Nematicidal Alkylene Resorcinols from *Lithraea molleoides*

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Four new alkylene resorcinols, (*Z,Z*)-5-(trideca-4,7-dienyl)resorcinol (**1**), (*Z,Z,Z*)-5-(trideca-4,7,10-trienyl)-resorcinol (**2**), (*Z,Z,E*)-5-(trideca-4,7,10-trienyl)resorcinol (**3**), and (*Z*)-5-(trideca-4-enyl)resorcinol (**4**), were isolated from the MeOH−CH$_2$Cl$_2$ extract of *Lithraea molleoides*. The structures of these compounds were determined by one- and two-dimensional NMR including selective decoupling experiments. In vitro all four compounds showed strong paralyzing effects on the nematode *Caenorhabditis elegans* at concentrations between 6 and 50 µg/mL, whereas the activity of compounds **1** and **2** against the nematode *Trichostrongylus colubriformis* was less pronounced and no activity against this nematode was observed for compounds **1**−**4** in a rodent model.

Members of the family Anacardiaceae are known to contain alkyl or alkylene derivatives of catechol, resorcinol, and phenol.[1−4] These groups of compounds are known for their vesicant properties and are responsible for the induction of contact dermatitis by poison ivy (*Rhus radicans*) or cashew nut shell liquid (*Anacardium occidentale*).[3,4] The small genus *Lithraea* consists of only four species that are distributed in the Australian and American continents.[5] The Chilean *L. caustica* (Molina) Hook. & Arn. is known for the severe dermatitis it causes, the painful swellings lasting many days.[5,6] Its active principle was identified as 3-(pentadec-10-enyl)catechol.[7] The species *L. molleoides* (Vell.) Engl. is distributed in Argentina, Uruguay, Paraguay, Bolivia, and Southern Brazil.[8] This species, with its common name "molle", is also known under several other synonyms, including *L. molleoides* var. *lorentziana* Lillo, *L. ternifolia* (Gill. Ex Hook) F.A. Barkley, *L. gilliesii* Griseb., *Schinus molleoides* Vell., and *S. ternifolia* Hook.[8,9] The available chemical information on this species includes GC studies of its essential oils and allergenic catechols.[10,11] We report here on the isolation, structural elucidation, and nematicidal activities of four new alkylene resorcinols from *L. molleoides*. This study was prompted by in vitro nematicidal activity observed for an extract of *L. molleoides* in both the model, nonpathogenic, nematode *Caenorhabditis elegans* and the infective larvae of *Trichostrongylus colubriformis*, a commercially important pathogen in sheep. To our knowledge this is the first report of nematicidal activity in the genus *Lithraea*.

