Two New Triterpenes from the Rhizome of *Dryopteris crassirhizoma*, and Inhibitory Activities of Its Constituents on Human Immunodeficiency Virus-1 Protease

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Two new hopane type triterpenes, named dryopteric acids A (1) and B (2), were isolated from the Rhizome of *Dryopteris crassirhizoma* (Aspiadaceae) together with sixteen known compounds (3—18). Of isolated compounds, ursolic acid (15), and dryopteric acid A (1) and B (2) showed potent inhibitory activities against HIV-1 protease with IC_{50} values of 8.9—44.5 μM. In addition, acetylated compounds 1 and 2 appreciably increased inhibitory activities with their IC_{50} values of 1.7 and 10.8 μM, respectively.

Key words *Dryopteris crassirhizoma*; human immunodeficiency virus (HIV)-1 protease; dryopteric acid A; dryopteric acid B; hopanoic acid

Human immunodeficiency virus (HIV)-1 has been reported as an aetiological agent of acquired immunodeficiency syndrome (AIDS) in humans. Through processing of precursor polypeptide in the life cycle of this virus infection, HIV-1 protease is an essential enzyme for maturation of the virus particles. Therefore, this enzyme (aspartate protease) is considered to be a promising target for the developments of new anti-HIV agents, and these enzyme inhibitors have also been clinically used as effective chemotherapeutic agents of this disease. However, due to the appearance of resistant viruses to these inhibitory agents, development of new type inhibitors are demanded for anti-AIDS agents.[1,2]

The rhizome of *Dryopteris crassirhizoma* (Aspiadaceae) has been used as a tapeworm remedy, a cold remedy, and a therapeutic agent of viral diseases in traditional Chinese medicine.[3] From this plant, phloroglucinols have been reported as anti-bacterial and anti-oxidative agents,[4,5] and our group has reported four kaempferol acetylrhamnosides as HIV-1 reverse transcriptase inhibitors.[6] In the screening of Mongolian herbal medicines for their inhibitory activity against HIV-1 protease, an MeOH extract of the Rhizome of *D. crassirhizoma* showed potent activity with 89.2% inhibition at 200 μg/ml.[7] However, compounds responsible for this HIV-1 protease inhibitory activity have not yet been reported. Therefore, we intended to isolate HIV-1 protease inhibitors from this plant. This paper reports the isolation and structure determination, and inhibitory activity of compounds from *D. crassirhizoma* against HIV-1 protease *in vitro*.

The $CHCl_3$-, EtOAc-, BuOH- and H_2O-soluble fractions obtained from the MeOH extract showed potent activities with 82.5, 73.3, 78.3 and 81.3% at 100 μg/ml, respectively.

Eighteen compounds (**1**—**18**) were isolated from each fraction (eleven, seven, and two) compounds from the $CHCl_3$-, EtOAc-, and BuOH-soluble fractions, respectively) using repeatedly normal and reversed phase silica gel column, Sephadex LH-20, Diaion HP-20, Sep-pak, and preparative HPLC. By comparing their spectral data with those of published values (Fig. 1), the isolated compounds **3**—**18** were determined to be dimethylflavanones [desmethoxymatteucinol (**3**), matteucinol (**4**), and methoxymatteucin (**5**)],[8—10] kaempferol rhamnosides [kaempferol 7-*O*-α-L-rhamnopyranoside (**6**), kaempferol 3-*O*-α-L-rhamnopyranoside (**7**), kaempferol 3-*O*-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**8**)], kaempferol acetylrhamnosides [kaempferol 3-*O*-α-L-(4-*O*-acetyl)rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**9**, sutchuenoside A), kaempferol 3-*O*-α-L-(2,4-di-*O*-acetyl)rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**10**, crassirhizomoside A), kaempferol 3-*O*-α-L-(3,4-di-*O*-acetyl)rhamnopyranoside-7-*O*-α-L-rhamnopyrano-

