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CYTOTOXIC AND ANTIMALARIAL ALKALOIDS FROM THE TUBERS OF *STEPHANIA PIERREI*[1]

KITTISAK LIKHITWITAYAWUID, CINDY K. ANGERHOFER, HEEBYUNG CHAI, JOHN M. PEZZUTO, GEOFFREY A. CORDELL,*

Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, Illinois 60612

and NIJSIRI RUANGRUNGSI

Department of Pharmacognosy, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand

ABSTRACT.—Biological evaluation of extracts prepared from the tubers of *Stephania pierrei* revealed cytotoxic and antimalarial activity. During the course of separation, two new aporphine alkaloids, (−)-asimilobine-2-*O*-β-D-glucoside [**2**] and (−)-nordicentrine [**8**], in addition to twenty-one known isoquinoline alkaloids, were isolated. Each isolate was assessed for cytotoxic and antimalarial activities. It was found that the cytotoxicity of *S. pierrei* was mainly due to the presence of the aporphine alkaloids containing the 1,2-methylenedioxy group **3–10**, whereas the antimalarial activity was attributed to the nonquaternary aporphine alkaloids **1**, **3–10** and the tetrahydroprotoberberines possessing a phenolic functionality, **13–15**, **18**. None of the isolates showed a degree of selectivity comparable to that of antimalarial drugs such as chloroquine, quinine, mefloquine, and artemisinin. Comparison of the alkaloid content of *S. pierrei* and *Stephania erecta* strongly suggested separate identities for the two plants.

In our previous investigation of the tubers of *Stephania erecta* Craib (Menispermaceae), we described the cytotoxic and antimalarial activities of some bisbenzylisoquinoline alkaloids, including the new alkaloid (+)-2-*N*-methyltelobine (2). However, the identity of *S. erecta* has been controversial, for it has been deemed to be identical with *Stephania pierrei* Diels (3–6), despite some differences in morphology and dissimilarities in their traditional medicinal uses. Although the two plants share several morphological characteristics, the former has been described as an herb with erect stem, while the latter has been recognized as a climbing plant, producing a gigantic tuber (3,4). In terms of medicinal reputation, these plants are also disparate, as reflected by the use of *S. erecta* tubers as a muscle relaxant and an analgesic and the claimed stimulating and aphrodisiac properties of *S. pierrei* tubers in Thai folkloric medicine (6). As a part of our continuing efforts to discover anticancer and antimalarial agents from natural sources, an EtOH extract of *S. pierrei* was tested and found to possess cytotoxic and antimalarial potential (ED_{50} values of 2.2 and 16.9 μg/ml against P-388 and KB cells, respectively; ED_{50} 136 and 161 ng/ml against the D-6 and W-2 strains of *Plasmodium falciparum* respectively). In an attempt to determine the compounds responsible for these biological activities and to clarify the taxonomic issue between these two *Stephania* species, a bioactivity-directed separation of *S. pierrei* tubers was initiated. Repetitive chromatography resulted in the isolation of 23 isoquinoline alkaloids, two of which are new aporphine alkaloids. In this paper, we report the structure elucidation of the new compounds, the cytotoxic and antimalarial activities of the isolates, and the chemical evidence which supports the treatment of the plants as two distinct species.

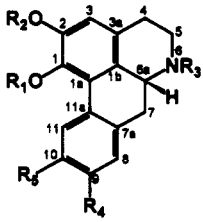
RESULTS AND DISCUSSION

The alkaloids obtained in this study included: (a) the aporphines (−)-asimilobine

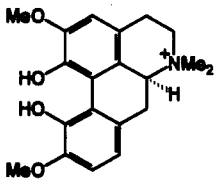
[1]Paper XXIII in the series "Traditional Medicinal Plants of Thailand." For paper XXII, see Likhitwitayawuid *et al.* (1).