Results and Discussion

The spectroscopic data of compounds **1**−**4** indicated four alkylene resorcinols with various degrees of unsaturations. In the ¹H and ¹³C NMR spectra of compound **1**, the resorcinol subunit was represented by two proton signals at δ_H 6.15 (d, *J* = 2 Hz, H-4/6) and at δ_H 6.07 (t, *J* = 2 Hz, H-2) and four carbon signals at δ_C 100.7 (C-2), 107.9 (C-4/6), 146.2 (C-5), and 158.8 (C-1/3) (Table 1). Positive HRFABMS showed the protonated molecular ion at *m/z* 289. The corresponding molecular formula C$_{19}$H$_{28}$O$_2$ and the overlapping signals of four olefinic protons δ_H 5.40−
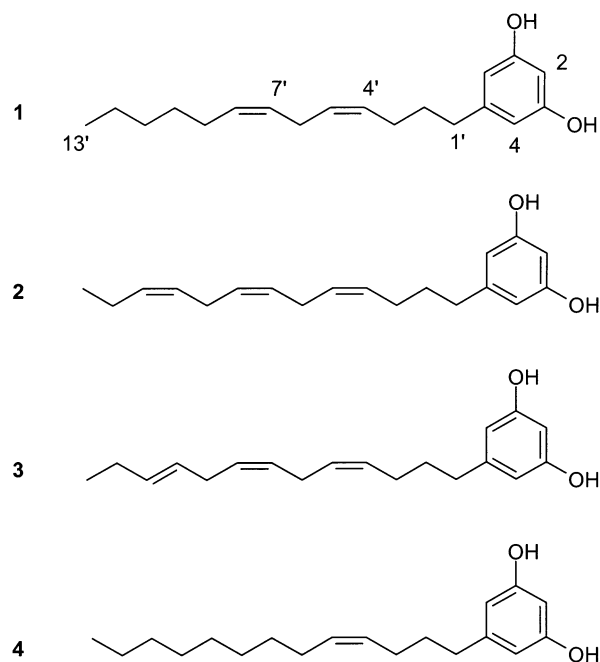
5.32 suggested a 13-carbon side chain with two double bonds. Edited HSQC spectra allowed the assignment of the multiplicity of all protonated carbons, which confirmed that the C$_{13}$ side chain was linear. HMBC data established the position of the double bonds at Δ⁴′ and Δ⁷′ with a bisallylic methylene group (δ_H 2.75, δ_C 26.2) located at position 6′ (Figure 1). Because the proton signals of the 1,2-disubstituted Δ⁴′ and Δ⁷′ double bonds were too complex and in part overlapping, coupling constants that would allow the assignment of the double bond configuration could not be observed directly. ¹H NMR selective decoupling experiments of the two allylic methylene groups in positions C-3′ and C-9′ and the bisallylic methylene group at C-6′ were carried out to simplify the multiplicity of the olefinic protons. These experiments allowed the observation of $J_{4',5'}$ and $J_{7',8'}$ coupling constants of 11 Hz each, which are in agreement with *Z* configurations of both double bonds. The ¹³C NMR resonances δ_C 27.4 and 27.7 for the two allylic methylenes at position 3′ and 9′, respectively, and δ_C 26.2 for the bisallylic methylene C-6′ are also in agreement with literature data reported for other alkylene resorcinols with

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Table 1. ^{13}C, ^{1}H NMR, HSQC, and HMBC Data of Compounds **1**, **2**, **3**, and **4** in CD_3CN^a

