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Antiviral Activity of Triterpenoid Derivatives

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3-Oxo or/and 11-oxo derivatives of natural 3-*hydroxy* triterpenes, *i.e.*, 3-oxoursolic acid **Ia**, 11-oxoursolic acid **Ib**, 3,11-dioxoursolic acid **Ic**, 3-oxobetulinic acid **IIa** and 3-oxopomolic acid **VIa** were exhibited to show an increased anti-HSV-1 activity *in vitro*, four to ten times as much as corresponding parent 3-*hydroxy* compounds.

Key words: 3-Oxoursolic acid, 3-Oxobetulinic acid, 11-Oxoursolic acid, 3-Oxopomolic acid, 3,11-Dioxoursolic acid, 3-Oxoglycyrrhetinic acid, Antiviral, HSV-1

INTRODUCTION

We have previously reported that ursolic acid **I**, betulinic acid **II**, and 2α,3α-dihydroxyurs-11-en-28-oic acid **III**, which were isolated from *Prunella vulgaris* (Labiatae), were exhibited a marked antiviral activity against HSV-1 (*Herpes simplex* virus type I) *in vitro* (Ryu *et al.*, 1992). On the continuing effort to the search for antivirally active compounds from natural resources especially from medicinal plants, we have succeeded to isolate another active ingredient, ziyu-glycoside 2 **V** from the root of *Sanguisorba officinalis* (Rosaceae). Even though all isolated active compounds were comprised in wellknown natural products belonging to a common triterpene series, it is interesting that all of them have a similar biological activity as well as some similarities on their structural features, *i.e.* each has a hydroxyl group at 3-position of A-ring and a carboxylic group at C/D ring of triterpene skeleton. It has been reported that several triterpenes such as aescin (Rao *et al.*, 1968), gymnemic acid (Sinsheimer *et al.*, 1968), protoprimulagenin (Amoros *et al.*, 1987) and glycyrrhizin (Ito *et al.*, 1987) were observed to exhibit antiviral activity *in vitro* and in some cases, *in vivo*, especially against HIV (human immunodeficiency virus) and HSV. Recently, Hirabayashi *et al.* (1991) reported that glycyrrhizin, which was a major saponin isolated from licorice root, showed an inhibitory activity against HIV-1 and HSV-1 and suggested that the 11-positioned oxo group of a glycyrrhizin or of glycyrrhetinic acid which was an unique structure of them, was predominantly or partially responsible for the activity. On the basis of this suggestion, we have tried to investigate the activity of some oxidative derivatives of **I**, **II** and **IV**, which were prepared by the conversion of 3-positioned hydroxyl group to oxo group or the insertion of new oxo group on 11-positioned carbon of the corresponding parent compound, together with for glycyrrhizin and its aglycone, a glycyrrhetinic acid, which were known as antiviral compounds and also as unique triterpenes that have 11-positioned oxo group.

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MATERIALS AND METHODS

^{1}H-NMR spectra were run at 300 MHz and and recorded by Bruker AM-300. MS(70 eV) were taken with a direct inlet and recorded by GC-MS QP-100 (Shimadzu) spectrometer. *Vero* cell and HSV-1 strain F were used for the assay of antiviral activity *in vitro*.

In vitro evaluation of anti HSV-1 activity

The anti-HSV-1 activity was evaluated by the plaque reduction assay (Hu *et al.*, 1989). The detailed procedures for the assay was described on the previous paper (Ryu *et al.*, 1992).

