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Cinnamic acid amides from *Chenopodium album*: effects on seeds germination and plant growth

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Abstract

Seven cinnamic acid amides have been isolated from *Chenopodium album*. The structures have been attributed by means of their spectral data. One of them, *N*-*trans*-4-*O*-methylferuloyl 4′-*O*-methyldopamine, is described for the first time. Their effects on germination and growth of dicotyledons *Lactuca sativa* L. (lettuce) and *Lycopersicon esculentum* L. (tomato) and of monocotyledon *Allium cepa* L. (onion) as standard target species have been studied in the range concentration 10^{-4}–10^{-7} M.

Keywords: *Chenopodium album*; *Lactuca sativa*; *Lycopersicon esculentum*; *Allium cepa*; Phytotoxic compounds; Cinnamic acid amides; Spectroscopic analysis; Toxicity test

1. Introduction

Allelochemicals involved in weed-crop interference may serve as source for natural herbicides or can be models for synthetic compounds. *Chenopodium album* is an odorless, branching, largely annual weed diffused in cultivated fields (Holm et al., 1977), commonly known as lambsquarters.

Mallik et al. (1994) reported the presence of growth inhibitory substances in this plant. They evidenced the aqueous extract inhibited the germination and growth of radish and wheat seeds, attributing the activity to the presence of phenols. In a previous study Horio et al. (1993) reported the isolation of a phenolic amide with attracting activity toward the zoospores of *Aphanomyces cochlioides*.

In a reinvestigation of *Chenopodium album* we have isolated seven cinnamic acid amides, which have been tested for their effects on seed germination and growth on *Lactuca sativa*, *Lycopersicon esculentum*, and *Allium cepa*.

2. Results and discussion

The methanol infusion of fresh plants of *Chenopodium album*, after removal of the solvent in vacuum, was suspended in water and precipitated with acetone. Crude aqueous fraction of lambsquarter reduced germination of *Lactuca sativa*, *Lycopersicon esculentum* and *Allium cepa* seeds: 50% inhibition was observed at 10 mg/ml concentration. The fraction was extracted with ethyl acetate and the organic layer was separated by conventional procedures into an acidic and a neutral fraction. The neutral portion was fractionated by silica gel column chromatography and the fractions were purified by preparative layer chromatography and HPLC yielding seven cinnamic amides **1**–**7**.

Compound **1** identified as *N*-*trans*-feruloyl 4′-*O*-methyldopamine, has been isolated from the roots of the same plant (Horio et al., 1993). Compounds **2**–**6** were already known: *N*-*trans*-feruloyl 3′-*O*-methyldopamine (**2**), *N*-*trans*-feruloyl tyramine (**3**) and *N*-*trans*-4-*O*-methylferuloyl 3′,4′-*O*-dimethyldopamine (**4**) have been isolated from *Spinacia oleracea* (Suzuki et al., 1981), from *Hypecoum* sp. (Hussain et al., 1982) and from *Zanthoxylum rubescens* (Adesina et al., 1989), respectively. *N*-*trans*-4-*O*-Methylcaffeoyl 3′-*O*-methyldopamine

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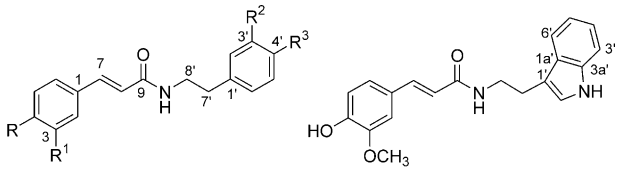
(**5**) and *N-trans*-feruloyl tryptamine (**6**) have been synthesized by Tanaka et al. (1989) and Ehmann (1974), respectively.



1 R = R^2 = OH R^1= R^3= OCH_3
2 R = R^3 = OH R^1= R^2= OCH_3
3 R = R^3 = OH R^1= OCH_3 R^2= H
4 R = R^1 = R^2 = R^3 = OCH_3
5 R = R^2 = OCH_3 R^1= R^3= OH
7 R = R^1 = R^3= OCH_3 R^2= OH