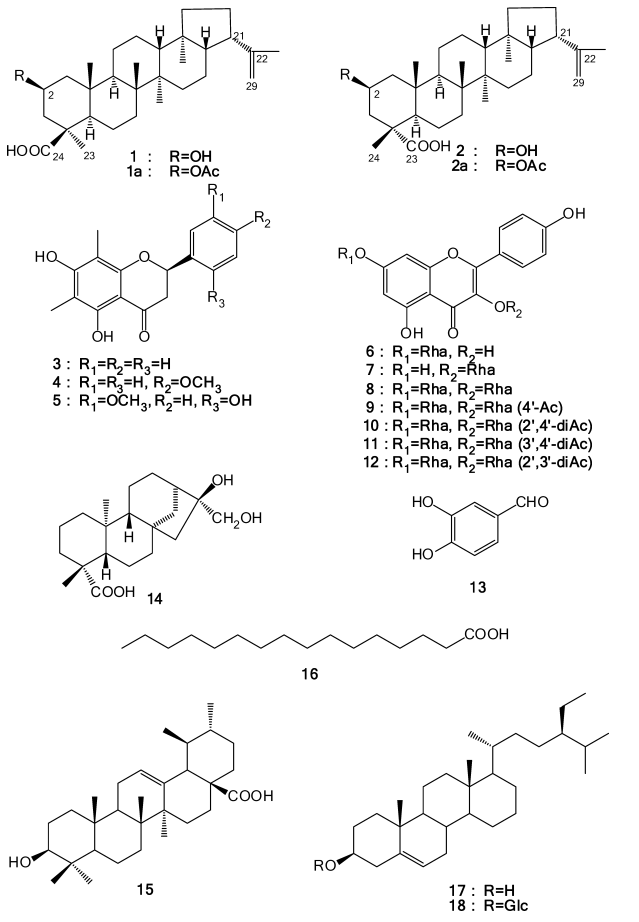

Fig. 1. Structures of Compounds **1**—**18** from the Rhizome of *D. crassirhizoma* and Acetylated Dryopteric Acids

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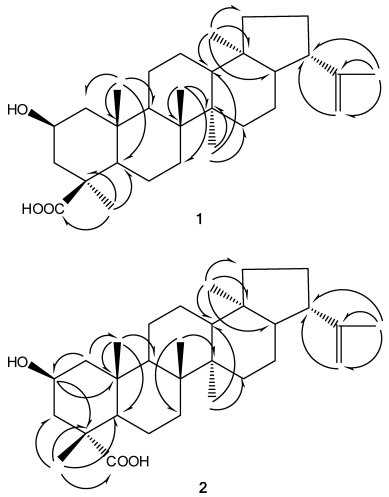

Fig. 2. HMBC Correlations of Compounds **1** and **2**

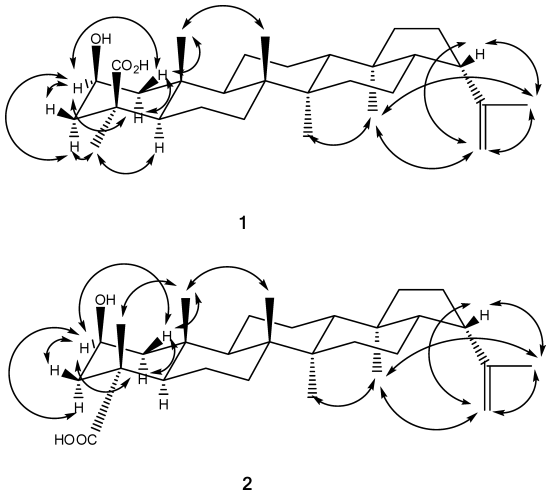

Fig. 3. Nuclear Overhauser Effects of Compounds **1** and **2**

side (**11**, crassirhizomoside B), kaempferol 3-*O*-α-L-(2,3-di-*O*-acetyl)rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**12**, crassirhizomoside C)],[7,11] 3,4-dihydroxybenzaldehyde (**13**), 16β,17-dihydroxykauran-18-oic acid (**14**),[12,13] ursolic acid (**15**), stearic acid (**16**), β-sitosterol (**17**), and β-sitosterol glucoside (**18**). Of the known compounds, **3**—**8** and **13**—**15** were isolated for the first time from this plant family. Compounds **1** and **2** were new, and their structures were determined as follows:

