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Isolation, Structure Determination, and Anti-HIV Evaluation of Tigliane-type Diterpenes and Biflavonoid from *Stellera chamaejasme*

Yoshihisa Asada[*,†], **Aya Sukemori**[‡], **Takashi Watanabe**[§], **Kuber J. Malla**[⊥], **Takafumi Yoshikawa**[‡], **Wei Li**[||,*], **Xinzhu Kuang**[||], **Kazuo Koike**[||], **Chin-Ho Chen**[▽], **Toshiyuki Akiyama**[°], **Keduo Qian**[°], **Kyoko Nakagawa-Goto**[°], **Susan L. Morris-Natschke**[°], **Yan Lu**[°], and **Kuo-Hsiung Lee**[°,•,*]

[†]Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba 278-8510, Japan

[‡]School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan

[§]Laboratory for the Studying of Complementary and Medicinal Resources, The Kochi University of Technology, Tosayamada-cho, Kochi, 782-8502, Japan

[⊥]Department of Plant Resources, G.P.O. Box 2270, Lalitpur, Kathmandu, Nepal

[||]Faculty of Pharmaceutical Sciences, Toho University, Miyama 2-2-1, Funabashi, Chiba 274-8510, Japan

[▽]Duke University Medical Center, Box 2926, SORF, Durham, North Carolina 27710, United States

[°]Natural Products Research Laboratories, UNC Eshelman School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27599-7568, United States

[•]Chinese Medicine Research and Development Center, China Medical University and Hospital, Taichung, Taiwan

Abstract

Five novel tigliane-type diterpenes, stelleracins A–E (**3**–**7**), a novel flavanone dimer, chamaeflavone A (**8**), and six known compounds were isolated from roots of *Stellera chamaejasme*. Their structures were elucidated by extensive spectroscopic analyses. The isolated compounds were evaluated for anti-HIV activity in MT4 cells. New compounds **3**–**5** showed potent anti-HIV activity (EC_{90} 0.00056–0.0068 μM) and relatively low or no cytotoxicity (IC_{50} 4.4–17.2 μM). These new compounds represent promising new leads for development into anti-AIDS clinical trial candidates.

Stellera chamaejasme L. (Thymelaeaceae) is a toxic perennial plant that grows at high altitudes in China and Nepal. Its roots have been used in Traditional Chinese Medicine as an

[*]Corresponding Authors: *Tel: +1-919-962-0066. Fax: +1-966-3893. khlee@unc.edu, asaday@rs.noda.tus.ac.jp, liwei@phar.toho-u.ac.jp.

ASSOCIATED CONTENT

Supporting information

Extraction procedures from *Stellera chamaejasme,* HPLC conditions for separation of compounds **1**–**10**, ^{1}H-NMR, ^{13}C-NMR COSY, HMBC, NOESY and ROESY data and spectra for **1**–**10.** Comparison of key HMBC/COSY (Fig. 1) and ROESY (Fig. 2) correlations in **5** and **6**. This material is available free of charge via the Internet at http://pubs.acs.org.

emulgent, anthelmintic, and dermatological agent. During our ongoing chemical investigations of Nepalese medicinal plants, a MeOH extract of *S. chamaejasme* showed potent anti-HIV activity. Investigation of the chemical constituents of this extract resulted in the isolation of eight daphnane-type diterpenes.[1] Two of these compounds, stelleralide A and gnidimacrin, showed extremely potent anti-HIV activity (EC_{90} 0.40 nM) with relatively low cytotoxicity (IC_{50} 4.3 μM) in MT4 cells.[1] Herein, we report a further phytochemical investigation of *S. chamaejasme*, including the isolation and structural determination of five novel tigliane-type diterpenes (**3**–**7**), a novel flavanone dimer (**8**), and six known compounds; stellerarin (**1**)[2], 12-*O*-benzoylphorbol 13-octanoate (**2**),[3] (−)-chamaejasmenin B (**9**),[4,5] (+)-chamaejasmenin C (**10**),[4,5] 1,5-diphenyl-2(*E*)-penten-1-one (**11**),[6,7] and 1-(ω-feruloyldocosanoyl)glycerol (**12**).[8] The anti-HIV activity of compounds **1**–**12** was also evaluated in our study.