[**1**] (7,8), (−)-asimilobine-2-*O*-β-D-glucoside [**2**], (−)-anonaine [**3**] (9), (−)-isolaureline [**4**] (10), (−)-xylopine [**5**] (10), (−)-roemeroline [**6**] (11), (−)-dicentrine [**7**] (12–14), (−)-nordicentrine [**8**], (−)-phanostenine [**9**] (15), cassythicine [**10**] (14,16), and magnoflorine [**11**] (17–20); (b) the tetrahydroprotoberberines (−)-tetrahydropalmatine [**12**] (21–23), (−)-capaurine [**13**] (24–27), (−)-thaicanine [**14**] (23), (−)-corydalmine [**15**] (25,26), (−)-*N*-methyltetrahydropalmatine [**16**] (28), (−)-xylopinine [**17**] (22), and (−)-tetrahydrostephabine [**18**] (29); (c) the tetrahydrobenzylisoquinolines (+)-reticuline [**19**] (30,31), (+)-codamine [**20**] (32,33), and (±)-oblongine [**21**] (34,35); (d) the hasubanan (−)-delavaine [**22**] (36) and the morphinan (−)-salutaridine [**23**] (37,38). Compounds **2** and **8** are hitherto unknown alkaloids.

Alkaloid **2**, in its positive hrfabms, exhibited a $[M+H]^+$ ion at *m/z* 430.1872, corresponding to the formula $C_{23}H_{28}NO_7$ (calcd 430.1866). The uv absorptions at 227, 272, and 306 nm were characteristic of a 1,2-disubstituted aporphine (39). This was further supported by the presence of H-11 at δ 8.29 (dd, J=7.4, 1.4 Hz), and H-8, H-9, and H-10 as multiplets in the region of δ 7.16 to 7.30 in the ^{1}H-nmr spectrum. Further examination of the ^{1}H-nmr spectrum of **2** revealed the presence of a sugar unit with its anomeric proton appearing at δ 4.93 (d, J=7.6 Hz), and, therefore, an aporphine glycoside structure was proposed. In addition, the ^{1}H-nmr spectrum of **2** displayed signals analogous to those of (−)-asimilobine [**1**] with its C-3 proton resonating at δ 7.00 (s) and 1-OMe protons at δ 3.68, suggesting that the aglycone part of **2** was derived from (−)-asimilobine [**1**] and that the sugar unit was attached to the C-2 of the aporphine skeleton. The location of the sugar moiety was confirmed by an nOe observed between H-1′ (δ 4.93) and H-3 (δ 7.00) and between the 1-OMe (δ 3.68) and H-11 (δ 8.29) in the NOESY spectrum. The sugar part was identified as β-D-glucose from the proton signals at δ 4.93 (d, J=7.6 Hz, H-1′), 3.92 (dd, J=12.0, 1.7 Hz, H_a-6′), 3.71 (dd, J=12.0, 5.7 Hz, H_b-6′), 3.54 (dd, J=8.1, 7.6 Hz, H-2′), 3.48 (dd, J=8.1, 8.1 Hz, H-3′), 3.45 (m, H-5′), and 3.40 (dd, J=8.1, 8.1 Hz, H-4′) in the ^{1}H-nmr spectrum, and from the carbon resonances at δ 102.72 (C-1′), 78.29 (C-3′ and C-5′), 75.00 (C-2′), 71.41 (C-4′), and 62.58 (C-6′) in the ^{13}C-nmr spectrum. In support of this, in the NOESY spectrum, nOe contours were displayed between H-1′ and H-3′, and between H-1′ and H-5′. Based on the above spectroscopic evidence and the optical rotation of the isolate, the structure of **2** was established as (−)-asimilobine-2-*O*-β-D-glucoside.



	R_1	R_2	R_3	R_4	R_5
1	Me	H	H	H	H
2	Me	Glc	H	H	H
3	-CH_2-		H	H	H
4	-CH_2-		Me	OMe	H
5	-CH_2-		H	OMe	H
6	-CH_2-		Me	OH	H
7	-CH_2-		Me	OMe	OMe
8	-CH_2-		H	OMe	OMe
9	-CH_2-		Me	OMe	OH
10	-CH_2-		Me	OH	OMe



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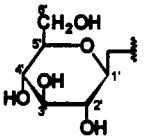
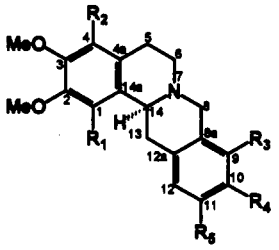
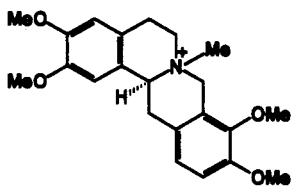
Glc=





	R_1	R_2	R_3	R_4	R_5
12	H	H	OMe	OMe	H
13	OH	H	OMe	OMe	H
14	H	OH	OMe	OMe	H
15	H	H	OMe	OH	H
17	H	H	H	OMe	OMe
18	OH	H	H	OMe	OMe



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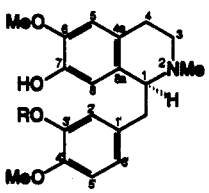
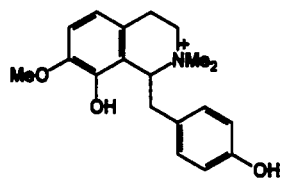
Assignment of the remaining proton signals of **2** was achieved through further analysis of the COSY and NOESY spectra, and the results are shown in Table 1.

