Thalibealine, a Novel Tetrahydroprotoberberine−Aporphine Dimeric Alkaloid from *Thalictrum wangii*[1]

Tawfeq A. Al-Howiriny,[†] Michael A. Zemaitis,[†] Cong-yuan Gao,[‡] Chad E. Hadden,[§] Gary E. Martin,[§] Fu-tyan Lin,[⊥] and Paul L. Schiff, Jr.*[,†]

Department of Pharmaceutical Sciences, School of Pharmacy, University of Pittsburgh, Pittsburgh, Pennsylvania 15261, Department of Spectral Analysis, Beijing Medical University, Beijing, 100083, People's Republic of China, Pharmaceutical Development, Pharmacia Corporation, Kalamazoo, Michigan 49001, and Department of Chemistry, Faculty of Arts and Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

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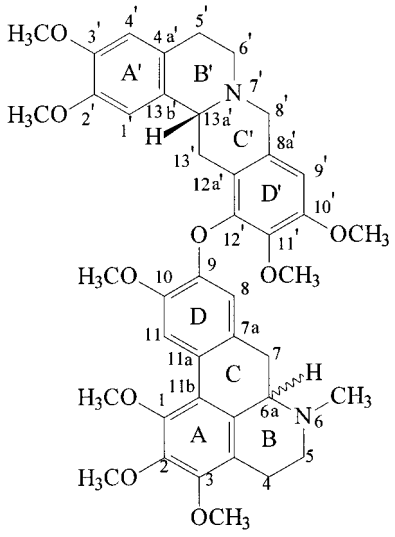
A novel tetrahydroprotoberberine−aporphine dimeric alkaloid, (−)-thalibealine (**1**), was isolated from the roots of *Thalictrum wangiii*, and its structure established via spectroscopic analysis. Three other alkaloids were isolated, including the benzyltetrahydroisoquinoline−aporphine dimer (+)-thalmelatidine, the aporphine (+)-magnoflorine, and the protoberberine berberine. This is the first reported isolation of a tetrahydroprotoberberine−aporphine dimer from nature, as well as the first reported isolation of constituents from *Thalictrum wangii*.

The *Thalictrum* alkaloids are principally phenylalanine/tyrosine-derived bases that occur as a variety of diverse but related monomeric or dimeric structural variants. Just 50 years ago, fewer than 10 alkaloids had been isolated from species of this genus, whereas today there are at least 250.[2,3] In a continuation of our studies on the alkaloids from this genus,[4] we have focused our attention on a study of the alkaloids of the Chinese plant *Thalictrum wangii* Boivin (Ranunculaceae). In this paper, we report the isolation and identification of (−)-thalibealine (**1**), the first tetrahydroprotoberberine−aporphine alkaloid described in nature. In addition, we report the isolation of the benzylisoquinoline−aporphine dimer (+)-thalmelatidine, as well as the aporphine (+)-magnoflorine and the protoberberine berberine. Thalmelatidine was first isolated from *T. minus* L. var. *elatum* Jacq. in 1970 and has been isolated from several other *Thalictrum* species including *T. minus* var. *microphyllum* Boiss., *T. cultratum* Wall, *T. honanense* W. T. Wang, *T. minus* var. *hypoleucum*, *T. minus* var. *minus* L., and *T minus* L. race C.[2,3] Magnoflorine and berberine are extremely common *Thalictrum* alkaloids, with magnoflorine being nearly ubiquitous to the genus.[2,3] To our knowledge, this is the first reported isolation of constituents from the species *T. wangii*.

Extraction of the powdered whole plant with EtOH and fractionation of the extract into nonquaternary and quaternary alkaloid fractions was achieved using conventional extraction and partition techniques.[4] Column chromatography of the nonphenolic nonquaternary alkaloid fraction, followed by preparative layer chromatography of selected fractions, afforded (−)-thalibealine (**1**) as an optically active light yellow residue. The UV spectrum showed absorption maxima at 210 nm (log ϵ 4.80), 223 (sh) (4.69), 281 (4.37), 300 (4.21), 303 (sh) (4.19), and 312 (4.17) and was characteristic of an aporphinoid system,[2,3,6] while the FTIR spectrum indicated the absence of hydroxy and carbonyl groups.[7] The dimeric nature of the alkaloid was suggested by a consideration of the ^{1}H NMR spectrum that showed



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the presence of nine sharp singlets representing eight aromatic methoxy groups (δ 3.74, 3.80, 3.83, 3.84, 3.88, 3.87, 3.94, and 4.01) and one *N*-methyl group (δ 2.48). Five aromatic protons were observed as four singlets at δ 6.40, 6.58, 6.59, 6.74, and 8.02, the last being characteristic of the H-11 proton in an aporphine.[2,3,6] The EIMS displayed the molecular ion at *m*/*z* 724 (70%) with other significant fragment ions at *m*/*z* 533 (25) (**2**), 518 (36) (**2**-Me), 502 (91) (**2**-OMe), 354 (20) (**3**), and 192 (100) (**4**). The HREIMS exhibited the molecular ion at *m*/*z* 724 (70%) (obsd 724.3334; calcd 724.3359 for $C_{42}H_{48}O_9N_2$), 533 (25) (obsd 533.2432; calcd 533.2413 for $C_{31}H_{35}O_7N$), 518 (36) (obsd 518.2167; calcd 518.2178 for $C_{30}H_{32}O_7N$), 502 (90) (obsd 502.2256; calcd 502.2229 for $C_{30}H_{32}O_6N$), and 192 (100) (obsd 192.1010; calcd 192.1024 for $C_{11}H_{14}O_2N$). A consideration of the UV and ^{1}H NMR spectra, particularly the low-field proton signal at δ 8.02 in the latter, suggested that thalibealine was dimeric, with one-half of the dimer being a C-11 unsubstituted aporphine.[2,3,6] A review of the dimeric *Thalictrum* alkaloids, particularly those that contain an aporphinoid as one of the monomeric halves, failed to uncover any dimeric class whose spectral properties were consistent with those reported above. In fact, only three classes of

