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Cinnamic acid amides and lignanamides from *Aptenia cordifolia*

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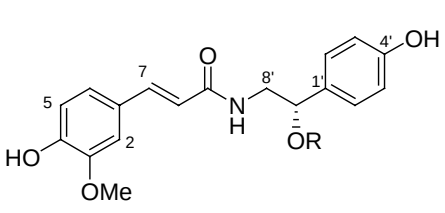
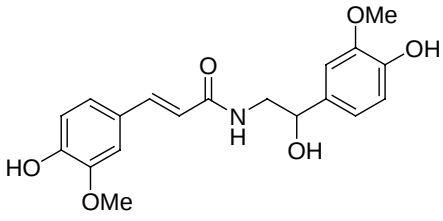
Dedicated to the memory of Professor Gaspare Barone (1943–2005)

Abstract—Examination of the hydroalcoholic extract of the leaves of *Aptenia cordifolia* has afforded three cinnamic acid amides and two lignanamides. Structures were established on the basis of spectroscopic data, including 2D-NMR analyses.

1. Introduction

As part of our ongoing research program on the identification of novel bioactive constituents from plants of the Mediterranean area, we have investigated *Aptenia cordifolia*. Previous chemical studies of the aerial part of *A. cordifolia* evidenced the presence of flavonoids.[1] A study of the leaves extract of *A. cordifolia* belonging to Aizoaceae family, led to the isolation and characterization of novel phytotoxic oxyneolignans.[2] Further examination of the hydroalcoholic extract of the leaves has afforded compounds with amide and lignanamide skeletons. Hydrocinnamic acid amides have been observed in several higher plant.[3] Some of this compounds were identified in the leaves of virus-infected tobacco and the authors[4] suggested that





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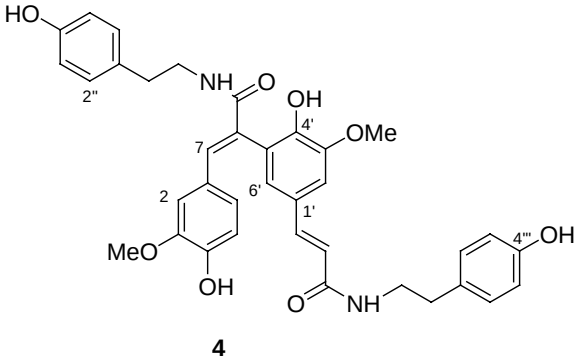

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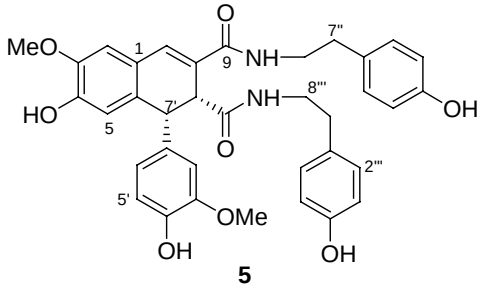

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Keywords: *Aptenia cordifolia*; Cinnamic acid amides; Lignanamides; Spectroscopic analysis.
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they have antiviral effect. Amides and lignanamides are common in *Cannabis sativa* and *Procelia macrocarpa*.[5]

2. Results and discussion

The hydroalcoholic infusion of fresh leaves of *A. cordifolia* was reduced in volume and precipitated with acetone. The acetone/water soluble part was fractionated by Amberlite XAD-2 column chromatography and fractions were purified by silica gel chromatography and HPLC, yielding five compounds **1**–**5**.

The molecular formula of compound **1** was deduced to be $C_{18}H_{19}NO_5$ as the HREIMS spectrum showed the $[M]^+$ ion at *m*/*z* 329.1267 (calcd for $[C_{18}H_{19}NO_5]^+$: 329.1263). The UV spectrum revealed bands at 222, 294, and 316 nm. Signals at δ 7.44 (1H, d, J=15.8 Hz, H-7), and 6.45 (1H, d, J=15.8 Hz, H-8), in the ^{1}H NMR spectrum, indicated the presence of a trans-substituted double bond. Signals at δ 7.12 (1H, d, J=1.9 Hz, H-2), 6.79 (1H, d, J=8.8 Hz, H-5), and 7.03 (1H, dd, J=8.8, 1.9 Hz, H-6) in the ^{1}H NMR spectrum suggested the presence of a 1,3,4-trisubstituted aromatic ring, as did signals at δ 111.7 (C-2), 116.1 (C-5), and 123.3 (C-6) in the ^{13}C NMR (DEPT) spectrum. In the HMBC spectrum, long-range correlations from the H-7 olefinic proton to the carbonyl carbon (δ 169.6, C-9) and the methine carbons C-2 and C-6 were observed, indicating the presence of a feruloyl group. Signals at δ 7.22 (2H, d, J=8.9 Hz, H-2′, H-6′), and 6.77 (2H, d, J=8.9 Hz, H-3′, H-5′) in the ^{1}H NMR spectrum, and signals at δ 128.4 (C-2′, C-6′), and 116.5 (C-3′, C5′), in the ^{13}C NMR (DEPT) spectrum, suggested the presence of a *p*-substituted aromatic ring. In the ^{1}H NMR spectrum, the signals at δ 4.72 (1H, dd, J=7.8, 4.9 Hz, H-7′), 3.53 (1H, dd, J=13.7, 4.9 Hz, H-8a′), 3.44 (1H, dd, J=13.7, 7.8 Hz, H-8b′), as well as signals at δ 73.5 (C-7′), and 48.1 (C-8′) in the ^{13}C NMR (DEPT) spectrum indicated the presence of a 2-amino ethanoyl chain. In the HMBC spectrum, long-range correlations from the H-8′ protons to the carbonyl carbon and the methine carbon (δ 73.5, C-7′) were observed, indicating the presence of a 2-hydroxy-2-(4-hydroxyphenyl) ethyl amine group (octopamine) linked to C-9 of the ferulic unit. The structure of compound **1** was *N*-[2-hydroxy-2-(4-hydroxyphenyl)ethyl] ferulamide. The optical rotation of **1** was found to be −3.0, establishing the *S* (−) absolute configuration at C-7′ chiral center.[6] The *R* (+) isomer was previously isolated from root bark of *Lycium chinense*[7] and it showed antifungal activity. This substance was synthesized in a combinatorial library of a small molecule that selectively induces apoptosis in cancer cells.[8]

