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Structure-Activity Relationship Study of Asiatic Acid Derivatives for New Wound Healing Agent

Byeong-Seon Jeong
College of Pharmacy, Seoul National University, Seoul 151-742, Korea

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Ten semi-synthetic derivatives of asiatic acid were prepared and their wound healing effects were evaluated by employing a tensile strength assay and a wound area assay. Among them, ethoxymethyl 2-oxo-3,23-isopropylidene-asiatate (**12**) showed the strongest and the fastest wound healing activity. Furthermore, it left the smallest scar after healing.

Key words: Asiatic acid, SAR, Wound healing

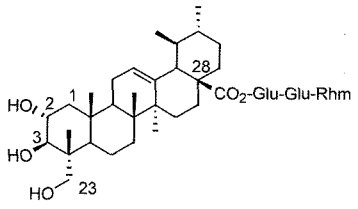
INTRODUCTION

Centella asiatica is an herbal plant used on different continents by diverse ancient cultures and tribal groups (Bontems, 1941). Historically, the extract has been used as a wound healing agent (Beljanski, 1972). The extract has three different triterpenoid ingredients: asiaticoside (**1**), asiatic acid (**2**), and madecassic acid (**3**) (Inamdar, 1996). It has been reported that its wound healing activity is associated with the modulation of collagen synthesis in the skin dermis (Bonte, 1995). Among the three triterpenes, asiatic acid (**2**) is the most essential ingredient for biological activity (Lawrence, 1967a, 1967b). The wound healing property of the extract has led to its commercial introduction under the trade name, Madecassol® (Pointel, 1987; Poizot, 1978).

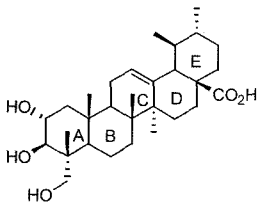
As part of our program toward the development of new wound healing agents, structure activity relationship (SAR) studies have been performed by modifying asiatic acid (**2**). The first SAR study indicated that generally lipophilic transformations, such as acetate formation with the three hydroxy groups in the A ring and ester formation with the C(28) carboxylic acid in the D ring (**4**, **5**), are relatively important to enhance the wound healing activity (Shim, 1996). This finding led us to pursue the SAR study further. In this communication, the extended SAR study of asiatic acid (**2**) for the development of an efficient wound healing agent is reported.

MATERIALS AND METHODS

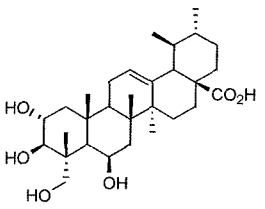
Infrared (IR) spectra were recorded on a JASCO FT/IR-300E and Perkin-Elmer 1710 FT spectrometer. Nuclear magnetic resonance (^{1}H-NMR) spectra were measured on JEOL JNM-LA 300 [300 MHz (^{1}H)] spectrometer, JEOL JNM-GSX 400 [400 MHz (^{1}H)] spectrometer using



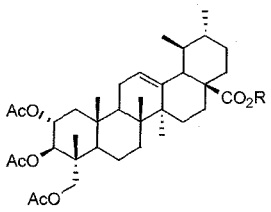
1
Asiaticoside



2
Asiatic acid



3
Madecassic acid



4 R = THP
5 R = CH_2OEt

Correspondence to: Byeong-Seon Jeong, College of Pharmacy, Seoul National University, Seoul 151-742, Korea
Tel: 82-2-880-7872, Fax: 82-2-872-9129
E-mail: ddongmak@naver.com

DMSO-d_6 or $CHCl_3$-*d* as solvents, and were reported in ppm relative to DMSO (δ 2.50) or $CHCl_3$ (δ 7.26) for ^{1}H-NMR. Coupling constants (*J*) in ^{1}H-NMR are in Hz. Low-resolution mass spectra (MS) were recorded on a VG Trio-2 GC-MS spectrometer. For thin-layer chromatography (TLC) analysis, Merck precoated TLC plate (silica gel 60 GF_{254}, 0.25 mm) were used. For flash column chromatography, E. Merck Kieselgel 60 (70~230 mesh) was used. All solvents and commercially available chemicals were used without additional purification.

