Sesquiterpene Alkaloids from *Tripterygium hypoglaucum* and *Tripterygium wilfordii*: A New Class of Potent Anti-HIV Agents

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Five new sesquiterpene pyridine alkaloids [triptonines A (**1**) and B (**2**), and wilfordinines A (**3**), B (**4**), and C (**5**)] and two known compounds (peritassine A and hypoglaunine C) were isolated from *Tripterygium hypoglaucum* and a clinically used extract of *Tripterygium wilfordii*. The structures of **1**−**5** were elucidated by spectroscopic methods. The anti-HIV activity of **1**, **2**, and several related compounds was evaluated. Triptonine B (**2**) demonstrated potent anti-HIV activity with an EC_{50} value of <0.10 μg/mL and an *in vitro* therapeutic index value of >1000.

Plants of the genus *Tripterygium* (Celastraceae) have been used in traditional Chinese medicine as a cancer treatment and as an insecticide for hundreds of years. Recently, an extract (the so-called "total multi-glycoside" or "T_{II} extract") derived from a water and chloroform partitioning of the roots of *T. wilfordii* Hook f. has been used in clinical treatment of rheumatoid arthritis, skin disorders, male-fertility control, and other inflammatory and autoimmune diseases.[1−3] In the course of our study of the sesquiterpene constituents of this genus, we have described the isolation of hyponines A−F[4,5] and hypoglaunines A−D[6] from the root bark of *T. hypoglaucum* (Levl.) Hutch. We have also described anti-HIV principles from *T. hypoglaucum*.[7] This paper deals with the isolation and structure determination of five new (**1**−**5**, Chart 1) and two known sesquiterpene alkaloids (peritassine A and hypoglaunine C) from *T. hypoglaucum* and *T. wilfordii* (T_{II} extract), along with the anti-HIV activities of the novel compounds and similar analogues.

Results and Discussion

Repeated column chromatography of the ethyl acetate-soluble fraction from the methanolic extract of root bark of *Tripterygium hypoglaucum* yielded two novel sesquiterpene derivatives, named triptonines A (**1**) and B (**2**). The powdered T_{II} extract of *T. wilfordii* was chromatographed repeatedly on Si gel to afford three new sesquiterpene alkaloids, named wilfordinines A (**3**), B (**4**), and C (**5**), as well as two known compounds, peritassine A and hypoglaunine C.

Triptonine A (**1**), colorless needles, mp 284.0−285.5 °C, had the molecular formula, $C_{45}H_{55}O_{21}N$ (HREIMS). The IR spectrum showed hydroxyl and ester carbonyl bands at 3438 and 1737 cm^{-1}, and the UV spectrum contained an aromatic moiety (224 and 264 nm). Its ^1H NMR spectral data revealed the presence of four acetyl groups (δ_H 1.75, 1.90, 2.10, and 2.18), two methylene groups [δ_H 4.21, 5.41 (2H, d, J = 14.2 Hz); 3.65, 5.95 (2H, d, J = 11.7 Hz)], and seven methine protons [δ_H 2.43, 4.65, 5.15, 5.25, 5.42, 5.46, and 7.02], as well as a 2,3-disubstituted pyridine [δ_H 7.20 (1H, dd, J = 4.9, 7.8 Hz), 8.01 (1H, dd, J = 1.5, 7.8 Hz), and 8.62 (1H, dd, J = 1.5, 4.9 Hz)], two secondary methyl groups [δ_H 1.10 and 1.32], and two coupled methine protons [δ_H 2.48 and 4.60]. The ^{13}C NMR spectral data of **1** were very similar to those of hyponine A (**9**) except for the ester functions (Table 2). Accordingly, **1** likely was a sesquiterpene pyridine alkaloid derived from dihydroagarofuran polyol esters such as hyponines A (**9**) and B (**18**).[4] The pyridine moiety of **1** was assigned as an evoninic acid. The $^1H-^1H$ COSY spectrum of **1** revealed two sets of separated spin−spin systems (H-1/H-2/H-3 and H-6/H-7/H-8) in the dihydroagarofuran core. The remaining dihydroagarofuran proton signal at δ_H 7.02 (H-5) was correlated with the carbon signals at δ_C 50.3 (C-6), 51.9 (C-9), 94.2 (C-10), and 84.7 (C-13) in the HMBC spectrum. In addition, the proton signal at δ_H 5.95 (Ha-15) correlated with the carbon signals at δ_C 84.7 (C-13) and 168.7 (C-12′), the proton signal at δ_H 4.65 (H-3) correlated with the carbon signal at δ_C 174.1 (C-11′), and the proton signal at δ_H 2.48 (H-8′) correlated with the carbon signals at δ_C 165.5 (C-2′), 36.5 (C-7′), and 174.1 (C-11′). From this evidence, the evoninic acid moiety was linked to the sesquiterpene unit at positions C-3 and C-15.

