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CYTOTOXIC AND ANTIMALARIAL BISBENZYLISOQUINOLINE ALKALOIDS FROM *STEPHANIA ERECTA*[1]

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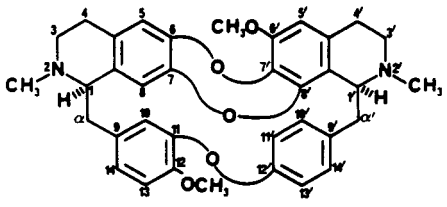
ABSTRACT.—(+)-2-*N*-Methyltelobine [**1**], a new alkaloid, together with twelve known bisbenzylisoquinolines, was isolated from the tubers of *Stephania erecta*. The structure determination and the complete ^{1}H- and unambiguous ^{13}C-nmr assignments of **1** were obtained through extensive use of several 1D and 2D nmr techniques. All alkaloids inhibited the growth of cultured *Plasmodium falciparum* strains D-6 and W-2 and displayed nonselective cytotoxicity with a battery of cultured mammalian cells. These data were used for the calculation of selectivity indices. Relative to known antimalarial agents, these bisbenzylisoquinoline alkaloids do not appear to be promising clinical candidates at the present time.

Stephania erecta Craib. (Menispermaceae) has been used in Thai folk medicine as a skeletal muscle relaxant and an analgesic; it is also known to produce a large number of alkaloids, including at least 17 bisbenzylisoquinolines (2). Several bisbenzylisoquinoline alkaloids have been shown to possess cytotoxicity against a number of human cancer cell lines (3), and recent studies have also disclosed the antimalarial activity of some members of this group of alkaloids (1,4,5). As described previously (6,7), there appears to be some merit in comparing the potential of a test substance to mediate a cytotoxic response with the potential of the same test substance to mediate an antimalarial response. Utilizing cultured KB cells and cultured *Plasmodium falciparum* strains D-6 and W-2, we have recently defined the ratio of the respective biological responses as a selectivity index (7). Whereas well-known antimalarial agents such as artemisinin were found to give a selectivity index >1000, generally cytotoxic agents produced ratios of <10. In the current report, we describe studies conducted with *S. erecta* to explore further the relationship between cytotoxic and antimalarial activities. This resulted in the bioactivity-directed isolation and characterization of twelve known bisbenzylisoquinolines and one new natural alkaloid, (+)-2-*N*-methyltelobine [**1**].

In the hrms, alkaloid **1** showed a molecular ion at 576.2619 mass units, corresponding to the molecular formula $C_{36}H_{36}N_2O_5$ (calcd 576.2624), and the uv absorptions at 207, 233, 280, and 305 nm were indicative of a bisbenzylisoquinoline structure (8). As evident from the resonances at δ 2.46, 2.51, 3.84, and 3.87 in the ^{1}H-nmr spectrum, the structure of **1** includes two *N*-Me and two aromatic MeO groups. In the aromatic region of the ^{1}H-nmr spectrum, three singlets were found at δ 5.97, 6.34, and 6.50, in addition to a doublet at δ 6.27 ($J = 1.8$ Hz) and a cluster of six aromatic protons at δ 6.82–6.88. These ^{1}H-nmr spectral features suggested that **1** was a tail-to-tail bisbenzylisoquinoline containing three diaryl ether bridges (9). The peaks at *m/z* 350 and 175 in the ms, together with the H-1 and H-1′ signals at δ 3.32 (dd, $J = 4.2$, 4.2 Hz) and 3.94 (dd, $J = 5.3$, 3.2 Hz) in the ^{1}H-nmr spectrum, strongly indicated that **1** belonged to the subgroup K (6–7′, 7–8′, 11–12′), with one MeO group on the

[1]Part XX in the series "Traditional Medicinal Plants of Thailand." For part XIX, see Lin *et al.* (1).



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upper and one on the lower part of the molecule (9). The full structural characterization of **1** was then accomplished by examination of the homonuclear COSY and NOESY spectra.

The MeO group resonating at δ 3.84 was located at C-6′ since H-5′ appeared as a singlet at δ 6.34. This was corroborated by the nOe contours observed between the 6′-MeO and H-5′, and between H-5′ and H-4′ (δ 2.74 ppm) in the NOESY spectrum. The proton at C-10 (δ 6.27, d, $J = 1.8$ Hz) was coupled to H-14 (δ 6.88, dd, $J = 8.1$, 1.8 Hz), which was further coupled to H-13 (δ 6.84, d, $J = 8.1$ Hz) in the homonuclear COSY spectrum. The second MeO group resonating at δ 3.87 should be located at C-12 since it displayed nOe enhancement with H-13. The broad singlet aromatic proton at δ 6.50, showing long-range coupling with the C-4 methylene protons at δ 2.54 (m) in the homonuclear COSY spectrum, was assigned to H-5. An nOe enhancement was also observed between H-5 and the C-4 protons in the NOESY spectrum. The signal at δ 5.97 (s) was assigned to H-8, according to its nOe correlation contour with H-10. In addition, nOe's were observed between H-1 and the 2*N*-Me group (δ 2.46), and between H-1′ and 2′-*N*-Me group (δ 2.51). Further analysis of the homonuclear COSY and NOESY spectra led to the complete assignment of all the remaining protons, as shown in Table 1.