RESULTS AND DISCUSSION

A MeOH extract from roots of *S. chamaejasme* collected in Nepal was partitioned between EtOAc and H_2O. The EtOAc fraction was fractionated by ODS and silica gel column chromatography, as well as preparative HPLC, to afford seven tigliane-type diterpenes (**1**–**7**), three flavanone dimers (**8**–**10**), and two phenolic compounds (**11** and **12**).

New compounds **3**–**7** were obtained as white powders, and their ^1H and ^{13}C NMR spectra showed characteristic resonances for the gem-dimethyl cyclopropane moiety of tigliane-type diterpenes (Table 1). A comparison of the NMR data of stelleracin A (**3**), and stelleracin B (**4**) with those of the known compounds **1** and **2** indicated that the four compounds had the same structures, except for the 13-acyl moiety.

The molecular formula of **3** was determined as $C_{37}H_{48}O_8$ by HR-FAB-MS. Thus, compound **3** has two fewer hydrogen atoms than **1,** suggesting the presence of a decenoate moiety in **3**. The olefinic position in this moiety was established at C-4′, based on HMBC correlations from δ_H 2.45 (H_2-2′) and 2.38 (H_2-3′) to δ_C 176.0 (C-1′), as well as ^1H -^1H COSY correlations between δ_H 2.38 (H_2-3′) and 5.33 (H-4′). The coupling constant between the olefinic protons was 10.5 Hz, indicating a *cis* relationship, and accordingly, the 13-acyl moiety in **3** was assigned as 4*Z*-decenoate.

The molecular formula of **4** ($C_{37}H_{46}O_8$ from HR-FAB-MS) contained two fewer hydrogen atoms than that of **3**, indicative of a decadienoate 13-acyl moiety. The ^1H NMR spectrum included resonances at δ_H 5.29 (1H, dt, J = 11.0, 7.5 Hz) and 5.34–5.44 (3H, m) for four olefinic protons, which had ^1H-^1H COSY correlations with H_2-9′, H_2-6′ and H_2-3′. Based on these data, the two double bonds were assigned at C-4′ and C-7′. The orientations of both olefins were established as *cis*, based on the chemical shifts of C-3′, C-6′ and C-9′ (δ_C 22.4, 25.5 and 20.5, respectively). Thus, the 13-acyl moiety in **4** was fully determined as 4*Z*, 7*Z*-decadienoate.

The molecular formula of stelleracin C (**5**) was determined as $C_{35}H_{46}O_9$ from HR-FAB-MS. Thus, compound **5** contains one more oxygen atom than **2**, which is consistent with the presence of an additional hydroxy group in **3**. Accordingly, the C-5 methylene resonances at δ_H 2.50, 2.60 and δ_C 38.9 in the ^1H and ^{13}C NMR spectra of **2** were replaced by hydroxymethylene resonances at δ_H 4.38 and δ_C 71.7 in **5**. A hydroxy moiety at C-5 was further confirmed by HMBC correlations from δ_H 5.73 (H-7) and 3.06 (H-10) to δ_C 71.7 (C-5). The β-orientation of this hydroxy moiety was deduced from a ROESY correlation between δ_H 3.06 (H-10) and 4.38 (H-5).

The molecular formula of stelleracin D (**6**) was determined by HR-FAB-MS as $C_{37}H_{50}O_{10}$, and contains two more oxygen atoms than that of **1**. A comparison of the ^{1}H and ^{13}C NMR spectroscopic data of **6** and **1** revealed large differences in the B-ring resonances. From detailed analyses of 2D NMR spectroscopic data, the olefinic proton resonance at δ_H 6.42 in **6** was assigned to H-5 based on HMBC correlations with δ_C 206.2 (C-3), 57.3 (C-10) and 66.5 (C-20). Thus, **6** contains a $\Delta^{5,6}$ olefin moiety. In addition, the oxymethine proton resonance at δ_H 4.81 in **6** was assigned to C-7, based on sequential ^{1}H-^{1}H COSY correlations between δ_H 1.70 (H-14), 2.94 (H-8) and 4.81 (H-7), and HMBC correlations from H-7 to δ_C 75.0 (C-9), 31.8 (C-14) and 66.5 (C-20). Based on the molecular formula and the chemical shift of the C-7 resonance at δ_C 83.1, the presence of a hydroperoxy moiety at C-7 was determined. The β-orientation of this moiety was established from ROESY correlations between H-7/H-10 and H-7/H-14.