2α-Hydroxy-3β,23-methylidenedioxyurs-12-ene-28-oic acid (6a)

To a solution of asiatic acid (2.53 g, 5.18 mmol) in THF (100 mL) were added DMSO (2.6 mL, 13.0 mmol) and TMSCl (1.7 mL, 13.0 mmol). The reaction mixture was refluxed for 3 days. After completion of the reaction, the reaction mixture was concentrated under reduced pressure and the residue was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20 : 1) to yield a white solid (2.0 g, 80%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.24 (t, *J* = 3.2 Hz, 1 H), 3.64 (dt, 1 H), 4.95 (dd, *J* = 5.9 Hz, 2 H), 3.87 (dt, *J* = 4.3, 10.0 Hz, 1 H), 3.23 (d, *J* = 10.0 Hz, 1 H), 3.04, 3.76 (Abq, *J* = 10.1 Hz, 2 H), 2.19 (d, *J* = 10.1 Hz, 1 H), 0.95 (d, *J* = 5.8 Hz, 3 H), 0.85 (d, *J* = 6.2 Hz, 3 H), 1.12 (s, 3 H), 1.08 (s, 3 H), 1.05 (s, 3 H), 0.75 (s, 3 H). IR (neat) 2932, 1698 cm^{-1}. Mass (EI) m/e 500 [M^+].

2α-Hydroxy-3β,23-ethylidenedioxyurs-12-ene-28-oic acid (6b)

To a solution of **1** (2.55 g, 5.18 mmol) and *p*-toluenesulfonic acid (50 mg) in dry dimethyl formamide (50 mL) was added 1,1-dimethoxyethane (1.5 mL, 13.0 mmol). The reaction mixture was stirred at room temperature for 2 h. After neutralization with 5% aq. NaOH to pH 7-8, the reaction mixture was diluted with ethyl acetate (250 mL) and washed with water (100 mL × 3) and saturated NaCl solution (80 mL). The organic layer was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent at reduced pressure gave a light yellow solid, which was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20 : 1) to yield a white solid (1.78 g, 66.2%).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.14 (t, *J* = 3.2 Hz, 1 H), 4.64 (q, *J* = 4.92 Hz, 1 H), 3.75 (m, 1 H), 3.63, 2.97 (ABq, *J* = 10.1 Hz, 2 H), 3.17 (d, *J* = 10.4 Hz, 1 H), 0.98 (s, 3 H), 0.95 (s, 3 H), 0.65 (s, 3 H), 0.85 (d, *J* = 5.50 Hz, 1 H), 0.75 (d, *J* = 6.4 Hz, 3 H). IR (neat) 2926, 1695 cm^{-1}. Mass (EI) m/e 514 [M^+].

2α-Hydroxy-3β,23-benzylidenedioxyurs-12-ene-28-oic acid (6c)

The synthetic method was same as **6b** except the ultization of 1,1-dimethoxymethyllbenzene instead of 1,1-dimethoxyethane (32% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.52-7.49 (m, 2H), 7.37-7.35 (m, 3H), 5.53 (s, 1H), 5.24 (t, *J* = 3.2 Hz, 1 H), 3.90, 3.30 (ABq, *J* = 10.1 Hz, 2 H), 3.47 (d, *J* = 10.4 Hz, 1 H), 2.18 (d, *J* = 11.5 Hz, 1 H), 1.19 (s, 3 H), 1.09 (s, 3 H), 1.07 (s, 3 H), 0.93(d, *J* = 6.1 Hz, 3 H), 0.85 (d, *J* = 6.4 Hz, 3 H), 0.77 (s, 3 H). IR (neat) 3437, 1696 cm^{-1}. Mass (EI) m/e 576 [M^+].

2α-Hydroxy-3β,23-isopropylidenedioxyurs-12-ene-28-oic acid (6d)

The synthetic method was same as **6b** except the ultization of 2,2-dimethoxypropane instead of 1,1-dimethoxyethane (87% yield).

^{1}H-NMR (250 MHz, $CDCl_3$) δ 5.24 (t, *J* = 3.2 Hz, 1 H), 3.78 (m, 1 H), 3.46, 3.51 (AB quartet, *J* = 10.8 Hz, 2 H), 3.32 (d, *J* = 9.6 Hz, 1 H), 2.18 (d, *J* = 11.2 Hz, 1 H), 0.95 (d, *J* = 6.4 Hz, 3 H), 0.85 (d, *J* = 6.4 Hz, 3 H), 1.46 (s, 3 H), 1.45 (s, 3 H), 1.09 (s, 3 H), 1.06 (s, 3 H), 1.04 (s, 3 H), 0.75 (s, 3 H). IR (neat) 3440, 1698, 1200 cm^{-1}. Mass (EI) m/e 528 [M^+].