Compound **2** was identified as *N*-feruloyl normetanephrine. It had molecular formula $C_{19}H_{21}NO_6$ as deduced from the molecular peak at *m*/*z* 359.1368 in the HREIMS spectrum. The UV spectrum revealed bands at 226, 298, and 320 nm. The ^{13}C NMR spectrum (Table 1) showed the presence of 17 signals. The DEPT experiment evidenced a methyl, a methylene, and nine methines. In the ^{1}H NMR spectra, signals corresponding to two 1,3,4-trisubstituted aromatic rings were present. The H-2, H-5 and H-6 of the ferulic moiety, in the ^{1}H NMR spectrum (Table 1), were at δ 7.13, 6.80, and 7.03, as a narrow doublet, a doublet, and a double doublet, respectively. The H-2′, H-5′, and H-6′ of the normetanephrine moiety were at δ 7.00, 6.77, and 6.83, respectively. Furthermore, the spectrum showed the H-7 and H-8 trans olefinic protons at δ 7.45 and 6.47, the H-8′ methylene as two double doublets at δ 3.54 and 3.44, and the H-7′ as a double doublet at δ 4.73. In a NOE experiment the protons of the methoxyl group at δ 3.88 had relation with the proton doublet at δ 7.13, and the protons of the methoxyls at δ 3.86 had relation with the protons at δ 7.00. Finally, the HMBC experiment evidenced the following

Table 1. ^{1}H and ^{13}C NMR data of compound **1**–**3** (CD_3OD, 500 MHz)[a]

No.	1		2		3	
	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C
1		128.4		128.7		128.8
2	7.12 (d, 1.9)	111.7	7.13 (d, 1.5)	112.0	7.15 (d, 2.5)	112.2
3		149.9		149.8		150.3
4		149.3		149.5		149.8
5	6.79 (d, 8.8)	116.1	6.80 (d, 8.5)	116.5	6.81 (d, 9.5)	117.0
6	7.03 (dd, 8.8, 1.9)	123.3	7.03 (dd, 8.5, 1.5)	123.8	7.05 (dd, 9.5, 2.5)	123.8
7	7.44 (d, 15.8)	142.3	7.45 (d, 16.0)	142.8	7.46 (d, 19.5)	142.7
8	6.45 (d, 15.8)	118.7	6.47 (d, 16.0)	119.1	6.49 (d, 19.5)	119.2
9		169.6		170.0		169.7
1′		134.5		128.7		132.7
2′	7.22 (d, 8.9)	128.4	7.00 (d, 1.5)	111.3	7.19 (d, 10.5)	129.6
3′	6.77 (d, 8.9)	116.5		147.6	6.82 (d, 10.5)	116.8
4′		158.1		150.5		158.8
5′	6.77 (d, 8.9)	116.5	6.77 (d, 8.6)	117.0	6.82 (d, 10.5)	116.8
6′	7.22 (d, 8.9)	128.4	6.83 (dd, 8.6, 1.5)	120.5	7.19 (d, 10.5)	129.6
7′	4.72 (dd, 7.8, 4.9)	73.5	4.73 (dd, 7.8, 4.9)	74.1	4.37 (dd, 8.2, 5.3)	81.5
8′	3.53 (dd, 13.7, 4.9), 3.44 (dd 13.7, 7.8)	48.1	3.54 (dd, 13.6, 4.8), 3.44 (dd, 13.6, 7.9)	48.8	3.51 (dd, 14.0, 5.3), 3.44 (dd 14.0, 8.2)	47.7
3-OMe	3.87 (s)	56.4	3.88 (s)	56.9	3.91 (s)	56.9
3′-OMe			3.86 (s)	56.9		
1″					3.28 (obscured)	72.1
2″					1.58 (ses, 7.6)	24.5
3″					0.92 (t, 7.6)	11.5

