Journal of Natural Products
Vol. 49, No. 2, pp. 253-258, Mar-Apr 1986

CONSTITUENTS OF *PARABAENA SAGITTATA*. TWO NEW TETRAHYDROPROTOBERBERINE ALKALOIDS[1]

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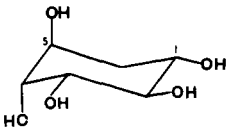
ABSTRACT.—The structural elucidation of five components isolated from *Parabaena sagittata* is described. The plant is a climber indigenous to northern Thailand and has been used by the hill tribes for medicinal purposes. Three of the components are known natural products: the cyclohexanepentol, (+)-protoquercitol (**1**); the tetrahydroprotoberberine, (−)-tetrahydropalmatine (**4**); and the protoberberine, berberine. The other two components are previously unreported tetrahydroprotoberberines and have been given the names (−)-*O*-methylthaicanine (**2**) and (−)-thaicanine (**3**). Their structures were assigned after detailed analysis of their ^{1}H- and ^{13}C-nmr spectra. Methylation of **3** was shown to yield **2**; **2** and **3** are the first reported examples of protoberberines with substituents at the 4-position.

Parabaena sagittata Miers (Menispermaceae) is a lofty climber indigenous to the northern part of Thailand and has been used by the hill tribes of this region for medicinal purposes (2). A decoction of stems and leaves affords a treatment for jaundice, indigestion, and painful intestinal disturbances. All parts of the plant may be used as a febrifuge and tonic. There have been no previous reports of any phytochemical or pharmacological studies on this species, but a recent tlc screening of *P. sagittata* Mooney suggested the presence of magnoflorine in the petiole (3). In this report, we describe the structural elucidation of five components isolated from the leaves of *P. sagittata* Miers. Two of these components are alkaloids not previously reported.

The colorless crystalline solid, **1**, was isolated as described in the Experimental section and was found to be the cyclohexanepentol (+)-protoquercitol (**1**) on the basis of the data reported below. Our 400 MHz ^{1}H-nmr spectrum of this cyclitol was essentially the same as that previously reported at 220 MHz (4). The ^{13}C-nmr spectrum of **1** was reported recently (5), but two of the six carbon resonances could not be assigned unambiguously. We have found that ^{1}H-^{13}C shift-correlated 2-D nmr spectroscopy (6,7) showed clearly that the multiplet at δ 3.59 ppm assigned to H-1 (4) was associated with the ^{13}C resonance at δ 68.9 ppm and that the doublet of doublets at δ 3.85 for H-5 was attached to the carbon resonating at δ 68.6. Thus, we have been able to assign all six resonances, and these are reported in the Experimental section. The isolation of (+)-protoquercitol from the family Menispermaceae has been reported previously (8,9).

The three components, **2, 3,** and **4,** which were isolated from the basic fraction of the leaf extracts, gave positive Dragendorff and Mayer's tests, suggesting they were alkaloids. Mass spectral analysis of the compounds indicated a close structural relation-



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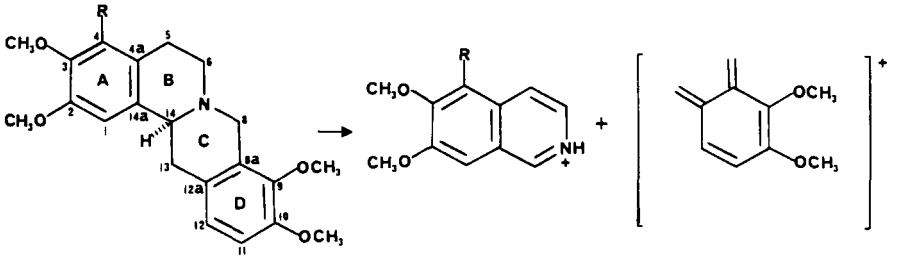
[1]Part II in the series of "Studies on Thai Medicinal Plants." For Part I, see Ruangrungsi *et al.* (1).

ship as all three exhibited the charcteristic retro Diels-Alder fragments associated with a tetrahydroprotoberberine skeleton possessing two methoxyl groups in the D ring (i.e., fragment *m/z* 164, Scheme 1) and at least two methoxyl groups in the A ring (10). These analyses also suggested that **2** contained an additional methoxyl group and **3** a hydroxyl group in ring A which was absent in **4** (*m/z* 220 vs. 206 vs. 190, respectively, Scheme 1).



		m/z	*m/z*
2	R=OCH_3	220	164
3	R=OH	206	164
4	R=H	190	164

SCHEME 1. Mass Spectral Fragmentations of **2**, **3**, and **4**.

