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Rapid Recognition and Targeted Isolation of Anti-HIV Daphnane Diterpenes from *Daphne genkwa* Guided by UPLC-MSⁿ

Hua-Ding Zhao^{†,‡}, Yan Lu^{†,‡}, Min Yan[†], Chin-Ho Chen[‡], Susan L. Morris-Natschke[§], Kuo-Hsiung Lee^{*,§,⊥}, Dao-Feng Chen^{*,†}

[†]School of Pharmacy, Institutes of Integrative Medicine, Fudan University, Shanghai 201203, People's Republic of China

[‡]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 40402, Taiwan

Abstract

Daphnane diterpenes with a 5/7/6-tricyclic ring system exhibit potent anti-HIV activity but are found in low abundance as plant natural products. In this study, an effective approach based on mass spectrometric fragmentation pathways was conducted to specifically recognize and isolate anti-HIV compounds of this type from *Daphne genkwa*. Briefly, the fragmentation pathways of reference analogues were elucidated based on characteristic ion fragments of m/z 323 $\rightarrow$ 295 $\rightarrow$ 267 or m/z 253 $\rightarrow$ 238 $\rightarrow$ 197 by ultra-high-performance liquid chromatography-ion trap tandem mass spectrometry (UPLC-IT-MSⁿ) and then applied to the differentiations of substances with or without an oxygenated group at C-12. Twenty-seven daphnane diterpenes were successfully recognized from a petroleum ether extract of *D. genkwa*, including some potential new compounds and isomers that could not be identified accurately only from the ion fragments. Further separation of these target compounds using high-speed countercurrent chromatography (HSCCC) and preparative HPLC led to the isolation of three new (**11**, **25**, and **27**) and 14 known compounds, whose structures were identified and confirmed based on MS, NMR, and electronic circular dichroism (ECD) spectroscopy. The isolates exhibited anti-HIV activities at nanomolar concentrations. The results demonstrated that this strategy is feasible and reliable to rapidly recognize and isolate daphnane diterpenes from *D. genkwa*.

Graphical Abstract

*Corresponding Authors: (K.-H. Lee): khlee@unc.edu. Tel: +1-919-962-0066. (D.-F. Chen): dfchen@shmu.edu.cn. Tel: +86-21-51980135.

#H.-D. Zhao and Y. Lu contributed equally.

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Supporting Information

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UPLC-IT-MSⁿ data of reference and 27 daphnane diterpenes, HSCCC chromatogram of isolation process, and UPLC-IT-MSⁿ, IR, and NMR spectra of **11**, **25**, and **27** (PDF)

Daphnane diterpenes with a 5/7/6-tricyclic ring system that occur mainly in the family Thymelaeaceae exhibit various biological activities including anti-HIV and antitumor effects.^{1–3} Of significance, many of these compounds, such as huratoxin, wikstroelide A, simplexin, and wikstrotoxin A, exhibit potent anti-HIV activity at nanomolar concentrations.⁴ However, such compounds occur in the plant of origin at very low concentration levels.^{5–79} It is time-consuming and laborious to isolate them from complex mixtures using conventional repeated column chromatography.^{8,9} Therefore, efficient recognition and target-guided isolation are urgently required to accelerate the research on daphnane diterpenes as anti-HIV drug leads.

In recent years, HPLC-MS/MS has become a very powerful tool for the identification of active trace constituents from a complex matrix.^{10,11} Especially, ion-trap MS provides multi-stage MS^n and generates many fragmentation ions, which is extremely suitable for the identification of target compounds. Thus far, different classes of natural compounds, such as flavonoids, phenolic acids, terpenoids, and saponins, have been analyzed and characterized by HPLC- MS^n based on fragmentation pathways.^{12–14} However, few reports have elucidated the MS fragmentation patterns of daphnane diterpenes in detail.

