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HIV-1 Integrase Inhibitory Phenylpropanoid Glycosides from *Clerodendron trichotomum*

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Seven phenylpropanoid glycosides named acteoside (**1**), acteoside isomer (**2**), leucosceptoside A (**3**), plantainoside C (**4**), jionoside D (**5**), martynoside (**6**), and isomartynoside (**7**) were isolated from *Clerodendron trichotomum*. Compounds **1** and **2** showed potent inhibitory activities against HIV-1 integrase with IC_{50} values of 7.8 ± 3.6 and 13.7 ± 6.0 μM, respectively.

Key words: AIDS, Antivirals, HIV-1 integrase inhibitors, *Clerodendron trichotomum*

INTRODUCTION

HIV (human immunodeficiency virus) requires three key enzymes, reverse transcriptase, protease, and integrase for viral replication inside a host cell. Inhibitors of the first two enzymes are targets which currently provide the basis for most AIDS therapies. To augment these approaches for AIDS therapies, inhibitors directed at new enzyme targets are needed. Integrase catalyzes the integration of HIV DNA copy into the host cell DNA and integration is essential for the production of progeny viruses. Therefore, a therapeutic agent that can interrupt this process should be an effective anti-HIV agent (Sakai *et al.*, 1993; Taddeo *et al.*, 1994; Engelman *et al.*, 1995). Despite extensive efforts directed at developing potent integrase inhibitors, no clinically useful inhibitors are available yet.

The search of new enzyme inhibitors from plants has led to the discovery of structurally diverse integrase inhibitors. In this regard, we recently reported several flavonol glycoside gallates as inhibitors of HIV-1 integrase from *Acer okamotoanum* (Kim *et al.*, 1998). In continuation of a search program for biologically active compounds from traditional medicine, we have found that a crude extract of the stems of *Clerodendron trichotomum* Thunb. (Verbenaceae) growing in Korea had strong inhibitory activity against HIV-1 integrase in our screening system. It has been known that the resin of this plant has diverse pharmacological activities such as blood pressure reduction, sedation, soothing, and paralysis activity (Ahn, 1998). In the previous paper, the isolation of three flavonoids (clerodendrin, clerodendroside and acacetin-7-β-D-glucorono-β-(1→2)-D-glucuronide) (Chen *et al.*, 1988; Morita *et al.*, 1977; Okigawa *et al.*, 1970), four diterpenes (clerodendren A, B, D and I) (Kato *et al.*, 1971; Nishida *et al.*, 1989; Kawai *et al.*, 1999), two blue pigments (trichotomine and trichotomine G_1) (Iwadare *et al.*, 1974) and phenylpropanoid glycoside named kusaginin (acteoside) (sakurai *et al.*, 1983) have been reported.

In this report, we investigated active constituents of HIV-1 integrase inhibitory effects in *C. trichotomum* (Table I). By means of bioassay-directed chromatographic fractionation, seven phenylpropanoid glycosides (**1**~**7**), which contain a 1-*O*-arylethyl-α-L-rhamnopyranosyl(1→3)-β-D-glucopyranoside as a basic skeleton, were isolated from *C. trichotomum* (Fig. 1). All compounds except **1** were isolated for the first time from *C. trichotomum*.

MATERIALS AND METHODS

General experimental procedures

Melting points were determined on a Thomas-Hoover capillary melting apparatus and are uncorrected. Optical rotations were determined on an Autopol III automatic polarimeter (Rudolph Research Co., Flanders, New Jersey, U.S.A.). UV spectra were taken with a Shimazu UV 240 UV-Visible Recording Spectrometer. IR spectra were

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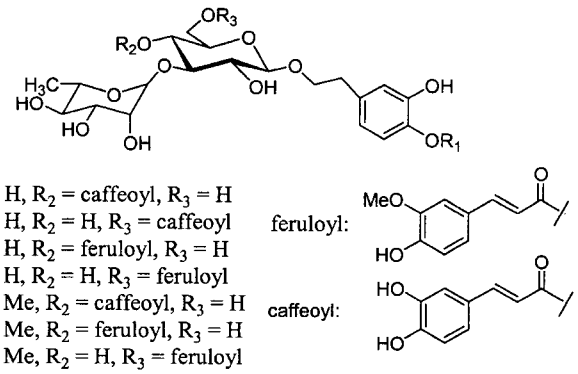
Table I. ^{13}C NMR Spectral data of Compounds **1-7** (CD_3OD)

