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Phenolic aporphine-benzylisoquinoline alkaloids from *Thalictrum faberi*

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Abstract

From the roots of *Thalictrum faberi*, six new phenolic aporphine-benzylisoquinoline alkaloids, 3-hydroxy-6′-desmethyl-9-*O*-methylthalifaboramine (**1**), 3-hydroxythalifaboramine (**2**), 6′-desmethylthalifaboramine (**3**); 3,5′-dihydroxythalifaboramine (**4**), 5′-hydroxythalifaboramine (**5**) and 3-hydroxy-6′-desmethylthalifaboramine (**6**) were isolated. Their structures were established through the use of one- and two-dimensional NMR techniques. All of the tested alkaloids showed potent cytotoxic and antimalarial activities.

Keywords: Thalictrum faberi; Ranunculaceae; Phenolic aporphine-benzylisoquinoline alkaloids; 3-Hydroxy-6′-desmethyl-9-*O*-methylthalifaboramine; 3-Hydroxythali-faboramine; 6′-Desmethylthalifaboramine; 3,5′-Dihydroxythalifaboramine; 5′-Hydroxy-thalifaboramine; 3-Hydroxy-6′-desmethylthalifaboramine; Spectral assignments; Biological evaluation; Cytotoxicity; Antimalarial activity

1. Introduction

In a previous report (Lin et al., 1994), we presented the isolation of thalifaberidine, a cytotoxic thalifaberine-type alkaloid from *Thalictrum faberi* Ulbr. (Ranunculaceae), a perennial herb indigenous to China, which is used in Chinese folk medicine as an antiphlogistic and for the treatment of stomach cancer. The plant produces several types of bisbenzylisoquinoline and aporphine-benzylisoquinoline alkaloids (Lin et al., 1980, 1981; Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987). The bisbenzylisoquinoline alkaloids are structurally and pharmacologically a very interesting alkaloid group and the aporphine-benzylisoquinoline alkaloids are a rare type of alkaloid (Buckingham et al., 1987). Continuing work on the alkaloids of this plant afforded several additional new aporphine-benzylisoquinoline alkaloids. In this report, we present the isolation and structure determination of six new phenolic thalifaberine-type aporphine-benzylisoquinoline alkaloids, 3-hydroxy-6′-desmethyl-9-*O*-methylthalifaboramine (**1**), 3-hydroxythalifaboramine (**2**), 6′-desmethylthalifaboramine (**3**), 3,5′-dihydroxythalifaboramine (**4**), 5′-hydroxythalifaboramine (**5**) and 3-hydroxy-6′-desmethylthalifaboramine (**6**), as well as an evaluation of the biological activity of these alkaloids. The structures and NMR spectral assignments of these alkaloids were made by using a combination of COSY, ROESY (Bothner-By et al., 1984; Summers, Marzilli, & Bax, 1986; Griesinger, & Ernst, 1987), HMQC (Bax, & Subramanian, 1986), and HMBC (Bax, & Summers, 1986; Bax et al., 1986) NMR techniques, together with mass spectrometry and a comparison of their NMR data with those of other thalifaberine-type alkaloids.

2. Results and discussion

The phenolic alkaloid extract of the roots of *Thalictrum faberi* displayed both cytotoxic and antimalarial activities and was subjected to column chromatography followed by preparative TLC to yield alkaloids **1**–**6**.

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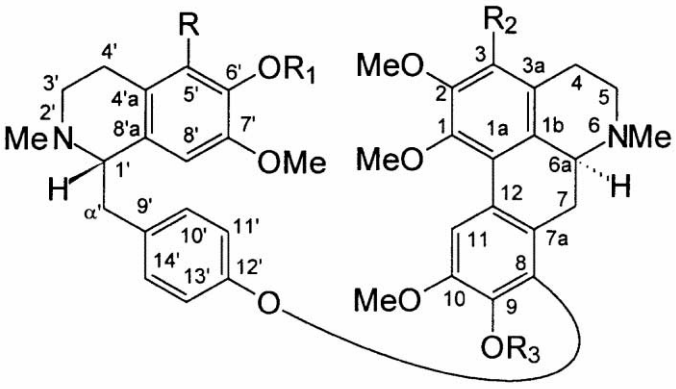