6

Compound **7** was identified as *N-trans*-4-*O*-methylferuloyl 4′-*O*-methyldopamine. It had a molecular formula $C_{20}H_{23}NO_5$ according to the molecular ion at *m*/*z* 357 in its EIMS spectrum and elemental analysis. The ^{13}C NMR spectrum (Table 1) showed the presence of only eighteen signals, with the methyls of the three methoxyl groups having almost the same chemical shift. The DEPT experiment defined the carbons as three methyls, two methylenes, eight methines and seven quaternary carbons. In the ^1H NMR spectrum the H-2′, H-5′ and H-6′ protons of the dopamine moiety were present as a narrow doublet, a large doublet and a double doublet at δ 6.84, 6.86 and 6.76, respectively, while those H-2, H-5 and H-6 of the ferulic moiety were at δ 7.06, 6.90 and 7.11, respectively. Furthermore, the spectrum showed the H-7′ and H-8′ methylenes as two triplets at δ 2.79 and 3.62 and the H-7 and H-8 olefinic protons as two doublets at δ 7.60 and 6.25. According to the structure, in a NOE experiment the protons of the methoxyl group at δ 3.88 had relation with the proton doublet at δ 6.86, and the protons of the methoxyls at δ 3.90 had relations with the protons doublets at δ 7.06 and 6.90. Finally the HMBC experiment evidenced the following 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-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.

Table 1
^{13}C NMR data of **1**, **2**, **4**–**7**

C	**1**[b]	**2**[a]	**4**[b]	**5**[a]	**6**[c]	**7**[b]
1	127.3	128.3	127.7	128.2	126.7	127.8
2	109.7	111.6	109.5	111.5	109.8	109.5
3	146.8	149.9	149.1	149.9	147.2	149.1
4	145.4	149.0	150.5	147.6	147.6	150.5
5	114.8	116.5	111.0	116.5	114.9	111.0
6	122.1	123.2	121.9	123.2	121.8	122.0
7	141.1	142.0	140.9	142.0	140.7	140.9
8	118.2	118.8	118.4	118.7	117.5	118.5
9	166.4	169.2	166.1	169.2	167.0	166.1
1′	132.1	132.1	131.4	133.5	111.9	132.1
2′	115.0	113.5	111.9	113.0	122.2	114.9
3′	145.4	149.3	149.1	149.3	111.1	145.7
4′	145.7	146.1	147.7	147.5	118.7	145.3
5′	110.9	116.2	111.3	116.4	121.4	110.9
6′	120.2	122.3	120.6	120.9	118.1	120.2
7′	34.9	36.2	35.1	36.0	24.9	35.0
8′	40.8	42.5	40.8	42.4	39.7	40.8
1a′					127.1	
3a′					136.2	
3-OMe	55.9	56.4	55.9		55.4	55.9
4-OMe			55.9	56.5		55.9
3′-OMe		56.4	55.9	56.5		
4′-OMe	56.0		55.9			55.9

[a] CD_3OD.
[b] $CDCl_3$.
[c] $CDCl_3$–CD_3OD (4:1).

Preliminary tests evidenced the phytotoxicity of the aqueous extract of *C. album* on dicotyledons, *Lactuca sativa* L. (lettuce), and *Lycopersicon esculentum* L. (tomato), and the monocotyledon *Allium cepa* L. (onion). These species were selected as representatives of main monocotyledon and dicotyleton commercial crops (Macias et al., 2000). The activity resembled that reported by Mallik et al. (1994) on radish and wheat seeds. The seven amides of *C. album* were tested on lettuce, tomato and onion to evaluate the effects on germination and seedling growth. The assays were performed according to the procedures optimised by Macias et al. (2000). The results are reported as percentage differences of germination (Fig. 1), root elongation (Fig. 2) and shoot elongation (Fig. 3) from the control.

Compounds **2**, **4**–**7** caused about 15% inhibition on germination of lettuce in the tested concentrations range and no dose dependence effect was observed (Fig. 1a). Compound **1** was not active, while compound **3** caused 45% inhibition at the highest concentration tested. Comparable effects were also found on tomato (Fig. 1b). The responses on onion germination were different: compounds **2**, **5** showed inhibitory effects, compounds **6**, **7** were inactive and compounds **1**, **3** stimulated the germination (Fig. 1c). The effects of amides on the root length of dicotyledons were quite small (Fig. 2a and b). At 10^{-4} M concentration about 15% inhibition was observed on lettuce, while on tomato only compounds **3**, **4** and **6** caused the same inhibition. On the contrary the root length of onion was stimulated by amides, with exception of **5** that causes 50% reduction at 10^{-5} M (Fig. 2c). The compounds stimulated the shoot length of lettuce, while tomato response was opposite with exception of compound **4** (Fig. 3a and b). Onion shoot length was inhibited by the compounds at all concentration tested (Fig. 3c). Bioactivity of cinnamic acid amides isolated from *C. album* showed a variable response on the tested species and for some compounds no dose dependence effects were observed. The reason for this response may be due to differences in seeds size,

