Anti-HIV-1 Protease Triterpenoid Saponins from the Seeds of *Aesculus chinensis*

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Received April 19, 1999

Eight bioactive triterpenoid saponins (**1**−**8**) were isolated from the seeds of *Aesculus chinensis*, four of which are novel compounds. The major saponins were identified as escin Ia (**1**), Ib (**2**), isoescin Ia (**3**) and Ib (**4**), while the new compounds were identified as 22α-tigloyl-28-acetylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (escin IVc, **5**), 22α-angeloyl-28-acetylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (escin IVd, **6**), 28-tigloylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (escin IVe, **7**), and 28-angeloylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (escin IVf, **8**). The structures were determined by chemical and spectroscopic methods. All the above compounds were evaluated for their inhibitory activity against HIV-1 protease.

Aesculus chinensis Bge. (Hippocastanaceae) is a medicinal plant widely distributed in northwestern China. Its dried ripe seeds have been used as a carminative, stomachic, and analgesic for the treatment of distention and pain in chest and abdomen.[1] The saponin mixtures extracted from the seeds are known as escins, which have distinguished antiinflammatory, antiedema, capillary-protective, hypoglycemic and ethanol absorption inhibitory activities.[2−4] In the course of our search for novel anti-HIV agents, escins were found to show moderate anti HIV-1 protease activity. Here we describe the isolation and structure elucidation of four novel triterpenoid saponins, escin IVc (**5**), IVd (**6**), IVe (**7**), and IVf (**8**). All of the isolated compounds were investigated for inhibitory activities against HIV-1 protease.

Results and Discussion

EtOH extracts of the seeds of Chinese horse chestnut were separated as described in the Experimental Section to yield compounds **1**−**8**. The four major active compounds were identified as 21*β*-tigloyl-22α-acetylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (escin Ia, **1**), 21*β*-angeloyl-22α-acetylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (escin Ib, **2**), 21*β*-tigloyl-28-acetylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (isoescin Ia, **3**), and 21*β*-angeloyl-28-acetylprotoaescigenin-3*β*-*O*-[*β*-D-glucopyranosyl (1−2)] [*β*-D-glucopyranosyl (1−4)]-*β*-D-glucopyranosiduronic acid (isoescin Ib, **4**) by comparison of their spectral data with the literature data.[4−6] A single-crystal X-ray analysis undertaken on escin Ia* supported the structure elucidated based on NMR evidences and further suggested its absolute configuration (Figures 1 and 2).

Compound **5** was isolated as an amorphous powder, with a compositions of $C_{55}H_{86}O_{24}$ as determined by negative-ion

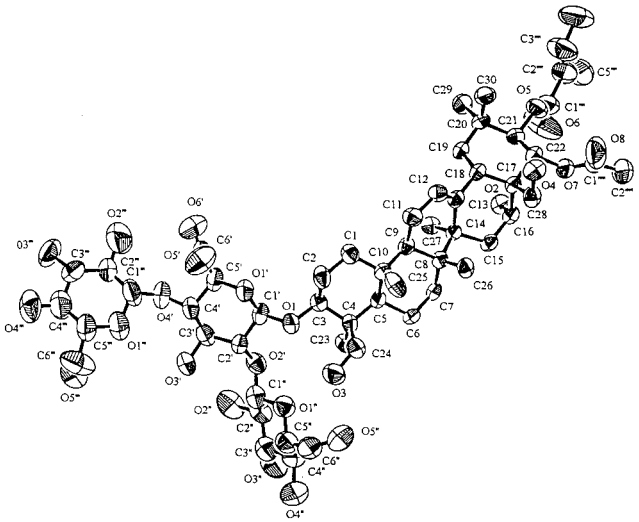

Figure 1. ORTEP drawing with atomic numbering of escin Ia.