Stelleracin E (**7**) and **6** had superimposable ^{1}H and ^{13}C resonances, except for those of the C-13 acyl moiety. The acyl group in **7** was established as octanoate, based on the molecular formula of $C_{35}H_{46}O_{10}$ and the integrated values of the relevant ^{1}H and ^{13}C NMR resonances.

Chamaeflavone A (**8**) was isolated as a white powder, $[\alpha]_D^{22}$ −319.0 (*c* 0.11, MeOH). Its molecular formula was determined as $C_{32}H_{26}O_{10}$ by positive-ion HRFABMS, and was the same as that of (−)-chamaejasmenin B (**9**). Compound **8**, **9**, and **10** had similar ^{1}H and ^{13}C resonance patterns, suggesting that **8** is also a 3,3″ biflavanone. Based on ROESY correlations between 7-OC*H*$_3$/H-6, H-8, and 4′-OC*H*$_3$/H-3′, H-5′, two methoxy moieties were present in the same flavanone monomer at C-7 and C-4′. Two fragment peaks at *m/z* 299 and 271 in the FABMS spectrum also supported this conclusion. The *trans* relationships of both H-2/H-3 and H-2″ H-3″ were determined from their coupling constant values (12.0 Hz) by ^{1}H NMR decoupling experiments, and contrasted with the *cis* relationships of the same hydrogens in **9** and **10** (coupling constant values of 0 Hz). In the CD spectrum, a negative Cotton effect at 311 nm and positive Cotton effect at 287 nm were consistent with 2*R* and 2″ *R* configurations.

The newly isolated compounds (**1–12**) were evaluated for anti-HIV-1 activity against NL4-3 virus in MT4 lymphocytes. The anti-HIV EC_{90} values, defined as the compound concentration that inhibited HIV-1 replication by 90%, are summarized in Table 3. Diterpenes **1**, **2**, and **3** were the most potent compounds, with comparable anti-HIV EC_{90} values of 0.50, 0.56, and 0.66 nM, respectively. All three compounds also demonstrated relatively low cytotoxicity (IC_{50} 4.4–5.1 μM). Structurally, these three compounds differ only in the C-13 ester substituent (decanoate in **1**, octanoate in **2**, and 4*Z*-decenoate in **3**). Compounds **4** and **5** showed lower, but still significant, anti-HIV potency with EC_{90} values of 6.8 and 3.8 nM, respectively. Compound **5** has a β-hydroxy group at C-5, while the more potent **1**, **2**, and **3** are unsubstituted at this position. Compound **4** showed lower anti-HIV potency, and has a 4*Z*,7*Z*-decadienoate ester rather than the less rigid ester side chains in **1**, **2**, and **3**. Compounds **6** and **7** with a C-7 hydroperoxy moiety and a $\Delta^{5,6}$ rather than $\Delta^{6,7}$ olefin (as in compounds **1–5**) showed significantly decreased anti-HIV activity with EC_{90} values of 0.49 and 1.52 μM, respectively. From the preliminary SAR, we can conclude that, in addition to the C-13 ester substituent, the B-ring also plays a critical role in the anti-HIV activity of tigliane-type diterpenes. Finally, the new flavanone dimer **8** and known phenolic compound **12** showed marginal anti-HIV activity with EC_{90} values of 4.21 and 3.63 μM, respectively.

In conclusion, seven tigliane-type diterpenes (**1**–**7**), three flavanone dimers (**8**–**10**), and two phenolic compounds (**11** and **12**) were isolated from roots of *Stellera chamaejasme* L. Six compounds, stelleracins A–E (**3**–**7**) and chamaeflavone A (**8**), were identified for the first

time. Three compounds (**1**, **2**, and **3**) exhibited potent anti-HIV EC_{90} values of less than 1 nM. The relevant structural pharmacophores were the ester side chain at C-13 and the substitution/unsaturation pattern at C-5 to C-7.

The tigliane-type diterpenes described in this study share some structural similarity to prostratin (12-deoxyphorbol-13-acetate) and DPP (12-deoxyphorbol-13-phenylacetate), as well as our previously discovered daphnane-type diterpenes,[1] stelleralide A and gnidimacrin. The anti-HIV-1 activity of prostratin, DPP, and gnidimacrin is due, at least in part, to an ability to activate protein kinase C and down-regulate HIV-1 cellular receptors, CD4 and chemokine receptors, such as coreceptor CCR5.[9–12] Due to their structural similarity, the new compounds' extremely potent anti-R5 HIV-1 activity likely results from the same mechanisms. Although gnidimacrin is a potent inhibitor of many HIV-1 R5 viruses, it was essentially inactive against X4 or dual tropic HIV-1 replication in peripheral blood mononuclear cells (PBMCs).[12] Therefore, it is likely that these new compounds will also be inactive under the same conditions.

