# An HIV RNase H Inhibitory 1,3,4,5-Tetragalloylapiitol from the African Plant *Hylodendron gabunensis*

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A new compound, 1,3,4,5-tetragalloylapiitol (**1**), was isolated from the aqueous extract of the plant *Hylodendron gabunensis* and was found to be a potent inhibitor of RNase H enzymatic activity. The structure of **1** was elucidated by NMR analyses to be an apiitol (**2**) sugar moiety substituted with four gallic acid residues. Optical rotation measurements of the free sugar following basic hydrolysis indicated that the 3*S* absolute configuration was the same as that of D-apiitol. Compound **1** inhibited HIV-1, HIV-2, and human RNase H with IC$_{50}$ values of 0.24, 0.13, and 1.5 $\mu$M, respectively, but it did not show inhibition of *E. coli* RNase H at 10 $\mu$M.

Human immunodeficiency virus-type 1 (HIV-1) reverse transcriptase (RT) has two distinct enzymatic domains, and these domains separately carry out a RNA-dependent/DNA-dependent DNA polymerization reaction and ribonuclease H (RNase H) hydrolytic activity. RNase H specifically hydrolyzes the RNA strand of a RNA/DNA heteroduplex.[1–3] The RNase H function of HIV RT is required to effectively incorporate viral genetic information into the host cell genome.[4,5] While both of the RT activities are critical for viral infectivity, only the polymerase activity has been successfully exploited as a target for commercial drugs.[6] Therefore, HIV-1 RNase H remains an attractive molecular target for developing new anti-HIV agents for potential chemotherapeutic applications.[7] As part of a screening campaign to find compounds that can inhibit RNase H activity, a library of natural product extracts ($n = 82\,067$) was screened by our group. Active extracts from the initial RNase H screen were confirmed and then prioritized using a series of secondary assays that included a rapid fluorescence-based capillary electrophoresis analysis, dose–response testing using a panel of different RNase H enzymes, and a cell-based anti-HIV assay.[8] In this paper we describe the isolation, structure elucidation, and biological characterization of a new compound from an extract of the plant *Hylodendron gabunensis* Taub. (Fabaceae) that has potent activity against HIV RNase H.

A 3 g portion of the organic solvent extract of *H. gabunensis* was partitioned between CH$_2$Cl$_2$ and H$_2$O, which concentrated the RNase H inhibitory activity into the H$_2$O-soluble fraction. The aqueous layer was then extracted with *n*-BuOH, and the active *n*-BuOH-soluble material was sequentially fractionated on ODS and on a polyamide resin. Final purification was carried out by ODS HPLC to yield compound **1** as the principal active component. The molecular formula of **1** was determined to be C$_{33}$H$_{28}$O$_{21}$ by HRESIMS measurement (obsd [M – H]$^-$ *m/z* 759.1050, calcd for C$_{33}$H$_{27}$O$_{21}$ 759.1045). Analysis of both the $^1$H NMR and HSQC spectroscopic data obtained in C$_5$D$_5$N showed an oxymethine ($\delta_H$ 6.44/$\delta_C$ 72.7) and three oxymethylene groups ($\delta_H$ 5.31, 5.07/$\delta_C$ 63.7, 5.11, 4.88/65.5, and 4.97, 4.92/65.5). A COSY spectrum revealed correlations between H-3 and H-4 and between the geminal methylene protons. HMBC correlations from H-1a, H-1b, H-3, H-5a, and H-5b to a nonprotonated carbon at $\delta$ 74.3 (C-2) implied that the sugar moiety was apiitol (**2**). The presence of four galloyl

groups in **1** was revealed by the presence of four singlet aromatic resonances ($\delta_H$ 7.85, 7.82, 7.80, 7.78) that each integrated for 2H, four ester carbonyl carbons ($\delta_C$ 167.6, 166.9, 166.9, 166.3), and four groups of $^{13}$C NMR resonances with characteristic chemical shifts that clustered around $\delta_C$ 147.4, 141.1, 120.6, and 110.3. This assignment was supported by the observation that each of the aromatic protons showed HMBC correlations to two phenolic carbon resonances, a nonprotonated aromatic carbon around $\delta$ 120.6, and an ester carbonyl. Further HMBC correlations from H-1 to C-1′, H-3 to C1″, H-4 to C-1‴, and H-5 to C-1⁗ indicated that the four galloyl groups were connected to C-1, C-3, C-4, and C-5 of the apiitol core.



