

## Isolation and Phytotoxicity of Apocarotenoids from *Chenopodium album*

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Received April 21, 2004

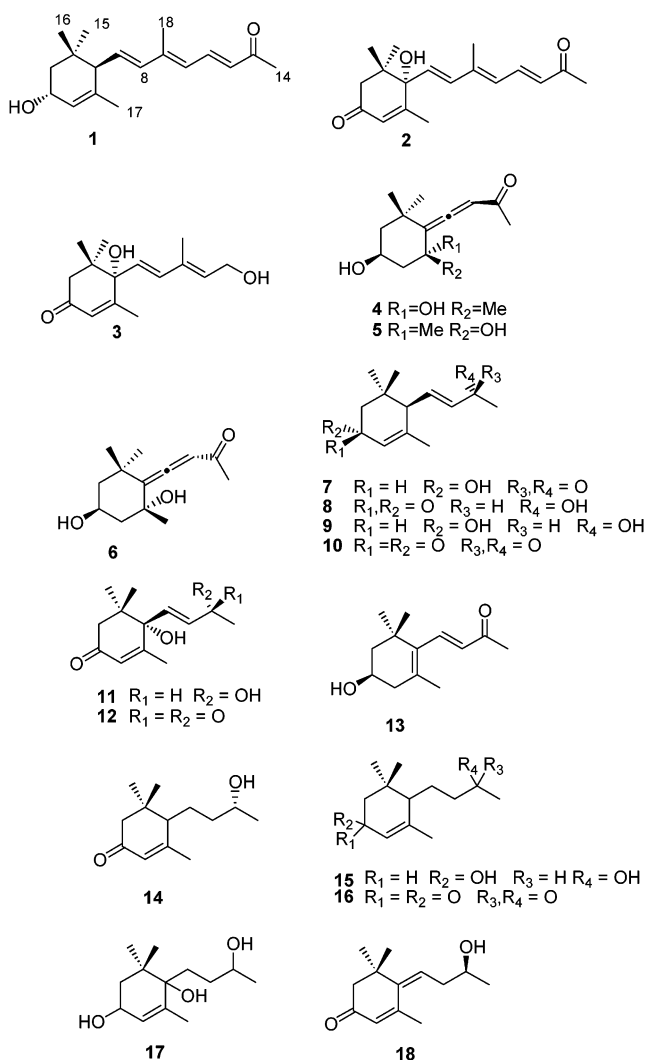
Two new compounds (**1**, **2**) and 16 apocarotenoids (**3**–**18**) were isolated from the weed *Chenopodium album*. The structures of new apocarotenoids were determined to be (3*R*,6*R*,7*E*,9*E*,11*E*)-3-hydroxy-13-apo- $\alpha$ -caroten-13-one (**1**) and (6*S*,7*E*,9*E*,11*E*)-3-oxo-13-apo- $\alpha$ -caroten-13-one (**2**) by spectroscopic, NMR, and MS analysis. Five of the known compounds (**5**, **6**, **13**, **15**, and **17**) were previously reported only as synthetic compounds. Effects of these compounds on germination and growth of *Lactuca sativa* (lettuce) were studied in the  $10^{-4}$ – $10^{-7}$  M concentration range.

*Chenopodium album* (Chenopodiaceae) is an odorless, branching, largely annual weed often found in cultivated fields and commonly known as lambsquarters.<sup>1</sup> Mallik et al.<sup>2</sup> reported the presence of growth inhibitory substances in this plant. They observed that the aqueous extract inhibited the germination and growth of radish and wheat seeds, attributing the activity to the presence of phenols. Horio et al.<sup>1</sup> reported the isolation of a phenolic amide with attractant activity toward zoospores of *Aphanomyces cochliformis*. In a reinvestigation of *C. album* we isolated seven cinnamic acid amides, which were tested for their effects on seed germination and growth of *Lactuca sativa*, *Lycopersicon esculentum*, and *Allium cepa*.<sup>3</sup> Analysis of an aqueous infusion has now led to the isolation of 18 apocarotenoids (**1**–**18**) identified on the basis of spectroscopic and chiroptic methods and comparison with literature data.