Isolation and derivatization

Ursolic acid **I**, betulinic acid **II** and 2α,3α-dihydroxyurs-12-en-28-oic acid **III** were isolated from *Prunella vulgaris* by the procedure described on the previous paper (Ryu *et al.*, 1992). Ziyu-glycoside 1 **IV** and ziyu-glycoside 2 **V** were isolated from *Sanguisorba officinalis* according to the scheme for the isolation of the antivirally active compounds from medicinal plants (Park *et al.*, 1992). Glycyrrhizin (**VII**, glycyrrhizic acid ammo-

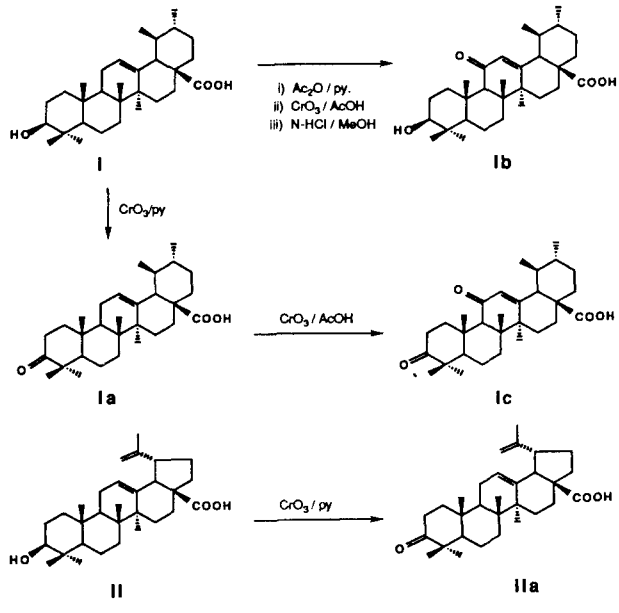
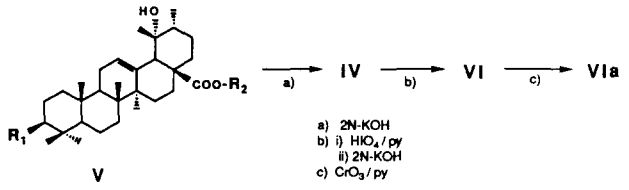
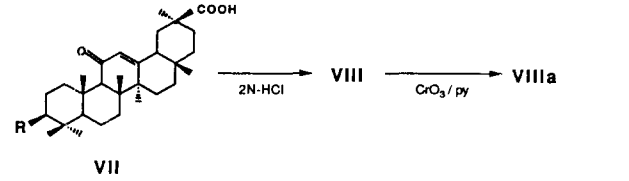


Fig. 1. The synthetic scheme of **Ia**, **Ib**, **Ic** and **IIIa**.



		R_2	R_2
V	(Ziyu-glycoside-1)	-O-α-L-ara	-β-D-glc
IV	(Ziyu-glycoside-2)	-O-α-L-ara	-H
VI	(Pomolic acid)	-OH	-H
VIa	(3-Oxopomolic acid)	=0	-H



		R
VII	(Glycyrrhizin)	-O-gln-O-gln
VIII	(Glycyrrhetinic acid)	-OH
VIIIa	(3-Oxoglycyrrhetinic acid)	=O

Fig. 2. The synthetic scheme of **VI**, **VIa** and **VIIIa**.

nium salt) was purchased from Sigma Co. (USA). Glycyrrhetinic acid (**VIII**, glycyrrhetic acid) was prepared by the acid hydrolysis of glycyrrhizin **VII** with 2-N HCl. 3-Oxo or/and 11-oxo derivatives was prepared by the CrO_3 oxidation in various conditions described below (Fig. 1 and 2).

Preparation of 3-oxoursolic acid (Ia, ursonic acid, 3-oxo-12-ursen-28-oic acid): A freshly prepared complex of CrO_3 (50 mg, 0.5 meq.) in 1.0 ml of pyridine was added to a solution of I (50 mg, 0.11 meq.) in 0.5 ml pyridine, and stirred at room temp. overnight (Han, B. H. *et al.*, 1984). The mixture was poured into an excess amount of cold water and then extracted with ether. The ethereal layer was washed with d-HCl followed by water and dried over Na_2SO_4, which was purified by the silica gel column chromatography eluting with CH_2Cl_2/MeOH, and yielded 35 mg of colorless amorphous powder **Ia**. It was identical with the authentic sample by the co-TLC as well as ^{1}H-NMR and MS datas were superimposible with reported ones (Poehland *et al.*, 1987).