Octyloxymethyl-2α-hydroxy-3β,23-methylidenedioxyurs-12-ene-28-oate (7a)

To a solution of **6a** (260 mg, 0.52 mmol) and N-diisopropylethylamine (186 mL, 1.04 mmol) in dry dimethyl formamide (5 mL) was added chloromethyloctyl ether (0.1 mL, 0.53 mmol) and the reaction mixture was stirred at 0°C for 30 min. The mixture was diluted with ethyl acetate (100 mL) and washed with water (50 mL × 2) and saturated NaCl solution (50 mL). The organic layer was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent at reduced pressure gave a light yellow solid, which was purified by silica gel column chromatography ($CHCl_3$: MeOH = 30 : 1) to yield a white solid (138 mg, 42%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.26 (t, *J* = 3.2 Hz, 1 H), 5.21, 5.24 (AB quartet, *J* = 5.9 Hz, 2 H), 4.94 (d, *J* = 9.0 Hz, 2 H), 3.58 (m, 2 H), 3.22 (d, *J* = 10.8 Hz, 1 H), 3.53 (s, 3 H), 3.04, 3.76 (ABq, *J* = 10.0 Hz, 2 H), 2.25 (d, *J* = 10.8 Hz, 1 H), 1.13 (s, 3 H), 1.09 (s, 3 H), 1.05 (s, 3 H), 0.95 (d, *J* = 6.4 Hz, 3 H), 0.88 (d, *J* = 5.6 Hz, 3 H), 0.76 (s, 3 H). IR (neat) 3475, 1734 cm^{-1}. Mass (EI) m/e 642 [M^+].

Octyloxymethyl-2α-hydroxy-3â,23-ethylidenedioxyurs-12-ene-28-oate (7b)

The synthetic method was same as **7a** except the ultization of **6b** instead of **6a** (85% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.22 (t, *J* = 3.2 Hz, 1 H), 5.20, 5.17 (AB quartet, *J* = 6.2 Hz, 2 H), 4.69 (q, *J* = 5.0 Hz, 1 H), 3.84-3.77 (m, 1 H), 3.69, 3.03 (ABq, *J* = 10.0 Hz, 2 H), 3.55 (m, 1H), 2.22 (d, *J* = 11.2 Hz, 1 H), 1.05 (s, 3

H), 1.00 (s, 3 H), 0.95 (s, 3 H), 0.84 (d, J = 2.56 Hz, 3 H), 0.82 (d, J = 2.2 Hz, 3 H), 0.72 (s, 3 H). IR (neat) 3481, 2927, 1732 cm^{-1}. Mass (EI) m/e 656 [M^+].

Octyloxymethyl-2α-hydroxy-3β,23-benzylidenedioxyurs-12-ene-28-oate (7c)

The synthetic method was same as **7a** except the ultization of **6c** instead of **6a** (92% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 7.50-7.48 (m, 2H), 7.35-7.33 (m, 3H), 5.52 (t, J = 3.2 Hz, 1 H), 5.45, 5.37 (ABq, J = 6.3 Hz, 2 H), 4.85 (q, J = 5.0 Hz, 1 H), 3.98-3.87 (m, 1 H), 3.79, 3.23 (ABq, J = 10.0 Hz, 2 H), 3.65 (m, 1H), 2.32 (d, J = 11.2 Hz, 1 H), 1.10 (s, 3 H), 1.05 (s, 3 H), 0.96 (s, 3 H), 0.84 (d, J = 2.60 Hz, 3 H), 0.82 (d, J = 2.2 Hz, 3 H), 0.73 (s, 3 H). IR (neat) 3697, 1730 cm^{-1}. Mass (EI) m/e 718 [M^++1].

Octyloxymethyl-2α-hydroxy-3β,23-isopropylidenedioxyurs- 12-ene-28-oate (7d)

The synthetic method was same as **7a** except the ultization of **6d** instead of **6a** (92% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.26 (t, J = 3.2 Hz, 1 H), 5.19, 5.16 (AB quartet, J = 6.2 Hz, 2 H), 3.73-3.82 (m, 1 H), 3.67, 3.01 (ABq, J = 10.0 Hz, 2 H), 3.56 (m, 1H), 2.25 (d, J = 11.2 Hz, 1 H), 1.46 (s, 3 H), 1.45 (s, 3H), 1.10 (s, 3 H), 1.07 (s, 3 H), 1.04 (s, 3 H), 0.85 (d, J = 2.55 Hz, 3 H), 0.82 (d, J = 2.2 Hz, 3 H), 0.76 (s, 3 H). IR (neat) 3469, 1733 cm^{-1}. Mass (EI) m/e 670 [M^+].