Daphne genkwa Sieb. et Zucc. (Thymelaeaceae) is a traditional medicinal herb with antitumor, diuretic, and antitussive effects.^{15,16} Phytochemical studies of its flower buds have led to the isolation of diterpenoids, coumarins, flavonoids, lignans, and sesquiterpenoids.^{17–19} A preliminary experiment in the present investigation indicated that a petroleum ether extract of the flower buds of *D. genkwa* possessed potent anti-HIV activity ($\text{IC}_{50} = 0.083 \mu\text{g/mL}$). Subsequently, a strategy based on MS fragmentation pathways was used for the rapid recognition and targeted isolation of selected diterpenoids from *D. genkwa* via ultra-high-performance liquid chromatography-ion trap tandem mass spectrometry (UPLC-IT- MS^n) and high-speed countercurrent chromatography (HSCCC). Altogether, 17 daphnane diterpenes, including three new compounds, were isolated and identified by using MS, NMR, and electronic circular dichroism (ECD) analysis. Also, the anti-HIV activities of nine isolated daphnane diterpenes were evaluated.

RESULTS AND DISCUSSION

Fragmentation Pathways and Recognition of Daphnane Diterpenes.

To rapidly recognize diterpenoids in a complex mixture, the fragmentation pathways of reference compounds (**S1–S8**) in Figures 1 and 2 were elucidated in detail based on characteristic ion fragments. Depending on the presence of an oxygenated group at C-12, these eight diterpenes could be classified into type A (**S1–S4**) in Figure 1 and type B (**S5–**

S8) in Figure 2. Subtoxin A (**S1**) and huratoxin (**S5**) were taken as examples to explain the fragmentation pathways for types A and B, respectively. For subtoxin A, the MS² fragmentation of the quasimolecular ion at m/z 643 $[M + H]^+$ yielded three prominent fragments at m/z 583, 419, and 359, corresponding to $[M - R_2H + H]^+$, $[M - R_1COOH + H]^+$, and $[M - R_2H - R_1COOH + H]^+$, respectively, as shown in Figure 1. Other fragments at m/z 625, 605, 565, 401, 341, and 323 were associated with the consecutive loss of H₂O. Owing to the formation of relatively stable conjugated structures, the ion at m/z 323 had the highest abundance and its MS³ fragmentation produced the ion at m/z 295 due to decarbonylation. The MS⁴ fragment ion of m/z 267 came from the decarbonylation of the ion at m/z 295. Similarly, the fragmentation of **S2–S4** produced mainly the fragments at m/z 359, 341, and 323 from the loss of fatty acid and H₂O and at m/z 295 and 267 from the consecutive decarbonylation (Table S1, Supporting Information). For recognition of type A daphnane diterpenes, the diagnostic ions are the fragment ions at m/z 323 $\rightarrow$ m/z 295 $\rightarrow$ m/z 267.

For huratoxin (**S5**), the protonated ion at m/z 585 produced fragments at m/z 567, 549, 531, 361, 343, 325, 307, 297, and 279 from the loss of fatty acid (RCOOH) and sequential dehydration and decarbonylation (Figure 2). The fragment at m/z 253 had the highest abundance and resulted from the loss of a C₂H₂ group from the MS² fragment ion at m/z 279. In MS³ and MS⁴, fragment ions at m/z 238 and 197 originated through the loss of CH₃ and C₃H₅, respectively, from the benzene ring. The fragmentation pathways of **S6–S8** were identical to those of **S5** (Table S1, Supporting Information). The fragment ions m/z 253 $\rightarrow$ m/z 238 $\rightarrow$ m/z 197 were selected as diagnostic ions for recognition of type B daphnane diterpenes.

The above diagnostic ions of types A and B were used for the rapid recognition of daphnane diterpenes with similar structures from the petroleum ether extract of *D. genkwa*. As a result, 27 peaks (Figure 3) were determined as daphnane diterpenes, including 25 peaks for type A and 2 peaks for type B (Table S2, Supporting Information). Among them, there were five groups of isomers, namely, peaks 7–10, peaks 11, 12, and 14, peaks 17–19, and 21, peaks 20 and 22, and peaks 23 and 24. Each group of isomers had the same fragment ions in MS^{1–4}, which were difficult to accurately identify simply by the fragmentation ions. Peak 25, $[M + H]^+$ at m/z 643, produced fragment ions at m/z 527, 461, and 359 in MS² corresponding to the loss of R₂H (102 Da), R₁COOH (168 Da), and R₂H + R₁COOH (270 Da), respectively, which generated characteristic ion fragments at m/z 323, 295, and 267 (Figure S3, Supporting Information). The molecular mass of the R₂ group was 14 Da greater than that (C₃H₇COO) of yuanhualine,²⁰ while the R₁ groups have the same molecular mass. Thus, the R₂ group was deduced as C₄H₉COO. Similarly, the R₂ group of peak 27 was deduced as C₅H₁₁COO (Figure S4, Supporting Information). Peaks 25 and 27 with the above structure units have not been reported before and were deduced as potential new diterpenes. Accordingly, it was necessary to obtain the isomers and new analogues to determine their structures and evaluate their anti-HIV activities.

