

Phytotoxicity of Secondary Metabolites from *Aptenia cordifolia*

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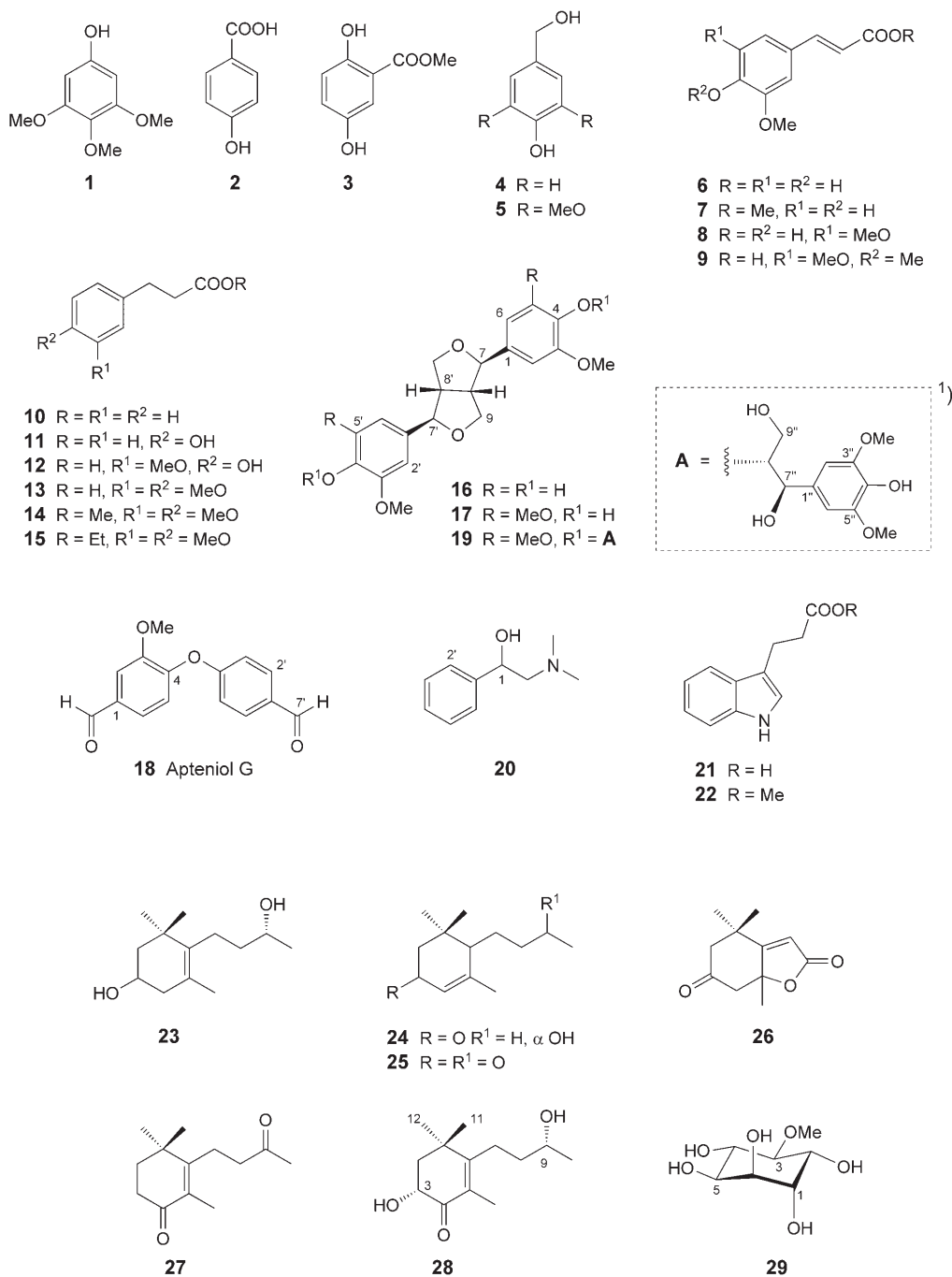
From the fresh leaves or twigs of *Aptenia cordifolia*, a total of 29 compounds were isolated, including the new tetranoroxyneolignan **18**, the new dilignan **19**, and the β -ionone derivative **27**, previously only known as a synthetic compound, together with 26 known compounds. The structures of the new products were determined by ¹H-, ¹³C-, and 2D-NMR, as well as HR-MS analyses. The phytotoxic effects of the isolates on the germination and growth of the dicotyledon *Lactuca sativa* L. (lettuce) were studied in the concentration range 10⁻⁴ to 10⁻⁷ M. Several constituents of *A. cordifolia* were found to be equally active as or superior to 4-hydroxybenzoic acid (HBA) used as positive control.

Introduction. – In the search of new phytotoxic natural compounds from Mediterranean plants, we previously reported studies of *Brassica fruticulosa* [1], *Chenopodium album* [2], and *Cestrum parqui* [3], which furnished many new biologically active compounds showing interesting effects on germination and for the development of standard species tests [4].

In pursuing the phytochemical investigation of spontaneous plants, we investigated *Aptenia cordifolia*. This plant, belonging to the Aizoaceae family, is a perennial herb native to South Africa and now largely diffused throughout Europe. A study of the leaf extract of *A. cordifolia* led to the isolation and characterization of novel phytotoxic oxyneolignans [5], as well as amides and lignanamides [6]. As a result of this study, we herein describe the isolation and identification of compounds **1–29**, including the new tetranoroxyneolignan **18**, the new dilignan **19**, and the new natural product **27**.

Results and Discussion. – 1. *Structure Elucidation.* The hydro-alcoholic infusion of the fresh leaves or twigs of *A. cordifolia* was reduced in volume and precipitated with acetone. The acetone/water-soluble part was fractionated by column chromatography on *Amberlite XAD-2* and silica gel, and further purified by HPLC to yield compounds **1–29**.