EXPERIMENTAL SECTION

General Experimental Procedures

The UV spectra were obtained with a Hitachi U-2800 spectrophotometer, and the IR spectra were measured with a Hitachi DFT/IR 460 (KBr disk) spectrometer. Optical rotations were measured with a Hitachi DIP-1000 polarimeter in a 0.5 dm cell. The ^{1}H and ^{13}C NMR measurements were recorded using a Varian XL-400 NMR spectrometer with TMS as the internal reference, and chemical shifts are expressed in δ (ppm). FABMS and HRFABMS were conducted using JEOL JMS-AX505 HA and JMS-700 M Station mass spectrometers. Silica gel (Kanto Chemical Corporation) and ODS (Senshu Scientific Co., Ltd.) were used for column chromatography. For HPLC, a Waters 515 HPLC system, equipped with Shodex RI-72 refractive index and Shimadzu SPD-10AV UV detectors, was used. TLC was carried out on Kieselgel 60 F254 Art.5715 (E. Merck) and RP-18 F254 Art.15389 (E. Merck).

Plant Material

The roots of *S. chamaejasme* were collected from Chele (Alt. 3115m) to Shyammochem (Alt. 3755m), upper Mustang, Mustang District, Nepal in July 2002, and identified by Dr. Takahide Kurosawa (Faculty of Symbiotic System Science, Fukushima University). A plant specimen [LOM-SP020729(047)] is deposited in the herbarium of the University of Tokyo.

Extraction and isolation

Roots of *S. chamaejasme* (4.2 kg) were extracted with MeOH. The methanolic extract was concentrated (840.2 g), suspended in H_2O, and then partitioned successively with EtOAc. The EtOAc layer was evaporated under reduced pressure below 40 °C to give a residue (471.6 g), which was passed over an ODS column, and washed with a gradient of MeOH-H_2O to give eight fractions (1–8). Fractions 2 and 3 were purified by repeated RP-HPLC to give **8** (15.6 mg), **9** (27.7 mg), **10** (348.4 mg), **11** (88.6 mg), and **12** (1.9 mg). Fraction 4 was purified by repeated RP-HPLC to give **1** (48.6 mg), **2** (114.3 mg), **3** (27.4 mg), **4** (5.6 mg), **5** (7.4 mg), **6** (2.2 mg), and **7** (2.0 mg).

Stelleracin A (3)—white powder; $[\alpha]_D^{23}$ +2.5 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.31) nm; CD (*c* 2.19 × 10^{-5}, MeOH) $\Delta\varepsilon^{24}$ +0.77 (307), 0 (285), −4.8 (258), 0 (241), +24.1 (225); IR (KBr) ν_{max} 3413, 2925, 1720, 1711, 1629 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive-ion FABMS *m/z* 621 $[M + H]^+$, 643 $[M + Na]^+$; positive-ion *m/z* HRFABMS 643.3270 $[M+Na]^+$(calcd for $C_{37}H_{48}O_8Na$, 643.3242).

Stelleracin B (4)—white powder; $[\alpha]_D^{23}$ +2.3 (*c* 0.14, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.33) nm; CD (*c* 2.26 × 10^{-5}, MeOH) $\Delta\varepsilon^{24}$ (nm) +1.4 (301), 0 (282), −4.2 (258), 0 (242), +23.9 (225); IR (KBr) ν_{max} 3414, 2925, 1717, 1710, 1629 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive-ion FABMS *m/z* 641 $[M + Na]^+$; positive-ion HRFABMS *m/z* 641.3088 $[M + Na]^+$(calcd for $C_{37}H_{46}O_8Na$, 641.3086).

Stelleracin C (5)—white powder; $[\alpha]_D^{23}$ −37 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.31) nm; CD (*c* 1.96 × 10^{-5}, MeOH) $\Delta\varepsilon^{24}$ (nm) +1.2 (302), 0 (280), −9.1 (251), 0 (240), +24.9 (225); IR (KBr) ν_{max} 3404, 2926, 1721, 1712, 1627, 1603 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive-ion FABMS *m/z* 633 $[M + Na]^+$; positive-ion HRFABMS *m/z* 633.3055 $[M + Na]^+$ (calcd for $C_{35}H_{46}O_9Na$, 633.3034).