		1			2			3			4	
position	δ_C	δ_H mult. J	HMBC	δ_C	δ_H mult. J	HMBC	δ_C	δ_H mult. J	HMBC	δ_C	δ_H mult. J	HMBC
1	158.8			158.8			158.9			158.8		
2	100.7	6.07 t 2	1,3,4,6	100.8	6.07 t 2	1,3,4,6	100.8	6.07 t 2	1,3,4,6	100.7	6.07 t 2	1,3
3	158.8			158.8			158.9			158.8		
4	107.9	6.15 d 2	2,3,6,1′	108.0	6.15 d 2	2,3,6,1′	108.0	6.14 d2	2,3,6,1′	107.9	6.14 d 2	2,3,6,1′
5	146.2			146.2			146.2			146.2		
6	107.9	6.15 d 2	1,2,4,1′	108.0	6.15 d 2	1,2,4,1′	108.0	6.14 d 2	1,2,4,1′	107.9	6.14 d 2	1,2,4,1′
1′	35.9	2.45 t 8	4,5,6,2′,3′	35.9	2.45 t 8	4,5,6,2′,3′	35.9	2.45 t 8	4,5,6,2′,3′	36.0	2.44 t 8	4,5,6,2′,3′
2′	31.8	1.60 quint 8	5,1′,3′,4′	31.8	1.60 quint 8	5,1′,3′,4′	31.8	1.60 quint 8	5,1′,3′,4′	32.0	1.58 quint 8	5,1′,3′,4′
3′	27.4	2.06 q 7	1′,2′,4′,5′	27.4	2.08 m	1′,2′,4′,5′	27.4	2.06 q 7	1′,2′,4′,5′	27.5	2.03 q 7	1′,2′,4′,5′
4′	130.4	5.40 m	3′,6′ ^{e}	130.7	5.41 m	3′,^{e}	130.6	5.40 m	e	130.2	5.37 m	2′,3′,6′
5′	129.2	5.36 m	3′,6′ ^{e}	129.0^b	5.37−5.34 m	e	129.0	5.41−5.35 m	e	131.2	5.37 m	3′,6′,7′
6′	26.2	2.75 t 7	e	26.2^c	2.79 t 6	4′,5′/7′/8′	26.2	2.76 t 6	4′,5′	27.8	1.99 q 7	4′,5′,7′
7′	128.7	5.32 m	6′ ^{e}	129.0^b	5.37−5.34 m	e	129.3	5.41−5.35 m	e	30.4	1.32 m	5′,6′,8′/9′
8′	131.0	5.37 m	6′,9′ ^{e}	129.1^b	5.37−5.34 m	e	128.8	5.41−5.35 m	e	29.9^d	1.29−1.27 m	e
9′	27.7	2.03 q 7	7′,8′,10′,11′	26.1^c	2.79 t 6	7′/8′	31.0	2.73 t 6	8′,10′,11′	30.0^d	1.29−1.27 m	e
10′	30.0	1.33 m	8′,9′,11′,12′	128.0	5.30 m	e	128.1	5.38 m	e	31.0^d	1.29−1.27 m	e
11′	32.1	1.27 m		132.7	5.38 m	e	133.2	5.48 m	e	32.7	1.26 m	e
12′	23.2	1.29 m	11′	21.1	2.06 m	10′,11′	26.2	1.98 quint 7	10′,11′	23.3	1.28 m	e
13′	14.3	0.88 t 7	11′,12′	14.5	0.94 dd 7,8	11′,12′	14.1	0.93 dd 7,8	11′,12′	14.3	0.87 t 7	11′,12′

^{a} Correlations between ^{1}H and ^{13}C based on HSQC spectra. ^{b,c,d} Assignments interchangeable. ^{e} Additional HMBC correlations that could not be interpreted were present for this δ_H.
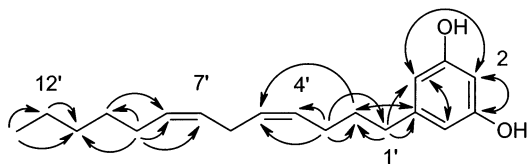
Figure 1. HMBC correlations of compound **1** (H → C).