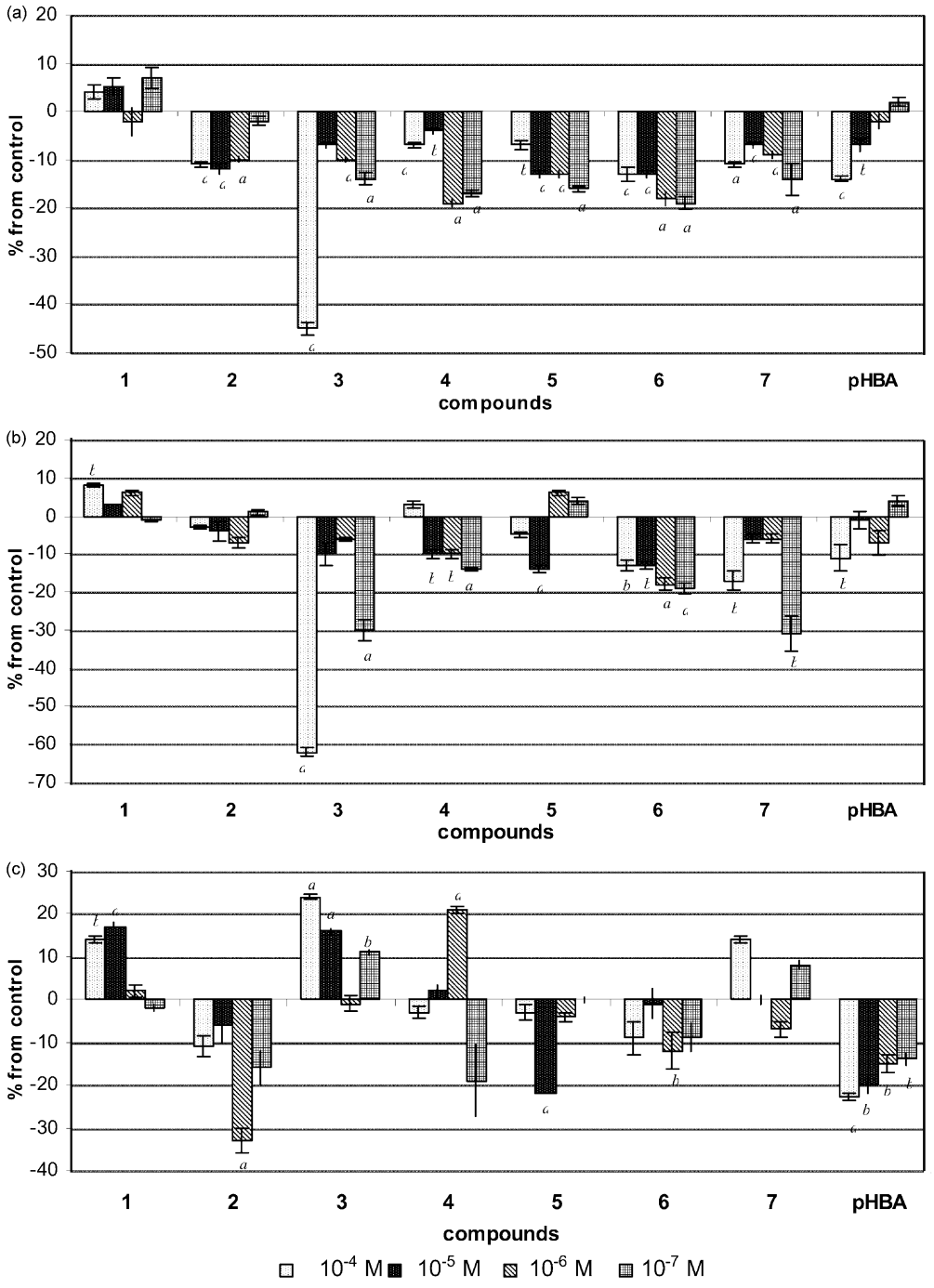


Fig. 1. Effect of compounds **1–7** and pHBA (4-hydroxybenzoic acid) on germination of *Lactuca sativa* (a), *Lycopersicon esculentum* (b), and *Allium cepa* (c). Value presented as percentage differences from control and are not significantly different with $P > 0.05$ for Student's *t*-test. *a*, $P < 0.01$; *b*, $0.01 < P < 0.05$.

