Cinnamic Ester Derivatives from *Oxalis pes-caprae* (Bermuda Buttercup)[#]

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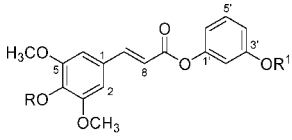
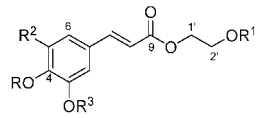
Seven new cinnamic ester derivatives (**1**–**7**) were isolated from a methanol extract of the fresh leaves and twigs of *Oxalis pes-caprae* (Bermuda buttercup). The structures of these new compounds were determined by spectroscopic data interpretation. The effects of compounds **1**–**7** on the germination and growth of *Lactuca sativa* (lettuce) were studied.

Bermuda buttercup, also known as *Oxalis pes-caprae* L. (Oxalidaceae), a native plant of South Africa, is a very successful, globally widely distributed, aggressive colonized invasive weed,[1] due to its ability to rapidly uptake limited resources and because it can maintain dense monocultures and high levels of allelopathy.[2] In the search for new potential allelochemicals from plants, we have studied the weed *O. pes-caprae*, which is now widely distributed in Italy and commonly found on cultivated lands. The above-ground biomass of *Oxalis* species contains oxalic acid and is toxic to large herbivores (livestock) when consumed in large quantities.[3] One of the most useful aspects of allelopathy in manipulated ecosystems is its role in agriculture.[4] In this paper, we report the characterization of seven new cinnamic ester derivatives (**1**–**7**) together with several known phenols and cinnamic acids, from the leaves and twigs of *O. pes-caprae.*



1 R = R^1 = CH_3
2 R = CH_3, R^1 = H
3 R = R^1 = H



4 R = R^3 = CH_3, R^1 = H, R^2 = OCH_3
5 R = R^1 = H, R^2 = OCH_3, R^3 = CH_3
6 R = R^3 = CH_3, R^1 = $COCH_3$, R^2 = OCH_3
7 R = R^1 = R^2 = R^3 = H

A crude methanolic infusion of fresh *O. pes-caprae* was partitioned between EtOAc and water. The EtOAc extract was fractionated by silica gel column chromatography, and the fractions were purified by preparative thin-layer chromatography and HPLC, yielding pure compounds. The known compounds were identified by direct comparison with authentic samples as caffeic acid, 4-hydroxybenzyl alcohol, 4-hydroxycinnamic acid, 3-methoxy-4-hydroxybenzoic acid, methyl 4-hydroxycinnamate, resorcinol, sinapic acid, and β-sitosterol.

Compound **1** was determined to have the molecular formula $C_{19}H_{20}O_6$ from the molecular ion at *m/z* 344.1256 in the HREIMS. The ^{1}H NMR and COSY spectra revealed two sets of 1,3,4,5-tetrasubstituted and *m*-disubstituted benzene rings, a disubstituted *trans*-double bond, and four methoxyls (Table 1). The ^{13}C NMR spectrum showed 15 carbon signals (Table 2). The DEPT spectrum showed three methyls and seven methines, which were correlated to the corresponding protons by a HSQC experiment. In a NOESY experiment, the protons of the methoxyl groups at δ 3.91 correlated with the proton singlet at δ 6.82, and the protons of the methoxyl at δ 3.86 correlated with the proton resonances at δ 6.73 and 6.79. In a HMBC experiment, the following correlations were observed: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-2/C-6, C-8, and C-9; H-8 with C-1 and C-9; H-2′ with C-3′ and C-4′; H-4′ and H-6′ with C-1′ and C-2′; and H-5′ with C-1′ and C-3′. Thus, the structure (*E*)-3-methoxyphenyl 3,4,5-trimethoxycinnamate was established for compound **1**.