Octyloxymethyl-2α-hydroxy-3â,23-methylidenedioxyurs-2-oxo-12-ene-28-oate (8a)

A solution of pyridinium dichromate (1.63 g, 7.56 mmol) and acetic anhydride (2.68 mL) in anhydrous CH_2Cl_2 (50 mL) was stirred 30 min at room temperature. To the mixture was added **7a** (6.08 g, 9.46 mmol) in anhydrous CH_2Cl_2 (10 mL) dropwise and refluxed for 2 h. The solution was diluted with ethyl acetate (300 mL), and the mixture was filtered to remove precipitate. The solution was washed with water (80 mL × 3) and saturated NaCl solution (60 mL). The organic phase was dried over anhydrous magnesium sulfate. Filtration and evaporation of solvent at reduced pressure gave a light yellow solid, which was purified by silica gel column chromatography ($CHCl_3$: MeOH = 30 : 1) to yield a white solid (1.45 g, 24.1%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.25 (t, J = 3.2 Hz, 1 H), 5.22, 5.28 (AB quartet, J = 6.0 Hz, 2 H), 4.94 (d, J = 9.0 Hz, 2 H), 4.45 (s, 1 H), 3.22 (d, J = 10.8 Hz, 1 H), 3.24, 3.96 (ABq, J = 10.0 Hz, 2 H), 2.27 (d, J = 10.8 Hz, 1 H), 1.17 (s, 3 H), 1.11 (s, 3 H), 1.07 (s, 3 H), 0.97 (d, J = 6.4 Hz, 3 H), 0.89 (d, J = 5.6 Hz, 3 H), 0.78 (s, 3 H). IR (neat) 3468, 1735 cm^{-1}. Mass (EI) m/e 640 [M^+].

Octyloxymethyl-2α-hydroxy-3β,23-isopropylidenedioxyurs-2-oxo-12-ene-28-oate (8d)

The synthetic method was same as **8a** except the ultization of **7d** instead of **7a** (65% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.26 (t, J = 3.2 Hz, 1 H), 5.19, 5.16 (AB quartet, J = 6.2 Hz, 2 H), 3.73-3.82 (m, 1 H), 3.67, 3.01 (ABq, J = 10.0 Hz, 2 H), 4.41 (s, 1H), 2.25 (d, J = 11.1 Hz, 1 H), 1.48 (s, 3 H), 1.47 (s, 3H), 1.12 (s, 3 H), 1.08 (s, 3 H), 1.06 (s, 3 H), 0.85 (d, J = 2.5 Hz, 3 H), 0.82 (d, J = 2.2 Hz, 3 H), 0.77 (s, 3 H). IR (neat) 3473, 1732 cm^{-1}. Mass (EI) m/e 668 [M^+].

Methyl-2α-hydroxy-3β,23-isopropylidenedioxyurs-12-ene-28-oate (9)

To a solution of **6d** (4.0 g, 7.56 mmol) in diethyl ether (50 mL) was added excess of diazomethane at 0°C and the reaction solution was stirred for 2 h. Evaporation of solvent at reduced pressure gave a light yellow solid, which was purified by silica gel column chromatography ($CHCl_3$: MeOH = 20 : 1) to yield a white solid (3.48 g, 85%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.18 (t, J = 3.2 Hz, 1 H), 3.72 (m, 1H), 3.53 (s, 3H), 3.41, 3.43 (ABq, J = 10.0 Hz, 2 H), 3.25 (d, J = 9.6 Hz, 1 H), 2.15 (d, J = 11.1 Hz, 1 H), 1.40 (s, 3 H), 1.39 (s, 3H), 1.02 (s, 3 H), 1.00 (s, 3 H), 0.97 (s, 3 H), 0.87 (d, J = 6.0 Hz, 3 H), 0.79 (d, J = 6.4 Hz, 3 H), 0.66 (d, J = 2.55 Hz, 3 H). IR (neat) 3466, 1724, 1201 cm^{-1}. Mass (EI) m/e 542 [M^+].

Methyl-2α-hydroxy-3β,23-isopropylidenedioxyurs-2-oxo-12-ene-28-oate (10)

The synthetic method was same as **8d** except the ultization of **9** instead of **7d** (65% yield).

^{1}H-NMR (400 MHz, $CDCl_3$) δ 5.19 (t, J = 3.2 Hz, 1 H), 4.42 (s, 1H), 3.61, 3.63 (ABq, J = 10.0 Hz, 2 H), 3.27 (d, J = 9.6 Hz, 1 H), 2.17 (d, J = 11.1 Hz, 1 H), 1.43 (s, 3 H), 1.40 (s, 3H), 1.06 (s, 3 H), 1.03 (s, 3 H), 0.98 (s, 3 H), 0.88 (d, J = 6.0 Hz, 3 H), 0.79 (d, J = 6.4 Hz, 3 H), 0.71 (d, J = 2.5 Hz, 3 H). IR (neat) 3458, 1722, 1201 cm^{-1}. Mass (EI) m/e 540 [M^+].