seed coat permeability, differential uptake and metabolism (Macias et al., 1997). To evaluate the potency of active compounds **1–7** it was compared with the value for 4-hydroxybenzoic acid, which is known to be an effective germination inhibitor (Sebeson et al., 1969; Mizutani, 1999) and data are reported in Fig. 1. The inhibition value at 10^{-4} M on lettuce for 4-hydroxybenzoic acid was comparable to that of amides **2**, **6** and **7** and lower for **3**. The effects on tomato at highest concentration tested were about the same for compounds **6** and **7** and amide **3** resulted six-fold more toxic than the control. Anti-germination effects on onion were higher for 4-hydroxybenzoic acid than amides. These resulted indicated the possible implication of these amides in inhibitory activity detected in the aqueous extract.

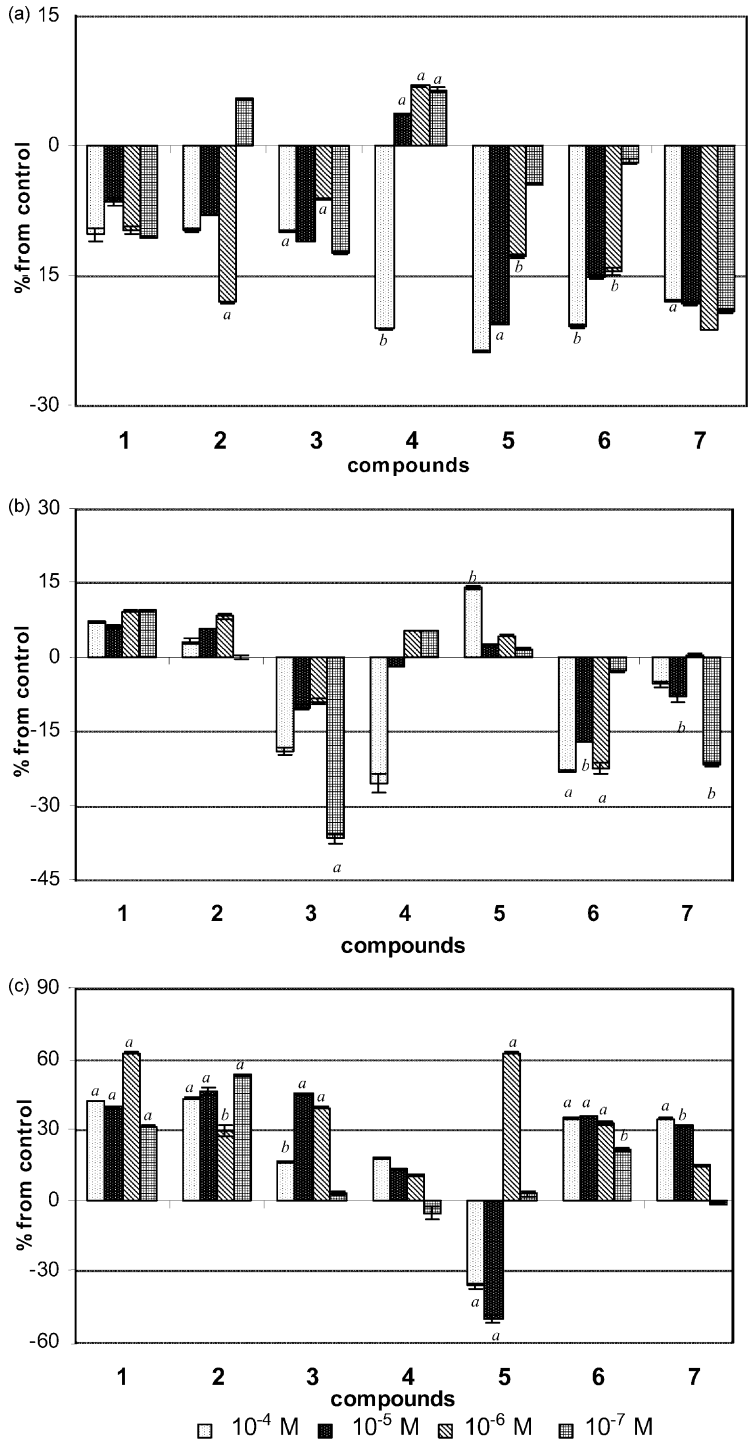


Fig. 2. Effect of compounds **1–7** on root length of *Lactuca sativa* (a), *Lycopersicon esculentum* (b), and *Allium cepa* (c). Value presented as percentage differences from control and are not significantly different with $P > 0.05$ for Student's *t*-test. *a*, $P < 0.01$; *b*, $0.01 < P < 0.05$.