By studying the $^1H-^1H$ COSY, $^{13}C-^1H$ COSY, and HMBC spectra of **1**, a monoterpene partial structure was determined (Figure 1). From the molecular formula, **1** contained 19 degrees of unsaturation, and 18 were readily accounted for (four acetyl groups = 4, monoterpene partial structure = 4, and the macrocyclic structure, including a dihydroagarofuran unit = 10). The remaining one degree of unsaturation indicated that compound **1** has another ring in its structure. In the HMBC spectrum, the proton signals at δ_H 4.69 (H-6″) and 5.41 (H-11) correlated with the carbon signal at δ_C 168.0 (C-9″), and the proton signals at δ_H 1.12 (H-10″) and 5.42 (H-7) correlated with the carbon signal at δ_C 175.9 (C-1″). These observations indicated that another ring was formed by an ester linkage between the monoterpene and sesquiterpene portions of the molecule at positions C-7 and C-11. The remaining four acetyl groups

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Table 1. ^{1}H NMR Spectral Data of Compounds **1**−**5**[a]

proton	**1**[b]	**2**[c]	**3**	**4**	**5**[d]
1	5.46 (d, 4.4)	5.58 (d, 5.4)	5.40 (d, 3.8)	5.58 (d, 3.8)	5.91 (d, 3.9)
2	5.15 (dd, 2.0, 4.4)	5.34 (dd, 2.4, 3.9)	4.08 (br, 2.5, 3.6)	5.33 (dd, 2.6, 3.8)	5.45 (dd, 2.5, 3.9)
3	4.65 (d, 2.0)	4.70 (d, 2.4)	4.76 (d, 2.5)	4.69 (d, 2.6)	4.78 (d, 2.5)
5	7.02 (s)	7.13 (s)	7.05 (s)	7.03 (s)	7.08 (s)
6	2.43 (d, 3.9)	2.52 (d, 3.4)	2.33 (d, 4.1)	2.41 (d, 3.8)	2.43 (d, 3.8)
7	5.42 (dd, 3.9, 5.9)	5.53 (dd, 3.4, 6.0)	5.52 (dd, 4.1, 6.0)	5.51 (dd, 3.8, 5.9)	5.53 (dd, 3.8, 6.0)
8	5.25 (d, 5.9)	5.32 (d, 6.0)	5.36 (d, 6.0)	5.34 (d, 5.9)	5.42 (d, 6.0)
11a	5.41 (d, 14.2)	5.49 (d, 14.2)	5.30 (d, 13.7)	5.13 (d, 13.5)	5.34 (d, 13.5)
11b	4.21 (d, 14.2)	4.29 (d, 14.2)	4.62 (d, 13.7)	4.48 (d, 13.5)	4.69 (d, 13.5)
12	1.44 (s)	1.53 (s)	1.58 (s)	1.54 (s)	1.58 (s)
14	1.75 (s)	1.63 (s)	1.69 (s)	1.61 (s)	1.66 (s)
15a	5.95 (d, 11.7)	5.49 (d, 11.2)	6.04 (d, 11.5)	5.11 (d, 11.4)	5.12 (d, 11.3)
15b	3.65 (d, 11.7)	4.28 (d, 11.2)	3.68 (d, 11.5)	4.27 (d, 11.4)	4.30 (d, 11.3)
2′		8.99 (s)	8.96 (s)	8.98 (s)	9.00 (s)
4′	8.01 (dd, 1.5, 7.8)				
5′	7.20 (dd, 4.9, 7.8)	7.81 (d, 5.4)	7.36 (d, 5.3)	7.83 (d, 5.3)	7.83 (d, 5.2)
6′	8.62 (dd, 1.5, 4.9)	8.69 (d, 5.4)	8.69 (d, 5.3)	8.68 (d, 5.3)	8.69 (d, 5.2)
7′	4.60 (q, 6.8)	4.24 (q, 6.8)	4.69 (q, 7.1)	4.23 (q, 7.1)	4.25 (q, 7.1)
8′	2.48 (q, 7.3)		2.42 (q, 7.1)		
9′	1.32 (d, 6.8)	1.19 (d, 6.8)	1.34 (d, 7.1)	1.18 (d, 7.1)	1.20 (d, 7.1)
10′	1.10 (d, 7.3)	1.34 (s)	1.04 (d, 7.1)	1.35 (s)	1.43 (s)
OAc-1	1.75 (s)	1.98 (s)	1.91 (s)	1.86 (s)	
OAc-2	2.10 (s)	1.85 (s)		2.19 (s)	2.19 (s)
OAc-5	2.18 (s)	2.24 (s)	2.20 (s)	2.20 (s)	2.22 (s)
OAc-7			2.15 (s)	2.17 (s)	2.14 (s)
OAc-8	1.90 (s)	2.20 (s)	2.01 (s)	2.00 (s)	1.45 (s)
OAc-11			2.31 (s)	2.31 (s)	2.34 (s)