1: R = R$_1$ = H, R$_2$ = OH, R$_3$ = Me

2: R = R$_3$ = H, R$_1$ = Me, R$_2$ = OH

3: R = R$_1$ = R$_2$ = R$_3$ = H

4: R = R$_2$ = OH, R$_1$ = Me, R$_3$ = H

5: R = OH, R$_1$ = Me, R$_2$ = R$_3$ = H

6: R = R$_1$ = R$_3$ = H, R$_2$ = OH

7: R = R$_2$ = R$_3$ = H, R$_1$ = Me

Alkaloid **1**, a yellow solid, $C_{39}H_{44}N_2O_8$ (HRMS), showed UV, ^{1}H, ^{13}C, DEPT, COSY and ROESY NMR spectra for a thalifaberine-type alkaloid with five methoxy and two phenolic functions. The base peak of the mass spectrum at *m/z* 192 and the H-8′ signal at δ 5.93, suggested that one phenolic function should be located at C-6′ and the other phenolic function at the C-9 or C-3 positions, with four methoxy functions at C-1, C-2, C-10, C-7′ and the remaining methoxy at either C-3 or C-9 (Lin et al., 1994; Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987; Guinaudeau, Leboeuf, & Cavé, 1979, 1984, 1988; Hussain et al., 1986). In the ROESY spectrum of **1**, a nOe cross peak between H-8′ and the methoxy signal at δ 3.53, led to the assignment of a methoxy function at the C-7′ position. The ROESY spectrum also showed nOe cross peaks between H-11 and the C_{10}-OMe (δ 3.92), H-11 and the 1-OMe (δ 3.78), H-8′ and H-1′ (δ 3.63), H-8′ and H-α′-B (δ 2.75), H-1′ and 2″-NMe (δ 2.48), and H-10′ and H-1′, which were used to assign these signals (Table 1). Integration of these data suggested that this alkaloid should be 3-hydroxy-6′-desmethyl-9-methylthalifaboramine (**1**). This structure was further confirmed by HMBC and HMQC techniques. The HMQC correlations led to the assignment of all of the proton-attached carbons from the above assigned proton signals (Table 1). The HMBC experiment led to the assignment of the carbons two- and three bonds away from the proton. In the HMBC spectrum, H-11 and a OMe group showed strong three-bond correlations with same oxygenated quaternary carbon, which led to the assignment of this carbon as C-9. This methoxy group at C_9-OMe could not be assigned by the ROESY technique. The proton at C-11 also showed three-bond HMBC cross peaks with C-1a and C-7a, and also a two-bond HMBC cross peak with C-12. H-6a showed HMBC correlations with C-3a, C-7a, 6-NMe and C-7, and H-7 showed HMBC correlations with C-1b, C-8 and C-12. The proton at C-5′ showed HMBC cross peaks with C-7′ and C-8′a, H-8′ showed HMBC correlations with C-6′ and C-4′a, H-1′ showed a HMBC correlation with C-9′, and H-10′ and H-14′ showed correlations with C-12′. Thus, the remaining

Table 1
^{1}H- and ^{13}C NMR data and the major results from the HMBC spectrum of 3-hydroxy-6′desmethyl-9-*O*-methylthalifaboramine (**1**)[a].