Stelleracin D (6)—white powder, $[\alpha]_D^{23}$ −6.9 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.33) nm; CD (*c* 1.99×10^{-5}, MeOH) $\Delta\varepsilon^{24}$ (nm) −25.5 (243), 0 (230), +22.6 (207); IR (KBr) ν_{max} 3388, 2925, 1719, 1628, 1603 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive-ion FABMS *m/z* 677 $[M + Na]^+$; negative-ion FABMS *m/z* 653$[M - H]^-$; positive-ion HRFABMS *m/z* 677.3292 $[M + Na]^+$(calcd for $C_{37}H_{50}O_{10}Na$, 677.3297).

Stelleracin E (7)—white powder, $[\alpha]_D^{22}$ −38 (*c* 0.12, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.26) nm; CD (*c* 1.91×10^{-5}, MeOH) $\Delta\varepsilon^{24}$ (nm) −15.0 (243), 0 (230), +13.7 (207); IR (KBr) ν_{max} 3390, 2926, 1719, 1626, 1603 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 1; positive-ion FABMS *m/z* 649 $[M + Na]^+$; negative-ion FABMS *m/z* 625 $[M-H]^-$; positive-ion HRFABMS *m/z* 649.2979 $[M + Na]^+$ (calcd for $C_{35}H_{46}O_{10}Na$, 649.2984).

Chamaeflavone A (8)—white powder; $[\alpha]_D^{22}$ −319 (*c* 0.11, MeOH); UV (MeOH) λ_{max} (log ε) 297 (4.25) nm; CD (*c* 1.93×10^{-5}, MeOH) $\Delta\varepsilon^{24}$ (nm) −25.4 (311), 0 (299), +32.3 (288), 0 (263), −4.6 (255), 0 (242), −35.4 (218); IR (KBr) ν_{max} 3431, 1638, 1611, 1517, 1459 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) and ^{13}C NMR ($CDCl_3$, 100 MHz), see Table 2; positive-ion FABMS *m/z* 571 $[M + H]^+$, 593 $[M + Na]^+$, 299 $[M - C_{15}H_{11}O_5]^+$, 271 $[M-C_{17}H_{15}O_5]^+$; positive-ion HRFABMS *m/z* 593.1448 $[M + Na]^+$(calcd for $C_{32}H_{26}O_{10}Na$, 593.1418).

Anti-HIV Assay

MT4 cells were infected with HIV-1 NL4-3 (multiplicity of infection = 0.001) in the presence of various concentrations of compounds. Fresh medium, which contained appropriate concentrations of the compounds, was added to the culture 48 h after infection to maintain normal cell growth. Virus replication was analyzed on day-4 post-infection using p24 ELISA kits from Perkin-Elmer. The compound concentration that inhibited HIV-1 replication by 90% (EC_{90}) was calculated by using the biostatistic software Calcusun (Biosoft).

Cytotoxicity Study

Cytotoxicity of the purified compounds toward MT4 cells was determined by using a cell viability kit provided by Promega. The CellTiter-Glo Luminescent Cell Viability Assay is a simple method of determining the viability of the cells in culture based on quantitation of ATP in metabolically active cells. The CellTiter-Glo reagent was added to MT4 cells that were cultured parallel to the antiviral assays. The compound concentration that decreased the cell viability by 50% (IC_{50}) was calculated by using Calcusun (Biosoft).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