The unequivocal ^{13}C-nmr assignments of **2** (Table 1) were obtained through a combination of APT, HETCOR, and selective INEPT experiments, as previously described (40,41). Examination of the APT and HETCOR spectra led to the assignments of all of the protonated carbons. A series of selective INEPT experiments was then applied to obtain the unambiguous assignments of the quaternary carbons. Irradiation of H-1′ (δ 4.93) resulted in the enhancement of the carbon resonance at δ 151.65, which was therefore assigned to C-2. As a result, the other oxygenated aromatic carbon at δ 146.78 should be assigned to C-1. The signal at δ 131.38 was assigned to C-1b since it was enhanced when H-3 (δ 7.00) was irradiated. Polarization transfer from H-5eq (δ 3.27) enhanced the signals of C-3a at δ 130.52 and C-6a at δ 54.77. Enhancement of the C-9 (δ 128.58), C-7a (δ 137.35) and C-1a (δ 127.76) carbon resonances was observed when H-11 (δ 8.29) was irradiated. Magnetization transfer from H-7eq resulted in the enhancement of the signals at δ 128.87 and 133.18, which were then assigned to C-8



19 R=H
20 R=Me



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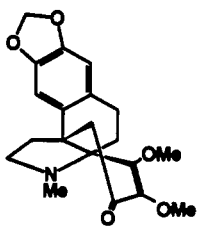

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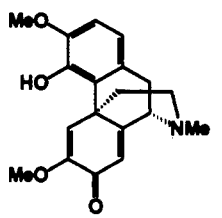

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TABLE 1. ^{1}H- and ^{13}C-nmr Assignments of (−)-Asimilobine-2-*O*-β-D-glucoside [**2**].[a]

Position	^{1}H[b]	^{13}C
1		146.78
1a		127.76
1b		131.38
2		151.65
3	7.00 (s)	117.39
3a		130.52
4ax	2.96 (ddd, 14.3, 3.8, 1.3)	29.49
4eq	2.69 (br d, 14.3)	
5ax	2.91 (ddd, 12.4, 3.8, 1.2)	43.81
5eq	3.27 (m)	
6a	3.66 (dd, 13.8, 4.6)	54.77
7ax	2.67 (dd, 13.8, 13.8)	37.80
7eq	2.83 (dd, 13.8, 4.6)	
7a		137.35
8	7.23 (m)	128.87
9	7.21 (m)	128.58
10	7.26 (ddd, 7.4, 7.4, 1.8)	127.91
11	8.29 (dd, 7.4, 1.4)	129.22
11a		133.18
1-OMe	3.68 (s)	61.15
1′	4.93 (d, 7.6)	102.72
2′	3.54 (dd, 8.1, 7.6)	75.00
3′	3.48 (dd, 8.1, 8.1)	78.29
4′	3.40 (dd, 8.1, 8.1)	71.41
5′	3.45 (m)	78.29
6′a	3.92 (dd, 12.0, 1.7)	62.58
6′b	3.71 (dd, 12.0, 5.7)	

[a]Recorded at 300 MHz for ^{1}H and 75.6 MHz for ^{13}C; chemical shifts are reported in ppm (δ) from TMS in CD_3OD.

[b]Signal multiplicity and coupling constants are in parentheses.

and C-11a, respectively, and this completed the ^{13}C-nmr assignments of (−)-asimilobine-2-*O*-β-D-glucoside [**2**].

Occurrence of aporphine glycosides is indeed rare; to date, only two glycosidic aporphine alkaloids have been reported. The first was floripavidine from *Papaver floribundum* (42), and the second was tuberosinone-*N*-β-D-glucoside from *Aristolochia tuberosa* (43). (−)-Asimilobine-2-*O*-β-D-glucoside [**2**] is the first, and thus far the only, representative of a glycosidic aporphine alkaloid in the family Menispermaceae.

