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Tetrahedron 61 (2005) 11924–11929
Tetrahedron

Apteniols A–F, oxyneolignans from the leaves of *Aptenia cordifolia*

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Received 5 July 2005; revised 5 September 2005; accepted 15 September 2005

Available online 6 October 2005

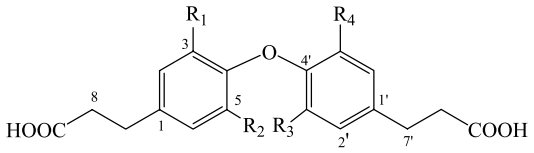
Abstract—Investigation of the organic extract of *Aptenia cordifolia* leaves revealed six new oxyneolignans named apteniols A–F. The structures were determined by means of spectroscopic methods. The C_6C_3 units are linked by an oxygen atom at C4–C4′ or C4–C2′ and they are dihydrophenylpropanoid acid units. Their effects on germination and growth of *Lactuca sativa* L. have been studied in the range concentration 10^{-4}–10^{-7} M.

1. Introduction

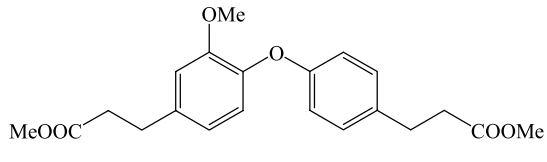
Aptenia cordifolia, belonging to the Aizoaceae family, is a perennial herb native to South Africa and has now largely spread throughout Europe. The most commonly grown plant, usually grown under the cultivar name of 'Red Apple', is considered by some botanists to be actually a hybrid between *Aptenia cordifolia* and the closely related *Platythyra Aptenia haeckeliana*.[1] Previous chemical studies of the aerial part of *A. cordifolia* evidenced the presence of flavonoids.[2] As part of our research on bioactive natural products isolated from spontaneous plants present in Italy and their use as natural herbicide models, we recently reported that some metabolites isolated from *Brassica fruticulosa* and *Chenopodium album* inhibited the germination and growth of some mono and dicotyledons.[3,4] Continuing the phytochemical study of common weeds widely distributed in the Mediterranean area, we have investigated *A. cordifolia*. The present paper reports the isolation and structure elucidation of six new oxyneolignans from the hydroalcoholic infusion of fresh leaves of *A. cordifolia*. The C_6C_3 units are linked by an oxygen atom at C4–C4′ or C4–C2′ and they are dihydrophenylpropanoid acid units. The phytotoxic activity of the compounds have been evaluated on the dicotyledon *Lactuca sativa*.

2. Results and discussion

Repeated column chromatographies and preparative HPLC of the methanol/water extract of leaves of the plants, after acetone precipitation, afforded compounds **1**–**6**. The



1 $R_1 = R_2 = R_3 = R_4 = H$
2 $R_1 =$ OMe $R_2 = R_3 = R_4 = H$
4 $R_1 = R_2 = R_3 =$ OMe $R_4 = H$
5 $R_1 = R_2 = R_3 = R_4 =$ OMe



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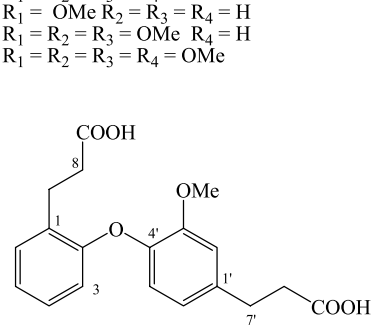

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Keywords: *Aptenia cordifolia*; Apteniols A–F; Oxyneolignans; Spectroscopic analysis; *Lactuca sativa*.
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doi:10.1016/j.tet.2005.09.054

Table 1. ^{1}H NMR Data of **1–6** (CD_3OD) at 500 MHz[a]