Phenols **1–5** were identified as 3,4,5-trimethoxyphenol, 4-hydroxybenzoic acid, methyl 2,5-dihydroxybenzoate, 4-(hydroxymethyl)phenol, and 4-(hydroxymethyl)-2,6-dimethoxyphenol, respectively. Compounds **6–9** were identified as ferulic acid, methyl ferulate, sinapic acid, and 3,4,5-trimethoxycinnamic acid, respectively. Compounds **10–15** were identified as 'dihydrocinnamic acid' (= 3-phenylpropanoic acid; **10**), '4-hydroxy-dihydrocinnamic acid' (= 3-(4-hydroxyphenyl)propanoic acid; **11**), dihydro-



¹) Arbitrary C-atom numbering. Due to the symmetry of the molecule, each atom has its primed or multiple-primed analogue (see chemical formula). For simplicity, only one atom each is referred to. Thus, when referring to, *e.g.*, C(1) or C(2''), the same applies to C(1') and C(2'''), resp.

ferulic acid (**12**), and ‘3,4-dimethoxy-dihydrocinnamic acid’ (= 3-(3,4-dimethoxyphenyl)propanoic acid; **13**) as well as the corresponding Me and Et esters **14** and **15**, respectively. Moreover, compounds **16** and **17** were identified as pinoresinol and syringaresinol, respectively. All these compounds were identified by comparison of their spectroscopic and mass-spectrometric data with those of authentic samples or literature data.

The new oxyneolignan **18**, named apteniol G, showed the M^+ peak at m/z 256 in the EI mass spectrum, with prominent fragments at m/z 255 ($[M-H]^+$) and 225 ($[M-MeO]^+$), in accord with the molecular formula $C_{15}H_{12}O_4$, as determined by HR-ESI-MS (m/z 256.0732 (M^+ ; calc. 256.0736)). The UV spectrum revealed an absorption maximum at 276 nm, and the IR spectrum showed bands typical of C=O (1687 cm^{-1}) and Ph (1604 cm^{-1}) groups. The structure of compound **18** was established by means of ^1H - and ^{13}C -NMR as well as 2D-NMR (COSY, NOESY, HMQC, HMBC) experiments.

The ^1H -NMR spectrum of **18** (Table 1) indicated the presence of four *ortho*-coupled H-atoms, two *doublets*, and one *singlet* in the aromatic region, as well a MeO *singlet* and two *singlets* due to formyl (CHO) groups. In the ^{13}C -NMR (DEPT) spectrum (Table 1), twelve resonances were evident, including one Me and seven aromatic CH groups. An HMQC experiment allowed the assignment of all H-atoms, with key HMBC cross-peaks of both H–C(2) and H–C(5) to C(3) and C(4), and of H–C(6) to C(1) and C(7). Furthermore H–C(2',6') coupled with C(4') and C(7'), H–C(7) showed a cross-peaks with C(1) and C(2,6), and H–C(7') interacted with C(1') and C(2',6'). Finally, the MeO group was placed at C(3), based on an NOE between C(3) and H–C(2). From these data, the structure of **18** was derived as 4-(4-formylphenoxy)-3-methoxybenzaldehyde.

The new compound **19** had the molecular formula $C_{44}H_{54}O_{18}$, based on the quasi-molecular ion at m/z 893 ($[M+Na]^+$) in the MALDI mass spectrum. In the ^{13}C -NMR (DEPT) spectrum of **19** (Table 2), only 16 signals were present, indicating a highly

Table 1. ^1H - and ^{13}C -NMR-Spectroscopic Data of **18**. At 500/125 MHz, resp., in CD_3OD ; δ in ppm, J in Hz.

Position	$\delta(\text{H})$	NOESY	$\delta(\text{C})$ (DEPT)	HMBC (H $\rightarrow$ C)
1			129.3 (s)	
2	7.43 (s)	7, MeO	111.7 (d)	1, 3, 4, 7
3			149.7 (s)	
4			156.0 (s)	
5	6.91 (d, $J=7.0$)		117.1 (d)	3, 4, 6
6	7.41 (d, $J=7.0$)	7	128.8 (d)	1, 2, 4, 7
7	9.72 (s)	2, 6	193.3 (d)	1, 2, 6
1'			133.1 (s)	
2'	7.77 (d, $J=8.8$)	7'	134.0 (d)	4', 6', 7'
3'	6.88 (d, $J=8.8$)		117.6 (d)	1', 4', 5'
4'			164.6 (s)	
5'	6.88 (d, $J=8.8$)		117.6 (d)	1', 3', 4'
6'	7.77 (d, $J=8.8$)	7'	134.0 (d)	2', 4', 7'
7'	9.75 (s)	2', 6'	193.3 (d)	1', 2', 6'
MeO	3.92 (s)	2	56.9 (q)	3

symmetric molecule. The ^1H -NMR and COSY spectra revealed the connectivities of four H-atoms characteristic of one 3,7-dioxabicyclo[3.3.0]octane and two propane-1,2,3-triol moieties. The ^1H - and ^{13}C -NMR spectra of **19** showed the presence of four aromatic rings, each one with two H-atoms in *meta* position relative to each other. The COSY spectrum enabled us to define the two glycerol moieties as $\text{C}(7'')\text{--C}(8'')\text{--C}(9'')$ [$\delta(\text{H})$ 4.99 (*d*, $J = 4.0$ Hz); 4.09–4.17 (*m*), 3.88 (obscured); 3.50 (*dd*, $J = 2.4, 12.0$ Hz)]. The coupling constant of 4.0 Hz between $\text{H--C}(7'')$ and $\text{H--C}(8'')$ indicated an *erythro* relative configuration. The ^{13}C -NMR (DEPT) signals of **19** showed two Me, two CH_2 , and five CH, as well as six quaternary C-atoms, which were connected by HMQC analysis. The functional groups were attached to the main skeleton with the aid of HMBC correlations. In the HMBC spectrum, $\text{H--C}(7)$ correlated with $\text{C}(2,6)$, $\text{C}(8)$, and $\text{C}(9)$. The *doublet* at $\delta(\text{H})$ 4.99 was attributed to $\text{H--C}(7'')$, with HMBC correlations to $\text{C}(1'')$, $\text{C}(2'',6'')$, and $\text{C}(9'')$. Finally, NOEs between the Me groups at $\delta(\text{H})$ 3.90 and 3.88 and both $\text{H--C}(2,6)$ and $\text{H--C}(2'',6'')$, respectively, defined the positions of the MeO groups. From these data, the structure of the new dilignan **19** was elucidated as ‘di-*erythro*-syringylglycerol- β -O-4,4'-syringaresinol ether'²⁾.