C	1	2	3	4	5	6	7
1	131.6	131.5	131.6	132.9	131.4	132.9	132.7
2	117.2	117.1	117.2	117.1	117.1	117.1	117.0
3	146.1	146.1	144.7	146.9	144.7	147.4	147.5
4	144.6	144.6	146.1	147.4	146.1	147.6	147.3
5	116.4	116.4	116.6	112.9	116.5	112.9	112.8
6	121.4	121.3	121.2	121.2	121.2	121.2	121.1
7	36.6	36.7	36.6	36.6	36.7	36.6	36.7
8	72.3	72.4	72.1	72.1	72.3	72.1	72.4
1'	104.2	104.4	104.3	104.2	104.4	104.3	104.4
2'	76.2	75.7	76.2	76.2	75.7	76.2	75.7
3'	81.7	84.1	81.5	81.7	84.0	81.5	84.1
4'	70.4	70.4	70.4	70.4	70.5	70.4	70.6
5'	75.9	75.4	76.0	76.1	75.4	76.1	75.4
6'	62.4	64.7	62.4	62.4	64.7	62.4	64.7
1''	103.0	102.7	102.9	103.1	102.7	102.9	102.8
2''	72.4	72.4	72.4	72.4	72.5	72.4	72.4
3''	72.1	72.3	72.3	72.1	72.3	72.1	72.4
4''	73.8	74.0	73.8	73.8	74.0	73.8	74.0
5''	70.6	70.1	70.7	70.6	70.0	70.7	70.1
6''	18.5	17.9	18.5	18.5	17.9	18.5	17.9
1'''	127.7	127.7	127.7	127.7	127.6	127.7	127.4
2'''	115.4	115.2	111.9	115.2	111.6	111.9	111.6
3'''	146.8	146.8	149.4	147.8	149.4	149.4	149.5
4'''	149.8	149.6	150.8	149.8	150.8	150.9	151.2
5'''	116.6	116.6	116.4	116.5	116.3	116.6	116.6
6'''	123.3	123.2	124.4	123.2	124.3	127.7	124.4
7'''	148.1	147.3	148.0	148.0	147.1	147.9	147.3
8'''	114.8	114.9	115.2	114.7	115.2	115.1	115.0
9'''	168.4	169.2	168.4	168.3	169.1	168.3	169.2
OMe			56.5	56.5	56.5	56.5	56.4



1. R_1 = H, R_2 = caffeoyl, R_3 = H
2. R_1 = H, R_2 = H, R_3 = caffeoyl
3. R_1 = H, R_2 = feruloyl, R_3 = H
4. R_1 = H, R_2 = H, R_3 = feruloyl
5. R_1 = Me, R_2 = caffeoyl, R_3 = H
6. R_1 = Me, R_2 = feruloyl, R_3 = H
7. R_1 = Me, R_2 = H, R_3 = feruloyl

Fig. 1. Structure of the compounds from *Clerodendron trichotomum* Thunb

recorded on a Perkin Elmer 16F-PC FT-IR and MIDAC 101025 instrument using KBr pellets. ^1H NMR spectra were recorded on a (300 mg) Bruker 500 spectrometer (500 MHz), using TMS as an internal standard. ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer (125 MHz) 300 mg. Electro-spray mass spectra were determined on an Api ES/MS (HP 59987A ES/5989A MS) mass spectrometer. Medium pressure liquid chromatography (MPLC) was performed on a pump model RP-SY (Fluid Metering Inc., Oyster Bay, N.Y., U.S.A) using a LiChroprep RP-18 (1.5 cm × 47 cm, 40-63 μm, Merck). TLC were carried out on a precoated silica gel 60 F_{254} (Merck, Art. 5715) and RP-18 $F_{254}S$ (Merck, Art. 15683). Curcumin was purchased from Aldrich® and L-chicoric acid was prepared by the known procedure (King *et al.*, 1999).

Plant material

The stems of *Clerodendron trichotomum* Thunb. were collected at Mt. Kodong, Korea, in October 1997. Voucher specimens (609-23C) have been deposited in Natural Product Chemistry Laboratory at Korea Institute of Science & Technology.

