Tripfordines A−C, Sesquiterpene Pyridine Alkaloids from *Tripterygium wilfordii,* and Structure Anti-HIV Activity Relationships of *Tripterygium* Alkaloids[#]

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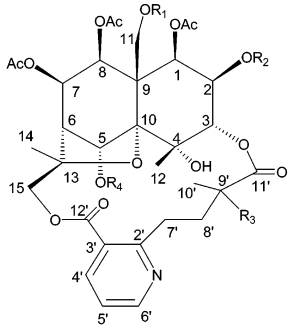
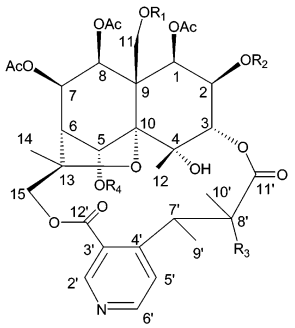
Three new sesquiterpene pyridine alkaloids, tripfordines A−C (**1**−**3**), were isolated from an ethanolic extract of the roots of *Tripterygium wilfordii*, along with eight known pyridine alkaloids, and tested for in vitro cytotoxic and anti-HIV activity. The structures of the new compounds were established on the basis of spectroscopic data interpretation. Anti-HIV structure−activity relationships (SAR) for this compound type are proposed on the basis of the screening results from the newly isolated compounds and prior data of known sesquiterpene pyridine alkaloids. The position of a carboxyalkyl chain on the pyridine moiety was not critical since both 2′- and 4′-substituted compounds exhibited high anti-HIV activity (EC_{50} 0.1 μg/mL). In contrast, a hydroxy group at C-8′ (carboxypropyl side chain) or C-9′ (carboxybutyl side chain) was found to affect anti-HIV activity.

Tripterygium wilfordii Hook.f. (Celastraceae) is a poisonous liana found in southern mainland China. The roots of the plant are known as "Lei Gong Teng" in Chinese folklore[1−3] and, because of their high toxicity, are used as an insecticide. Since the 1960s, various laboratories have found that this plant has anti-inflammatory, immunosuppressive, antitumor, and anti-AIDS activities. Consequently, much effort has been devoted to clarify the active principles,[4,5] leading to the isolation of dihydro-β-agarofuran sesquiterpenes,[6] tricyclic diterpenes,[7,8] triterpenes,[9,10] and sesquiterpene pyridine alkaloids.[11−13] Recently, an extract of the roots of *T. wilfordii*, designated T_{II}, has been used for the treatment of various diseases, including dermatitis, rheumatoid arthritis, systemic acne rosacea, and nephritis.[14] As part of our continuing study on the constituents of Chinese medicinal plants, we report herein on the isolation and structural elucidation of three new sesquiterpene alkaloids, **1**−**3**, from *T. wilfordii*. In addition, these compounds were screened for in vitro cytotoxic and anti-HIV activity, and the anti-HIV screening results were combined with prior data from known sesquiterpene pyridine alkaloids to generate initial structure−activity parameters.





1: R_1 = Ac, R_2 = H, R_3 = OH, R_4 = Ac

2: R_1 = Ac, R_2 = Ac, R_3 = R_4 = H

4: R_1 = Ac, R_2 = Ac, R_3 = OH, R_4 = Ac

6: R_1 = Ac, R_2 = Bz, R_3 = H, R_4 = Ac

7: R_1 = Ac, R_2 = Fu, R_3 = H, R_4 = Ac

8: R_1 = Ac, R_2 = Bz, R_3 = OH, R_4 = Ac

9: R_1 = Ac, R_2 = Fu, R_3 = OH, R_4 = Ac

3: R_1 = R_2 = Ac, R_3 = H, R_4 = H

5: R_1 = Ac, R_2 = Fu, R_3 = H, R_4 = Ac

10: R_1 = Fu, R_2 = Ac, R_3 =OH, R_4 = Ac

11: R_1 = Ac, R_2 = Fu, R_3 = OH, R_4 = Ac

12: R_1 = Fu, R_2 = Ac, R_3 =H, R_4 = Ac

Results and Discussion

The air-dried roots of *T. wilfordii* were extracted with EtOH to afford a crude extract. After evaporation of the solvent, the crude extract was re-extracted with $CHCl_3$. The $CHCl_3$ extract was chromatographed on silica gel using benzene−EtOAc−*n*-hexane, CH_2Cl_2−acetone, acetone, $CHCl_3$−MeOH−H_2O, and MeOH, successively, to give five fractions. Further purification of fraction 3 using silica gel chromatography followed by repeated preparative HPLC gave the three new alkaloids **1**−**3**.

