

## Identification of a New Fatty Acid from the Seeds of *Coix lachryma-jobi* var. *ma-yuen*

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Received December 5, 2012, Accepted January 17, 2013

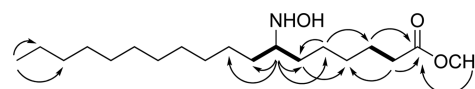
**Key Words :** *Coix lachryma-jobi* L. var. *ma-yuen* Stapf., Gramineae, Fatty acid

The hulled seeds of *Coix lachryma-jobi* L. var. *ma-yuen* Stapf. (Gramineae) have been used as a traditional herbal medicine for treating inflammation, asthma, cough, stomach disorder, diarrhea, and diabetes.<sup>1</sup> There have been previous phytochemical studies reporting on fatty acids,<sup>2,3</sup> triglyceride,<sup>4</sup> phenolic acids,<sup>5-7</sup> flavonoids,<sup>5-7</sup> lactams,<sup>8</sup> and polysaccharides<sup>9</sup> as constituents of *C. lachryma-jobi* var. *ma-yuen*. The extracts and some isolates of this plant have showed various activities including *anti-obesity*,<sup>10</sup> *anti-inflammatory*,<sup>11</sup> *anticancer*,<sup>3-5,8</sup> *antimutagenic*,<sup>6</sup> *gastroprotective*,<sup>7</sup> and *hypoglycemic* activities<sup>9</sup> in the literatures.

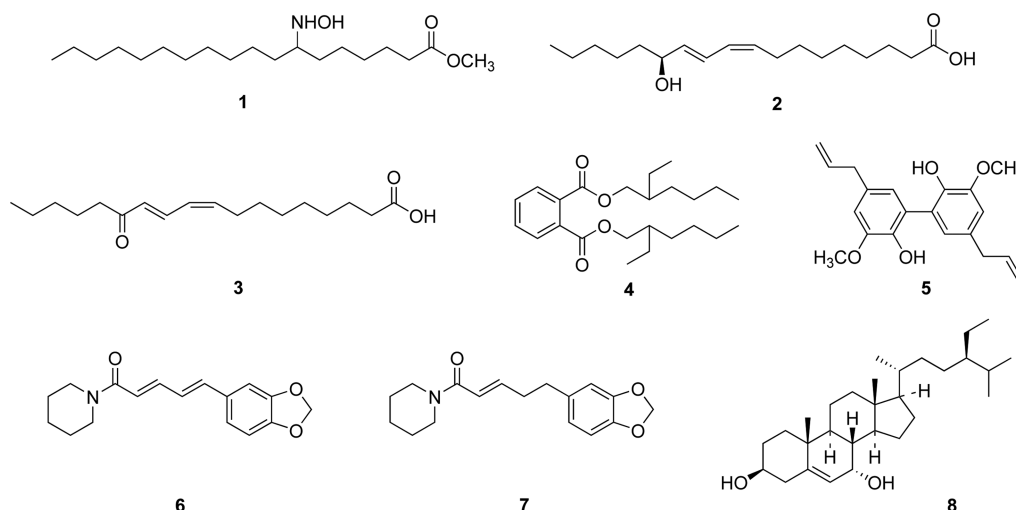
As a part of our ongoing research to find bioactive compounds from traditional herbal medicines, the hulled seeds of *C. lachryma-jobi* var. *ma-yuen* were investigated, affording a new fatty acid, (+)-7-hydroxyamino-octadecanoic acid (**1**) together with seven known compounds. The structure of **1** was elucidated by physical and spectroscopic data analysis (Figure 1).

Compound **1** was obtained as colorless oil. Its HR-ESI-MS gave a molecular ion peak at  $m/z$  330.3000  $[M+H]^+$ , corresponding to an elemental formula of  $C_{19}H_{40}NO_3$ . The IR spectrum showed absorption bands at  $3628\text{ cm}^{-1}$  for a