Table 2. ^1H - and ^{13}C -NMR-Spectroscopic Data of **19**. At 500/125 MHz, resp., in CDCl_3 ; δ in ppm, J in Hz. Asterisks (*) denote interchangeable assignments.

Position	$\delta(\text{H})$	NOESY	$\delta(\text{C})$ (DEPT)	HMBC ($\text{H} \rightarrow \text{C}$)
1/1'			134.2 (<i>s</i>)	
2/2'	6.65 (<i>s</i>)	7/7', 3/3'-MeO	102.9 (<i>d</i>)	1/1', 3/3', 4/4', 7/7'
3/3'			153.5 (<i>s</i>)	
4/4'			137.6 (<i>s</i>)	
5/5'			153.5 (<i>s</i>)	
6/6'	6.65 (<i>s</i>)	7/7', 5/5'-MeO	102.9 (<i>d</i>)	1/1', 4/4', 5/5', 7/7'
7/7'	4.79 (<i>d</i> , $J = 2.8$)	2/2', 6/6', 9/9'	85.8 (<i>d</i>)	2/2', 8/8', 9/9', 6/6'
8/8'	3.20–3.10 (<i>m</i>)		54.5 (<i>d</i>)	
9/9'	4.36 (<i>dd</i> , $J = 7.0, 14.8$)			
	3.99 (obscured)		72.0 (<i>t</i>)	7/7', 8/8'
1''/1'''			130.4 (<i>s</i>)	
2''/2'''	6.58 (<i>s</i>)	3''/3'''-MeO	102.6 (<i>d</i>)	4''/4''', 7''/7'''
3''/3'''			147.1 (<i>s</i>)	
4''/4'''			136.0 (<i>s</i>)	
5''/5'''			147.1 (<i>s</i>)	
6''/6'''	6.58 (<i>s</i>)		102.6 (<i>d</i>)	4''/4''', 7''/7'''
7''/7'''	4.99 (<i>d</i> , $J = 4.0$)		87.1 (<i>d</i>)	1''/1''', 2''/2''', 6''/6''', 9''/9'''
8''/8'''	4.17–4.09 (<i>m</i>)		72.7 (<i>d</i>)	
9''/9'''	3.88 (obscured)			
	3.50 (<i>dd</i> , $J = 2.4, 12.0$)		60.6 (<i>t</i>)	7''/7'''
3,5- and 3',5'-MeO	3.90 (<i>s</i>)	2/2', 6/6'	56.3 (<i>q</i>)*	3/3', 5/5'
3'',5''- and 3''',5'''-MeO	3.88 (<i>s</i>)	2''/2''', 6''/6'''	56.4 (<i>q</i>)*	3''/3'''

Compound **20** was identified as 2-(dimethylamino)-1-phenylethanol. EI-MS showed the M^+ peak at m/z 165, with a prominent fragment at m/z 150 ($[M - \text{Me}]^+$),

²⁾ For systematic names, see *Exper. Part*.

in accord with the molecular formula $C_{10}H_{15}NO$. The structure of **20** was established by means of 1H - and ^{13}C -NMR (DEPT) as well as 2D-NMR analyses, including COSY, HMQC, and HMBC experiments. The 1H -NMR spectrum indicated the presence of five aromatic H-atoms, three double *doublets* at $\delta(H)$ 4.86 ($J=9.5, 3.5$), 2.81 ($J=12.8, 9.5$), and 2.66 ($J=12.8, 3.5$ Hz), and two Me *singlets* at $\delta(H)$ 2.35. In the ^{13}C -NMR (DEPT) spectrum, seven resonances were evident due to one Me, one CH_2 , and four different types of CH groups. An HMQC experiment allowed the assignment of the H-atoms to the corresponding C-atoms, and the HMBC spectrum showed cross-peaks of both H–C(1) and H–C(2) to C(1'), and of H–C(2',6') to C(1) and C(3',5'). These data confirmed the structure of **20**, which had been isolated before from *Dolichothale uberiformis* as the corresponding hydrochloride [7], exhibiting an $[\alpha]_D$ value of -74 . In contrast, **20** isolated from *A. cordifolia* had an $[\alpha]_D$ value of $+1.1$.

Compounds **21** and **22** were identified as 3-(1*H*-indol-3-yl)propanoic acid and its Me ester, respectively.

Compounds **23–28** were identified as C_{13} norisoprenoids. Compound **23** corresponds to 3-hydroxy-7,8-dihydro- β -ionone, and has been previously isolated from the fruits of *Prunus* species [8]. Compounds **24** and **25** gave rise to spectroscopic data corresponding to (9*R*)-9-hydroxymegastigm-4-ene-3-one and megastigm-4-ene-3,9-dione, in agreement with those isolated from *Chenopodium album* [2]. Compound **26** was identified as dehydrolololide, previously isolated from *Vitis vinifera* [9]. Finally, the structure of **27** was determined by spectroscopic comparison as 4-oxo-7,8-dihydro- β -ionone, a synthetic intermediate previously obtained during the preparation of canthaxanthin [10].

