Thalifaberidine, a Cytotoxic Aporphine-Benzylisoquinoline Alkaloid from Thalictrum faberi

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THALIFABERIDINE, A CYTOTOXIC APORPHINE-BENZYLISOQUINOLINE ALKALOID FROM *THALICTRUM FABERI*

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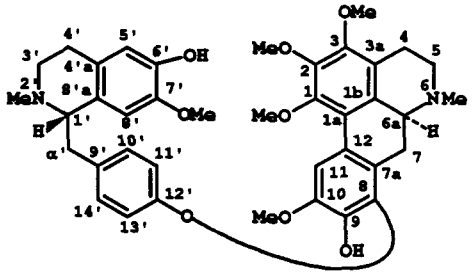
ABSTRACT.—From *Thalictrum faberi*, thalifaberidine [**1**], a new aporphine-benzylisoquinoline alkaloid, together with four known alkaloids, thalifaramine [**2**], thalifaricine [**3**], thalifarazine [**4**], and thalifaronine [**5**], were isolated. Thalifaberidine [**1**] was identified as 6′,8-desmethylthalifaberine, and its ^{1}H- and ^{13}C-nmr data were completely assigned through the use of one- and two-dimensional nmr techniques. Thalifaberidine [**1**], thalifaberine [**6**], and thalifasine [**7**] showed cytotoxic activity against several human cancer cell lines, as well as antimalarial activity.

Thalictrum faberi Ulbr. (Ranunculaceae), a perennial herb indigenous to China, is used in Chinese folk medicine as an antiphlogistic and in the treatment of stomach cancer. Previous phytochemical studies have led to the isolation of 12 aporphine-benzylisoquinoline alkaloids and about ten bisbenzylisoquinoline alkaloids (1–6). Continuing study of the root constituents of this plant led to the isolation of about 20 additional alkaloids. In this report, we present the isolation and structure determination of one new aporphine-benzylisoquinoline alkaloid, thalifaberidine [**1**], together with the isolation of four known alkaloids thalifaramine [**2**], thalifaricine [**3**], thalifarazine [**4**], as well as an evaluation of the biological activity of **1**, and of thalifaberine [**6**] and thalifasine [**7**], the major alkaloids from this plant. The structures and ^{1}H- and ^{13}C-nmr assignments of **1** were made by using the DEPT, COSY, ROESY (7–9), HETCOR, FLOCK (10), and selective INEPT (11,12) nmr techniques, together with mass spectrometry.

RESULTS AND DISCUSSION

The phenolic alkaloid extract of the roots of *T. faberi* was subjected to cc followed by prep. tlc to yield **1** and **3**. The non-phenolic alkaloid extract was treated in the same way to yield **2**, **4**, and **5**. Thalifaberidine [**1**], a yellow solid, $C_{39}H_{44}N_2O_8$ (hrms), $[\alpha]_D$ +87° (c=0.01, MeOH), showed uv absorptions (282, 310 nm) for an aporphine-benzylisoquinoline alkaloid. The ^{1}H-, ^{13}C-, DEPT, COSY, and HETCOR nmr spectra of **1** showed signals for the existence of two *N*-methyl groups, five methoxy groups, two aliphatic methines, seven aromatic methines, six methylenes, and sixteen quaternary carbon atoms, suggesting a benzylisoquinoline-aporphine alkaloid with five methoxy and two phenolic functions. Furthermore, with four aromatic proton signals appearing as an AA′BB′ system at δ 6.77 and 6.93, and the H-11 signal at δ 7.86, the isolate was considered a member of the thalifaberine group, which has an ether linkage between C-8 of the aporphine moiety and C-12′ of the benzylisoquinoline unit (13–15).

The base peak of the mass spectrum at m/z 192, and the H-8′ signal at δ 5.92, 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 five methoxy functions at C-1, C-2, C-10, C-7′, and C-3 or C-9. In the ROESY spectrum of **1**, a nOe contour between H-8′ and the methoxy signal at δ 3.50 led to the assignment of a methoxy function at the C-7′



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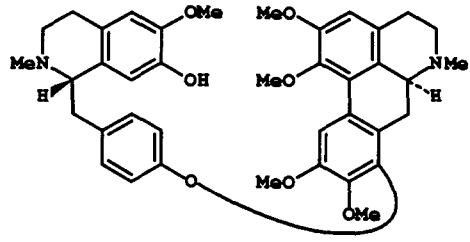