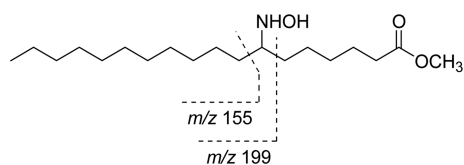
free O-H group as a weak sharp peak and at  $1740\text{ cm}^{-1}$  for an aliphatic ester group as a very strong peak.<sup>12</sup> The  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR spectra of **1** showed signals for a  $C_{14}$  aliphatic methylene group at  $\delta_{\text{H}}$  1.62-1.27 (28H, m) and  $\delta_{\text{C}}$  32.3-22.9, indicating the presence of a long aliphatic chain of fatty acid moiety. A methyl ester group appeared at  $\delta_{\text{H}}$  3.67/ $\delta_{\text{C}}$  51.7 (OCH<sub>3</sub>) and  $\delta_{\text{C}}$  174.5 (C-1) which were correlated in the HMBC (OCH<sub>3</sub>/C-1) experiment (Figure 2). The peaks for a hydroxyaminated methine group of **1** resonated at  $\delta_{\text{H}}$  2.65 (1H, t,  $J = 4.7$  Hz) and  $\delta_{\text{C}}$  59.1 which were shifted to upfield and downfield, respectively, compared to the values of a methine with a free amine group.<sup>13</sup> A methylene group appeared at  $\delta_{\text{H}}$  2.30 (2H, t,  $J = 7.2$  Hz)/ $\delta_{\text{C}}$  34.3 and a methyl group was observed at  $\delta_{\text{H}}$  0.88 (3H, t,  $J = 6.8$  Hz)/ $\delta_{\text{C}}$  14.3. The above data suggested that **1** was possibly a hydroxyaminated octadecanoic acid methyl ester. To determine the



**Figure 2.** Selected  $^1\text{H}$ - $^1\text{H}$  COSY (—) and  $^1\text{H}$ - $^{13}\text{C}$  HMBC (---) correlations of compound **1**.



**Figure 1.** Structures of the isolates **1-8** from the seeds of *C. lachryma-jobi* var. *ma-yuen*.



**Figure 3.** MS fragmentation of compound **1**.

position of the hydroxyamino group, EI mass spectrometric data of **1** were utilized. Two fragment ion peaks at 199 [ $M-(CH_2)_5COOCH_3$ ] and 155 [ $M-OHNH(CH_2)_6COOCH_3$ ] indicated the position of the hydroxyamino group at C-7 (Figure 3). Although the aliphatic secondary N-H group was not observed in the IR spectrum of **1**, the presence of the hydroxy amine group could not be doubted because this type of amine peak was usually expected to show at about  $3300\text{ cm}^{-1}$  as a vanishingly weak peak or not to be observed.<sup>12</sup> Compound **1** was optically active ( $[\alpha]_D^{25} +54.3$ ), however, the absolute configuration at C-7 could not be resolved. Thus, the structure of **1** was determined as a new compound, namely, (+)-7-hydroxyamino-octadecanoic acid methyl ester. The hydroxylamine containing structure is very rare as a natural product.<sup>14</sup> However, structure of **1** could be considered as a possible natural product related to the major constituents of *C. lachryma-jobi* var. *ma-yuen* such as fatty acids,<sup>2,3</sup> oleic acid and linoleic acid, and triglycerides,<sup>4</sup> glyceryl trioleate (olein). Compounds **2** and **3** were identified as 13*S*-hydroxy-9*Z*,11*E*-octadecadienoic acid<sup>15</sup> and 13-oxo-9*Z*,11*E*-octadecadienoic acid,<sup>16</sup> respectively, which had very similar structural skeletons with **1**, providing another possible evidence for a natural occurrence of **1**.

The other known compounds **4-8** were identified as bis(2-ethylhexyl)phthalate,<sup>17</sup> dehydrodieugenol,<sup>18</sup> piperine,<sup>19</sup> piperanine,<sup>19</sup> and 7 $\alpha$ -sitosterol,<sup>20</sup> respectively, by analyses of their physical and spectroscopic data as well as by comparison of their data with the published values. **2-8** were found in the *Coix* species for the first time. Moreover, **3** and **5-8** have never been isolated from the family Gramineae (Poaceae).

### Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a P-1010 polarimeter (JASCO, Japan) at 20 °C. IR spectrum was recorded on Nicolet iS10 FT-IR spectrometer (Thermo Fisher Scientific, MA). 1D and 2D NMR experiments were performed on a UNITY INOVA 400 MHz FT-NMR instrument (Varian, CA) with tetramethylsilane (TMS) as internal standard. EI-Mass spectrum was obtained on Py-GC/MS system composed of Agilent 6890N gas chromatograph and Agilent 5975i MSD mass spectrometer. HR-ESI mass spectrometric analyses were performed with Waters ACQUITY UPLC system coupled to a Micromass Q-ToF Micro mass spectrometer and Agilent 6220 Accurate-Mass TOF LC/MS system. Silica gel (230-400 mesh, Merck, Germany) and RP-18 (YMC gel ODS-A, 12 nm, S-150  $\mu\text{m}$ ) were used for column chromatography.