Compound **1** was obtained as a colorless, amorphous powder. Its IR spectrum showed the presence of hydroxyl (3600 cm^{-1}), ester carbonyl (1740 cm^{-1}), α,β-unsaturated carbonyl (1675 cm^{-1}), and pyridine ring (1640, 1550 cm^{-1}) absorptions. Its molecular formula was established as $C_{36}H_{45}O_{18}N$ from the HREIMS (*m*/*z* 779.2628) and NMR spectroscopic data. The UV spectrum of **1** showed an absorption maximum at 260 nm due to the presence of a pyridine ring.

The ^1H NMR spectrum of **1** disclosed the presence of three quaternary methyl groups [δ 1.41 (H-10′), 1.62 (H-14), and 1.61 (H-12)], five acetyl methyl groups [δ 1.96 (OAc-1), 2.01 (OAc-2), 2.18 (OAc-7), 2.19 (OAc-5), and 2.29 (OAc-11)], two methylene groups connected to oxygen atoms [δ 4.61 and 5.42 (H-11), 3.70 and 5.83 (H-15)], a methine group connected to an oxygen atom [δ 3.99 (H-2)], five methine groups connected to ester oxygen atoms [δ 5.02 (H-3), 5.35 (H-8), 5.50 (H-1), 5.52 (H-7), and 6.91 (H-5)], and three pyridine protons [δ 7.21 (H-5′), 8.11 (H-4′), and 8.68 (H-6′)]. The relationships between proton signals in **1** were established by $^1H-^1H$ COSY examination. The ^{13}C and DEPT NMR spectra showed the presence of seven ester carbonyls [δ 168.0 (C-12′), 169.2 (OAc-8), 169.8 (OAc-1), 169.8 (OAc-5), 169.9 (OAc-7), 170.2 (OAc-11), and 173.0 (C-11′)], five pyridine ring carbons [δ 120.6 (C-5′), 125.7 (C-3′), 137.7 (C-4′), 152.2 (C-6′),

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and 164.7 (C-2′)], three methyl carbons [δ 17.8 (C-14), 22.7 (C-12), and 27.6 (C-10′)], two methylene carbons [δ 31.3 (C-7′) and 38.6 (C-8′)], two methylene carbons connected to ester oxygen atoms [δ 60.5 (C-11) and 70.0 (C-15)], five methine carbons connected to oxygen atoms [δ 69.9 (C-7), 71.0 (C-8), 73.7 (C-5), 75.2 (C-1), and 78.9 (C-3)], and a methine carbon connected to a hydroxyl group [δ 69.0 (C-2)]. The ^{1}H and ^{13}C NMR spectroscopic data of **1** were similar to those of **4**,[15] indicating that these compounds have the same basic skeleton. However, spectroscopic comparison also revealed the following differences. The ^{1}H and ^{13}C NMR spectra of **1** lacked signals for one acetyl methyl group found in **4**. Additionally, the H-2 (δ 3.99) signal in **1** appeared at higher field by approximately 1.15 ppm than that of **4**. These observations and the HREIMS data suggested that compound **1** has a hydroxyl group at C-2. In the HMBC spectrum of **1**, cross-peaks were observed between H-2/C-4; H-3/C-1, C-4, C-12; H-5/C-10, C-13; H-6/C-5, C-10; H-7/C-8; H-8/C-1, C-9, C-11; H-11/C-8, C-9, C-10; H-14/C-6; and H15/C-13, which confirmed the partial structure of a dihydroagarofuran skeleton attached to a pyridine alkaloid ring moiety at C-3 to C-15. Cross-peaks between H-4′/C-2′, C-6′, C-12′; H-5′/C-3′; H-7′/C-2′, C-9′; and H-10′/C-8′ supported the assignments of the pyridine alkaloid moiety. Cross-peaks that appeared between δ 3.70 (H-15) and 168.0 (C-12′) and between δ 5.02 (H-3) and 173.0 (C-11′) indicated that the pyridine alkaloid moiety was connected with ester linkages at C-3 and C-15 of the dihydroagarofuran skeleton. The positions of the acetate esters in **1** were confirmed by long-range correlations of the ester carbonyl carbon signals (1, 5, 7, 8, and OAc-11 at δ 169.8, 169.8, 169.9, 169.2, and 170.2) with the respective ring protons (H-1 at δ 5.50, H-5 at δ 6.91, H-7 at δ 5.52, H-8 at δ 5.35, and H-11 at δ 4.61 and 5.42) in the HMBC spectrum. The relative stereochemistry of **1** was confirmed by NOE correlations. From these data, the structure of **1** was established as 1β,5α,7β,8β,11-pentaacetoxy-2β,4α-dihydroxy-3α,15-[2′-methyl-2′-hydroxy-3′-(3″-carboxy-2″-pyridyl)propanoic acid]dicarbolactone dihydroxyagarofuran and has been given the trivial name tripfordine A.