A variety of spectroscopic techniques was employed to determine the positions of the substituents in rings A and D. The presence of a C-9 methoxyl group in all three alkaloids was indicated by the significant relative intensity of the ($M-OCH_3$) fragment in the mass spectra of **2**, **3**, and **4** (18, 12, and 12%, respectively) (11). Further evidence for this substitution was obtained from the ^{1}H-nmr signal for the C-8 methylene group. In compounds with no C-9 oxygen substituent, this methylene appears as a broad singlet, but when a methoxyl group is present on C-9, the C-8 pseudo axial proton is shifted upfield to become a doublet ($J \approx 16$ Hz) at about 3.65 ppm, and the pseudo equatorial proton is deshielded to about 4.35 ppm (12, 13). Examination of the ^{1}H spectra of these three alkaloids (Table 1) shows clearly that the latter situation exists for the protons at C-8 [e.g., in **2** δ 3.55 (d, J= 15.7 Hz), 4.25 (d, J= 15.7 Hz)]. Placement of the second methoxyl group in ring D at C-10 was indicated by the *ortho* coupling (J=8.1 Hz) between H-11 and H-12. Thus, we can conclude that the two methoxyl groups in ring D of these compounds are located at C-9 and C-10.

Both ^{1}H- and ^{13}C-nmr were employed to determine the substitution patterns in ring A of the compounds. A 400 MHz ^{1}H-nmr spectrum of **4** showed singlets for the two aromatic protons in ring A, which suggested that they were *para* to each other (i.e., at the 1- and 4-positions) and thus that the two methoxyl groups were at C-2 and C-3. Comparison of the ^{13}C-nmr spectrum of **4** with that of tetrahydropalmatine (14,15), a known tetrahydroprotoberberine with methoxy groups at the 2,3,9, and 10-positions, established that the compounds were the same. Further comparison of other physical properties (see Experimental section) confirmed that the isolate was indeed **4**. Although portions of the ^{1}H-nmr spectrum of **4** have been reported (16), in Table 1 we have indicated the assignment for each resonance in the 400 MHz spectrum. Determination of the optical rotation of **4** established that it was (−)-tetrahydropalmatine with the (*S*) absolute configuration as depicted in Scheme 1 (17,18).

Mass spectral analyses established that **2** was a pentamethoxyl derivative which contained an additional OCH_3 group in ring A as compared with **4** and that of **3** had an additional OH group in this ring. Reaction of **3** with MeI and K_2CO_3 in DMF yielded **2** and, thus, showed that **2** was the methyl ether of **3**. On biosynthetic grounds, the three methoxyl groups in ring A of **2** would be expected at the 1-, 2- and 3-positions,

TABLE 1. ^{1}H 400 MHz NMR Spectra of **2**, **3**, and **4**[a]

Hydrogen	Compound		
	2	**3**	**4**[b]
1	6.56 s	6.37 s	6.75 s
4	—	5.84 br. s (*OH*)	6.64 s
5 ax.	2.87 m	2.84 m	3.17 m
5 eq.	2.56 m	2.58 m	2.67 m
6 ax.	2.87 m	2.84 m	2.67 m
6 eq.	3.22 m	3.22 m	3.21 m
8 ax.	3.55 d (15.7)	3.55 d (15.9)	3.58 d (15.8)
8 eq.	4.25 d (15.7)	4.25 d (15.9)	4.26 d (15.8)
11	6.79 d (8.1)	6.80 d (8.1)	6.81 d (8.1)
12	6.89 d (8.1)	6.87 d (8.1)	6.88 d (8.1)
13 ax.	2.87 m	2.84 m	2.84 dd (16.0, 12.0)
13 eq.	3.24 dd (10.9, 4.0)	3.26 dd (10.5, 4.4)	3.28 dd (16.0, 4.0)
14	3.52 m	3.51 m	3.55 dd (12.0, 4.0)
OCH_3's	3.850	3.849	3.850
	3.853	3.852	3.856
	3.871	3.874	3.870
	3.877	3.892	3.892
	3.883		

[a]Chemical shifts are in ppm from TMS and coupling constants are in parentheses in Hertz.
[b]See Tourwé *et al.* (16) for a discussion of some of these assignments.

but comparison of the ^{13}C spectrum of **2** (Table 2) with that of *O*-methylcapaurine (15,19), the known tetrahydroproberberine with methoxyls at the 1,2,3,9, and 10-positions, showed that they were not the same.