Preparation of 11-oxoursolic acid (Ib, 3β-hydroxy-11-oxo-12-ursen-28-oic acid): The ursolic acid **I** was converted to acetylursolic acid **Id** by the conventional manner using Ac_2O/pyridine. One hundred mg of CrO_3 (1.0 meq.) in 2 ml of AcOH were added slowly into **Id** 50 mg (0.1 meq.) dissolved in 1 ml of AcOH, stirred for 4 hours at room temp. The reaction mixture was diluted with cold water and extracted with EtOAc. EtOAc layer was dried over Na_2SO_4 and evaporated to dryness, which was chromatographyed on silica gel and finally given 40 mg of 3-O-acetyl-11-oxoursolic acid **Ie**. It was dissolved in N HCl in MeOH and refluxed for 2 hours. Then the reaction mixture was evaporated to dryness and purified by silica gel column chromatography to give 28 mg of colorless amorphous powder **Ib** (Siddiqui *et al.*, 1990). UV λ_{max} 250(MeOH). EIMS (70 eV): m/z(rel. int.); 470(M^+, 66%), 455(31%), 303(100%), 262(98%). ^{1}H-NMR (DMSO, δ): 0.68. 0.85, 0.88, 0.98, 1.25 (each 3H, s, 23, 24, 25, 26 and 27-CH_3), 0.80 and 0.90 (each 3H, d, J=7.5 and 8.0 Hz, 29 and 30-CH_3), 2.05 (1H, m, 19-H), 2.28 (1H, d, J=12 Hz, 18-H), 2.34 (1H, s, 9-H), 5.38(1H, s, 12-H).

Preparation of 3,11-dioxoursolic acid Ic: One hundred mg of CrO_3 (1.0 meq.) in AcOH was added slowly into the solution of Ia 50 mg (0.11 meq.) in 1.0 ml AcOH. The reaction mixture was stirred for 4 hours at room temp, then diluted with an excess amount of water and extracted with EtOAc. The EtOAc layer was concentrated to dryness and subjected to silica gel column chromtography to give 40 mg of colorless amorphous powder **Ic** (Zaletova *et al.*, 1986). UV λ_{max} 250(MeOH). EIMS (70 eV): m/z(rel. int.); 468(M^+, 52%), 453(32%), 303(98%), 262(100%). ^{1}H-NMR ($CDCl_3$, δ): 0.88. 0.94, 0.98, 1.22 and 1.30 (each 3H, s, 23, 24, 25, 26 and 27-CH_3), 0.85 and 0.90 (each 3H, d, J=7.5 and 8.0 Hz, 29 and 30-CH_3), 2.48 (1H, s, 9-H), 2.46 (1H, d, J=12 Hz, 18-H), 5.61(1H, s, 12-H).

Preparation of pomolic acid VI: The pomolic acid **VI** was prepared by the HIO4 oxidation of **IV**, follo-

Table I. Anti-HSV-1 activity of triterpenoid derivatives *in vitro*

		EC_{50} (μg/ml)[a]
I	(ursolic acid)	80
Ia	(3-oxoursolic acid)	8
Ib	(11-oxoursolic acid)	15
Ic	(3,11-dioxoursolic acid)	18
II	(betulinic acid)	30
IIa	(3-oxobetulinic acid)	5
III	(2,3-dihydroxyurs-12-en-28-oic acid)	8
IV	(ziyu-glycoside-1)	>100
V	(ziyu-glycoside-2)	10
VI	(pomolic acid)	50
VIa	(3-oxopomolic acid)	10
VII	(glycyrrhizin)	100
VIII	(glycyrrhetinic acid)	30
VIIIa	(3-oxoglycyrrhetinic acid)	30

[a]The 50% effective concentration (EC_{50}) was defined as the concentration of test material that caused 50% reduction of plaque number compared with that of virus control.

wed by the alkaline hydrolysis with 2-N KOH (Han *et al.*, 1985).

Preparation 3-oxobetulinic acid (IIa, *betulonic acid*), 3-oxopomolic acid VIa and 3-oxoglycyrrhetinic acid VIIIa: II, VI and **VIII** were treated with the CrO_3 respectively with the same manner mentioned above as for the preparation of **Ia** from **I**.