Compound **2** was obtained as a colorless, amorphous solid. The IR spectrum indicated the presence of hydroxyl (3450 cm^{-1}), ester carbonyl (1740 cm^{-1}), α,β-unsaturated carbonyl (1710 cm^{-1}), and pyridine ring (1650, 1540 cm^{-1}) moieties. Its molecular formula was established as $C_{36}H_{45}O_{17}N$ from the HREIMS (*m*/*z* 763.2693) and NMR spectroscopic data. Its UV spectrum showed an absorption maximum at 267 nm due to a conjugated aromatic ring. The ^{1}H NMR spectrum of **2** revealed the presence of two quaternary methyls, one tertiary methyl, five acetyl methyls, three aromatic protons, two methylenes connected to oxygens, five methines connected to ester oxygens, and one methine connected to a hydroxyl group. The ^{13}C and DEPT NMR spectroscopic data indicated the presence of seven ester carbonyl groups, five pyridine ring carbons, three methyls, two methylenes, two methylenes connected to ester oxygen atoms, six methines, and two quaternary carbons connected to oxygen atoms. ^{1}H−^{1}H COSY and HMBC experiments established the carbon connectivity patterns. Although the ^{1}H and ^{13}C NMR spectra of **2** were similar to those of **1** and **4**,[15] demonstrating that these three compounds have the same skeleton, comparison of their spectroscopic data showed the following differences. Like **1**, compound **2** has only five acetyl methyl groups rather than the six found in **4**. A hydroxyl rather than an acetate group is found at C-5 in **2**, on the basis of an approximately 1.55 ppm upfield shift of H-5 (δ 5.33) in **2** relative to **4**. In addition, compounds **2** and **4** have different substituents at C-9′ (H in **2**, OH in **4**) on the basis of the following data. In **4**, the 10′-methyl proton signal (δ 1.46) appeared as a singlet, while in **2**, this signal (δ 1.16) was observed as a doublet at slightly higher field (0.3 ppm). Moreover, in the ^{13}C NMR spectrum of **2**, the C-9′ carbon signal appeared at δ 38.1, and this tertiary carbon signal correlated with the H-10′ proton signal at δ 2.33 in the HMBC spectrum of **2**. These observations and the HREIMS data suggested that compound **2** has a hydroxyl group at C-5 and a tertiary methyl group at C-9′.

The HMBC correlations between H-3/C-1, C-10; H-5/C-13; H-6/C-7; H-7/C-5, C-8; H-8/C-1; H-11/C-8, C-10; and H-15/C-13 confirmed the partial structure of **2** as a dihydroagarofuran skeleton attached to a pyridine alkaloid ring at C-3 to C-15. Assignments of the pyridine alkaloid moiety were supported by the following cross-peaks: H-3/C-11′; H-15/C-12′; H-4′/C-2′, C-6′; H-7′/C-9′; H-10′/C-8′, C-9′, and C-11′. The ester carbonyl carbon signals (1, 2, 7, 8, and OAc-11 at δ 169.6, 168.7, 170.1, 169.0, and 169.7) showed long-range HMBC correlations with ring protons (H-1 at δ 5.62, H-2 at δ 5.18, H-7 at δ 5.50, H-8 at δ 5.34, and H-11 at δ 4.51 and 5.21). The relative stereochemistry of **2** was determined by analysis of NOE correlations. From these data, the structure of **2** was established as 1β,2β,7β,8β,11-pentaacetoxy-4α,5α-dihydroxy-3α,15-[2′-methyl-3′-(3″-carboxy-2″-pyridyl)propanoic acid]-dicarbolactone dihydroxyagarofuran, and the compound has been given the trivial name tripfordine B.

Compound **3** was obtained as a colorless, amorphous powder. As in **1** and **2**, the IR spectrum of **3** showed the presence of hydroxyl (3600 cm^{-1}), ester carbonyl (1695 cm^{-1}), α,β-unsaturated carbonyl (1680 cm^{-1}), and pyridine (1640, 1535 cm^{-1}) groups. Its molecular formula was established as $C_{36}H_{45}O_{17}N$ from the HREIMS (*m*/*z* 763.2679) and NMR spectroscopic data. Its UV spectrum showed an absorption maximum at 237 nm due to a conjugated aromatic ring.