	^{13}C	^{1}H	HMBC (H)
1	148.35	–	1-OMe
1a	118.42	–	11
1b	131.68	–	7
2	138.48	–	2-OMe
3	146.05	–	–
3a	116.30	–	6a
4	23.27	2.84 (m), 2.75 (m)	–
5	52.72	2.99 (m), 2.37 (m)	6-NMe, (4)
6-NMe	43.86	2.31 (s)	5, 6a
6a	62.42	2.81 (m)	6-NMe, (7)
7	29.29	3.19 (m), 1.99 (t, 14.0)	(6a)
7a	122.69	–	6a, 11
8	144.88	–	7
9	140.53	–	9-OMe
10	151.85	–	10-OMe
11	108.81	8.01	–
12	128.19	–	7, (11)
1′	65.05	3.63 (m)	2′-NMe
2′-NMe	42.45	2.48 (s)	1′
3′	46.70	3.16 (m), 2.73 (m)	2′-NMe
4′	26.69	2.73 (m), 2.50 (m)	–
4′a	126.31	–	8′
5′	114.19	6.55 (s)	–
6′	143.94	–	8′
7′	144.16	–	5′
8′	110.55	5.93 (s)	–
8′a	128.19	–	5′, α′
α′	40.42	3.07 (m), 2.75 (m)	–
9′	133.02	–	1′, 11′, 13′
10′	130.91	6.95 (d, 8.5)	α′, (11′), 14′
11′	114.83	6.76 (d, 8.5)	(10′), 13′
12′	157.12	–	10′, 14′
13′	114.83	6.76 (d, 8.5)	11′, (14′)
14′	130.91	6.95 (d, 8.5)	α′, 10′, (13′)
1-OMe	60.32	3.78 (s)	–
2-OMe	61.13	3.96 (s)	–
9-OMe	60.97	3.78 (s)	–
10-OMe	56.23	3.92 (s)	–
7′-OMe	55.68	3.53 (s)	–

[a] Recorded in C*D*Cl$_3$, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^{1}H, and 125.8 MHz for ^{13}C; signal multiplicity, coupling constants (Hz), and two-bond correlations of proton and carbon are shown in parentheses.

Table 2
^{1}H- and ^{13}C NMR spectral data and the major results from the HMBC spectrum of 3-hydroxythalifaboramine (**2**)[a].

	^{13}C	^{1}H	HMBC (H)
1	148.70	–	1-OMe
1a	118.58	–	11
1b	131.22	–	7
2	138.49	–	2-OMe
3	145.64	–	–
3a	116.23	–	6a
4	23.20	2.87 (m), 2.73 (m)	–
5	52.68	3.01 (dd, 5.5, 10.0), 2.37 (dd, 4.0, 11.5)	6-NMe
6-NMe	43.73	2.28 (s)	5, 6a
6a	62.36	2.82 (m)	6-NMe, (7)
7	29.28	3.21 (m), 2.01 (t, 14.0)	(6a)
7a	122.78	–	6a, 11
8	138.31	–	7
9	137.35	–	9-OMe
10	146.15	–	10-OMe
11	107.70	7.81	–
12	124.25	–	7, (11)
1′	64.97	3.66 (m)	2′-NMe
2′-NMe	42.21	2.52 (s)	1′
3′	46.48	3.18 (m), 2.84 (m)	2′-NMe
4′	26.66	2.75 (m), 2.59 (dt, 16.0, 4.0)	–
4′a	125.12	–	8′
5′	111.17	6.54 (s)	–
6′	147.52	–	8′
7′	146.42	–	5′
8′	111.17	5.98 (s)	–
8′a	128.17	–	5′, α′
α′	40.42	3.14 (dd, 4.5, 13.0), 2.72 (m)	–
9′	132.86	–	1′, 11′, 13′
10′	130.99	6.97 (d, 8.5)	α′, (11′),14′
11′	114.80	6.81 (d, 8.5)	(9′), 13′
12′	156.70	–	10′, 14′
13′	114.80	6.81 (d, 8.5)	11′, (14′)
14′	130.99	6.97 (d, 8.5)	α′, 10′, (13′)
1-OMe	60.49	3.75 (s)	–
2-OMe	61.14	3.98 (s)	–
10-OMe	56.36	3.96 (s)	–
6′-OMe	55.76	3.82 (s)	–
7′-OMe	55.61	3.55 (s)	–

[a] Recorded in C*D*Cl$_3$, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^{1}H, and 125.8 MHz for ^{13}C; signal multiplicity, coupling constants (Hz), and two-bond correlations of proton and carbon are shown in parentheses.

oxygenated aromatic quaternary carbon should be C-3 bearing a phenolic function. An analysis (Table 1) of the correlations in the HMBC spectrum confirmed the structure deduced from the analysis of ROESY, MS and proton NMR data, and led to the complete ^{13}C NMR assignments as shown in Table 1.