Compound **28** was identified as (3*R*,9*R*)-3,9-dihydroxymegastigm-5-en-4-one, previously identified in the metabolism of astaxanthin, a carotenoid non-provitamin A [11]. EI-MS showed the M^+ ion at m/z 226, which, together with the elemental-analysis data, was in agreement with the molecular formula $C_{13}H_{22}O_3$ typical for a bisnorsesquiterpene. In the 1H -NMR spectrum of **28** (Table 3), there were three Me *singlets* at $\delta(H)$ 1.23, 1.29, and 1.83, one Me *doublet* at $\delta(H)$ 1.28, three CH_2 groups at $\delta(H)$ 2.15/1.77 ($CH_2(2)$), 2.50/2.30 ($CH_2(7)$), and 1.60 ($CH_2(8)$), and a double *doublet* at $\delta(H)$ 4.30 (H–C(3)) as well as a *multiplet* at 3.89 (H–C(9)) due to two oxygenated methines. The ^{13}C -NMR (DEPT) spectrum (Table 3) showed 13 signals, including four Me, three CH_2 , two CH, and four quaternary C-atoms, which were assigned on the basis of HMQC experiments.

1H , 1H -COSY Experiments with **28** showed a correlation series beginning with $\delta(H)$ 3.85–3.94 (H–C(9)), which was coupled to the Me *doublet* at $\delta(H)$ 1.28 (Me(10)) and to $CH_2(8)$ at $\delta(H)$ 1.59–1.66. The latter, in turn, was correlated with $CH_2(7)$ at $\delta(H)$ 2.45–2.54/2.26–2.34. The signal at $\delta(H)$ 4.30 was correlated to the methylene H-atoms at $\delta(H)$ 2.15/1.77. The C=O group was placed in 4-position, and the OH groups were attached to C(3) and C(9), respectively, on the basis of HMBC experiments. HMBC correlations of $CH_2(2)$ and Me(13) with C(4), of $CH_2(2)$ with C(3), and of $CH_2(8)$ and Me(10) with C(9) confirmed the above assignments. In the NOESY spectrum of **28**, spatial interactions between the Me groups at $\delta(H)$ 1.23 and H–C(3), and between the Me group at $\delta(H)$ 1.29 and $CH_2(2)$ were observed, which allowed us to differentiate between Me(11) and Me(12).

Table 3. ^1H - and ^{13}C -NMR-Spectroscopic Data of **28**. At 500/125 MHz, resp., in CDCl_3 ; δ in ppm, J in Hz.

Position	$\delta(\text{H})$	NOESY	$\delta(\text{C})$ (DEPT)	HMBC ($\text{H} \rightarrow \text{C}$)
1			37.5 (s)	
2	2.15 (dd, $J=5.8, 12.5$) 1.77 (br. t, $J=13.0$)	11	45.3 (t)	1, 3, 4, 6, 11
3	4.30 (dd, $J=5.8, 14.0$)	12	69.1 (d)	1, 2, 4
4			200.2 (s)	
5			127.9 (s)	
6			166.1 (s)	
7	2.54–2.45 (m) 2.34–2.26 (m)		27.1 (t)	5, 6, 8
8	1.66–1.59 (m)	11	37.7 (t)	7, 9
9	3.94–3.85 (m)		68.2 (d)	7
10	1.28 (d, $J=6.4$)		23.8 (q)	8, 9
11	1.23 (s)		29.8 (q)	1, 2, 6, 12
12	1.29 (s)		25.6 (q)	1, 2, 6, 11
13	1.83 (s)		11.9 (q)	4, 5, 6

The absolute configuration of **28** was established by Mosher's method [12] upon conversion of **28** into the diastereoisomeric Mosher diesters. ^1H , ^1H -COSY Experiments allowed us to identify the signals for $\text{CH}_2(2)$ and $\text{CH}_2(8)$ of the two diastereoisotopic diesters. Comparison of the chemical shifts of these H-atoms in both the (*R*)- and the (*S*)-Mosher derivatives, and calculation of the corresponding shift differences, expressed as $\Delta\delta = \delta(R) - \delta(S)$, were in agreement with an (*R*)-configuration at both C(9) and C(3), as further confirmed by a positive $\Delta\delta$ value for Me(13), and negative ones for Me(11) and Me(12) [13].

Compound **29** was identified as 3-*O*-methyl-*chiro*-inositol. ESI-MS showed the $[M + \text{H}]^+$ signal at m/z 195, in agreement with the molecular formula $\text{C}_7\text{H}_{14}\text{O}_6$. The ^1H -NMR spectrum showed a MeO group at $\delta(\text{H})$ 3.35, and six methine H-atoms at $\delta(\text{H})$ 3.91, 3.89, 3.73, 3.62, 3.58, and 3.25. The ^{13}C -NMR (DEPT) spectrum showed signals due to seven C-atoms, including one Me and six CH groups. Analysis of the ^1H , ^1H -COSY and HSQC spectra of **29** suggested the presence of a sequence of six consecutive CH groups, and their ^1H -NMR coupling constants were in good agreement with those previously reported [14]. The MeO group was positioned at C(3), on the basis of HMBC correlations between C(3) and H–C(1), H–C(2), H–C(4), H–C(5), and the Me H-atoms, respectively.

2. Biological Activity. The phytotoxicities of compounds **2**, **8**, **11**, **12**, **16**, **24**, and **25** on the seeds of *Lactuca sativa* were reported previously [1–3]. We, thus, tested compounds **1**, **3**, **4**, **6**, **7**, **9**, **10**, **13–15**, **17–23**, and **26–29** for their activities towards the seeds of *L. sativa*. Aqueous solutions of the compounds, ranging from 10^{-4} to 10^{-7} M, were investigated in terms of germination, root length, and shoot length of treated lettuce seeds. The results are summarized in Fig. 1.

