Anti-herpes Virus Type 1 Activity of Oleanane-Type Triterpenoids[1)]

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The anti-herpes simplex virus type 1 (HSV-1) activity of 15 oleanan-type triterpenoides including glycyrrhizin and its sapogenol was examined and their structure–activity relationships were discussed. Although glycyrrhizin which exhibited *in vivo* efficacy against HSV-1 replication showed moderate *in vitro* anti-HSV-1 activity, its sapogenol, glycyrrhetic acid, showed 10 times greater action than glycyrrhizin. Therefore, the *in vivo* anti-HSV-1 activity of glycyrrhizin administered orally could be reasonably attributed to glycyrrhetic acid generated by hydrolysis by intestinal bacteria. Since the activity of soyasapogenol A was less than 1/20 of that of soyasapogenol B, the hydroxylation at C-21 seemed to reduce anti-HSV-1 activity. Since kudzusapogenol A, abrisapogenols B and C lacked the activity, the C-29 hydroxy group would eliminate anti-HSV-1 activity. On the other hand, since the methylesters of kudzusapogenol B and glycyrrhetic acid exhibited greater action, a methoxy carboxy group at C-20 might enhance activity.

Key words anti-herpes simplex virus activity; glycyrrhizin; oleanane-type triterpenoid; structure–activity relationship; Leguminosae

Some triterpenoidal saponins have been reported to show anti-herpes simplex virus type 1 (HSV-1) activity.[2—4)] In a preceding paper[5)] we reported the *in vitro* anti-HSV-1 activity of some leguminous triterpenoidal saponins.

Very recently, Cinatl *et al*. reported that a leguminous saponin, glycyrrhizin, inhibited replication of the severe acute respiratory syndrome (SARS)-associated virus.[6)] Glycyrrhizin also showed potent increase in the survival rate against murine HSV-1 encephalitis.[7)] Further, Kurokawa *et al*. proved that a triterpene sapogenol (non-saccharide part of saponin) itself exhibited oral therapeutic anti-HSV-1 activity in mice.[8)] These reports have prompted us to examine the anti-HSV-1 action of triterpenoidal sapogenols together with glycyrrhizin and its sapogenol (glycyrrhetic acid).

Herein, we describe the anti-HSV-1 activity of 15 oleanane-type triterpenoids and discuss their structure–activity relationships.

MATERIALS AND METHODS

Materials Glycyrrhizin (**1**) and glycyrrhetic acid (**2**) were purchased from Tokyo Chemical Ind. Co. (Tokyo, Japan). Compound **3** was prepared in the usual manner.[9)] Compounds **4**—**15** were prepared as described in previous papers.[10,11)] Fetal calf serum (FCS) was purchased from Gibco BRL (Grand Island, NY, U.S.A.). Sulfonated γ-globulin (Venilon) was supplied by the Chemo-Sero Therapeutic Institute.

Virus and Cells HSV-1 strain KOS and Vero cells were provided by the Chemo-Sero-Therapeutic Institute.

Antiviral Assays The antiviral activity of test samples on HSV-1 (KOS) was measured by the plaque reduction assay.[12)] The detailed procedure was described in a previous paper.[13)]

Cytotoxic Assays The anticellular activity was examined as described previously.[13)]

RESULTS AND DISCUSSION

The results of anti-HSV-1 activity, cytotoxicity and a selective index are listed in Table 1.