The mol wt of alkaloid **8**, $C_{19}H_{19}NO_4$ (calcd 325.1314), was deduced from the molecular ion at *m/z* 325.1316 in the hreims, and the uv absorptions at 220, 281, and 305 nm were indicative of a 1,2,9,10-oxygenated aporphine (39). The $[M-29]^+$ ion at *m/z* 296 in the eims indicated a secondary amine functionality (44), and this was corroborated by the absence of NMe resonances in the ^{1}H-nmr spectrum. Since a methylenedioxy group was situated at C-1 and C-2, as evident from the well-separated proton signals at δ 6.02 and 5.87 (1H each, d, J=1.3 Hz) (45,46), the MeO groups (δ 3.87 and 3.86) must be located at C-9 and C-10. The above spectral evidence suggested a plane structure identical with (+)-nordicentrine (47). However, since **8** was levorotatory, it must have the 6a*R* configuration (48). Although (+)-nordicentrine, the enantiomer of **8**, was previously identified from several plants (47, 49–52), neither natural nor synthetic (−)-nordicentrine was known. This study is the first report on the

structure determination of (−)-nordicentrine [**8**]. The ^{1}H assignments of **8**, as illustrated in Table 2, were obtained through examination of the homonuclear COSY and NOESY spectra.

Similar to **2**, unambiguous assignment of the carbon resonances of (−)-nordicentrine [**8**] (Table 2) was accomplished through the use of the APT, HETCOR, and selective INEPT techniques. Examination of the APT and HETCOR spectra allowed the assignments of all protonated carbons. Assignment of the quaternary carbons was then achieved by application of the selective INEPT experiments. In short, 3-bond connectivity was observed for the following pairs of protons and carbons: H-11/C-1a, H-11/C-7a, and H-11/C-9; H-8/C-10, H-8/C-11a, and H-8/C-7; H-3/C-1, H-3/C-1b, H-3/C-4; H-5eq/C-3a, H-5eq/C-6a; H-7/C-1b, H-7/C-11a, and H-7/C-8; OCH_2O/C-1 and OCH_2O/C-2.

Each of the isolates was assessed for cytotoxic and antimalarial potential according to established methods (2). Except for the aporphine alkaloids, all other alkaloids, including the tetrahydroprotoberberines **12–18**, the tetrahydrobenzylisoquinolines **19–21**, the hasubanan alkaloid **22**, and the morphinandienone **23**, were devoid of cytotoxicity, as demonstrated by their ED_{50} values of > 5 μg/ml in the P-388 cell line and >20 μg/ml in a variety of mammalian cancer cell lines (data not shown). Among the aporphine alkaloids (Table 3), the secondary and tertiary bases with the 1,2-methylenedioxy group, such as **3–10**, displayed general cytotoxicity in nearly all cancer cell lines tested, while those without this functionality, for example **1** and **2**, showed no inhibitory effect on the growth of the cancer cells. The enhanced activity of **3** relative to **1** (which was inactive) implies that the 1,2-methylenedioxy substituent is required for the expression of cytoxicity of the aporphine alkaloids. Similarly, the lack of activity of

TABLE 2. ^{1}H- and ^{13}C-nmr Assignments of (−)-Nordicentrine [**8**].[a]

Positive	^{1}H[b]	^{13}C
1		141.55
1a		116.19
1b		126.98
2		146.60
3	6.47 (s)	107.09
3a		126.47
4ax	2.94 (m)	29.07
4eq	2.59 (m)	
5ax	2.94 (m)	43.14
5eq	3.32 (m)	
6a	3.89 (dd,14.3, 5.3)	53.54
7ax	2.71 (dd, 14.3, 14.3)	36.38
7eq	2.80 (dd, 14.3, 5.3)	
7a		127.88
8	6.69 (s)	111.00
9		148.10
10		147.48
11	7.62 (s)	110.45
11a		123.46
OCH_2O	5.87 (d, 1.3)	100.48
	6.02 (d, 1.3)	
9-OMe	3.86 (s)	55.81
10-OMe	3.87 (s)	55.98

[a]Recorded at 300 MHz for ^{1}H and 75.6 MHz for ^{13}C; chemical shifts are reported in ppm (δ) from TMS in $CDCl_3$.

[b]Signal multiplicity and coupling constants are in parentheses.

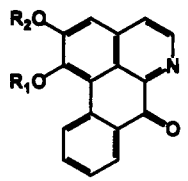
TABLE 3. Evaluation of the Cytotoxic Activity of the Aporphine Alkaloids from *Stephania pierrei*.