The molecular formula of **2** was determined to be $C_{18}H_{18}O_6$ by HREIMS ([M]$^+$, *m/z* 330.1100), which was consistent with the disappearance of a methyl signal in the ^{1}H and ^{13}C NMR spectra compared with those of **1**. The H-2 and H-6 resonances of the 3,4,5-trimethoxycinnamoyl moiety, in the ^{1}H NMR spectrum (Table 1), appeared at δ 6.82 as a singlet. In turn, the H-5′, H-4′, H-6′, and H-2′ signals of the 3-hydroxyphenyl moiety were observed at δ 7.25, 6.74, 6.72, and 6.68, respectively. Furthermore, the ^{1}H NMR spectrum showed resonances of the H-7 and H-8 *trans*-olefinic protons at δ 7.78 and 6.53 and three methoxyl groups at δ 3.91. In a NOESY experiment, the protons of the methoxyl groups correlated with the proton singlet at δ 6.82. The HMBC experiment demonstrated the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H-2′ with C-3′ and C-4′; H-4′ and H-6′ with C-1′ and C-2′; and H-5′ with C-1′ and C-3′. On the basis of these observations, compound **2** was assigned as (*E*)-3-hydroxyphenyl 3,4,5-trimethoxycinnamate.

The molecular formula of **3** was determined to be $C_{17}H_{16}O_6$ by HREIMS ([M]$^+$, *m/z* 316.0943). Comparison of its ^{1}H and ^{13}C NMR data with those of **2** suggested that the methyl group at C-4 was missing. The H-2 and H-6 signal of the sinapoyl moiety in the ^{1}H NMR spectrum (Table 1) was at δ 6.82 as a singlet, and the H-5′, H-4′/H-6′, and H-2′ signals of the 3-hydroxyphenyl moiety were at δ 7.22, 6.70, and 6.66, respectively. The H-7 and H-8 *trans*-olefinic protons resonated at δ 7.77 and 6.47, respectively, and two methoxyl groups were observed at δ 3.92. In a NOESY experiment, the protons of the methoxyl groups correlated with the proton singlet at δ 6.82. In a HMBC study, the correlations were comparable to those of **2**. Accordingly, compound **3** was identified as 3-hydroxyphenyl sinapate.

It is noteworthy that *m*-substituted aromatic products, like compounds **1**–**3**, as potential products of shikimic pathway origin, are very rare.[5] Instead, *m*-substituted benzene rings are common in natural structures of polyketide origin.[6]

Compound **4** was identified as (*E*)-2-hydroxyethyl 3,4,5-trimethoxycinnamate. It gave a molecular formula of $C_{14}H_{18}O_6$, as deduced from the molecular ion peak at *m/z* 282.1100 in the HREIMS. The ^{13}C NMR spectrum (Table 2) showed the presence of 11 signals, with a DEPT experiment revealing signals of one methyl group, two methylenes, and three methines. In the ^{1}H NMR spectrum, signals corresponding to a 1,3,4,5-tetrasubstituted benzene ring were present. The H-2 and H-6 signal of the 3,4,5-trimethoxycinnamoyl moiety resonated in the ^{1}H NMR spectrum (Table 1) as

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Table 1. ^{1}H NMR Data (δ) of **1–7** (500 MHz, $CDCl_3$)[a]

position	1	2	3	4	5	6	7[b]
2	6.82 s	6.82 s	6.82 s	6.75 s	6.77 s	6.76 s	7.04 d (2.0)
5							6.77 d (7.5)
6	6.82 s	6.82 s	6.82 s	6.75 s	6.77 s	6.76 s	6.95 dd (2.0, 7.5)
7	7.78 d (15.9)	7.78 d (16.0)	7.77 d (16.2)	7.63 d (16.0)	7.64 d (15.6)	7.63 d (16.0)	7.58 d (16.0)
8	6.53 d (15.9)	6.53 d (16.0)	6.47 d (16.2)	6.38 d (16.0)	6.38 d (15.6)	6.37 d (16.0)	6.29 d (16.0)
1′				4.35 m	4.35 m	4.42 m	4.24 m
2′	6.73 t br (2.2)	6.68 t br (2.0)	6.66 d (2.1)	3.90 m	3.87 m	4.36 m	3.79 m
4′	6.79 dd (2.2,8.2)	6.74[c] dd (2.0, 8.5)	6.70 d br (8.4)				
5′	7.30 t (8.2)	7.25 t (8.5)	7.22 t (8.4)				
6′	6.77 dd (2.0, 8.2)	6.72[c] dd (2.0, 8.5)	6.70 d br (8.4)				
OCH_3-3	3.91 s	3.91 s	3.92 s	3.88 s	3.91 s	3.89 s	
OCH_3-4	3.91 s	3.91 s		3.88 s		3.89 s	
OCH_3-5	3.91 s	3.91 s	3.92 s	3.88 s	3.91 s	3.89 s	
OCH_3-3′	3.86 s						
OAc-3′						2.11 s	

[a] Assignments are based on COSY, HSQC, HMBC, and NOESY. [b] Recorded in CD_3OD. [c] Values are exchangeable.