Alkaloid **2**, a yellow solid, $C_{39}H_{44}N_2O_8$ (HRMS) and a minor thalifaberine-type alkaloid of this plant, showed a base peak at *m*/*z* 206 in its mass spectrum, indicating that two methoxy groups were located at C-6′ and C-7′. Thus the remaining three methoxy groups might be at the C-1, C-10 and C-2 positions and two phenolic functions might be located at the C-9 and C-3 positions (Lin et al., 1994; Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987; Guinaudeau, Leboeuf, & Cavé, 1979, 1984, 1988; Hussain et al., 1986). The ^{1}H, COSY and ROESY NMR spectra of this isolate further supported this suggestion. In the ROESY spectrum of **2**, a nOe cross peak between H-8′ and the methoxy signal at δ 3.55, led to the assignment of a methoxy function at the C-7′ position. The ROESY spectrum also showed nOe cross peaks between H-11 and the C_{10}-OMe (δ 3.96), H-11 and the C_1-OMe (δ 3.75), H-8′ and H-1′ (δ 3.66), H-8′ and H-α′-B (δ 2.72), H-1′ and 2′-NMe (δ 2.52) and H-10′ and H-1′, which were used to assign these resonances (Table 2) and identified this alkaloid as 3-hydroxythalifaboramine (**2**). The HMQC spectrum directly confirmed the presence of one phenolic function at the C-9 position. Analysis of the HMBC (Table 2) and HMQC spectra of this isolate led to the establishment of the structure from the ROESY, MS and proton NMR data, and to the complete carbon NMR assignments of this alkaloid as shown in Table 2.

6′-Desmethylthalifaboramine (**3**), a yellow solid, $C_{38}H_{42}N_2O_7$ (HRMS), showed UV, ^{1}H, ^{13}C, DEPT, COSY, ROESY and mass spectra for a thalifaberine-type alkaloid with four methoxy and two phenolic functions (Lin et al., 1994; Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987; Guinaudeau, Leboeuf, & Cavé, 1979, 1984, 1988; Hussain et al., 1986). Similarly to alkaloid **1**, the base peak at *m*/*z* 192 and the H-8′ signal at δ 5.92, suggested that one of the phenolic functions should be located at C-6′. From the presence of an aromatic proton signal δ 6.59 (H-3), the remaining phenolic function should be located to C-9. Thus, the four methoxy functions are at the C-1, C-2, C-10 and C-7′ positions. In the HMBC spectrum, H-8′ showed a correlation with the C-6′ carbon, but this carbon showed no correlations with any of the methoxy groups, thereby placing a phenolic function at C-6′. Unlike alkaloids **1** and **2**, this isolate has a H-3 proton which showed a HMBC correlation with C-1 (δ 144.19), which was also correlated the C-1 methoxy signal at δ 3.70. The major results of the HMBC spectrum of **3** are listed in Table 3, together with complete NMR assignments.

Alkaloid **4**, a yellow solid, $C_{39}H_{44}N_2O_9$ (HRMS), is also a thalifaberine-type alkaloid with two *N*-methyl and five methoxy groups and three phenolic functions based on the analysis of its UV, ^{1}H NMR, COSY and ROESY and mass spectra. The base peak at *m*/*z* 222 and the H-8′ signal at δ 5.69, suggested that one phenolic function should be located at C-5′, with the other phenolic functions at C-9 and C-3, and the five methoxy functions at C-1, C-2, C-10, C-6′ and C-7′ (Lin et al., 1994; Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987; Guinaudeau, Leboeuf, & Cavé, 1979, 1984, 1988; Hussain et al., 1986). In the ROESY spectrum of **4**, a nOe cross peak between H-8′ and the methoxy signal

Table 3
^{1}H- and ^{13}C spectral NMR data and the major results from the HMBC spectrum of 6′-desmethylthalifaboramine (**3**)[a].