Position	1	2	3[b]	4[b]	5[b]	6
2	6.92d (8.5)	6.72d (1.5)	6.70d (1.5)	6.44s	6.42s	
3	6.63d (8.5)					7.21[c]
4						7.23[c]
5	6.63d (8.5)	6.82d (8.2)	6.83d (8.0)			7.12m
6	6.92d (8.5)	6.70dd (8.2, 1.5)	6.68dd (8.0, 1.5)	6.44s	6.42s	7.21[c]
7	2.84t (8.1)	2.89t (8.0)	2.88t (7.5)	2.90t (7.5)	2.88t (8.0)	2.89t (7.6)
8	2.66t (8.1)	2.66t (8.0)	2.59t (7.5)	2.67t (7.5)	2.61t (8.0)	2.44t (7.6)
2′	6.92d (8.5)	7.05d (8.5)	7.05d (9.0)	6.72s	6.42s	6.82d (2.0)
3′	6.63d (8.5)	6.74d (8.5)	6.75d (9.0)			
5′	6.63d (8.5)	6.74d (8.5)	6.75d (9.0)	6.82d (8.5)		6.67d (8.0)
6′	6.92d (8.5)	7.05d (8.5)	7.05d (9.0)	6.70d (8.5)	6.42s	6.63dd (8.0, 2.0)
7′	2.84t (8.1)	2.89t (8.0)	2.88t (7.5)	2.90t (7.5)	2.88t (8.0)	2.82t (7.8)
8′	2.66t (8.1)	2.66t (8.0)	2.61t (7.5)	2.67t (7.5)	2.61t (8.0)	2.41t (7.8)
3-OMe		3.86s	3.87s	3.87s	3.88s	
5-OMe				3.87s	3.88s	
3′-OMe				3.87s	3.88s	3.87s
5′-OMe					3.88s	
9-OMe			3.67s			
9′-OMe			3.67s			

[a] *J* values (in Hz) in parentheses.
[b] Recorded in $CDCl_3$ 500 MHz.
[c] Multiplicity was not determined due to overlapping.

structure of these compounds was elucidated by their spectral data.

Apteniol A (**1**) showed a molecular peak at *m/z* 337 $[M+Na]^+$ in the MALDI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{18}H_{18}O_5$. The UV spectrum revealed band at 278 nm. The ^{1}H NMR and the ^{13}C NMR spectra indicated a highly symmetric molecule. In the ^{1}H NMR spectrum (Table 1) eight aromatic protons were present as two *ortho*-coupled protons and eight methylene protons as two triplets in aliphatic region. The ^{13}C NMR spectrum (Table 2) showed only seven carbon signals. The ^{1}H and ^{13}C resonances of **1** were assigned by combination of COSY, DEPT, HMQC and HMBC experiments. The DEPT spectrum showed two methylenes, and two methines. According to the structure in the HMBC spectrum both the H-7/H-7′ and H-8/H-8′ protons were correlated to the C-9/C-9′ at δ 180.6 and C-1/C-1′ at δ 129.6; furthermore the H-3/H-3′ protons were correlated to C-1/C-1′, and C-4/C-4′ carbons at δ 157.4.

The molecular ion at *m/z* 390 $[M+2Na]^+$ in the MALDI-MS spectrum along with the elemental analysis, defined the molecular formula $C_{19}H_{20}O_6$ of apteniol B (**2**). Its ^{1}H NMR spectra (Table 1) indicated the presence of four *ortho*-coupled protons, two doublet protons, and one double doublet proton in the aromatic region; a methyl singlet of a methoxyl group, and two triplets in the aliphatic region. In the ^{13}C NMR spectrum (Table 2) fourteen carbons were evident. The DEPT spectrum indicated the presence of a