[a] $CDCl_3$ was used as solvent, and TMS as internal standard. [b] **1**: 2.54 (m, H-2″), 1.90, 1.77 (2H, m, H-3″), 3.43, 2.91 (2H, m, H-4″), 4.69 (dd, 5.9, 9.3, H-6″), 3.02 (dd, 9.3, 17.6, H-7″), 2.90 (dd, 5.9, 17.6, H-7″), 1.12 (3H, d, 6.8, H-10″), 3.61 (s, OMe). [c] **2**: 2.62 (m, H-2″), 1.91, 2.06 (2H, m, H-3″), 2.99, 3.27 (2H, m, H-4″), 4.74 (t, 7.3, H-6″), 3.01 (2H, d, 7.3, H-8″), 1.22 (3H, d, 6.8, H-10″), 3.68 (s, OMe). [d] **5**: 1-Bz, 7.84 (2H, d, 8.1, *ortho*), 7.42 (2H, br t, 7.8, *meta*), 7.56 (t, 7.5, *para*).

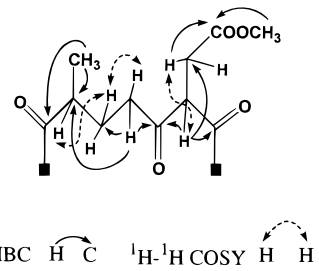


Figure 1. The monoterpene partial structure of **1**.

were assigned at C-1, C-2, C-5, and C-8 from the HMBC spectral data. In the NOESY spectrum, the proton signal at δ_H 5.41 (H-11a) correlated with the signals at δ_H 7.02 (H-5) and 1.44 (H_3-12). In turn, the proton signal at δ_H 5.46 (H-1) correlated with the signals at δ_H 5.15 (H-2) and 5.25 (H-8), and the proton signal at δ_H 5.25 (H-8) correlated with the signals at δ_H 5.42 (H-7) and 5.46 (H-1). Thus, the relative configurations of the ester groups were 1β, 2β, 5α, 7β, and 8β. Therefore, the structure of compound **1** was formulated as 7,11{1′-[(methoxycarbonyl)methyl]-5′-methyl-2′-oxopentane-1′,5′-dicarboxylic acid}dicarbolactone-7-deoxo-11-deacetoxyevonine.

Triptonine B (**2**), $C_{45}H_{55}O_{22}N$, contained four acetyl groups and the same monoterpene partial structure as **1**. Its ^{1}H and ^{13}C NMR spectral data were very similar to those of **1**, except for the pyridine unit (Tables 1 and 2). The coupling pattern suggested that the pyridine unit in **2** was 3,4-substituted [δ_H 8.99 (s), 8.69, and 7.81 (each 1H, d, J = 5.4 Hz)]. Further, in the HMBC spectrum of **2**, the proton signal at δ_H 8.99 (H-2′) correlated with the signals at δ_C 127.5 (C-3′), 167.7 (C-12′), and 152.7 (C-6′), and the proton signal at δ_H 7.81 (H-5′) correlated with the signals at δ_C 127.5 (C-3′), 151.8 (C-4′), and 41.9 (C-7′), while the methyl proton signal at δ_H 1.19 (H-9′) correlated with the signals at δ_C 151.8 (C-4′), 41.9 (C-7′), and 76.8 (C-8′). Thus, the presence of a 3,4-substituted pyridine was confirmed. In turn, the proton signal at δ_H 1.34 (H_3-10′) correlated with the signals at δ_C 41.9 (C-7′), 76.8 (C-8′), and 175.2 (C-12′), and furthermore, no HMBC correlation from the H_3-10′ methyl proton signal to the carbon signal (C-4′) of the pyridine ring was observed. From the above observations, the pyridyl moiety was assigned as 2-hydroxy-2,3-dimethyl-3(3′-carboxy-4′-pyridyl)-propanoic acid. In addition, the proton signal at δ_H 4.70 (H-3) correlated with the signal at δ_C 175.2 (C-11′), the proton signal at δ_H 5.49 (H-15) correlated with the signal at δ_C 167.7 (C-12′), the proton signals at δ_H 5.53 (H-7) and 1.22 (H-10″) correlated to the carbon signal at δ_C 175.4 (C-1″), and the proton signals at δ_H 5.49 (H-11) and 4.74 (H-6″) correlated with the carbon signal at δ_C 168.1 (C-9″). These observations indicated that the pyridyl moiety was linked to the sesquiterpene molecule at positions C-3 and C-15, and the monoterpene moiety was bonded to the sesquiterpene at positions C-7 and C-11. The remaining four acetyl groups were located at C-1, C-2, C-5, and C-8 due to their HMBC correlations. The relative stereochemistry of **2** was determined from the ^{1}H NMR coupling constants and the NOESY spectral data. ^{1}H and ^{13}C NMR spectral data assignments were confirmed by 2D NMR spectra as shown in Tables 1 and 2. Therefore, the structure of triptonine B (**2**) was determined as 1β,2β,5α,8β-tetraacetoxy-3α,15[2′-hydroxy-2′,3′-dimethyl-3′(3″-carboxy-4″-pyridyl)-propanoic acid]dicarbolactone-4α-hydroxy-7β,11{1′-[(methoxycarbonyl)methyl]-5′-methyl-2′-oxopentane-1′,5′-dicarboxylic acid}dicarbolactone dihydroagarofuran.