The ^{1}H NMR spectrum of **3** disclosed the presence of two quaternary methyls, two tertiary methyls, five acetyl methyls, three aromatic protons, two methylenes connected to oxygens, five methines connected to ester oxygens, and a methine connected to a hydroxyl group. The ^{13}C and DEPT NMR spectroscopic data indicated the presence of seven ester carbonyl groups, five pyridine ring carbons, four methyl carbons, two methylene carbons connected to oxygen, six methine carbons connected to oxygen, two quaternary carbons connected to oxygen, and two methine carbons. The ^{1}H−^{1}H COSY and HMBC experiments established the connectivity of these carbons. The ^{1}H and ^{13}C NMR spectra of **3** were similar to those of **5**,[16,17] suggesting that these compounds have the same hypoglaunine-type pyridine alkaloid structure. However, the ^{1}H and ^{13}C NMR spectra of **3** lacked the furanoyl group found in **5**. Furthermore, the H-5 (δ 5.46) signal in **3** appeared at higher field by approximately 1.41 ppm than that of **5**. In the HMBC spectrum of **3**, correlations between H-5 (δ 5.46) and both C-7 (δ 69.1) and C-10 (δ 92.9) were observed. These findings suggest that **3** has a hydroxyl group at C-5. The HMBC correlations between H-1/C-9, C-8, C-11; H-3/C-2, C-1; H-5/C-7, C-10, C-13; H-7/C-8, C-9; H-8/C-9, C-11; H-11/C-1, C-8, C-9; H-12/C-3, C-4; and H-14/C-13 again confirmed a dihydroagarofuran structure with a pyridine alkaloid ring attached at C-3 to C-15. Assignments of the pyridine alkaloid moiety were supported by the following cross-peaks: H-3/C-11′; H-14/C-15; H-15/C-12′; H-2′/C-12′; H-5′/C-3′, C-6′; H-6′/C-5′; H-7′/C-4′; H-9′/C-4′, C-7′, C-8′; H-10′/C-7′,C-8′, and C-11′. The acetate esters in **3** were assigned at C-1, -2, −7, -8, and -11 on the basis of the correlations of the ester carbonyl carbons with ring protons (1, 2, 7, 8, and OAc-11 at δ 169.3, 168.9, 170.0, 168.6, and 169.8 with H-1 at δ 5.55, H-2 at δ 5.28, H-7 at δ 5.49, H-8 at δ 5.34, and H-11 at δ 4.53 and 5.10, respectively). The molecular formula and the unsaturation number obtained by HREIMS supported the above conclusions. The relative stereochemistry of **3** was determined by analysis of NOE correlations. From these data, the structure of **3** was established as 1β,2β,7β,8β,11-pentaacetoxy-4α,5α-dihydroxy-3α,15-[2′,3′-dimethyl-3′-(3″-carboxy-4″-pyridyl)-propanoic acid]dicarbolactone dihydroxyagarofuran and has been given the trivial name tripfordine C.

Eight known compounds [evonine, alatusinine, wilforjine, 4-hydroxy-7-epi-chuchuhusnine E-V, euonymine (**20**), tripterifordin,

Table 1. Anti-HIV Activity of **1**−**3** and Related Compounds from Prior Screening[13,16,22,23]

compound	IC_{50} (μg/mL)[a]	EC_{50} (μg/mL)[b]	TI[c]
tripfordine A (**1**)	>25	13.4	1.9
tripfordine B (**2**)	>25	NS[d]	
tripfordine C (**3**)	>25	NS	
wilforine (**6**)[16]	>100	NS	
wilforgine (**7**)[16]	>100	NS	
wilfordine (**8**)[16]	20.0	<0.10	>200
wilfortrine (**9**)[16]	>100	<0.10	>1000
hypoglaunine A (**10**)[16]	>100	0.13	>769
hypoglaunine B (**11**)[16]	>100	0.10	>1000
hypoglaunine D (**12**)[16]	22.2	NS	
triptonine A (**13**)[13]	>100	2.5	>39.4
triptonine B (**14**)[13]	>100	< 0.1	>1000
hyponine A (**15**)[22]	27.7	1.0	27.7
hyponine D (**16**)[23]	20.2	NS	
hyponine F (**17**)[23]	>100	35.2	>2.8
forrestine (**18**)[23]	>100	0.48	>208
cangoronine E-1 (**19**)[22]	56.8	0.90	63.4
euonymine (**20**)[16]	22.8	0.20	113
neoeuonymine (**21**)[23]	>100	0.88	>113
hyponine B (**22**)[22]	>100	0.10	>1000
AZT	500	0.012[e]	41 667

[a] IC_{50} = concentration of test sample that was toxic to 50% of the mock-infected cells. [b] EC_{50} = concentration of the test sample that was able to suppress HIV replication by 50%. [c] Therapeutic index (TI) = ratio of the IC_{50} to EC_{50}. [d] NS = no suppression. [e] This EC_{50} value is the mean of 65 EC_{50} values for AZT.