### Results and Discussion

An aqueous infusion of fresh *C. album* plants was reduced in volume and treated with acetone. The crude aqueous fraction was extracted with CH<sub>2</sub>Cl<sub>2</sub>. The CH<sub>2</sub>Cl<sub>2</sub> extract was fractionated by silica gel column chromatography, and the fractions were purified by preparative layer chromatography and HPLC, yielding 18 compounds, **1**–**18**.

Compound **1** showed an absorption maximum at 329 nm, suggesting the presence of a carotenal type chromophore.<sup>4</sup> The molecular formula was determined to be C<sub>18</sub>H<sub>26</sub>O<sub>2</sub> by HREIMS. Of the two oxygen functions, one was ascribed to a secondary hydroxyl group ( $\delta_{\text{H}}$  4.26,  $\delta_{\text{C}}$  65.8) and the remaining one was attributed to a carbonyl group ( $\delta_{\text{C}}$  198.6) using <sup>1</sup>H NMR and <sup>13</sup>C NMR data. The structure of **1** was established using <sup>1</sup>H NMR and <sup>13</sup>C NMR including COSY, ROESY, HMQC, and HMBC experiments. The <sup>1</sup>H–<sup>1</sup>H COSY experiment showed a correlation series beginning with the olefinic methine at  $\delta$  5.58, assigned to H-4, which was coupled with a carbinol methine at  $\delta$  4.26 (H-3), which was also coupled with two double doublets at  $\delta$  1.84 and 1.40 assigned to the H-2 methylene. The proton signal at  $\delta$  6.20 assigned to H-8 was correlated with the H-7 proton ( $\delta$  5.69), which was coupled with the proton at  $\delta$  2.44 (H-6). The double doublet at  $\delta$  7.58, assigned to H-11, was correlated with H-12 and H-10 at  $\delta$  6.20 and 6.18, respectively. Present also in the <sup>1</sup>H NMR were five singlet



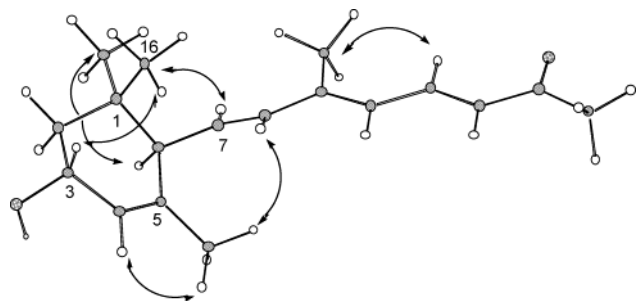
methyls. ROESY correlations CH<sub>3</sub>-15/H-6, CH<sub>3</sub>-16/H-3 and H-7, CH<sub>3</sub>-17/H-4 and H-8 revealed the stereostructure of **1** as shown in Figure 1. The CD spectrum showed a positive Cotton effect,  $\Delta\epsilon_{330.0} +12.0$ , showing that C-6 has the *R* configuration.<sup>5</sup> Therefore, the structure of **1** was deduced to be (3*R*,6*R*,7*E*,9*E*,11*E*)-3-hydroxy-13-apo- $\alpha$ -caroten-13-one.