3-Oxobetulinic acid (IIa, betulonic acid, Gonalez *et al.*, 1983). mp. 230-235℃. EIMS (70 eV): m/z(rel. int.); 454(M^+, 76%), 408(48%), 248(100%), 235(54%), 205(72 %). ^{1}H-NMR ($CDCl_3$, δ): 0.88. 0.90, 0.92, 0.94 and 1.02 (each 3H, s, 23, 24, 25, 26 and 27-CH_3), 1.65(3H, s, 30-CH_3), 2.95 (1H, m, 19-H), 4.58 and 4.68 (each 1H, m, 29-H).

3-Oxopomolic acid. VIa. (Cheng *et al.*, 1992) mp. 195-197℃. EIMS (70 eV): m/z(rel. int.); 470(M^+, 16%), 452(12%), 424(100%), 352(54%), 146(98%). ^{1}H-NMR ($CDCl_3$, δ): 0.78. 0.95, 0.98, 1.00 and 1.23 (each 3H, s, 23, 24, 25, 26 and 27-CH_3), 0.90 (3H, d, J=6.6 Hz, 30-CH_3), 1.27 (3H, s, 29-CH_3), 2.50 (1H, s, 18-H), 5.30 (1H, t, J=3.5 Hz, 12-H).

3-Oxoglycyrrhetinic acid. VIIIa. (Hattori *et al.*, 1983) mp. >300℃. UV λ_{max}; 250(MeOH). EIMS (70 eV): m/z (rel. int.); 468(M^+, 57%), 453(30%), 440(34%), 303(98%), 262(100%), 135(64%). ^{1}H-NMR($CDCl_3$, δ): 0.82. 1.03, 1.07, 1.18, 1.23, 1.28 and 1.40 (each 3H, s, 23, 24, 25, 26, 27, 28 and 30-CH_3), 2.42 (1H, s, 9-H), 5.73 (1H, brs, 12-H).

RESULTS AND DISCUSSION

For the purpose of finding a more effective compound against HSV-1, we have tried to investigate the activity of six kinds of synthesized 3-oxo or/and 11-oxo-derivatives of terpenoids known as antiviral compounds. All of them were identified by the direct comparison with authentic sample or by reported spectral properties of them. The bioassay for the antiviral activity of them against the anti-HSV-1 *in vitro* was revealed that 3-oxo-compounds **Ia**, **IIa** and **Va** as well as 11-oxo-ursolic acid **Ib** and 3,11-*dioxo*-ursolic acid **Ic** were 4 to 10 times as potent as the corresponding parent 3-*hydroxy*-compounds, whereas the other 3, 11-dioxo-compound, 3-*oxo*-glycyrrhetinic acid **VIIIa** retained the activity of glycyrrhetinic acid **VIII** (Table I). **Ia** was exhibited the activity as much as with the similar potency compared with that of the reported (Poehland *et al.*, 1987), whereas, glycyrrhizin **VII** was shown a poor activity at least by the current bioassay system in spite of its prominency as an promising antiviral compound (Hirabayashi *et al.*, 1991). Therefore, it seemed to be hard to tell the real activity or the potency of them just by the results obtained by the present experiments, because of the difference of the bioassay methods evaluating the antiviral activity. One of the interesting thing was that 3-*oxo* group instead of 3-*hydroxyl* group of the mentioned triterpenes were probably much contributive to the activty of them, therefore, for example, **Ia** and **IIa** could become more recommendable than **I** and **II** for the drug development. On the previous paper (Ryu *et al.*, 1992), it had been suggested that the 3-*hydroxyl* group and 28-*carboxylic* group might be an active site for the antiviral activity of **II** and **III**, because of the fact that the lack or diminution of activity was resulted from the protection of each group with acetyl or methyl group. However, such an assumption was still vague because that the 3-oxo compounds were shown much stronger activity than the corresponding 3-*hydroxyl* compounds.

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