More than 50 macrocyclic sesquiterpene pyridine alkaloids have been isolated from Celastraceae plants, but cathedulin-K 20,[9] which has a di-macrocyclic structure, is the only example of a compound related to triptonine A (**1**). Triptonines A (**1**) and B (**2**) have a monoterpene moiety bonded to a sesquiterpene moiety by ester linkage and are the first compounds of this structural type to be isolated and characterized.

Table 2. ^{13}C NMR Spectral Data of Compounds **1**−**5** and **9**[a]

carbon	**1**[b]	**2**[c]	**3**	**4**	**5**[d]	**9**
1	73.7 d	73.3 d	75.7 d	73.0 d	73.1 d	73.2 d
2	68.8 d	70.8 d	69.2 d	68.0 d	68.0 d	68.7 d
3	75.9 d	77.8 d	78.5 d	77.4 d	77.4 d	75.7 d
4	70.7 s	70.6 s	70.7 s	70.4 s	70.5 s	70.6 s
5	73.9 d	74.6 d	73.9 d	74.2 d	74.3 d	73.9 d
6	50.3 d	50.6 d	50.6 d	50.6 d	50.6 d	50.3 d
7	69.8 d	69.7 d	69.1 d	68.9 d	68.9 d	68.9 d
8	71.1 d	68.1 d	70.9 d	70.6 d	71.3 d	70.6 d
9	51.9 s	52.3 s	52.6 s	52.3 s	52.7 s	52.1 s
10	94.2 s	93.3 s	94.7 s	93.2 s	93.3 s	93.7 s
11	61.6 t	61.7 t	60.4 t	59.8 t	59.9 t	60.0 t
12	22.6 q	22.0 q	23.0 q	22.3 q	22.4 q	22.9 q
13	84.7 s	83.6 s	84.1 s	83.3 s	83.5 s	84.1 s
14	18.7 q	18.8 q	18.5 q	18.6 q	18.6 q	18.5 q
15	70.0 t	69.8 t	70.3 t	69.7 t	69.9 t	69.8 t
2′	165.5 s	151.5 d	150.7 d	151.3 d	151.5 d	165.0 s
3′	125.1 s	127.5 s	125.7 s	127.4 s	127.5 s	125.2 s
4′	138.0 d	151.8 s	156.3 s	151.7 s	151.7 s	137.5 d
5′	121.3 d	123.6 d	121.6 d	123.5 d	123.5 d	121.1 d
6′	151.7 d	152.7 d	152.6 d	152.5 d	152.6 d	151.5 d
7′	36.5 d	41.9 d	33.3 d	41.8 d	41.9 d	36.4 d
8′	45.2 d	76.8 s	45.7 d	76.8 s	76.7 s	44.9 d
9′	11.9 q	17.3 q	11.4 q	17.2 q	17.3 q	11.8 q
10′	9.8 q	24.1 q	9.6 q	24.0 q	24.0 q	9.5 q
11′	174.1 s	175.2 s	174.1 s	174.9 s	175.1 s	173.9 s
12′	168.7 s	167.7 s	167.9 s	167.6 s	167.7 s	169.1 s
1-OAc	20.5 q	20.5 q	20.8 q	20.3 q		20.5 q
	169.1 s	168.7 s	169.5 s	169.2 s		169.0 s
2-OAc	21.1 q	20.4 q		21.0 q	20.9 q	21.0 q
	168.6 s	169.1 s		168.5 s	168.3 s	168.0 s
5-OAc	21.9 q	21.8 q	21.7 q	21.6 q	21.7 q	
	170.3 s	169.9 s	169.9 s	169.5 s	169.6 s	
7-OAc			21.1 q	21.0 q	21.0 q	21.0 q
			170.1 s	169.9 s	169.9 s	170.2 s
8-OAc	20.6 q	21.0 q	20.6 q	20.4 q	20.0 q	21.4 q
	168.9 s	168.5 s	169.2 s	168.9 s	168.8 s	168.8 s
11-OAc			21.5 q	21.3 q	21.5 q	21.4 q
			170.3 s	170.2 s	170.4 s	170.1 s

[a] $CDCl_3$ was used as solvent, and TMS as internal standard. [b] **1**: 175.9 (s, C-1″), 37.4 (d, C-2″), 28.2 (t, C-3″), 42.3 (t, C-4″), 204.5 (s, C-5″), 52.1 (d, C-6″), 32.8 (t, C-7″), 171.9 (s, C-8″), 168.0 (s, C-9″), 18.2 (q, C-10″), 52.1 (q, OMe). [c] **2**: 175.4 (s, C-1″), 38.1 (d, C-2″), 28.4 (t, C-3″), 42.1 (t, C-4″), 203.3 (s, C-5″), 52.1 (d, C-6″), 32.4 (t, C-7″), 171.9 (s, C-8″), 168.1 (s, C-9″), 18.3 (q, C-10″), 52.0 (q, OMe). [d] **5**: 1-Bz, 129.0 (s, *ipso*), 129.6 (d, *ortho*), 128.7 (d, *meta*), 133.7 (d, *para*), 164.6 (s).