TABLE 1. ^{1}H- and ^{13}C-nmr Assignments of (+)-2-*N*-Methyltelobine [**1**].[a]

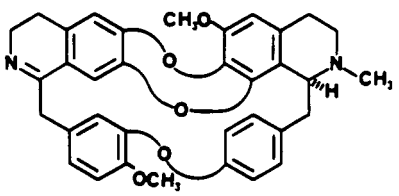
Position	^{1}H	^{13}C	Position	^{1}H	^{13}C
1	3.32 (dd, 4.2, 4.2)	64.91	1′	3.94 (dd, 5.3, 2.2)	58.86
3	2.60 (m)	48.38	3′	2.85 (m)	44.81
	3.02 (m)			3.28 (m)	
4	2.54 (m, 2H)	26.29	4′	2.74 (m)	24.62
				2.95	
4a		129.76	4a′		127.78
5	6.50 (s)	116.22	5′	6.34 (s)	107.08
6		139.25	6′		146.06
7		138.39	7′		146.06
8	5.97 (s)	114.59	8′		139.25
8a		131.99	8a′		128.80
α	2.89 (m, 2H)	37.25	α′	2.68 (dd, 12.8, 5.3)	40.10
				3.19 (dd, 12.8, 2.2)	
9		131.44	9′		134.64
10	6.27 (d, 1.8)	120.67	10′	6.86 (m)	130.29
11		147.28	11′	6.86 (m)	120.24
12		148.56	12′		155.32
13	6.84 (d, 8.1)	111.82	13′	6.86 (m)	120.24
14	6.88 (dd, 8.1, 1.8)	124.53	14′	6.86 (m)	130.29
2-*N*Me	2.46 (s)	43.30	2′-*N*Me	2.51 (s)	42.45
12-OMe	3.87 (s)	56.04	6′-OMe	3.84 (s)	56.20

[a]Chemical shifts are reported in ppm from TMS in $CDCl_3$; multiplicity and coupling constants in Hz are in parentheses.

The relative stereochemistry between H-1 and H-1′ was deduced as a *syn* configuration from the following observations: (1) a small chemical shift difference (0.05 ppm) between the two *N*-Me groups; (2) a characteristic chemical shift value of H-10 (δ 6.27); and (3) the appearance of the resonances for H-10′, H-11′, H-13′, and H-14′ as ill-defined absorptions in the region δ 6.84 to 6.88 (9). The positive sign of the specific optical rotation of **1** (+226°) indicated that **1** had the absolute configuration 1*R*,1′*S* (10). Based on the above spectroscopic evidence, it was concluded that **1** was a 2*N*-Me derivative of (+)-telobine. The ^{1}H-nmr and specific optical rotation data of **1** were in good agreement with those of synthetic (+)-2-*N*-methyltelobine (9,11).

In order to confirm further the structure of **1**, as well as to obtain its unequivocal ^{13}C-nmr assignments (Table 1), a combination of 1D and 2D nmr techniques, including the APT, HETCOR, selective INEPT (12,13), COLOC (14), and selective proton decoupling experiments, was applied. Examination of the APT and HETCOR spectra yielded the complete assignment of all protonated carbons. The assignment of the quaternary carbons was achieved by the application of the selective INEPT, COLOC, and selective proton decoupling techniques. Selective INEPT irradiation of H-5 enhanced C-7 (δ 138.39) and C-8a (δ 131.99). Although the C-8a signal was not enhanced when the C-α protons were irradiated in the selective INEPT experiment, a small, long-range coupling was displayed between C-8a and the C-α protons (δ 2.89, m, 2H) in the COLOC spectrum, supporting the assignment of C-8a. Magnetization transfer from H-8 resulted in the enhancement of C-4a (δ 129.76) and C-6 (δ 139.35). A two-bond coupling between the C-4 protons and C-4a was demonstrated in the COLOC spectrum. During the selective INEPT irradiation of H-10, apart from the enhancement of the resonances of C-12 (δ 148.56) and C-14 (δ 124.53), enhancement of C-11 (δ 147.28) was also observed. Polarization transfer from H-5′ to C-4′, C-7′, and C-8a′ was displayed when H-5′ was irradiated. From the COLOC spectrum, the 12-MeO protons showed a three-bond coupling with C-12, whereas those of the 6′-MeO group were coupled to the signal at δ 146.06 (C-6′). The proton H-1′ exhibited three-bond couplings with C-4a′ (δ 127.78) and C-8′ (δ 139.25) on selective INEPT irradiation, leaving the last oxygenated carbon signal at δ 155.32 ppm to be assigned to C-12′. The resonance of C-9′ (δ 134.64), in the COLOC spectrum, was shown to be coupled to H-10′ (14′) and H-11′ (13′), thereby confirming the assignment of C-9′ obtained by the selective INEPT irradiation of H-1′. The remaining signals to be assigned were those of C-10′ (14′) and C-11′ (13′). Distinction of these resonances proved to be somewhat more difficult since these carbon resonances appeared as broad singlets at δ 130.29 and 120.24. In our experiments, neither the selective INEPT irradiation of H-α′ with varying coupling constants, nor the COLOC pulse sequence, could elicit the coupling between the C-α′ protons and C-10′ (14′), probably due to the very small magnitude of the coupling. This problem was resolved by a low power selective proton decoupling experiment. When the H-α′ at δ 2.68 was selectively irradiated, the complex doublet at δ 130.29 in the fully proton-coupled carbon spectrum became a relatively simplified doublet, while the complex doublet at δ 120.24 remained undisturbed. Hence, the former signal was assigned to C-10′ (14′), and the latter to C-11′ (13′), and this therefore completed the unambiguous ^{13}C nmr assignment of **1**, as shown in Table 1.