The following evidence unambiguously established that the third methoxyl group in ring A of **2** was at the 4-position and that no substituent was on C-1. Tetrahydroprotoberberines such as **4**, which contain no methoxyl group on C-1, have been shown to exist in a *trans*-conformation with respect to the B/C ring fusion (19,20). The observation of a high field (δ 3.5-4.0 ppm) H-14 resonance is one indication of this *trans*-conformation (e.g., 3.55 ppm in **4**) (16,19). In **2** and **3**, the H-14 resonance was found in this region (Table 1), indicating that both compounds have no substituent on C-1 and that they exist in the *trans*-conformation. Further, in similar compounds with no substituent on C-1, the C-6 resonance in the ^{13}C-nmr spectrum appears at about 51 ppm, while in compounds with an oxygen substituent at this position, the C-6 signal appears in the region 47-49 ppm (19,21). In **2** and **3**, this signal appeared at 51.2 and 50.9 ppm, (Table 2) respectively, as expected for compounds with no C-1 substituent.

It could be assumed that because there is no substituent at C-1 in either of these alkaloids, the three substituents in ring A must be at the 2,3, and 4-positions. But because substitution at the 4-position in protoberberines is without precedent, evidence will be presented to establish clearly this substiution pattern. In compounds without an oxygen substituent at the 4-position, the ^{13}C resonance for C-5 appears at about 30 ppm (e.g., 29.1 ppm in **4**) (15). In both **2** and **3**, this carbon is strongly shielded (23.6 and 23.1 ppm, respectively) because of the presence of a C-4 oxygen substituent. A similar shielding of C-5 has been reported for simple tetrahydroisoquinolines (22,23) and for more complex systems (24,25) possessing a methoxyl group at the 4-position. Also, the ^{13}C resonance for all carbons in the A ring of **3** (Table 2) showed excellent agreement with a similarly substituted tetrahydroisoquinoline system (24). ^{1}H 2-D nOe spectroscopy (NOESY) (26) was also employed to confirm the substitution pattern in ring A. For **2**, this technique showed that one of the methoxyl groups was in close

TABLE 2. ^{13}C-nmr Spectra of **2**, **3**, and **4**[a]

Carbon	Compound		
	2	**3**	**4**[b]
1	104.6	100.6	108.9
2	150.8	150.4	147.6
3	140.5	133.5	147.6
4	151.8	146.3	111.5
4a	121.4	114.8	127.8
5	23.6	23.1	29.1
6	51.2	50.9	51.5
8	54.0	54.1	54.0
8a	127.7	127.7	126.9
9	150.3	150.2	150.3
10	145.1	145.0	145.2
11	111.0	111.0	111.1
12	123.8	123.7	123.7
12a	128.7	128.6	128.7
13	36.4	36.2	36.4
14	59.5	59.5	59.3
14a	133.6	133.7	129.9
2-OCH_3	55.9	55.9	55.8
3-OCH_3	60.6[c]	61.0	55.8
4-OCH_3	60.9[c]	—	—
9-OCH_3	60.2	60.2	60.1
10-OCH_3	56.2	55.9	56.1

[a]Chemical shifts are in ppm from TMS.
[b]Data taken from Hughes and MacLean (15).
[c]Assignments may be interchanged.

proximity to H-5_{ax} (δ 2.87), a result that is only possible if a methoxyl group is on C-4. The NOESY technique was even more informative with **3**, as it showed that the hydroxyl proton at δ 5.84 was in close proximity to H-5_{eq} (δ 2.58). This result established that the hydroxyl group in ring A must be at the 4-position. In making the ^{1}H-nmr assignments for all protons in **2** and **3** (Table 1), we were assisted by a ^{1}H homonuclear shift-correlated 2-D experiment (COSY) (27) performed with **2**.