Wilfordinine A (**3**), isolated from *T. wilfordii*, was assigned a molecular formula of $C_{36}H_{45}O_{17}N$ via HREIMS (*m*/*z* 763.2654). The ^1H NMR spectrum revealed the presence of five acetyl groups (δ_H 1.91, 2.01, 2.15, 2.20, and 2.31), two sets of methylene groups [δ_H 4.62, 5.30 (2H, d, J = 13.7 Hz); 3.68, 6.04 (2H, d, J = 11.5 Hz)], a 3,4-substituted pyridine ring [δ_H 8.96 (s); 8.69, 7.36 (2H, d, J = 5.3 Hz)], and six methine protons (δ_H 2.33, 4.08, 4.76, 5.36, 5.40, and 5.52). Its ^{13}C NMR spectral data were similar to those of **2**, except for the ester groups and in the C-7′−C-10′ region (Table 2). Compound **3** was proposed as an isomeric evonine-type sesquiterpene alkaloid, with the same pyridyl unit as that of hypoglaunine D[6] (**8**). In the HMBC spectrum, the proton signals at δ_H 5.40 (H-1), 7.05 (H-5), 5.52 (H-7), 5.36 (H-8), and 4.62 (H-11) were correlated with the carbonyl carbons of acetyl groups at δ_C 169.5, 169.9, 170.1, 169.2, and 170.3, respectively. Thus, five acetyl groups could be assigned at positions C-1, C-5, C-7, C-8, and C-11. Therefore, the structure of wilfordinine A was determined as 1β,5α,7β,8β,11-pentaacetoxy-2β,4α-hydroxy-3α,15[2′,3′-dimethyl-3′(3″-carboxy-4″-pyridyl)-propanoic acid]dicarbolactone dihydroagarofuran.

Wilfordinine B (**4**), $C_{38}H_{47}O_{19}N$, contained six acetyl groups (δ_H 1.86, 2.00, 2.17, 2.19, 2.20, and 2.31) and a 3,4-substituted pyridyl moiety (δ_H 8.98, 8.68, and 7.83), similar to compound **3**. It was elucidated as 2-acetyl-8′-hydroxy-wilfordinine A (**3**) based upon comparison of ^1H and ^{13}C NMR spectral data with those of compound **3** (Table 2). In the HMBC spectrum, the proton signal at δ_H 1.35 (H_3-10′) was correlated with the carbon signals at δ_C 41.8 (C-7′), 76.8 (C-8′), and 174.9 (C-12′), thus the hydroxyl group was located at C-8′. The dihydroagarofuran proton signals at δ_H 5.58 (H-1), 5.33 (H-2), 7.03 (H-5), 5.51 (H-7), 5.34 (H-8), and 5.13 (H-11) were correlated with the carbonyl carbons of the acetyl groups at δ_C 169.2, 168.5, 169.5, 169.9, 168.9, and 170.2, respectively. Therefore, six acetyl groups were assigned at positions C-1, -2, -5, -7, -8, and -11, respectively, and **4** was established as 1β,2β,5α,7β,8β,11-hexaacetoxy-3α,15[2′-hydroxy-2′,3′-dimethyl-3′(3″-carboxy-4″-pyridyl)-propanoic acid]dicarbolactone-4α-hydroxy dihydroagarofuran.

Wilfordinine C (**5**), $C_{43}H_{49}O_{19}N$, contained five acetyl groups (δ_H 1.45, 2.14, 2.19, 2.22, and 2.34) and one benzoyl group [δ_H 7.42 (2H, br t, 7.8), 7.84 (2H, d, 8.1), 7.56 (t, 7.5)]. It was also a macrocyclic sesquiterpene alkaloid with a 3,4-substituted pyridine moiety linked to the sesquiterpene molecule at positions C-3 and C-15. In the HMBC spectrum, the proton signal at δ_H 5.91 (H-1) was correlated with the carbonyl carbon of the benzoyl group at δ_C 164.6, and thus the benzoyl group could be assigned at position C-1. The remaining acetyl groups were assigned in the same manner as described above for **3** and **4**. Therefore, the structure of wilfordinine C was assigned as 1β-benzoyl-2β,5α,7β,8β,11-pentaacetoxy-3α,15[2′-hydroxy-2′,3′-dimethyl-3′(3″-carboxy-4″-pyridyl)-propanoic acid]dicarbolactone-4α-hydroxy dihydroagarofuran.