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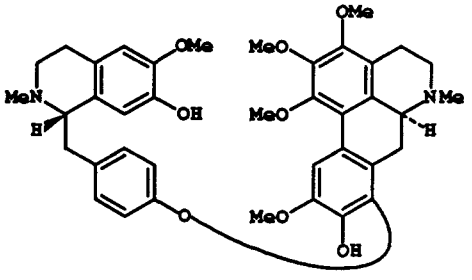

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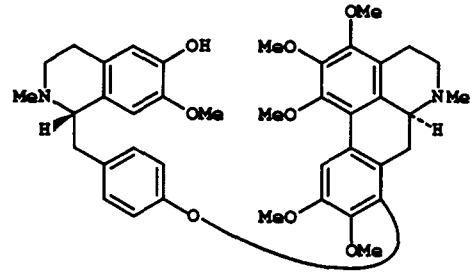

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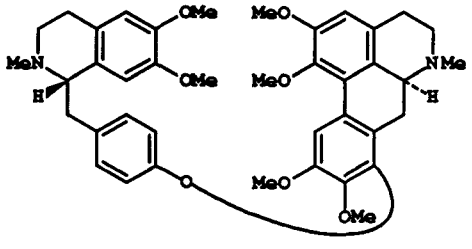

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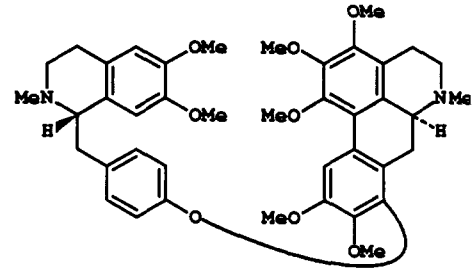

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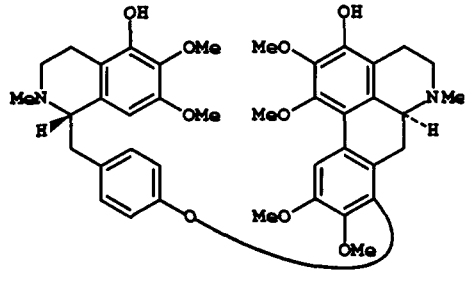

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position. The ROESY spectrum also showed nOe contours between H-11 and the OMe-10 (δ 3.92), H-11 and OMe-1 (δ 3.76), H-8′ and H-1′ (δ 3.66), H-8′ and Hα′-B (δ 2.78), H-1′ and NMe-2′ (δ 2.48), and H-10′ (and/or H-11′) and H-1′, which were used to assign these signals. The above assignments were confirmed by selective INEPT and FLOCK nmr experiments, which also led to the assignment of all the sixteen quaternary carbon atoms. The results of these two experiments are shown in Table 1, and a short explanation of the assignments is presented here.

TABLE 1. ^{1}H- and ^{13}C-Nmr Data and Major Observations from the FLOCK and Selective INEPT Nmr Spectra of Thalifaberidine [**1**].[a,b]