'3,4-Dimethoxy-dihydrocinnamic acid' (**13**) and methyl 3-(1*H*-indol-3-yl) propionate (**22**) were found to be most active, reducing the germination by 90%, and the radical and shoot growth by 100% at 10^{-4} and 10^{-5} M, respectively, relative to 4-hydroxybenzoic acid (HBA). The other compounds showed slight inhibition of

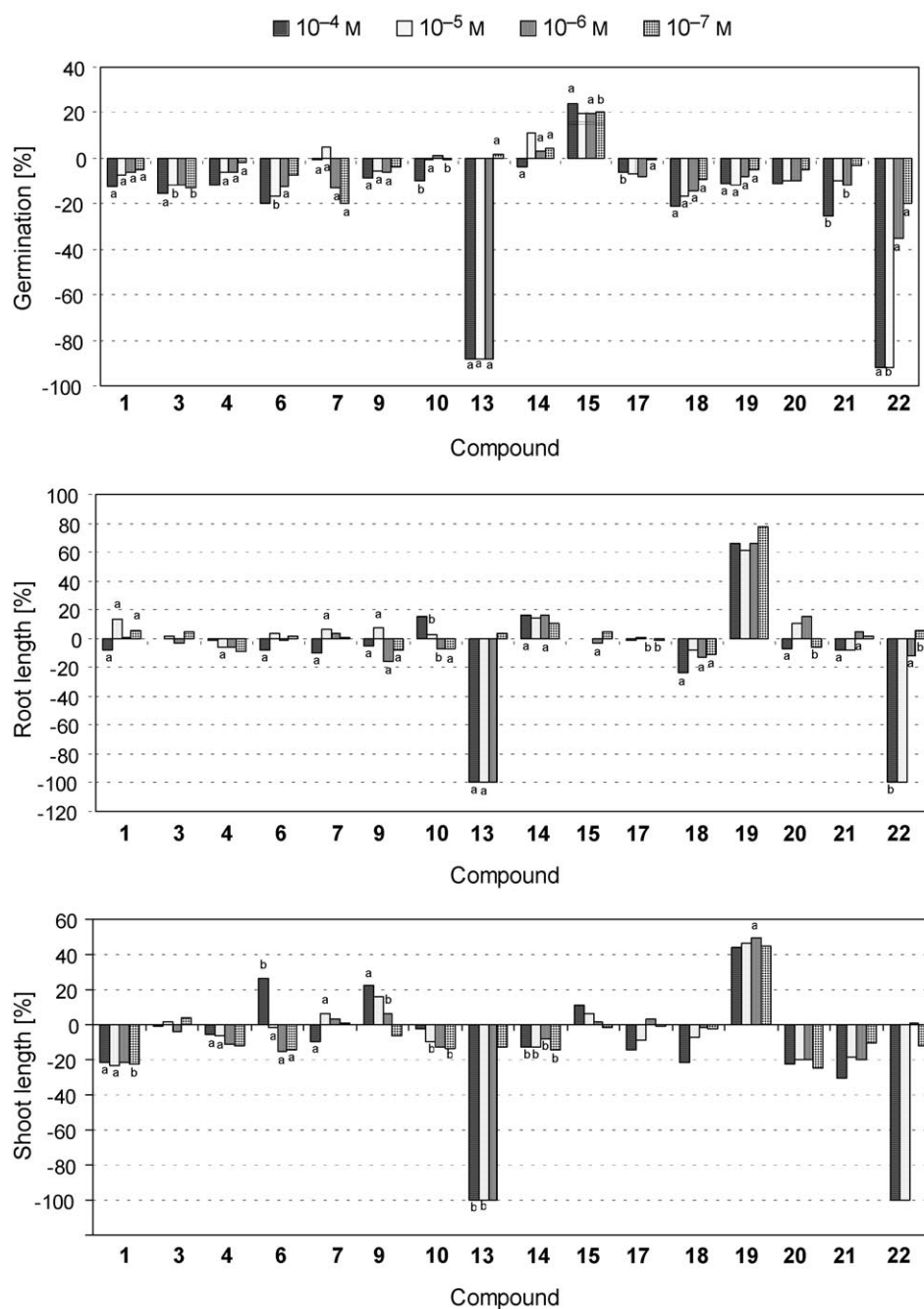


Fig. 1. Effect of Compounds 1, 3, 4, 6, 7, 9, 10, 13–15, and 17–22 from *Aptenia cordifolia* on the Germination (top), Root Length (middle), and Shoot Length (bottom) of *Lactuca sativa* L. Values represent the percent deviation from control (=0%), negative and positive values indicating inhibition and stimulation, resp. Significance: $P < 0.01$ (a) and $0.01 < P < 0.05$ (b).

germination, with an activity of 10–30% at the highest concentration tested, with the exception of compounds **23** and **29**, which reduced the germination by 80 and 45% at 10^{-4} M, respectively. Exceptional was also compound **15**, which gave rise to a stimulation of germination at all concentrations tested. Further, the dilignan **19** showed a stimulation of root and shoot length at all concentrations tested. Finally, compounds **23**, **26**, **27**, and **29** reduced the root and shoot length by 50–80% at the highest concentration tested.

The activities of the above compounds isolated from *A. cordifolia* were compared with that of 4-hydroxybenzoic acid (HBA), a well-known and effective germination inhibitor [15]. The results are shown in Fig. 2. The inhibition value for many compounds isolated from *A. cordifolia* were, at a concentration of 10^{-4} M, comparable to that of HBA. The bioactivities of the compounds tested showed a variable response on lettuce, and for some compounds, no dose-dependence effects were observed. The reason for this response could be due to differences in seed size, seed-coat permeability, differential uptake, and metabolism [16].