Two known compounds were also isolated. These were identified by spectral comparison with peritassine A[8] and hypoglaunine C.[6]

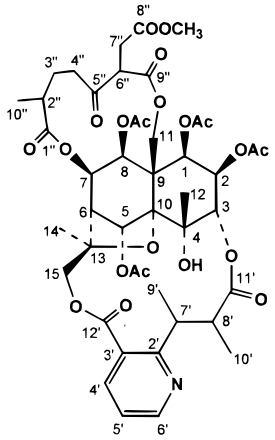
In searching for natural products as potential anti-AIDS agents, several compound series, such as coumarins,[10] diterpenoids,[11] triterpenoids,[12] a variety of tannins,[13,14] flavonoids,[15,16] and a variety of alkaloids[17] have been reported to have anti-HIV activity. Plant-derived natural products and their analogues as anti-HIV agents have been reviewed recently.[18] In this paper, we report a new class of potent anti-HIV agents. Compounds **1** and **2** and related sesquiterpene alkaloids (**9**−**22**), isolated from *T. hypoglaucum*, exhibited anti-HIV activity as shown in Table 3.

Table 3. Anti-HIV Activity of Compounds **1**, **2**, and Their Analogues

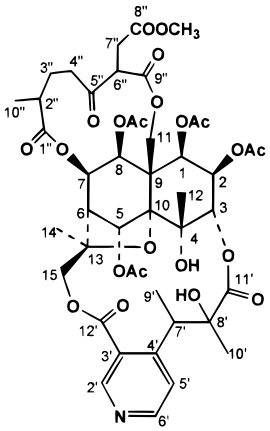
compound	IC_{50} (μg/mL)	EC_{50} (μg/mL)	TI
triptonine A (**1**)	>100	2.54	>39.4
triptonine B (**2**)	>100	<0.10	>1000
hypoglaunine A[6] (**6**)	>100	0.13	>769
hypoglaunine B[6] (**7**)	>100	0.10	>1000
hypoglaunine D[6] (**8**)	22.2	no	suppression
hyponine A[4] (**9**)	27.7	1.00	27.7
hyponine D[5] (**10**)	20.2	no	suppression
hyponine E[5] (**11**)	1.95	0.17	11.3
hyponine F[5] (**12**)	>100	35.2	>2.84
forrestine[5] (**13**)	>100	0.48	>208
cangoronine E−1[4] (**14**)	56.8	0.9	63.4
euonymine[6] (**15**)	22.8	0.203	113
neoeuonymine[5] (**16**)	>100	0.884	>113
evonine[4] (**17**)	21.2	0.503	42.1
hyponine B[4] (**18**)	>100	0.10	>1000
wilforine[6] (**19**)	>100	no	suppression
wilforgine[6] (**20**)	>100	no	suppression
wilfordine[6] (**21**)	20.0	<0.10	>220
wilfortrine[6] (**22**)	>100	<0.10	>1000
AZT	500	0.012[a]	41,667

[a] This EC_{50} value is the mean of 65 EC_{50} values for AZT.

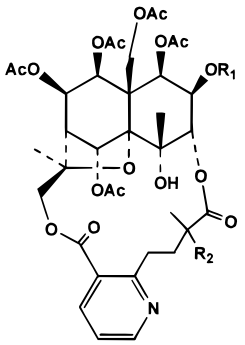
Chart 1



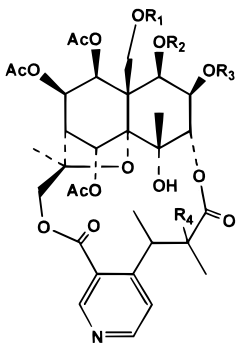
1



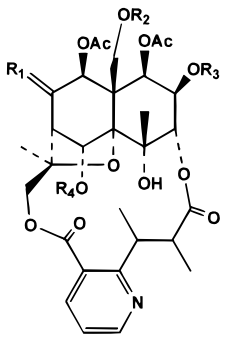
2



	R_1	R_2
19	Bz	H
20	Fu	H
21	Bz	OH
22	Fu	OH



	R_1	R_2	R_3	R_4
3	Ac	Ac	H	H
4	Ac	Ac	Ac	OH
5	Ac	Bz	Ac	OH
6	Fu	Ac	Ac	OH
7	Ac	Ac	Fu	OH
8	Fu	Ac	Ac	H