	^{13}C	^{1}H	HMBC (H)
1	144.19	–	3, 1-OMe
1a	123.67	–	11
1b	127.15	–	3, 7
2	151.81	–	2-OMe
3	110.49	6.59	–
3a	128.69	–	6a
4	24.99	2.84 (m), 2.75 (m)	–
5	53.13	2.99 (m), 2.35 (m)	6-NMe
6-NMe	43.81	2.30 (s)	5, 6a
6a	62.09	2.81 (m)	6-NMe, (7)
7	26.85	3.22 (dd, 4.0, 14.0), 2.00 (t, 14.0)	(6a)
7a	122.69	–	11
8	138.37	–	7
9	138.15	–	11
10	146.13	–	10-OMe, (11)
11	108.36	8.01	–
12	123.62	–	(11)
1′	64.90	3.63 (m)	2′-NMe
2′-NMe	42.43	2.48 (s)	1′
3′	46.52	3.13 (m), 2.72 (m)	2′-NMe
4′	29.12	2.71 (m), 2.50 (m)	–
4′a	126.18	–	8′
5′	114.23	6.54 (s)	–
6′	143.83	–	8′
7′	144.14	–	5′
8′	110.55	5.92 (s)	–
8′a	128.13	–	5′, α′
α′	40.31	3.08 (m), 2.75 (m)	–
9′	133.34	–	1′, 11′, 13′
10′	130.68	6.93 (d, 8.5)	α′, (11′),14′
11′	114.63	6.77 (d, 8.5)	(10′), 13′
12′	156.50	–	10′, 14′
13′	114.63	6.77 (d, 8.5)	11′, (14′)
14′	130.68	6.93 (d, 8.5)	α′, (13′), 14′
1-OMe	60.21	3.70 (s)	–
2-OMe	55.76	3.89 (s)	–
10-OMe	56.14	3.92 (s)	–
7′-OMe	55.55	3.53 (s)	–

[a] Recorded in C*D*Cl$_3$, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^{1}H, and 125.8 MHz for ^{13}C; signal multiplicity, coupling constants (Hz), and two-bond correlations of proton and carbon are shown in parentheses.

at δ 3.57, led to the assignment of a methoxy function at the C-7′ position. The ROESY spectrum also showed nOe cross peaks between H-11 and the C_{10}-OMe (δ 3.97), H-11 and the C_1-OMe (δ 3.76), H-8′ and H-1′ (δ 3.62), H-8′ and H-α′-B (δ 2.76), H-1′ and 2′-NMe (δ 2.49), and H-10′ and H-1′, which were used to assign these resonances (Table 4). This alkaloid is therefore 3,5′-dihydroxythalifaboramine (**4**).

Alkaloid **5**, a yellow solid, $C_{39}H_{44}N_2O_8$ (HRMS), was assigned the structure 5′-hydroxythalifaboramine (**5**) by comparison of its 1D and 2D proton NMR spectra with those of **4** and the parent compound thalifaboramine (**7**). Both alkaloids have the same base peak at *m*/*z* 222, indicating they have the same isoquinoline moiety, *i.e.*, a phenolic function at C-5′ and methoxy groups at the C-6′ and C-7′ positions (Lin et al., 1994; Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987; Guinaudeau, Leboeuf, & Cavé, 1979, 1984, 1988; Hussain et al., 1986). This alkaloid has one aromatic proton signal more and one phenolic function less than that of **4**, indicating that no hydroxy function is present at the C-3 position. The ROESY spectrum of this isolate showed nOe cross peaks between H-3 and C_2-OMe, H-8′ and 7′-OMe, H-11 and C_{10}-OMe and C_1-OMe, H-8′ and H-1′ (δ 3.68), H-8′ and H-α′-B (δ 2.76), H-1′ and 2′-NMe (δ 2.52), and H-10′ and H-1′. Integration of these data led to the unambiguous assignment of these resonances (Table 4) and to structure **5** for this alkaloid.

Alkaloid **6**, a yellow solid, $C_{38}H_{42}N_2O_8$, is a very minor alkaloid of this plant with a base peak of *m*/*z* 192, like **1** and **2**, suggesting that the presence of a hydroxy group at the C-6′ position (Lin et al., 1994;

Table 4
^{1}H NMR spectral data of alkaloids **4**–**6** from *Thalictrum faberi*[a,b].