Z geometry.[12] On the basis of the spectroscopic data compound **1** was elucidated as (*Z,Z*)-5-(trideca-4,7-dienyl)-resorcinol. Positive HRFABMS of compounds **2** and **3** showed protonated molecular ions at *m*/*z* 287 and established the molecular formula to be $C_{19}H_{26}O_2$ for both molecules. Their ^{1}H and ^{13}C NMR spectra were similar to those of **1** and indicated two additional linear C_{13} alkylene resorcinols. In contrast to the ^{1}H NMR spectrum of **1**, in the spectra of **2** and **3** the olefinic signals between δ_H 5.43−5.29 and 5.51−5.33, respectively, were integrated to 6 protons each, representing three double bonds in the alkylene chains in **2** and **3**. The ^{1}H NMR spectra of both compounds also revealed resonances of two bisallylic methylene groups, suggesting that in both molecules the three double bonds were separated by methylene groups. Edited HSQC and HMBC experiments allowed the assignment of all protonated carbons and showed that in both molecules the double bonds are placed at $\Delta^{4'}$, $\Delta^{7'}$, and $\Delta^{10'}$. ^{1}H NMR selective decoupling experiments of allylic methylene H-3′ at δ_H 2.08 and bisallylic methylenes H-6′/9′ at δ_H 2.79 allowed the assignment of *Z* configurations for $\Delta^{4'}$ and $\Delta^{10'}$ ($J_{4',5'} = J_{10',11'} = 11$ Hz) in **2**. For compound **3**, selective decoupling of allylic methylene H-12′ at δ_H 1.98 and bisallylic methylene H-6′ at δ_H 2.76 led to the assignment of a *Z* configuration for $\Delta^{4'}$ ($J_{4',5'} = 11$ Hz) and an *E* configuration for $\Delta^{10'}$ ($J_{10',11'} = 16$ Hz). Due to overlapping ^{1}H NMR signals, selective decoupling experiments did not allow unambiguous assignment of the configuration of $\Delta^{7'}$ in compounds **2** and **3**. However, in the ^{1}H NMR spectrum of **2**, the signals of the two bisallylic methylene groups in positions 6′ and 9′ were completely overlapping at δ_H 2.79 (t, *J* = 6 Hz, 4H). In the case of **3**, the two bisallylic methylene groups were well separated at δ_H 2.76 (t, *J* = 6 Hz, H-6′a/b) and 2.73 (t, *J* = 6 Hz, H-9′a/b). In addition, the ^{13}C NMR resonances of bisallylic methylenes C-6′ and C-9′ and of the two allylic methylenes C-3′ and C-12′ in **2** were 26.2, 26.1, 27.4, and 21.1 ppm, respectively, and those in **3** were 26.2, 31.0, 27.4, and 26.2 ppm, respectively. When compared to literature data,[12,13] these ^{1}H and ^{13}C NMR chemical shifts are in agreement with an all-*Z* geometry for compound **2** and a *Z,Z,E* configuration for compound **3**. On the basis of the spectroscopic data compound **2** was elucidated as (*Z,Z,Z*)-5-(trideca-4,7,10-trienyl)resorcinol and compound **3** was elucidated as (*Z,Z,E*)-5-(trideca-4,7,-10-trienyl)resorcinol. The ^{1}H and ^{13}C NMR spectral data of compound **4** were also very similar to those of **2** and indicated a fourth unbranched C_{13} alkylene resorcinol. Positive HRFABMS established the molecular formula to be $C_{19}H_{30}O_2$. The molecular mass of 290 Da and a multiplet in the ^{1}H NMR spectrum at δ_H 5.37 integrated to two protons pointed toward a monounsaturated alkylene side chain in **4**. HSQC and HMBC experiments unambiguously allowed the assignment of a $\Delta^{4'}$ double bond. ^{1}H NMR selective decoupling in deuterated benzene of overlapping allylic methylenes H-3′ and H-6′ at δ_H 2.05 allowed the observation of the coupling constant $J_{4',5'} = 11$ Hz, which is in agreement with a *Z* configuration of the $\Delta^{4'}$ double bond. ^{13}C NMR chemical shifts of 27.5 and 27.8 ppm for allylic C-3′ and C-6′ confirmed the *Z* geometry in compound **4**. On the basis of the spectroscopic data compound **4** was elucidated as (*Z*)-5-(trideca-4-enyl)resorcinol.

Rapid (<4 h) and complete paralysis of the model, nonpathogenic, nematode *C. elegans* was observed at a concentration of 2400 μg/mL crude MeOH−CH$_2$Cl$_2$ extract obtained from *L. molleoides*. In three separate *C. elegans* assays with the four purified compounds (assayed at 50, 25, 13, 6, and 3 μg/mL), all gave very rapid activity (the results of one representative assay are shown in Table 2). At 50 μg/mL, compounds **1**, **2**, and **4** produced 95−100% paralysis at times ranging from 10 to 25 min. Compound **3** gave a less rapid onset of activity, but by 1 h showed potency similar to those of the other three compounds. The activities of all four compounds declined after 2 h. At the lowest concentration of 3 μg/mL, none of the compounds showed activity beyond an occasional, transient (1−4 h) slowing of movement. The potencies of the compounds against *C. elegans* were rather similar, with differences of 2−3-fold at most and some variability between assays. The initial paralyzing activities of the compounds against *C. elegans* were partly transient. This is best illustrated by analysis of a representative experiment (Table 2; note that Table 2 shows "number of worms moving" in order to highlight subtle differences between the compounds and