HRSIMS, identical with **1**−**4**. The ^{1}H and ^{13}C NMR signals of **5** showed a close resemblance to those of **1** and **3**,[4,6] with presences of a protoaescigenin skeleton, a trisaccharide moiety, a tigloyl group, and an acetyl group. The significant differences in ^{13}C and ^{1}H NMR spectra from those of **1** and **3** were chemical shifts of C-21 (δ 76.2) and C-22 (δ 77.9) with corresponding protons at δ 4.95 and 5.92. Otherwise, minor changes were also observed for C-17 (δ 45.9), C-18 (δ 41.4), and H-18 (δ 2.75). Compared with **1**−**4**,[4,6] the stereochemistry of C-21 and C-22 remained unchanged as deduced from NOESY spectrum: Me-29 showed a strong NOE correlation with H-21 while Me-30 correlated with H-22 strongly. These indicated the α-configuration of H-21 and the *β*-configuration of H-22. The coupling constant of H-21 and H-22 (J = 9.5 Hz) also supported the proposed structure. The attachments of the tigloyl group at C-22 and the acetyl group at C-28 were derived from a HMBC experiment, which correlated the carbonyl carbons of the tigloyl and the acetyl groups to H-22 and H-28, respectively. ^{1}H and ^{13}C NMR signals of the trisaccharide moiety were coincident with those of **1**−**4**, and acid hydrolysis of **1** also

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10.1021/np990180u CCC: $18.00
Published on Web 09/23/1999

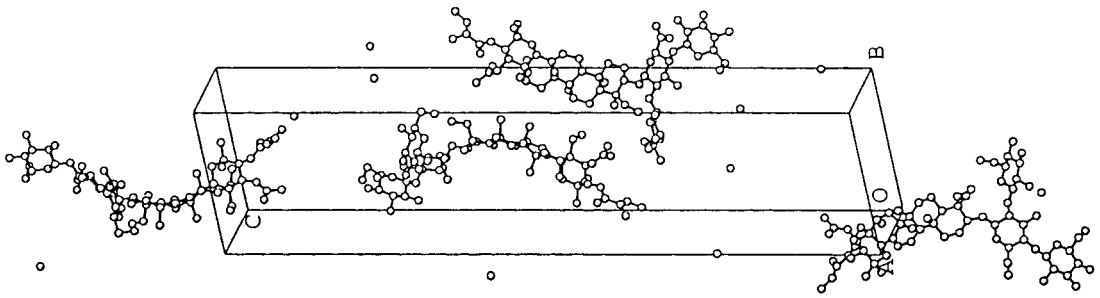


Figure 2. Stereoview of the unit-cell packing of escin Ia.

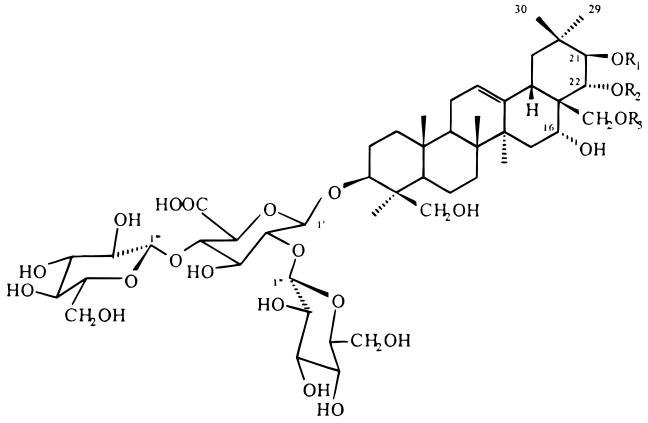
yielded glucose and glucuronic acid. Furthermore, their sequences and linkage sites were confirmed by HMBC correlations between the following pairs: C-3 (δ 90.9) and H-1′ (δ 4.87); C-2′ (δ 79.5) and H-1″ (δ 5.58); and C-4′ (δ 81.7) and H-1‴ (δ 5.18). Hence, **5** was established as 22α-tigloyl-28-acetylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1−2)] [β-D-glucopyranosyl (1−4)]-β-D-glucopyranosiduronic acid and named escin IVc.