Ethyloxymethyl-2α-hydroxy-3β,23-isopropylidenedioxyurs-12-ene-28-oate (11)

The synthetic method was same as **7d** except the ultization of chloromethylethyl ether instead of chloromethyloctyl ether (85% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.25 (t, J = 3.2 Hz, 1 H), 5.21 (s, 2H), 3.81-3.72 (m, 1H), 3.64 (q, J = 7.1 Hz, 2 H), 3.49, 3.44 (ABq, J = 10.5 Hz, 2 H), 3.30 (d, J = 9.8 Hz, 1 H), 1.43 (s, 3 H), 1.42 (s, 3H), 1.08 (s, 3 H), 1.05 (s, 3 H), 1.00 (t, J = 7.1 Hz, 3 H), 0.92 (d, J = 5.3 Hz, 3 H), 0.84 (d, J = 6.3 Hz, 3 H), 0.74 (s, 3 H). IR (neat) 3440, 2921, 1755 cm^{-1}. Mass (EI) m/e 586 [M^+].

Ethyloxymethyl-2α-hydroxy-3β,23-isopropylidenedioxyurs-2-oxo-12-ene-28-oate (12)

The synthetic method was same as **8d** except the ultization of **11** instead of **7d** (85% yield).

^{1}H-NMR (300 MHz, $CDCl_3$) δ 5.26 (t, *J* = 3.2 Hz, 1 H), 5.23 (s, 2H), 4.40 (s, 1 H), 3.59, 3.69 (ABq, *J* = 10.7 Hz, 2 H), 3.66 (q, *J* = 7.1 Hz, 1 H), 1.52 (s, 3 H), 1.43 (s, 3H), 1.15 (s, 3 H), 1.05 (s, 3 H), 1.01 (t, *J* = 7.1 Hz, 3 H), 1.27 (t, *J* = 7.1 Hz, 3 H), 0.95 (d, *J* = 6.1 Hz, 3 H), 0.87 (d, *J* = 6.6 Hz, 3 H), 0.77 (s, 3 H). IR (neat) 2924, 1727, 755 cm^{-1}. Mass (EI) m/e 584 [M^+].

Biological activity evaluation

Incisional wounds

Female Sprague-Dawley rats weighting 200 ± 20 g were used in these studies. The animals were kept under controlled conditions and were given solid stock food and tap water as desired. The animals were anesthetized with ether and their backs were shaved with animal clippers and cleaned with 70% ethanol. On operation day under ether anesthesia, a 2 cm dorsal linear skin incision was made. The wounds were closed with continuous sutures with stitches 0.5cm apart. Black polyethylene surgical thread and curved needles were used. The rats were maintained in separate cages following wounding. The suture was removed 4 days after the operation. The animals were killed after 6 days, the skin of the back was removed and a strip of dorsal skin measuring 1 cm in length and 3 with was excised from the area around the wound. Tensile strength was measured by a Rheometer with digital readout in gram force. Asiaticoside derivatives were administered topically at sites of the incision for 6 consecutive days starting from the day of operation. The samples were applied as ointment with a cotton swab (approximately 50 mg/site).

Open wounds

Backs of female Sprague-Dawley rats (200 ± 20 g) were shaved and cleaned, and two square open wounds of 0.8×0.8 cm were made. After the wounds were made on their backs, they were gently cleaned with 70% ethanol. The rats were maintained in separate cages following wounding. The surface area of wound was measured with caliper at 2 days intervals until the wound was completely healed. The time for 50% of the wound in each treatment group to be healed is 50% healing time (HT_{50}) and the time for complete healing of the wound is healing time (HT_{100}). Each rat was treated with asaticoside derivatives daily until the wound was completely healed. The samples were applied as ointment with a cotton swab (approximately 50 mg/site).