[a] J values are in parentheses and reported in Hz; chemical shifts are given in ppm; assignments were confirmed by COSY, HSQC, and HMBC experiments.

correlations: H-2′ with C-4′, H-5′ with C-1′ and C-3′, H-6′ with C-4′ and C-7′, H-8′ with C-9 and C-1′, H-2 with C-4, H-5 with C-1 and C-3, H-6 with C-4 and C-7, H-7 with C-9 and H-8 with C-1. This is the first report of *N*-feruloyl normetanephrine from plant extract. It was previously identified during the cloning and expression of a potato cDNA encoding hydroxycinnamoyl-CoA:tyramine *N*-(hydroxycinnamoyl) trasferase.[9]

The new compound **3** had the molecular formula $C_{21}H_{25}NO_5$ as deduced from the molecular peak at *m/z* 371.1730 in the HREIMS spectrum. The general features of its MS and NMR spectra closely resembled those of **1**, except that for the presence of 42 mass unit more than **1**, and three signals in the ^{1}H (δ 3.28, 1.58, and 0.92) and ^{13}C NMR (δ 72.1, 24.5, and 11.5) spectra attributed to an *n*-propyl group. The long-range correlations, in the HMBC spectrum, from the H-7′ protons to the C-8′ carbon and the methine carbon (δ 72.1, C-1″) were observed, indicating that the *n*-propyl group was linked at the alcoholic hydroxyl.

The new compound **4** showed the molecular ion peak at *m/z* 624 $[M]^+$ and significant fragments at *m/z* 488 $[M-136]^+$, 459 $[M-165]^+$ in the MALDI-MS spectrum. Its molecular formula was deduced to be, from the elemental analysis and NMR spectral data, $C_{36}H_{36}N_2O_8$. The UV spectrum revealed bands at 246, 315, and 336 nm. The ^{1}H and ^{13}C resonances of **4** were assigned by combination of COSY, DEPT, HMQC and HMBC experiments (Table 2). Two doublets at δ 7.40 and 6.38 (J=16.0 Hz), in the ^{1}H NMR spectrum, indicated the presence of a trans-substituted double bond. Furthermore, an olefinic proton singlet at δ 7.59 was evident. Signals at δ 6.52 (1H, d, J=2.0 Hz, H-2), 6.65 (1H, d, J=8.0 Hz, H-5), and 6.74 (1H, dd, J=8.0, 2.0 Hz, H-6), in the ^{1}H NMR spectrum, suggested the presence of a trisubstituted aromatic ring, as did correlations with the signals at δ 113.6 (C-2), 116.4 (C-5), and 116.8 (C-6) in the HSQC spectrum. The ^{1}H NMR spectrum showed two narrow doublets at δ 7.19 (1H, d, J=2.0 Hz, H-2′), 6.83 (1H, d, J=2.0 Hz, H-6′), of a 1,3,4,5-tetrasubstituted aromatic ring, correlated in the HSQC experiment at δ 112.2 and 125.1 carbon signals, respectively. Furthermore, eight *ortho*-coupled protons of two disubstituted aromatic rings were present as doublets at δ 7.04 (2H, d, J=8.0 Hz, H-2″, H-6″), 6.70 (2H, d, J=8.0 Hz, H-3″, H-5″), 6.91 (2H, d, J=8.0 Hz, H-2‴, H-6‴), and 6.62 (2H, d, J=8.0 Hz, H-3‴, H-5‴), correlated in the HSQC experiment to the carbon signals at δ 131.2 and 116.8. Signals at δ 3.47 (2H, t, J=6.8 Hz, H-8″), 2.75 (2H, t, J=6.8 Hz, H-7″), 3.43 (2H, t, J=6.8 Hz, H-8‴), 2.65 (2H, t, J=6.8 Hz, H-7‴), correlated in the HSQC experiment to the carbons at δ 43.0 (C-8″, C-8‴), 36.2 (C-7″), and 35.9 (C-7‴), indicated the presence of two 2-amino ethyl chains of a tyramine group. Long-range correlations between the H-7 olefinic proton and the carbonyl carbon (δ 170.4, C-9), the C-2 and C-6 methine carbons, and the C-1 and C-5′ quaternary carbons in the HMBC spectrum indicate the presence of a 8-linked feruloyl group. Long-range correlations from the H-7′ proton to the carbonyl carbon (δ 169.5), the C-2′, C-6′, C-8′ methine carbons, and the C-1′ quaternary carbon, in the HMBC spectrum indicating the presence of a 5-linked feruloyl group. These correlations were consistent with a 8-5′ neolignan structure. The correlations from the H-8″ protons to the C-9, and from the H-8‴ protons to the C-9′ indicate the presence of two tyramine groups linked to C-9, and C-9′ carbons. The analysis of the NOESY spectrum (Table 2) evidenced NOEs between the H-7 proton with H-2 and H-6, the H-2 proton with 3-OMe, the H-2′ with H-8′ and 3′-OMe, the H-7″ and H-8″ with H-2″ and H-6″, and the H-7‴ and H-8‴ with H-2‴ and H-6‴. These data confirmed the structure of compound **4** as depicted.