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Experimental Part

1. *General*. Anal. TLC: *Kieselgel 60 F₂₅₄* plates (0.2-mm; *Merck*); visualization under UV light and by spraying with $\text{H}_2\text{SO}_4/\text{AcOH}/\text{H}_2\text{O}$ 1:20:4, followed by heating for 5 min at 110° . Prep. TLC: *Kieselgel 60 F₂₅₄* plates (0.5 or 1 mm; *Merck*). Flash chromatography (FC): *Kieselgel 60* (230–400 mesh; *Merck*), at medium pressure. Anal. HPLC: *Agilent-1100* system with UV detector. Prep. HPLC: *RP-18* (*LiChrospher*, 10 μm ; 250×10 mm i.d.; *Merck*) column. NMR Spectra: *Varian-INOVA* spectrometer, at 500/125 MHz for ^1H and ^{13}C , resp., at 25° ; δ in ppm, J in Hz. HMQC and HMBC experiments were optimized for $^1J(\text{H,C}) = 140$ Hz and $^nJ(\text{H,C}) = 8$ Hz, resp. EI-MS: *HP-6890* mass spectrometer equipped with an MS 5973-N detector. MALDI-MS: *Voyager-DE MALDI-TOF* mass spectrometer. HR-ESI-MS/MS: *Q-TRAP* model *API-2000 LC/MS/MS* system equipped with a heated nebulizer source and using the *Analyst* software of *Applied Biosystem*; in m/z .

2. *Plant Material*. Plants of *Aptenia cordifolia* were collected in Campania, Italy, during August 2004, and identified by Prof. Antonino Pollio, Dipartimento di Biologia Vegetale, University Federico II, Naples, Italy. A voucher specimen (HERBNAPY 680) was deposited at the herbarium of the University Federico II.

3. *Extraction and Isolation*. 3.1. *Leaf Extract*. The fresh leaves (12.0 kg) of *A. cordifolia* were powdered and then extracted with $\text{H}_2\text{O}/\text{MeOH}$ 9:1 at 25° for 7 d. Cold acetone (1.0 l) was added to the aq. suspension (800 ml) of the crude extract (450 g), and the mixture was placed on a stirring plate in a cold room (-18°) overnight. Addition of acetone gave rise to heavy precipitation, consisting mostly of protein material, which was removed by centrifugation. The acetone was removed by evaporation, and the resulting clear aq. extract was reduced to a volume of 150 ml, and then subjected to FC (*Amberlite XAD-2*), eluting with H_2O (W), MeOH (M), and acetone (A).

The fraction eluted with H_2O was lyophilized (1.9 g), and the crude extract was resubjected to FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}/\text{H}_2\text{O}$ 14:6:1) to afford eight subfractions: *Fr. W1–W8*. From *Fr. W1* (60 mg), compound **29** (14 mg) was obtained by HPLC (*RP-18*; $\text{MeOH}/\text{H}_2\text{O}$ 9:1).

The fraction eluted with MeOH was dried by evaporation (85.0 g), and then extracted with $\text{H}_2\text{O}/\text{BuOH}$. The BuOH was removed by evaporation (45.0 g), and the crude extract was purified by FC (SiO_2) to afford 13 subfractions: *Fr. M1–M13*. *Fr. M4* (13.5 g), eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 4:1, was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ gradient) to give fractions *M4.A–M4.G*. *Fr. M4.A* (20 mg), eluted with CH_2Cl_2 , was purified by FC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 3:2:5) to afford **18** (3 mg). *Fr. M4.E*

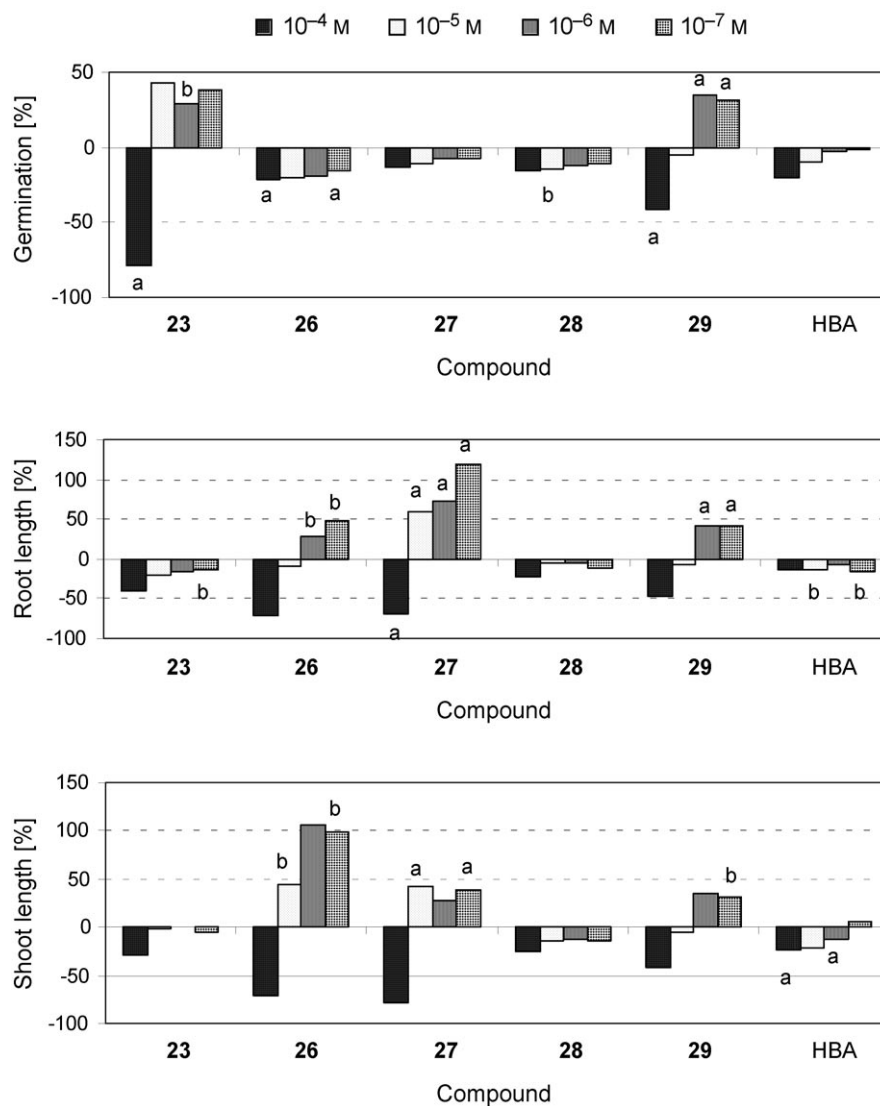


Fig. 2. Effect of Compounds **23** and **26–29** from *Aptenia cordifolia* on the Germination (top), Root Length (middle), and Shoot Length (bottom) of *Lactuca sativa* L. Relative to 4-Hydroxybenzoic Acid (HBA) as Positive Control. Values represent the percent deviation from control (=0%), negative and positive values indicating inhibition and stimulation, resp. Significance: $P < 0.01$ (a) and $0.01 < P < 0.05$ (b).