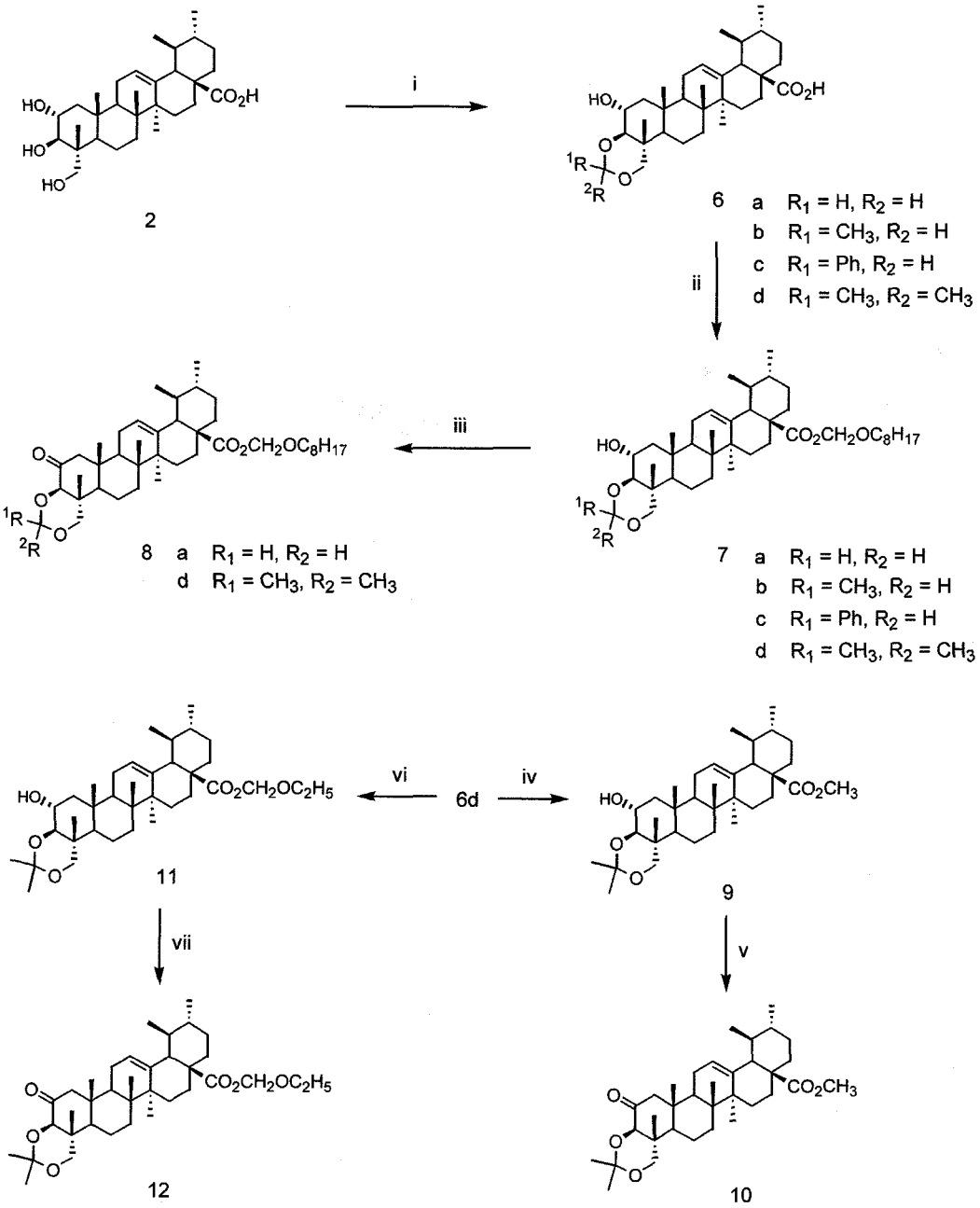
Visual assessment

A visual assessment of the quality of recovery was made for each completely healed wound. Each wound was given a score ranging from 0 to 3, with 3 corresponding to almost no scar and 0 corresponding to large scar. The scores were assigned separately by two of the investigators and averaged for analysis. In open wounds, on one, seven and fifteen days after wounding, photographs were taken at a standard distance. In incisional wounds, photographs were taken on six days after wounds. The data were analyzed for significance using Student's *t* test.

RESULTS AND DISCUSSION

Structurally, asiatic acid (**2**) has three kinds of functional groups: three hydroxy groups at C(2), C(3), and C(23); an olefin group at C(12); and a carboxylic acid group at C(28). The chemical modifications were planned to extend the lipophilicity. Among these functional groups, the C(3)-OH and C(23)-OH were modified to ketal groups and the C(2)-OH was transformed to a ketone. Also, the C(28)-carboxylic acid was converted to an ester moiety. Ten compounds were prepared from **2**, which could be easily obtained from the titrated extract of *Centella asiatica* (Scheme 1). We adapted the known procedures for the synthesis of **7d**, **11**, **12** (Jew, 2000). The ketal formation of C(3)-OH and C(23)-OH of **2**, by using the corresponding dimethoxyketals in the presence of *p*-toluenesulfonic acid, followed by octyloxymethylation of C(28)-CO_2H, gave **7a-d**. Compounds **8a** and **8d** were prepared from **7a** and **7d**, respectively, by the oxidation using pyridinium dichromate (PDC). Compounds **10** and **12** were also prepared by PDC oxidation of the corresponding alcohols, **9** and **11**, respectively obtained from **6d** by methylation using an excess of diazomethane and ethyloxymethylation, respectively.