Table 2. ^{13}C NMR Data of **1–6** (CD_3OD) at 125 MHz

Position	1	2	3[a]	4[a]	5[a]	6
1	129.6	132.1	132.7	133.1	132.0	135.7
2	130.7	111.0	110.2	104.8	104.9	113.5
3	116.6	146.4	146.7	146.9	147.0	148.6
4	157.4	144.0	144.2	131.9	145.2	145.8
5	116.6	114.4	114.6	146.9	147.0	116.4
6	130.7	120.8	121.0	104.8	104.9	122.0
7	35.7	30.3	30.9	30.7	31.2	34.0
8	42.9	35.9	36.3	35.7	36.1	41.9
9	180.6	178.9	173.8	177.8	178.4	182.2
1′	129.6	132.1	130.0	131.2	132.0	127.1
2′	130.7	129.4	129.6	110.8	104.9	144.2
3′	116.6	115.4	115.5	146.9	147.0	129.2
4′	157.4	154.1	154.4	144.0	145.2	129.2
5′	116.6	115.4	115.5	114.3	147.0	126.6
6′	130.7	129.4	129.6	120.7	104.9	129.2
7′	35.7	30.3	30.9	30.2	31.2	34.4
8′	42.9	35.8	36.3	35.7	36.1	41.6
9′	180.6	178.9	173.8	177.8	178.4	182.2
3-OMe		55.8	56.1	56.1	56.3	56.5
5-OMe				56.1	56.3	
3′-OMe				55.7	56.3	
5′-OMe					56.3	
9-OMe			51.9			
9′-OMe			51.9			

[a] Recorded in $CDCl_3$.

methyl, three methylenes, and five methines. The HMQC experiment allowed the assignment of the protons to the corresponding carbons. The HMBC spectrum showed cross-peaks of both the H-2 and H-5 protons with the C-3 and C-4 carbons and the H-6 with the C-1 and C-7. Furthermore the H-2′/H-6′ protons gave cross peaks with the C-4′ and C-7′ carbons, the H-7′ and the H-8′ protons gave cross peaks with the C-1′ and C-9′ carbons. These data led to the structure **2** as depicted. The assignment of the methoxyl at C-3 was confirmed by NOE between the methoxyl and the H-2 proton.

Apteniol C (**3**) had the molecular formula $C_{21}H_{24}O_6$ as deduced from the molecular peak at *m/z* 372 in the MALDI-MS spectrum. The general features of its IR and NMR spectra (Tables 1 and 2) closely resembled those of **2**, except for the presence of two methoxyl signals in the NMR spectra. The structure of compound **3** was also deduced from the NOE observed between the methoxyls at δ 3.67 and the H-8/H-8′ protons in the NOESY experiment. Compound **3** was also obtained by treatment of Apteniol B with ethereal CH_2N_2.

Apteniol D (**4**) had the molecular formula $C_{21}H_{24}O_8$, as established by the ion at *m/z* 427 $[M+Na]^+$ in the MALDI-MS spectrum and elemental analysis. The ^1H NMR spectrum (Table 1) showed the presence of one aromatic ring with three coupled protons in a ABX system, an aromatic ring with two protons *meta* coupled, two methylenes as triplets and a methyl singlet of three methoxyls. In the ^{13}C NMR spectrum (Table 2) fifteen carbon signals were present, and the DEPT experiment evidenced two methyls, two methylenes, and four methines. The ^1H and ^{13}C resonances of apteniol C were assigned by