Glycyrrhizin (**1**) which exhibited *in vivo* efficacy[7)] against

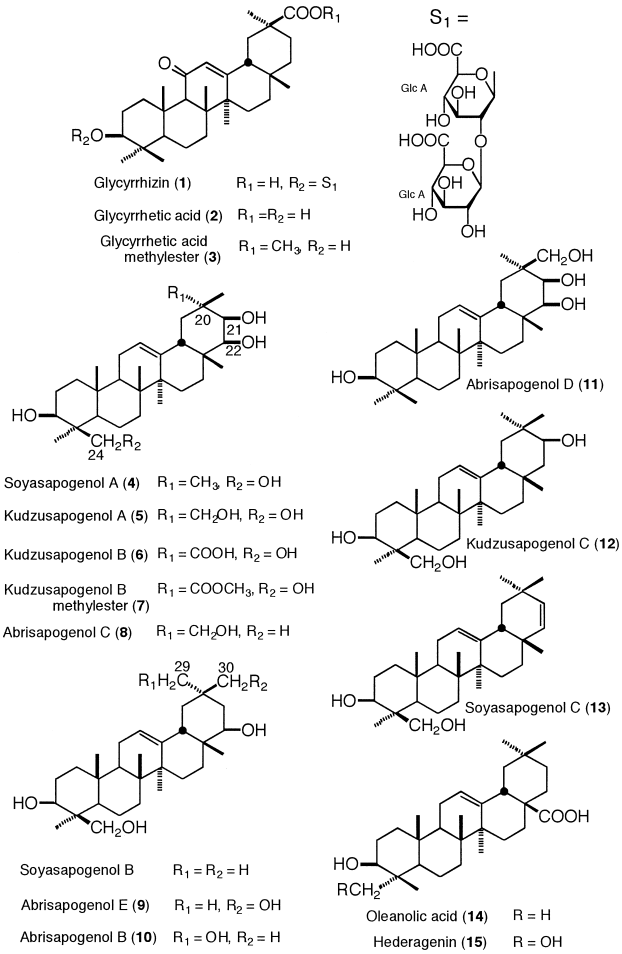

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Table 1. Anti-HSV-1 Activity, Cytotoxicity and Selectivity Index

Sample	Anti-HSV-1 activity (IC_{50}; μM)	Cytotoxicity (CC_{50}; μM)	Selectivity index (CC_{50}/IC_{50})
Glycyrrhizin (**1**)	225±24.1	>608	>2.7
Glycyrrhetic acid (**2**)	21.7±0.6	84.0±2.8	3.9
Glycyrrhetic acid methylester (**3**)	8.1±0.2	>207	>26
Soysapogenol A (**4**)	124±6.8	169±24	1.4
Kudzusapogenol A (**5**)	>204	>1020	—
Kudzusapogenol B (**6**)	32.7±3.4	219±29	6.7
Kudzusapogenol B methylester (**7**)	12.9±0.8	290±49	22
Abrisapogenol C (**8**)	>105	>2640	—
Abrisapogenol E (**9**)	11.5±0.3	71.3±13	6.2
Abrisapogenol B (**10**)	>105	320±88	—
Abrisapogenol D (**11**)	>105	>2640	—
Kudzusapogenol C (**12**)	84.1±4.6	1570±264	19
Soyasapogenol C (**13**)	18.9±1.0	921±131	49
Oleanolic acid (**14**)	18.2±1.1	172±29	9.4
Hederagenin (**15**)	29.2±1.7	108±2.1	3.7
Soysapogenol B[5]	5.6±0.6	116±29	20.7
Soyasaponin I[5]	>75.0	—	—
Acyclovir (positive control)	1.1±0.09	>444	>400

The results are expressed as mean±S.E. (n=2).

HSV-1 replication showed moderate *in vitro* anti-HSV-1 activity. On the contrary, its sapogenol, glycyrrhetic acid (**2**), showed greater action than **1**. The potency was 10 times higher. Further, the potency of its methylester (**3**) was about three times greater than that of **2**. The IC_{50} value (8.1 μM) of **3** was equal to that of soyasapogenol B (5.6 μM) which showed the highest activity in the previous experiment.[5]

The activity of soyasapogenol A (**4**) was less than 1/20 of that of soyasapogenol B. Since **4** was a hydroxy derivative of **3** at C-21, the hydroxylation at C-21 would reduce anti-HSV-1 activity. Similarly, kudzusapogenol A (**5**), a hydroxy derivative of **4** at C-29, did not show any activity. On the other hand, kudzusapogenol B (**6**) having an α-carboxy group at C-20 exhibited greater action than **4**. Further, the potency of its methylester (**7**) was about 2.5 times greater than that of **6**. Although the configurations for the carboxy groups of **3** and **7** were opposite, a methoxy carboxy group at C-20 might enhance activity.