Structure Elucidation of Isolated Daphnane Diterpenes.

Seventeen diterpenes were isolated using HSCCC, a liquid-liquid partition chromatography with high selectivity,^{21–23} and further purified by preparative HPLC, including three new compounds (**11**, **25**, and **27**) and 14 known compounds [yuanhuafine (**1**),²⁴ yuanhuaoate C (**2**),²⁵ genkwaphnine (**4**),²⁶ yuanhuaoate E (**6**),²⁷ yuanhuagine (**10**),²⁸ isoyuanhuadine (**12**),²⁹ yuanhuadine (**14**),²⁸ excoecariatoxin (**15**),³⁰ yuanhuahine (**16**),¹⁸ genkwadane D (**20**),³¹ yuanhuajine (**21**),²⁸ yuanhualine (**22**),²⁰ isoyuanhuacine (**23**),³² and yuanhuacine (**24**)²⁸].

Compound **11** (yuanhuamine A), a white amorphous powder, showed 12 degrees of unsaturation based on the molecular formula ($C_{32}H_{42}O_{10}$), which was determined by the HRESIMS ion at m/z 587.2846 $[M + H]^+$ (calcd for $C_{32}H_{42}O_{10}$, 587.2851). In the IR spectrum, the absorption of 1701, 1734, and 3387 cm^{-1} revealed the presence of hydroxy and carbonyl groups, respectively. The UPLC-MSⁿ fragments (m/z 323, 295, and 267) indicated compound **11** is a daphnane diterpene (Figure S2, Supporting Information). The ^1H NMR spectrum revealed two AB system protons at δ_{H} 3.78 (1H, d, $J = 12.4$, H-20a) and 3.97 (1H, d, $J = 12.4$, H-20b), two olefinic protons at δ_{H} 4.99 (1H, s, H-16) and 5.03 (1H, s, H-16), and five methyl groups at δ_{H} 0.88 (3H, t, H-10'), 1.99 (3H, s, H-2''), 1.84 (3H, s, H-17), 1.31 (3H, d, $J = 7.2$, H-18), and 1.79 (3H, s, H-19) in Table 1. The ^{13}C NMR spectrum indicated the presence of an ester group [δ_{C} 169.8 (C-1'')], an epoxy group [δ_{C} 60.5 (C-6) and 64.5 (C-7)], an α,β -unsaturated cyclopentanone [δ_{C} 209.7 (C-3) and 160.8 (C-1)], and an orthoester group [δ_{C} 117.6 (C-1')]. All the NMR signals were typical for a daphnane diterpene.²⁸ In the HMBC spectrum, the correlated peaks (H-7/C-8, H-17/C-16, H-17/C-13, H-18/C-12, H-5/C-6, H-5/C-20, and H-14/C-1') confirmed **11** was a daphnane diterpene (Figure 4). The similarity of the NMR signals of **11** to those of yuanhuadine (**14**), except for the conjugated diene signals (H-2' to H-5'), suggested that the two compounds are geometric isomers. Based on the coupling constant (J) in the ^1H NMR spectrum, **11** contains a *cis,cis*-conjugated diene, while **14** contains a *trans,trans*-conjugated diene unit. The correlations (H-2' to H-10') in the ^1H - ^1H COSY spectrum indicated that the R₂ group is a straight chain. Its location at C-14 was supported by the HMBC correlation between H-14/C-1'. In the NOESY spectrum, the correlation between H-18/H-12 revealed H-11 and H-12 to have a *trans*-orientation. The correlations of H-8/H-11, H-8/H-14, and H-7/H-14 suggested β -orientations for these protons, while the correlation between H-5/H-10 indicated α -orientations for H-5 and H-10.