Thin-layer chromatographic (TLC) analysis was performed on Kieselgel 60 F 254 (silica gel, 0.25 mm layer thickness, Merck, Germany) and RP-18 F 254s (Merck, Germany) plates, with visualization under UV light (254 and 365 nm) and 10% (v/v) sulfuric acid spray followed by heating (120 °C, 5 min).

**Plant Material.** The seeds of *C. lachryma-jobi* var. *ma-yuen* were collected in Sangju, Gyeongsangbuk-do, Korea, in January 2012 and identified by one of the authors (J.-H. Lee). A voucher specimen (No. EAB326) has been deposited at the College of Pharmacy, Ewha Womans University.

**Extraction and Isolation.** The hulled seeds of *C. lachryma-jobi* var. *ma-yuen* (12 kg) were extracted with MeOH ( $3 \times 5\text{ L}$ ) overnight at room temperature. The solvent was evaporated *in vacuo* to afford a MeOH extract (230 g), which was then suspended in H<sub>2</sub>O (1.5 L), and partitioned with hexanes ( $3 \times 1.5\text{ L}$ ), EtOAc ( $3 \times 1.5\text{ L}$ ), and *n*-BuOH ( $3 \times 1.5\text{ L}$ ), sequentially. The hexanes-soluble extract (174 g) was subjected to silica gel column chromatography (CC;  $\phi 9\text{ cm}$ ; 60-250 mesh, 1 kg), using gradient mixtures of hexanes-EtOAc (99:1  $\rightarrow$  0:1) as mobile phases, affording fifteen fractions (F1 – F15). The fraction F2 (69 g) eluted with hexanes-EtOAc (99:1) from the first CC, was subjected to silica gel CC ( $\phi 9\text{ cm}$ ; 230-400 mesh, 1 kg) with hexanes-acetone (99:1  $\rightarrow$  9:1) as a solvent system, yielding six sub-fractions (F2-1 – F2-6). Sub-fraction F2-1 (37 g) eluted with hexanes-acetone (99:1), was separated by silica gel CC ( $\phi 6\text{ cm}$ ; 230-400 mesh, 700 g), using gradient mixtures of hexanes-acetone (10:0  $\rightarrow$  9:1), affording nine sub-fractions (F2-1-1 – F2-1-9). Sub-fraction F2-1-5 (1.6 g) eluted with hexanes-acetone (98:2), was applied to reversed-phase CC ( $\phi 3\text{ cm}$ ; ODS-A, 200 g), using an isocratic mixture of acetonitrile-H<sub>2</sub>O (95:5) to afford **1** (3 mg). Sub-fraction F2-2 (14 g) eluted with hexanes-acetone (98:2), was separated by silica gel CC ( $\phi 4\text{ cm}$ ; 230-400 mesh, 600 g), using gradient mixtures of hexanes-acetone (99:1  $\rightarrow$  4:1), providing eight sub-fractions (F2-2-1 – F2-2-8). Sub-fractions F2-2-1 (2.7 g), was further separated by reversed-phase CC ( $\phi 3\text{ cm}$ ; ODS-A, 200 g), using 100% MeOH as solvent system to give **4** (5 mg). The fraction F3 (16 g) eluted with hexanes-EtOAc (95:5) from the first separation, was subjected to silica gel CC ( $\phi 4.5\text{ cm}$ ; 230-400 mesh, 700 g) with gradient mixtures of hexanes-EtOAc (9:1  $\rightarrow$  1:1), yielding nine sub-fractions (F3-1 – F3-9). Sub-fraction F3-8 (80 mg) eluted with hexanes-EtOAc (1:1), was further purified by reversed-phase CC ( $\phi 2\text{ cm}$ ; ODS-A, 130 g), using gradient mixtures of acetonitrile-H<sub>2</sub>O (2:1  $\rightarrow$  4:1), furnishing **5** (1 mg). Sub-fraction F3-9 (100 mg) eluted with 100% EtOAc, was subjected to reversed-phase CC ( $\phi 2\text{ cm}$ ; ODS-A, 130 g), using an isocratic mixture of acetonitrile-H<sub>2</sub>O (2:1) as a solvent system, affording **2** (5 mg) and **3** (2 mg). The fraction F9 (2.5 g) eluted with hexanes-EtOAc (2:1) from the first separation, was subjected to silica gel CC ( $\phi 4\text{ cm}$ ; 230-400 mesh, 500 g) with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (10:0  $\rightarrow$  9:1), providing eighteen sub-fractions (F9-1 – F9-18). Sub-fraction F9-9 (21 mg) eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (95:5), was chromatographed on Sephadex LH-20 (100% MeOH), providing **6** (2 mg).