Compound **6** was isolated as an amorphous powder. It was determined to be an isomer of **5** by HRMALDIMS with the molecular formula of $C_{55}H_{86}O_{24}$. The only differences in their ^{1}H and ^{13}C NMR spectra were signals due to an angeloyl group [^{13}C NMR: δ 168.1 (C-1⁗), 129.1 (C-2⁗), 136.6 (C-3⁗), 15.7 (C-4⁗), and 20.6 (C-5⁗). ^{1}H NMR: δ 5.83 (H-3⁗), 1.43 (Me-4⁗), and 1.92 (Me-5⁗)] replaced those due to a tigloyl group. Similar HMBC and NOE correlations were also present for **6**. Therefore, **6** was identified as 22α-angeloyl-28-acetylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1−2)] [β-D-glucopyranosyl (1−4)]-β-D-glucopyranosiduronic acid, and named escin IVd. It is a geometrical isomer of **5** (Figure 3).

Compound **7** was isolated as an amorphous powder. It has a composition of $C_{53}H_{84}O_{23}$ as shown by HRMALDIMS. Compared with **1**−**4** and **5**, ^{1}H and ^{13}C NMR signals belonging to an acetyl group disappeared. Long-range correlation between the carbonyl carbon of the tigloyl group and H-28 (δ 4.15) characterized the presence of a tigloyl group at C-28. Otherwise, ^{1}H and ^{13}C NMR signals due to the sugar moiety remained unchanged. On the basis of the evidence above, **7** was established as 28-tigloylprotoaescigenin-3β-*O*-[β-D-glucopyranosyl (1−2)] [β-D-glucopyranosyl (1−4)]-β-D-glucopyranosiduronic acid and named escin IVe.

Compound **8** was isolated as an amorphous powder. Compounds **7** and **8** were determined to be isomeric, with a composition of $C_{53}H_{84}O_{23}$ as shown by negative-ion HRSIMS. Compared with **7**, ^{1}H and ^{13}C NMR signals assignable to an angeloyl group [^{13}C NMR: δ 167.6 (C-1⁗), 128.0 (C-2⁗), 138.1 (C-3⁗), 15.8 (C-4⁗), and 20.8 (C-5⁗). ^{1}H NMR: δ 5.84 (H-3⁗), 1.94 (Me-4⁗), and 1.85 (Me-5⁗)] replaced those due to a tigloyl group. The position of the angeloyl group and the sequence of the trisaccharide were consistent with **7** as determined by HMBC spectrum. Acid hydrolysis of **8** yielded glucose and glucuronic acid. On the basis of the above evidences, **8** was established as 28-angeloyl-protoaescigenin-3β-*O*-[β-D-glucopyranosyl (1−2)] [β-D-glucopyranosyl (1−4)]-β-D-glucopyranosiduronic acid and named escin IVf. Compounds **7** and **8** are also geometrical isomers.

Escins inhibited 86.1 ± 0.2% of the HIV-1 protease activity at 100μM. The inhibition percentages of mixtures of its main components, **1**+**2** (2:1) and **3**+**4** (2:1), were 89.9 ± 1.1% and 50.8 ± 0.4% at 100 μM. Of the isolated compounds (**1**−**8**) that were tested, compounds **1** and **2** showed inhibitory activity against HIV-1 protease with IC_{50} values of 35 and 50 μM, respectively. The inhibition



	R_1	R_2	R_3
1	Tig	Ac	H
2	Ang	Ac	H
3	Tig	H	Ac
4	Ang	H	Ac
5	H	Tig	Ac
6	H	Ang	Ac
7	H	H	Tig
8	H	H	Ang

Figure 3. Structures of compounds **1**−**8**.

percentages of **3**−**8** at 100 μM were 39.0 ± 4.4%, 14.7 ± 0.7%, 35.2 ± 2.3%, 34.0 ± 9.4%, 15.9 ± 0.5%, and 12.5 ± 1.7%, respectively. This is the first report of their anti-HIV-1-protease activity.