Compound **25** (yuanhuamine B) was obtained as a white amorphous powder. Its HRESIMS ion at m/z 629.3315 $[M + H]^+$ (calcd for $C_{35}H_{48}O_{10}$, 629.3320) indicated 12 degrees of hydrogen deficiency. The IR spectrum indicated the presence of hydroxy (3425 cm^{-1}) and carbonyl (1708 and 1752 cm^{-1}) moieties. The NMR data indicated one more methylene [δ_{C} 22.5 (C-4'')] in **25** compared with **22**.³⁰ In the ^1H - ^1H COSY spectrum of **25**, the correlations between H-2'', H-3'', H-4'', and H-5'' indicated the presence of an *n*-butyl fragment (Figure S5, Supporting Information), which was consistent with the results from UPLC-MSⁿ. Furthermore, a $C_4H_9\text{COO}$ group was assigned at C-12 based on the correlations between H-12/C-1'' and H-2''/C-1'' in the HMBC spectrum. In the NOESY spectrum, correlations were observed between H-12/H-18, H-8/H-11, H-8/H-14, H-7/H-14, and H-5/H-10, which were analogous to those for **11**.

The molecular formula of compound **27** (yuanhuamine C), obtained as an amorphous powder, was assigned as $C_{36}H_{50}O_{10}$ according to its HRESIMS ($[M + H]^+$ at m/z 643.3471, calcd for $C_{36}H_{50}O_{10}$), with 12 degrees of hydrogen deficiency. The IR spectrum revealed the presence of hydroxy (3491 cm^{-1}) and carbonyl (1749 and 1711 cm^{-1}) groups. Based on the ^1H and ^{13}C NMR spectra, compounds **27** and **25** have the same skeleton and differ only in the C-12 side chain. In the ^1H - ^1H COSY spectrum (Figure S6, Supporting Information), the correlations between H-2'', H-3'', H-4'', H-5'', and H-6'' proved the presence of an *n*-pentyl chain in **27**. Accordingly, the presence of a $C_5H_{11}COO$ group at C-12 was confirmed by the HMBC correlations between H-12/C-1'' and H-2''/C-1''. The correlations between H-12/H-18, H-8/H-11, H-8/H-14, H-7/H-14, and H-5/H-10 in the NOESY spectrum were consistent with those of **11**.

To determine the absolute configuration of compounds **11**, **25**, and **27**, their experimental ECD data were obtained. All three compounds showed the same Cotton effect (Figure 5A). The calculated ECD spectra of (4*S*,5*R*,6*S*,7*S*,8*S*,9*R*,–10*S*,11*R*,12*R*,13*S*,14*R*)-**25** and (4*R*,5*S*,6*R*,7*R*,8*R*,9*S*,–10*R*,11*S*,12*S*,13*R*,14*S*)-**25** were obtained from the B3LYP/6–311+G(d,p) (Figure 5B), and the former was consistent with the experimental ECD curve of **25**. Therefore, the structure and absolute configuration of **25** were established.

Nine diterpenoids, including isomers (**12** and **14**), the new compounds (**11**, **25**, and **27**), the most abundant compounds (**4**, **16**, and **24**), and compound **2**, were evaluated for their anti-HIV activity and cytotoxicity, with the results presented in Table 2. Most of the compounds, except **2** and **16**, showed extremely potent anti-HIV activity at nanomolar concentrations. Compared with the positive control AZT (zidovudine), the diterpenoids tested were more potent with higher selectivity indexes. Structurally, the main difference between the more potent (**4**, **11**, **12**, **14**, **24**, **25**, and **27**) and the less potent (**2**) indicated that the orthoester group at C-9, C-13, and C-14 might be responsible for the enhanced anti-HIV activity, which is consistent with our previous study.⁴

EXPERIMENTAL SECTION

General Experimental Procedures.