and **7** (1 mg). The fraction F11 (400 mg) eluted with hexanes-EtOAc (1:1) from the first separation, was subjected to silica gel CC ( $\phi$  2 cm; 230-400 mesh, 200 g), using gradient mixtures of CH<sub>2</sub>Cl<sub>2</sub>-MeOH (99:1  $\rightarrow$  95:5), yielding eleven sub-fractions (F11-1 – F11-11). Sub-fraction F11-5 (39 mg) eluted with CH<sub>2</sub>Cl<sub>2</sub>-MeOH (99:1), was purified by reversed-phase CC ( $\phi$  2 cm; ODS-A, 130 g), using an isocratic mixture of MeOH-H<sub>2</sub>O (9:1) as a solvent system to give **8** (2 mg).

**(+)-7-Hydroxyamino-octadecanoic Acid Methyl Ester (1):** Colorless oil.  $[\alpha]_D^{25}$  +54.3 (*c* 0.08, CHCl<sub>3</sub>); IR (KBr)  $\nu_{\max}$  cm<sup>-1</sup>: 3628, 2926, 2341, 1740, 1457, 1171, 668; <sup>1</sup>H-NMR (CDCl<sub>3</sub>, 400 MHz)  $\delta$  3.67 (3H, s, OCH<sub>3</sub>), 2.65 (1H, t, *J* = 4.7 Hz, H-7), 2.30 (2H, t, *J* = 7.2 Hz, H-2), 1.62 (2H, t, *J* = 7.2 Hz, H-3), 1.50 (4H, br t, *J* = 4.7 Hz, H-6 and H-8), 1.43 (4H, br t, *J* = 7.0 Hz, H-5 and H-9), 1.37-1.27 (18H, m, H-4, H-10~17), 0.88 (3H, t, *J* = 6.8 Hz, H-18); <sup>13</sup>C-NMR (CDCl<sub>3</sub>, 100 MHz)  $\delta$  174.5 (C-1), 59.1 (C-7), 51.7 (OCH<sub>3</sub>), 34.3 (C-2), 32.3 and 32.3 (C-6, C-8), 32.1 (C-16), 29.8, 29.7, 29.6, 29.5, 29.4, 29.3, and 29.2 (C-4, C-10, C-11, C-12, C-13, C-14, C-15), 26.3 and 26.2 (C-5, C-9), 25.1 (C-3), 22.9 (C-17), 14.3 (C-18); EIMS *m/z* = 199.2 [M-(CH<sub>2</sub>)<sub>5</sub>COOCH<sub>3</sub>], 155.2 [M-NHOH(CH<sub>2</sub>)<sub>6</sub>COOCH<sub>3</sub>], 74.2 [M-(CH<sub>2</sub>)<sub>4</sub>NHOH(CH<sub>2</sub>)<sub>11</sub>CH<sub>3</sub>]; HRESIMS (positive mode) *m/z* = 330.3000 [M+H]<sup>+</sup> (calcd for C<sub>19</sub>H<sub>40</sub>NO<sub>3</sub>: 330.3003).

**13S-Hydroxy-9Z,11E-octadecadienoic Acid (2):**  $[\alpha]_D^{25}$  +8.80 (*c* 0.1, CHCl<sub>3</sub>; literature values: +9.6, *c* 0.6, CHCl<sub>3</sub><sup>15</sup>).

**Acknowledgments.** This research was funded by a grant (12172KFDA989) from Korea Food & Drug Administration in 2012. The work was also supported by the National Research Foundation of Korea(NRF) grant funded by the Korea Government (MEST) (No. 2012-0000952) and in part by the Ewha Global Top5 Grant 2013 of Ewha Womans University.

**Supporting Information.** The spectral data of compound **1** are available on request from the correspondence author.

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