3. Experimental

3.1. General experimental procedures

NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Varian INOVA spectrometer at 25 °C. Proton-detected heteronuclear correlations were measured using HMQC (optimised for $^{1}J_{HC} = 160$ Hz) and HMBC (optimised for $^{1}J_{HC} = 8$ Hz). MS spectra were obtained with a HP 6890 spectrometer equipped with a MS 5973 N detector. HPLC was performed on an Agilent 1100 by using an UV detector. TLC was performed on a Merck Kiesegel 60 F_{254} with 0.2 mm layer thickness. Preparative HPLC was performed using

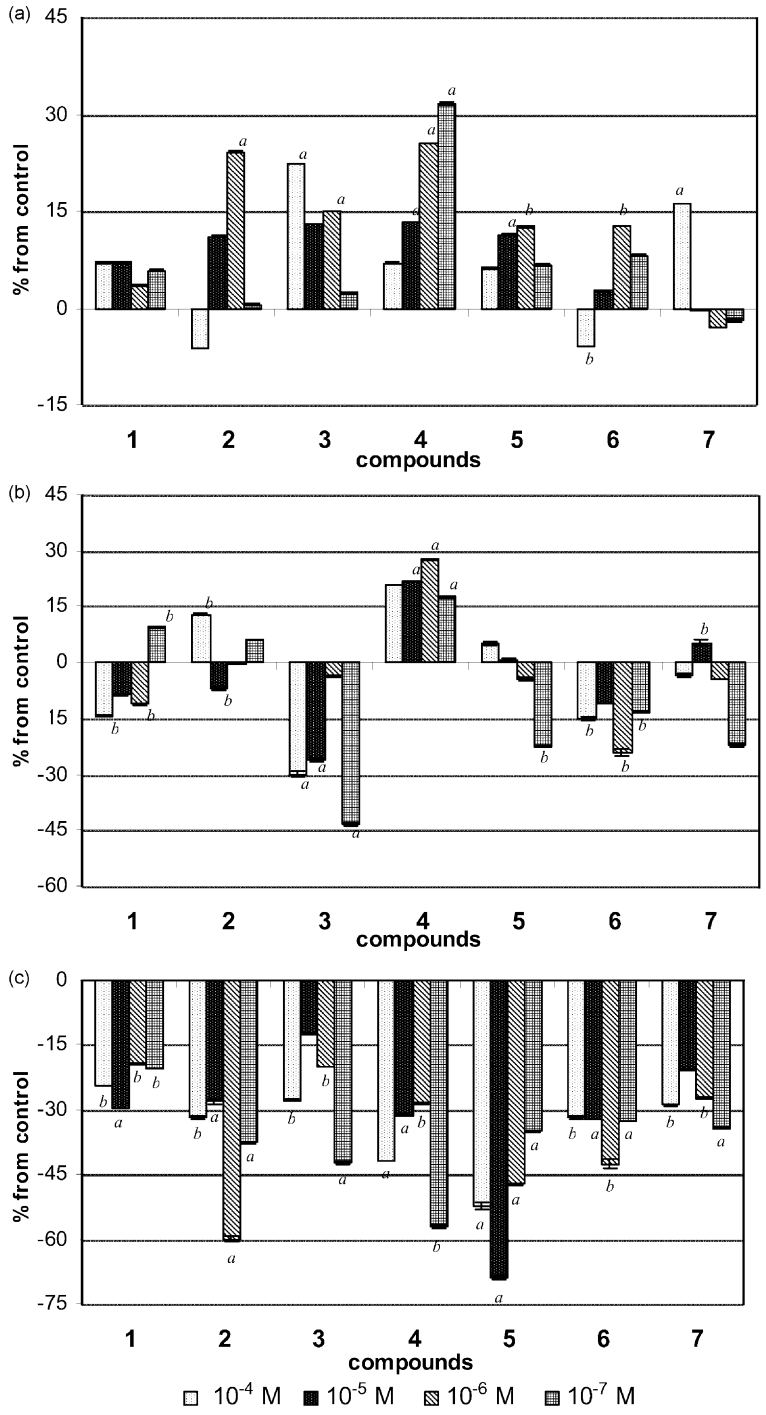


Fig. 3. Effect of compounds **1–7** on shoot length of *Lactuca sativa* (a), *Lycopersicon esculentum* (b), and *Allium cepa* (c). Value presented as percentage differences from control and are not significantly different with $P > 0.05$ for Student's *t*-test. *a*, $P < 0.01$; *b*, $0.01 < P < 0.05$.