Table 2. ^{13}C NMR Data (δ) of **1–7** (125 MHz, $CDCl_3$)[a]

position	1	2	3	4	5	6	7[b]
1	129.5	129.6	130.0	129.7	129.6	129.8	128.2
2	105.6	105.5	105.4	105.3	105.1	105.4	115.5
3	153.6	153.5	151.6	153.4	147.2	153.5	150.1
4	153.6	140.8	137.6	141.8	138.8	142.0	147.3
5	153.6	153.5	151.6	153.4	147.2	153.5	115.6
6	105.6	105.5	105.4	105.3	105.1	105.4	123.4
7	146.6	146.7	147.3	145.4	145.7	145.5	147.6
8	116.5	116.3	114.7	116.8	115.2	116.7	117.0
9	165.3	165.4	166.0	167.2	167.4	166.7	169.8
1′	151.8	152.1	147.3	66.2	66.1	62.3	67.4
2′	107.7	109.2	109.3	61.4	61.5	62.3	61.7
3′	160.6	156.6	156.9				
4′	111.7[c]	113.0[d]	113.2[e]				
5′	129.9	130.1	130.0				
6′	113.9[c]	113.7[d]	113.4[e]				
OCH_3-3	56.2	56.2	56.3	56.1	56.3	56.2	
OCH_3-4	61.0	61.0		60.9		61.0	
OCH_3-5	56.2	56.2	56.3	56.1	56.3	56.2	
OCH_3-3′	55.4						
OAc-3′						170.8	
						20.9	

[a] Assignments are based on HSQC and HMBC. [b] Recorded in CD_3OD. [c] Values with same superscript are exchangeable. [d] Values with same superscript are exchangeable. [e] Values with same superscript are exchangeable.

a singlet at δ 6.75. The signals H-2′ and H-3′ of the hydroxyethyl chain were at δ 4.35 and 3.90, respectively. Furthermore, the H-7 and H-8 *trans*-olefinic protons occurred at δ 7.63 and 6.38, and three methoxyl groups were observed at δ 3.88. In a NOESY experiment, the protons of the methoxyl groups correlated with the proton singlet at δ 6.75. The HMBC experiment exhibited the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H-1′ with C-9 and C-2′; H-2′ with C-1′.

Compound **5** was identified as 2-hydroxyethyl sinapate. It showed the molecular formula $C_{13}H_{16}O_6$ from the molecular ion peak at *m*/*z* 268.0950 in the HREIMS. The ^{13}C NMR spectrum (Table 2) exhibited 10 signals, including from a DEPT experiment one methyl, two methylenes, and three methines. In the ^{1}H NMR spectrum (Table 1), signals corresponding to a 1,3,4,5-tetrasubstituted benzene ring were present. The H-2 and H-6 signal of the sinapoyl moiety occurred at δ 6.77 as a singlet, with the H-1′ and H-2′ signals of the 2-hydroxyethyl moiety at δ 4.35 and 3.87, respectively, the H-7 and H-8 *trans*-olefinic protons at δ 7.64 and 6.38, and two methoxyl groups at δ 3.91. In a NOESY experiment, the protons of the methoxyl groups correlated with the proton singlet at δ 6.77. A HMBC experiment gave the following correlations: H-2/H-6 with C-3/C-5, C-4, and C-7; H-7 with C-1, C-2/C-6, and C-9; H-8 with C-1 and C-9; H-1′ with C-9 and C-2′; H-2′ with C-1′.

Compound **6** was identified as the acetyl derivative of compound **4**. Its ^{1}H NMR spectrum (Table 1) showed the presence of an additional methyl group signal at δ 2.11, and the ^{13}C NMR spectrum (Table 2) showed a methyl and carbonyl at δ 20.9 and 170.8, respectively. This identification was confirmed by the HREIMS that exhibited a molecular ion peak at *m*/*z* 324.1205, according to the molecular formula $C_{16}H_{20}O_7$.