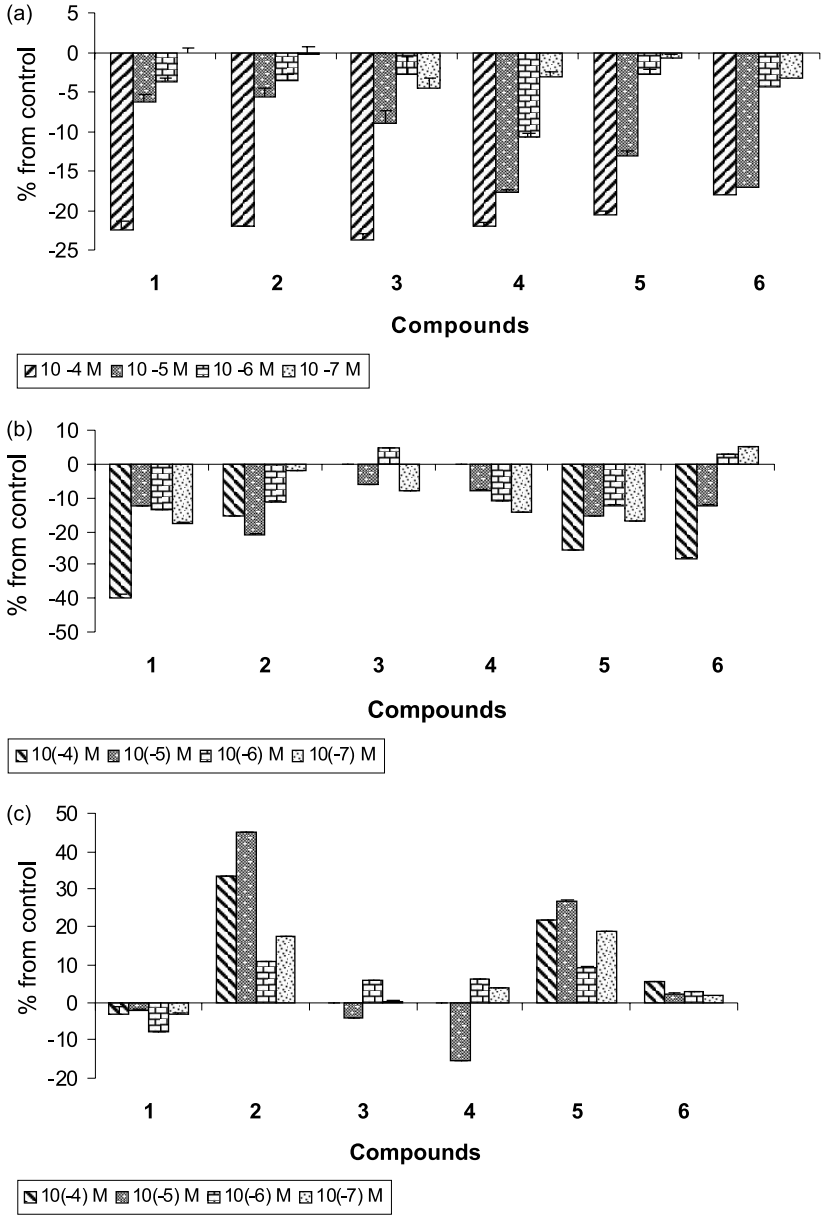

Figure 1. (A) Effect of compounds **1–6** on germination of *Lactuca sativa* L. Value presented as percentage differences from control. (B) Effect of compounds **1–6** on root length of *Lactuca sativa* L. Value presented as percentage differences from control. (C) Effect of compounds **1–6** on shoot length of *Lactuca sativa* L. Value presented as percentage differences from control.

combination of COSY, DEPT, HMQC and HMBC experiments. The HMBC spectrum of compound **4** showed cross-peaks of both the H-2 and H-6 with C-4 and C-7, and both the H-2′ and the H-5′ protons with the C-1′ and C-4′ carbons. NOEs between the signal at δ 3.87 and the H-2, H-6 and H-2′ protons allowed to assign the methoxyls at C-3, C-5, and C-5′ positions.

Apteniol E (**5**) showed a molecular peak at *m/z* 480 $[M+2Na]^+$ in the MALDI-MS spectrum suggesting, along with the elemental analysis, a molecular formula $C_{22}H_{26}O_9$. The UV spectrum revealed bands at 322, 279 and 244 nm. The ^{1}H NMR spectrum (Table 1) showed only one singlet aromatic signal, two triplets in aliphatic region, and a methyl signals. In the ^{13}C NMR spectrum (Table 2) only eight carbon signals were present indicating a highly symmetric molecule. The ^{1}H and ^{13}C resonances of **5** were assigned by combination of COSY, DEPT, HMQC and HMBC experiments. The DEPT spectrum showed one methyl, two methylenes, and one methine. According to the structure in the HMBC spectrum both the H-7/H-7′ and the H-8/H-8′ protons were correlated to the C-9/C-9′ at δ 178.4 and the C-1/C-1′ at δ 132.0; furthermore the H-2, H-6/H-2′, H-6′ protons were correlated to the C-1/C-1′ and C-4/C-4′ carbons. The NOE of the signal at δ 3.88 with H-2/H-6 and H-2′/H-6′ allowed assignment of the methoxyls at the C-3, C-3′, C-5, and C-5′ positions.

