional interaction and conclusive evidence of the stereochemical conformation was provided by an alternative of the 1D version of the NOESY experiment, the so-called GOESY 1D experiment, with pulsed gradients (PFG).[17] A selective excitation at δ_H 2.56 (t, H-8) gave a clean 1D spectrum, with only one of the methyl protons at δ 1.12 (s, H-14α). An HMBC experiment also confirmed the chemical shifts of the remaining protons (Table 1). Treatment of **1** with diazomethane furnished a methyl ester **2**, for which the ^{1}H NMR spectrum exhibited a carbomethoxy singlet at δ_H 3.88. Accordingly the structure of **1** was assigned as {3-[*rel*-(8*R*,9*R*-9-hydroxy-9,13,-13-trimethyl-12-oxo-10-cyclohexenyl)methyl]-4-methoxybenzoic acid}, to which the trivial name *rel*-(8*R*,9*R*)-carrizaloic acid has been accorded.

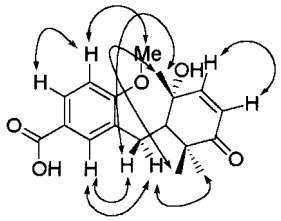

Figure 1. NOE interactions observed for compound **1**.

rel-(8*R*,9*R*)-Carrizaloic acid (**1**) can be considered to be biogenetically derived from the cyclization of the side chain of an aromatic geranyl compound.[3] The chemical composition of *H. huascoense* resin exudate falls within the chemical pattern found for other Chilean *Heliotropium* species. Several flavonoids have been isolated from the resinous exudates of *H. stenophyllum*, *H. chenopodiaceum*, and *H. filifolium*, and aromatic geranyl derivatives were obtained from the exudates of *H. stenophyllum* and *H. filifolium*.[3,16,18,19]

The major resin component **1**, its methyl derivative **2**, and the biogenetically related filifolinol (**3**) did not exhibit antifeedant activity against the polyphagous aphid *Myzus persicae*. Table 2 shows the antifeedant and toxic effects

Table 2. Antifeedant (%FI = [1-(T/C)] × 100, Where T = Consumption of Treated Disks and C = Consumption of Control Disks at 50 μg/cm^2) and Toxic (% mortality at 72 h) Effects of the Test Compounds on *L. decemlineata* Adults. Represented Are Mean Values ± Standard Error

compound	%FI choice	EC_{50}[a]	%FI no-choice	EC_{50}[a] (CL)[b]	% mortality[c]
1	37.0 ± 16.8	>50	56.1 ± 9.5	~50	0
2	55.4 ± 16.5	~50	81.82 ± 10.4	24.19 (16.9, 34.6)	0
3	16.82 ± 13.0	>50	42.4 ± 9.5	>50	20
silphinene[d]		0.27		2.7	24

[a] EC_{50} = concentration needed to produce 50% feeding inhibition (μg/cm^2). [b]95% confidence limits of estimation (lower, upper). [c]Corrected according to Abbott.[29] [d]From González-Coloma et al.[30]

Table 3. Antifeedant (%FI and EC_{50}) and Nutritional Effects (Oral Administration of 20 μg/Insect) of the Test Compounds on *S. littoralis* L6 Larvae Performance

compound	%FI[a] (50 μg/cm^2)	EC_{50}[a] (μg/cm^2)	weight gain (% control)	consumption (% control)
1	0.0 ± 0.0	>100	126.2 ± 3.8	113.1 ± 17.1
2	71.8 ± 7.6	17.4 (10.3, 29.8)	66.1 ± 4.5[c]	75.9 ± 19.8[c]
3[b]	34.6 ± 16.5	>100	120.9 ± 4.0	120.2 ± 17.6

[a] As in Table 2. [b]Tested at 100 μg/cm^2. [c]Significant difference from the control, $p < 0.05$, LSD test.