Compound **1** was obtained as a white amorphous powder. The molecular formula $C_{30}H_{48}O_3$ was assigned by high-resolution electron impact mass spectrum (HR-EI-MS). The ^{1}H-NMR spectrum showed the presence of six singlet methyl groups [δ 1.43, 1.39, 0.98, 0.94, 0.73, 1.75 (H-23, 25, 26, 27, 28, 30)], and an isopropenyl group [δ 4.89 (m), 1.75 (s) (H-29, 30)]. An ion peak at *m*/*z* 189 in the EI-MS spectrum exhibited a characteristic triterpene of hopane or lupane series, possessing an isopropenyl substituent, fragment peaks revealed the presence of hydroxyl and carboxylic acid groups in A–B rings, and an isopropenyl group in C–D–E rings.[14] The ^{13}C-NMR spectrum was indicative of a triterpene of 21βH-hop-22(29)-ene series by comparing the reported values of hopane and isohopane (Table 1).[14—17] Specifically, the signals at δ 46.7 (C-21), and δ 25.2 (C-30) were characteristic of the 21βH series, while signals at δ 48.0, 19.7 (C-21, C-30) corresponded with the 21αH series. In the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum, the correlations at δ 4.34 (H-2α) and δ 1.19 (H-1α), 2.30 (H-1β), 1.56 (H-3α), and 2.62 (H-3β) exhibited the presence of the hydroxyl group at C-2 position. In the nuclear Overhauser enhanced spectroscopy (NOESY) spectrum, **1** showed correlation between the proton signals δ 1.43 (H-23) and δ 1.56, 2.16 (H-3α, 5α), but failed to display any correlations of proton signals between the δ 1.43 (H-23) and δ 1.39 (H-25). The configuration at C-2 was revealed by the coupling constants, δ 2.62 (1H, dd, J=15.0, 4.0 Hz, H-3β) and 2.30 (1H, dd, J=13.5, 5.5 Hz, H-1β) to be 2β position. The presence of 2β-hydroxyl and 24-carboxylic acid groups were confirmed by ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, and heteronuclear multiple bonding correlation (HMBC) spectral data. Therefore, the chemical structure of compound **1** was elucidated as 2β-hydroxy-21βH-hop-22(29)-ene-24-oic acid.

Table 1. ^{13}C-NMR Spectral Data of Compounds **1** and **2** from the Rhizome of *D. crassirhizoma*

	1[a]	**2**[a]
1	47.4	47.4
2	66.7	66.7
3	43.4	43.4
4	44.4	47.2
5	56.4	49.4
6	19.8	19.7
7	33.5	33.1
8	42.1	42.4
9	50.7	51.5
10	37.9	37.5
11	22.0	21.9
12	24.3	24.3
13	49.7	49.8
14	42.4	42.4
15	33.7	33.8
16	21.7	21.6
17	54.9	54.9
18	44.9	44.9
19	41.9	42.1
20	27.8	27.6
21	46.7	46.7
22	148.7	148.3
23	29.7	182.3
24	183.1	20.1
25	16.3	19.2
26	16.8	16.8
27	16.7	16.9
28	16.4	16.3
29	110.7	110.6
30	25.2	25.1

a) Measured in pyridine-d_5. 125 MHz. The chemical shifts were assigned on the basis of ^{1}H–^{1}H COSY, HMQC, HMBC, and NOE experiments.

Compound **2** was also purified as a white amorphous powder. The HR-MS spectrum of compound **2** also showed a molecular ion at *m*/*z* 456.3611, which was in agreement with the molecular formula $C_{30}H_{48}O_3$. Spectral data of **2** were quite similar to those of **1**, suggesting that **2** was the same planar structure of **1**. The C, D, and E rings of these two compounds were identical by comparison of 1D and 2D NMR spectra (Table 1). In the NOESY spectrum, **2** did not show any correlation between proton signals of δ 1.43 (H-23) and δ 1.56,

Table 2. Inhibitory Effects of Compounds Isolated from the Rhizome of *D. crassirhizoma* against HIV-1 protease *in Vitro*