The wound healing activity of the prepared derivatives (**7a-d**, **8a**,**d**, **9-12**) along with asiatic acid (**2**) was evaluated by measuring the tensile strength of skin strips from the wound segment according to the published method (Table I, Schulte, 1967). As shown in Table I, unexpectedly, asiatic acid (**2**) itself did not show enhanced wound healing activity over the control. Generally, the ketal derivatives (**7a-d**) showed comparable or slightly higher tensile strength than the control. Especially, the dimethylketal derivative (**7d**; 337 g/cm^2) showed 10% higher activity than the control (305 g/cm^2). The wound healing effect is also clearly affected by the alkyl group in the C(28)-CO_2R group. The bulky ester reduced the wound-healing activity (**7d**: 337 g/cm^2; **11**: 365 g/cm^2; **9**: 365 g/cm^2), which is consistent with our previous result. When the C(2)-OH is converted to a ketone moiety, the variation of wound healing effect was dependent upon the alkyl group of



Scheme 1. Reagents and conditions: i) TMSCl, DMSO, THF or RR'C$(OCH_3)_2$, PTSA(cat), DMF, 2h-72h, 32-87%, ii) $C_8H_{17}OCH_2Cl$, *i*-Pr_2NEt, DMF, 0°C, 0.5 h, 42-92%, iii) PDC, CH_2Cl_2, reflux, 2 h, 24-65%, iv) excess CH_2N_2, 0°C, 1 h, 85%, v) PDC, CH_2Cl_2, reflux, 2 h, 65%, vi) $C_2H_5OCH_2Cl$, *i*-Pr_2NEt, DMF, 0°C, 0.5 h, 85%, vii) PDC, CH_2Cl_2, reflux, 2 h, 85%.

C(28)-CO_2R. The octyloxymetyl (**7d**) and methyl ester derivatives (**9**) had 7% and 9% less activity, respectively, but that of ethyloxymethyl ester derivative (**12**) increased by 3%. The enhanced wound healing effects of **12** along with those of **9**-**11** were confirmed by the wound area method (Hunt, 1969). As shown in Table 2, there were no significant differences in the healing times except for **12** (HT_{50}: 5.67 days), which could heal 8% faster than the control (HT_{50}: 6.18 days). The commercial wound healing agent, Madecassol®, is popularly used for the prevention of scarring caused by the excess production of collagen in the wound healing process. Since not only the fast and strong wound healing activity but also less scarring after healing is quite important with respect to cosmetic aspects, we also employed a visual assessment test for the selection of the candidate (Tavakkol, 1998). The visual assessments of the derivatives in both assays are shown in Tables I and II. Interestingly, **12** exhibited the highest

Table I. Effects of asiatic acid derivatives on tensile strength and healing appearance of wounds on the 5th postoperative day

Compound[a]	N	Tensile strength[b] (g/cm^2)	Relative value	Visual assessment[c]
control	25	305 ± 19	100	1.33 ± 0.08
2	21	293 ± 21	96	1.96 ± 0.12*
7a	23	322 ± 19	106	2.42 ± 0.20*
7b	19	311 ± 24	102	2.06 ± 0.14*
7c	21	323 ± 18	106	1.83 ± 0.14
7d	22	337 ± 20	110	1.28 ± 0.14
8a	18	367 ± 23*	120	2.23 ± 0.12**
8d	21	310 ± 27	102	1.77 ± 0.11
9	19	365 ± 27	120	2.41 ± 0.15**
10	23	333 ± 18	109	2.30 ± 0.14*
11	21	365 ± 15*	120	2.00 ± 0.13*
12	22	377 ± 32*	124	2.82 ± 0.14**

[a]Each group contained 20 or more rats. One skin incision was made in each rat, and was treated topically with 50 mg of a 1% ointment of the tested compounds once a day during the experimental period.

[b]Tensile strength was measured by a Rheometer (Fudoh Kogyo, Japan) with a digital readout. Each value represents the mean ± S.E. *P<0.05, **P<0.01.

[c]Photographs of each completely healed wound were given scores ranging from 0 to 3, with 3 corresponding to almost no scar and 0 corresponding to a large scar. The scores were assigned separately by two of the investigators and were averaged for analysis. Each value represents the mean ±S.E. *P<0.05, **P<0.01.