Experimental Section

General Experimental Procedures. Melting points are uncorrected. IR studies were made with KBr disks. NMR studies employed a Varian INOVA-500 Spectrometer operating at 500 MHz for ^{1}H and 125 MHz for ^{13}C including COSY, HMQC, HMBC, and NOESY, and chemical shifts are given in δ relative to TMS as internal standard. MALDIMS (matrix-assisted laser desorption ionization MS) was taken on BIFLEX III (Bruker) and SIMS was taken on APEX II (Bruker). For preparative HPLC (pump, P2000; detector, UV 3000; software, PC 1000; Thermo Separation Products, U.S.A.) separation, an ODS column [Phenomenex LUNA 10μ C_{18} (2) (250 × 21.2 i.d.), U.S.A.] column was used. A YMC-Pack ODS-AP (4.6 × 150 mm, YMC, Kyoto, Japan) was used for anti-HIV-1 protease assay. The integrator was a C-R1A Chromatopac (Shimadzu, Japan) product.

Plant Material. The seeds of *A. chinensis* Bge. were collected at Lueyang County, Shaanxi Province, P. R. China, in September 1997. The voucher specimens are deposited at the National Laboratory of Natural and Biomimetic Drugs, Beijing Medical University (Beijing, People's Republic of China).

Extraction and Isolation. The powdered seeds (8 kg) were extracted four times with 70% EtOH under reflux for 2 h. After removal of the solvent in vacuo, the extract (2 kg) was further partitioned between H_2O and EtOAc to give H_2O-soluble (1.9 kg) and EtOAc-soluble (118.7 g) fractions. The H_2O-soluble (1.9 kg) fraction was subjected to D-101 macroreticular resin column and eluted successively with H_2O, 20% EtOH, 70% EtOH, and 95% EtOH, giving four saponin fractions of 20 g (0.25%), 22 g (0.28%), 195 g (2.44%), and 3 g (0.04%), respectively. A 10 g amount of the third fraction was separated repeatedly by preparative HPLC [MeCN−1% HOAc aq. (7:3, v/v)] to yield **5** (26 mg, 0.063‰), **6** (22 mg, 0.055‰), **7** (59 mg, 0.144‰), **8** (0.1 g, 0.244‰), **1** (3.0 g, 0.73%), **2** (2.0 g, 0.48%), **3** (1.2 g, 0.29%), and **4** (0.9 g, 0.22%).

Acid Hydrolysis of Saponins. A solution of saponin (10 mg) in H_2O (1 mL) was treated with 20% aqueous H_2SO_4 (1 mL), and the mixture was heated under reflux for 4 h. It was then neutralized by saturated $NaHCO_3$ and extracted three times with EtOAc. The water layer was then condensed and subjected to PC (n-BuOH−H_2O−HOAc, 4:2:1) together with authentic glucose and glucuronic acid. The hydrolysates were coincident with authentic samples.

Crystallographic Analysis of Escin Ia (1). Transparent colorless plate crystals were grown from MeOH and a crystal with the size of 0.2 × 1.0 × 1.0 mm was used for X-ray diffraction work. All reflection data were collected on MAC DIP2030K Image Plate diffractometer with the Mo Kα radiation (λ = 0.710 73 Å) using a graphite monochromator at the temperature of 23 ± 1 °C, the voltage 50 kV, and the current 90 mA. The distance between the crystal and the image plate was 100 mm. A total of 5987 unique reflections were collected using the ω scan from 0−180° at the speed of 1.2°/min with oscillational angle 3°, $2\theta_{max}$ = 50°. 5003 reflections with $|F|^2 \geq 6.0\sigma|F|^2$ were considered as observed and were used for structure determination. Empirical formula, $C_{55}H_{86}O_{24}\cdot(H_2O)_{1.5}$; M_r = 1158.27, crystal system, orthorhombic; space group, $P2_12_12_1$; a = 7.155(1) Å, b = 14.837(1) Å, c = 62.326(3) Å, V = 6616.4(6) Å^3, Z = 4, D_c = 1.166 g/cm^3, μ = 0.09 mm^{-1}. The structure was solved by direct method (SIR92). All of the non-hydrogen atoms were refined by the block least-squares matrix. Owing to the high flexibility of the molecule and disorder of hydroxyl groups, R_F = 0.111 and R_w = 0.112.