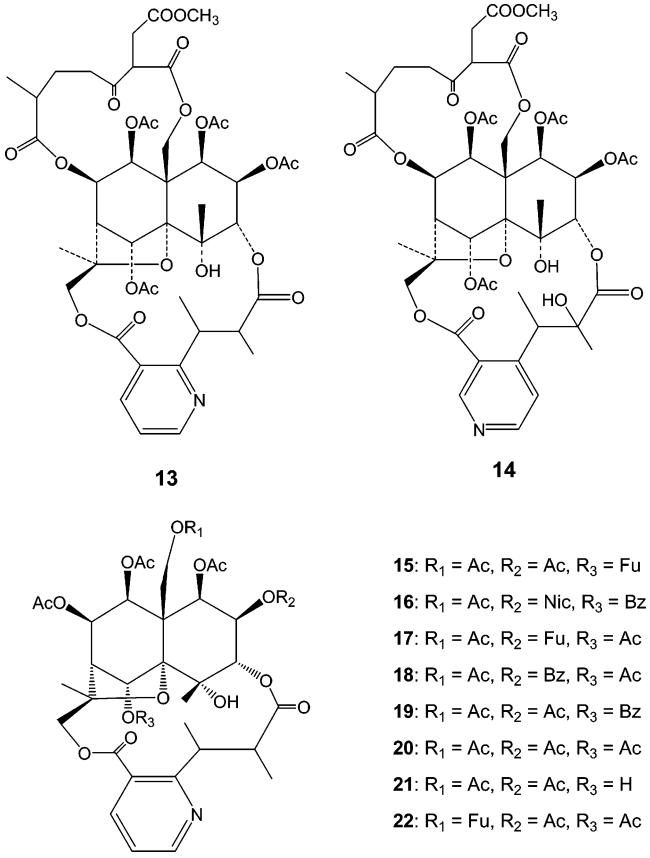
wilforine (**6**), and wilfordine (**8**)] also insolated were identified by comparing their physical and spectroscopic data with those in the literature.[16−20]

The three new compounds, **1**−**3**, were evaluated in an in-house cytotoxicity bioassay[21] against six different human cancer cell lines [A549 (lung), HCT-8 (ileocecal), A431 (epidermoid), AR751 (breast), LN-CaP (prostate), PC-3 (prostate)], but were not active (IC_{50} > 4 μg/mL).

Because we previously reported anti-HIV data for related sesquiterpene pyridine alkaloids (**6**−**22**) from the genus *Tripterygium*,[13,16,22,23] compounds **1**−**3** were also screened in HIV-1-infected H9 lymphocytes (Table 1). Among these compounds, 12 alkaloids (**8**−**11**, **13**−**15**, and **18**−**22**) showed anti-HIV activity with EC_{50} values from 2.54 to <0.10 μg/mL, while the remaining compounds (**1**−**3**, **6**, **7**, **12**, **16**, and **17**) were inactive. The following SAR observations were drawn from these data.

This alkaloid class contains a sesquiterpene skeleton, a nicotinic acid moiety, and a propionic or butyric acid at position-2′ or -4′ of the nicotinic acid, with the two latter components linking the sesquiterpene C-3 and C-15. Among the various sesquiterpene substituents, a C-5 acetyl group was found most commonly among the active compounds, and the rank order of potency was 5-acetyl (**20**) > 5-hydroxy (**21**) > 5-benzyloxy (**19**) > 5-furanoyl (**15**), on the basis of comparison of the EC_{50} values of **15** and **19**−**21**. In addition, the active triptonine B (**14**) has a monoterpene moiety linked to positions-7 and -11 by ester bonds. However, compounds with a nicotinic ester (**16**) or hydroxy (**1**) group at C-2 were inactive.

The position of the carboxypropyl or carboxybutyl chain on the nicotinic moiety is not critical since both compounds **9** (2′-substituted) and **11** (4′-substituted) exhibited high activity (EC_{50} 0.10 μg/mL). In addition, compounds with a tertiary hydroxy group in the propionic (C-8′) or butyric (C-9′) acid side chain showed anti-HIV activity (e.g., **8**, **9**, **10**, **11**, **14**), while corresponding compounds without this OH were inactive (**6**, **7**, **12**) or much less active (**13**), respectively. However, although compound **22** does not contain an OH-8′, it was highly active; thus, other structural parameters also contribute to activity. More data are needed to elucidate other structure−activity parameters.