Twelve additional bisbenzylisoquinolines from this plant were identified by comparing their physical and spectral properties (mp, uv, ir, $[\alpha]^{20}_D$ and ^{1}H nmr) with those reported earlier (1, 15–17). (+)-1,2-Dehydrotelobine [**2**] was another tail-to-tail (6–7′, 7–8′, 11–12′) bisbenzylisoquinoline isolated. The remaining alkaloids are comprised of two subgroups. The first group is the tail-to-tail (8–7′, 11–12′) bisbenzylisoquinolines which include (+)-2-norisotetrandrine [**3**], (+)-isotetrandrine [**4**], (+)-



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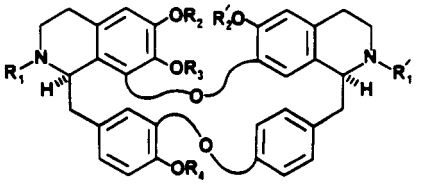
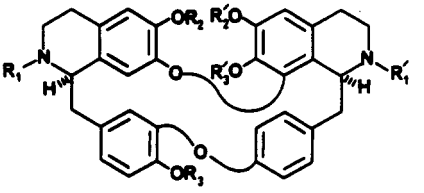


	R_1	R_2	R_3	R_4	R_1'	R_2'
3	H	Me	Me	Me	Me	Me
4	Me	Me	Me	Me	Me	Me
5	H	Me	H	Me	Me	Me
6	Me	Me	H	Me	Me	Me



	R_1	R_2	R_3	R_1'	R_2'	R_3'
7	Me	Me	Me	Me	Me	H
8	Me	Me	Me	Me	H	Me
9	H	Me	Me	Me	Me	H
10	H	Me	Me	Me	$-CH_2-$	
11	Me	Me	Me	Me	$-CH_2-$	
12	H	Me	Me	Me	Me	Me
13	Me	Me	Me	Me	Me	Me

2-northalrugosine [**5**], and (+)-thalrugosine [**6**]. The second group is composed of seven tail-to-tail (7–8′, 11–12′) bisbenzylisoquinolines which are (+)-homoaromaline [**7**], (+)-stephibaberine [**8**], (+)-daphnandrine [**9**], (+)-2-norcepharanthine [**10**], (+)-cepharanthine [**11**], (+)-2-norobaberine [**12**], and (+)-obaberine [**13**].

As summarized in Table 2, the antimalarial potential of each of these alkaloids was investigated with cultured *P. falciparum*. Although the intensity of the response was not equivalent to the known antimalarial agents investigated, appreciable activity was

TABLE 2. Evaluation of the Antimalarial Activity of Bisbenzylisoquinoline Alkaloids from *Stephania erecta*.

Compound	*Plasmodium falciparum* strain (ED_{50}, ng/ml)	
	D-6	W-2
(+)-*N*-Methyltelobine [**1**]	97.4	255.7
(+)-1,2-Dehydrotelobine [**2**]	306.7	256.4
(+)-2-Norisotetrandrine [**3**]	66.1	45.3
(+)-Isotetrandrine [**4**]	165.1	54.6
(+)-2-Northalrugosine [**5**]	68.6	125.1
(+)-Thalrugosine [**6**]	120.6	229.7
(+)-Homoaromoline [**7**]	104.6	288.3
(+)-Stephibaberine [**8**]	130.0	310.0
(+)-Daphnandrine [**9**]	63.0	223.2
(+)-2-Norcepharanthine [**10**]	46.6	129.4
(+)-Cepharanthine [**11**]	140.4	294.8
(+)-2-Norobaberine [**12**]	45.9	93.7
(+)-Obaberine [**13**]	231.0	216.0
Chloroquine	2.5	31.7
Quinine	7.6	30.8
Mefloquine	9.0	1.2

demonstrated with compounds **1–13**. In general, the concentration of test agent required to inhibit the growth by 50% was roughly equivalent or greater in the drug-resistant strain W-2 as compared with strain D-6, with the exception of the compound **4** which demonstrated greater activity with strain W-2.