Escin IVc (5): white powder; $[\alpha]^{25}_D$ −11.2° (*c* 1.25, MeOH); IR (KBr) ν_{max} 3417, 1740, 1711, 1654, 1640, 1606, 1382, 1076 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ_{ppm} 0.60 (3H, s, Me-25), 0.68 (1H, m, H-1a), 0.75 (1H, d, *J* = 12.0 Hz, H-5), 0.81 (3H, s, Me-26), 1.21 (1H, m, H-1b), 1.26 (3H, s, Me-23), 1.27 (3H, s, Me-29), 1.33 (3H, s, Me-30), 1.34 (1H, dd, *J* = 13.5, 13.5 Hz, H-19a), 1.43 (3H, d, *J* = 7.0 Hz, Me-4‴), 1.58 (1H, d, *J* = 12.5 Hz, H-9), 1.59 (1H, d, *J* = 13.5 Hz, H-15a), 1.76 (3H, s, Me-5‴), 1.78 (3H,s, Me-27), 1.82 (1H, d, *J* = 13.5 Hz, H-15b), 2.05 (3H, s, OAc-Me), 2.75 (1H, dd, *J* = 9.0, 15.0 Hz, H-18), 3.00 (1H, dd, *J* = 13.5, 13.5 Hz, H-19b), 3.26 (1H, d, *J* = 11.0 Hz, H-24a), 3.34 (1H, dd, *J* = 4.5, 13.8 Hz, H-3α), 3.63 (1H, d, *J* = 9.5 Hz, H-6″a), 4.02 (2H, d, *J* = 8.0 Hz, H-28ab), 4.16 (1H, d, *J* = 11.0 Hz, H-24b), 4.65 (1H, br s, H-16*β*), 4.87 (1H, d, *J* = 7.5 Hz, H-1′), 4.95 (1H, d, *J* = 9.5 Hz, H-21), 5.18 (1H, d, *J* = 8.0 Hz, H-1‴), 5.36 (1H, br s, H-12), 5.58 (1H, d, *J* = 8.0 Hz, H-1"), 5.92 (1H, d, *J* = 9.5 Hz, H-22), 6.94 (1H, dq-like, H-3‴). ^{13}C NMR, data see Table 1; negative-ion HRSIMS *m/z* [M − H$^+$]$^-$ 1129.5438 (calcd for $C_{55}H_{85}O_{24}$, 1129.5436).

Escin IVd (6): white powder; $[\alpha]^{25}_D$ −26.4° (*c* 1.25, MeOH); IR(KBr) ν_{max} 3401, 1741, 1719, 1645, 1611, 1382, 1076 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ_{ppm} 0.59 (3H, s, Me-25), 0.68 (1H, m, H-1a), 0.74 (1H, d, *J* = 11.5 Hz, H-5), 0.81 (3H, s, Me-26), 1.21 (1H, m, H-1b), 1.24 (3H, s, Me-23), 1.25 (3H, s, Me-29), 1.32 (3H, s, Me-30), 1.34 (1H, dd, *J* = 13.5, 13.5 Hz, H-19a), 1.43 (3H, d, *J* = 7.0 Hz, Me-4‴), 1.58 (1H, d, *J* = 12.0 Hz, H-15a), 1.59 (1H, d, *J* = 12.5 Hz, H-9), 1.77 (3H, s, Me-27), 1.82 (1H, d, *J* = 12.0 Hz, H-15b), 1.92 (3H, s, Me-5‴), 2.07 (3H, s, OAc-Me), 2.73 (1H, dd, *J* = 9.0, 15.0 Hz, H-18), 3.00 (1H, t, *J* = 13.5 Hz, H-19b), 3.26 (1H, d, *J* = 11.0 Hz, H-24a), 3.33 (1H, dd, *J* = 4.5, 10.5 Hz, H-3α), 4.02 (2H, d, *J* = 9.0 Hz, H-28ab), 4.16 (1H, d, *J* = 11.0 Hz, H-24b), 4.63 (1H, br s, H-16*β*), 4.86 (1H, d, *J* = 8.0 Hz, H-1′), 4.93 (1H, d, *J* = 9.5 Hz, H-21), 5.17 (1H, d, *J* = 7.5 Hz, H-1‴), 5.36 (1H, br s, H-12), 5.57 (1H, d, *J* = 7.0 Hz, H-1″), 5.83 (1H, dq-like, H-3‴), 5.94 (1H, d, *J* = 9.5 Hz, H-22). ^{13}C NMR data, see Table 1; HRMALDI MS *m/z* [M + Na]$^+$ 1153.5406 (calcd for $C_{55}H_{86}O_{24}$-Na, 1153.5401).