Table 2. Effect of Compounds **1**–**4** on *C. elegans*[a]

	number of worms moving															
	1 h				2 h				4 h				24 h			
	concentration (μg/mL)				concentration (μg/mL)				concentration (μg/mL)				concentration (μg/mL)			
compound	50	25	13	6	50	25	13	6	50	25	13	6	50	25	13	6
1	0/0	4/0	3/3	30/25	0/1	3/1	13/3	28/27	3/0	2/1	30/18	>30/>30	3/1	16/8	>30/>30	NE[b]
2	0/0	1/5	1/5	25/13	0/0	0/0	0/0	20/15	1/0	2/3	8/14	18/>30	0/0	3/2	18/>30	NE[b]
3	0/0	1/1	11/7	>30/>30	0/0	4/7	10/10	NE[b]	2/1	7/6	11/21	>30/>30	0/0	12/>30	>30/>30	NE[b]
4	0/0	0/2	25/12	NE[b]	0/0	5/2	17/9	NE[b]	2/1	15/7	>30/28	NE[b]	17/13	>30/>30	>30/>30	NE[b]

[a] Details of the bioassay are described in the Experimental Section. The number of worms per well in this experiment was 243 ± 29 (SD). The numbers of nonparalyzed (motile) worms in each of two duplicate wells are shown for each compound, concentration, and time point. Thus "0" indicates 100% paralysis. The term ">30" indicates that the number of motile worms was beyond the limit for accurate counting. At 3 μg/mL only occasional and transient (1–4 h) slowing of movement was observed. [b] No discernible effect on mortality.

also because % paralysis could not be calculated when the number of active worms exceeded the limit for accurate counting). Compound **2** produced 100% paralysis by 2 h at all concentrations down to 13 μg/mL and also gave 90% paralysis at 6 μg/mL. By 4 h, there was noticeable recovery, especially at 13 and 6 μg/mL. By 24 h, significant effects were no longer seen at 6 μg/mL, and the effect at 13 μg/mL was greatly diminished from the 100% paralysis seen at 2 h. However the 50 and 25 μg/mL doses produced sustained effects, at or near 100% paralysis/death for 24 h (sustained paralysis produced a darkened, refractile appearance characteristic of dead worms). The other three compounds also showed this general trend. It is interesting to note that only the smallest sized larvae (L1/L2; ∼0.1–0.2 mm length = 10–20% of adult length) showed recovery from the initial paralyzing effects of the compounds. It is unclear whether recovery from paralysis is due to instability of the test compounds, to physiological features of the smaller larvae, or to the mode of action. Compounds **1** and **2** were also tested in vitro against infective larvae of *Trichostrongylus colubriformis*, an economically important, pathogenic parasite in sheep. Compound **1** gave nearly 100% paralysis at 100 μg/mL: the normally rapidly moving larvae were completely immobile or showed barely discernible movement. At 50 μg/mL, the larvae were about 50% immobile, with the rest showing very slow movement. At 25 μg/mL, the larvae were moving but at obviously slower rates than the untreated controls. There was no effect at 13 μg/mL. Compound **2** was somewhat less potent. Its effects at 100 and 50 μg/mL were equivalent to compound **1** at 50 and 25 μg/mL. For both compounds, the onset of activity occurred later in *T. colubriformis* than in *C. elegans*. With compounds **1** and **2**, the maximum effects were apparent by about 2 h, in contrast to the time frame of 10–25 min required for *C. elegans*. Also in contrast to *C. elegans*, *T. colubriformis* showed no evidence of recovery from paralysis at later time points (up to 24 h after treatment). All four *Lithraea* compounds **1**–**4** were inactive in vivo against *T. colubriformis* in a rodent test model at a dose of 50 μg/g body weight. Several factors including enzymatic degradation, biodistribution, and life stage of the parasite differentiate the in vivo from the in vitro assay. Therefore, it is impossible to account for this lack of in vivo activity without further investigation. There were no signs of toxicity to the host animals. To the limited extent that we have tested, these compounds show some selectivity for nematodes and, therefore, are not general biocides. In addition to the lack of host toxicity in the in vivo nematicide assay, larvae of *Lucilia serricata* (blowfly) were unaffected by concentrations up to 200 μg/mL in a feeding assay. In assays of this type, *L. serricata* larvae are sensitive to commercial insecticides at concentrations as low as 0.1 μg/mL.[14]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker DRX 600 spectrometer in deuterated acetonitrile. Solvent signals at δ_H 7.19 and δ_C 123.5 were used to reference the spectra. Carbon multiplicities were established by edited HSQC experiments. Gradient selected HSQC and HMBC experiments were used. HRFABMS were recorded on a JEOL HX 110 spectrometer with a resolution of 10 000 using a mixed matrix consisting of glycerol, thioglycerol, and m-NBA. Preparative reversed-phase HPLC was carried out on a Varian ProStar system with a UV/vis detector using a 21 mm Dynamax C18 column. Solvent gradient A was 75–90% CH_3-CN in 0.15% aqueous HCOOH over 20 min at 15 mL/min. Solvent gradient B was 75–80% CH_3CN in 0.15% aqueous HCOOH over 20 min at 15 mL/min. TLCs were sprayed with 0.5% anisaldehyde, 10% HOAc, and 5% H_2SO_4 in MeOH and heated until colored spots appeared.