	^{1}H	^{13}C	FLOCK (Carbon)	Selective INEPT (Carbon)
1	—	149.14	—	—
1a	—	122.36	—	—
1b	—	130.81	—	—
2	—	145.11	—	—
3	—	149.61	—	—
3a	—	122.03	—	—
4A	2.84 (m)	23.41	n.o	3, 1b, (3a), (5)
4B	2.75 (m)		n.o	3, 1b, (3a), (5)
5A	2.99 (m)	52.69	4	3a, (4), 6-NMe, 6a
5B	2.35 (m)		n.o	3a, (4), 6-NMe, 6a
6-NMe	2.28 (s)	43.70	5, 6a	5, 6a
6a	2.81 (m)	62.33	(7)	5, (7), 1a, 3a, 7a
7A	3.20 (dd, 4.0, 14.0)	26.38	8, 12, 1b, (6a), (7a)	8, 12, 1b, (6a), (7a)
7B	2.00 (t, 14.0)		8, 12, 1b, (6a), (7a)	8, 12, 1b, (6a), (7a)
7a	—	122.03	—	—
8	—	138.49	—	—
9	—	137.99	—	—
10	—	146.37	—	—
11	7.86 (s)	107.85	9, (10), (12), 1a, 7a	9, (10), (12), 1a, 7a
12	—	123.55	—	—
1′	3.63 (m)	64.75	(α′)	(8′), 2′-NMe, 4′a, (8′a), (α′)
2′-NMe	2.48 (s)	43.03	1′, 3′	1′, 3′
3′A	3.13 (m)	46.11	1′	1′, 4′a, 2′-NMe
3′B	2.72 (m)		n.o	1′, 4′a, 2′-NMe
4′A	2.71 (m)	24.52	n.o	(3′), 5′, (4′a), 8′a
4′B	2.50 (m)		n.o	(3′), 5′, (4′a), 8′a
4′a	—	125.67	—	—
5′	6.51 (s)	114.38	4′, 7′, 8′a	4′, 7′, 8′a
6′	—	144.02	—	—
7′	—	144.25	—	—
8′	5.92 (s)	110.58	1′, 6′, 4′a	1′, 6′, 4′a
8′a	—	127.48	—	—
α′A	3.08 (dd, 4.5, 13.5)	40.19	n.o	(1′), (9′), 10′, 14′, 8′a
α′B	2.78 (m)		n.o	(1′), (9′), 10′, 14′, 8′a
9′	—	133.00	—	—
10′	6.93 (d, 8.5)	114.58	(11′), (12′), (α′)	(11′), 12′, (α)
11′	6.77 (d, 8.5)	130.84	9′, (10′)	9′, (10′)
12′	—	156.55	—	—
13′	6.77 (d, 8.5)	130.84	9′, (14′)	9′, (14′)
14′	6.93 (d, 8.5)	114.58	12′, (13′), (α′)	12′, (13′), (α′)
1-OMe	3.76 (s)	60.54	1	1
2-OMe	3.95 (s)	60.90	2	2
3-OMe	3.86 (s)	60.26	3	3
10-OMe	3.92 (s)	56.10	10	10
7′-OMe	3.50 (s)	55.38	7′	7′

[a]Recorded in $CDCl_3$, 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; n.o. indicates no clear correlations with carbons were observed on the irradiation of this proton.

[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 (13–15).

The alkaloid has an aporphine and a benzylisoquinoline unit, and each unit has one N-Me, three methylenes and one aliphatic methine function, which were distinguished by the above-mentioned nmr techniques. Starting from H-11 of the aporphine moiety, which showed a characteristic down-field resonance at δ 7.86, the OMe-1 and OMe-10

were assigned by the ROESY nmr experiment, and C-7a, C-9, C-1b, C-12, and C-10 were attributed by selective INEPT and FLOCK experiments. Carbon-10 was unambiguously assigned by the selective INEPT technique and the FLOCK correlation with OMe-10.

Both C-7a and C-12 showed selective INEPT correlations with one of the methylene signals at δ 2.00, which was quite separate from the other aliphatic proton signals, and could be assigned as H-7A. This proton showed COSY correlations with its geminal partner H-7B at δ 3.20 and with H-6a at δ 2.81. From H-6a, the ^{13}C-nmr signal for NMe-6 was assigned, and the ^{1}H-nmr signal of NMe-6 was thus attributed from the HETCOR spectrum. The NMe-6 showed FLOCK and selective INEPT correlations with C-5, and this led to the assignment of the C-5 and H-5 methylene proton signals; the latter showed COSY correlations with the H-4 methylene protons. From an analysis of the selective INEPT correlations between these protons and carbons, as shown in Table 1, eleven quaternary carbons of the aporphine moiety were unambiguously assigned. For example, C-2 and C-3 are oxygen-attached quaternary carbons, and only C-3 exhibited selective INEPT correlations with the H-4 protons, and thus, was distinguished from C-2. Five non-oxygen-attached carbons were distinguished according to their correlations with different protons, i.e., C-1a showed correlations with H-11 and H-6a, C-1b showed correlations with H-6a, H-7, and the H-4 protons, C-3a showed correlations with the H-4, H-5, and H-6a protons, C-12 showed correlations with H-11 and the H-7 protons, and C-7a showed correlations with the H-11, H-7, and H-6a protons, and thus these carbons were assigned unambiguously. Consequently, this unit has four methoxy functions at the C-1, C-2, C-3, and C-10 positions, one phenolic function at C-9, and the ether linkage at C-8, as do all of the thalifaberine-type alkaloids (14,15). Treated in the same way, the remaining ^{1}H- and ^{13}C-nmr resonances were unambiguously assigned to the protons and carbons of the benzylisoquinoline unit, as shown in Table 1. This unit has a methoxy at C-7′, a phenolic function at C-6′ and the ether linkage at the C-12′ position. Thus, this isolate is 6′,8-demethylthalifaberine, and was named thalifaberidine, with the structure **1**.