	R_1	R_2	R_3	R_4
9	β-OAc, α-H	Ac	Ac	Fu
10	β-OAc, α-H	Ac	Nic	Bz
11	β-ONic, α-H	Ac	Ac	Fu
12	β-OAc, α-H	Ac	Fu	Ac
13	β-OAc, α-H	Ac	Bz	Ac
14	β-OAc, α-H	Ac	Ac	Bz
15	β-OAc, α-H	Ac	Ac	Ac
16	β-OAc, α-H	Ac	Ac	H
17	O	Ac	Ac	Ac
18	β-OAc, α-H	Fu	Ac	Ac

Triptonine B (**2**) inhibited HIV replication in H9 lymphocytes with an EC_{50} value of <0.10 μg/mL and inhibited uninfected H9 cell growth with an IC_{50} value of >100 μg/mL, thus the in vitro therapeutic index (TI) value was >1000. In general, a TI >5.0 is considered significant. Compounds **7**, **18**, and **22** also showed promising anti-HIV activity with TI values of >1000. It should also be noted that diterpenes were isolated previously from *T. wilfordii* as anti-HIV principles.[19,20]

Experimental Section

General Experimental Procedures. Optical rotations were measured with a JASCO DIP-370 digital polarimeter. UV spectra were run on an UV 2100 UV−vis recording spectrometer (Shimadzu). IR spectra were recorded on a 1720 infrared Fourier transform spectrometer (Perkin-Elmer). NMR experiments were run on a Bruker ARX-400 instrument using TMS as internal standard. NMR spectra were recorded at 400 MHz (^{1}H) and 100 MHz (^{13}C). Mass spectra were obtained on a JEOL JMSD-300 instrument. Column chromatography was performed using Si gel 60 (Merck) or Sephadex LH-20 (Pharmacia). HPLC was carried out as follows: Si gel HPLC (Hibar RT 250−25, LiChrosorb Si 60), ODS_1 (YMC SH-345-5, S-5, 120A, AM, Yamamura), ODS_2 (YMC SH-345-5, S-5, 120A, CN, Yamamura), ODS_3 (INERTSIL PREP ODS, 20.0 × 250 mm, GL Sciences Inc.).

Plant Material. The root bark of *T. hypoglaucum* was purchased in March 1995, in Kunming, Yunnan Province, People's Republic of China, and identified by Prof. Dr. Dao-

Feng Cheng (Shanghai Medical University, People's Republic of China). A powdered extract of *T. wilfordii* (T_{II}) was purchased in 1997, from the School of Pharmacy, Shanghai Medical University, Shanghai, People's Republic of China. This extract was prepared from the root xylem with water then with chloroform and by column chromatographic separation (Si gel, $CHCl_3$−MeOH, 95:5). Samples of *T. hypoglaucum* (TH950331), T_{II}, and the original plant (*T. wilfordii*, TW940930) are deposited in the Faculty of Pharmaceutical Sciences, University of Tokushima, Japan.

Extraction and Isolation. The root bark (15.3 kg) of *T. hypoglaucum* was crushed and extracted three times with MeOH (50 L each) at 60 °C for 6 h. The combined MeOH extracts were concentrated in vacuo (860 g), and then the residue was partitioned between EtOAc and H_2O. The EtOAc layer was concentrated to give a residue (314 g), which was chromatographed on a Si gel (1.6 kg) column (90 × 850 mm). The column was eluted with solvents of increasing polarity [hexanes−EtOAc [3:1 (4 L), 3:2 (3 L), 1:1 (4 L), 1:2 (3 L), 1:4 (3 L)], EtOAc, EtOAc−MeOH (19:1, 9:1, 4:1), and MeOH to give 22 fractions (fractions 1−22). Combined fractions 14 and 15 (60 g) were chromatographed over a Si gel column (800 g, 60 × 700 mm) eluting with $CHCl_3$−MeOH (19:1, 9:1, 8:2) and MeOH to give 10 further fractions (fractions 14.1−14.10). Fraction 14.2 (18 g) was chromatographed by medium-pressure liquid chromatography on a silica column (50 × 500 mm) eluting with a $CHCl_3$−MeOH system to give nine fractions (fractions 14.2.1−14.2.9). Fraction 14.2.7 (1 g) was separated using ODS_1 and ODS_2 (MeOH−H_2O, 8:2) to give **2** (13 mg). Fraction 14.1 (1.5 g) was separated using ODS_3 and Si gel HPLC to give **1** (98 mg).

The extract (T_{II}, 54 g) prepared from *T. wilfordii* was chromatographed over a Si gel column (1.0 kg, 11 × 90 cm) and eluted with solvents of increasing polarity [$CHCl_3$−MeOH (99:2, 95:5, 9:1, MeOH)] to give 10 fractions (fractions 1−10). Fraction 5 (16.5 g) was chromatographed on a Si gel column (6 × 80 cm) by elution with hexanes−EtOAc (1:1, 1:2, 1:4) to give 12 further fractions (fractions 5.1−5.12). Fraction 5.9 (3 g) was chromatographed over Sephadex LH-20 (MeOH) to give another five fractions (fractions 5.9.1−5.9.5). Fraction 5.9.1 (2 g) was chromatographed on using ODS_3 (MeOH−H_2O, 8:2) to give eight fractions (fractions 5.9.1.1−5.9.1.8), with fraction 5.9.1.1 separated again over ODS_3 (MeOH−H_2O, 8:2 and then 7:3) to give **3** (8 mg) and **4** (66 mg). Fraction 5.9.1.2 was isolated by Si gel HPLC and ODS_3 (MeOH−H_2O, 7:3) to give **5** (9 mg) and peritassine A (110 mg). Fraction 5.9.1.4 was separated by ODS_3 (MeOH−H_2O, 7:3) and Si gel HPLC to give hypoglaunine C (13 mg).