Experimental Section

General Experimental Procedures. Melting points were determined on an MRK air bath-type melting point apparatus. Specific rotations were obtained on a JASCO DIP-370 digital polarimeter (l = 0.5 dm). IR and UV spectra were recorded on JASCO IR-810 and Hitachi 320-S spectrophotometers, respectively. ^{1}H and ^{13}C NMR spectra were determined on JEOL JNM-A400 and JEOL LA 500 instruments in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on a JEOL SX102A instrument. Silica gel (Merck, type 60, 70−320 mesh) was used for column chromatography. Analytical HPLC was performed on a Tosoh liquid chromatograph equipped with a UV detector at 254 nm and a reversed-phased column (YMC A-303) using a solvent mixture of MeOH−H_2O. Preparative HPLC was carried out on Tosoh or Gilson liquid chromatographs equipped with a reversed-phase column (YMC-Pack, ODS-A) at 254 nm using the same solvents as employed for analytical HPLC.

Plant Material. The roots of *Tripterygium wilfordii* were collected in Fujian Province, People's Republic of China, and verified by Pharmacognosy Associate Professor Guan-Yun Gu, Vice-Chairman of Scientific and Technical Archives of Shanghai Medical University, Shanghai, China. A voucher specimen is deposited at the Department of Chemistry of Natural Drugs, School of Pharmacy, Shanghai Medical University, Shanghai, China.

Extraction and Isolation. The root bark of *T. wilfordii* was extracted with $CHCl_3$. The $CHCl_3$ extract (925 g) was obtained by evaporation of the solvent. The $CHCl_3$ extract was dissolved in MeOH and chromatographed on a silica gel column (3.5 kg, 11 × 150 cm). The MeOH solution was eluted with benzene−EtOAc−*n*-hexane (14:5:6) to give fraction 1 (64.5 g), CH_2Cl_2−acetone (1:0 to 1:4) to give fraction 2 (41.1 g), acetone to give fraction 3 (120.3 g), $CHCl_3$−MeOH−H_2O (14:50:3, 65:35:10) to give fraction 4 (63.6 g), and MeOH to give fraction 5 (106.1 g). Each fraction was checked by TLC and HPLC. Fraction 3 (120.3 g) was chromatographed on a LH-20 silica gel column (1.9 kg, 11 × 150 cm) (MeOH) and eluted with $CHCl_3$−MeOH (95:5, 9:1, 8:2, 7:3, 1:1, 0:100) to afford six fractions (fractions 3-1: 3.8 g, 3-2: 8.4 g, 3-3: 37.8 g, 3-4: 20.1 g, 3-5: 3.9 g, and 3-6: 23.3 g). Fraction 3-2 (8.4 g) was separated by MPLC (Lichroprep RP-18, MeOH−H_2O, 7:3, 100:0) to gave three fractions (fractions 3-2-1 and 3-2-2 and MeOH fraction). Fraction 3-2-1 was separated by preparative HPLC (YMC-Pack, ODS-A, MeOH−H_2O, 1:1) to provide three new sesquiterpene pyridine alkaloids, **1** (1.1 mg), **2** (1.7 mg), and **3** (6.3 mg), along with five known alkaloids, wilforjine (9.1 mg),[17] alatusinine

(5.7 mg),[15] evonine (9.2 mg),[19] 4-hydroxy-7-*epi*-chuchuhusnine E-V (3.3 mg),[18] and **20** (20.0 mg).[15] These five known compounds were obtained as colorless, amorphous powders. Si gel chromatography of fraction 2 (41.1 g) eluting with $CHCl_3$−MeOH (99:1, 95:1, and 90:1) and MeOH afforded nine fractions (fractions 2-1: 5.8 g, 2-2: 664.7 mg, 2-3: 475.9 mg, 2-4: 3.67 g, 2-5: 6.58 g, 2-6: 4.92 g, 2-7: 4.34 g, 2-8: 3.02 g, and 2-9: 8.56 g). Further purification of fraction 2-1 using repeated preparative HPLC (Lichroprep RP-18, MeOH−H_2O, 7:1) gave **20** (7.0 mg)[20] and tripterifordin (2.3 mg).[10] Repeated purification of fraction 2-4 yielded **6** (3.3 mg)[16] and **8** (3.2 mg).[16] These four known compounds were obtained as colorless, amorphous powders.