The cytotoxic potential of these agents was then measured with a battery of cultured mammalian cells (Table 3). Each of the alkaloids was found to demonstrate a general cytotoxic response with no discernable cell-type selectivity. However, ten of these alkaloids showed greater cytotoxic activity with vinblastine-resistant KB cells (KB-V1) as compared with KB cells. For example, the cytotoxic activity of (+)-2-*N*-methyltelobine [**1**] against the KB-V1 cells was about 10-fold greater than against the KB cells (Table 3). A similar phenomenon was observed with the three known antimalarial agents tested in these assays. It should be mentioned that cepharanthine [**11**] has been shown to reverse multi-drug resistance of KB cells by suppressing the function of the P-glycoprotein (22).

With regard to the structures of these compounds, it is of interest to note that for each pair of 2-nor alkaloid and its di-*N*-Me counterpart, the 2-nor alkaloid, which has only one Me group on the 2′-*N*, was always more active against either strain of *Plasmodium* as compared with the di-*N*-Me derivative, which bears Me groups on both nitrogens. For example, the ED_{50} values of (+)-2-northalrugosine [**5**] (68.6 and 125.1 ng/ml) were lower than those of (+)-thalrugosine [**6**] (120.6 and 229.7 ng/ml). Similar observations were noted between (+)-2-norisotetrandrine [**3**] and (+)-isotetrandrine [**4**], (+)-2-norcepharanthine [**10**] and (+)-cepharanthine [**11**], and (+)-2-norobaberine [**12**] and (+)-obaberine [**13**]. Although this relationship also held true for the cytotoxic response demonstrated with certain cell lines such as HT-1080 and Col-1 (Table 3), other cell lines demonstrated no appreciable differences.

Finally, based on the data described above, it is possible to compare certain elements of the antimalarial and cytotoxic potential of these compounds. As shown in Table 3, the selectivity indices of chloroquine (158 to >8000), quinine (162 to

TABLE 3. Cytotoxic Activity of Alkaloids **1–13** and their Cytotoxicity/Antimalarial Activity Ratios.[a]

Compound	Cell line tested[b] (ED_{50}, μg/ml)			
	BCA-1	HT-1080	LUC-1	MEL-2
(+)-2-*N*-Methyltelobine [1]	2.3 (24, 9)[b]	2.2 (23, 9)	7.7 (79, 30)	8.7 (89, 34)
(+)-1,2-Dehydrotelobine [2]	4.6 (15, 18)	2.1 (7, 8)	1.7 (6, 7)	9.9 (32, 39)
(+)-2-Norisotetrandrine [3]	4.4 (67, 97)	7.5 (114, 166)	9.8 (148, 216)	8.2 (124, 181)
(+)-Isotetrandrine [4]	5.4 (33, 99)	11.0 (67, 202)	10.0 (61, 183)	19.5 (118, 357)
(+)-2-Northalrugosine [5]	4.1 (60, 33)	2.2 (32, 18)	8.4 (123, 67)	15.4 (225, 23)
(+)-Thalrugosine [6]	5.0 (42, 22)	10.0 (83, 44)	9.7 (80, 42)	14.9 (124, 65)
(+)-Homoaromoline [7]	3.6 (34, 13)	9.0 (86, 31)	9.7 (93, 34)	9.0 (86, 31)
(+)-Stephibaberine [8]	4.7 (9, 97)	7.2 (14, 149)	8.6 (17, 177)	12.8 (25, 264)
(+)-Daphandrine [9]	4.6 (73, 21)	6.9 (110, 31)	9.0 (143, 40)	13.4 (213, 60)
(+)-2-Norcepharanthine [10]	1.7 (36, 13)	1.9 (41, 15)	2.0 (43, 16)	10.5 (225, 81)
(+)-Cepharanthine [11]	4.7 (34, 16)	6.1 (44, 21)	7.3 (52, 25)	14.5 (103, 49)
(+)-2-Norobaberine [12]	3.2 (70, 34)	7.4 (161, 79)	8.8 (192, 94)	14.5 (316, 155)
(+)-Obaberine [13]	4.6 (20, 21)	10.4 (45, 48)	9.7 (42, 45)	20.0 (87, 93)
Chloroquine	>20 (>8000, >631)	6.5 (2600, 205)	>20 (>8000, >631)	>20 (>8000, >631)
Quinine	>20 (>2631, 649)	>20 (>2631, 649)	>20 (>2631, 649)	>20 (>2631, 649)
Mefloquine	5.3 (588, 4417)	2.3 (256, 1917)	3.7 (411, 3083)	8.9 (989, 7416)

[a]The numbers in the parentheses are the ratios of cytotoxicity/antimalarial activity against the D-6 and the W-2 strains, respectively.

[b]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 Prostate Cancer; ZR-75-1 = Hormone Dependent Human Breast Cancer.