Table 4. Analysis of Variance and Covariance (Consumption as the Covariate) Summarizing the Effects of Compound **2** on Consumption (I) and Biomass Gain (B-gain) of *S. littoralis* Larvae (Df, degrees of freedom; p, Probability Level)

	compound	source	Df	p
2	I (mg) (ANOVA)	Trtm	1	0.003
	B-gain (ANOVA)	Trtm	1	0.01
	B-gain (ANCOVA)	covariate	1	<0.001
		Trtm	1	0.89

of compounds **1**−**3** on *L. decemlineata* adults. Only the derivative **2** had significant antifeedant effects on this insect in no-choice tests, with a potency (EC_{50} values) 10 times lower than the positive control silphinene, a model CPB antifeedant.[20] None of these compounds significantly increased beetle mortality at the dose tested. Mendel et al.[21] found that citrus limonoids were more potent CPB deterrents in no-choice tests, suggesting that such compounds act primarily as post-ingestive toxins acting on the centers that control feeding. Therefore, this could be the case for compound **2**.

Table 3 shows the antifeedant and nutritional effects of the test compounds on *S. littoralis* larvae. Among them, **2** had significant antifeedant effects on this insect in choice tests. An ANOVA analysis of food consumption and biomass gains of orally injected larvae showed that both parameters significantly decreased with **2** ($p < 0.003$ for consumption and $p < 0.001$ for biomass gains, Table 4). An ANCOVA analysis performed on larval biomass gains with food consumption as covariate showed that the treatment effect disappeared following covariance adjustment (ANCOVA $p > 0.05$, Table 4), indicating that much of the original variation in biomass gains was due to differences in consumption rates.[22,23] Similar post-ingestive antifeedant effects have been observed for azadirachtin and neoclerodane diterpenes on *S. littoralis* and have been attributed to a direct action on the centers that control feeding and metabolism,[24,25] in agreement with the no-choice effects of **2** on CPB.

Among the compounds tested, filifolinol (**3**) had a significant inhibitory effect when tested against *Fusarium moniliforme* and moderate activity against *Aspergillus niger* (61% and 36% mycelial growth inhibition at 0.5 mg/mL, respectively), while **1** gave negative results against these fungal species.

Prenylated benzoic acid-type derivatives have a wide range of activities including antibacterial, antifungal, antiinflammatory, molluscicidal, and plant-growth inhibitory effects,[26−31] as well as toxic effects on mosquito larvae. However, this is the first report on antifeedant and post-ingestive effects for this class of compounds.

As a general trend, the methylated derivative **2** was more active than the parent acid **1**. A senecionate derivative of filifolinol (**3**) had increased antimicrobial activity,[32] and the antifungal activity of several prenylated benzoic acid-type compounds was higher for the methylated derivatives,[27] suggesting that esterification of the carboxylic acid moiety in the benzene acid plays a key role in the antifeedant and antimicrobial/antifungal activity of these compounds.

Resin exudates are proposed to play a defensive role in *Heliotropium* species. However, carrizaloic acid (**1**), the major component of *H. huascoense* resin, did not have significant biological effects. Further research is needed in order to understand the possible defensive role that stress-related plant esterases may play in the biotransformation of **1**.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a Perkin-Elmer 137 polarimeter. IR spectra were obtained on KBr disks on a Bruker IFS66V, and UV spectra were recorded in CH_2Cl_2 using a Varian Carey 1E spectrometer. NMR spectra were obtained in $CDCl_3$ on a Bruker AMX2 500 MHz spectrometer with a pulsed field gradient. The programs used for DEPT, ^{1}H COSY, HMQC, HMBC (J = 7 Hz), and GOESY (mixing time, ι_m = 600 ms) experiments were those furnished with the manufacturer's software. Exact mass measurements and EIMS were recorded on an Autospect instrument at 70 eV. Separations were performed by flash column chromatography Si gel (63−200 mesh, Merck). Fractions obtained from column chromatography were monitored by TLC (Si gel 60 HF_{254}). Preparative TLC was carried out on Merck Si gel GF_{254} plates (20 × 20 mm, 0.5 mm thickness).

Plant Material. *Heliotropium huascoense* J.M. Johnston was collected during the flowering season in October 1995, in Carrizal Bajo, Chile (III Region 29° 57′ S, 71° W) and identified by Dr. Sebastian Teiller from the Museo de Historia Natural de Santiago de Chile. A voucher specimen is deposited in the Herbarium of this Museum (number ST 2570).