Compound **7** was identified as 2-hydroxyethyl caffeate. It was assigned the molecular formula $C_{11}H_{12}O_5$, as deduced from the molecular ion peak at *m*/*z* 224.0688 in the HREIMS. The ^{13}C NMR spectrum (Table 2) showed the presence of 11 signals, assigned by a DEPT experiment as one methyl group, two methylenes, and five methines. In the ^{1}H NMR spectrum, signals corresponding to a 1,3,4-trisubstituted benzene ring were evident. The H-2, H-5, and H-6 signals for the caffeoyl moiety, in the ^{1}H NMR spectrum (Table 1), were observed at δ 7.04, 6.77, and 6.95, as a narrow doublet, a doublet, and a double doublet, respectively. The H-1′ and H-2′ resonances of the 2-hydroxyethyl moiety were at δ 4.24 and 3.79, respectively. Furthermore, the spectrum showed the H-7 and H-8 *trans*-olefinic protons at δ 7.58 and 6.29. In a NOESY experiment, the protons of the methoxyl group corrrelated with the narrow proton doublet at δ 7.04. Finally, the HMBC experiment gave the following correlations: H-2 with C-3, C-4, and C-7; H-6 with C-2, C-4, and C-5; H-7 with C-1, C-2, C-6, and C-9; H-8 with C-1 and C-9; H-1′ with C-9 and C-2′; and H-2′ with C-1′.

The phytotoxicity of various known compounds on the seeds of the dicotyledonous *Lactuca sativa* L. (lettuce) has been reported previously.[7–9] The new compounds **1–7** were tested against *L. sativa* to evaluate the inhibitory or stimulatory effects on germination, root length, and shoot length of the tested seeds, and the results expressed in Figure 1 are reported as percentage differences from the control. The activities of compounds **1–7** were compared with that of pendimethalin (**P**), a commercial pre-emergence herbicide used widely in agriculture. Aqueous solutions of the compounds, ranging from 10^{-4} to 10^{-7} M, were investigated in accordance with the procedures optimized by Macias et al.[10]

The effects of **1–7** on the inhibition of germination of *L. sativa* seeds showed a variable behavior within 10–20% at higher concentrations and were more active than pendimethalin. The results reported in Figure 1B show greater phytotoxic activities on lettuce root length by compounds **2**, **3**, **6**, and **7**, with respect to this standard herbicide. The test compounds revealed ≥80% inhibition at 10^{-4} M concentration, and compound **7** was found to be completely active up to 10^{-7} M. Compound **7** showed a radical and shoot inhibition of 100% at all concentrations tested. This compound was tested at lower concentrations (10^{-8} and 10^{-9} M), and it showed no relevant effects on germination and shoot length, while slight stimulatory effects were observed on root length (results not shown). Finally, all compounds reduced shoot length by 60–80% at the highest concentration tested (Figure 1C), with the exception

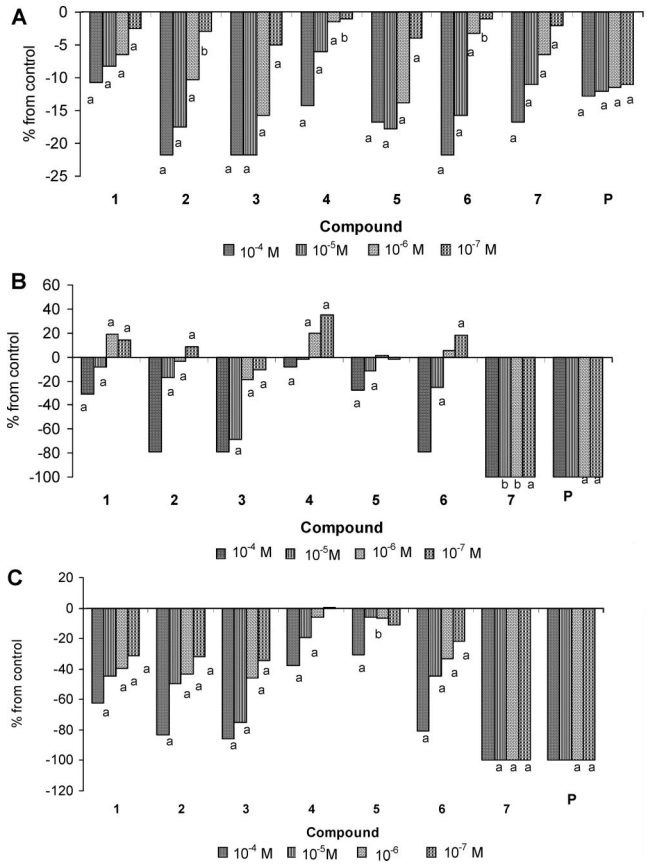

Figure 1. Effects of compounds **1**–**7** and pendimethalin (**P**) on germination (A), root length (B), and shoot length of *Lactuca sativa* L. Values are presented as percentage differences from control and are not significantly different with $P > 0.05$ for Student's t test. (a) $P < 0.01$; (b) $0.01 < P < 0.05$.

of compounds **4** and **5**, which reduced shoot length by 30–40% at 10^{-4} M, and compound **7**, demonstrating a full inhibitory effect at all concentrations tested.