Alkaloid	Cell line tested (ED_{50}, μg/ml)[a]											
	BCA-1	HT-1080	LUC-1	MEL-2	COL-1	KB	KB-V1	P-388	A-431	LNCaP	ZR-75-1	U-373
1,2-Substituted Aporphines												
(−)-Asimilobine [1]	>20	>20	>20	>20	>20	>20	>20	>5	>20	>20	>20	>20
(−)-Asimilobine-2-*O*-β-D-glucoside [2]	>20	>20	>20	>20	>20	>20	>20	>5	>20	>20	>20	>20
(−)-Anonaine [3]	3.3	2.4	1.8	15.8	4.0	2.6	1.8	>5	4.2	2.9	2.7	1.5
1,2,9-Substituted Aporphines												
(−)-Isolaureline [4]	2.9	1.8	12.2	>20	3.2	2.5	1.2	>5	1.3	2.9	2.6	1.3
(−)-Xylopine [5]	3.4	2.2	2.3	12.6	4.1	2.0	0.9	2.2	4.6	2.4	2.4	1.9
(−)-Roemeroline [6]	8.9	7.6	6.9	>20	11.9	4.1	1.2	>5	6.7	7.3	13.6	4.9
1,2,9,10-Substituted Aporphines												
(−)-Dicentrine [7]	2.2	1.7	6.1	3.8	2.1	1.6	0.9	0.6	1.7	1.9	2.8	1.5
(−)-Nordicentrine [8]	2.0	1.7	13.2	3.3	1.7	0.8	0.7	0.6	0.8	1.5	1.7	0.6
(−)-Phanostenine [9]	2.5	2.9	3.1	18.8	4.1	1.8	1.2	0.4	12.6	1.9	2.1	1.7
(−)-Cassythicine [10]	6.8	7.6	6.6	8.9	7.3	3.0	1.2	>5	10.5	6.1	9.8	4.8

[a]BCA-1=Human breast cancer, HT-1080=Human fibrosarcoma, LUC-1=Human lung cancer, MEL-2=Human melanoma, COL-1=Human colon cancer, KB=Human oral epidermoid carcinoma, KB-V1=Vinblastine-resistant KB, P-388=Murine lymphoid neoplasm, A-431=Human epidermoid carcinoma, LNCaP=Hormone-dependent human prostatic cancer, ZR-75-1=Hormone-dependent human breast cancer, U-373=Human glioblastoma.

2 may be due to the absence of the 1,2-methylenedioxy or due to its polarity which attenuated the ability to penetrate the cell membrane. The latter may also account for the absence of biological activity of **11**, as well as of the other quaternary alkaloids. The 1,2-methylenedioxy substituent appears to play a similar role in the oxo-aporphine alkaloids. For instance, liriodenine [**24**] displayed cytotoxicity in KB cells (ED_{50}, 3.1 μg/ml), but this activity was lost in lysicamine [**25**] (ED_{50} >10 μg/ml) (53).

Regarding antimalarial activity (Table 4), none of the isolates demonstrated strong activity. Nevertheless, for the tetrahydroprotoberberines, the presence of a phenolic functionality on either ring A or ring D seemed to enhance the antimalarial activity. For



24 R_1,R_2=-CH_2-
25 $R_1=R_2$=Me

example, in the 1,2,9,10-substituted tetrahydroprotoberberine series, alkaloid **12** was inactive, whereas appreciable activity was observed for alkaloids **13–15**. A similar relationship was also obtained for the 1,2,10,11-series, where **18** was more active than **17**. Most of the aporphine alkaloids in this investigation displayed significant antimalarial activity. In general, however, antimalarial activity paralleled cytotoxicity, with only one exception, (−)-asimilobine [**1**]. Despite the lack of cytotoxicity of **1**, perhaps due to the absence of the 1,2-methylenedioxy substituent, the compound demonstrated appreciable antimalarial activity, showing ED_{50} values of 950 and 470 ng/ml in the D-6 and W-2 strains, respectively. This observation may provide a good starting point for segregation of these two biological properties within the aporphine alkaloids. By retaining the functionalities on ring A and modifying substituents on, or the structures of, rings B, C, and D, one might obtain analogues with more potent and more selective antimalarial activity.