Extraction and isolation

The air-dried stems of *C. trichotomum* (4.8 kg) were cut into small pieces and extracted three times with MeOH at room temperature to afford dark-brown residue (254 g) upon removal of solvent under reduced pressure. The methanol extract was suspended in water and then partitioned in turn with CH_2Cl_2, EtOAc and *n*-

BuOH. The active EtOAc extract was evaporated under reduced pressure to yield 10.1 g of a residue. This residue was divided into four fractions (Fr. A~Fr. D) by column chromatography on silica gel (480 g) using the following solvent mixtures; CH_2Cl_2:MeOH:H_2O (6:1.5:0.1→5:1.5:0.1→3.5:1.5:0.1), and MeOH wash. Active fraction D (2.1 g) was further purified by column chromatography over LiChroprep® RP-18 (40~63 μm, Art. 113900, Merck) with *i*-PrOH:MeOH:H_2O (5:25:75→5:35:65→5:40:60) to give acteoside (**1**, 880.8 mg) and acteoside isomer (**2**, 141.2 mg). Fraction B was chromatographed on silica gel column (134 g) eluting with CH_2Cl_2:MeOH:H_2O (7:1:0.1→6:1:0.1→3.5:1:0.1→3.5:1.5:0.1), and MeOH to give eight fractions (Fr. B1~Fr. B8). Fraction B4 was rechromatographed over LiChroprep® RP-18 column eluting with *i*-PrOH:MeOH:H_2O (5:30:70) to yield martynoside (**6**, 675.9 mg). Fraction B8 was chromatographed on a silica gel column (65 g) eluting with CH_2Cl_2-MeOH-H_2O (6:1.2:0.1) to give six fractions (Fr. B8a~Fr. B8f). Fraction B8b was purified by LiChroprep® RP column eluting with *i*-PrOH:MeOH:H_2O (5:30:70) to afford leucosceptoside A (**3**, 103.9 mg). Fraction B8d was purified by preparative TLC on RP-18 (1 mm, 20 × 20 cm, Merck) developed with MeOH:H_2O (50:50) to afford plantainoside C (**4**, 12.9 mg).

Fraction C (3.4 g) was chromatographed on LiChroprep® RP-18 column eluting with MeOH:H_2O (38:62→60:40) gradient system to give four fractions (Fr. C1~Fr. C4). Fraction C4 was further submitted to medium pressure liquid chromatography on a RP-18 (1.5 cm × 47 cm). The solvent (*i*-PrOH:MeOH: H_2O=5:30:70) was eluted with a chromatography pump model RP-SY (Fluid Metering Inc., Oyster Bay, N.Y., U.S.A) at a rate of 6 ml/min. Separation was monitored by TLC on RP-18 (Art.115683) to yield jionoside D (**5**, 6.4 mg) and isomartynoside (**7**, 29.8 mg).

Compound 1 (acteoside): $[\alpha]_D$ -106.0° (*c* 2.1, MeOH); UV (MeOH): λ_{max} nm 247, 292, 332; ESMS: m/z 647 $(M+Na)^+$; ^{1}H NMR (500 MHz, CD_3OD): δ 7.59 (1H, d, *J*=15.9 Hz, H-7‴), 7.05 (1H, d, *J*=2.0 Hz, H-2‴), 6.94 (1H, dd, *J*=8.1, 2.0 Hz, H-6‴), 6.78 (1H, d, *J*=8.1 Hz, H-5‴), 6.70 (1H, d, *J*=2.0 Hz, H-2), 6.68 (1H, d, *J*=8.1 Hz, H-5), 6.56 (1H, d, *J*=8.1, 2.0 Hz, H-6), 6.27 (1H, d, *J*=15.9 Hz, H-8‴), 5.19 (1H, d, *J*=1.5 Hz, H-1″), 4.92 (1H, t, *J*=9.3 Hz, H-4′), 4.37 (1H, d, *J*=7.9 Hz, H-1′), 4.02 (1H, dd, *J*=15.9, 7.7 Hz, H-8), 3.93 (1H, d, *J*=1.5 Hz, H-2″), 3.81 (1H, t, *J*=9.3 Hz, H-3′), 3.71 (1H, dd, *J*=15.9, 8.1 Hz, H-8), 3.60 (1H, m, H-6′), 3.58 (2H, m, H-3″, 5″), 3.52 (1H, m, H-6′), 3.52 (1H, t, *J*=9.3 Hz, H-5′), 3.39 (1H, dd, *J*=9.0, 7.9 Hz, H-2′), 3.30 (1H, m, H-4″), 2.79 (2H, m, H-7), 1.09 (1H, d, *J*=6.2 Hz, H-6″); ^{13}C NMR (125 MHz, CD_3OD): see Table I.