Escin IVe (7): white powder; $[\alpha]^{25}_D$ +8.6° (*c* 1.05, MeOH); IR(KBr): ν_{max} 3409, 1700, 1695, 1603, 1381, 1074 cm^{-1}; ^{1}H NMR(500 MHz, pyridine-d_5) δ_{ppm} 0.57 (3H, s, Me-25), 0.75 (1H, d, *J* = 11.0 Hz, H-5), 0.87 (3H, s, Me-26), 1.24 (3H, s, Me-23), 1.29 (3H, s, Me-29), 1.30 (1H, dd, *J* = 12.0, 13.0 Hz, H-19a), 1.37 (3H, s, Me-30), 1.51 (3H, d, *J* = 5.5 Hz, Me-4‴), 1.53 (1H, m, H− 9), 1.58 (1H, t, *J* = 13.0 Hz, H-15a), 1.76 (3H, s, Me-5‴), 1.77 (3H, s, Me-27), 1.86 (1H, t, *J* = 13.0 Hz, H-15b), 2.90 (1H, d, *J* = 12.0 Hz, H-18), 3.01 (1H, dd, *J* = 12.0, 13.0 Hz, H-19b), 3.24 (1H, d, *J* = 10.5 Hz, H-24a), 3.32 (1H, d, *J* = 11.0 Hz, H-3α), 3.62 (1H, d, *J* = 9.0 Hz, H-6″a), 4.14 (1H, d, *J* = 10.5 Hz, H-24b), 4.15 (1H, d, *J* = 8.5 Hz, H-28a), 4.19 (1H, d, *J* = 11.0 Hz, H-6″b), 4.39 (1H, d, *J* = 9.0 Hz, H-22), 4.44 (1H, d, *J* = 8.5 Hz, H-28b), 4.77 (1H, br s, H-16*β*), 4.78 (1H, d, *J* = 9.0 Hz, H-21), 4.84 (1H, d, *J* = 7.0 Hz, H-1′), 5.16 (1H, d, *J* = 8.0 Hz, H-1‴), 5.40 (1H, br s, H-12), 5.56 (1H, d, *J* = 7.5 Hz, H-1″), 6.94 (1H, dq-like, H-3‴). ^{13}C NMR data, see Table 1; HRMALDIMS *m/z* [M + Na]$^+$ 1111.5251 (calcd for $C_{53}H_{84}O_{23}$-Na, 1111.5295).