Finally, comparison of the alkaloid content of *S. pierrei* with that of *S. erecta* (2) indicated that different types of isoquinoline alkaloids were produced by the two plants. It was clear that while bisbenzylisoquinolines were the major alkaloids of *S. erecta*, aporphines and tetrahydroprotoberberines represented the major constituents of *S. pierrei*. The qualitative difference in the alkaloid production of the two plants suggests that each possesses distinct enzymatic systems for the biosynthesis of secondary metabolites, such as alkaloids. Based on this chemical evidence, it can be concluded that the two *Stephania* species are not identical and, from the chemotaxonomic point of view, should be treated as separate species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's were determined on a Kofler hot plate and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. Uv spectra were obtained on a Beckman DU-7 spectrometer, and the ir spectra were measured on a Nicolet MX-1 FT-IR (KBr) interferometer. ^{1}H-nmr, homonuclear COSY, ^{13}C-nmr, APT, and HETCOR spectra were recorded in $CDCl_3$ with TMS as internal standard, employing a Varian XL-300 instrument. Standard Varian pulse sequences were used. Selective INEPT experiments were performed at 90.8 MHz using a Nicolet NMC-360 spectrometer. Data sets of 16K covering a spectral width of 10 MHz were acquired. Proton pulse widths were calibrated using a sample of HOAc in 10% C_6D_6 (^1J=6.7 Hz) in a 5-mm nmr tube. The radio frequency

TABLE 4. Evaluation of the Antimalarial Activity of the Isoquinoline Alkaloids from *Stephania pierrei*.

Alkaloid	*Plaspodium falciparum* strain (ED_{50}, ng/ml)[a]			
	D-6		W-2	
1,2-Substituted Aporphines				
(−)-Asimilobine [1]	950	(>21)[b]	470	(>42)
(−)-Asimilobine-2-*O*-β-D-glucoside [2]	>10000		>10000	
(−)-Anonaine [3]	1290	(5)	1900	(3)
1,2,9-Substituted Aporphines				
(−)-Isolaureline [4]	2560	(1)	1610	(1)
(−)-Xylopine [5]	440	(>45)	2270	(>9)
(−)-Roemeroline [6]	3150	(2)	1780	(4)
1,2,9,10-Substituted Aporphines				
(−)-Dicentrine [7]	1260	(4)	2550	(2)
(−)-Nordicentrine [8]	470	(2)	1030	(1)
(−)-Phanostenine [9]	2010	(1)	2880	(1)
(−)-Cassythicine [10]	2290	(2)	2260	(3)
1,2,10,11-Substituted Aporphines				
(+)-Magnoflorine [11]	>10000		>10000	
2,3,9,10-Substituted Tetrahydroprotoberberines				
(−)-Tetrahydropalmatine [12]	>10000		5020	
(−)-Capaurine [13]	4340	(>5)	1910	(>10)
(−)-Thaicanine [14]	1610	(>12)	550	(>36)
(−)-Corydalmine [15]	2840	(>7)	840	(>24)
(−)-*N*-Methyltetrahydropalmatine [16]	>10000		>10000	
2,3,10,11-Substituted Tetrahydroprotoberberines				
(−)-Xylopinine [17]	>10000		>10000	
(−)-Tetrahydrostephabine [18]	2230	(>9)	1940	(>10)
Tetrahydrobenzylisoquinolines				
(+)-Reticuline [19]	4050		4470	
(+)-Codamine [20]	5800		4010	
(±)-Oblongine [21]	>10000		>10000	
Others				
(−)-Delavaine [22]	>10000		>10000	
(−)-Salutaridine [23]	>10000		>10000	
Controls				
Chloroquine	1.3	(13380)	11.2	(1550)
Quinine	9.4	(>2130)	24.5	(>820)
Mefloquine	7.3	(480)	1.6	(2190)
Artemisinin	0.6	(>33300)	0.5	(40000)

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

[b]The number in parentheses is the ratio of ED_{50} in KB cells to the ED_{50} in the parasites (selectivity index).

field strength for the soft proton pulse was on the order of 25 Hz for these experiments. Eight Hz was used as $^3J_{CH}$ for aromatic protons, and 6 and 3 Hz for aliphatic protons. The ms was obtained with a Varian MAT 112S instrument operating at 70 eV. Unless stated otherwise, preparative and analytical tlc were performed on Si gel plates.

PLANT MATERIAL.—The tubers of *S. pierrei* were collected in Prachin Buri Province, Thailand, in August 1990. The plant was identified through direct comparison with herbarium specimens in the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperatives, Bangkok, Thailand. A voucher specimen is deposited in the herbarium of the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.