Compound	IC_{50} value (μM)[a)]
Dryopteric acid A (**1**)	26.5
2-*O*-Acetyldryopteric acid A (**1a**)	1.7
Dryopteric acid B (**2**)	44.5
2-*O*-Acetyldryopteric acid A (**2a**)	10.8
Desmethoxymatteucinol (**3**)	105.9
Matteucinol (**4**)	64.3
Methoxymatteucin (**5**)	100.9
Kaempferol 7-*O*-α-L-rhamnopyranoside (**6**)	146.6
Kaempferol 3-*O*-α-L-rhamnopyranoside (**7**)	>300
Kaempferol 3-*O*-α-L-rhamnopyranoside-7-*O*-α-L-rhamnopyranoside (**8**)	>300
Sutchuenoside A (**9**)	88.0
Crassirhizomoside A (**10**)	>300
Crassirhizomoside B (**11**)	>300
Crassirhizomoside C (**12**)	100.1
3,4-Dihydroxybenzaldehyde (**13**)	27.5
16β,17-Dihydroxykauran-18-oic acid (**14**)	>300
Ursolic acid (**15**)	8.9
Stearic acid (**16**)	>300
β-Sitosterol (**17**)	>300
β-Sitosterol glucoside (**18**)	>300
Acetyl-pepstatin[b)]	0.09

a) The IC_{50} value was defined as a concentration of compounds needed to reduce 50% of a *p*-NO_2-Phe-bearing hydrolysate peak relative to control. The data are mean values of three experiments performed in triplicate. *b*) Acetyl-pepstatin as positive control.

2.16 (H-3α, 5α), but did show a correlation of the methyl signals at the 24 and 25 positions [δ 1.81 (H-24) and δ 1.42 (H-25)], which suggested the presence of carboxylic acid group at the 23 position. Therefore, the chemical structure of compound **2** was elucidated as 2β-hydroxy-21βH-hop-22(29)-ene-23-oic acid.

Compounds **1** and **2** were labeled as new natural products, dryopteric acids A and B.

Compounds **1**, **2**, **13**, and **15** inhibited HIV-1 protease with IC_{50} values of 8.9—44.5 μM (Table 2). Dryopteric acid A (**1**) bearing a carboxylic acid group at 24 position (IC_{50}, 26.5 μM) showed more potent inhibitory activity than dryopteric acid B (**2**) bearing a carboxylic acid group at 23 position (IC_{50}, 44.5 μM). Interestingly, the activities of **1** and **2** were abruptly increased by a factor of 15 and 4 times by acetylating the 2β hydroxyl group, respectively (**1a**, IC_{50}, 1.7 μM, **2a**, IC_{50}, 10.8 μM). In the results, 2β-acetoxy-21βH-hop-22(29)-ene-24-oic acid (**1a**) showed the most potent activity (IC_{50}, 1.7 μM) of all the compounds examined. These results proposed that the carboxylic group at the 24 position, and the acetyl group at the 2 position could play key roles in the inhibition of HIV-1 protease in these triterpene acids of the hopane series. In addition, 6,8-dimethylflavanones (**3**—**5**) moderately inhibited enzyme activity with IC_{50} values of 105.9 (**3**), 64.3 (**4**), and 106.9 (**5**) μM, and kaempferol rhamnosides (**6**, **9**, **12**) exhibited weak inhibitory activities (IC_{50}, 146.6, 88.0, 100.1 μM) against HIV-1 protease. 3,4-Dihydoxybenzaldehyde (**13**), and ursolic acid (**15**) also inhibited HIV-1 protease with IC_{50} values of 27.5 and 8.9 μM.

Experimental

General Optical rotations were measured with a Jasco DIP-1000 automatic polarimeter (Jasco Co., Tokyo, Japan). IR spectra were measured with a Jasco FT/IR-230 infrared spectrometer. UV spectra were measured with a UV-2200 UV–VIS recording spectrometer (Shimadzu Co., Kyoto, Japan). NMR spectra were taken on either a Varian Unity 500 (^{1}H, 500 MHz; ^{13}C, 125 MHz) or a JEOL JNA-LAA 400 EB-FT (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. MS spectra were obtained on a VG Trio-2 spectrometer. TLC was carried out on Silica gel 60 F_{254} and RP-18 F_{254}S plates (0.25 mm, Merck, Darmstadt, Germany). Column chromatography was performed on Silica gel 60 (70—230 mesh, Merck), Chromatorex-ODS DM1020T (Fuji Silysia Co., Nagoya, Japan), Sephadex LH-20 (Pharmacia, Uppsala, Sweden) and Diaion HP-20 (Mitsubishi Kasei Co., Tokyo, Japan). Preparative HPLC was carried out on a Gilson HPLC system (pump: model 305 and 306; detector: 119 UV/VIS detector).