Plant Material. Aerial parts of *Lithraea molleoides* (Vell.) Engl. were collected and identified by Renée H. Fortunato in September 1998 in the district of Valle Fértil of the Argentinean province of San Juan. A specimen has been deposited in the herbarium of the Instituto Nacional de Tecnología Agropecuaria (INTA), Castelar, Buenos Aires, Argentina (no. RHF 5943). Intellectual Property Rights Agreements for plant collections and collaborative research have been fully executed between The University of Arizona and INTA.

Biological Assay. A mixed population of larval and adult *Caenorhabditis elegans* (L1–L4 larvae and adults; size range from ∼0.1–1.0 mm), grown on agar plates with feeder layers of *E. coli*, was prepared as described by Wood et al.[15] For assays, the worms were grown for 3 days after passage to fresh agar plates. Then they were transferred into liquid medium ("M9" medium[15]) by washing the plates with M9. Fresh *E. coli* were added to provide nutrients and stimulate motility. Test compounds were solubilized at 100 times the desired final test concentrations in DMSO–MeOH (1:1) and added in 0.001 mL to individual wells of a 96-well, polystyrene, microtiter plate. Duplicate samples at each concentration were assayed. Control wells received 0.001 mL of DMSO–MeOH. The MeOH was allowed to evaporate, and then 0.1 mL of worms in M9 medium was added to each well. The numbers of worms per well in three experiments and standard deviation based on counting 4–8 wells were 243 ± 29, 248 ± 53, and 210 ± 31. At 1, 2, 4, and 24 h of incubation at 20 °C, the numbers of motile worms in each test well were counted, up to a maximum of 30 worms (the limit for accurate counting). Motility of the untreated, control worms ranged from >90% at early time points to 50% at 24 h.

