Antiviral Triterpenes from *Prunella vulgaris*

Shi Yong Ryu*, Chong-Kyo Lee, Chong Ock Lee, Hae Soo Kim and Ok Pyo Zee

Korea Research Institute of Chemical Technology, Taejeon 305-606, Korea

(Received August 10, 1992)

**Abstract** Two triterpenes 1 and 2 with antiviral activity against *Herpes simplex* virus type 1 *in vitro* were isolated from *Prunella vulgaris*. Each compound caused a significant reduction in viral cytopathic effect when vero cells were exposed to them for 72 hours after viral challenge. They were identified as betulinic acid (1) and 2α,3α-dihydroxyurs-12-en-28-oic acid (2) on the basis of their spectroscopic properties. The antiviral activity of them was estimated as EC<sub>50</sub> = 30 μg/ml (1) and 8 μg/ml (2), respectively by plaque reduction assay.

**Keywords** *Prunella vulgaris*, Labiatae, betulinic acid, 2α,3α-dihydroxyurs-12-en-28-oic acid, antiviral, *Herpes simplex*.

We have been searching for the new class of antiviral agents from natural resources especially from plant materials. For this purpose, more than fifty kinds of medicinal plants were screened for the antiviral activity against *Herpes simplex* virus type 1 (HSV-1) and 2 (HSV-2), *in vitro*. Among these, chloroform extracts of the whole herb of *Prunella vulgaris* (Labiatae), of the fruit of *Forsythia koreana* (Oleaceae) and the root of *Sanguisorba officinalis* (Rosaceae) were observed to exhibit activity in our *in vitro* antiherpes bioassay, which were chosen as candidates for active component isolation studies.

*Prunella vulgaris* is a perennial herb which is widely distributed throughout Korea, Japan and China, and has been used in the folk medicine as a diuretic or an astringent. It was recently reported that aqueous extract of *Prunella vulgaris* showed antiviral activity against Human immunodeficiency virus (HIV-1)<sup>1</sup>, and more recently, the prunellin which is a polysaccharide isolated from *Prunella vulgaris*, was reported to exhibit anti-HIV activity<sup>2</sup>. The present paper deals with the isolation and identification of active components of *Prunella vulgaris*, which showed significant anti-HSV-1 activities *in vitro*, by the activity-guided fractionation.

**EXPERIMENTAL**

1H-NMR spectra were run at 300 MHz and 13C-NMR at 75 MHz and recorded by Bruker AM-300. MS (70 eV) were taken with a direct inlet and recorded by GC-MS QP-100 (Shimadzu) spectrometer.

**In vitro evaluation of anti-HSV-1 activity**

The anti-HSV-1 activity was evaluated by plaque reduction assay<sup>4</sup>. Confluent monolayers of vero cells in 24-well plates were inoculated with 0.2 ml of HSV-1 strain F at the M.O.I. (multiplicity of infection) of 100 PFU (plaque forming unit) per well. After 1 hour adsorption at 37°C in CO<sub>2</sub> incubator, unadsorbed viruses were aspirated off and test material at various concentrations prepared in overlay-medium (Dulbecco's modified essential medium (Gibco): 2% heat inactivated fetal bovine serum (Gibco): 0.8% gum tragacanth; 4 μg/ml gentamycin) was applied in a volume of 0.5 ml per well in duplicate. In case of necessity, the test material was dissolved in small amount of dimethylsulfoxide (DMSO), but the final concentration of DMSO in
test material-medium mixture was not exceeded 1.0%. Each plate contained duplicated cell control (no virus and no test material added) and virus control (no test material added). After 3 days incubation at 37°C in CO2 incubator, medium was removed and cells were fixed and stained with dye-fixer solution (5% formalin: 50% ethanol: 0.5% crystal violet: saline). Plaque numbers obtained in cultures containing test material at varying concentrations were compared with those of virus control which was considered as 100%. The 50% effective concentration (EC50) was defined as the concentration of test material that caused 50% reduction of plaque number compared with that of virus control.