^{1}H and ^{13}C NMR Spectroscopic Data (δ, $CDCl_3$) for **3**–**7**

position	3		4		5		6		7	
	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)	δ_C, type	δ_H (J in Hz)
1	160.8, CH	7.6, dq (2.5,1.5)	160.7, CH	7.61, dq (2.5,1.5)	161.9, CH	7.68, dq (2.5,1.5)	160.3, CH	7.66, dq (2.5,1.5)	160.2, CH	7.66, dq (3.0,1.5)
2	132.9, C		132.9, C		134.1, C		134.1, C		134.1, C	
3	209.0, C		208.9, C		209.6, C		206.2, C		206.2, C	
4	73.7, C		73.7, C		73.4, C		72.5, C		72.5, C	
5	38.6, CH	2.50, d (19.5)	38.6, CH	2.50, d (19.0)	71.7, CH	4.38, s	133.7, CH	6.42, d (2.0)	133.8, CH	6.42, d (2.0)
		2.60, d (19.5)		2.60, d (19.0)						
6	140.5, C		140.5, C		140.2, C		150.6, C		150.6, C	
7	129.2, CH	5.72, d (5.0)	129.2, CH	5.72, br.d (5.5)	132.8, CH	5.73, d (5.5)	83.1, CH	4.81, dd (9.0,2.0)	83.1, CH	4.80, dd (9.0,2.0)
8	39.1, CH	3.34, dd (5.0,5.0)	39.1, CH	3.32, dd (5.5,5.5)	38.9, CH	3.54, dd (5.5,5.5)	46.0, CH	2.94, dd (9.0,5.5)	46.0, CH	2.93, dd (9.0,5.5)
9	78.3, C		78.3, C		77.3, C		75.0, C		74.9, C	
10	56.2, CH	3.29, dd (2.5,2.5)	56.2, CH	3.29, dd (2.5,2.5)	53.8, CH	3.06, dd (2.5,2.5)	57.3, CH	3.02, dd (2.5,2.5)	57.3, CH	3.02, dd (3.0,3.0)
11	43.3, CH	3.32, dq (10.0,6.5)	43.3, CH	2.32, m	44.1, CH	2.24, dq (10.0,6.5)	44.7, CH	2.35, dq (10.0,6.5)	44.7, CH	2.36, dq (10.0,6.5)
12	77.6, CH	5.67, d (10.0)	77.6, CH	5.66, d (10.0)	77.6, CH	5.64, d (10.0)	77.7, CH	5.71, d (10.0)	77.7, CH	5.72, d (10.0)
13	65.6, C		65.7, C		65.4, C		65.8, C		65.8, C	
14	36.6, CH	1.14, d (5.0)	36.6, CH	1.14, d (5.5)	36.7, CH	1.12, d (5.5)	31.8, CH	1.70, d (5.5)	31.8, CH	1.70, d (5.5)
15	26.0, C		26.0, C		26.2, C		26.1, C		26.1, C	
16	23.8, CH_3	1.21, s	23.8, CH_3	1.21, s	23.7, CH_3	1.20, s	23.8, CH_3	1.28, s	23.8, CH_3	1.28, s
17	17.0, CH_3	1.38, s	17.0, CH_3	1.38, s	17.0, CH_3	1.35, s	16.8, CH_3	1.36, s	16.8, CH_3	1.36, s
18	14.5, CH_3	0.95, d (6.5)	14.5, CH_3	0.95, d (7.5)	15.0, CH_3	0.98, d (6.5)	14.8, CH_3	0.97, d (6.5)	14.8, CH_3	0.98, d (6.5)
19	10.1, CH_3	1.77, dd (2.5,1.5)	10.1, CH_3	1.77, dd (2.5,1.5)	10.0, CH_3	1.78, dd (2.5,1.5)	10.2, CH_3	1.80, dd (2.5,1.5)	10.2, CH_3	1.80, dd (3.0,1.5)
20	68.1, CH_3	4.01, d (13.0)	68.0, CH_3	4.01, d (13.0)	67.8, CH_2	4.23, s	66.5, CH_3	4.25, d (11.0)	66.6, CH_3	4.25, d (11.0)
		4.07, d (13.0)		4.07, d (13.0)				4.48, d (11.0)		4.49 d (11.0)
1′	176.0, C		175.9, C		176.7, C		176.9, C		176.8, C	
2′	34.5, CH_2	2.45, m	34.3, CH_2	2.44, dd (7.5,7.5)	34.4, CH_2	2.33, ddd (15.0,7.5,7.5)	34.4, CH_2	2.34, ddd (16.0,8.0,8.0)	34.4, CH_2	2.34, ddd (16.0,8.0,8.0)
					2.40, ddd (15.0,7.5,7.5)		2.40, ddd (16.0,8.0,8.0)		2.40, ddd (16.0,8.0,8.0)	
3′	22.4, CH_2	2.38, m	22.4, CH_2	2.43, dd (7.5,1.5)	24.5, CH_2	1.64, ddd (15.0,7.5,7.5)	24.6, CH_2	1.65, ddd (16.0,8.0,8.0)	24.6, CH_2	1.65, ddd (16.0,8.0,8.0)