>2631), or mefloquine (22 to 4417) were generally superior to the values calculated with compounds **1–13** (1 to 357). With a given alkaloid, the ratio varied considerably from one cancer cell line to another. As an example, (+)-isotetrandrine [**4**] yielded ratios ranging from 9 up to 357. In addition, although certain of these compounds demonstrated greater activity with drug-resistant KB cells as compared with the parent cell line, this relationship did not hold for the drug-resistant *P. falciparum* strain W-2. Although further studies are needed, the current results suggest that equivalent information can be gained with regard to selectivity index using a limited panel of cultured mammalian cells. One cell line (such as KB) may be sufficient, and promising leads can be subjected to more intensive investigation. Based on the data presented herein, the bisbenzylisoquinoline alkaloids do not appear to be promising candidates as antimalarial agents. However, elucidation of their mechanisms would reveal if these two biological activities are inextricably related, and it is possible that structure modifications could yield species capable of demonstrating selectivity.

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. The COLOC experiment was carried out at 75.6 MHz on a Varian XL-300 nmr spectrometer, using relaxation delay 1.0 sec, acquisition time 0.112 sec, $\Delta_1 = 25$ msec, $\Delta_2 = 30$ msec. 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 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 were obtained with a Varian MAT 112S instrument operating at 70 eV.

PLANT MATERIAL.—The tubers of *S. erecta* were collected in Prachin Buri Province, Thailand, in

TABLE 3. Continued

Cell line tested[b] (ED_{50}, μg/ml)						
COL-1	KB	KB-V1	P-388	A-431	LNCaP	ZR-75-1
3.6(37, 14)	4.8(49, 19)	0.4(4, 2)	5.6(57, 22)	2.6(27, 10)	2.1(22, 8)	1.2(12, 5)
5.0(16, 20)	2.8(9, 11)	6.8(22, 27)	3.1(10, 12)	6.0(20, 23)	2.0(7, 8)	0.7(2, 3)
7.6(115, 168)	6.3(95, 139)	2.8(42, 62)	4.7(71, 104)	4.7(71, 104)	4.1(62, 91)	1.8(27, 40)
10.5(64, 192)	6.6(40, 121)	1.5(9, 28)	5.6(34, 103)	8.4(51, 154)	9.4(57, 172)	5.0(30, 92)
4.7(69, 38)	6.3(92, 50)	8.1(118, 65)	0.1(2, 1)	4.2(61, 34)	6.6(97, 53)	1.6(23, 13)
11.1(92, 48)	6.5(54, 28)	3.7(31, 16)	7.4(61, 32)	4.9(41, 21)	5.9(49, 26)	2.5(21, 11)
10.0(96, 35)	6.3(60, 22)	3.1(30, 11)	3.7(35, 13)	8.9(85, 31)	3.4(33, 12)	2.0(19, 7)
7.5(15, 155)	6.4(13, 132)	7.4(15, 153)	3.7(7, 76)	8.9(18, 184)	8.3(16, 171)	2.1(4, 43)
10.3(164, 46)	5.8(92, 26)	2.2(35, 10)	2.0(32, 9)	8.3(132, 37)	5.8(92, 26)	1.8(29, 8)
3.1(67, 24)	3.9(84, 30)	1.4(30, 11)	0.1(2, 1)	1.8(39, 14)	3.0(64, 23)	0.9(19, 7)
9.0(64, 31)	5.9(42, 20)	0.9(6, 3)	0.3(2, 1)	2.1(15, 7)	5.6(40, 19)	1.0(7, 3)
5.5(120, 59)	6.0(131, 64)	2.0(44, 21)	3.5(76, 37)	6.1(133, 65)	3.7(81, 40)	1.9(41, 20)
10.8(47, 50)	8.6(37, 40)	2.6(11, 12)	4.1(18, 19)	8.7(38, 40)	5.0(22, 23)	4.0(17, 19)
10.1(4040, 319)	17.4(6960, 549)	7.8(3120, 246)	>5(>2000, 158)	>20(>8000, >631)	14.3(5720, 451)	15.8(6320, 498)
>20(>2631, 649)	>20(>2631, 649)	5.6(737, 182)	>5(>658, 162)	>20(>2631, 649)	>20(>2631, 649)	>20(>2631, 649)
1.0(111, 833)	3.5(389, 2916)	1.1(122, 917)	0.2(22, 166)	3.0(333, 2500)	2.3(256, 1917)	2.7(300, 2250)

August 1990. Authentication was achieved by 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 minced fresh tubers of *S. erecta* (10 kg) were exhaustively extracted with EtOH. The EtOH extract was then partitioned between $CHCl_3$ and H_2O. The H_2O extract was considered biologically inactive as its ED_{50} values were greater than 100 μg/ml against the P-388 and KB cell lines and greater than 5000 ng/ml against the D-6 and W-2 strains of *P. falciparum*. The $CHCl_3$ extract demonstrated ED_{50} values of 3.3 and 10.7 μg/ml in the P-388 and KB cell lines, and was active against the D-6 and W-2 strains of *P. falciparum*, showing ED_{50} values of 218 and 247 ng/ml, respectively. Rough separation of alkaloids in the $CHCl_3$ extract was achieved by open column chromatography, varying polarity, using $CHCl_3$ and MeOH as the solvents. This led to the separation of the $CHCl_3$ extract into 4 fractions designated A, B, C, and D.