Compound **2** also showed an absorption maximum at 323 nm. The molecular formula was determined to be C<sub>18</sub>H<sub>24</sub>O<sub>3</sub>

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**Figure 1.** Selected NOE interactions of compound **1**.

by HREIMS. Of the three oxygen functions, one was ascribed to a tertiary hydroxyl group ( $\delta_c$  80.1) and the remaining two were attributed to carbonyl groups ( $\delta_c$  198.4). The partial structure of the 3-oxo- $\alpha$ -end group and the polyene chain in **2** were characterized by  $^1\text{H}$  NMR and  $^{13}\text{C}$  NMR including COSY, ROESY, HMQC, and HMBC experiments. Present in the  $^1\text{H}$  NMR spectrum of **2** were five methyls as singlets, two aliphatic protons as doublets, and six olefinic protons as one singlet, four doublets, and one double doublet. The  $^1\text{H}$ - $^1\text{H}$  COSY experiment showed correlation of the olefinic methine at  $\delta$  5.96, assigned to H-7, with H-8 at  $\delta$  6.47. The double doublet at  $\delta$  7.55, assigned to H-11, was correlated with H-10 and H-12 at  $\delta$  6.26 and 6.20, respectively. The  $^{13}\text{C}$  NMR spectrum showed 16 carbon signals, identified, by a DEPT experiment, as five methyls, one methylene, eight olefinic carbons, two of them tetrasubstituted, two quaternary carbons, and two carbonyls. All the carbons were correlated to the corresponding protons on the basis of an HMQC experiment. The tertiary hydroxyl group was positioned at C-6 on the basis of an HMBC experiment that showed correlations between C-6 and the H-7, H-8, H-15, H-16, and H-17 protons. The CD spectrum showed a positive Cotton effect,  $\Delta\epsilon_{320.0} +43.0$ , suggesting C-6 to have the *S* configuration.<sup>5</sup> Furthermore, ROESY correlations  $\text{CH}_3$ -15/H-7,  $\text{CH}_3$ -16/H-2<sub>eq</sub> and H-7, and  $\text{CH}_3$ -17/H-4 and H-8 confirmed the stereostructure of **2**. Therefore, the structure of **2** was deduced to be (6*S*,7*E*,9*E*,11*E*)-3-oxo-13-apo- $\alpha$ -caroten-13-one.

Compound **3** was identified as *S*-(+)-abscisic alcohol by comparison with previously reported spectroscopic data.<sup>6</sup>

The spectral data of compounds **4**–**6** indicated the presence of an allenic group in these molecules. The  $^1\text{H}$  NMR spectra presented the olefinic proton at  $\delta$  5.84, 5.86, and 5.97, respectively, and in the  $^{13}\text{C}$  NMR spectrum two  $\text{sp}^2$  and one  $\text{sp}$  carbon were present in each. The data of compound **4** well matched those of grasshopper ketone isolated from ant-repellant secretions of a large flightless grasshopper, *Romalea microptera*.<sup>7</sup> The isomeric **5** and **6** were identified by comparison with previously reported spectroscopic data of the synthetic allenic zeaxanthin end group.<sup>8,9</sup>

Compounds **7**–**9**, **14**, and **18** showed spectral data identical with those reported for (3*R*,6*R*,7*E*)-3-hydroxy-4,7-megastigmadien-9-one, (6*R*,7*E*,9*R*)-9-hydroxy-4,7-megastigmadien-3-one, (3*R*,6*R*,7*E*,9*R*)-3,9-dihydroxy-4,7-megastigmadiene, (6*R*,9*R*)-9-hydroxy-4-megastigmen-3-one, and (6*Z*,9*S*)-9-hydroxy-4,6-megastigmadien-3-one C-13 nor-terpenes isolated from *Cestrum parqui*.<sup>10</sup> Compound **10**, (6*R*,7*E*)-4,7-megastigmadien-3,9-dione, was previously isolated from Greek tobacco.<sup>11</sup> Compounds **11** and **12** were identified as blumenol A<sup>12,13</sup> and (+)-dehydrovomifoliol,<sup>14</sup> respectively, on the basis of their spectral data. Compound **13** showed spectral data identical with those reported for synthetic *S*-(+)-3-hydroxy- $\beta$ -ionone.<sup>15</sup> Compound **15** was

identified as 3,9-dihydroxy-4-megastigmenone, and its spectral data were in agreement with those synthesized by Prestwich et al.<sup>16</sup> Compound **16** was identified as 4-megastigmen-3,9-dione, and its spectral data were in agreement with those of **16** synthesized by Aasen et al.<sup>17</sup> and previously identified in honey.<sup>18</sup> Compound **17** showed spectral data identical with those reported for 3,6,9-trihydroxy-4-megastigmenone<sup>19</sup> obtained by reduction of dehydroionone.