position	3		4		5		6		7	
	δ_C, type	**δ_H (*J* in Hz)**	**δ_C, type**	**δ_H (*J* in Hz)**	**δ_C, type**	**δ_H (*J* in Hz)**	**δ_C, type**	**δ_H (*J* in Hz)**	**δ_C, type**	**δ_H (*J* in Hz)**
4′	127.0, CH	5.33, dtt (10.5,7.0,1.5)	127.3,[a] CH	c	28.9,[a] CH_2	b	29.1,[a] CH_2	b	28.9,[a] CH_2	b
5′	131.8, CH	5.42, dt (10.5,7.0)	129.9,[a] CH	c	29.0,[a] CH_2	b	29.2,[a] CH_2	b	29.0,[a] CH_2	b
6′	27.2, CH_2	2.03, dt (7.0,7.0)	25.5, CH_2	2.80, dd (7.5,7.5)	31.6,[a] CH_2	b	29.3,[a] CH_2	b	31.6, CH_2	b
7′	29.2, CH_2	b	126.8, CH	5.29, dt (11.0,7.5)	22.6,[a] CH_2	b	29.4,[a] CH_2	b	22.6, CH_2	b
8′	31.5, CH_2	b	132.1,[a] CH	c	14.0, CH_3	0.88, t (6.5)	31.8, CH_3	b	14.1, CH_2	0.88, t (7.0)
9′	22.5, CH_2	b	20.5, CH_2	2.06, dq (7.5,7.5)			22.7, CH_2	b		
10′	14.0, CH_2	0.88, t (7.0)	14.2, CH_3	0.96, t (7.5)		0.88, d (6.5)	14.1, CH_3	0.88, t (7.0)		
1″	166.2, C		166.3, C		166.2, C		166.3, C		166.3, C	
2″	130.1, C		130.1, C		130.0, C		130.2, C		130.2, C	
3″, 7″	129.7, CH	8.03, dd (7.5,1.5)	129.7, CH	8.03, dd (7.5,1.5)	129.7, CH	8.02, dd (7.5,1.5)	129.7, CH	8.03, dd (7.5,1.5)	129.8, CH	8.04, dd (7.5,1.5)
4″, 6″	128.4, CH	7.45, dd (7.5,7.5)	128.4, CH	7.45, dd (7.5,7.5)	128.5, CH	7.45, dd (7.5,7.5)	128.4, CH	7.45, dd (7.5,7.5)	128.4, CH	7.45, dd (7.5,7.5)
5″	133.0, CH	7.58, tt (7.5,1.5)	133.0, CH	7.58, tt (7.5,1.5)	133.1, CH	7.58, tt (7.5,1.5)	133.0, CH	7.57, tt (7.5, 1.5)	133.0, CH	7.58, tt (7.5,1.5)

[a]These resonances in the same column are interchangeable,

[b]1.21~1.38 (m),

[c]5.34~5.44 (3H, m)

Table 2

^{1}H and ^{13}C NMR Spectroscopic Data (δ, $CDCl_3$) for **8**

position	δ_C	δ_H (J in Hz)
2	83.4	5.93, d (12.0)[a]
3	49.7	2.82, dd (12.0,1.5)
4	196.5	
5	164.3	
6	94.1	5.95, d (2.0)
7	167.9	
8	95.2	6.06, d (2.0)
9	162.6[b]	
10	103.2[c]	
1′	128.5	
2′,6′	129.2	7.04, d (8.0)
3′,5′	114.3	6.87, d (8.0)
4′	160.4	
7-OCH_3	55.7	3.77, s
4′-OCH_3	55.4	3.85, s
2″	83.4	5.92, d (12.0)[a]
3″	49.7	2.80 dd (12.0,1.5)
	4″	
	5″	
6″	96.7	5.98, d (2.0)
7″	164.1	
8″	95.3	5.86, d (2.0)
9″	162.9[b]	
10″	103.1[c]	
1‴	128.7	
2‴,6‴	129.4	6.98, d (8.0)
3‴,5‴	115.7	6.80, d (8.0)
4‴	156.5	
5,5″-OH		11.89, d (14.5)

[a,b,c] Interchangeable signals

Table 3

Anti-HIV Data of **1–12**[a]

compound	anti-HIV (NL4-3) EC_{90} (μM)	cytotoxicity (MT-4) IC_{50} (μM)
1	0.00050	5.1
2	0.00056	4.4
3	0.00066	4.7
4	0.0068	7.8
5	0.0038	17.2
6	0.49	3.5
7	1.52	15.8
8	4.21	17.5
12	3.63	16.2

Compounds **9**–**11** were inactive.