Extraction and Isolation. Air-dried and ground aerial parts of *L. molleoides* (200 g) were extracted three times with MeOH–CH_2Cl_2 (1:1) at room temperature. The dried extract (11.5 g) was subjected to VLC on reversed-phase Si gel C18 with a CH_3CN/H_2O gradient of increasing CH_3CN content. The fraction eluting with 60–80% CH_3CN was found responsible for the biological activity and applied to preparative reversed-phase HPLC with gradient A, yielding compounds **1** (185 mg) and **4** (48 mg) and a mixture of **2** and **3**. This mixture was

repurified by reversed-phase HPLC using gradient B and yielded 18 mg of **2** and 12 mg of **3**.

(*Z,Z*)-5-(Trideca-4,7-dienyl)resorcinol (1): colorless oil; ^{1}H, ^{13}C, and 2D NMR data, Table 1; HRFABMS obsd *m/z* 289.2163 [M + H]$^+$, calcd for $C_{19}H_{29}O_2$ 289.2167.

(*Z,Z,Z*)-5-(Trideca-4,7,10-trienyl)resorcinol (2): colorless oil; ^{1}H, ^{13}C, and 2D NMR data, Table 1; HRFABMS obsd *m/z* 287.2003 [M + H]$^+$, calcd for $C_{19}H_{27}O_2$ 287.2011.

(*Z,Z,E*)-5-(Trideca-4,7,10-trienyl)resorcinol (3): colorless oil; ^{1}H, ^{13}C, and 2D NMR data, Table 1; HRFABMS obsd *m/z* 287.2014 [M + H]$^+$, calcd for $C_{19}H_{27}O_2$ 287.2011.

(*Z*)-5-(Trideca-4-enyl)resorcinol (4): colorless oil; ^{1}H, ^{13}C, and 2D NMR data, Table 1; HRFABMS obsd *m/z* 291.2340 [M + H]$^+$, calcd for $C_{19}H_{31}O_2$ 291.2324.

References and Notes

(1) Backer, H. J.; Haack, N. H. *Recl. Trav. Chim. Pays-Bas* **1938**, *57*, 225−232.

(2) Symes, W. F.; Dawson, C. R. *Nature* **1953**, *171*, 841−842.

(3) Keil, H.; Wasserman, D.; Dawson, C. R. *Ind. Med.* **1945**, *14*, 825−830.

(4) Backer, H. J.; Haack, N. H. *Recl. Trav. Chim. Pays-Bas* **1941**, *60*, 661−677.

(5) Montenegro, G. *Chile, Nuestra Flora Útil;* Montenegro, G., Timmermann, B. N., Eds.; Ediciones Universidad Católica de Chile, 2000; pp 154−155.

(6) Mabberley, D. J. *The Plant-Book*, 2nd ed.; Cambridge University Press: Cambridge, 1998; p 416.

(7) Gambaro, V.; Chamy, M. C.; von Brand, E.; Garbarino, J. A. *Planta Med.* **1986**, 20−22.

(8) Munhoz, D. *Flora Fanerogamica Argent.* **2000**, *65*, 6.

(9) *Catalogo de las Plantas Vasculares de la República Argentina II. Acanthaceae−Euphorbiaceae (Dicotyledoneae)*; Zuloaga, F. O., Morrone, O., Eds.; Missouri Botanical Garden Press: St. Louis, 1999; pp 42−44.

(10) Leandro Montes, A. *An. Soc. Cient. Argent.* **1969**, *187*, 21−48.

(11) Alé, S. I.; Ferreira, F.; Gonzáles, G.; Epstein, W. *J. Am. Contact Dermat.* **1997**, *8*, 144−149.

(12) Barrow, R. A.; Capon, R., J. *Aust. J. Chem.* **1991**, *44*, 1393−1405.

(13) Orjala, J.; Mian, P.; Rali, T.; Sticher, O. *J. Nat. Prod.* **1998**, *61*, 939−941.

(14) Roxburgh, N. A.; Shanahan, G. J. *Bull. Ent. Res.* **1973**, *63*, 99−102.

(15) Wood, W. B., Ed. *The Nematode C. elegans*; Cold Spring Harbor Laboratory Press: Plainview, NY, 1988.