^{1}H	**4**	**5**	**6**
3	–	6.60 (s)	–
4A	2.83 (m)	2.95 (m)	2.85 (m)
4B	2.78 (m)	2.73 (m)	2.73 (m)
5A	3.02 (dd, 5.0, 13.0)	2.98 (m)	2.98 (m)
5B	2.38 (m)	2.44 (m)	2.37 (m)
6-NMe	2.28 (s)	2.28 (s)	2.31 (s)
6a	2.85 (m)	2.86 (m)	2.82 (m)
7A	3.19 (m)	3.22 (m)	3.20 (dd,4.0, 14.0)
7B	1.98 (t, 13.5)	2.01 (t, 14.5)	2.01 (t, 14.0)
11	7.81 (s)	8.02 (s)	7.82 (s)
1′	3.62 (m)	3.68 (m)	3.66 (m)
2′-NMe	2.49 (s)	2.52 (s)	2.51 (s)
3′A	3.16 (m)	3.16 (m)	3.18 (m)
3′B	2.74 (m)	2.82 (m)	2.83 (m)
4′A	2.74 (m)	2.82 (m)	2.74 (m)
4′B	2.45 (m)	2.48 (m)	2.58 (m)
5′	–	–	6.59 (s)
8′	5.69 (s)	5.65 (s)	5.96 (s)
α′A	3.08 (dd, 5.5, 14.0)	3.16 (m)	3.14 (dd,4.5, 13.0)
α′B	2.76 (m)	2.76 (m)	2.78 (m)
10′, 14′	7.00 (d, 8.5)	7.00 (d, 8.0)	6.98 (d, 8.0)
11′, 13′	6.80 (d, 8.5)	6.81 (d, 8.0)	6.81 (d, 8.0)
1-OMe	3.76 (s)	3.70 (s)	3.77 (s)
2-OMe	3.99 (s)	3.89 (s)	3.97 (s)
3-OMe	–	–	–
9-OMe	–	–	–
10-OMe	3.97 (s)	3.94 (s)	3.99 (s)
6′-OMe	3.84 (s)	3.84 (s)	–
7′-OMe	3.57 (s)	3.55 (s)	3.57 (s)

[a] Recorded in C*D*Cl$_3$, chemical shift values are reported as δ values (ppm) from TMS at 500.1 MHz for ^{1}H NMR; signal multiplicity and coupling constants (Hz) are shown in parentheses. [b] In order to distinguish the numbering of protons and carbons with letters, the suffixes A and B are used for the geminal protons of one carbon, and suffixes a and b are used to express some carbons for this kind of alkaloid (Bax et al., 1986; Guinaudeau, Leboeuf, & Cavé, 1979; Guinaudeau, Leboeuf, & Cavé, 1984; Guinaudeau, Leboeuf, & Cavé, 1988).

Table 5
Evaluation of the cytotoxic activity of alkaloids **1**–**5** and the phenolic alkaloid extract (PAE) of *Thalictrum faberi*[a,b].

Cell lines	LU-1	KB	(ED_{50}, μg/ml) KB-V (+VLB)	LNCap	ZR-75-1
Test samples					
PAE	0.8	4.8	8.4	5.1	5.1
1	4.6	2.7	1.3	4.7	1.9
2	3.3	1.8	3.1	6.2	2.8
3	5.4	1.8	1.8	4.0	2.3
4	2.9	0.5	4.1	9.2	3.4
5	3.8	0.8	1.7	11.2	3.0
Control samples					
Colchicine	0.2	0.2	0.6	0.06	0.1
Ellipticine	0.2	0.04	0.2	0.8	0.9

[a] LU-1 = Human Lung Cancer; KB = Human Oral Epidermoid Carcinoma; KB-V (+VLB) = Vinblastine-resistant KB in the presence of VLB; LNCaP = Hormone-dependent Human Prostatic Cancer; ZR-75-1 = Hormone-dependent Human Breast Cancer.