Triptonine A (**1**): colorless needles; mp 284.0−285.5 °C, $[\alpha]^{25}_D$ −24.1° (*c* 1.0, MeOH), UV (MeOH) λ_{max} (log ϵ) 224 (3.96), 264 (3.58) nm; IR (KBr) ν_{max} 3590, 3570, 3438, 2926, 2854, 2366, 2346, 1737, 1655, 1639, 1562, 1459, 1376, 1234, 1117, 715 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 945 $[M]^+$ (93), 914 (10), 886 (19), 857 (60), 262 (10), 220 (21), 206 (100), 178 (40), 161 (27), 150 (14), 134 (18), 107 (69), 95 (22), 43 (46); HREIMS *m/z* 945.3254 (calcd for $C_{45}H_{55}O_{21}N$, 945.3267).

Triptonine B (**2**): amorphous powder; $[\alpha]^{25}_D$ +15.5° (*c* 0.6, MeOH); UV (MeOH) λ_{max} (log ϵ) 261 (3.40) nm; IR (KBr) ν_{max} 3622, 3570, 3437, 2929, 2365, 2346, 1742, 1656, 1639, 1460, 1376, 1234, 1158, 1041 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 961 $[M]^+$ (100), 946 (9), 929 (9), 902 (12), 873 (35), 814 (7), 225 (15), 208 (15), 194 (14), 176 (32), 165 (8), 150 (45), 134 (41), 107 (26), 95 (22), 43 (51); HREIMS *m/z* 961.3235 (calcd for $C_{45}H_{55}O_{22}N$, 961.3216).

Wilfordinine A (**3**): amorphous powder; $[\alpha]^{25}_D$ −11.0° (*c* 0.9, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (3.50), 220 (3.89) nm; IR (KBr) ν_{max} 3570, 3436, 2930, 2371, 2346, 1746, 1656, 1639, 1630, 1562, 1460, 1371, 1238, 1122, 1099, 1057, 885, 600 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 763 $[M]^+$ (18), 748 $[M - Me]^+$ (13), 704 $[M - OAc]^+$ (23), 690 (13), 644 (9), 236 (12), 224 (11), 206 (35), 191 (10), 178 (48), 160 (38), 146 (18), 134 (16), 132 (12), 107 (100), 43 (42); HREIMS *m/z* 763.2654 (calcd for $C_{36}H_{45}O_{17}N$, 763.2687).

Wilfordinine B (**4**): amorphous powder; $[\alpha]^{25}_D$ +43.5° (*c* 1.1, MeOH); UV (MeOH) λ_{max} (log ϵ) 262 (3.39) nm; IR (KBr) ν_{max} 3475, 2981, 2367, 1752, 1656, 1639, 1594, 1459, 1372, 1235, 1157, 1122, 1097, 1049, 1010, 977, 945, 906, 603 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 821 $[M]^+$ (100), 806 $[M - Me]^+$ (6), 762 $[M - OAc]^+$ (18), 748 (11), 658 (6), 222 (9), 208 (7), 194 (10), 176 (22), 150 (26), 134 (16), 124 (7), 107 (13), 83 (8), 43 (20); HREIMS *m/z* 821.2747 (calcd for $C_{38}H_{47}O_{19}N$, 821.2742).

Wilfordinine C (**5**): amorphous powder; $[\alpha]^{25}_D$ +39.1° (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 263 (3.50), 228 (4.11) nm; IR (KBr) ν_{max} 3570, 3449, 2929, 2371, 1752, 1656, 1649, 1639, 1630, 1595, 1459, 1372, 1245, 1157, 1098, 1053, 714 cm^{-1}; ^1H NMR ($CDCl_3$), see Table 1; ^{13}C NMR ($CDCl_3$), see Table 2; EIMS *m/z* 883 $[M]^+$ (100), 868 $[M - Me]^+$ (6), 824 (21), 810 (11), 222 (12), 206 (15), 194 (14), 176 (32), 160 (14), 150 (32), 134 (22), 124 (12), 105 (91), 93 (10), 43 (27); HREIMS *m/z* 883.2852 (calcd for $C_{43}H_{49}O_{19}N$, 883.2899).

Biological Testing. Anti-HIV assays were performed as described previously.[12−14]

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