Apteniol F (**6**) had the same molecular formula as **2** as deduced from a molecular peak at *m/z* 343 $[M-H]^+$ in the MALDI-MS spectrum, and the elemental analysis. The ^{1}H NMR spectrum (Table 1) showed the presence of one aromatic ring with three coupled protons in a ABX system, an aromatic ring with four adjacent protons, two methylenes as triplets and a methyl singlet of a methoxyl group. In the ^{13}C NMR spectrum (Table 2) sixteen carbon signals were present, and the DEPT experiment showed one methyl, four methylenes, and five methines. The ^{1}H and ^{13}C resonances of apteniol F were assigned by a combination of COSY, DEPT, HMQC and HMBC experiments. The HMBC spectrum of compound **6** showed cross-peaks of H-7 with C-2, C-6 and C-9, H-8 with C-1, and both the H-2′ and the H-5′ protons with the C-1′ and C-4′ carbons. NOEs between the signal at δ 3.87 and H-2′ allowed assignment of the methoxyl at the C-3′ position.

All of the compounds isolated from *A. cordifolia* were tested for their phytotoxicity on the seeds of *Lactuca sativa*.[5] This species was selected as representative of main dicotyledon commercial crops.[5] It has been used extensively as a test organism because of its fast germination and high sensitivity, and allows comparison of bioassay results for many different compounds.[6,7] Aqueous solution of oxyneolignans **1**–**6**, ranging between 10^{-4} and 10^{-7} M, were tested on germination, root length and shoot length of treated lettuce seeds (Fig. 1). All the tested compounds reduced the germination by 25% at 10^{-4} M, and dose dependence effects were observed. They reduced the root elongation by 20% at 10^{-4} M, but compound **1** was the most active (40%) when compared to the control and compound **3** was almost completely inactive. Amongst compounds **1**–**6**, only oxyneolignan **4** reduced shoot elongation by 15% at the highest concentration and no important effects were observed for compounds **1**, **3**, and **6**, while compounds **2** and **5** showed stimulatory effects within 20–50%.

Lignans, found widely throughout the plant kingdom, exhibit interesting antimicrobic, antiviral, herbicidal, or antifeedant activities that are thought to participate in plant defence mechanisms against biotic stresses.[8–11] Cancer protective effects of dietary lignans have been also demonstrated.[12,13] In contrast, neolignans are distributed in the limited plant families, as Pinaceae and Cupressaceae.[14,15] The presence of dihydrocinnamic acid residues, in oxyneolignans isolated from *A. cordifolia* is peculiar because, to the best of our knowledge, the more common compounds isolated contain a reduced carboxylic group as alcohol or alkane.

3. Experimental

3.1. General procedures

NMR spectra were recorded at 25 °C on a Varian UNITY INOVA-500 spectrometer, operating at 500 and 125 MHz for ^{1}H and ^{13}C, respectively. Matrix assisted laser desorption ionization (MALDI) mass spectra were recorded using a Voyager-DE MALDI-TOF mass spectrometer. UV–vis spectra were recorded in methanol on a Perkin-Elmer Lambda 7 spectrophotometer. Liquid chromatography over silica gel (230–400 mesh) was performed at medium pressure. Preparative HPLC was run on a Agilent 1100 equipped with an UV detector and using SiO_2 (LiChrospher Silica 10 μm, 250×10 mm i.d., Merck), RP-18 (LiChrospher 10 μm, 250×10 mm i.d., Merck) columns. Analytical TLC was performed on precoated Merck aluminum sheet (DC-Alufolien Kielselgel 60 F_{254}, 0.2 mm) or RP-18 F_{254} plates with 0.2 mm film thickness. The spots were visualized by UV light or by spraying with H_2SO_4–AcOH–H_2O (1:20:4). The plates were then heated for 5 min at 110 °C. Prep. TLC was performed on Merck Kiesegel 60 F_{254} plates, with 0.5 or 1 mm film thickness.