Chemicals. Filifolinol (**3**) has been previously isolated from *Heliotropium filifolium*[3] and silphinene from *Senecio palmensis*.[34]

Insect Bioassays. *Leptinotarsa decemlineata*, *Spodoptera littoralis*, and *Myzus persicae* colonies were reared on potato foliage, artificial diet,[34] 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 Feeding Assays. These experiments were conducted with adult *L. decemlineata*, newly emerged fifth-instar *S. littoralis* larvae, and *M. persicae* apterous adults. Percent feeding inhibition (%FI) and percent settling inhibition (%SI) were calculated as described in ref 35. Compounds with an FR/SI > 50% were tested in a dose−response experiment to calculate their relative potency (EC_{50} values, the effective dose for 50% feeding reduction), which was determined from linear regression analysis (%FR or %SI on log dose).

Oral Cannulation. This experiment was performed with preweighed newly molted *S. littoralis* L6-larvae as previously described.[35] 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. A second ANCOVA analysis was performed on biomass gains with food consumption as covariate to test for post-ingestive effects.[22,23]

Hemolymph Injection. DMSO solutions of the test compounds (10 μg/insect) were injected into 20 adult *L. decemlineata* beetles as described in ref 35. Beetle mortality was recorded up to 3 days after injection. Percent mortality was analyzed with contingency tables and corrected according to Abbott.[33]

Antifungal Activity Assays. The antifungal activity of the substances was tested at a single dose (0.5 mg/mL) against the plant pathogens *Fusarium moniliforme* and *Aspergillus niger* and estimated as mycelial growth inhibition.[4]

Extraction and Isolation. The resinous exudates of *H. huascoense* were obtained by dipping 200 g of each fresh plant in cold CH_2Cl_2 for 15 to 20 s. The extracts were concentrated to afford 18.2 g of a residue.

A portion of the resinous exudate of *H. huascoense* (9.7 g) was fractioned by flash column chromatography on Si gel, using CH_2Cl_2 with increasing amounts of MeOH to obtain 133 fractions, each of 100 mL. The fractions were monitored by TLC on Si gel, using the systems *n*-hexanes−EtOAc (3:1), CH_2Cl_2−MeOH (9:1), and CH_2Cl_2−MeOH (8:2). Visualization was effected with H_2SO_4 (25% v/v) and heat. The eluate obtained with CH_2Cl_2−MeOH (3:1) gave a mixture of flavonoids. Further purification using preparative TLC over Si gel eluted with $CHCl_3$−MeOH (8:2) gave 3-*O*-methylgalangin (12 mg), 3,7-*O*-dimethylgalangin (15 mg), and (−)-alpinone (14 mg). The eluate obtained with CH_2Cl_2−MeOH (3:2) gave carrizaloic acid **1** (200 mg).

***rel*-(8*R*,9*R*)-Carrizaloic acid (1)**: amorphous solid; $[\alpha]_D$ +19.4° (*c* 0.072, CH_2Cl_2); UV (CH_2Cl_2) 288 λ_{max} (log ϵ) 288 (3.6) nm; IR (KBr) ν_{max} 3435, 2975, 1679, 1651 (sh) cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; EIMS *m/z* $[M]^+$ 318 (19), 300 (8), 285 (15), 257 (18), 221 (85), 203 (32), 177 (21), 165 (64), 135 (11), 98 (100), 57 (27), 55 (26); HREIMS *m/z* $[M]^+$ 318.1443, calcd for $C_{18}H_{22}O_5$ 318.1467, $[M - H_2O]^+$ 300.1353, calcd for $C_{18}H_{20}O_4$ 300.1361, $[M - CH_5O]^+$ 285.1114, calcd for $C_{17}H_{17}O_4$ 285.1126, $[M-C_2H_5O_2]^+$ 257.1252, calcd for $C_{16}H_{17}O_3$ 257.1177, $[M - C_5H_5O_2]^+$ 221.1218, calcd for $C_{13}H_{17}O_3$ 221.1177, $[M - C_7H_9O_3]^+$ 177.0977, calcd for $C_{11}H_{13}O_2$ 177.0915, $[M - C_9H_{13}O_2]^+$ 165.0578, calcd for $C_9H_9O_3$ 165.0551.