EXTRACTION AND ISOLATION.—The fresh minced tubers of *S. pierrei* (20 kg) were exhaustively extracted with 95% EtOH (15, 10, and 5 liters). Evaporation of the organic solvent gave a brownish syrupy residue which was then partitioned between $CHCl_3$ (12 liters) and H_2O (3 liters). The $CHCl_3$ fraction, after removal of the solvent and drying over Na_2SO_4, afforded a $CHCl_3$ extract (35 g). Lyophilization of the H_2O fraction

gave an aqueous extract (67 g). The $CHCl_3$ extract showed cytotoxicity against the KB (ED_{50} 3.6 μg/ml) and P-388 (ED_{50} 0.8 μg/ml) cell systems. It also demonstrated antimalarial activity against the D-6 (ED_{50} 1540 ng/ml) and W-2 (ED_{50} 3130 ng/ml) strains of *Pl. falciparum*. The H_2O fraction was inactive in KB cells (ED_{50} >20 μg/ml) but active in P-388 cells (ED_{50} 1.4 μg/ml). It exhibited antimalarial activity against the D-6 (ED_{50} 790 ng/ml) and W-2 (ED_{50} 3990 ng/ml) strains of *Pl. falciparum*. The $CHCl_3$ extract was triturated with 2% HOAc (120 ml) and filtered. The filtrate was then basified with $NaHCO_3$ and extracted with $CHCl_3$ (1.2 liters) to give, after evaporation of the solvent, fraction A (15 g). The insoluble material was dried in a vacuum desiccator to afford fraction B (20 g).

Fraction A was subjected to cc on Si gel using $CHCl_3$ and MeOH as the solvents in a polarity gradient fashion. Thirty-six 500-ml fractions were collected. Monitoring of the separation was done by tlc analysis of each fraction. Fractions showing the same tlc behavior were pooled, evaporated, and dried in vacuo. Fractions 19 to 22 were pooled, dried in vacuo, and recrystallized from Me_2CO to give (−)-tetrahydropalmatine [**12**] (8.4 g). Fraction 24 was further purified by preparative tlc eluting with toluene-diethylamine (DEA) (96:4) to afford (−)-xylopinine [**22**] (15 mg). Fractions 25 to 31 were combined and subjected to preparative tlc, using cyclohexane-EtOAc-DEA (5:4:1) as the solvent. The lower band, after usual workup, afforded (−)-delavaine [**22**] (25 mg). The upper band was removed and further separated by preparative tlc eluting with cyclohexane-EtOAc-DEA (7:2:1) to give (−)-capaurine [**13**] (9 mg) and (−)-thaicanine [**14**] (12 mg). Fractions 32 to 36 were combined, dried, and further separated by preparative tlc eluting with toluene-DEA (94:6) to yield (−)-tetrahydrostephabine [**18**] (16 mg) and (−)-corydalmine [**15**] (35 mg).

Fraction B was chromatographed over Si gel and eluted with a series of $CHCl_3$/MeOH combinations in a polarity-gradient manner. Seventy-eight fractions (500 ml each) were collected. Fractions 6 and 7 gave (−)-tetrahydropalmatine [**12**] (1.1 g). Fraction 10, after removal of the solvent and drying in vacuo, was recrystallized from Me_2CO to afford (−)-dicentrine [**7**] (1.2 g). Fractions 11 to 36 were combined, dried, and recrystallized from Me_2CO to give crystals identified as (−)-dicentrine [**7**] (120 mg) and a mother liquor which was dried and further purified by preparative tlc eluting with toluene-DEA (98:2) to afford (−)-isolaureline [**4**] (130 mg). Preparative tlc of fractions 37 to 39 using EtOAc-MeOH (6:1) as the eluent afforded (−)-phanostenine [**9**] (42 mg). Fractions 40 and 41 were pooled, dried, and subjected to preparative tlc eluting with cyclohexane-EtOAc-DEA (2:7:1). The more polar band was removed and extracted to give xylopine [**5**] (7 mg). The less polar band, after normal workup, yielded (−)-anonaine [**1**] (6 mg). Fractions 42 to 52 were combined, dried, and then chromatographed over a Si gel column, using EtOAc-MeOH (5:1) as the eluting solvent. Twenty 50-ml fractions were collected. Fractions 5 to 9 were pooled and evaporated to give a residue which was identified as (−)-cassythicine [**10**] (11 mg). Fractions 11 to 13 were combined and further purified by preparative tlc eluting with cyclohexane-EtOAc-DEA (4:5:1) to afford (−)-nordicentrine [**8**] (140 mg), (−)-salutaridine [**23**] (28 mg), and (+)-codamine [**20**] (11 mg). Fractions 55 to 69 from fraction B were combined and further separated by preparative tlc, using cyclohexane-EtOAc-DEA (1:8:1) as the solvent to give (+)-reticuline [**19**] (11 mg) and (−)-asimilobine [**2**] (51 mg).