The other four isolates were identified as thalifaramine [**2**], thalifaricine [**3**], thalifarazine [**4**], and thalifaronine [**5**] by comparison of their specific rotations, uv, ^{1}H-nmr, and mass spectra to literature values (15,16).

The phenolic alkaloid extract and thalifaberidine [**1**] were subjected to biological evaluation for cytotoxic, antimalarial, and HIV-reverse transcriptase inhibitory activities (17–19). All displayed significant cytotoxic activities as shown in Table 2. In order to obtain some information about structural modification and biological activity, thalifaberine [**6**] and thalifasine [**7**], both of which were previously isolated from the same plant and have the same structural skeleton (3,4), were subjected to the above evaluation, and also showed demonstrable cytotoxicity (Table 2). Among the alkaloids tested, thalifasine [**7**] shows the strongest antimalarial activity (Table 3). The extract showed weak activity (IC_{50} = 139 μg/ml) in the HIV-reverse transcriptase inhibitory test (19), but alkaloids **1**, **6**, and **7** were inactive. Furthermore, alkaloids **2**–**7** and the other six thalifaberine-type alkaloids, that is, thalifabine, thalifarapine, thalifabatine, dehydrothalifaberine, thalifaboramine, thalifalandine (3–6) isolated from this plant, showed cytotoxic activity against the P-388 cell line with ED_{50} values in the range 1.0–1.5 μg/ml (J.-L. Yang, J.-H. Han, and L.-Z. Lin, unpublished results). From the preliminary results, there were no remarkable differences of activity evident in the exchange of phenolic and methoxy functions, or in the presence of oxygenated functions at the C-3 and C-5′ positions. Additional studies on alkaloids of this class are currently underway.

TABLE 2. Evaluation of the Cytotoxic Activity of Thalifaberidine [**1**], Thalifaberine [**6**], Thalifasine [**7**], and the Phenolic Alkaloid Extract (PAE) of *Thalictrum faberi*.[a]

Cell Lines[a]	Compound (ED_{50}, μg/ml)			
	1	**6**	**7**	PAE
BCA-1	3.9	5.4	2.4	n.t.[b]
HT-1080	3.1	2.6	1.3	n.t.
Lu-1	5.4	16.3	2.0	0.8
Col-2	3.2	1.6	2.1	n.t.
KB	5.7	15.5	4.6	4.8
KB-V (+VLB)	17.7	4.6	5.7	8.4
KB-V (−VLB)	7.8	1.2	1.6	>20
P-388	0.6	1.3	1.2	n.t.
A431	6.7	4.9	8.2	n.t.
LNCaP	3.7	5.7	3.3	5.1
ZR-75-1	1.5	3.7	1.2	5.1
U373	1.5	4.1	2.2	n.t.

[a]BCA-1=human breast cancer; HT-1080=human fibrosarcoma; Lu-1=human lung cancer; Col-2=human colon cancer; KB=human oral epidermoid carcinoma; KB-V=vinblastine-resistant KB tested in the presence (+VLB) or absence (−VLB) of 1 μg/ml vinblastine; P-388=murine lymphoid leukemia; A431=human epidermoid carcinoma; LNCaP=hormone-dependent human prostatic cancer; ZR-75-1=hormone-dependent human breast cancer; U373=human glioblastoma.

[b]n.t. indicates the test was not performed with the phenolic alkaloid extract.

Thus far, about 60 oxygen-bonded aporphine-benzylisoquinoline dimers have been reported in the literature without ^{13}C-nmr assignments (13–15). Except for a preliminary report about the ^{13}C-nmr assignments of thalifaberine (21), this is the first report of the complete ^{13}C-nmr assignments for a member of this alkaloid class.

TABLE 3. Evaluation of the Antimalarial Activity of the Alkaloids Thalifaberidine [**1**], Thalifaberine [**6**], and Thalifasine [**7**], and the Phenolic Alkaloid Extract (PAE) of *Thalictrum faberi*.[a]

Compounds	*P. falciparum* clone (ED_{50}, ng/ml)		Selectivity Index[b]	
	D-6	W-2	D-6	W-2
Test Materials				
Thalifaberidine [**1**]	880	122	6.5	47
Thalifaberine [**6**]	5,090	441	3.0	35
Thalifasine [**7**]	238	49.3	19	93
PAE	184	203	26.1	23.6
Control Compounds				
Quinine	20.8	59.2	>960	>340
Chloroquine	3.5	36.5	4,970	480
Mefloquine	8.9	1.9	393	1,840
Artemisinin	1.1	0.4	>1,820	>50,000

[a]Chloroquine-sensitive (D-6) and chloroquine-resistant (W-2) clone of *Plasmodium falciparum*.