Plant Materials The rhizome of *Dryopteris crassirhizoma* Nakai (Aspiadaceae) was purchased from Zhongshanlu Drug Store of Hohhot, Inner Mongolia of the People's Republic of China, and were identified by Dr. Katsuko Komatsu, Institute of Natural Medicine, University of Toyama. A voucher specimen (TMPW No. 19159) is deposited at the Museum of Materia Medica of the University of Toyama.

Extraction and Isolation The rhizome of *D. crassirhizoma* (5.0 kg) was extracted with 80% MeOH in an ultrasonic apparatus for 3 h to yield an 80% MeOH extract (85.4 g). The extract was suspended in water and then extracted with the respective organic solvents to give $CHCl_3$ (31.4 g), EtOAc (32.6 g), BuOH (12.1 g) and H_2O (13.2 g) extracts. The $CHCl_3$ extract was subjected to column chromatography on silica gel (500 g, $CHCl_3$–MeOH). The fractions obtained were further purified by Sephadex LH-20, and repeated column chromatography, to give compounds **1** (8.2 mg), **2** (32.5 mg), **3** (2.5 mg), **4** (8.0 mg), **5** (13.1 mg), **13** (15.1 mg), **14** (54.5 mg), **15** (10.1 mg), **16** (300.5 mg), **17** (50.2 mg), and **18** (64.0 mg). The EtOAc extract was chromatographed on columns of normal and reversed phase silica gel, followed by prep HPLC on TSK-gel ODS-80TM, to produce compounds **6** (12.1 mg), **7** (66.3 mg), **8** (200.5 mg), **9** (2.6 mg), **10** (2.5 mg), **11** (1.5 mg) and **12** (2.1 mg). The BuOH extract was chromatographed on columns of Diaion HP-20 and reversed phase silica gel, followed by Sephadex LH-20 column chromatography to afford compounds **7** (5.2 mg) and **8** (35.1 mg).

2β-Hydroxy-21βH-hop-22(29)-ene-24-oic Acid (1, Dryopteric Acid A) White amorphous powder, $[\alpha]_D^{24}$ +34.0° (c=1.0, $CHCl_3$: MeOH=7 : 1), IR (KBr) cm^{-1}: 3271, 2924, 1644, 1537, 1449, 1373, 1241, 1196, 881, 754, UV λ_{max} ($CHCl_3$: MeOH=7 : 1) nm (log ε): 242 (0.60), 254 (0.48) 272 (0.70), 275 (0.15), ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 4.89 (2H, m, H-29), 4.34 (1H, m, H-2α), 2.72 (1H, t, J=5.0 Hz, H-21), 2.62 (1H, dd, J=15.0, 4.0 Hz, H-3β), 2.30 (1H, dd, J=13.5, 5.5 Hz, H-1β), 2.16 (1H, m, H-5α), 1.75 (3H, s, CH_3-30), 1.56 (1H, m, H-3α), 1.43 (3H, s, CH_3-23), 1.39 (3H, s, CH_3-25), 1.19 (1H, m, H-1α), 0.98 (3H, s, CH_3-26), 0.94 (3H, s, CH_3-27) 0.73 (3H, s, CH_3-28); ^{13}C-NMR (pyridine-d_5, 125 MHz): Table 1. MS m/z (rel. int.): 456 (M^+, 10), 437 (18), 352 (18), 326 (18), 258 (5), 218 (25), 188 (88), 175 (30). HR-MS m/z: 456.3641 (Calcd for $C_{30}H_{48}O_3$: 456.3603).