Methylation of Compound 1. To a solution of **1** (5 mg) in diethyl ether (2 mL), was added ethereal CH_2N_2 at 0 °C. After 2 days the solvent was removed to afford a crude residue that was purified on a Si gel column. Similar fractions were combined to give 3.8 mg of pure methylated compound (73%): ^1H NMR ($CDCl_3$, 500 MHz) δ 7.93 (H, s, H-2), 7.91 (H, d, J = 8.9 Hz, H-6), 6.88 (H, d, J = 8.8 Hz, H-5), 6.77 (H, d, J = 10.0 Hz, H-10), 5.84 (H, d, J = 10.1 Hz, H-11), 3.91 (3H, s, OMe), 3.88 (3H, s, COOMe), 3.05 (H, dd, J = 6.5, 14.5 Hz, H-7b) 2.92 (H, dd, J = 5.6, 14.0 Hz, H-7a), 2.57 (H, t, J = 6.0 Hz, H-8, 1.50 (3H, s, H-16β), 1.17 (3H, s, H-15β), 1.13 (3H, s, H-14α); EIMS *m/z* 332 (9), 304 (7), 301 (7), 257 (7), 235 (60), 105 (100), 98 (53), 55 (45); HREIMS *m/z* $[M]^+$ 332.1573, calcd for $C_{19}H_{24}O_6$ 332.1622; $[M - C_3H_7O_3]^+$ 257.1119, calcd for $C_{16}H_{17}O_3$ 257.1177; $[M - C_5H_5O_3]^+$ 235.1318, calcd for $C_{14}H_{19}O_3$ 235.1334.

Acknowledgment. This work has been partially supported by FONDECYT-Chile (1961241), DICYT (Universidad de Santiago de Chile, 49541 VV), DGICYT-Spain (PB97-1226), and a Collaborative Research Grant (CSIC, Spain-USACH, Chile).