Table 2. NMR (CD_3OD, 500 MHz) data for compound **4**

No.	^{13}C (ppm)[a]	^{1}H δ (m, *J*/Hz)	NOESY	HMBC[b]
1	127.0			
2	113.6	65.2 (d, 2.0)	3-OMe	1, 3, 4, 7
3	150.6			
4	149.3			
5	116.4	6.65 (d, 8.0)		4
6	116.8	6.74 (dd, 8.0, 2.0)		2, 4, 7
7	139.1	7.59 (s)	2, 6	1, 2, 9, 5′
8	129.4			
9	170.4			
3-OMe	56.2	3.38 (s)	2	3
1′	128.8			
2′	112.2	7.19 (d, 2.0)	3′-OMe, 8′	3′, 6′, 7′
3′	149.9			
4′	148.7			
5′	129.7			1′, 4′
6′	125.1	6.83 (d, 2.0)	8′	1′, 2′, 4′, 7′
7′	141.9	7.40 (d, 16.0)		1′, 2′, 6′, 8′, 9′
8′	120.2	6.38 (d, 16.0)		1′, 9′
9′	169.5			
3′-OMe	57.2	3.96 (s)	2′	3′
1″	131.8			
2″	131.2	7.04 (d, 8.0)	7″, 8″	1″, 4″
3″	116.8	6.70 (d, 8.0)		1″, 2″, 4″, 6″
4″	157.3			
5″	116.8	6.70 (d, 8.0)		1″, 3″, 4″
6″	131.2	7.04 (d, 8.0)	7″, 8″	2″, 4″, 7″
7″	36.2	2.75 (t, 6.8)	2″, 6″	1″, 2″, 6″, 8″
8″	43.0	3.47 (t, 6.8)	2″, 6″	1″, 7″, 9
1‴	131.6			
2‴	131.2	6.91 (d, 8.0)	7‴, 8‴	1‴, 3‴, 4‴
3‴	116.8	6.62 (d, 8.0)		1‴, 2‴, 4‴
4‴	157.3			
5‴	116.8	6.62 (d, 8.0)		1‴, 3‴, 4‴
6‴	131.2	6.91 (d, 8.0)	7‴, 8‴	2‴, 4‴, 7‴
7‴	35.9	2.65 (t, 6.8)	2‴, 6‴	8‴
8‴	43.0	3.43 (t, 6.8)	2‴, 6‴	9′, 1‴, 7‴

[a] ^{13}C NMR assignments are supported by a DEPT experiment.
[b] HMBC correlations from H to C.

Compound **5** showed the molecular ion peak at *m/z* 624 $[M]^+$ and significant fragments at *m/z* 501 $[M-123]^+$, 488 $[M-136]^+$, 460 $[M-164]^+$ and 352 $[M-272]^+$ in the MALDI-MS spectrum. Its molecular formula was deduced to be, from the elemental analysis and NMR spectral data, $C_{36}H_{36}N_2O_8$. The ^{1}H and ^{13}C resonances of **5** were assigned by combination of COSY, DEPT, HMQC and HMBC experiments (Table 3). The ^{1}H NMR spectrum of **5** showed the presence of two tyramine moieties, six aromatic and/or olefinic protons, two methoxy signals and two methine protons signals, which were coupled with each other. The ^{1}H and ^{13}C NMR data of **5** were almost coincident with those reported by Sakakibara et al.[10] for the dihydronaphthalene lignan, cannabisin D. The authors indicated a trans configuration of the phenyl group at C-7′ and the