Compound 2 (acteoside isomer): $[\alpha]_D$ -55.3° (*c* 1.64, MeOH); UV (MeOH): λ_{max} nm 245, 291, 330; ESMS: m/z 647 $(M+Na)^+$; ^{1}H NMR (500 MHz, CD_3OD): δ 7.55 (1H, d, *J*=15.9 Hz, H-7‴), 7.04 (1H, d, *J*=2.0 Hz, H-2‴), 6.88 (1H, dd, *J*=8.1, 2.0 Hz, H-6‴), 6.76 (1H, d, *J*=8.1 Hz, H-5‴), 6.66 (1H, d, *J*=2.0 Hz, H-2), 6.63 (1H, d, *J*=8.0 Hz, H-5), 6.52 (1H, d, *J*=8.0, 2.0 Hz, H-6), 6.28 (1H, d, *J*=15.9 Hz, H-8‴), 5.17 (1H, d, *J*=1.5 Hz, H-1″), 4.49 (1H, dd, *J*=11.9, 2.1 Hz, H-6′), 4.34 (1H, dd, *J*=11.9, 5.9 Hz, H-6′), 4.32 (1H, d, *J*=7.9 Hz, H-1′), 3.99 (1H, dd, *J*=9.5, 6.2 Hz, H-5″), 3.95 (2H, m, H-8, 2″), 3.70 (2H, m, H-8, 3″), 3.53 (1H, t, *J*=9.0 Hz, H-3′), 3.53 (1H, m, H-5′), 3.39 (2H, t, *J*=9.5 Hz, H-4′, 4″), 3.30 (1H, dd, *J*=9.0, 7.9 Hz, H-2′), 2.77 (2H, td, *J*=6.3, 1.9 Hz, H-7), 1.23 (1H, d, *J*=6.3 Hz, H-6″); ^{13}C NMR (125 MHz, CD_3OD): see Table I.

Compound 3 (leucosceptoside A): $[\alpha]_D$ -103.3° (*c* 3.4, MeOH); UV (MeOH): λ_{max} nm 233, 291, 326; ESMS: m/z 661 $(M+Na)^+$; ^{1}H NMR (300 MHz, CD_3OD): δ 7.65 (1H, d, *J*=15.9 Hz, H-7‴), 7.18 (1H, d, *J*=1.7 Hz, H-2‴), 7.07 (1H, dd, *J*=8.2, 1.6 Hz, H-6‴), 6.81 (1H, d, *J*=8.2 Hz, H-5‴), 6.70 (1H, d, *J*=2.5 Hz, H-2), 6.68 (1H, d, *J*=8.2 Hz, H-5), 6.55 (1H, d, *J*=8.0, 1.9 Hz, H-6), 6.38 (1H, d, *J*=15.9 Hz, H-8‴), 5.20 (1H, d, *J*=1.4 Hz, H-1″), 4.82 (1H, t, *J*=9.3 Hz, H-4′), 4.37 (1H, d, *J*=7.8 Hz, H-1′), 4.03 (1H, dd, *J*=16.7, 7.6 Hz, H-8), 3.92 (1H, dd, *J*=2.9, 1.6 Hz, H-2″), 3.87 (3H, s, OCH_3), 3.82 (1H, t, *J*=9.2 Hz, H-3′), 3.72 (1H, m, H-8), 3.60 (1H, m, H-3″), 3.59 (1H, m, H-6′), 3.56 (2H, m, H-6′, 4″), 3.53 (1H, m, H-5′), 3.39 (1H, dd, *J*=9.0, 8.1 Hz, H-2′), 3.29 (1H, t, *J*=9.6 Hz, H-5″), 2.78 (2H, t, *J*=7.1 Hz, H-7), 1.10 (1H, d, *J*=6.0 Hz, H-6″); ^{13}C NMR (75 MHz, CD_3OD): see Table I.