All of the compounds isolated from *C. album*, with the exception of **13** and **17**, were tested for their phytotoxicity on the seeds of *Lactuca sativa*.<sup>20</sup> Aqueous solutions of apocarotenoids **1**–**12**, **14**–**16**, and **18**, ranging between  $10^{-4}$  and  $10^{-7}$  M, were tested on germination, root length, and shoot length of treated lettuce seeds. The new apocarotenoids (**1**, **2**) reduced the germination by 20% at  $10^{-4}$  M. Compounds **7** and **10** showed about 30% inhibition at higher concentrations, while the abscisic alcohol (**3**) showed a reduction of germination at  $10^{-4}$  M. Among compounds **4**–**6**, only the allene **6** reduced the shoot length (30%) and root length (20%) at  $10^{-7}$  M when compared to the control. No important effects were observed on root and shoot elongation, with the exception of compounds **10**–**12**, which inhibited plant development by about 30%.

Bioactivity of apocarotenoids 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 seed size, seed coat permeability, differential uptake, and metabolism.<sup>21</sup> These results indicated the possible implication of the apocarotenoids' inhibitory activity detected in aqueous extracts. The growth inhibitory substances in this plant showed activity comparable with that of 4-hydroxybenzoic acid, which is known to be an effective germination inhibitor.<sup>3</sup>

## Experimental Section

**General Experimental Procedures.**  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were run on a Varian INOVA 500 NMR spectrometer at 500 and 125 MHz, respectively, in  $\text{CDCl}_3$ . MS spectra were obtained with a HP 6890 spectrometer equipped with a MS 5973 N detector. UV-vis spectra were recorded in  $\text{CHCl}_3$  on a Perkin-Elmer Lambda 7 spectrophotometer. The CD curves were measured with a Jasco J-715 dichrograph. HPLC was performed on an Agilent 1100 by using an UV detector. Silica gel 60 (230–400 mesh, E. Merck) was used for CC, and preparative TLC was performed on silica gel (UV-254 pre-coated) plates with 0.5 and 1.0 mm thickness (E. Merck). Preparative HPLC was performed using an RP-18 (LiChrospher 10  $\mu\text{m}$ , 250  $\times$  10 mm i.d., Merck) column.

**Plant Material.** The aerial part of plants of *C. album* was collected near Caserta (Italy) during autumn 2002 and identified by Professor Antonino Pollio of the Dipartimento di Biologia Vegetale of the University of Naples. Voucher specimens (HERBNAPY620) are deposited at the Dipartimento di Biologia Vegetale of University Federico II of Naples.

**Extraction and Isolation.** Fresh leaves (5.0 kg) of the plant were powdered and extracted with  $\text{H}_2\text{O}$  at room temperature for 7 days. To an aqueous suspension (700 mL) of the crude aqueous extract (108 g) was added cold acetone (900 mL), and the mixture was placed on a stir plate overnight in a cold room. The acetone addition produced heavy precipitation of materials, which were removed by centrifugation. The acetone was removed and the aqueous extract was partitioned between  $\text{CH}_2\text{Cl}_2$  and  $\text{H}_2\text{O}$ . The crude  $\text{CH}_2\text{Cl}_2$  residue (18 g) was chromatographed on a silica gel column with a  $\text{EtOAc}$ - $\text{CH}_2\text{Cl}_2$ -petroleum ether gradient as eluent to give fractions A–T. Fraction A (1.0 g) contained **1**, purified using HPLC with  $\text{MeOH}$ - $\text{CH}_3\text{CN}$ - $\text{H}_2\text{O}$  (2:1:2) (7 mg), and **2** on a silica gel column [ $\text{CHCl}_3$ -acetone (9:1), 8 mg]. Fraction B (1.2 g)