Tripfordine A (1): colorless, amorphous solid; mp 172−174 °C; $[\alpha]^{25}_D$ +4.5 (*c* 0.034, MeOH); UV (MeOH) λ_{max} (log ϵ) 260 (2.91) nm; IR (KBr) ν_{max} 3600, 1740, 1725, 1675, 1640, 1550, 1535, 1500, 1450 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.41 (3H, H-10′), 1.61 (3H, s, H-12), 1.62 (3H, s, H-14), 1.96 (3H, s, CH_3COO-1), 2.01 (3H, s, CH_3COO-8), 2.18 (3H, s, CH_3COO-7), 2.19 (3H, s, CH_3COO-5), 2.29 (3H, s, CH_3COO-11), 2.35 (1H, d, J = 3.6 Hz, H-6), 2.84 (1H, m, H-7′a), 3.70 (1H, d, J = 12.0 Hz, H-15a), 3.99 (1H, dd, J = 2.8, 2.8 Hz, H-2), 4.61 (1H, d, J = 13.6 Hz, H-11a), 5.02 (1H, d, J = 2.8 Hz, H-3), 5.35 (1H, d, J = 6.0 Hz, H-8), 5.42 (1H, d, J = 13.6 Hz, H-11b), 5.50 (1H, d, J = 3.6 Hz, H-1), 5.52 (1H, dd, J = 4.0 Hz, 5.8, H-7), 5.83 (1H, d, J = 12.0 Hz, H-15b), 6.91 (1H, s, H-5), 7.21 (1H, dd, J = 4.8 Hz, 8.0, H-5′), 8.11 (1H, dd, J = 1.8, 7.8 Hz, H-4′), 8.68 (1H, dd, J = 1.8, 5.0 Hz, H-6′); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.8 (C-14), 20.6 (CH_3COO-8), 20.8 (CH_3COO-1), 21.0 (CH_3COO-7), 21.4 (CH_3COO-11), 21.6 (CH_3COO-1), 22.7 (C-12), 27.6 (C-10′), 31.3 (C-7′), 38.6(C-8′), 51.0 (C-6), 52.3 (C-9), 60.5 (C-11), 69.0 (C-2), 69.8 (C-4), 69.9 (C-7), 70.0 (C-15), 71.0 (C-8), 73.7 (C-5), 75.2 (C-1), 77.6 (C-9′), 78.9 (C-3), 84.6 (C-13), 94.6 (C-10), 120.6 (C-5′), 125.7 (C-3′), 137.7 (C-4′), 152.2 (C-6′), 164.7 (C-2′), 168.0 (C-12′), 169.2 (CH_3COO-8), 169.8 (CH_3COO-1), 169.8 (CH_3COO-5), 169.9 (CH_3COO-7), 170.2 (CH_3COO-11), 173.0 (C-11′); EIMS *m*/*z* 779 $[M]^+$ (5), 250 (7), 194 (26), 176 (33), 106 (22), 77 (17), 43 (100); HREIMS *m*/*z* 779.2628 (calcd for $C_{36}H_{45}O_{18}N$. 779.2617).

Tripfordine B (2): colorless, amorphous solid; mp 132−133 °C; $[\alpha]^{25}_D$ −15.4 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ϵ) 267 (3.55) nm; IR (KBr) ν_{max} 3450, 1740, 1710, 1650, 1635, 1540, 1460 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.16 (3H, d, J = 6.8 Hz, H-10′), 1.65 (3H, s, H-14), 1.85 (3H, s, CH_3COO-1), 1.86 (3H, s, H-12), 1.95 (3H, s, CH_3COO-8), 2.13 (3H, s, CH_3COO-2), 2.16 (3H, s, CH_3COO-7), 2.16 (3H, s, CH_3COO-11), 2.33 (1H, m, H-8′a), 2.33 (1H, m, H-9′), 2.43 (1H, d, J = 3.2 Hz, H-6), 2.87 (1H, m, H-7′a), 3.72 (1H, d, J = 12.4 Hz, H-15a), 4.08 (1H, m, H-7′b), 4.51 (1H, d, J = 13.2 Hz, H-11a), 5.00 (1H, d, J = 2.4 Hz, H-3), 5.18 (1H, dd, J = 2.8, 2.8 Hz, H-2), 5.21 (1H, d, J = 12.4 Hz, H-11b), 5.33 (1H, d, J = 2.8 Hz, H-5), 5.34 (1H, d, J = 5.6 Hz, H-8), 5.50 (1H, dd, J = 4.0, 5.6 Hz, H-7), 5.62 (1H, d, J = 3.6 Hz, H-1), 5.85 (1H, d, J = 12.4 Hz, H-15b), 5.99 (1H, d, J = 3.6 Hz, OH-5), 6.37 (1H, brs, OH-4), 7.28 (1H, 1H, dd, J = 4.8, 8.0 Hz, H-5′), 8.36 (1H, dd, J = 2.0, 8.0 Hz, H-4′), 8.76 (1H, dd, J = 2.0, 4.8 Hz, H-6′); ^{13}C NMR ($CDCl_3$) δ 18.1 (C-14), 19.0 (C-10′), 20.4 (CH_3COO-1), 20.6 (CH_3COO-8), 21.0 (CH_3COO-11), 21.0 (CH_3COO-2), 21.4 (CH_3COO-7), 23.4 (C-12), 33.0 (C-7′), 33.4 (C-8′), 38.1 (C-9′), 50.8 (C-6), 52.4 (C-9), 60.7 (C-11), 69.2 (C-2), 69.3 (C-7), 71.2 (C-8), 71.2 (C-15), 71.8(C-4), 73.6 (C-1), 74.1 (C-5), 75.1(C-3), 85.0 (C-13), 92.7 (C-10), 121.3 (C-5′), 123.8 (C-3′), 138.7 (C-4′), 153.6 (C-6′), 165.1 (C-2′), 167.1 (C-12′), 168.7 (CH_3COO-2), 169.0 (CH_3COO-8), 169.6 (CH_3COO-1), 169.7 (CH_3COO-11), 170.1 (CH_3COO-7), 175.4 (CH_3COO-11′); EIMS *m*/*z* 763 $[M]^+$ (37), 705 (22), 690 (23), 632 (22), 530 (31), 218 (29), 206 (100), 178 (98), 161 (36), 93 (86), 43 (96); EIMS *m*/*z* 763 $[M]^+$ (37), 705 (22), 690 (23), 632 (22), 530 (31), 218 (29), 206 (100), 178 (98), 161 (36), 93 (86), 43 (96); HREIMS *m*/*z* 763.2693 (calcd for $C_{36}H_{45}O_{17}N$, 779.2700).

