A Dimeric Lactone from *Ardisia japonica* with Inhibitory Activity for HIV-1 and HIV-2 Ribonuclease H

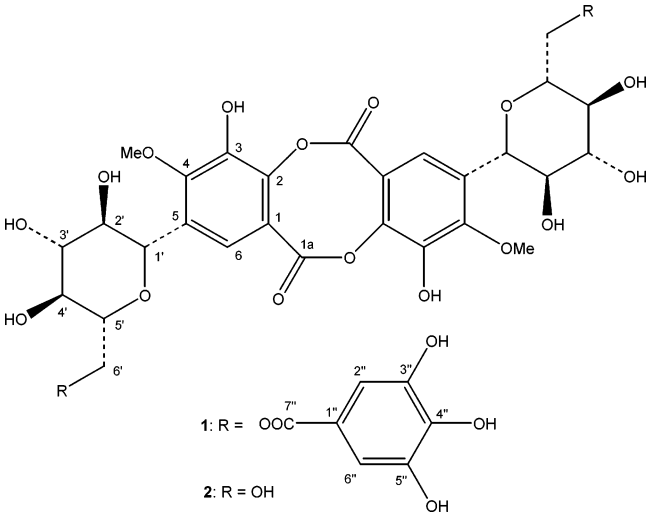
Nguyen Tien Dat,[†] KiHwan Bae,[†] Antony Wamiru,[‡,⊥] James B. McMahon,[‡] Stuart F. J. Le Grice,[§] Marion Bona,[§,⊥] John A. Beutler,[‡] and Young Ho Kim*[,†,‡,⊥]

College of Pharmacy, Chungnam National University, Daejeon 305-764, Korea, and Molecular Targets Development Program, HIV Drug Resistance Program, and SAIC-Frederick, National Cancer Institute, Building 1052, Frederick Maryland 21702

Received July 21, 2006

A new dimeric lactone, ardimerin digallate (**1**), was isolated from the whole plants of *Ardisia japonica*, along with six known constituents. The structure of **1** was established on the basis of spectroscopic analysis including 1D- and 2D-NMR techniques. Compound **1** inhibited HIV-1 and HIV-2 RNase H in vitro with IC_{50} values of 1.5 and 1.1 μM, respectively.

Ardisia japonica (Thunb.) Blume (Myrsinaceae) has been used in Oriental traditional medicine as an antitussive and as a diuretic agent and also to stop uterine bleeding.[1,2] The chemical constituents and biological activities of *A. japonica* have been reported and include triterpenoid saponins,[2,3] bergenin and analogues with anti-HIV activity,[4] benzoquinones,[1,5,6] and a dimeric lactone with radical-scavenging activity.[7] As part of an ongoing molecularly targeted screening program to develop anticancer and antiviral drugs from natural products, we have isolated a new inhibitor of HIV RNase H, ardimerin digallate (**1**), along with six known compounds, namely, ardimerin (**2**),[7] quercitrin,[8] friedelin,[9] bauerenyl acetate,[10] epifriedelinol, and bauerenol,[11] from the whole plants of *A. japonica.*



Compound **1** was obtained as white crystals, with a strong absorption band at 1700 cm^{-1} in the IR spectrum attributed to a lactone ring. The positive HRFABMS revealed an ion at *m*/*z* 983.1706 corresponding to the molecular formula $C_{42}H_{40}O_{26}Na$. The ^1H NMR spectrum showed a broad methoxy singlet at δ 3.93 and two peaks at δ 7.10 and 7.12 due to aromatic protons. Signals characteristic for a glucose moiety were observed from an anomeric proton doublet at δ 5.03 (J = 10.5 Hz) and a cluster of peaks from δ 3.56 to 4.38, which were all linked in a single spin system from the COSY spectrum. The ^{13}C NMR and DEPT spectra indicated the presence of gallic acid (δ 120.0, 109.2, 139.0, 145.5, and 176.1) and a *C*-glucose (δ 73.2, 79.6, 74.4, 70.8, 80.3, and 63.6) moiety.[12] The portions of the NMR data not due to gallic acid moieties were similar to those of a dimeric lactone, ardimerin (**2**), previously reported from this plant.[7] The HMBC NMR spectrum showed coupling between the carboxylic acid carbon of the gallic acid unit and proton H-6′ of the glucose moiety (Figure 1), which implied that the gallic acid unit was attached at C-6′ of glucose. The molecular mass of **1** was 304 Da greater than that for **2**, which implied two additional gallic acid units. Further analysis of the HMBC spectra found correlations from the H-6 proton to C-2, C-4, and the lactone carbonyl (C-1a) at δ 164.7 and from the methoxyl proton to C-4 at δ 141.2, establishing the location of the methoxyl group at C-4. COSY and DEPT spectroscopic analysis of the carbohydrate moiety confirmed the NMR assignments in that portion of the molecule. Thus compound **1** was established as a dibenzodioxocine C-glycoside derivative and named ardimerin digallate.