Table 3. NMR (CD_3OD, 500 MHz) data for compound **5**

No.	^{13}C (ppm)[a]	^{1}H δ (m, *J*/Hz)	NOESY	HMBC[b]
1	125.3			
2	113.7	6.88 (s)	7, 3-OMe	3, 4, 6, 7
3	148.7			
4	147.2			
5	117.7	6.52 (s)	7′	1, 3, 7′
6	133.0			
7	135.1	7.21 (s)	2	2, 8, 9, 8′
8	128.0			
9	170.0			
3-OMe	57.1	3.90 (s)	2	3
1′	136.4			
2′	113.0	6.69 (d, 1.0)	3′-OMe, 7′	4′, 6′, 7′
3′	150.1			
4′	146.0			
5′	116.2	6.66 (d, 8.0)		1′, 4′
6′	121.9	6.41 (dd, 8.0, 2.0)		2′, 4′, 7′
7′	48.1	4.36 (d, 3.9)	5, 2′	1, 5, 6, 8, 1′, 2′, 6′, 6′, 9′
8′	50.3	3.70 (d, 3.9)		6, 7, 8, 9, 8′, 9′
9′	175.0			
3′-OMe	56.8	3.75 (s)	2′	3′
1″	131.9			
2″	131.3	6.97 (d, 8.5)	7″	1″, 3″, 4″, 7″
3″	116.7	6.68 (d, 8.5)		1″, 4″, 5″
4″	157.4			
5″	116.7	6.68 (d, 8.5)		1″, 3″, 4″
6″	131.3	6.97 (d, 8.5)	7″	1″, 4″, 5″, 7″
7″	36.2	2.65 (t, 6.8)	2″, 6″	2″, 6″, 8″
8″	43.0	3.42 (t, 6.8)		9, 1″, 7″
1‴	131.6			
2‴	131.2	6.82 (d, 8.5)	7‴	1‴, 4‴, 7‴
3‴	116.7	6.65 (d, 8.5)		1‴, 4‴, 5‴
4‴	157.4			
5‴	116.7	6.65 (d, 8.5)		1‴, 3‴, 4‴
6‴	131.2	6.82 (d, 8.5)	7‴	1‴, 4‴, 7‴
7‴	35.9	2.50 (t, 6.8)	2‴, 6‴	2‴, 6‴, 8‴
8‴	42.9	3.23 (t, 6.8)		9′, 1‴

[a] ^{13}C NMR assignments are supported by a DEPT experiment.
[b] HMBC correlations from H to C.

amide carbonyl at C-8′ supported by the coupling constant between H-7′ and H-8′. Compound **5** showed, in the ^{1}H NMR spectrum, the relative protons as two doublets at 4.36 and 3.70 ($J=3.9$ Hz). The coupling constant between H-7′ and H-8′ indicates that the corresponding dihedral angle should be ca. 45°. The minimized structure obtained by MM2 calculation[11] was used to generate dihedral angles and an angle of 52° was measured, which is compatible with a cis configuration (Fig. 1).[12] Correspondingly, the NOESY spectrum of **5** showed NOE between H-7′ with H-5 and H-2′, so the quasi-equatorial orientation of bond C-7′–H-7′ was supposed, and quasi-axial orientation of C-8′–H-8′.

The compounds isolated from *A. cordifolia* were tested for their phytotoxicity on the seeds of *Lactuca sativa*.[13] This species was selected as representative of main dicotyledon commercial crops.[13] 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.[14,15] Aqueous solution of compounds **1**–**5**, ranging between 10^{-4} and 10^{-7} M, were tested on germination, root length and shoot length of treated lettuce seeds (Fig. 2). Compounds **2** and **3** reduced the germination by 20% compared to the control at 10^{-4} M,

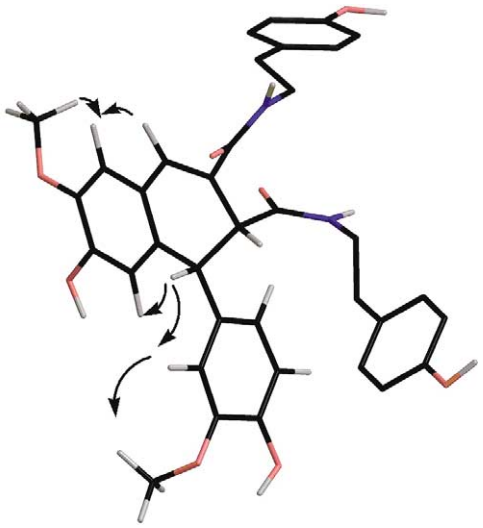

Figure 1. Selected NOEs of minimized structure of **5**.

and dose dependence effects were observed. Compounds **1** and **4** were inactive, and compound **5** reduced the germination by 11% at highest concentration tested. The root elongation of *L. sativa* was not affected by compounds tested, with exception of lignanamide **4** that showed 25% of inhibition at 10^{-4} M. Amongst compounds **1**–**5**, only amide **2** stimulated shoot elongation at all concentrations tested and no important effects were observed for compounds **1**, **3**, **4** and **5**.