Compound 4 (plantainoside C): $[\alpha]_D$ -50.9° (*c* 0.5, Me OH); UV (MeOH): λ_{max} nm 232, 288, 327; ESMS: m/z 661 $(M + Na)^+$; ^{1}H NMR (300 MHz, CD_3OD): δ 7.62 (1H, d, *J*=15.9 Hz, H-7‴), 7.15 (1H, d, *J*=1.9 Hz, H-2‴), 7.01 (1H, dd, *J*=8.4, 1.9 Hz, H-6‴), 6.79 (1H, d, *J*=8.1 Hz, H-5‴), 6.66 (1H, d, *J*=1.9 Hz, H-2), 6.62 (1H, d, *J*=8.0 Hz, H-5), 6.52 (1H, d, *J*=8.0, 1.9 Hz, H-6), 6.38 (1H, d, *J*=15.9 Hz, H-8‴), 5.17 (1H, d, *J*=1.4 Hz, H-1″), 4.51 (1H, dd, *J*=12.0, 1.9 Hz, H-6′), 4.36 (1H, dd, *J*=12.0, 5.8 Hz, H-6′), 4.33 (1H, d, *J*=7.7 Hz, H-1′), 4.00 (1H, dd, *J*=9.8, 6.2 Hz, H-5″), 3.93 (1H, dd, *J*=3.3, 1.8 Hz, H-2″), 3.88 (1H, m, H-8), 3.86 (3H, s, OCH_3), 3.75 (1H, m, H-8), 3.70 (1H, dd, *J*=9.5, 3.3 Hz, H-3″), 3.57 (1H, m, H-5′), 3.53 (1H, t, *J*=8.8 Hz, H-3′), 3.40 (1H, t, *J*=9.6 Hz, H-4″), 3.39 (1H, t, *J*=9.6 Hz, H-4′), 3.30 (1H, m, H-2′), 2.77 (2H, t, *J*=7.4 Hz, H-7), 1.24 (1H, d, *J*=6.1 Hz, H-6″); ^{13}C NMR (75 MHz, CD_3OD): see Table I.

Compound 5 (jionoside D): $[\alpha]_D$ -95.0° (*c* 0.2, MeOH); UV (MeOH): λ_{max} nm 233, 289, 331; ESMS: m/z 661 $(M+Na)^+$; ^{1}H NMR (300 MHz, CD_3OD): δ 7.58 (1H, d, *J*=15.9 Hz, H-7‴), 7.04 (1H, d, *J*=1.7 Hz, H-2‴), 6.94 (1H, dd, *J*=8.2, 1.8 Hz, H-6‴), 6.82 (1H, d, *J*=8.1 Hz, H-

5), 6.76 (1H, d, J=8.2 Hz, H-5‴), 6.73 (1H, d, J=1.9 Hz, H-2), 6.68 (1H, d, J=8.1, 1.9 Hz, H-6), 6.26 (1H, d, J=15.9 Hz, H-8‴), 5.19 (1H, br s, H-1″), 4.91 (1H, t, J=9.3 Hz, H-4′), 4.37 (1H, d, J=7.8 Hz, H-1′), 4.06 (1H, m, H-8), 3.91 (1H, m, H-2″), 3.80 (3H, s, OCH_3), 3.77 (1H, t, J=9.0 Hz, H-3′), 3.72 (1H, m, H-8), 3.63 (2H, m, H-6′, 5″), 3.58 (1H, m, H-3″), 3.54 (1H, m, H-6′), 3.51 (1H, m, H-5′), 3.38 (1H, t, J=9.0 Hz, H-2′), 3.28 (1H, m, H-4″), 2.81 (2H, dd, J=7.4, 7.1 Hz, H-7), 1.08 (1H, d, J=6.0 Hz, H-6″); ^{13}C NMR (75 MHz, CD_3OD): see Table I.