(33 mg), eluted with CH₂Cl₂/MeOH 3:1, was purified by HPLC (RP-18; MeOH/MeCN/H₂O 3:1:6) to give **6** (10 mg) and **7** (3 mg). Fr. M4.F (103 mg), eluted with CH₂Cl₂/MeOH 7:3, afforded **20** (103 mg). Fr. M5 (5.5 g), eluted with CH₂Cl₂/MeOH 4:1, was purified by FC (SiO₂; CH₂Cl₂/AcOEt/MeOH gradient) to afford Fr. M5.A–M5.I. Fr. M5.B (210 mg), eluted with AcOEt/MeOH 17:3, was purified by prep. TLC (SiO₂; CH₂Cl₂/MeOH 9:1) to afford **4** (20 mg). Fr. M5.F (52 mg), eluted with AcOEt/MeOH

4:1, was purified by prep. TLC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 3:1) to give **3** (6 mg). *Fr. M13* (5.5 g), eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 4:1, was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ gradient) to give *Fr. M13.A–M13.E*. *Fr. M13.C* (36 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 47:3, was purified by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 3:2:5) to afford **19** (4 mg).

The fraction eluted with acetone (50.0 g) was purified by FC (SiO_2) to give ten subfractions, *Fr. A1–A10*. *Fr. A1* (2.0 g), eluted with CH_2Cl_2 , was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ gradient) to provide *Fr. A1.A–A1.M*. *Fr. A1.M* (45 mg), eluted with MeOH, was purified by prep. TLC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{acetone}$ 4:1) to yield **16** (2 mg) and **17** (3 mg). *Fr. A2* (1.5 g), eluted with $\text{CH}_2\text{Cl}_2/\text{acetone}$ 4:1, was purified by FC (SiO_2 ; petroleum ether (PE)/acetone gradient) to give fractions *A2.A–A2.O*. *Fr. A2.D* (103 mg), eluted with PE/acetone 4:1, was purified by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 2:2:3) to give **24** (2 mg) and **25** (4 mg). *Fr. A2.G* (103 mg), eluted with PE/acetone 3:1 was purified by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 1:1:3) to give **23** (8 mg) and **28** (7 mg). *Fr. A3* (2.0 g), eluted with $\text{CH}_2\text{Cl}_2/\text{acetone}$ 3:2, was purified by FC (SiO_2 ; PE/acetone gradient) to give *Fr. A3.A–A3.Q*. *Fr. A3.H* (56 mg), eluted with PE/acetone 3:1, was purified by prep. TLC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{acetone}$ 9:1) to afford **9** (28 mg). *Fr. A6* (5.0 g), eluted with $\text{CHCl}_3/\text{MeOH}$ 1:1, was purified by FC (SiO_2 ; $\text{CHCl}_3/\text{MeOH}$ gradient) to afford *Fr. A6.A–A6.Q*. *Fr. A6.O* (2.9 g), eluted with MeOH, was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{AcOEt}/\text{AcOH}$ gradient) to give nine fractions: *Fr. A6.O1–A6.O9*. *Fr. A6.O1* (23 mg), eluted with CH_2Cl_2 , was purified by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}/\text{AcOH}$ 1:5:4:0.1) to afford **22** (10 mg). *Fr. A6.O2* (13 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 9:1, contained **21** (13 mg). *Fr. A6.O3* (60 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 17:3, contained **11**. *Fr. A6.O4* (160 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 3:1, provided **10** (52 mg) after purification by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 3:1:6). *Fr. A6.O5* (250 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{AcOEt}$ 1:1, gave **12** (10 mg), **13** (14 mg), and **14** (18 mg) after purification by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 3:2:5).

3.2. *Twig Extract*. The twigs of *A. cordifolia* (1.5 kg) were powdered and then extracted with $\text{H}_2\text{O}/\text{MeOH}$ 9:1 at 25° for 7 d. To an aq. suspension (100 ml) of the crude extract (45.0 g), cold acetone (100 ml) was added, and the mixture was placed on a stirring plate in a cold room (–18°) overnight. Addition of acetone led to the precipitation of mostly proteinaceous material, which was removed by centrifugation. The acetone was removed by evaporation, and the resulting clear aq. extract was reduced to a volume of 150 ml, and then purified by FC (*Amberlite XAD-2*; 1. H_2O , 2. MeOH, 3. acetone). The fraction eluted with MeOH (12.0 g) was purified by FC (SiO_2) to afford ten major fractions: *Fr. M'1–M'10*. *Fr. M'1* (1.8 g), eluted with PE/acetone 3:1, was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{acetone}/\text{Et}_2\text{O}$ gradient) to give six subfractions: *Fr. M'1.1–M'1.6*. *Fr. M'1.1* (16 mg) was purified by HPLC (*RP-18*; $\text{MeOH}/\text{MeCN}/\text{H}_2\text{O}$ 2:1:2) to afford **26** (2 mg), **2** (1 mg), and **27** (2 mg). *Fr. M'2* (206 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{Me}_2\text{CO}$ 4:1, was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ gradient) to give *Fr. M'2.1–M'2.5*. *Fr. M'2.3* (35 mg) was purified by prep. TLC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{Et}_2\text{O}$ 4:1) to provide **1** (8 mg) and **8** (3 mg). *Fr. M'2.4* (110 mg), eluted with $\text{CH}_2\text{Cl}_2/\text{acetone}$ 7:3, was purified by FC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 99:1) to afford subfractions *M'2.4.A–M'2.4.F*. *Fr. M'2.4.B* (48 mg) was purified by prep. TLC (SiO_2 ; $\text{CH}_2\text{Cl}_2/\text{MeOH}$ 19:1) to give **5** (8 mg) and **15** (9 mg).