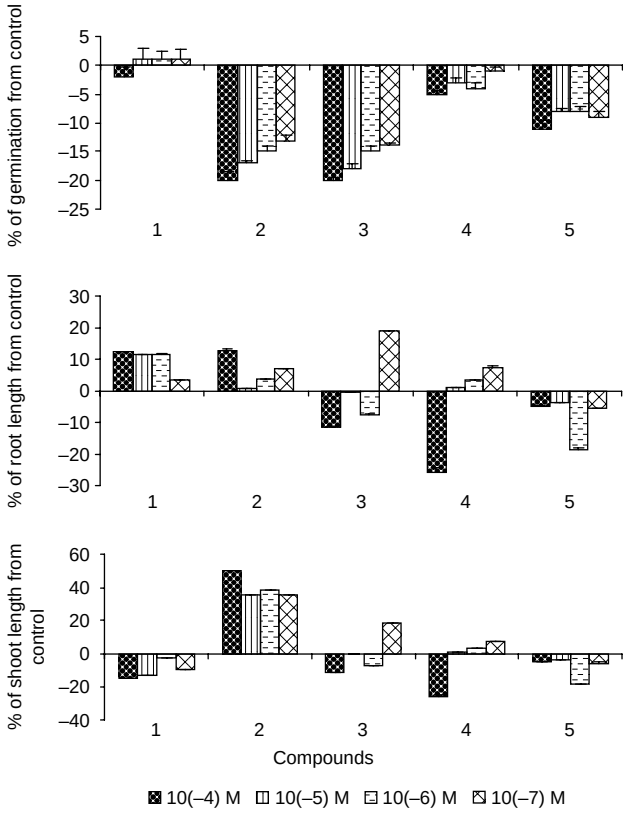

Figure 2. Effect of compounds **1**–**5** on *Lactuca sativa* L. Value presented as percentage differences from control.

The compounds **1**–**5** isolated from *A. cordifolia* could be considered as natural product hybrids.[16] This class of compounds usually exhibit a different biological activity to that of single components. Thus, they could represent a promising approach for the development of new lead structures for bioactive compounds for medicine and agriculture.[16]

3. Experimental

3.1. General experimental procedures

^{1}H and ^{13}C NMR spectra were run on a Varian INOVA 500 NMR spectrometer at 500 and 125 MHz, respectively, in CD_3OD. Matrix assisted laser desorption ionization (MALDI) mass spectra were recorded using a Voyager-DE MALDI-TOF mass spectrometer. MS spectra were obtained with a HP 6890 spectrometer equipped with a MS 5973 N detector. IR spectra were recorded in $CHCl_3$ on a Nicolet 5700 FT-IR spectrometer. UV–vis spectra were recorded in CH_3OH on a Perkin-Elmer Lambda 7 spectrophotometer. HPLC was performed on an Agilent 1100 by using an UV detector. Silica gel 60 (230–400 mesh, E. Merck) or Sephadex LH-20 (Pharmacia) was used for CC, and preparative TLC was performed on silica gel (UV-254 precoated) plates with 0.5 and 1.0 mm thickness (E. Merck). Preparative HPLC was performed using RP-18 (LiChrospher 10 μm, 250×10 mm i.d., Merck) column.

3.2. Plant material

Leaves of *A. 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

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 crude extract (450 g), cold CH_3COCH_3 (1.0 l) was added, and the mixture was placed on a stir plate in a cold room (−18 °C) overnight. The CH_3COCH_3 addition produced heavy precipitation consisting mostly of proteinaceous material, which was removed by centrifugation. The CH_3COCH_3 was removed by evaporation and the clear aqueous extract, reduced to 150 ml, was chromatographed on Amberlite XAD-2, with H_2O, CH_3OH and, CH_3COCH_3 to give six fractions.

The fraction eluted with CH_3COCH_3 (50.0 g) was rechromatographed on silica gel column to give 11 fractions.

Fraction 3 (4.3 g), eluted with AcOEt, was rechromatographed on SiO_2 flash column eluting with CH_2Cl_2–CH_3COCH_3 gradient to afford fractions A–I. Fraction D (51 mg), eluted with CH_2Cl_2–CH_3COCH_3 (9/1), was purified by preparative TLC [$CHCl_3$–CH_3COCH_3 (9/1)] and, reversed-phase HPLC column [CH_3OH–CH_3CN–H_2O (3/1/6)], to give **2** (8 mg). Fraction E (28 mg) eluted with CH_2Cl_2–CH_3COCH_3 (7/3) was purified by reversed-phase HPLC column [CH_3OH–CH_3CH–H_2O (6/3/11)], to give **1** (11 mg), **5** (2 mg) and, **4** (3 mg), respectively.

Fraction 8 (16.0 g), eluted with CH_3OH, was rechromatographed on SiO_2 flash column eluting with CH_2Cl_2–CH_3OH gradient to afford fractions A–G. Fraction F (20 mg) eluted with CH_2Cl_2–CH_3OH (11/9) was purified by reversed-phase HPLC column [H_2O–CH_3OH (1/1)], to give **3** (5 mg).