Fraction A was further chromatographed on a Si gel column, using EtOAc-diethylamine (DEA) (95:5) as the solvent, to yield (+)-cepharanthine [**11**] (920 mg), R_f [toluene-DEA (95:5)] 0.42, and crude alkaloid **2** (15 mg). Alkaloid **2** was further purified [preparative tlc, Si gel, cyclohexane-EtOAc-DEA (5:4:1)] to give (+)-1,2-dehydrotelobine [**2**] (10 mg) (R_f 0.17). Fraction B was further separated [preparative tlc, Si gel, toluene-DEA (9:1)] to afford (+)-homoaromaline [**7**] (20 mg) (R_f 0.25), (+)-2-northalrugosine [**5**] (11 mg) (R_f 0.40), (+)-stephibaberine [**8**] (4 mg) (R_f 0.38), and (+)-daphnandrine [**9**] (6 mg) (R_f 0.36). Preparative tlc of fraction C [Si gel, cyclohexane-EtOAc-DEA (8:1:1)] yielded (+)-obaberine [**13**] (13 mg) (R_f 0.31), (+)-isotetrandrine [**4**] (28 mg) (R_f 0.28), and (+)-norcepharanthine [**10**] (10 mg) (R_f 0.26). Fraction D was first separated on preparative tlc [Si gel, cyclohexane-$CHCl_3$-DEA (7:2:1)] to give (+)-2-*N*-methyltelobine [**1**] (13 mg) (R_f 0.65) and two other alkaloid fractions. The first fraction was purified [preparative tlc, Si gel, EtOAc-Me_2CO-NH_4OH (50:40:2)] to give (+)-thalrugosine [**6**] (22 mg) (R_f 0.31). Separation of the other fraction [preparative tlc, Si gel, Me_2CO-NH_4OH (90:3)] resulted in the isolation of (+)-norisotetrandrine [**3**] (15 mg) (R_f 0.29) and (+)-2-norobaberine [**12**] (13 mg) (R_f 0.20).

(+)-2-N-Methyltelobine [**1**].—Mp 172°; uv λ max (MeOH) 207 (log ε 4.65), 233 (4.56), 280 (3.65), 305 (3.47) nm; ir ν max (KBr) 1618, 1587, 1504, 1273, 1210, 1127, 771; ^1H and ^{13}C nmr, see Table 1; ms *m/z* (rel. int.) $[M]^+$ 576 (60), 350 (23), 349 (100), 335 (33), 175 (9); hrms found 576.2619 (calcd for $C_{36}H_{36}N_2O_5$ 576.2624); $[\alpha]^{20}D$ +226° (*c* = 0.8, $CHCl_3$).

The identification of the known alkaloids was accomplished by comparison of their mp's, uv, ir, specific optical rotations, and ^1H-nmr data with those reported previously (1, 15–17).

ASSAYS OF ANTIMALARIAL ACTIVITY.—Cultures of *P. falciparum* (chloroquine-sensitive strain D-6 derived from CDC Sierra Leone, and chloroquine-resistant strain W-2 derived from CDC Indochina III) were maintained in human erythrocytes in vitro according to established methods (18). Parasites were inoculated into type A+ human erythrocytes at a hematocrit of 6% in RPMI-1640 culture medium (GIBCO Laboratories, Grand Island, NY) supplemented with 32 mM $NaHCO_3$ (GIBCO), 25 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid, Sigma Chemical Co., St. Louis, MO), and 10% heat-inactivated human plasma type A+. Parasitemia was maintained below 4% under an atmosphere of 5% O_2, 5% CO_2, and 90% N_2 in 25-cm^2 culture flasks at 37°.

The antimalarial activity of test compounds was assessed with an in vitro radioisotope-incorporation method (19,20). In brief, a suspension (200 μl) of *P. falciparum*-infected red blood cells (0.5–1.0% parasitemia, 1.0% cell hematocrit) was added to wells of a standard 96-well tissue culture plate containing 25 μl of drug to be tested. Each test compound was assayed in duplicate over a concentration range of 10,000–14 ng/ml. In addition, the known antimalarial drugs quinine, chloroquine, mefloquine, and artemisinin were tested in each experiment over a range of 250–0.3 ng/ml. Microtiter plates were incubated for 24 h at 37° in a sealed chamber under an atmosphere of 5% CO_2, 5% O_2, and 90% N_2. After this incubation period, 0.5 μCi of [^{3}H(G)]hypoxanthine (New England Nuclear Research Products, Boston, MA) was added to each well (25 μl of 20 μCi/ml), and the microtiter plate was returned to the sealed chamber at 37° for an additional 18 h incubation. The assay was terminated by harvesting the contents of each microtiter plate onto a glass fiber filter using a Skatron model 11021 semi-automatic cell harvester. Filters were dried, and the radioactivity from individual wells was excised from the filter and placed in 4-ml vials with toluene-based scintillation cocktail. Radioactivity was determined with a Beckman LS 5801 liquid scintillation counter. Concentrations of both test compounds and positive controls that inhibited parasite-specific incorporation of [^{3}H]hypoxanthine by 50% (IC_{50}) were determined by non-linear regression analysis. Zero-drug controls defined 100% incorporation.