Tripfordine C (3): colorless, amorphous solid; mp 137−141 °C; $[\alpha]^{25}_D$ −5.8 (*c* 0.022, MeOH); UV (MeOH) λ_{max} (log ϵ) 237 (2.93) nm; IR (KBr) ν_{max} 3600, 1695, 1680, 1640, 1535, 1500, 1450 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.08 (3H, d, J = 7.2 Hz, H-10′), 1.36 (3H, d, J = 7.2 Hz, H-9′), 1.68 (1H, s, H-14), 1.82 (3H, d, J = 1.2 Hz, H-12), 1.83 (3H, s, CH_3COO-1), 1.95 (3H, s, CH_3COO-8), 2.14 (3H, s, CH_3COO-7), 2.15 (3H, s, CH_3COO-2), 2.20 (3H, s, CH_3COO-11), 2.45 (1H, d, J = 2.4 Hz, H-6), 3.73 (1H, d, J = 12.0 Hz, H-15a), 4.53 (1H, d, J = 13.2 Hz, H-11a), 4.73 (1H, d, J = 2.4 Hz, H-3), 4.79 (1H, q, J = 7.2 Hz, H-7′a), 5.10 (1H, d, J = 13.2 Hz, H-11b), 5.28 (1H, dd, J = 2.8, 3.6 Hz, H-2), 5.34 (1H, d, J = 6.0 Hz, H-8), 5.46 (1H, d, J = 2.4 Hz, H-5), 5.49 (1H, dd, J = 3.8, 5.8 Hz, H-7), 5.55 (1H, d, J = 3.8 Hz, H-1), 5.98 (1H, d, J = 1.2 Hz, OH-5), 6.02 (1H, brs, OH-4), 6.04 (1H, d, J = 12.0 Hz, H-15b), 7.38 (1H, d, J = 8.8 Hz, H-5′), 8.72 (1H, d, J = 5.2 Hz, H-6′), 9.04 (1H, s, H-2′); ^{13}C NMR ($CDCl_3$) δ 9.8 (C-9′), 11.2 (C-10′), 18.6 (C-14), 20.4 (CH_3COO-8), 20.4 (CH_3COO-1), 21.0 (CH_3COO-2), 21.0 (CH_3COO-11), 21.4 (CH_3COO-7), 23.1 (C-12), 33.0 (C-7′), 46.0 (C-8′), 51.1 (C-9), 51.9 (C-6), 60.4 (C-11), 68.5 (C-2), 69.1 (C-7), 70.8 (C-8), 71.0 (C-15), 72.5 (C-4), 73.3 (C-1), 74.3 (C-5), 75.1 (C-3), 84.7 (C-13), 92.9 (C-10), 121.5 (C-5′),124.6 (C-3′), 151.4 (C-2′), 153.1 (C-6′), 168.4 (C-12′), 168.6 (CH_3COO-8), 168.9 (CH_3COO-2), 169.3 (CH_3COO-1), 169.8 (CH_3COO-11), 170.0 (CH_3COO-7), 173.4 (C-11′); HREIMS *m*/*z* 763.2679 (calcd for $C_{36}H_{45}O_{17}N$, 763.2668).

Biological Assays. The details of the in vitro cytotoxicity and anti-HIV assays have been reported previously.[13,21]

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Supporting Information Available: HMBC correlations for **1**−**3**, major HMBC correlations of **1**−**3** (Figures 1−3), and major NOESY correlations of **1** (Figure 4). This material is available free of charge via the Internet at http://pubs.acs.org.

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