3.3.1. (2*S*,*E*)-*N*-[2-Hydroxy-2-(4-hydroxyphenyl)ethyl] ferulamide (1). Colourless oil; HREIMS *m/z* 329.1267 $[M]^+$ (calcd for $C_{18}H_{19}NO_5$ 329.1263); $[\alpha]_D^{25}$ −3.0 (*c* 0.12, CH_3OH); ν_{max} (CH_2Cl_2) 3580, 3400, 2940, 1705, 1670, 1592, 1424, 1334, 1019 cm^{-1}; UV λ_{max} (CH_3OH) nm (log ε): 222 (3.9), 294 (2.5), 316 (2.4); MALDI-MS *m/z* (%): 329 (40), 192 (10), 177 (30), 137 (100); δ_H (500 MHz, CD_3OD) 7.44 (1H, d, J=15.8 Hz, H-7), 7.22 (2H, d, J=8.9 Hz, H-2′, H-6′), 7.12 (1H, d, J=1.9 Hz, H-2), 7.03 (1H, dd, J=8.8, 1.9 Hz, H-6), 6.79 (1H, d, J=8.8 Hz, H-5), 6.77 (2H, d, J=8.9 Hz, H-3′, H-5′), 6.45 (1H, d, J=15.8 Hz, H-8), 4.72 (1H, dd, J=7.8, 4.9 Hz, H-7′), 3.87 (3H, s, 3-OMe), 3.53 (1H, dd, J=13.7, 4.9 Hz, H-8′a), 3.44 (1H, dd, J=13.7, 7.8 Hz, H-8′b); δ_C (125 MHz, CD_3OD) 169.6, 158.1, 149.9, 149.3, 142.3, 134.5, 128.4, 123.3, 118.7, 116.5, 116.1, 111.7, 7.35, 56.4, 48.1.

3.3.2. (*E*)-*N*-[2-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-ethyl] ferulamide (2). Colourless oil; HREIMS *m/z* 359.1368 $[M]^+$ (calcd for $C_{19}H_{21}NO_6$ 359.1269); $[\alpha]_D^{25}$ 0.0 (*c* 0.10, CH_3OH); ν_{max} (CH_2Cl_2) cm^{-1} 3676, 3433, 3046, 2940, 1663, 1601, 1512, 1424, 1057; UV λ_{max} (CH_3OH) nm (log ε): 226 (2.9), 298 (1.2), 320 (1.1); MALDI-MS *m/z* (%): 359 (15), 192 (20), 182 (40), 167 (100); δ_H (500 MHz, CD_3OD) 7.45 (1H, d, J=16.0 Hz, H-7), 7.13 (1H, d, J=1.5 Hz, H-2), 7.03 (1H, dd, J=8.5, 1.5 Hz, H-6), 7.00 (1H, d, J=1.5 Hz, H-2′), 6.83 (1H, dd, J=8.6, 1.5 Hz, H-6′), 6.80 (1H, d, J=8.5 Hz, H-5), 6.77 (1H, d, J=8.6 Hz, H-5′), 6.47 (1H, d, J=16.0 Hz, H-8), 4.73 (1H, dd, J=7.8, 4.9 Hz, H-7′), 3.88 (3H, s, 3-OMe), 3.86 (3H, s, 3′-OMe), 3.54 (1H, dd, J=13.6, 4.8 Hz, H-8′a), 3.44 (1H, dd, J=13.6, 7.9 Hz, H-8′b); δ_C (125 MHz, CD_3OD) 170.0, 150.5, 149.8, 149.5, 147.6, 142.8, 128.7, 123.8, 120.5, 119.1, 117.0, 116.5, 112.0, 111.3, 74.1, 56.9, 48.8.

3.3.3. (*E*)-*N*-[2-(4-Hydroxyphenyl)-2-propoxyethyl] ferulamide (3). Colourless oil; HREIMS *m/z* 371.1730 $[M]^+$ (calcd for $C_{21}H_{25}NO_5$ 371.1733); $[\alpha]_D^{25}$ −2.0 (*c* 0.08, CH_3OH); ν_{max} ($CHCl_3$) 3625, 3522, 3400, 2943, 1705, 1673, 1594, 1512, 1425, 1334, 1019 cm^{-1}; UV λ_{max} (CH_3OH) nm (log ε): 280 (3.1); MALDI-MS *m/z* (%): 372 (18), 254 (30), 209 (100), 163 (40); δ_H (500 MHz, CD_3OD) 7.46 (1H, d, J=19.5 Hz, H-7), 7.19 (2H, d, J=10.5 Hz, H-2′, H-6′), 7.15 (1H, d, J=2.5 Hz, H-2), 7.05 (1H, dd, J=9.5, 2.5 Hz, H-6), 6.82 (2H, d, J=10.5 Hz, H-3′, H-5′), 6.81 (1H, d, J=9.5 Hz, H-5), 6.49 (1H, d, J=19.5 Hz, H-8), 4.37 (1H, dd, J=8.2, 5.3 Hz, H-7′), 3.91 (3H, s, 3-OMe), 3.51 (1H, dd, J=14.0, 5.3 Hz, H-8′a), 3.44 (1H, dd, J=14.0, 8.2 Hz, H-8′b), 3.28 (2H, m, H-1″), 1.58 (2H, ses, J=7.6 Hz, H-2″), 0.92 (3H, t, J=7.6 Hz, H-3″); δ_C (125 MHz, CD_3OD) 169.7, 158.8, 150.3, 149.8, 142.7, 132.7, 129.6,

128.8, 123.8, 119.2, 117.0, 116.8, 112.2, 81.5, 72.1, 56.9, 47.7, 24.5, 11.5.