3.2. Plant material

Leaves of *Aptenia cordifolia* were collected in Italy (Campania) during the summer (August) and identified by Professor Pollio of the Dipartimento di Biologia Vegetale of University Federico II of Napoli. A voucher specimen (HERBNAPY680) has been deposited in the herbarium at the University Federico II.

3.3. Extraction and isolation of metabolites

Fresh leaves (12 kg) of the plants were powdered and extracted with H_2O–CH_3OH (9/1) at room temperature (25 °C) for 7 days. To an aqueous suspension (800 ml) of the H_2O/CH_3OH extract (450 g), cold $(CH_3)_2CO$ (1.0 l) was added, and the mixture was placed on a stirring plate overnight at −18 °C. The $(CH_3)_2CO$ addition led to heavy precipitation consisting mostly of proteinaceous material, which was removed by centrifugation. The $(CH_3)_2CO$ was removed and the aqueous solution was chromatographed on Amberlite XAD-2 with H_2O, CH_3OH and $(CH_3)_2CO$ to give

3 fractions. The fraction eluted with CH_3OH (45.0 g) was rechromatographed on silica gel column to give 13 fractions. Fraction 5 (5.5 g) eluted with CH_2Cl_2–CH_3OH (4/1) was rechromatographed on SiO_2 flash column eluting with CH_2Cl_2–AcOEt–$(CH_3)_2CO$ gradient to afford 9 subfractions. Subfraction 1 (91 mg) eluted with CH_2Cl_2–$(CH_3)_2CO$ (4/1) was purified by preparative TLC [petrol ether–$(CH_3)_2CO$ (3/1)], to give **3** (8 mg).

Fraction eluted with $(CH_3)_2CO$ (50.0 g) was rechromatographed on silica gel column to give 11 fractions. Fraction 6 (5.0 g) eluted with CH_2Cl_2–CH_3OH (1/1) was rechromatographed on SiO_2 flash column eluting with CH_2Cl_2–$(CH_3)_2CO$–CH_3OH gradient to afford 15 subfractions. Subfraction 10 (498 mg) eluted with CH_2Cl_2–CH_3OH (4/1) was rechromatographed on SiO_2 flash column eluting with AcOEt–$(CH_3)_2CO$–CH_3OH gradient to afford the fractions A–M. Fraction B (52 mg) eluted with AcOEt–$(CH_3)_2CO$ (9/1) was purified by reversed-phase HPLC column (LiChrosphere RP-18 10 μm, 250×10 mm i.d., Merck) [H_2O–CH_3OH (1/1)], to give **6** (4 mg). Subfraction 13 (2.3 g) eluted with $(CH_3)_2CO$–CH_3OH (1/1) was rechromatographed on SiO_2 flash column, eluting with CH_2Cl_2–$(CH_3)_2CO$–CH_3OH gradient to afford the fractions A–I. Fraction C (46 mg) eluted with CH_2Cl_2–$(CH_3)_2CO$ (4/1) was purified by preparative TLC [Petrol ether 40–60–$(CH_3)_2CO$ (3/1)], to give **5** (4 mg). Fraction D (30 mg) eluted with CH_2Cl_2–$(CH_3)_2CO$ (3/1) was purified by reversed-phase HPLC column [H_2O–CH_3OH (1/1)], to give **4** (9 mg) and **2** (11 mg). Fraction 8 (16.0 g) eluted with CH_3OH was rechromatographed on SiO_2 flash column eluting with $(CH_3)_2CO$–CH_3OH gradient to afford 7 subfractions. Subfraction 6 (292 mg) eluted with $(CH_3)_2CO$–CH_3OH (1/1) was rechromatographed on SiO_2 flash column eluting with $(CH_3)_2CO$–CH_3OH (7/3) to afford the fractions A–G. Fraction B (76 mg) was purified by preparative TLC [$(CH_3)_2CO$–CH_3OH–H_2O (35/14/1)], to give **1** (8 mg).