contained **13**, purified using HPLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (2:1:2) (1 mg). Fraction E (750 mg) contained **10**, purified by semipreparative TLC [CHCl<sub>3</sub>–EtOAc (19:1), 10 mg]. Fraction F (6.0 g) was extracted with 2 N NaOH, and the organic phase was neutralized. The crude residue (320 mg) was repeatedly chromatographed on a silica gel column with an acetone–CHCl<sub>3</sub>–petroleum ether gradient to afford a mixture of eight compounds. **16** (12 mg) was purified by semipreparative TLC [petroleum ether–CHCl<sub>3</sub>–acetone (8:11:1)]. **7** (3 mg) was purified by semipreparative TLC [petroleum ether–acetone (4:1)]. **12** (2 mg) was purified using HPLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (5:1:4). **17** (1 mg) and **15** (2 mg) were purified by semipreparative TLC [CHCl<sub>3</sub>–acetone (4:1)]. **9**, **14**, and **18** were separated using HPLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O [(3:2.5), 6, 5, and 4 mg, respectively]. The aqueous alkaline solution was acidified in the cold with concentrated HCl, liberated solids were extracted with EtOAc, washed with H<sub>2</sub>O, and dried, and the solvent was removed. The crude residue (315 mg) was repeatedly chromatographed on a silica gel column with an acetone–CH<sub>2</sub>Cl<sub>2</sub> gradient to afford a mixture of four compounds. **3** and **11** were purified by HPLC with MeOH–H<sub>2</sub>O (9:1) (13 and 10 mg). **5** (5 mg) was purified using HPLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (2:1:2). **8** (10 mg) was purified using HPLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (4:1:5). Fraction G (700 mg) contained **4** and **6**, which were purified using HPLC with MeOH–CH<sub>3</sub>CN–H<sub>2</sub>O (6:1:13) (12 and 8 mg).

**(3R,6R,7E,9E,11E)-3-Hydroxy-13-apo- $\alpha$ -caroten-13-one (1):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +251° (c 0.006, CHCl<sub>3</sub>); UV (CHCl<sub>3</sub>)  $\lambda_{\max}$  (log  $\epsilon$ ) 329 (4.3) nm; CD (CHCl<sub>3</sub>)  $\lambda$  ( $\Delta\epsilon$ ) 313.0 (+12.0) nm; <sup>1</sup>H NMR  $\delta$  7.58 (1H, dd,  $J$  = 11.5, 15.0 Hz, H-11), 6.20 (2H, d,  $J$  = 15.0 Hz, H-8, 12), 6.18 (1H, d,  $J$  = 11.5 Hz, H-10), 5.69 (1H, dd,  $J$  = 9.5, 15.0 Hz, H-7), 5.58 (1H, br s, H-4), 4.26 (1H, br s, H-3), 2.44 (1H, d,  $J$  = 9.5 Hz, H-6), 2.30 (3H, s, H-14), 2.01 (3H, s, H-18), 1.84 (1H, dd,  $J$  = 6.4, 17.5 Hz, H-2<sub>eq</sub>), 1.62 (3H, s, H-17), 1.40 (1H, dd,  $J$  = 5.8, 17.5 Hz, H-2<sub>ax</sub>), 1.01 (3H, s, H-15), 0.86 (3H, s, H-15); <sup>13</sup>C NMR  $\delta$  198.6 (C-13), 144.5 (C-9), 139.0 (C-11), 137.2 (C-5), 136.9 (C-8), 133.6 (C-7), 129.8 (C-12), 127.8 (C-10), 125.0 (C-4), 65.8 (C-3), 54.9 (C-6), 44.5 (C-2), 34.0 (C-1), 29.5 (C-15), 27.7 (C-14), 22.8 (C-17), 13.5 (C-18); EIMS  $m/z$  274 [M]<sup>+</sup> (60), 259 [M – Me]<sup>+</sup> (40), 256 [M – H<sub>2</sub>O]<sup>+</sup> (100); HREIMS  $m/z$  274.1929 (calcd for C<sub>18</sub>H<sub>26</sub>O<sub>2</sub>, 274.1933).