Escin IVf (8): white powder; $[\alpha]^{25}_D$ −21.8° (*c* 1.1, MeOH); IR(KBr): ν_{max} 3389, 1730, 1710, 1653, 1640, 1605, 1383, 1073, 1040 cm^{-1}; ^{1}H NMR (500 MHz, pyridine-d_5) δ_{ppm} 0.53 (3H, s, Me-25), 0.63 (1H, m, H-1a), 0.72 (1H, d, *J* = 9.5 Hz, H-5), 0.83 (3H, s, Me-26), 1.14 (1H, m, H-7a), 1.19 (3H, s, Me-23), 1.24 (3H, s, Me-29), 1.30 (1H, dd, *J* = 12.0, 13.0 Hz, H-19a), 1.30 (3H, s, Me-30), 1.53 (1H, m, H-9), 1.57 (1H, t, *J* = 13.0 Hz, H-15a), 1.63 (1H, m, H-7a), 1.73 (3H, s, Me-27), 1.74 (1H, d, H-9), 1.84 (1H, dd, *J* = 13.0, 13.0 Hz, H-15b), 1.85 (3H, s, Me-

Table 1. ^{13}C NMR Data (δ values) for Compounds **5**−**8**[a]

C	5	6	7	8	C	5	6	7	8
1	38.2	38.2	38.3	38.3	1′	104.4	104.3	104.4	104.5
2	26.3	26.3	26.3	26.3	2′	79.5	79.4	79.4	79.5
3	90.9	90.8	90.9	90.8	3′	76.2	76.2	76.3	76.6
4	43.4	43.4	43.4	43.4	4′	81.7	81.4	81.7	82.7
5	55.8	55.8	55.9	55.9	5′	75.5	75.5	75.7	75.4
6	18.3	18.3	18.3	18.3	6′	171.8	172.0	172.2	174.4
7	32.9	32.8	33.0	33.0	1″	104.0	104.0	104.0	104.0
8	39.6	39.6	39.7	39.7	2″	75.5	75.5	75.5	75.4
9	46.5	46.5	46.6	46.5	3″	78.1	78.0	78.1	78.0
10	36.1	36.1	36.2	36.2	4″	69.5	69.5	69.5	69.5
11	23.8	23.8	23.9	23.9	5″	77.8	77.8	77.8	77.8
12	123.0	123.0	123.0	123.2	6″	61.3	61.3	61.3	61.4
13	142.3	142.2	143.1	143.1	1‴	104.4	104.3	104.4	104.2
14	41.5	41.5	41.7	41.7	2‴	74.7	74.7	74.7	74.8
15	34.4	34.4	34.5	34.6	3‴	78.3	78.3	78.3	78.2
16	67.7	68.0	67.9	68.0	4‴	71.3	71.3	71.3	71.2
17	45.9	45.7	46.6	46.6	5‴	77.8	77.8	77.8	77.6
18	41.4	41.3	40.7	40.7	6‴	62.2	62.2	62.1	62.0
19	47.2	47.2	47.5	47.5	1⁗	168.1	168.1	167.7	167.6
20	36.6	36.7	36.1	36.1	2⁗	129.6	129.1	129.0	128.0
21	76.2	76.1	78.4	78.4	3⁗	136.2	136.6	136.9	138.1
22	77.9	77.8	73.4	73.4	4⁗	13.8	15.7	14.0	15.8
23	22.3	22.2	22.3	22.3	5⁗	12.2	20.6	12.1	20.8
24	63.1	63.0	63.1	63.1	1′′′′′	170.7	170.7		
25	15.4	15.3	15.4	15.4	2′′′′′	20.6	20.7		
26	16.5	16.5	16.7	16.7					
27	27.2	27.3	27.2	27.3					
28	68.4	68.2	66.7	66.4					
29	30.0	30.0	30.4	30.4					
30	19.2	19.2	19.2	19.2					

[a] Obtained in pyridine-d_5, 125 MHz. Assignments made by a combination of NOESY, HMQC, and HMBC data and comparison with the literature data.