CELL LINES FOR CYTOTOXICITY ASSAYS.—Human breast cancer (UISO-BCA-1), colon cancer (UISO-COL-1), lung cancer (UISO-LUC-1), and melanoma (UISO-MEL-2) cell lines were established from primary human tumors in the Specialized Cancer Center, University of Illinois College of Medicine at

Chicago. Each of these cell lines has been shown to grow after sc injection of $1–4 \times 10^6$ cultured cells in male or female (breast cancer only) athymic mice, and diagnosis of the original patient specimen was consistent with analysis of the cultured cells by electron microscopy and analysis of nude mouse tumors by light microscopy. Each cell type has also been found to contain human isozymes when analyzed using the Corning Authentic Kit electrophoresis system. Fibrosarcoma (HT-1080) cells were purchased from the American Type Culture Collection (Rockville, MD), as were P-388 cells. KB-3 and a multidrug-resistant cell line, KB-V1, which was established by treating KB-3 cells with a chronic sublethal dose of vinblastine (21), were supplied by Dr. I.B. Roninson (Department of Genetics, University of Illinois College of Medicine at Chicago, Chicago, IL), and A-431 (human squamous cell carcinoma), LNCaP (human prostatic cancer), and ZR-75-1 (human breast cancer) cell lines were supplied through the courtesy of Dr. R.M. Tait, Glaxo Group Research, Greenford, UK. UISO-BCA-1, and UISO-COL-2 were cultured in Eagle's minimal essential medium with Earle's salts (MEME) (GIBCO Laboratories, Grand Island, NY) supplemented with 15% fetal bovine serum (FBS) (Biofluids, Rockville, MD), 100 units/ml penicillin G, 100 μg/ml streptomycin sulfate, 0.25 μg/ml amphotericin B (Fungizone) (PSF) (GIBCO), and 1% nonessential amino acids (NAA) (Sigma); HT-1080 and UISO-LUC-1 were maintained in MEME (GIBCO) supplemented with 10% FBS, PSF, and 1% NAA; KB-3 and A-431 were cultured in Dulbecco's modified minimum essential medium (D-MEM) (GIBCO) supplemented with 10% FBS and PSF; KB-V1 was cultured in D-MEM (GIBCO) supplemented with 10% FBS, PSF and 1 μg/ml vinblastine (Sigma); UISO-MEL-2 was grown in minimal essential medium with Hanks' salts (MEMH) (GIBCO) supplemented with 10% FBS and PSF. ZR-75-1 and LNCaP were grown in RPMI 1640 media (phenol red-free) supplemented with 10% FBS (hormone-free) and PSF. The medium for ZR-75-1 cells was further supplemented with 0.1 nM estradiol, and the medium for LNCaP cells was also supplemented with 0.1 nM testosterone. P-388 cells were cultured in Fisher medium containing 10% FBS and PSF. All cell lines were cultured at 37° in 100% humidity with a 5% CO_2 atmosphere in air, except the melanoma cell line, which was kept at 37° in closed tissue culture flasks.

EVALUATION OF CYTOTOXIC POTENTIAL.—Cells were typically grown to 60%–70% confluence; the medium was then changed and the cells were used for test procedures one day later. In each case, 96-well tissue culture plates were used. Test samples were initially dissolved in DMSO and then diluted 10-fold with H_2O. Serial dilutions were performed using 10% aqueous DMSO as the solvent, and 10 μl were added to the wells. In general, five concentrations were tested (in triplicate), and preliminary studies were conducted to determine test concentrations that were above and below the ED_{50} of the samples. Control groups were also added in which 10 μl of 10% DMSO were added to wells. After the plates were prepared, cells were removed from the tissue culture flasks by treatment with trypsin, enumerated, and diluted with fresh media. Various quantities of cells (in 190 μl of media) were then added to the 96-well plates, and incubations were performed for various periods of time, as follows [cell number; incubation time (d)]: UISO-BCA-1 (10×10^4; 3), UISO-COL-1 (6.5×10^4; 3), UISO-LUC-1 (5×10^4; 3), UISO-MEL-2 (10×10^4; 3), HT-1080 (5×10^4; 3), KB-3 (5×10^4; 3), KB-V1 (6×10^4; 3), A-431 (6×10^4; 3), LNCaP (6.5×10^4; 3), and ZR-75-1 (7×10^4; 3). All incubations were performed at 37° in a CO_2 incubator with the plates capped in the normal fashion, except for UISO-MEL-2, for which the plates were sealed with Parafilm.

After the incubation period, cells were fixed to the plastic substratum by the addition of 50 μl of cold 50% aqueous trichloroacetic acid. The plates were incubated at 4° for 1 h, washed with tap H_2O (4×), and air-dried. The trichloroacetic-acid-fixed cells were stained by the addition of 0.4% sulforhodamine B (w/v) dissolved in 1% HOAc (30 min). Free sulforhodamine B solution was then removed by washing with 1% aqueous HOAc (4×). The plates were then air-dried, and the bound dye was solubilized by the addition of 10 mM unbuffered Tris base, pH 10 (200 μl). The plates were placed on a shaker for 5 min, and the absorption was determined at 515 nm using an ELISA plate reader. In each case, a zero-day control was performed by adding an equivalent number of cells to several wells of the 96-well plates and incubating at 37° for a period of 10 min. The cells were then fixed with trichloroacetic acid and processed as described above.