Experimental Section

General Experimental Procedures. IR spectra were recorded on a JASCO FT/IR-430 instrument. HPLC was performed on an Agilent 1100 instrument using a UV detector. ^{1}H and ^{13}C NMR spectra were run on a Varian INOVA 500 NMR spectrometer at 500 and 125 MHz, respectively, in $CDCl_3$. Mass spectra were obtained with a HP 6890 spectrometer equipped with a MS 5973 N detector. Silica gel 60 (230–400 mesh, E. Merck) or Sephadex LH-20 (Pharmacia) was used for column chromatography, and preparative TLC was performed on silica gel (UV-254 precoated) plates of 0.5 and 1.0 mm thickness (E. Merck). Preparative HPLC was performed using a RP-18 (LiChrospher 10 μm, 250 × 10 mm i.d., Merck) column.

Plant Material. Leaves and twigs of *Oxalis pes-caprae* were collected from Bacoli-Naples, Italy, in April 2005, and identified by a botanist, Prof. Antonino Pollio, Dipartimento di Biologia Vegetale, University of Naples. A voucher specimen (HERBNAPY-126) was deposited at the Botanical Gardens of the University of Federico II of Naples, Italy.

Extraction and Isolation. Fresh leaves and twigs (21.0 kg) of the plant were powdered and extracted with MeOH at room temperature (25 °C) for 7 days. The solution was concentrated and partitioned between EtOAc and H_2O. The crude EtOAc residue (125 g) was chromatographed on silica gel eluting with mixtures of petroleum ether, ethyl ether, CH_2Cl_2, EtOAc, Me_2CO, MeOH, and H_2O to give 35 fractions.

Fraction 4 (480 mg), eluted with petroleum ether, was rechromatographed on silica gel, eluting with petroleum ether and Me_2CO, to give five fractions. Fraction 2 (40 mg), eluted with petroleum ether–Me_2CO (9:1), was subjected to preparative TLC (petroleum ether–Me_2CO, 4:1) to give resorcinol (1,3-dihydroxybenzene) (4.5 mg) and compound **6** (6.9 mg): IR ($CHCl_3$) ν_{max} 3000, 2971, 1741, 1711, 1638, 1575, 1243 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 324 $[M]^+$ (33), 238 $[M - C_4H_6O_2]^+$ (50); HREIMS m/z 324.1205 (calcd for $C_{16}H_{20}O_7$, 324.1209).

Fraction 10 (5.8 g), eluted with petroleum ether–ethyl ether (9:1), was rechromatographed on silica gel eluting with petroleum ether, ethyl ether, EtOAc, and MeOH to give 16 fractions. Fraction 1 (30 mg), eluted with petroleum ether–ethyl ether (17:3), was subjected to preparative TLC (petroleum ether–Me_2CO, 4:1) to give methyl 4-hydroxycinnamate (18.5 mg) and compound **1** (5.1 mg): IR ($CHCl_3$) ν_{max} 3003, 2961, 2941, 1719, 1639, 1584, 1137 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 344 $[M]^+$ (40), 313 $[M - OCH_3]^+$ (30), 221 $[M - C_7H_7O_2]^+$ (60); HREIMS m/z 344.1256 (calcd for $C_{19}H_{20}O_6$, 344.1260).

Fraction 11 (850 mg), eluted with petroleum ether–ethyl ether (4:1), was rechromatographed on silica gel, eluting with petroleum ether and ethyl ether to give seven fractions. Fraction 3 (34 mg), eluted with petroleum ether–ethyl ether (9:1), was subjected to preparative TLC (petroleum ether–Me_2CO, 3:1) to give 4-hydroxybenzyl alcohol (1.5 mg), β-sitosterol (5.0 mg), and compound **3** (4.1 mg): IR ($CHCl_3$) ν_{max} 3527, 3030, 2932, 2854, 1724, 1636, 1513, 1146, 1249 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 316 $[M]^+$ (35), 207 $[M - C_6H_5O_2]^+$ (38); HREIMS m/z 316.0943 (calcd for $C_{17}H_{16}O_6$, 316.0947).