Table II. Effect of asiatic acid derivatives on the healing of experimentally opened wound in rats

Compound[a]	HT_{50} (day)[b]	Visual assessment[c]
control	6.18 ± 0.24	1.50 ± 0.08
2	5.99 ± 0.12	1.77 ± 0.08*
9	6.11 ± 0.20	2.62 ± 0.11**
10	6.01 ± 0.09	2.33 ± 0.10**
11	6.07 ± 0.08	2.04 ± 0.11**
12	5.67 ± 0.15	2.67 ± 0.10**

[a]Each group contained 20 or more rats. A square, open wound of 0.8 × 0.8 cm was made in each rat, and was treated topically with 50 mg of a 1% ointment of the tested compounds once a day during the experimental period.

[b]The surface area of the wound was measured with calipers at daily intervals until the wound was completely healed. The time for the 50% of the wound in each treatment group to be healed is the 50 % healing time (HT_{50}). Each value represents the mean ± S.E. *P<0.05, **P<0.01.

[c]Photographs of each completely healed wound were given scores ranging from 0 to 3, with 3 corresponding to almost no scar and 0 corresponding to a large scar. The scores were assigned separately by two of the investigators and were averaged for analysis. Each value represents the mean ± S.E. *P<0.05, **P<0.01.

score in both assays. Considering that the excess production of collagen in the healing process leaves a scar, the high evaluation of **12** in the visual assessment might be due to the control of collagen biosynthesis.

In conclusion, the second SAR study was performed with semi-synthetic derivatives of asiatic acid (**2**). Among them, **12** showed the highest tensile strength and the fastest wound healing activity. Furthermore it leaves the smallest scar. This finding encourages us to develop **12** as a candidate for a new wound healing agent. A mechanistic study associated with collagen biosynthesis and preclinical studies are currently underway.

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REFERENCES

Beljanski, M. and Vapaille, N., Role of triterpenes in the binding of L-amino acids by template RNA. Rev. *Eur. Etud. Clin. Biol.*, 16, 897-905 (1971).

Bonte, F., Dumas, M., Chaudagne, C., and Meybeck, A., Asiaticoside and madecassoside comparative activities on human fibroblast type I and III collagen secretion. *Ann. Pharm. Fr.*, 53, 38-42 (1995)

Bontems, J. E., A new heteroside, asiaticoside, isolated from Hydrocotyle asiatica L. (Umbelliferae) *Bull. Sci. Pharmacol.*, 49, 186-191 (1941).

Hunt, T. K., Ehrlich, H. P., Garcia, J. A., and Dunphy, J. E., Effect of vitamin A on reversing the inhibitory effect of cortisone on healing of open wounds in animals and man. *Ann. Surg.*, 170, 633-641 (1969).

Inamdar, P. K., Yeole, R. D., Ghogare, A. B., and de Souza, N. J., Determination of biologically active constituents in Centella asiatica. *J. Chromatography*, 742, 127-130 (1996).

Jew, S. S., Yoo, J. H., Lim, D. Y., Kim, H., Mook-Jung, I., Jung, M., Choi, H., Jung, Y.-h., Kim, H., and Park, H.-G., Structure-activity relationship study of Asiatic acide derivatives against β, amyloid (Aβ)-induced neurotoxicity. *Bioorg. & Med. Chem. Lett.*, 10, 119-121 (2000).

Lawrence, J. C., The effect of asiaticoside on guinea pig skin. *J. Invest. Dermatol.*, 49, 95-96 (1967a).

Lawrence, J. C., The morphological and pharmacological effects of asiaticoside upon skin *in vitro* and *in vivo*. *Eur. J. Pharmacol.*, 414-424 (1967b).

Pointel, J. P., Boccalon, H., Cloarec, M., and Ledevehat, J. M.,

Titrated extract of Centella asiatica (TECA) in the treatment of venous insufficiency of the lower limbs. *Angiology, 38*, 46-50 (1987).

Poizot, A., Dumez, D. C. R., Modification of the kinetics of healing after iterative exeresis in the rat. Action of a triterpenoid and its derivatives on the duration of healing. *Acad. Sci. [D]*, 286, 789-792 (1978).

Schulte, R. and Domenjoz, R., Methode zur Bestimmuny der Reifestigkeit heilender Hautwunden bei Ratten. *Med. Pharmacol. Exp.*, 16, 453 (1967).

Shim, P.-J., Park, J.-H., Chang, M.-S., Lim, M.-J., Kim, D.-H., Jung, Y.-H., Jew, S.-s., Park, E.-H., and Kim, H.-D., Asiaticoside mimetics as wound healing agent. *Bioorg. Med. Chem. Lett.*, *6*, 2937-2940 (1996).

Tavakkol, A., Klingman, L., Morrison, B. M., Jr., and Polefka, T. G., The effects of prolonged use of surfactants on the skin of normal and photo-exposed hairless mice. *Contact Dermatitis*, 39, 231-239 (1998).