3.3.1. 4,4′-Oxyneolign-9,9′-dioic acid (1). UV λ_{max} (CH_3OH) nm: 278 (log ε 3.2). MALDI-MS *m/z* (%): 337 (70), 277 (10), 255 (100). Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.78, H, 5.77. Found: C, 69.00, H, 5.79. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

3.3.2. 3-Methoxy-4,4′-oxyneolign-9,9′-dioic acid (2). UV λ_{max} (CH_3OH) nm: 280 (log ε 2.2). MALDI-MS *m/z* (%): 390 (15), 344 (10), 329 (20), 300 (30), 179 (100). Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.27, H 5.85. Found: C, 66.45, H, 5.95. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

3.3.3. Dimethyl 3-methoxy-4,4′-oxyneolign-9,9′-dioate (3). UV λ_{max} (CH_3OH) nm: 280 (log ε 3.1). MALDI-MS *m/z* (%): 372 (18), 254 (30), 209 (100), 163 (40). Anal. Calcd for $C_{21}H_{24}O_6$: C, 67.73, H, 6.50. Found: C, 67.91, H, 6.48. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

3.3.4. 3,3′,5-Trimethoxy-4,4′-oxyneolign-9,9′-dioic acid (4). UV λ_{max} (CH_3OH) nm: 321 (log ε 0.9), 280 (2.6), 242 (2.5). MALDI-MS *m/z* (%): 427 (10), 409 (20), 331 (10), 226 (20), 273 (100). Anal. Calcd for $C_{21}H_{24}O_8$: C, 76.18, H, 4.79. Found: C, 76.10, H, 4.50. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

3.3.5. 3,3′,5,5′-Tetramethoxy-4,4′-oxyneolign-9,9′-dioic acid (5). UV λ_{max} (CH_3OH) nm: 322 (log ε 1.5), 279 (3.0), 244 (2.8). MALDI-MS *m/z* (%): 507 (20), 344 (20), 240 (100). Anal. Calcd for $C_{22}H_{26}O_9$: C, 60.82, H, 6.03. Found: C, 61.00, H, 6.05. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

3.3.6. 3′-Methoxy-2,4′-oxyneolign-9,9′-dioic acid (6). UV λ_{max} (CH_3OH) nm: 280 (log ε 1.4). MALDI-MS *m/z* (%): 343 (20), 272 (30), 179 (100), 196 (35). Anal. Calcd for $C_{19}H_{20}O_6$: C, 66.27, H, 5.85. Found: C, 66.91, H, 5.90. ^1H and ^{13}C NMR data are listed in Tables 1 and 2.

3.4. Bioassays

Seeds of *Lactuca sativa* L. (cv. Cavolo di Napoli, collected during 2003, were obtained from Ingegnoli Spa (Milan, Italy). All undersized or damaged seeds were discarded and the assay seeds were selected for uniformity. For the bioassays we used Petri dishes of 50 mm diameter with one sheet of Whatman No. 1 filter paper as support. In four replicate experiments, germination and growth were conducted in aqueous solutions at controlled pH. Test solutions (10^{-4} M) were prepared using MES (2-[*N*-morpholino]ethanesulfonic acid, 10 mM, pH 6) and the rest (10^{-5}–10^{-7} M) were obtained by dilution. Parallel controls were performed. After adding 25 seeds and 2.5 ml test solutions, Petri dishes were sealed with Parafilm® to ensure closed-system models. Seeds were placed in a growth chamber KBW Binder 240 at 25 °C in the dark. Germination percentage was determined daily for 5 days (no more germination occurred after this time). After growth, plants were frozen at −20 °C to avoid subsequent growth until the measurement process. Data are reported as percentage differences from control in the graphics and tables. Thus, zero represents the control, positive values represent stimulation of the parameter studied and negative values represent inhibition.

Acknowledgements

NMR experiments have been performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche of University Federico II of Naples on a 500 MHz spectrometer of Consortium INCA.

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