**(6S,7E,9E,11E)-3-Oxo-13-apo- $\alpha$ -caroten-13-one (2):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +165° (c 0.008, CHCl<sub>3</sub>); UV (CHCl<sub>3</sub>)  $\lambda_{\max}$  (log  $\epsilon$ ) 323 (3.3) nm; CD (CHCl<sub>3</sub>)  $\lambda$  ( $\Delta\epsilon$ ) 320.0 (+43.0) nm; <sup>1</sup>H NMR  $\delta$  7.55 (1H, dd,  $J$  = 11.5, 15.5 Hz, H-11), 6.47 (1H, d,  $J$  = 15.9 Hz, H-8), 6.26 (1H, d,  $J$  = 11.5 Hz, H-10), 6.24 (1H, d,  $J$  = 15.5 Hz, H-12), 5.96 (1H, d,  $J$  = 15.9 Hz, H-7), 5.94 (1H, s, H-4), 2.50 (1H, d,  $J$  = 17.5 Hz, H-2<sub>eq</sub>), 2.30 (1H, d,  $J$  = 17.5 Hz, H-2<sub>ax</sub>), 2.30 (3H, s, H-14), 2.02 (3H, s, H-18), 1.91 (3H, s, H-17), 1.11 (3H, s, H-15), 1.03 (3H, s, H-16); <sup>13</sup>C NMR  $\delta$  198.4 (C-3, C-13), 161.0 (C-5), 143.2 (C-9), 138.4 (C-11), 134.6 (C-8), 132.0 (C-7), 130.3 (C-10, C-12), 127.1 (C-4), 80.1 (C-6), 49.7 (C-2), 41.6 (C-1), 28.5 (C-14), 24.3 (C-16), 23.1 (C-15), 18.9 (C-17), 14.0 (C-18); EIMS  $m/z$  288 [M]<sup>+</sup> (50), 273 [M – Me]<sup>+</sup> (100), 245 [M – MeCO]<sup>+</sup> (30); HREIMS  $m/z$  288.1725 (calcd for C<sub>18</sub>H<sub>24</sub>O<sub>3</sub>, 288.1721).

**(+)-Abscisic alcohol (3):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +210° (c 0.012, CHCl<sub>3</sub>); EIMS  $m/z$  250 [M]<sup>+</sup> (80), 235 [M – Me]<sup>+</sup> (100).

**Grasshopper ketone (4):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> –62° (c 0.012, CHCl<sub>3</sub>); EIMS  $m/z$  224 [M]<sup>+</sup> (10), 209 [M – Me]<sup>+</sup> (100); 191 [M – Me – H<sub>2</sub>O]<sup>+</sup> (20).

**Compound 5:** [ $\alpha$ ]<sup>25</sup><sub>D</sub> –32° (c 0.018, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.16 (3H, s, H-11), 1.38 (3H, s, H-12), 1.43 (3H, s, H-13), 2.19 (3H, s, H-10), 1.42 and 2.00 (2H, m, H-4), 1.48 and 2.29 (2H, m, H-2), 4.34 (1H, m, H-3), 5.85 (1H, s, H-8); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  209.7 (C-9), 198.3 (C-7), 118.8 (C-6), 100.8 (C-8), 72.3 (C-5), 63.9 (C-3), 49.0 (C-2), 48.7 (C-4), 36.1 (C-1), 31.7 (C-12), 30.9 (C-13), 29.1 (C-11), 26.3 (C-10); EIMS  $m/z$  224 [M]<sup>+</sup> (30), 209 [M – Me]<sup>+</sup> (100); 191 [M – Me – H<sub>2</sub>O]<sup>+</sup> (40).