Optical rotations were measured using an Autopol V Plus instrument. UV and ECD spectroscopic data were obtained with a Brighttime Chirscan spectrometer. IR spectra were detected by a PE Spectrum RXI spectrophotometer. NMR data were recorded on a Bruker Ultrashield Plus 600 MHz instrument in $CDCl_3$ with tetramethylsilane as an internal standard. HRESIMS data were acquired with an AB SCIEX Triple TOF 5600+ mass spectrometer. Isolation was performed on a TBE-300A HSCCC and preparative Agilent 1200 HPLC.

Eight daphnane diterpenoids, subtoxin A (**S1**), stelleralide I (**S2**), wikstroelide B (**S3**), wikstroelide J (**S4**), huratoxin (**S5**), wikstroelide A (**S6**), simplexin (**S7**), and wikstroelide M (**S8**), were isolated previously from *Stellera chamaejasme* in our laboratory, and their structures were established from MS and comprehensive NMR experiments with literature values.⁴ The purities of all compounds were determined to be no less than 95% by HPLC.

Plant Material.

The flower buds of *D. genkwa* were purchased from Tianma Medical Company (Anhui, People's Republic of China) in June 2016 and authenticated by Prof. Daofeng Chen. A voucher specimen (DFC-ZHD-DG-2016-09) has been deposited in the Department of Pharmacognosy, School of Pharmacy, Fudan University.

UPLC-IT-MS Analyses of Daphnane Diterpenoids from *D. genkwa*.

Analyses were conducted using a Dionex Ultimate 3000 UPLC coupled with a Thermo LTQ Velos Pro ion trap mass spectrometer via an electrospray ionization interface. A YMC-Triart C₁₈ column (150 mm × 2.1 mm, 1.9 μm) was applied for the separation with a flow rate of 0.3 mL/min at 25 °C. A mobile phase consisted of eluent A (acetonitrile) and B (water) programmed as follows: 0–2 min, 50% A; 2–42 min, 50–90% A; 42–45 min, 90–100% A; 45–70 min, 100% A. The operating conditions were optimized as follows: positive-ion mode, auxiliary gas (N₂) flow, 10 arb; sheath gas (N₂) flow, 40 arb; collision gas (He); source voltage, 3.5 kV; capillary temperature, 350 °C; capillary voltage, 35 V. A data-dependent scan mode was selected for MSⁿ analysis.

Extraction and Isolation.

Dried powders of *D. genkwa* flower buds (1.0 kg) were extracted with methanol. The filtrates were concentrated to obtain a crude MeOH extract (202.0 g), which was partitioned by liquid-liquid extraction with petroleum ether and water. The petroleum ether fraction (23.2 g) was repeatedly subjected to HSCCC. Petroleum ether-ethyl acetate-methanol-water 2:1:1.5:1.5, 2:1:1.8:1.2, and 2:1:2.1:0.9 (v/v/v/v) were selected for stepwise separation in the period of 0–250, 250–500, and 500–700 min, respectively, to afford fractions a–g (Figure S1, Supporting Information). Subsequently, the fractions were purified by means of preparative HPLC with a Phenomenex-Luna C₁₈ column (250 mm × 10 mm, 5 μm) using CH₃CN–H₂O (3 mL/min). Fraction a (21.1 mg) was purified with a gradient elution (60% to 70% CH₃CN in 20 min). Finally, **1** (2.1 mg) and **2** (2.8 mg) were obtained. Fraction b (22.8 mg) was purified by isocratic elution (75% CH₃CN in 25 min) and afforded **4** (3.7 mg) and **6** (6.3 mg). Fraction c (37.5 mg) was purified using linear gradient elution (65% to 90% CH₃CN in 30 min) to obtain **10** (4.4 mg), **11** (1.7 mg), and **12** (1.9 mg). Fractions d (53.6 mg) and f (33.5 mg) were purified using isocratic elution (79% CH₃CN in 35 min) to yield **14** (12.0 mg) and **20** (1.7 mg), **22** (1.9 mg), **24** (16.1 mg), respectively. Fraction e (29.0 mg) was purified by linear gradient elution (75% to 95% CH₃CN in 35 min) and yielded **15** (2.7 mg), **16** (3.5 mg), **21** (6.0 mg), and **23** (2.1 mg). Fraction g (31.0 mg) was purified with a linear gradient elution (80% to 95% CH₃CN in 35 min) and yielded **25** (2.4 mg) and **27** (2.7 mg).