3.3.4. (*E*,*E*)-*N*,*N*-Dityramin-4,4′-dihydroxy-3,5′-dimethoxy-β,3′-bicinnamamide (4). Amorphous powder; UV λ_{max} (CH_3OH) nm (log ε): 336 (0.9), 315 (2.6), 246 (3.2); ν_{max} ($CHCl_3$) 3684, 3595, 2927, 2857, 1740, 1598, 1509, 1455, 1057 cm^{-1}; MALDI-MS *m/z* (%): 624 (100), 488 (40), 459 (20), 136 (50). Anal. Calcd for $C_{36}H_{36}N_2O_8$: C, 69.22, H, 5.81, N, 4.48. Found: C, 68.96, H, 5.84, N, 4.35. δ_H (500 MHz, CD_3OD) 7.59 (1H, s, H-7), 7.40 (1H, d, J=16.0 Hz, H-7′), 7.19 (1H, d, J=2.0 Hz, H-2′), 7.04 (2H, d, J=8.0 Hz, H-2″, H-6″), 6.91 (2H, d, J=8.0 Hz, H-2‴, H-6‴), 6.83 (1H, d, J=2.0 Hz, H-6′), 6.74 (1H, dd, J=8.0, 2.0 Hz, H-6), 6.70 (2H, d, J=8.0 Hz, H-3″, H-5″), 6.65 (1H, d, J=8.0 Hz, H-5), 6.62 (2H, d, J=8.0 Hz, H-3‴, H-5‴), 6.52 (1H, d, J=2.0 Hz, H-2), 6.38 (1H, d, J=16.0 Hz, H-8′), 3.96 (3H, s, 3′-OMe), 3.47 (2H, t, J=6.8 Hz, H-8″), 3.43 (2H, t, J=6.8 Hz, H-8‴), 3.38 (3H, s, 3-OMe), 2.75 (2H, t, J=6.8 Hz, H-7″), 2.65 (2H, t, J=6.8 Hz, H-7‴); δ_C (125 MHz, CD_3OD) 170.4, 169.5, 157.3, 150.6, 149.9, 149.3, 148.7, 141.9, 139.1, 131.8, 131.6, 131.2, 129.7, 129.4, 128.8, 127.0, 125.1, 120.2, 116.8, 116.4, 113.6, 112.2, 57.2, 56.2, 43.0, 36.2, 35.9.

3.3.5. 7-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-*N*^{2},*N*^{3}-bis(4-hydroxyphenethyl)-6-methoxy-1,2-dihydronaphthalene-2,3-dicarboxamide (5). Amorphous powder; $[\alpha]_D^{25}$ 0.0 (*c* 0.20, CH_3OH); ν_{max} ($CHCl_3$) 3684, 3595, 3414, 3043, 2981, 1660, 1605, 1513, 1431, 1308, 945 cm^{-1}; UV λ_{max} (CH_3OH) nm (log ε): 223 (3.9), 284 (1.2), 333 (1.1); MALDI-MS *m/z* (%): 624 (100), 501 (10), 488 (30), 460 (10), 352 (10). Anal. Calcd for $C_{36}H_{36}N_2O_8$: C, 69.22, H, 5.81, N, 4.48. Found: C, 68.86, H, 5.78, N, 4.40. δ_H (500 MHz, CD_3OD) 7.21 (1H, s, H-7), 6.97 (2H, d, J=8.5 Hz, H-2″, H-6″), 6.88 (1H, s, H-2), 6.82 (2H, d, J=8.5 Hz, H-2‴, H-6‴), 6.69 (1H, d, J=1.0 Hz, H-2′), 6.68 (2H, d, J=8.5 Hz, H-3″, H-5″), 6.66 (1H, d, J=8.0 Hz, H-5′), 6.65 (2H, d, J=8.5 Hz, H-3‴, H-5‴), 6.52 (1H, s, H-5), 6.41 (1H, dd, J=8.0, 2.0 Hz, H-6′), 4.36 (1H, d, J=3.9 Hz, H-7′), 3.90 (3H, s, 3-OMe), 3.75 (3H, s, 3′-OMe), 3.70 (1H, d, J=3.9 Hz, H-8′), 3.42 (2H, t, J=6.8 Hz, H-8″), 3.23 (2H, t, J=6.8 Hz, H-8‴), 2.65 (2H, t, J=6.8 Hz, H-7″), 2.50 (2H, t, J=6.8 Hz, H-7‴); δ_C (125 MHz, CD_3OD) 175.0, 170.0, 157.4, 150.1, 148.7, 147.2, 146.0, 136.4, 135.1, 133.0, 131.9, 131.6, 131.3, 131.2, 128.0, 125.3, 121.9, 117.7, 116.7, 116.2, 113.7, 113.0, 57.1, 56.8, 50.3, 48.1, 43.0, 42.9, 36.2, 35.9.

3.4. Bioassays

Seeds of *L. 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.

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