**Compound 6:** [ $\alpha$ ]<sup>25</sup><sub>D</sub> –28° (c 0.052, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.10 (3H, s, H-11), 1.39 (3H, s, H-12), 1.41 (3H, s, H-13), 2.25 (3H, s, H-10), 1.98 (2H, m, H-2), 1.91 and 2.20 (2H, m, H-4), 4.30 (1H, m, H-3), 5.97 (1H, s, H-8); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  209.4 (C-9), 199.1 (C-7), 118.7 (C-6), 100.1 (C-8), 72.4 (C-5), 63.9 (C-3), 48.7 (C-2 and C-4), 35.9 (C-1), 31.9 (C-12), 30.9 (C-

13), 29.1 (C-11), 27.1 (C-10); EIMS  $m/z$  224 [M]<sup>+</sup> (30), 209 [M – Me]<sup>+</sup> (100); 191 [M – Me – H<sub>2</sub>O]<sup>+</sup> (10).

**(6R,7E)-4,7-Megastigmadien-3,9-dione (10):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +200° (c 0.012, CHCl<sub>3</sub>); EIMS  $m/z$  206 [M]<sup>+</sup> (10), 108 [C<sub>7</sub>H<sub>8</sub>O]<sup>+</sup> (100).

**(+)-3-Hydroxy- $\beta$ -ionone (13):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +19° (c 0.080, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.09 (3H, s, H-11), 1.11 (3H, s, H-12), 1.78 (3H, s, H-13), 1.49 (1H, t,  $J$  = 12.0 Hz, H-2<sub>ax</sub>), 1.80 (1H, ddd,  $J$  = 12.0, 3.9, 2.0 Hz, H-2<sub>eq</sub>), 2.08 (1H, dd,  $J$  = 16.4, 10.0 Hz, H-4<sub>ax</sub>), 2.30 (3H, s, H-10), 2.44 (2H, dd,  $J$  = 16.4, 8.0 Hz, H-4<sub>eq</sub>), 4.05 (1H, m, H-3), 6.14 (1H, d,  $J$  = 16.3 Hz, H-8), 7.20 (1H, d,  $J$  = 16.3 Hz, H-7); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  198.4 (C-9), 142.2 (C-7), 135.5 (C-6), 132.2 (C-8), 132.0 (C-5), 64.3 (C-3), 48.2 (C-2), 42.6 (C-4), 36.7 (C-1), 29.9 (C-11), 28.4 (C-12), 21.4 (C-13), 27.2 (C-10); EIMS  $m/z$  208 [M]<sup>+</sup> (20), 193 [M – Me]<sup>+</sup> (100); 175 [M – Me – H<sub>2</sub>O]<sup>+</sup> (50).

**3,9-Dihydroxy-4-megastigmene (15):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +80° (c 0.080, CH<sub>3</sub>OH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.95 (3H, s, H-11), 1.08 (3H, s, H-12), 1.20 (3H, d,  $J$  = 6.6 Hz, H-10), 1.49 (1H, t,  $J$  = 12.0 Hz, H-2<sub>ax</sub>), 1.80 (1H, ddd,  $J$  = 12.0, 3.9, 2.0 Hz, H-2<sub>eq</sub>), 1.81 (3H, s, H-13), 2.08 (1H, dd,  $J$  = 16.4, 10.0 Hz, H-4<sub>ax</sub>), 2.44 (2H, dd,  $J$  = 16.4, 8.0 Hz, H-4<sub>eq</sub>), 3.72 (1H, m, H-9), 4.18 (1H, m, H-3), 5.45 (1H, m, H-4), 7.20 (1H, d,  $J$  = 16.3 Hz, H-7); <sup>13</sup>C NMR (CD<sub>3</sub>OD)  $\delta$  198.4 (C-9), 142.2 (C-7), 135.5 (C-6), 132.2 (C-8), 132.0 (C-5), 64.3 (C-3), 48.2 (C-2), 42.6 (C-4), 36.7 (C-1), 29.9 (C-11), 28.4 (C-12), 21.4 (C-13), 27.2 (C-10); EIMS  $m/z$  208 [M]<sup>+</sup> (20), 193 [M – Me]<sup>+</sup> (100); 175 [M – Me – H<sub>2</sub>O]<sup>+</sup> (50).