1 2
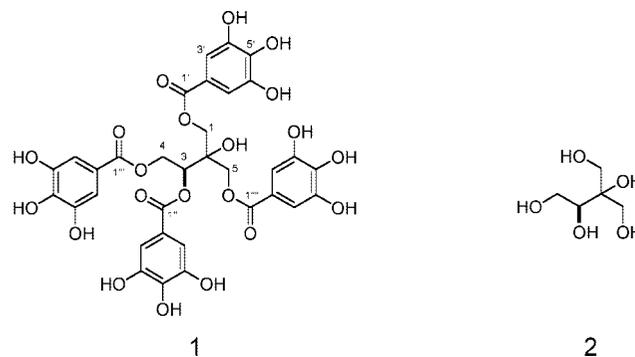
Compound **1** was hydrolyzed with 2 N NaOH/MeOH (1:1) for 12 h to liberate the free sugar moiety, and the hydrolysate was then purified by a combination of anion and cation exchange column chromatography to afford apiitol (**2**). The $^1$H and $^{13}$C NMR data of this material showed good consistency with the literature data for **2**, which was previously isolated from the plant *Torillis japonica*.[9] The specific rotation of **2** ($[\alpha]^{20}_D$ –5.5) that we recovered from the hydrolysate of **1** indicated that the absolute configuration of C-3 was *S* (lit. value $[\alpha]^{26}_D$ –4.0)[9] rather than *R* (lit. value $[\alpha]^{21}_D$ +4.3).[9] Thus the central core of **1** consists of D-apiitol, which has previously been shown to be biosynthesized via reduction of D-apiose (3-C-hydroxymethyl-*aldehydo*-D-*glycero*-tetrose).[10] While D-apiose is widely distributed in plant cell wall polysaccharides,[11] compound **1** is the first secondary metabolite reported that contains the reduced sugar apiitol.

Compound **1** was tested for *in vitro* activity against RNase H from HIV-1, HIV-2, human, and *E. coli* using a fluorescence resonance energy transfer (FRET) based assay.[8] It potently inhibited both HIV-1 and HIV-2 RNase H with IC$_{50}$ values of 0.24 and 0.13

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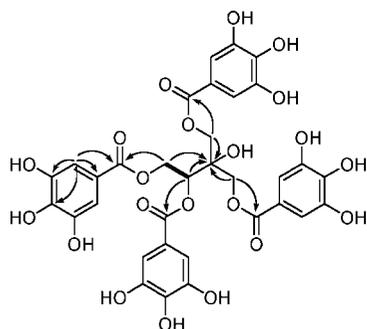[⊥] Proteomics and Mass Spectroscopy Facility, NIDDK, Bethesda, MD.

**Figure 1.** COSY and HMBC correlations of **1**. The bold line and the arrows represent a COSY cross-peak and HMBC correlations, respectively.
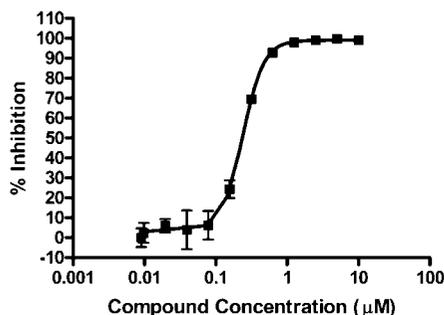


**Figure 2.** Dose–response curve for compound **1** tested against HIV-1 RNase H. Data were collected at a substrate concentration of 125 nM, over an inhibitor concentration range of 0 to 10 $\mu$M in 10 2-fold dilution steps.

$\mu$M, respectively (Figure 2). Compound **1** showed significantly weaker inhibition against human RNase H (IC$_{50}$ = 1.5 $\mu$M) and no detectable inhibition of the *E. coli* enzyme at the highest test concentration (10 $\mu$M). To assess whether the observed inhibition resulted from nonspecific formation of compound–enzyme aggregates, the dose–response experiment was repeated in the presence of 0.1% Triton X-100 detergent, a known suppressor of small molecules that work via this mechanism.[12] Addition of Triton X-100 to the reaction buffer produced no significant effect on the observed IC$_{50}$ or Hill slope for compound **1** (data not shown). This suggests that the activity of **1** is not due to compound aggregation or some other nonspecific hydrophobic interaction with RNase H. In a cell-based assay using virus strain HIV-1$_{RF}$ compound **1** did not afford any cytoprotective effects at concentrations up to 40 $\mu$M.[13]

### Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 241 polarimeter, and UV spectra were obtained on a Beckman DU640 spectrophotometer. $^1$H and $^{13}$C spectra and all 2D NMR data were recorded on a Varian INOVA NMR spectrometer at 500 MHz for $^1$H and 125 Hz for $^{13}$C. Spectra were acquired at 27 °C, and chemical shifts were referenced to the residual solvent resonances for C$_5$D$_5$N: $\delta_H$ 7.19 and $\delta_C$ 123.3. Electrospray ionization (ESI) mass spectra were obtained on a Waters LCT Premier time-of-flight (TOF) mass spectrometer. Fluorescence measurements for the FRET bioassay were made using a Tecan Safire fluorescence plate reader.

**Plant Material.** Leaf samples of *Hylodendron gabunensis* Taub. (Fabaceae) were collected in January 1988 in the Southern Bakundu Reserve Forest, Cameroon. The voucher number for this collection is Q66R75, and a voucher sample is maintained at the Smithsonian Institution in Washington, D.C.