Yuanhuamine A (11): white, amorphous power; $[\alpha]_D^{25} +9.0$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 235 (4.06) nm; ECD (MeOH) $\lambda_{\max}$ (ϵ) 228 (–10.28), 246 (–0.86), 253 (–1.84) nm; IR (KBr) $\nu_{\max}$ 3387, 1734, 1701, 1377, 1227 cm^{–1}; ¹H and ¹³C NMR data, see Table 1; HRESIMS m/z 587.2846 [M + H]⁺ (calcd for C₃₂H₄₂O₁₀, 587.2851).

Yuanhuamine B (25): white, amorphous power; $[\alpha]_D^{25} +13.0$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 234 (3.61) nm; ECD (MeOH) $\lambda_{\max}$ (ϵ) 227 (–12.53), 246 (+2.90), 259

(−0.46) nm; IR (KBr) $\nu_{\max}$ 3425, 1752, 1708 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 629.3315 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{35}\text{H}_{48}\text{O}_{10}$, 629.3320).

Yuanhuamine C (**27**): white, amorphous power; $[\alpha]_D^{25} +14.0$ (c 0.10, MeOH); UV (MeOH) $\lambda_{\max}$ (log ϵ) 233 (3.62) nm; ECD (MeOH) $\lambda_{\max}$ (ϵ) 226 (−8.63), 246 (+1.43), 261 (−0.78) nm; IR (KBr) $\nu_{\max}$ 3491, 1749, 1711 cm^{-1} ; ^1H and ^{13}C NMR data, see Table 1; HRESIMS m/z 643.3471 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{36}\text{H}_{50}\text{O}_{10}$, 643.3477).

Anti-HIV and Cytotoxicity Assays.

Compounds were evaluated by anti-HIV and cytotoxicity assays according to published procedures.³³

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
IT-MS¹⁻⁴ spectra of subtoxin A (**S1**) and the proposed fragmentation patterns of **S1-S4** reference compounds.

Figure 2. IT-MS¹⁻⁴ spectra of huratoxin (S5) and the proposed fragmentation patterns of S5-S8 reference compounds.

Figure 3.
UPLC-IT-MSⁿ total ion current in the positive-ion mode (A) and UPLC-UV at 233 nm (B)
of the petroleum ether extract of *D. genkwa*.

Figure 4.
Key ^1H - ^1H COSY, HMBC, and NOESY correlations of **11**.

Figure 5. Experimental ECD spectra of **11**, **25**, and **27** in MeOH (A) and calculated ECD spectra of **25** (B).

1	$R_1 = C_6H_5$	$R_2 = OCOCH_3$	2	$R = OCOCH_3$
4	$R_1 = C_6H_5$	$R_2 = OCOC_6H_5$	6	$R = OCOC_6H_5$
10	$R_1 = (CH^E=CH)_3(CH_2)_2CH_3$	$R_2 = OCOCH_3$		
11	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH_3$		
12	$R_1 = (CH^E=CH)(CH^Z=CH)(CH_2)_4CH_3$	$R_2 = OCOCH_3$		
14	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH_3$		
15	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = H$		
16	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH_2CH_3$		
20	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH(CH_3)_2$		
21	$R_1 = (CH^E=CH)_3(CH_2)_2CH_3$	$R_2 = OCOC_6H_5$		
22	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH_2CH_2CH_3$		
23	$R_1 = (CH^E=CH)(CH^Z=CH)(CH_2)_4CH_3$	$R_2 = OCOC_6H_5$		
24	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOC_6H_5$		
25	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH_2CH_2CH_2CH_3$		
27	$R_1 = (CH^E=CH)_2(CH_2)_4CH_3$	$R_2 = OCOCH_2CH_2CH_2CH_2CH_3$		

Chart 1.

Table 1.