In a procedure similar to that described above for cells capable of attaching to the surface of the culture dishes, P-388 cells were enumerated and diluted to a concentration of 10×10^4 cells/ml, 190 μl were added to the wells containing the test substances, and the incubation was performed at 37° in a CO_2 incubator for 2 days. After the incubation period, the plates were centrifuged (10 min, 2000 rpm) and the supernatant fractions were carefully removed. The cells were treated with 100 μl of 20% aqueous trichloroacetic acid (4°) and incubated at 4° for 1 h. The cells were rinsed with H_2O (4×), dried, and treated with sulforhodamine B as described above.

Finally, the absorption values obtained with each of the treatment procedures were averaged, and the average value obtained with the zero day control was subtracted. These values were then expressed as a percentage, relative to the solvent-treated control incubations, and ED_{50} values were calculated using nonlinear regression analysis (percent survival versus concentration). These experimental conditions were es-

tablished in preliminary studies wherein it was shown (a) there is at least a 7-fold increase in cell number relative to the amount of cells added to the plates at time zero, (b) the resulting absorption values were in a range to assure reading accuracy (i.e., <1.4 A_{515} units), and (c) the cell number attained during the incubation period did not reach a plateau region on the growth curve.

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LITERATURE CITED

1. L.-Z. Lin, H.-L. Shieh, C.K. Angerhofer, J.M. Pezzuto, G.A. Cordell, L. Xue, M.E. Johnson, and N. Ruangrungsi, *J. Nat. Prod.*, **56**, 22 (1993).
2. B. Tantisewie, S. Amurrio, H. Guinaudeau, and M. Shamma, *J. Nat. Prod.*, **52**, 846 (1989).
3. K.T. Buck, in: "The Alkaloids." Ed. by A. Brossi, Academic Press, New York, 1987, Vol. 30, pp. 1–222.
4. Z.G. Ye, and K. Van Dyke, *Biochem. Biophys. Res. Commun.*, **159**, 242 (1989).
5. K. Pavanand, H.K. Webster, K. Yongvanitchit, and T. Dechavongse, *Phytother. Res.*, **3**, 215 (1989).
6. S.J. Partridge, P.F. Russell, M.M. Anderson, C.W. Wright, J.D. Phillipson, G.C. Kirby, D.C. Warhurst, and P.L. Schiff Jr., *J. Pharm. Pharmacol.*, **42** (suppl.), 97 (1990).
7. C.K. Angerhofer, G.M. König, A.D. Wright, O. Sticher, W.K Milhous, G.A. Cordell, N.R. Farnsworth, and J.M. Pezzuto, "Advances in Natural Product Chemistry." Ed. by Atta-ur-Rahman, Harwood Academic Publishers, Amsterdam, 1992, in press.
8. K.P. Guha and B. Mukherjee, *J. Nat. Prod.*, **49**, 1 (1979).
9. H. Guinaudeau, A.J. Freyer, and M. Shamma, *Nat. Prod. Rep.*, 477 (1986).
10. B.K. Cassels and M. Shamma, *Heterocycles*, **14**, 211 (1980).
11. I.R.C. Bick and S. Sotheeswaran, *Aust. J. Chem.*, **31**, 2077 (1978).
12. G.A. Cordell, *Phytochem. Anal.*, **2**, 49 (1991).
13. G.A. Cordell and A.D. Kinghorn, *Tetrahedron*, **47**, 3521 (1991).
14. H. Kessler, C. Griesinger, J. Zarbock, and H.R. Loosli, *J. Magn. Reson.*, **57**, 331 (1984).
15. P.L. Schiff Jr., *J. Nat. Prod.*, **46**, 1 (1983).
16. P.L. Schiff Jr., *J. Nat. Prod.*, **50**, 529 (1987).
17. P.L. Schiff Jr., *J. Nat. Prod.*, **54**, 645 (1991).
18. W.K. Milhous, N.F. Weatherly, J.H. Bowdre, and R.E. Desjardins, *Antimicrob. Agents Chemother.*, **27**, 525 (1985).
19. R.E. Desjardins, C.J. Canfield, D.M. Haynes, and J.D. Chulay, *Antimicrob. Agents Chemother.*, **16**, 710 (1979).
20. L.B.S. Kardono, C.K. Angerhofer, S. Tsauri, K. Padmawinata, J.M. Pezzuto, and A.D. Kinghorn, *J. Nat. Prod.*, **54**, 1360 (1991).
21. D.-W. Shen, A. Fojo, J.E. Chin, I.B. Roninson, N. Richert, I. Pastan, and M.M. Gottesman, *Science*, **232**, 643 (1986).
22. A. Akiyama, M.M. Cornwell, M. Kuwano, I. Pastan, and M.M. Gottesman, *Mol. Pharmacol.*, **33**, 144 (1988).

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