**4-Megastigmen-3,9-dione (16):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> –1.5° (c 0.050, CHCl<sub>3</sub>); <sup>1</sup>H NMR (CDCl<sub>3</sub>)  $\delta$  1.01 (3H, s, H-11), 1.05 (3H, s, H-12), 1.24 (2H, m, H-7), 1.74 (1H, m, H-6), 1.99 (3H, s, H-13), 2.15 (3H, s, H-10), 2.29 and 2.52 (2H, d,  $J$  = 8.4 Hz, H-2), 2.29 and 2.54 (2H, m, H-8), 5.84 (1H, s, H-4); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  207.0 (C-9), 199.1 (C-3), 164.8 (C-5), 125.6 (C-4), 50.1 (C-6), 47.0 (C-2), 42.5 (C-1), 28.8 (C-10 and C-11), 27.3 (C-12), 24.6 (C-7) 23.4 (C-8 and C-13); EIMS  $m/z$  208 [M]<sup>+</sup> (40), 193 [M – Me]<sup>+</sup> (100); 165 [M – MeCO]<sup>+</sup> (50).

**3,6,9-Trihydroxy-4-megastigmene (17):** [ $\alpha$ ]<sup>25</sup><sub>D</sub> +66° (c 0.020, CH<sub>3</sub>OH); <sup>1</sup>H NMR (CD<sub>3</sub>OD)  $\delta$  0.90 (3H, s, H-11), 0.98 (3H, s, H-12), 1.15 (3H, s, H-10), 1.24 (2H, m, H-7), 1.40 and 1.53 (2H, m, H-2), 1.20–1.25 (2H, m, H-8), 1.47–1.53 (2H, m, H-7), 1.73 (3H, s, H-13), 2.29 and 2.52 (2H, d,  $J$  = 8.4 Hz, H-2), 2.29 and 2.54 (2H, m, H-8), 3.65 (1H, m, H-9), 4.10 (1H, m, H-3), 5.35 (1H, s, H-4); <sup>13</sup>C NMR (CDCl<sub>3</sub>)  $\delta$  141.2 (C-5), 125.6 (C-4), 69.7 (C-6), 69.4 (C-9), 67.5 (C-3), 40.9 (C-2), 36.1 (C-1), 29.5 (C-11), 28.6 (C-12), 28.4 (C-7) 23.9 (C-8), 24.0 (C-10), 23.5 (C-13); EIMS  $m/z$  228 [M]<sup>+</sup> (20), 213 [M – Me]<sup>+</sup> (100); 195 [M – Me – H<sub>2</sub>O]<sup>+</sup> (40).

**Bioassays.** Seeds of *L. sativa* L. (cv Napoli V. F.) collected during 2001 were obtained from Ingegnoli S.p.a. 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<sup>–4</sup> M) were prepared in MES, and the rest (10<sup>–5</sup>–10<sup>–7</sup> 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 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 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  $P < 0.05$ .

**Acknowledgment.** NMR experiments have been performed at Centro Interdipartimentale di Metodologie Chimico-Fisiche di University Federico II of Naples on a 500 MHz spectrometer of Consortium INCA (project P0 L488/92, Cluster 11A).

**Supporting Information Available:** Phytotoxic activity, on *Lactuca sativa*, of compounds **1–12**, **14–16**, and **18** (Figures 1S). Tables of NMR data for **1** and **2** (Tables 1S, 2S). This material is available free of charge via the Internet at <http://pubs.acs.org>.

## References and Notes

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NP049857Q