SiO_2 (LiChrospher Silica 10 μm, 250×10 mm i.d., Merck) and RP-18 (LiChrospher 10 μm, 250×10 mm i.d., Merck) columns. Analytical TLC was performed on Merck Kieselgel 60 F_{254} or RP-18 F_{254} plates with 0.2 mm film thickness. 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 a Merck Kiesegel 60 F_{254} plates, with 0.5 or 1 mm film thickness. Flash column chromatography was performed on Merck Kiesegel 60 (230–400 mesh) at medium pressure.

3.2. Plant material

Aerial part of plants of *Chenopodium album* were collected near Caserta (Italy) during the autumn and

identified by Professor Antonino Pollio of the Dipartimento di Biologia Vegetale of University of Naples. A voucher specimen (HERBNAPY620) is deposited at the Dipartimento di Biologia Vegetale of University Federico II of Naples.

3.3. Extraction and isolation

The plant material (2.4 kg) was sequentially extracted with H_2O–MeOH (9:1) and methanol at room temperature for 7 days. The extracts were frozen and stored at −80 °C until used. The phytotoxicity of these extracts were determined by the bioassay, described in the experimental section, with *Lactuca sativa*, *Lycopersicon esculentum* and *Allium cepa*.

3.3.1. Methanol extract fractionation

To an aqueous suspension (700 ml) of the MeOH extract (150 g), cold acetone was added (1.0 l), and the mixture was placed on a stir plate overnight in a cold room. The acetone addition produced heavy precipitation consisting mostly of proteinaceous materials, which was removed by centrifugation. The acetone was removed by evaporation and the clear aqueous extract, reduced to 200 ml, was extracted with EtOAc. The organic layer was extracted with 2 N HCl and the organic phase was neutralized. After removal of the solvent, the crude residue (32 g) was chromatographed on silica gel column to give fractions A–Z.

Fraction P (455 mg) eluted with $CHCl_3$–MeOH (9:1) was filtered on Sephadex LH-20 using hexane–$CHCl_3$–MeOH (1:3:1) to give fraction 1–3. Fraction 1 (68 mg) consisted in a mixture of **4** and **7**, which were separated by TLC [$CHCl_3$–acetone (22:3), 15 and 8 mg, respectively].

Fraction 2 (132 mg), was rechromatographed on silica gel column. The fractions eluted with $CHCl_3$–MeOH (95:5) gave the crude of **1**, **3** and **5**. Compound **3** was purified by preparative TLC [petrol–acetone (3:2), 10 mg]. Compound **1** was purified by flash column chromatography [$CHCl_3$–MeOH (9:1), 12 mg]. Compound **5** was purified by reverse phase C-18 HPLC with MeOH–CH_3CN–H_2O (3:2:5) (7 mg). Fraction 3 (214 mg), was rechromatographed on silica gel column. The fraction eluted with $CHCl_3$–acetone (17:3) consisted in a mixture of **2** and **6**, which was resolved by reverse phase C-18 HPLC with MeOH–CH_3CN–H_2O (3:2:5) to give pure **2** (4 mg) and pure **6** (5 mg).

3.3.2. Compound characterisation

N-trans-Feruloyl 3′-*O*-methyldopamine (**2**). Colourless oil; ^{1}H NMR spectral data (CD_3OD): δ 7.44 (1H, *d*, J = 15.8 Hz, H-7), 7.12 (1H, *d*, J = 1.8 Hz, H-2), 7.03 (1H, *dd*, J = 7.7, 1.8 Hz, H-6), 6.82 (1H, *d*, J = 1.8 Hz, H-2′), 6.80 (1H, *d*, J = 7.7 Hz, H-5), 6.73 (1H, *d*, J = 8.2 Hz, H-5′), 6.67 (1H, *dd*, J = 8.2, 1.8 Hz, H-6′), 3.90 (3H, *s*, 3-OMe), 3.82 (3H, *s*, 4′-OMe), 3.49 (2H, *t*, J = 7.1 Hz, H-8′) 2.77 (2H, t, J = 7.1, H-7′). ^{13}C NMR: see Table 1. EI-MS: *m*/*z* 337.