On the basis of the above evidence, we propose the illustrated structures for **2** and **3**. As neither of these compounds has been reported previously,[2] we suggest the names (−)-*O*-methylthaicanine for **2** and (−)-thaicanine for **3**. The names reflect the binational nature of the project as well as the country where the substances originated. Closely related levorotatory substances are of the (*S*) configuration (17, 18), so we propose the absolute configurations for **2** and **3** are as depicted in Scheme 1. An unusual biosynthetic feature of the two new alkaloids is the presence of oxygen substituents at C-4. Of the more than 70 known protoberberines (20), to our knowledge these are the first which have been reported with substituents at the 4-position.

The fifth isolate, a yellow crystalline solid, was shown to be identical to a previously isolated (1) sample of berberine by comparison of their tlc behavior as well as their uv, 400 MHz ^{1}H-nmr, and mass spectra. It is of interest from a biosynthetic standpoint that the substitution pattern of this protoberberine is similar to that of tetrahydropalmatine (**4**).

[2]A Chemical Abstracts Service Online search by structure of the more than 7 million compounds in the data base failed to find a match with structure **3**.

This is the first reported study of the constituents present in the Menispermaceae species *P. sagittata* Miers. Berberine possesses antimicrobial activity against a wide variety of organisms including Gram-positive and Gram-negative bacteria, fungi, and protozoa (28,29) and has been shown to be effective in the treatment of patients suffering from cholera and severe diarrhea (30,31). It is worthy of note that the Thai hill tribes use *P. sagittata* for intestinal disorders. (−)-Tetrahydropalmatine (**4**) exhibits strong analgesic, sedative, and hypnotic effects while the (+) enantiomer does not have these effects (29). As alkaloids **2** and **3** have not been reported previously, their pharmacological properties are unknown.

EXPERIMENTAL

INSTRUMENTS.—^{1}H- and ^{13}C-nmr spectra were recorded on a Bruker WH 400 and AM 250 spectrometer, respectively, with TMS (δ=0) as internal standard and $CDCl_3$ as solvent except for PS-1 where the solvent was D_2O. Ir spectra were obtained on a Perkin-Elmer 1330 spectrophotometer, uv spectra on a Varian DMS 90 spectrophotometer, and mass spectra on a Varian MAT CH7 or VG Micromass 7070F spectrometer. Optical rotations were performed in a Bendix-NPL automatic polarimeter. Tlc analyses were performed on silica gel GF 254 plates of thickness 0.25 mm.

PLANT MATERIAL.—The leaf material of *P. sagittata* used in this study was collected in Chiang Mai Province, Thailand, during the period May-June 1983. Authentication was achieved by comparison with herbarium specimens at the Botany Section, Technical Division, Department of Agriculture, Ministry of Agriculture and Cooperative, Thailand.

EXTRACTION AND PURIFICATION.—The powdered, dried leaves (1.2 kg) were extracted thoroughly by percolation with 95% EtOH (20 liters), and the percolate was concentrated under reduced pressure. During the concentration, **1** precipitated as colorless crystals which were removed by filtration. The filtrate was further evaporated to dryness (580 g). Recrystallization of **1** from 50% aqueous EtOH yielded colorless prisms (2.38 g). The residue from above (580 g) was acidified with 5% aqueous HOAc (1.5 liters) and filtered. The filtrate was basified with NH_4OH and extracted with $CHCl_3$ (10 liters). The $CHCl_3$ extract was washed with H_2O (1 liter), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to yield the syrupy crude bases (11.8 g). These were divided into five portions, and each portion was chromatographed on a silica gel column (70 g, 5×7 cm) with $CHCl_3$ as the eluent. Fractions of 20 ml were collected and monitored by tlc. Fractions 2-8 yielded 34 mg of yellow prisms (**2**), fractions 10-14 gave 32 mg of fine off-white needles (**3**), and fractions 19-25 yielded 64 mg of **4** as colorless needles. The polarity of the eluent was increased to 20% EtOH in $CHCl_3$, and fractions 33-39 yielded 54 mg of yellow crystals which were shown to be berberine by comparison with a sample isolated in a previous study (1).