Although the IC_{50} value (11.5 μM) of abrisapogenol E (**9**) having a hydroxy group at C-30 was about one-half of that of soyasapogenol B, the hydroxy derivative (abrisapogenol B, **10**) of soyasapogenol B at C-29 did not show any activity. Since abrisapogenol C (**8**) also lacked the activity, the C-29 hydroxy group would eliminate anti-HSV-1 activity. Abrisapogenol D (**11**) did not show any activity, indicating that not only the hydroxylation at C-21 but also the dehydroxylation at C-24 might reduce anti-HSV-1 activity, since the potency of soyasapogenol B having a hydroxy group at C-24 was about 7 times that of sophoradiol.[5]

The activity of kudzusapogenol C (**12**) was slightly more effective than that of **4**. Further, soyasapogenol C (**13**) having a double bond at C-21 and C-22 instead of the hydroxy groups, showed moderate activity. The potency of oleanolic acid (**14**) and its hydroxy derivative (**15**) was almost comparable with that of **13**. Hence, the hydroxy groups at C-21 and C-22 seemed to not contribute to anti-HSV-1 activity.

The obtained structure–anti-HSV-1 activity relationships are summarized in Fig. 1.

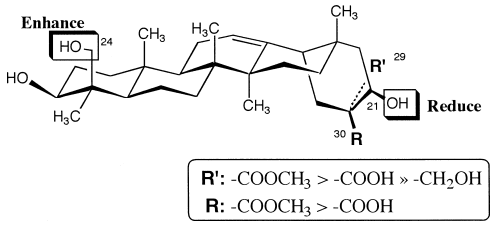

Fig. 1. Structure Anti-HSV-1 Activity Relationships of Oleanane-Type Triterpenoides

Usually, when crude drugs comprising saponins are adminstered orally, the saponins are metabolized into their sapogenols by enteric bacteria. The sapogenols produced by enteric bacteria are absorbed through the intestinal membranes. Therefore, the *in vivo* anti-HSV-1 activity[7] of glycyrrhizin (**1**) could be reasonably attributed to glycyrrhetic acid (**2**) generated by hydrolysis by intestinal bacteria. So far, soyasapogenol B was the most potent compound in the tested compounds. Since soyasapogenol B is the main sapogenol of soybean saponins, the antiviral activity of soyasapogenol B may also contribute to the health-promoting effect of soybeans.

REFERENCES AND NOTES

1) Part 4 in a series of studies on antiherpetic drugs. Part 3: Tanaka T., Ikeda T., Kaku M., Zhu X.-H., Okawa M., Yokomizo K., Uyeda M., Nohara T., *Chem. Pharm. Bull.*, **52**, 1242—1245 (2004).
2) Ushio U., Abe H., *Planta Medica*, **58**, 171—173 (1992).
3) Sindambiwe J. B., Calomme M., Geerts S., Pieters L., Vlietinck A. J., Vanden Berghe D. A., *J. Nat. Prod.*, **61**, 585—590 (1998).
4) Simoes C. M. O., Amoros M., Girre L., *Phytother. Res.*, **13**, 323—328 (1999).
5) Kinjo J., Yokomizo K., Hirakawa T., Shii Y., Nohara T., Uyeda M., *Biol. Pharm. Bull.*, **23**, 887—889 (2000).
6) Cinatl J., Morgenstern B., Bauer G., Chandra P., Rabenau H., Doerr H. W., *Lancet*, **361**, 2045—2046 (2003).
7) Sekizawa T., Yanagi K., Itoyama Y., *Acta Virologica*, **45**, 51—54 (2001).
8) Kurokawa M., Basnet P., Ohsugi M., Hozumi T., Kadota S., Namba T., Kawana T., Shiroki K., *J. Pharmacol. Exp. Ther.*, **289**, 72—78 (1999).
9) Hirooka M., Morishima N., Kaji E., Mori Y., Zen S., *Yakugaku Zasshi*,

109, 544—559 (1989).
10) Kinjo J., Miyamoto I., Murakami K., Kida K., Tomimatsu T., Yamasaki M., Nohara T., *Chem. Pharm. Bull.*, **33**, 1293—1296 (1985).
11) Takeshita T., Yokoyama K., Ding Y., Kinjo J., Nohara T., *Chem. Pharm. Bull.*, **39**, 1908—1910 (1991).
12) Schinazi R. F., Peters J., Williams C. C., Chance D., Nahmias A. J., *Antimicrob. Agent Chemother.*, **22**, 499—507 (1982).
13) Ikeda T., Ando J., Miyazono A., Zhu X., Tsumagari H., Nohara T., Yokomizo K., Uyeda M., *Biol. Pharm. Bull.*, **23**, 363—364 (2000).