2β-Hydroxy-21βH-hop-22(29)-ene-23-oic Acid (2, Dryopteric Acid B) White amorphous powder; $[\alpha]_D^{24}$ +33.8° (c=1.0, $CHCl_3$: MeOH=7 : 1), IR (KBr) cm^{-1}: 3272, 2924, 1635, 1537, 1449, 1370, 1241, 1192, 881, 753, UV λ_{max} ($CHCl_3$: MeOH=7 : 1): nm (log ε): 242 (0.58), 254 (0.36) 272 (0.71), 275 (0.15), ^{1}H-NMR (pyridine-d_5, 500 MHz) δ: 4.88 (2H, m, H-29), 4.68 (1H, m, H-2α), 2.60 (1H, t, J=4.5 Hz, H-21), 2.57 (1H, dd, J=13.5, 4.0 Hz, H-3α), 2.24 (1H, dd, J=13.5, 5.5 Hz, H-3β), 2.15 (1H, dd, J=12.0, 2.0 Hz, H-1α), 1.81 (3H, s, CH_3-24), 1.74 (3H, s, CH_3-30), 1.52 (1H, m, H-1β), 1.42 (3H, s, CH_3-25), 1.02 (3H, s, CH_3-27), 0.91 (3H, s, CH_3-26), 0.72 (3H, s, CH_3-28); ^{13}C-NMR (pyridine-d_5, 125 MHz): Table 1. MS m/z (rel. int.): 456 (M^+, 10), 437 (20), 352 (18), 282 (18), 258 (60), 240 (15), 189 (88), 175 (41). HR-MS m/z: 456.3611 (Calcd for $C_{30}H_{48}O_3$: 456.3603).

Acetylation of Compounds 1 and 2 Dryopteric acid A (**1**, 4.6 mg) and B (**2**, 5.1 mg) were acetylated by Ac_2O in pyridine at room temperature to yield the respective acetates: 2β-acetoxy-21βH-hop-22(29)-ene-24-oic acid (**1a**, 3.4 mg), and 2β-acetoxy-21βH-hop-22(29)-ene-23-oic acid (**2a**, 3.5 mg), respectively.

2β-Acetoxy-21βH-hop-22(29)-ene-24-oic Acid (1a) White amorphous powder, FAB-MS m/z: 499 $[M+H]^+$. ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 5.04 (1H, m, H-2α), 4.78 (2H, m, H-29), 2.72 (1H, t, J=15.3 Hz, H-3β), 2.69 (1H, t, J=4.5 Hz, H-21), 2.15 (1H, d, J=13.4 Hz, H-1β), 1.98 (3H, s, $OCOCH_3$-2), 1.74 (3H, s, CH_3-30), 1.30 (3H, s, CH_3-25), 1.01 (3H, s, CH_3-23), 0.93 (3H, s, CH_3-26), 0.92 (3H, s, CH_3-27), 0.71 (3H, s, CH_3-28).

2β-Acetoxy-21βH-hop-22(29)-ene-23-oic Acid (2a) White amorphous powder, FAB-MS m/z: 499 $[M++H]^+$. ^{1}H-NMR (pyridine-d_5, 400 MHz) δ: 5.53 (1H, m, H-2α), 4.87 (2H, m, H-29), 2.60 (1H, t, J=4.5 Hz, H-21), 2.44 (1H, dd, J=13.5, 4.0 Hz, H-3α), 2.15 (1H, dd, J=13.5, 5.5 Hz, H-3β), 2.10 (1H, dd, J=12.0, 2.0 Hz, H-1β), 2.02 (3H, s, $OCOCH_3$-2), 1.74 (3H, s, CH_3-

30), 1.62 (3H, s, CH_3-24), 1.18 (3H, s, CH_3-25), 0.98 (3H, s, CH_3-26), 0.87 (3H, s, CH_3-27), 0.68 (3H, s, CH_3-28).