**Extraction and isolation**

The whole herb of *Prunella vulgaris* was purchased at market and 1.8 kg of dried material was extracted with methanol at room temperature for 2 weeks to give an extract 85g, which was suspended in water and extracted with chloroform to give a chloroform extract 38g. One part of chloroform extract (4g) was dissolved in CHC13/MeOH (10:1) solvent and subjected to pass through neutral alumina (Al2O3, activity 1, Merck) gel (200g), which were eluted with CHCl3/MeOH (10:1) 5/ and washed with additional 51 of MeOH. The eluate and wash were pooled up and evaporated to dryness to give Fr.N 2.4g. The alumina gel was further eluted with 2.5% NH3/MeOH 51 and the eluate were collected and evaporated to dryness to give Fr.A 1.5g. The Fr.A was divided into five portions (Fr.a1 to Fr.a5) by SiO2 column chromatography. Two of which, Fr.a2 and Fr.a4 were showed marked anti-HSV-1 activity. Fr.a2 was purified by repeated SiO2 column chromatography using CHCl3/MeOH as a gradient elution followed by activity monitoring to give 52 mg of compound 1.

**Compound 1**

White needles (in MeOH). mp 300~308°, [α]D = +10.2 (c=0.5, CHCl3), MS (1b, 70 eV): m/z (rel. int.): 470(M+, missing), 411 (M+-COOCH3, 16%), 262 (65%), 207 (72%), 189 (100%). 1H-NMR (1b; CDCl3): δ 0.72, 0.80, 0.90, 0.95 and 0.96 (each 3H, s, 23, 24, 25, 26 and 27-CH3). 1.65 (3H, brs, 30-CH3), 2.22 (1H, dd, J=8.8, 4.4 Hz, 18-H), 2.95 (1H, m, 19-H), 3.15 (1H, dd, J=11.0, 5.2 Hz, 3-H), 3.58 (3H, s, COOCH3), 4.58 and 4.70 (each 1H, m, 29-H). 13C-NMR (1b; CDCl3): δ 38.9 (1-C), 27.5 (2-C), 79.1 (3-C), 38.8 (4-C), 55.2 (5-C), 18.2 (6-C), 34.2 (7-C), 40.8 (8-C), 50.5 (9-C), 37.2 (10-C), 20.8 (11-C), 25.7 (12-C), 38.2 (13-C), 42.2 (14-C), 29.9 (15-C), 32.2 (16-C), 56.5 (17-C), 49.4 (18-C), 47.0 (19-C), 150.5 (20-C), 30.7 (21-C), 36.8 (22-C), 280 (23-C), 15.5 (24-C), 16.0 (25-C), 15.9 (26-C), 14.9 (27-C), 176.5 (28-C), 109.5 (29-C), 19.5 (30-C), 51.4 (-COOCH3).

Fra4 was also repeated SiO2 column chromatography using CHCl3/MeOH as gradient elution to give compound 2 43 mg.

**Compound 2**

White needles (in MeOH). mp 186-188°, [α]D = +55 (c=0.2, CHCl3), MS (2b; 70 eV): m/z (rel. int.): 486 (M+, 1), 427 (M+- COOMe, 4), 262 (100), 223 (15), 203 (68). 1H-NMR (2b; CDCl3): δ 0.72 (3H, s, 26-CH3), 0.83 (3H, s, 24-CH3), 0.86 and 0.91 (each 3H, d, J=7.5 and 8.2 Hz, 29 and 30-CH3), 0.93 (3H, s, 25-CH3), 1.00 (3H, s, 24-CH3), 1.06 (3H, s, 27-CH3), 2.22 (1H, d, J=11 Hz, 18-H), 3.40 (1H, d, J=2.6 Hz, 3-H), 3.58 (3H, s, -COOCH3), 3.98 (1H, m, 2-H), 5.22 (1H, m, 12-H). 13C-NMR (2b; CDCl3): δ 54.17 (1-C), 66.5 (2-C), 78.9 (3-C), 39.0 (4-C)*, 48.1 (5-C), 180 (6-C), 327 (7-C), 396 (8-C)*, 47.3 (9-C), 38.2 (10-C), 23.3 (11-C), 125.3 (12-C), 138.2 (13-C), 42.1 (14-C), 27.9 (15-C), 24.2 (16-C), 48.1 (17-C), 52.6 (18-C), 38.6 (19-C), 38.3 (20-C), 30.6 (21-C), 36.6 (22-C), 28.5 (23-C), 216 (24-C), 164 (25-C), 17.0 (26-C), 23.7 (27-C), 178.1 (28-C), 16.9 (29-C), 21.2 (30-C), 51.4 (-COOCH3)*. Assignments may be reversed.

Compound 3 380 mg and compound 4 8 mg were yielded from Fra3 and Fra1, respectively by re-