¹H (600 MHz) and ¹³C NMR (150 MHz) Spectroscopic Data of Compounds 11, 25, and 27 in CDCl₃

position	11			25			27		
	< δ_c	< δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	δ_c	δ_H (J in Hz)	
1	160.8	7.57, s	160.7	7.57, s	160.6	7.60, s			
2	137		137		137				
3	209.7		209.7		209.7				
4	72.4		72.3		72.3				
5	72.2	4.26, s	72.2	4.25, s	72.2	4.27, s			
6	60.5		60.6		60.6				
7	64.5	3.59, s	64.4	3.55, s	64.4	3.51, s			
8	35.6	3.51, d (2.5)	35.7	3.47, d (2.5)	35.6	3.47, d (2.6)			
9	78.3		78.1		78.2				
10	47.6	3.84, s	47.6	3.82, m	47.6	3.84, m			
11	44.1	2.37, m	44.3	2.37, m	44.2	2.37, m			
12	78.4	4.98, s	78.3	5.00, s	78.3	5.00, s			
13	83.9		83.9		83.9				
14	80.2	4.83, d (2.5)	80.5	4.76, d (2.5)	80.5	4.78, d (2.5)			
15	143.1		143.1		143.2				
16	113.5	4.99, s; 5.03, s	113.5	4.98, s; 4.95, s	113.5	4.98, s; 4.95, s			
17	18.7	1.84, s	18.8	1.8, s	18.8	1.83, s			
18	18.5	1.31, d (7.2)	18.4	1.37, d (7.2)	18.4	1.33, d (7.2)			
19	10	1.79, s	10	1.79, s	10	1.79, s			
20	65.4	3.78, d (12.4)	65.2	3.79, d (12.6)	65.2	3.81, d (12.5)			
		3.97, d (12.4)		3.94, d (12.5)		3.95, d (12.5)			
1'	117.6		117.1		117.1				
2'	120.2	5.44, d (11.3)	122.4	5.63, d (15.4)	122.4	5.65, d (15.5)			
3'	136.2	6.23, dd (15.6, 11.3)	135.2	6.65, dd (15.4, 10.7)	135.2	6.68, dd (15.4, 10.7)			
4'	126.7	6.86, dd (15.0, 11.8)	128.7	6.04, dd (15.2, 10.7)	128.7	6.06, dd (15.1, 10.7)			
5'	140.2	5.82, m	139.5	5.85, m	139.5	5.87, m			
6'	32.8	2.10, m	32.8	2.06, m	32.8	2.10, m			

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position	11		25		27	
	< δc	δ_H (<i>J</i> in Hz)	δc	δ_H (<i>J</i> in Hz)	δc	δ_H (<i>J</i> in Hz)
7'	28.5	1.40, m	28.9	1.41, m	28.9	1.40, m
8'	31.6	1.27, m	31.5	1.26, m	31.4	1.26, m
9'	22.6	1.26, m	22.6	1.27, m	22.6	1.27, m
10'	14.2	0.88, t	14.2	0.88, t	14.2	0.88, t
1''	169.8		172		172.7	
2''	21.3	1.99, s	36.1	2.22, m	34.5	2.25, m
3''			27.4	1.65 m	24.6	1.65, m
4''			22.5	1.27, m	27.4	1.27, m
5''			14.3	0.98, t	22.5	1.26, m
6''					14	0.98, t

Table 2.Anti-HIV Activity and Cytotoxicity of Nine Daphnane Diterpenes^a

	anti-HIV	cytotoxicity	selectivity index
compound	IC ₅₀ (nM)	CC ₅₀ (μM)	CC ₅₀ /IC ₅₀
2	62.9 ± 14.7	>6.6	>105
4	2.0 ± 0.5	>6.6	>3320
11	<0.9	>6.8	>8035
12	<0.9	>6.8	>8035
14	5.3 ± 1.8	>6.8	>1288
16	805.0 ± 164.5	>6.7	>8
24	1.2 ± 0.3	>6.2	>5144
25	<0.8	>6.4	>7962
27	<0.8	>6.2	>7987
AZT ^b	20.0 ± 5.6	>15.0	>750

^aValues are mean ± SD (*n* = 3).^bPositive control substance.