N-trans-4-O-Methylcaffeoyl 3′-O-methyldopamine (**5**). Colourless oil; ^{1}H NMR spectral data (CD_3OD): δ 7.45 (1H, *d*, J = 15.5 Hz, H-7), 7.13 (1H, *d*, J = 1.6 Hz, H-2′), 7.04 (1H, *dd*, J = 8.0, 1.6 Hz, H-6′), 6.95 (1H, *d*, J = 2.0 Hz, H-2), 6.85 (1H, *d*, J = 8.0 Hz, H-5), 6.81 (1H, *d*, J = 8.0 Hz, H-5′), 6.69 (1H, *dd*, J = 8.0, 2.0 Hz, H-6), 6.42 (1H, *d*, J = 15.5 Hz, H-8), 3.89 (3H, *s*, 4′-OMe), 3.83 (3H, *s*, 3-OMe), 3.48 (2H, *t*, J = 7.6 Hz, H-8′), 2.76 (2H, t, J = 7.6, H-7′). ^{13}C NMR: see Table 1. EI-MS: *m*/*z* 337.

N-trans-Feruloyl tryptamine (**6**). Colourless oil; ^{1}H NMR spectral data ($CDCl_3$/CD_3OD 4/1): δ 7.51 (1H, *dd*, J = 7.6, 2.0 Hz, H-6′), 7.36 (1H, *d*, J = 15.6 Hz, H-7), 7.29 (1H, *dd*, J = 7.4, 2.2 Hz, H-3′), 7.07 (1H, *m*, H-5′), 6.98 (1H, *m*, H-4′), 6.97 (1H, *s*, H-2′), 6.90 (1H, *dd*, J = 8.6, 1.4 Hz, H-6), 6.88 (1H, *d*, J = 1.4 Hz, H-2), 6.72 (1H, *d*, J = 8.6 Hz, H-5), 6.13 (1H, d, J = 15.6 Hz, H-8), 3.77 (3H, *s*, 3-OMe), 3.56 (2H, *t*, J = 7.8 Hz, H-8′), 2.92 (2H, *t*, J = 7.8, H-7′). ^{13}C NMR: see Table 1. EI-MS: *m*/*z* 336.

N-trans-4-O-Methylferuloyl 4′-O-methyldopamine. (**7**). Colourless oil; ^{1}H NMR spectral data ($CDCl_3$): δ 7.60 (1H, *d*, J = 15.0 Hz, H-7), 7.11 (1H, *dd*, J = 8.0, 1.5 Hz, H-6), 7.06 (1H, *d*, J = 1.5 Hz, H-2), 6.90 (1H, *d*, J = 8.0 Hz, H-5), 6.86 (1H, *d*, J = 7.8 Hz, H-5′), 6.84 (1H, *d*, J = 1.5 Hz, H-2′), 6.76 (1H, *dd*, J = 7.8, 1.5 Hz, H-6′), 6.25 (1H, *d*, J = 15.0 Hz, H-8), 3.90 (6H, *s*, 3-OMe, 4-OMe), 3.88 (3H, *s*, 4′-OMe), 3.62 (2H, *t*, J = 7.0 Hz, H-8′), 2.79 (2H, *t*, J = 7.0, H-7′). ^{13}C NMR: see Table 1. EI-MS: *m*/*z* 357. Elemental analysis: found: C, 67.10; H, 6.36, N, 3.98. $C_{20}H_{23}NO_5$ requires: C, 67.21; H, 6.49; N, 3.92%.

3.4. Bioassays

Seeds of *Lactuca sativa* L. (cv. Cavolo di Napoli), *Lycopersicon esculentum* L. (cv. Napoli V. F.) and *Allium cepa* L. (cv. Ramata di Milano), collected during 2001, 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 in two sizes: 90 (tomato and onion) and 50 (lettuce) 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 solns. (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 5 ml test solutions for 90 mm dishes and 2.5 ml test solns for 50 mm dishes, Petri dishes were sealed with Para-

film® 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 for lettuce and tomato and for seven days for onion (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 control; positive values represent stimulation of the parameter studied and negative values represent inhibition.

3.5. 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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