The aqueous extract was redissolved in H_2O (600 ml) and then treated with Mayer's reagent. The precipitates were collected, resuspended in MeOH (450 ml), and the solution was filtered. The filtrate was designated as fraction C, and the precipitate fraction D. The filtrate (Fraction C) was passed through an Amberlite IRA-400 (chloride form) column, evaporated, and dried to afford a residue (1 g). This residue was then subjected to cc using $CHCl_3$ and MeOH as the solvents with increasing polarity. Twenty 40-ml fractions were collected. Further purification of fraction 4 was carried out by preparative tlc eluting with MeOH-NH_4OH (99:1) to give (−)-roemeroline [**6**] (5 mg). Preparative tlc of fraction 7 using MeOH-NH_4OH (99:1) as the eluent afforded (−)-asimilobine [**2**] (14 mg). Fractions 10 to 13 were combined and evaporated to dryness to give (−)-asimilobine-2-*O*-β-D-glucoside [**3**] (20 mg). Fractions 15 to 17 were further purified by preparative tlc in $CHCl_3$-MeOH (9:1) to yield *N*-methyltetrahydropalmatine [**16**] (125 mg). Separation of fractions 18 to 20 by preparative tlc eluting with MeOH-NH_4OH (90:10) afforded (±)-oblongine [**21**] (6 mg).

Fraction D was resuspended in Me_2CO-MeOH-H_2O (6:2:1) (400 ml) and filtered. The filtrate was then subjected to ion-exchange chromatography (Amberlite IRA-400, chloride form), evaporated under reduced pressure, and dried. Preparative tlc of the obtained residue in $CHCl_3$-MeOH-NH_4OH (14:5:1) afforded magnoflorine [**11**] (21 mg).

(−)-Asimilobine-2-O-β-D-glucoside [**2**].—Mp 158°; $[\alpha]^{20}D$ −107° (*c*=0.1, MeOH); R_f [Me_2CO-MeOH (1:1)] 0.13; uv λ max (MeOH) 211 (log ε 4.43), 227 (4.20), 262 (4.03) 272 (4.11), 306 (3.23) nm; ir ν max (KBr) 3432, 2911, 1593, 1427, 1314, 1256, 1073 cm^{-1}; ^1H and ^{13}C nmr, see Table 1; eims *m/z* (rel. int.) 268 (14), 267 (78), 266 (100), 265 (7), 256 (11), 253 (5), 252 (21), 251 (23), 238 (14), 237 (8), 236 (14), 223 (10), 178 (14), 165 (11); positive hrfabms $[M+H]^+$ 430.1872 (calcd for $C_{23}H_{28}NO_7$, 430.1866).

(−)-Nordicentrine [**8**].—Mp 248° (dec.); $[\alpha]^{20}D$ − 34° (*c*=0.2, MeOH); R_f [EtOAc-MeOH (6:1)] 0.10; uv λ max (MeOH) 220 (log ε 4.42), 231 (4.37), 272 (4.05), 281 (4.17), 305 (4.14) nm; ir ν max (KBr) 2928,

1607, 1516, 1462, 1215, 1113, 1051 cm^{-1}; ^1H and ^{13}C nmr see Table 2; eims *m/z* (rel. int.) $[M]^+$ 325 (85), 324 (100), 323 (18), 310 (13), 309 (13), 308 (17), 296 (12), 293 (20), 266 (10), 265 (12), hreims 325.1316 (calcd for $C_{19}H_{19}NO_4$, 325.1314).

Detailed spectral information for the alkaloids **1**, **3–7**, and **9–23** is available on request from the authors (54).

ASSAYS FOR CYTOTOXIC AND ANTIMALARIAL POTENTIAL.—Determinations for cytotoxic and antimalarial activities were performed as described previously (2).

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