All of the isolated compounds except bauerenol were tested for inhibition of RNase H enzymatic activity. Of these compounds, only ardimerin digallate (**1**) showed inhibitory activity against both HIV-1 and HIV-2 RNase H in vitro with IC_{50} values of 1.5 and 1.1 μM, respectively, while it did not inhibit either human or *E. coli* RNase H at 200 μM, demonstrating that selective inhibition of the retroviral enzyme can be achieved. Ardimerin (**2**), which lacks a galloyl moiety, did not show any inhibition of RNase H nor did the other compounds (quercitrin, friedelin, bauerenyl acetate, and epifriedelinol) tested, suggesting that the inhibitory effects of **1** are mediated in part due to the presence of the galloyl unit. Several reports have shown that the galloyl group is an important pharmacophore for HIV activity;[13−16] however, gallic acid alone was ineffective in inhibiting reverse transcriptase.[13] We found the isopropyl ester of gallic acid to inhibit RNase H, although the HIV-1 enzyme was more sensitive, with an IC_{50} of 1.3 μM, while the HIV-2 enzyme was less sensitive, with an IC_{50} of 8.0 μM. Most HIV-1 RT inhibitors have been shown to have no effect or lower potency in HIV-2 RT activity.[17−19] Interestingly, our results showed that **1** was active on both HIV-1 and HIV-2 RNase H at approximately equal concentrations. Thus, compounds targeting the RNase H domain of the protein may have greater potential for use as drug candidates directed at both HIV-1 and HIV-2 infection.

Compounds **1** and **2** were tested for inhibition of the DNA polymerase activity of reverse transcriptase, but they had no effect at a concentration of 50 μM. The anti-HIV activity of **1** was evaluated in an XTT-based cell viability assay using the human

* To whom correspondence should be addressed. Tel: +82-42-821-5933. Fax: +82-42-823-6566. E-mail: yhk@cnu.ac.kr.
[†] Chungnam National University.
[‡] Molecular Targets Development Program, NCI.
[§] HIV Drug Resistance Program, NCI.
[⊥] SAIC-Frederick, NCI.

10.1021/np060359m CCC: $37.00
Published on Web 03/31/2007

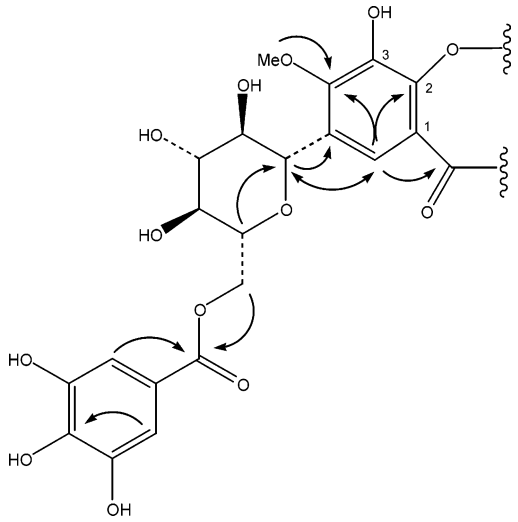


Figure 1. Selected HMBC correlations observed for **1**.

T-cell line CEM-SS infected with HIV-1_{RF}.[20] After a 6-day incubation period, **1** did not inhibit the cytopathic effect of HIV-1 infection.

Table 1. NMR Spectroscopic Data (CD_3OD) for Ardimerin Digallate (**1**)

position	δ_C	δ_H (*J* in Hz)
1a	164.7	
1	118.4	
2	151.3	
3	148.4	
4	141.2	
5	116.3	
6	110.0	7.10 (2H, s)
1′	73.2	5.03 (2H, d, 10.5)
2′	80.4	4.12 (2H, t, 9.9)
3′	74.4	3.97 (2H, m)
4′	70.8	3.56 (2H, t, 9.9)
5′	80.3	3.88 (2H, m)
6′	63.6	4.38 (2H, m)
		3.95 (2H, m)
1″	120.0	
2″	109.2	7.12 (2H, s)
3″	139.0	
4″	145.5	
5″	139.0	
6″	109.2	7.12 (2H, s)
7″	167.1	
OCH_3	59.9	3.93 (6H, s)

Experimental Section

General Experimental Procedures. Melting points were measured using a Yanagimoto micro hot-stage melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO P-1020 digital polarimeter. UV spectra were obtained with a JASCO V-550 UV/vis spectrometer. IR spectra were run on a JASCO 100 IR spectrophotometer. NMR experiments were performed on Bruker DRX-300 and -600 NMR spectrometers. FABMS were obtained from a JMS-HX/HX 110A tandem mass spectrometer.