Fraction 12 (2.2 g), eluted with petroleum ether–Me_2CO (17:3), was rechromatographed on silica gel, eluting with petroleum ether, ethyl ether, and Me_2CO, to give 17 fractions. Fraction 3 (80 mg), eluted with ethyl ether–Me_2CO (4:1), was subjected to preparative TLC (petroleum ether–Me_2CO, 7:3) to give 3-methoxy-4-hydroxybenzoic acid (8.5 mg), 4-hydroxycinnamic acid (4.5 mg), and compound **5** (4.3 mg): IR ($CHCl_3$) ν_{max} 3600, 3020, 1725, 1590, 1218 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 268 $[M]^+$ (50), 207 $[M - C_2H_5O_2]^+$ (65); HREIMS m/z 268.0950 (calcd for $C_{13}H_{16}O_6$, 268.0947).

Fraction 14 (134 mg), eluted with petroleum ether–Me_2CO (4:1), was rechromatographed on silica gel, eluting with petroleum ether, EtOAc, and Me_2CO, to give six fractions. Fraction 2 (65 mg), eluted with petroleum ether–EtOAc (4:1), was subjected to passage over a Sep-Pak column (Waters, Millford, MA) (MeCN–MeOH–H_2O, 2:1:2) to give compound **2** (4.0 mg): IR ($CHCl_3$) ν_{max} 3596, 3010, 2926, 1729, 1579, 1513, 1217 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 330 $[M]^+$ (28), 229 $[M - OCH_3]^+$ (60), 221 $[M - C_6H_5O_2]^+$ (62); HREIMS m/z 330.1100 (calcd for $C_{18}H_{18}O_6$, 330.1103).

Fraction 20 (3.2 g), eluted with CH_2Cl_2–EtOAc (8:2), was rechromatographed on silica gel eluting with CH_2Cl_2, Me_2CO, and MeOH to give 15 fractions. Fraction 2 (50 mg), eluted with CH_2Cl_2–Me_2CO (19:1), was subjected to preparative TLC (petroleum ether–Me_2CO, 7:3) to give sinapic acid (28.5 mg) and compound **4** (11.3 mg): IR ($CHCl_3$) ν_{max} 3606, 2938, 1712, 1637, 1578, 1457 cm^{-1}; ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 282 $[M]^+$ (42), 221 $[M - C_2H_5O_2]^+$ (52); HREIMS m/z 282.1100 (calcd for $C_{14}H_{18}O_6$, 282.1103).

Fraction 25 (40 mg), eluted with CH_2Cl_2 – MeOH (9:1), was subjected to preparative TLC (CH_2Cl_2–MeOH, 22:3) to give caffeic acid (16.5 mg) and compound **7** (5.1 mg): ^{1}H NMR and ^{13}C NMR data, see Tables 1 and 2; MS m/z 224 $[M]^+$ (30), 163 $[M - C_2H_5O_2]^+$ (63); HREIMS m/z 224.0688 (calcd for $C_{11}H_{12}O_5$, 224.0685).

Bioassays. Seeds of *Lactuca sativa* L. (cv. Napoli V. F.), collected during 2005, were obtained from Ingegnoli S.p.a, Milan, Italy. All undersized or damaged seeds were discarded, and the assay seeds were selected for uniformity. Bioassays used Petri dishes (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, using MES (2-[*N*-morpholino]ethanesulfonic acid, 10 mM, pH 6). Test solutions (10^{-4} M) were prepared in MES, and the remainder (10^{-5}–10^{-7} M) were obtained by dilution. Parallel controls were performed. After adding 25 seeds and 5 mL of test solutions, Petri dishes were sealed with Parafilm to ensure closed-system models. Seeds were placed in a KBW Binder 240 growth chamber at 25 °C in the dark. Germination percentage was determined daily for five 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 figures. Thus, zero represents the control; positive

values represent stimulation of the control; positive values represent stimulation of the parameter studied; and negative values represent inhibition.

Statistical Treatment. The statistical significance of differences between groups was determined by a 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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