4. *Compound Characterization*. 4.1. 4-(4-Formylphenoxy)-3-methoxybenzaldehyde (**18**). ^1H - and ^{13}C -NMR: see Table 1. HR-ESI-MS: 256.0732 (M^+ , $\text{C}_{15}\text{H}_{12}\text{O}_4$; calc. 256.0736).

4.2. 'Di-erythro-syringylglycerol- β -O-4,4'-syringaresinol Ether' (= (1*R*,2*R*,1'*R*,2'*R*)-2,2'-[(1*R*,3*aS*,4-*R*,6*aS*)-Tetrahydro-1*H*,3*H*-furo[3,4-*c*]furan-1,4-diylbis[(2,6-dimethoxybenzene-4,1-diyl)oxy]]bis[1-(4-hydroxy-3,5-dimethoxyphenyl)propane-1,3-diol]; **19**). $[\alpha]_D^{25} = +3.1$ ($c = 0.05$, CH_2Cl_2). ^1H - and ^{13}C -NMR: see Table 2. MALDI-MS: 893 ($[M + \text{Na}]^+$). Anal. calc. for $\text{C}_{44}\text{H}_{54}\text{O}_{18}$: C 60.68, H 6.25; found: C 60.68, H 6.29.

4.3. 2-(Dimethylamino)-1-phenylethanol (**20**). $[\alpha]_D^{25} = +1.1$ ($c = 0.08$, CH_2Cl_2). ^1H -NMR: 7.40 (*d*, $J = 7.0$, H–C(2',6'))); 7.35 (*t*, $J = 7.0$, H–C(3',5'))); 7.28 (*t*, $J = 7.0$, H–C(4'))); 4.86 (*dd*, $J = 9.5$, 3.5, H–C(1)); 2.81 (*dd*, $J = 12.8$, 9.5, H_a –C(2)); 2.66 (*dd*, $J = 12.8$, 3.5, H_b –C(2)); 2.35 (*s*, 2 Me). ^{13}C -NMR: 144.5 (C(1')); 130.0 (C(3',5'))); 129.3 (C(4'))); 127.6 (C(2',6'))); 71.8 (C(1)); 67.9 (C(2)); 45.9 (2 Me). EI-MS: 165 (M^+), 150 ($[M - \text{Me}]^+$).

4.4. (3*R*,9*R*)-3,9-Dihydroxymegastigm-5-en-4-one (**28**). $[\alpha]_D^{25} = -76.0$ ($c = 0.1$, CH_2Cl_2). ^1H - and ^{13}C -NMR: see Table 3. EI-MS: 226 (M^+), 211 ($[M - \text{Me}]^+$), 193 ($[M - \text{H}_2\text{O} - \text{Me}]^+$). Anal. calc. for $\text{C}_{13}\text{H}_{22}\text{O}_3$: C 68.99, H 9.80; found: C 69.00, H 9.87.

4.5. 3-O-Methyl-chiro-inositol (= (1R,2R,3S,4R,5S,6R)-6-Methoxycyclohexane-1,2,3,4,5-pentol; **29**). $[\alpha]_D^{25} = +56$ ($c=0.3$, CHCl_3). $^1\text{H-NMR}$: 3.91 (*dd*, $J=2.0, 9.5$, $\text{H-C}(2)$); 3.89 (*d*, $J=2.0$, $\text{H-C}(6)$); 3.73 (*dd*, $J=2.0, 9.5$, $\text{H-C}(1)$); 3.72 (*dd*, $J=2.0, 9.5$, $\text{H-C}(5)$); 3.58 (*t*, $J=9.5$, $\text{H-C}(4)$); 3.35 (*s*, MeO); 3.25 (*d*, $J=9.0, 9.5$, $\text{H-C}(3)$). $^{13}\text{C-NMR}$: 85.4 ($\text{C}(3)$); 74.8 ($\text{C}(4)$); 74.2 ($\text{C}(1)$); 74.0 ($\text{C}(6)$); 73.0 ($\text{C}(5)$); 72.5 ($\text{C}(2)$); 61.2 (Me). ESI-MS: 195 ($[M+H]^+$). Anal. calc. for $\text{C}_7\text{H}_{14}\text{O}_6$: C 43.30, H 7.27; found: C 43.26, H 7.30.

5. *Bioassays*. Seeds of *Lactuca sativa* L. (cv Napoli V. F.) collected during 2003, were obtained from Ingegnoli S.p.a. All undersized or damaged seeds were discarded, and the seeds to be assayed were selected for uniformity. For the assays, Petri dishes (50-mm diameter) with one sheet of filter paper (Whatman No. 1) as support were used. In four replicate experiments, repeated three times for three different periods, germination and growth experiments were conducted in aq. soln. at controlled pH. Test solns. (10^{-4} M) were prepared using 10 mM MES buffer (= (2-[N-morpholino]ethanesulfonic acid; pH 6). Concentrations of 10^{-5} to 10^{-7} M were adjusted by dilution. Parallel controls were performed. After the addition of 25 seeds and 2.5 ml of test soln., the Petri dishes were sealed with Parafilm to ensure closed-system models. The seeds were placed in a growth chamber (KBW Binder 240) at 25° in the dark. The percentage of germination was determined daily over 5 d, after which no more germination was observed. After growth, plants were frozen at -20° to avoid subsequent growth until measurement. Graphical data are reported as differences (in %) from the controls (0%). Thus, pos. values represent stimulation of the parameter studied, and negative values represent inhibition.

6. *Statistical Treatment*. The statistical significance of differences between groups were determined by Student's *t*-test, calculating mean values for every parameter (germination average, shoot and root elongation) and their population variance within a Petri dish. The level of significance was set at $P < 0.05$.

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