[b]KB ED_{50} (in ng/ml)/malaria IC_{50} (in ng/ml); see Likhitwitayawuid *et al.* (18) for details.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler hot-stage apparatus and are uncorrected. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. Uv spectra were taken in MeOH on a Beckman DU-7 spectrometer. Ir spectra were recorded in a KBr pellet on a Midac Ft-ir interferometer. Solutions in $CDCl_3$ were used for all the nmr studies. ^{1}H-Nmr, COSY, and ROESY nmr

spectra were recorded at 500.1 MHz with a GE Omega 500 instrument, using GE standard programs; ^{13}C-nmr and DEPT spectra were recorded at 125.8 MHz; HETCOR and FLOCK spectra were obtained at 500.1/125.8 MHz with a GE Omega 500 instrument, using standard programs from the GE library, and $^{n}J_{CH}=6$ Hz was used in the FLOCK experiment. Selective INEPT spectra were taken on a Nicolet NT-360 spectrometer operating at 90.8 MHz with $J=8$ or 6 Hz for aromatic protons and $J=6$ Hz for aliphatic protons. Ei mass spectra and high-resolution mass spectra were recorded on a Finnigan MAT-90 instrument.

PLANT MATERIAL.—The plant material of *T. faberi* was collected in Huangshan Mountain, Anwei Province, People's Republic of China, in September 1986, and identified by Dr. X.-L. Huang, Department of Phytochemistry, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China. A voucher sample is deposited in the herbarium of the Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai, People's Republic of China.

EXTRACTION AND SEPARATION.—The dry powdered roots (20 kg) of *T. faberi* were percolated with EtOH (240 liters) at room temperature. Evaporation of the solvent under reduced pressure afforded a thick residue which was repeatedly triturated with 1% HCl (ca. 20 liters) until the extract showed a weak reaction with Dragendorff's reagent. The acidic solution, after extraction with $CHCl_3$ (10 liters×2), was basified to pH 9 with NH_3 solution, extracted with $CHCl_3$ (10 liters×4). The combined $CHCl_3$ extract was washed twice with H_2O, dried over Na_2SO_4, and evaporated to dryness to yield a crude base (140 g). The base was dissolved in $CHCl_3$ (4 liters), and extracted with 5% NaOH (2 liters×4), and the combined NaOH solution was treated with solid NH_4Cl, then extracted with $CHCl_3$ four times, and the combined $CHCl_3$ solution was washed, dried, and evaporated to give the phenolic base fraction (12 g). This extract was subjected to cc, eluted with $CHCl_3$ and $CHCl_3$/MeOH mixtures of increased polarity (1%, 2%, 5%, 10%, 20%, 50%, and 100% MeOH), and finally 1% HCl solution, monitored by tlc, and the combined fractions were separated by prep. tlc, using cyclohexane-EtOAc-diethylamine (6:4:1 to 4:8:1, v/v) to yield **1** (20 mg, 0.00001%) and **3** (4 mg, 0.000002%). The non-phenolic alkaloid extract was treated in the same way to yield alkaloids **2** (5 mg, 0.0000025%), **4** (4 mg, 0.000002%), and **5** (50 mg, 0.000025%). Compounds **2–5** showed the same physical and spectral data as reported (15,16).

Isolation of thalifaberidine [**1**].—Alkaloid **1** was obtained as a yellow powder (20 mg, 0.00001%); $[\alpha]_D$ +87° (c=0.1, MeOH); uv (MeOH) λ max (log ε) 284 (4.10), 314 sh (3.90) nm; ^{1}H-nmr data, see Table 1; ^{13}C-nmr data, see Table 1; eims *m/z* 666 (M^+-H, 0.3), 446 (3), and 192 (100); hrms: observed 667.3012; calcd for $C_{39}H_{43}N_2O_8$ 667.3020.

Cytotoxicity, Antimalarial, and HIV-Reverse Transcriptase Inhibitory Assays.—The biological evaluation for cytotoxity, antimalarial, and HIV-reverse transcriptase inhibitory activities of the alkaloids were carried out in vitro according to established protocols (17–19).

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