(+)-PROTOQUERCITOL (**1**).—Mp 228-230°, lit. (8) mp 235-237°; $[\alpha]^{20}D$ +23° (H_2O), lit. (8) $[\alpha]^{20}D$ +25.6° (H_2O); ir ν max (KBr) 3320 (st., br.), 1070, 1050 cm^{-1}; ^{1}H nmr, as reported previously (4); ^{13}C nmr (D_2O) δ 33.3 (C-6), 68.6 (C-5), 68.9 (C-1), 71.0 (C-3), 72.3 (C-4), 74.6 (C-2); eims *m/z* (rel. int.) 165 (M^++1, 1), 128(10), 102(18), 99(23), 86(24), 74(53), 73(100).

(−)-*O*-METHYLTHAICANINE (**2**).—Mp 119-120° (MeOH/$CHCl_3$); $[\alpha]^{20}D$ −259° ($CHCl_3$); ir ν max ($CHCl_3$) 3040, 2940, 2850, 1495, 1120, 910 cm^{-1}; uv λ max (EtOH) 208 nm (ϵ 26,000), 229 sh (ϵ 15,000), 278 (ϵ 1700); ^{1}H and ^{13}C nmr, see Tables 1 and 2; eims *m/z* (rel. int.) 385 (M^+, 69), 384(39), 354(18), 220(26), 165(24), 164(100), 149(61); tlc (50% EtOAc/30-60° petroleum ether) Rf 0.44. Calcd for $C_{22}H_{27}NO_5$: C, 68.55; H, 7.06; N, 3.63. Found: C, 68.41; H, 7.40; N, 3.87.

(−)-THAICANINE (**3**).—Mp 144-146° (MeOH/$CHCl_3$); $[\alpha]^{20}D$ −243° ($CHCl_3$); ir ν max ($CHCl_3$) 3520, 3020, 2830, 2750, 1495, 1120, 910 cm^{-1}; uv λ max (EtOH) 208 nm (ϵ 22,000), 221 sh (ϵ 8100), 280 (ϵ 1300); with added OH^- 216 (ϵ 32,000), 280 (ϵ 3200); ^{1}H and ^{13}C nmr, see Tables 1 and 2; eims *m/z* (rel. int.) 371 (M^+, 60), 370(29), 340(12), 206(16), 165(23), 164(100), 149(57); tlc (50% EtOAc/30-60° petroleum ether) Rf 0.35. Calcd for $C_{21}H_{25}NO_5$: C, 67.91; H, 6.78; N, 3.77. Found: C, 67.75; H, 6.30; N, 3.80.

(−)-TETRAHYDROPALMITINE (**4**).—Mp 139-141°, lit. (32) mp 141°; $[\alpha]^{20}D$ −203° ($CHCl_3$), −236° (EtOH), lit. (32) $[\alpha]^{28}D$ −258°, −271° (EtOH); ir ν max ($CHCl_3$) 3020, 2830, 2750, 1595, 1255, 910 cm^{-1}; uv λ max (EtOH) 205 nm, 225 sh, 281; ^{1}H and ^{13}C nmr spectra, see Tables 1 and 2 and references (16) and (15), respectively; eims *m/z* (rel. int.) 355 (M^+, 67), 354(41), 324(12), 190(24), 165(23), 164(100), 149(162).

CONVERSION OF **3** TO **2**.—To a solution of **3** (24.7 mg) in DMF (1.5 ml) was added 24 mg of finely

powdered K_2CO_3. To this suspension was introduced 8.3 μl of MeI, and the reaction mixture was stirred at room temperature under an atmosphere of N_2 for 2.5 h. H_2O (10 ml) was added to the reaction mixture, and the product was extracted with $CHCl_3$ (3 times). The combined organic extracts were washed once with brine, dried (anhydrous $MgSO_4$), and the solvent removed at reduced pressure to give a yellow oil. Purification of the crude product by preparative tlc (50% EtOAc/30-60° petroleum ether, Rf 0.44) gave 16.4 mg (64%) of a compound identical to **2**.

ACKNOWLEDGMENTS

G.L.L. acknowledges financial support from the Natural Sciences and Engineering Research Council of Canada.

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Received 9 August 1985