Compound 6 (martynoside): $[\alpha]_D$ -86.2° (*c* 1.2, MeOH); UV (MeOH): λ_{max} nm 232, 288, 330; ESMS: m/z 675 $(M+Na)^+$; ^1H NMR (300 MHz, CD_3OD): δ 7.64 (1H, d, J=15.9 Hz, H-7‴), 7.19 (1H, d, J=1.9 Hz, H-2‴), 7.07 (1H, dd, J=8.2, 1.9 Hz, H-6‴), 6.82 (1H, d, J=8.2 Hz, H-5‴), 6.80 (1H, d, J=8.2 Hz, H-5), 6.73 (1H, d, J=1.9 Hz, H-2), 6.68 (1H, dd, J=8.1, 2.0 Hz, H-6), 6.37 (1H, d, J=15.9 Hz, H-8‴), 5.19 (1H, d, J=1.7 Hz, H-1″), 4.91 (1H, t, J=9.3 Hz, H-4′), 4.36 (1H, d, J=7.9 Hz, H-1′), 4.04 (1H, dd, J=16.4, 7.7 Hz, H-8), 3.91 (3H, s, OCH_3), 3.90 (1H, dd, J=3.3, 1.7 Hz, H-2″), 3.81 (1H, t, J=9.2 Hz, H-3′), 3.80 (3H, s, OCH_3), 3.74 (1H, m, H-8), 3.60 (1H, m, H-6′), 3.58 (2H, m, H-3″, 5″), 3.55 (1H, m, H-6′), 3.53 (1H, m, H-5′), 3.39 (1H, dd, J=8.8, 8.1 Hz, H-2′), 3.29 (1H, m, H-4″), 2.81 (2H, dd, J=7.4, 7.2 Hz, H-7), 1.09 (1H, d, J=6.0 Hz, H-6″); ^{13}C NMR (75 MHz, CD_3OD): see Table I.

Compound 7 (isomartynoside): $[\alpha]_D$ -44.6° (*c* 1.4, Me OH); UV (MeOH): λ_{max} nm 231, 287, 326; ESMS: m/z 675 $(M+Na)^+$; ^1H NMR (300 MHz, CD_3OD): δ 7.62 (1H, d, J=15.8 Hz, H-7‴), 7.14 (1H, d, J=1.9 Hz, H-2‴), 7.01 (1H, dd, J=8.2, 1.9 Hz, H-6‴), 6.79 (1H, d, J=8.2 Hz, H-5‴), 6.79 (1H, d, J=8.2 Hz, H-5), 6.68 (1H, d, J=1.9 Hz, H-2), 6.65 (1H, d, J=8.2, 1.9 Hz, H-6), 6.38 (1H, d, J=15.8 Hz, H-8‴), 5.17 (1H, d, J=1.6 Hz, H-1″), 4.50 (1H, dd, J=11.7, 1.9 Hz, H-6′), 4.37 (1H, dd, J=12.0, 6.2 Hz, H-6′), 4.32 (1H, d, J=7.9 Hz, H-1′), 3.99 (1H, dd, J=9.6, 6.3 Hz, H-5″), 3.94 (1H, dd, J=3.2, 1.8 Hz, H-2″), 3.85 (3H, s, OCH_3), 3.83 (1H, m, H-8), 3.77 (1H, m, H-8), 3.74 (3H, s, OCH_3), 3.70 (1H, dd, J=9.5, 3.3 Hz, H-3″), 3.57 (1H, ddd, J=12.3, 6.3, 2.4 Hz, H-5′), 3.53 (1H, t, J=8.7 Hz, H-3′), 3.39 (1H, t, J=9.4 Hz, H-4″), 3.31 (1H, t, J=8.4 Hz, H-4′), 3.30 (1H, m, H-2′), 2.79 (2H, t, J=7.4 Hz, H-7), 1.24 (1H, d, J=6.2 Hz, H-6″); ^{13}C NMR (125 MHz, CD_3OD): see Table I.

Bioassays

HIV-1 integrase. Recombinant human immunodeficiency virus type 1 (HIV-1) integrase was expressed in *Escherichia coli* and purified using nickel-chelated column in one-step manner, as described previously (Oh *et al.*, 1996). Aliquots of HIV-1 integrase of 0.5 mg/mL as stock solutions were stored at -70°C until used.

Oligonucleotide substrates. Two 20-mer oligonucleotides whose sequences resemble the end of U5-LTR were obtained from Korea Biotech.Inc., namely K16 (U5-LTR, +strand), 5′-TGTGGAAAATCTCTAGCAGT-3′ and K17 (U5-LTR, -strand), 5′-ACTGCTAGA-GATTTTCCACA-3′. The oligonucleotides were purified using 20% polyacrylamide gel before use. In order to construct the oligonucleotide substrate, oligonucleotide K16 (15 pmol) was labeled at the 5′ end, using [γ-^{32}P]-ATP of 250 mCi (3,000 Ci/mmol; 1 Ci=37 GBq; Amersham Life Science, Illinois, U.S.A.) and T4 polynucleotide kinase (T4 PNK, New England Biolabs, Beverly, Massachusetts, U.S.A.) of 10 units in 40 μL of reaction buffer (70 mM Tris-HCl [pH 7.6], 10 mM $MgCl_2$, 5 mM dithiothreitol) for 15 min at 37°C. The labelling reaction was subjected to 10 mM EDTA, and heated to 85°C for 15 min to inactivate T4 PNK. After the addition of complementary oligonucleotide K17 of 30 pmol, the reaction mixture was boiled for 3 min and cooled down slowly. Labelled substrate was separated from unincorporated nucleotide by passage through a Biospin 6 (Bio-Rad, Hercules, California, U.S.A.).