HIV-1 Protease Activity Assay[18] The inhibitory activity of test samples against HIV-1 protease was determined by an HPLC method using synthetic peptide [His-Lys-Ala-Arg-Val-Leu-(pNO_2-Phe)-Glu-Ala-Nle-Ser-NH_2] as a substrate, which was purchased from Bachem Feinchemikalien AG (Bubendorf, Switzerland). The fused recombinant HIV-1 PR was obtained as previously reported. A reaction mixture (25 μl) containing a substrate (2.5 μg), 2.5 μl of a test compound dissolved in DMSO, and 2.5 μl of recombinant HIV-1 PR (0.175 μg protein), in 50 mM acetate buffer (pH 5.0) was incubated at 37 °C for 15 min. The reaction was stopped by the addition of 2.5 μl of 10% trifluoroacetic acid. The hydrolysate and remaining substrate were quantitatively analyzed by HPLC. The HPLC system was composed of an LC9A liquid chromatograph, an SPD-6A UV spectrophotometric detector, an SLC-6B autoinjector and an integrator C-R6A Chromatopac (Shimadzu Co., Kyoto, Japan). Five microliters of the reaction mixture was injected into a reversed phased ODS column (4.6×150 mm, YMC) and eluted with a gradient of acetonitrile (15—40%) in 0.1% trifluoroacetic acid at a flow rate of 1.0 ml/min. The elution profile was monitored at 280 nm. The substrate and *p*-NO_2-Phe-bearing hydrolysate were eluted at 11.9 and 7.3 min, respectively. Acetyl-pepstatin, which was widely used as positive control, showed an IC_{50} of 0.09 μM under these conditions.

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References

1) Gallo R. C., Slalahuddin S. Z., Popovic M., Shearer G. M., Kaplan M., Haynes B. F., Palker T. J., Redfield R., Oleske J., Safai B., White G., Foster P., Markham P. D., *Science*, **224**, 500—503 (1984).
2) Kohl N. E., Emini E. A., Schleif W. A., Davis L. J., Heimbach J. C., Dixon R. A. F., Scolnick E. M., Sigal I. S., *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 4686—4690 (1998).
3) Jung B. S., Shin M. G., "Encyclopedia of Crude Drugs," Younglim-Sa, Seoul, 1990, pp. 77—79.
4) Do S. D., Min B. S., Bae K. H., *Yakhak Hoeji*, **40**, 478—481 (1996).
5) Lee S. M., Na M. K., An R. B., Min B. S., Lee H. K., *Biol. Pharm. Bull.*, **26**, 1354—1356 (2003).
6) Min B. S., Tomiyama M., Ma C. M., Nakamura N., Hattori M., *Chem. Pharm. Bull.*, **49**, 546—550 (2001).
7) Ma C. M., Miyashiro H., Masao H., Shimotohno K., *J. Trad. Med.*, **12**, 418—419 (1995).
8) Wada H., Tanaka N., Murakami T., Uchida T., Kozawa K., Saiki Y., Chen C. M., *Yakugaku Zasshi*, **108**, 740—744 (1988).
9) Tanaka N., Murakami T., Wada H., Gutierrez A. B., Saiki Y., Chen C. M., *Chem. Pharm. Bull.*, **33**, 5231—5238 (1985).
10) Mohri K., Takemoto T., Kondo Y., *Yakugaku Zasshi*, **102**, 310—312 (1982).
11) Mizuno M., Iinuma M., Tanaka T., Yamamoto H., Tu Z. B., *J. Nat. Prod.*, **54**, 1427—1429 (1991).
12) Tan R. X., Hu Y. H., Liu Z. L., *J. Nat. Prod.*, **56**, 1917—1922 (1993).
13) Herz W., Kulanthaivel P., *Phytochemistry*, **23**, 1453—1459 (1984).
14) Ageta H., Shiojima K., Suzuki H., Nakamura S., *Chem. Pharm. Bull.*, **41**, 1939—1943 (1993).
15) Nakatani M., Miyazaki Y., Iwashita T., Naoki H., Hase T., *Phytochemistry*, **28**, 1479—1482 (1989).
16) Rowan D. D., Russell G. B., *Phytochemistry*, **31**, 702—703 (1992).
17) Lopes D., Villela C. T., Kaplan M. A. C., Carauta J. P. P., *Phytochemistry*, **34**, 279—280 (1993).
18) Ma C., Nakamura N., Miyashiro H., Hattori M., Shimotohno K., *Chem. Pharm. Bull.*, **47**, 141—145 (1999).