[b] Positive limits: $ED_{50} < 5.0$ μg/ml for LU-1, KB, KB-V (+VLB), LNCaP and ZR-75-1.

Wagner, Lin, & Seligman, 1984a, 1984b; Lin et al., 1986; Lin, Li, & Wagner, 1987; Guinaudeau, Leboeuf, & Cavé, 1979, 1984, 1988; Hussain et al., 1986). Analysis of the ^{1}H, COSY and ROESY NMR spectra of **6** indicated two phenolic functions at C-9 and C-3, similar to like alkaloids **3** and **4**, suggesting this alkaloid is 3-hydroxy-6′-desmethylthalifaboramine (**6**). A comparison of the NMR spectra of this compound with the spectra of alkaloids **1**–**5**, led to the assignment of the ^{1}H NMR spectra of **6**, which are shown in Table 4.

Alkaloids **1**–**5** were subjected to HIV-1 reverse transcriptase inhibitory and cytotoxicity evaluation, and alkaloids **1**, **2** and **3** were also subjected to antimalarial testing. All of the tested alkaloids showed activity in the latter two tests (see Table 5 and Table 6), however, none showed activity in the HIV-1 RT test. Similar to other thalifaberine-type aporphine-benzylisoquinoline alkaloids isolated from this plant, nearly all of these alkaloids showed cytotoxicity, although there appear to be no substantial differences between their activities and structures which would infer structure activity relationships. Moderate selectivity indices (Likhitwitayawuid et al., 1993) were observed for the alkaloids **1**–**3** when the antimalarial and cytotoxic activities were compared.

Table 6
Evaluation of the antimalarial activity of the alkaloids **1**–**3** and the phenolic alkaloid extract (PAE) of *Thalictrum faberi*[a].

Compounds	ED_{50}[c]	D-6	W-2	Selectivity index[b] D-6	W-2
Test materials					
PAE		184	203	26.1	23.6
1	2,700	112	24.2	41.1	65.7
2	1,800	176	10.2	54.2	33.2
3	1,800	152	11.2	192	9.4
Control compounds					
Quinine	> 20,000	11.1	37.2	> 1,800	> 540
Chloroquine	> 17,400	3.5	83.3	5,440	210
Mefloquine	3,500	3.7	0.9	950	3,890
Artemisinin	> 20,000	1.7	1.5	> 11,760	> 13,330

[a] Chloroquine-sensitive (D-6) and chloroquine-resistant (W-2) clones of *Plasmodium falciparum*. IC_{50} in ng/ml.

[b] KB ED_{50} (in ng/ml)/malaria IC_{50} (in ng/ml); see (Likhitwitayawuid et al., 1993) for details.

[c] ED_{50} values for KB are expressed in ng/ml.

3. Experimental

The optical rotations were measured in MeOH. UV spectra were taken in MeOH. IR spectra were recorded in a KBr pellet. Solutions in $CDCl_3$ were used for all of the NMR studies. ^{1}H NMR, COSY and ROESY spectra were recorded at 500.1 MHz. ^{13}C NMR and DEPT spectra were recorded at 125.8 MHz. HMQC and HMBC spectra were obtained at 500.1/125.8 MHz and $^nJ_{CH} = 7.5$ Hz was used in the HMBC experiments.

3.1. Plant material

The plant material of *T. faberi* was collected in the Huangshan Mountain region, Anwei Province, P.R. China, in September, 1986 and was identified by Dr. X.-L. Huang, Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P.R. China. A voucher sample is deposited in the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, P.R. China.

3.2. Extraction and separation

A phenolic base extract (12 g) was obtained from the roots of this plant according to the procedure described previously (Lin et al., 1994; Wagner, Lin, & Seligman, 1984). This extract was subjected to cc, eluted with $CHCl_3$ and $CHCl_3$–MeOH mixtures of increasing polarity (1, 2, 5, 10, 20, 50 and 100% MeOH) and, finally, 1% HCl soln, monitored by TLC and the combined fractions were sepd by prep TLC, using cyclohexane–EtOAc–diethylamine (6:4:1 to 4:8:1, v/v) to yield **1**, **2**, **3**, **4**, **5**, **6** and several other alkaloids reported separately.