5‴), 1.94 (3H, d, J = 7.0 Hz, Me-4‴), 2.84 (1H, d, J = 14.0 Hz, H-18), 2.97 (1H, dd, J = 12.0, 13.0 Hz, H-19b), 3.20 (1H, d, J = 10.0 Hz, H-24a), 3.26 (1H, d, J = 11.0 Hz, H-3α), 3.58 (1H, d, J = 8.5 Hz, H-6″a), 4.12 (1H, d, J = 8.0 Hz, H-28a), 4.15 (1H, d, J = 10.5 Hz, H-24b), 4.22 (1H, d, J = 11.0 Hz, H-6″b), 4.35 (1H, d, J = 9.0 Hz, H-22β), 4.38 (1H, d, J = 8.5 Hz, H-28b), 4.71 (1H, br s, H-16β), 4.74 (1H, d, J = 9.0 Hz, H-21α), 4.75 (1H, d, J = 7.0 Hz, H-1′), 5.10 (1H, d, J = 7.5 Hz, H-1‴), 5.38 (1H, br s, H-12), 5.56 (1H, d, J = 7.5 Hz, H-1″), 5.84 (1H, dq-like, H-3‴). ^{13}C NMR data, see Table 1; negative-ion HRSIMS m/z [M − H$^+$]$^-$ 1087.5319 (calcd for $C_{83}H_{83}O_{23}$, 1087.5330).

HIV-Protease Assay. A 25-μL amount of HIV-PR assay buffer (50 mM NaOAc, pH 4.9) containing 2.5 μg of substrate [His-Lys-Ala-Arg-Val-Leu-(*p*NO_2-Phe)-Glu-Ala-Nle-Ser-NH_2] was mixed with 2.5 μL of a compound (**1**−**8**) solution (using DMSO as solvent), and then 6.25 μL of recHIV-PR (0.25 μg of protein from Bachem) was added into this mixture. The reaction mixture was incubated for 15 min at 37 °C and then terminated by addition of 2.5 μL of 10% trifluoroacetic acid (TFA). The hydrolysate (*p*NO_2-Phe-Glu-Ala-Nle-Ser-NH_2) and remaining substrate were quantitatively analyzed by reversed-phase HPLC with a gradient of acetonitrile (20−40% in 26 min) in 0.1% TFA at a flow rate of 1.0 mL/min. The elution profile was monitored at 280 nm. The substrate and the hydrolysate were eluted at 8.1 and 3.5 min, respectively. The peak areas were measured with an integrator. The inhibitory activity of a compound of HIV−PR was calculated as follows: %inhibition = ($A_{control}$ − A_{sample}) × 100/$A_{control}$ (where A is a relative peak area of the hydrolysate). Acetylpepstatin was used as a positive control, IC_{50} = 0.30 μM.

Acknowledgment. We thank Prof. Yang Lu, Dr. Nan Wu and Prof. Qi-Tai Zheng (Institute of Materia Medica, Chinese Academy of Medical Sciences, Beijing, China) for the single-crystal X-ray analysis.

Supporting Information Available: Tables of X-ray data for escin Ia, including hydrogen bonds, atomic parameters, bond lengths and angles, and anisotropic paraamters. This material is available free of charge via the Internet at http://pubs.acs.org. Crystallographic data for the structure reported in this paper have been deposited with the Cambridge Crystallographic Data Centre. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (Fax: +44 (0)1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

References and Notes

(1) Qian, X. Z. *Colored Illustrations of Chinese Herbs*; People's Health Press: Beijing, 1996; Part II, p 231.
(2) Bombardelli, E.; Morazzoni, P. *Fitoterapia* **1996**, *L67*, 483−511.
(3) Matsuda, H.; Li, Y.; Murakami, T.; Ninomiya, K.; Yoshikawa, M. *Biol. Pharm. Bull.* **1997**, *20*, 1092−1095.
(4) Yoshikawa, M.; Mulakami, T.; Matsuda, H.; Yamahara, J.; Murakami, N. *Chem. Pharm. Bull.* **1996**, *44*, 1454−1464.
(5) Yoshikawa, M.; Mulakami, T.; Yamahara, J.; Matsuda, H. *Chem. Pharm. Bull.* **1998**, *46*, 1764−1769.
(6) Zhao, J.; Yang, X. W.; Cui, Y. X.; Liu, X. H. *Chin. Chem. Lett.* 1999, *10*(4): 291−294.

NP990180U