References and Notes

(1) Villarroel, L.; Torres, R.; Urzúa, A.; Modak, B.; Henriquez, J.; Salgado, I. *Rev. Latinoamer. Quím.* **1997**, *25*, 109−116.
(2) Urzúa, A.; Modak, B.; Villarroel, L.; Torres, R.; Andrade, L. *Biochem. Syst. Ecol.* **1998**, *26*, 127−130.
(3) Torres, R.; Villarroel, L.; Urzúa, A.; Delle Monache, F.; Delle Monache, G.; Gacs-Baitz, E. *Phytochemistry* **1994**, *36*, 249−250.
(4) Reina, M.; González-Coloma, A.; Gutiérrez, C.; Cabrera, R.; Henríquez, J.; Villarroel, L. *Phytochemistry* **1997**, *46*, 845−853.
(5) Reina, M.; González-Coloma, A.; Gutiérrez, C.; Cabrera, R.; Henríquez, J.; Villarroel, L. *J. Nat. Prod.* **1998**, *61*, 1418−1420.
(6) Navas Bustamante, L. E. *Flora de la Cuenca de Santiago de Chile, Tomo III*; Ediciones de la Universidad de Chile: Santiago, 1974; p 46.
(7) Johnston, J. M. *Contrib. Gray Herbarium, Harvard Univ.* **1928**, *81*, 34.
(8) Dell, B.; McComb. *Adv. Bot. Res.* **1978**, *6*, 277−316.
(9) Kelsey, R. G.; Reynolds, G. W.; Rodriguez, E. In *Biology and Chemistry of Plant Trichomes*; Rodriguez, E., Healy, P., Metha, I., Eds.; Plenum Press: New York, 1984; Chapter 8, pp 187−241.
(10) Johnson, N. D. *Biochem. Syst. Ecol.* **1983**, *11*, 211−215.
(11) Bleir, W.; Chirikdjian, J. J. *Planta Med.* **1972**, *22*, 145−151.
(12) Wollenweber, E.; Yatskievych, G. *J. Nat. Prod.* **1982**, *45*, 216−219.
(13) Nshimo, C. M.; Pezzutto, J. M.; Kinghorn, A. D.; Farnsworth, N. R. *Int. J. Pharmacogn.* **1993**, *31*, 77−81.
(14) Urzúa, A.; Villarroel, L.; Torres, R.; Teiller, S. *Biochem. Syst. Ecol.* **1993**, *21*, 744−749.
(15) Shawl, A.; Kumar, T. *Phytochemistry* **1992**, *31*, 1399−1401.
(16) Urzúa, A.; Modak, B.; Villarroel, L.; Torres, R.; Andrade, L.; Mendoza, L.; Wilkens, M. *Bol. Soc. Chil. Quím.* **2000**, *45*, 23−29.
(17) Stonehouse, J.; Adell, P.; Keeler, J.; Shaka, A. J. *J. Am. Chem. Soc.* **1994**, *116*, 6037−6038.
(18) Villarroel, L.; Urzúa, A. *Bol. Soc. Chil. Quím.* **1990**, *35*, 309−311.
(19) Villarroel, L.; Urzúa, A.; Torres, R. *Bol. Soc. Chil. Quím.* **1991**, *36*, 169−174.
(20) Mullin, C. A.; González-Coloma, A.; Gutiérrez, C.; Reina, M.; Eichenseer, H.; Hollister, B.; Chyb, S. *J. Chem. Ecol.* **1997**, *23*, 1851−1866.
(21) Mendel, M. J.; Alford, A. R.; Rajab, M. S.; Benthey, M. D. *J. Econ. Entomol.* **1991**, *84*, 1158−1162.
(22) Horton, R. D.; Redak, R. A. *Entomol. Exp. Appl.* **1993**, *69*, 263−275.
(23) Raubenheimer, D.; Simpson, S. J. *Entomol. Exp. Appl.* **1992**, *62*, 221−231.
(24) Martinez, S. S.; van Emdem, H. F. *Bull. Entomol. Res.* **1999**, *89*, 65−71.
(25) González-Coloma,A.; Gutiérrez, C.; del Corral, J. M.; Gordaliza, M.; de la Puente, M. L.; San Feliciano, A. *J. Agric. Food Chem.* **2000**, *48*, 3677−3681.
(26) Orjala, J.; Erdelmeier, C. A. J.; Wright, A. D.; Rali, T.; Sticher, O. *Phytochemistry* **1993**, *34*, 813−818.
(27) Pereda-Miranda, R.; Bernard, C.; Durst, T.; Arnason, J. T. *J. Nat. Prod.* **1997**, *60*, 282−284.
(28) Terreaux, C.; Gupta, M. P.; Hostettmann, K. *Phytochemistry* **1998**, *49*, 461−464.
(29) Baldoqui, D. C.; Kato, M. J.; Cavalheiro, A. J.; Bolzani, V.; Young, M. C. M.; Furlan, M. *Phytochemistry* **1999**, *51*, 899−902.
(30) Dong, M.; Nagaoka, M.; Miyazaki, S.; Iriye, R.; Hirota, M. *Biosci., Biotechnol., Biochem.* **1999**, *63*, 1650−1653.
(31) Mizushina, Y.; Miyazaki, S.; Ohta, K.; Hirota, M.; Sakaguchi, K. *Biochim. Biophys. Acta* **2000**, *1475*, 1−4.
(32) Modak, B.; Mendoza, L.; Torres, R.; Arrieta, A.; Urzua, A. *XII Annual Meeting ISCE*; Los Andes, Chile, October 2−6, 1995, Abstract 127.
(33) Abbott, W. S. *J. Econ. Entomol.* **1925**, *18*, 265−267.
(34) González-Coloma, A.; Reina, M.; Cabrera, R.; Castañera, P.; Gutiérrez, C. *J. Chem. Ecol.* **1995**, *21*, 1255−1270.
(35) Reina, M.; González-Coloma, A.; Gutiérrez, C.; Cabrera, R.; Rodriguez, M. L.; Fajardo, V.; Villarroel, L. *J. Nat. Prod.* **2001**, *64*, 6−11.

NP000608L