**Isolation of 1,3,4,5-Tetragalloylapiitol (1).** The dried *H. gabunensis* leaves (349 g dry weight) were ground to a fine powder and extracted overnight with approximately 1000 mL of CH$_2$Cl$_2$–MeOH (1:1). After draining off the solvent, the plant material was washed with 100% MeOH, which was then combined with the earlier solvent mixture and

**Table 1.** NMR Spectroscopic Data (500 MHz C$_5$D$_5$N) for 1,3,4,5-Tetragalloylapiitol (**1**)

| position | $\delta_H$ (*J* in Hz) | $\delta_C$ | gCOSY | gHMBC |
|---|---|---|---|---|
| 1a | 4.88 d, (11.6) | 65.5 | H1b | C1′ |
| 1b | 5.11 d, (11.6) | | H1a | C1′ |
| 2 | | 74.3 | | |
| 3 | 6.44 brd, (8.0) | 72.7 | H4a, H4b | C1″ |
| 4a | 5.07 dd, (8.0, 12.0) | 63.7 | H4b, H3 | C1‴ |
| 4b | 5.31 brd, (12.0) | | H4a, H3 | C1‴ |
| 5a | 4.92 d, (10.6) | 65.5 | H5b | C1⁗ |
| 5b | 4.97 d, (10.6) | | H5a | C1⁗ |
| galloyl 1 | | | | |
| 1′ | | 166.9 | | |
| 2′ | | 120.6 | | |
| 3′ | 7.82 s$^a$ | 110.3 | | C1′,2′,3′,4′,5′ |
| 4′ | | 147.4 | | |
| 5′ | | 141.1 | | |
| galloyl 2 | | | | |
| 1″ | | 166.3 | | |
| 2″ | | 120.6 | | |
| 3″ | 7.80 s | 110.3 | | C1″,2″,3″,4″,5″ |
| 4″ | | 147.4 | | |
| 5″ | | 141.1 | | |
| galloyl 3 | | | | |
| 1‴ | | 167.6 | | |
| 2‴ | | 120.6 | | |
| 3″ | 7.78 s | 110.3 | | C1‴,2‴,3‴,4‴,5‴ |
| 4‴ | | 147.4 | | |
| 5‴ | | 141.1 | | |
| galloyl 4 | | | | |
| 1⁗ | | 166.9 | | |
| 2⁗ | | 120.6 | | |
| 3⁗ | 7.85 s$^a$ | 110.3 | | C1⁗,2⁗,3⁗,4⁗,5⁗ |
| 4⁗ | | 147.4 | | |
| 5⁗ | | 141.1 | | |

$^a$ These assignments may be reversed.

evaporated to give 56.5 g of organic extract. A 5 g portion of the extract was partitioned between H$_2$O and CH$_2$Cl$_2$. The aqueous layer was extracted with *n*-BuOH, and the *n*-BuOH-soluble material was separated on an ODS column to afford a potent active fraction (eluted with 50% aqueous MeOH). The active fraction was passed through polyamide resin with MeOH, and the eluant was purified by reversed-phase C$_{18}$ HPLC (Cosmosil AR-II i.d. 10 × 250 mm) with a gradient elution from 10% to 30% aqueous MeOH to give **1** (12.5 mg).

**1,3,4,5-Tetragalloylapiitol (1):** pale purple solid; [$\alpha$]$^{20}_D$ −18.6 (*c* 0.1, MeOH); UV (MeOH) $\lambda_{max}$ (log $\varepsilon$) 219 (4.96), 278 (4.58) nm; HRESIMS [M − H]$^-$ *m/z* 759.1045 (calcd for C$_{33}$H$_{27}$O$_{21}$, 759.1050, $\Delta$ −0.5 mmu); $^1$H and $^{13}$C NMR data, see Table 1.

**Hydrolysis of 1.** A 5 mg portion of **1** was hydrolyzed with 2 N NaOH–MeOH (1:1) at room temperature for 12 h. The reaction mixture was neutralized with HCl and then passed through an anion exchange column (DEAE Sephadex A-50) and then a cation exchange column (BIO-RAD AG 50W-X2) to afford **2** in the eluate, which was isolated as a colorless solid: [$\alpha$]$^{20}_D$ −5.5 (*c* 0.09, MeOH); $^1$H NMR (C$_5$D$_5$N) $\delta$ 4.61 (H-3), 4.51 (H-4a), 4,45 (H-4b), 4.44 (H-1), 4.40 (H-5); $^{13}$C NMR (C$_5$D$_5$N) $\delta$ 76.4 (C-2), 74.9 (C-3), 65.1 (C-5), 64.1 (C-1), 63.3 (C-4).

**RNase H FRET Assay.** This assay was performed with DMSO solutions of the chromatography fractions and pure compounds, as previously described.[8] Data were collected at a final substrate concentration of 125 nM (approximately 5 × $K_m$ value), over a 12-point inhibitor concentration range of 0 to 10 $\mu$M in 2-fold dilution steps. DMSO was present at a final concentration of 1% in all of the samples. The reaction was initiated through the addition of 2 nM enzyme and allowed to run for up to 60 min at 25 °C. Dose–response rate data were calculated using GraphPad Prism software version 4.00 for Windows, GraphPad Software, San Diego CA, www.graphpad.com.

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**Supporting Information Available:** ¹H, ¹³C, and 2D NMR spectra for compound **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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