Plant Material. The whole plants of *A. japonica* were collected in October 2002 and identified by one of us (K.B.). A voucher specimen (CNU02018) was deposited at the herbarium of the College of Pharmacy, Chungnam National University.

Extraction and Isolation. The dried samples (1.9 kg) were powdered and extracted with hot methanol (6 L × 3 times). The combined extract was evaporated in vacuo, giving a gummy residue (170 g), which was suspended in water and then extracted with dichloromethane. The aqueous fraction was filtered through a Diaion HP20 column to eliminate salts and inorganic components by eluting with water. The adsorbed sample on the resin was washed with methanol and the eluate concentrated to yield 61.5 g of a brown powder. This powder was chromatographed on a silica gel column using $CHCl_3$−MeOH (5:1 v/v) as eluent, giving six fractions (A−F). Fraction A was purified by means of silica gel column chromatography with $CHCl_3$−MeOH (5:1), affording **1** (11.9 mg). Ardimerin (**2**, 1500 mg) and quercitrin (368.0 mg) were obtained from fraction D through reversed-phase chromatography eluted with MeOH−H_2O (1:2). The dichloromethane fraction (101 g) was chromatographed over silica gel eluted with a hexane−ethyl acetate (10:1 to 5:1) gradient solvent system to give nine fractions (G−O). Repeated silica gel column chromatography of fraction H with hexane−ethyl acetate (10:1) yielded friedelin (134.0 mg), bauerenyl acetate (82.0 mg), and epifriedelinol (18.2 mg), respectively. Bauerenol (10.5 mg) was purified from fraction M by reversed-phase chromatography with MeOH−H_2O (20:1) as eluent.

Ardimerin digallate (1): white crystals, mp 178−180 °C; $[\alpha]_D^{25}$ +76.9 (*c* 0.3, MeOH); UV (MeOH) λ_{max} (log ϵ) 275 (3.15), 215 (3.96) nm; IR (KBr) ν_{max} 3400 (OH), 1700 (C═O), 1610, 1480 (C═C ring) cm^{-1}; ^1H NMR (CD_3OD, 600 MHz) and ^{13}C NMR (CD_3OD, 150 MHz), see Table 1; FABMS *m/z* 983 $[M + Na]^+$; HRFABMS *m/z* 983.1706 (calcd for $C_{42}H_{40}O_{26}Na$, 983.1706).

Ribonuclease H and Polymerase Assays. Assays for RNase H activity were performed as previously described.[21,22] IC_{50} values were determined from triplicate assays in 2-fold dilutions of compound. Assays for DNA-dependent DNA polymerase (DDDP assay) were carried out as previously described.[23] Briefly, a synthetic 71-base DNA template with a 5′ ^{32}P-labeled 36-base DNA primer annealed to its 3′ end was used as substrate for the polymerization assay (primer extension), with 1.5 pmol of substrate incubated with 0.1 pmol of enzyme for 5 min at room temperature. The reaction was started by adding deoxynucleotide phosphates at 0.2 mM and run for 20 min at room temperature. The reaction buffer was 50 mM Tris pH 8, 80 mM NaCl, 8 mM $MgCl_2$, and 5 mM DTT. Cleavage was visualized by autoradiography of polyacrylamide electrophoresis gels.

HIV Cytopathic Assay. This assay was conducted as previously reported, with CEM-SS cells infected with strain RF of HIV-1.[20]

Acknowledgment. This work was supported by Korea Research Foundation Grant (KRF-2005-013-E00045). This project has been funded in part with federal funds from the National Cancer Institute, National Institutes of Health, Bethesda, MD, under contract N01-CO-12400. The content of this publication does not necessarily reflect the views or polices of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. This research was supported in part by the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research. We are grateful to Korea Basic Science Institute for NMR spectroscopic measurements and J. Wilson for cytopathicity assays.

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NP060359M