HIV-1 Integrase Assay. A standard assay of endonucleolytic activity was carried out in the presence of potential inhibitor containing 0.1 pmol of duplex oligonucleotide substrate and 15 pmol of HIV-1 integrase in 15 mM Tris-HCl (pH 7.4), 100 mM NaCl, 1 mM $MnCl_2$, 2 mM 2-mercaptoethanol, 2.5 mM CHAPS, 0.1 mM EDTA, 0.1 mM PMSF, 1% glycerol, and 10 mM imidazole in a total volume of 10 mL. Inhibitors or drugs were dissolved in 100% DMSO and added to the reaction mixture 5% DMSO in the final volume. Reaction mixtures were incubated at 33°C for 90 min and stopped by the addition of 4 μL of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol FF. The reactions were heated to 90°C for 3 min and electrophoresed on a 20% denaturing polyacrylamide gel. Reaction products were visualized by autoradiography of the wet gel. IC_{50} was calculated by scanning bands on Kodak-5 film (Image Master VDS, Pharmacia Biotech., Piscataway, U.S.A.)

RESULTS AND DISCUSSION

Bioassay-guided isolation of ethyl acetate fraction afforded seven phenylpropanoid glycosides. Their structures were determined by physico-chemical and spectral data. They were identified as acteoside (**1**), acteoside isomer (**2**), leucosceptoside A (**3**), plantainoside C (**4**), jionoside D (**5**), martynoside (**6**), and isomartynoside (**7**) by comparison with those reported in the literatures (Andary, *et al.*, 1982; Miyase *et al.*, 1982; Ismail *et al.*, 1995; Miyase *et al.*, 1991; Sasaki *et al.*, 1989; Calis *et al.*, 1984). Table I summarizes the results of the inhibitory activities of isolated phenylpropanoid glycosides (**1**~**7**) against HIV-1

Table II. HIV-1 Integrase Inhibitory Activities of Compounds **1-7**

Compounds	IC_{50} (μM)[a]
1 (acteoside)	7.8 ± 3.6
2 (acteoside isomer)	13.7 ± 6.0
3 (leucosceptoside A)	29.4 ± 4.5
4 (plantainoside C)	39.9 ± 15.2
5 (jionoside D)	60.9 ± 12.8
6 (martynoside)	>100
7 (isomartynoside)	>100
curcumin	54.3 ± 7.8
L-chicoric acid	21.0 ± 6.1

[a]IC_{50} values with standard deviations are from at least three independent experiments.

integrase. The activity data of curcumin and L-chicoric acid were included as standards for comparison. Among the isolated compounds, acteoside (**1**) and acteoside isomer (**2**) showed the most potent HIV-1 integrase inhibition with IC_{50} values of 7.8 ± 3.6 and 13.7 ± 6.0 μM, respectively. The inhibitory activities of these compounds were more potent than curcumin and comparable to L-chicoric acid. It was recently reported that acteoside (**1**) showed antiviral activities against respiratory syncytial virus (EC_{50}=1.28 μM) (Kernan *et al.*, 1998). The inhibitory activity of compounds showed a tendency to decrease with increasing methoxy groups on aromatic ring. The HIV-1 integrase inhibitory activity in the isomeric series decreased in the order of acteoside isomer (**2**), plantainoside C (**4**), and isomartynoside (**7**) (IC_{50}=13.7, 39.9, and > 100 μM), respectively. The location of the feruloyl or caffeoyl substituent in the sugar part was not important on the activity. These results suggest that two catechol groups are required for the inhibition of HIV-1 integrase in the phenylpropanoid glycosides. However, these compounds were inactive against HIV-1 replication in cell-based assays (data not shown).

In conclusion, compounds **1** and **2**, the major constituents of active ethyl acetate fraction of *C. trichotomum*, play the dominant role in HIV-1 integrase inhibitory activity. Structural modification of these phenylpropanoid glycosides are undergoing in our laboratory to develop more potent and less toxic anti-HIV compounds.

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