3.3. Isolation of 3-hydroxy-6′-desmethyl-9-O-methylthalifaboramine (**1**)

Alkaloid **1** was obtained as a yellow powder (22 mg, 0.00001%), $[\alpha]_D$ +85.6° (c, 0.005, MeOH), UV (MeOH) λ_{max} (log ϵ): 284 (4.00), 314sh (3.85) nm; ^{1}H and ^{13}C NMR data, see Table 1; EIMS *m/z* (100%): 667 (M^+–H, 0.3), 476 (3%) and 192 (100); HRMS: obsvd. 667.3012 for $C_{39}H_{43}N_2O_8$, calc. 667.3020.

3.4. Isolation of 3-hydroxythalifaboramine (**2**)

Alkaloid **2** was obtained as a yellow powder (9 mg, 0.0000045%), $[\alpha]_D$ +92.3° (c, 0.007, MeOH), UV (MeOH) λ_{max} (log ϵ): 282 (4.07), 314sh (3.85) nm; ^1H and ^{13}C NMR data, see Table 2; EIMS *m/z* (100%): 667 (M^+–H, 0.3, for $C_{39}H_{44}N_2O_8$), 462 (3%) and 206 (100).

3.5. Isolation of 6′-desmethylthalifaboramine (**3**)

Alkaloid **3** was obtained as a yellow powder (20 mg, 0.00001%), $[\alpha]_D$ +65.5° (c, 0.005 MeOH), UV (MeOH) λ_{max} (log ϵ): 284 (4.05), 314sh (3.80) nm; ^1H and ^{13}C NMR data, see Table 3; EIMS *m/z* (100%): 638 (M^+, 0.3), 446 (3%) and 192 (100); HRMS: obsvd. 638.3012 for $C_{38}H_{42}N_2O_7$, calc. 638.3020.

3.6. Isolation of 3,5′-dihydroxythalifaboramine (**4**)

Alkaloid **4** was obtained as a yellow powder (12 mg, 0.000006%), $[\alpha]_D$ +89.8° (c, 0.008, MeOH), UV (MeOH) λ_{max} (log ϵ): 285 (4.08), 314sh (3.88) nm; ^1H NMR data, see Table 4; EIMS *m/z* (100%): 683 (M^+–H, 0.3), 463 (3%) and 222 (100); HRFAB: obsvd. 683.2956 for $C_{39}H_{43}N_2O_9$, calc. 683.2957.

3.7. Isolation of 5′-hydroxythalifaboramine (**5**)

Alkaloid **5** was obtained as a yellow powder (10 mg, 0.000005%), $[\alpha]_D$ +97.3° (c, 0.008, MeOH), UV (MeOH) λ_{max} (log ϵ): 283 (4.06), 314sh (3.82) nm; ^1H NMR data, see Table 4; EIMS *m/z* (100%): 667 (M^+–H, 0.3), 446 (3%) and 222 (100); HRMS: obsvd. 667.3057 for $C_{39}H_{43}N_2O_8$, calc. 667.3020.

3.8. Isolation of 3-hydroxy-6′-desmethylthalifaboramine (**6**)

Alkaloid **6** was obtained as a yellow powder (4 mg, 0.000002%), $[\alpha]_D$ +76.2° (c, 0.002, MeOH), UV (MeOH) λ_{max} (log ϵ): 285 (4.01), 314sh (3.78) nm; ^1H NMR data, see Table 4; EIMS *m/z* (100%): 654 (M^+–H, 0.3, for $C_{38}H_{42}N_2O_8$), 462 (3%) and 192 (100).

3.9. Cytotoxicity, antimalarial and HIV-reverse transcriptase inhibitory assays

The biological evaluations for cytotoxicity, antimalarial and HIV–RT inhibitory activities of the alkaloids were carried out according to established protocols (Lin et al., 1993; Likhitwitayawuid et al., 1993; Tan, Pezzuto, & Kinghorn, 1991).

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