* To whom correspondence should be addressed. Tel: (412) 648-8462. Fax: (412) 383-7436. E-mail: pschiff+@pitt.edu.
[†] School of Pharmacy, University of Pittsburgh.
[‡] Beijing Medical University.
[§] Pharmacia Corporation.
[⊥] Department of Chemistry, University of Pittsburgh.

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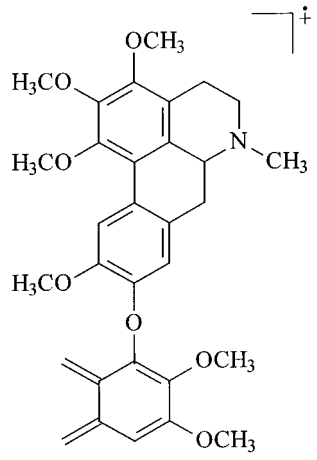
aporphinoid dimers have been reported in nature previously, with each of these classes being almost completely represented by alkaloids from *Thalictrum* species. These classes include the aporphine−benzylisoquinoline class, the aporphine−pavine class, and the dehydroaporphine−benzylisoquinoline class.[2,3,6,8−10] There were two spectral features that suggested the identity of the nonaporphine half of thalibealine (**1**) as a tetrahydroprotoberberine. First, the UV spectra of tetrahydroprotoberberines are simple, usually being characterized by a single prominent maximum at 282−289 nm (log ϵ 3.7−4.0).[2,3,11] This being the case, any absorption due to the tetrahydroprotoberberine half of an aporphine−tetrahydroprotoberberine dimer could be hidden under or combined with the corresponding aporphine absorption. Second, the mass spectral fragment ion at *m*/*z* 192 is a well-recognized ion in certain ring systems that are found in *Thalictrum* alkaloids and tends to arise either via the fragmentation of the isoquinoline portion of a benzyltetrahydroisoquinoline ring or via retro-Diels−Alder fragmentation of ring C′ of a tetrahydroprotoberberine.[2,3,11] Because the EIMS fragmentation pattern of the alkaloid was not characteristic of that expected for a dimer containing at least one benzyltetrahydroisoquinoline ring,[2,3,11] it was more likely that the other half of the dimer was a tetrahydroprotoberberine. Consistent with this premise, if the assumption was made that each half of the dimer contained four methoxy groups (which would be consistent with the established existence of numerous benzyltetrahydroisoquinoline-derived monomers)[2,3] and that two of the methoxy groups in the tetrahydroprotoberberine half were in ring A′, then the fragment ions at *m*/*z* 533, 518, 502, and 354 would be strongly supportive of the alkaloid as being an aporphine−tetrahydroprotoberberine dimer.[2,3] Even a superficial inspection of the ^{1}H NMR spectrum revealed that all of the protons were represented as uncoupled singlets, thereby suggesting the absence of any *ortho*- or *meta*-relationship of these protons to each other in either monomeric ring. This fact, coupled with the presence of a highly characteristic low-field H-11 aporphine singlet at δ 8.02, and with the knowledge that 2,3,10,11-oxygenation is common in the protoberberine series, strongly suggested that thalibealine was a dimer that contained a 1,2,3,9,10-pentaoxygenated aporphine linked to a 2,3,10,-11,12-pentaoxygenated tetrahydroprotoberberine through a diaryl ether bridge in the D ring of each monomer.[2,3] Although the termini of the diaryl ether bridge remained to be fixed, the genesis of the EIMS ions **2**, **3**, and **4** via retro-Diels−Alder fragmentation of ring C′ was readily apparent.

The structure of thalibealine (**1**) was ultimately elucidated via the concerted interpretation of the GCOSY, GHSQC, GHMBC, and ROESY NMR data. The GCOSY allowed for all ^{1}H−^{1}H couplings defining the different spin systems. These resonances were then correlated to their respective carbons via the GHSQC experiment. The GHMBC data afforded all of the long-range ^{1}H−^{13}C connectivities. These data were used to connect the different spin systems together. The skeleton of the tetrahydroberberine portion of the molecule was assembled on the basis of the GHMBC and ROESY correlations, with important correlations being shown in Table 1. The stereochemical orientation of H-6a could not be determined from any of the ROESY correlations, as there is no unique reference point. Strong ROESY correlations were observed from the H-13a′ resonance to H-6′ (2.64 ppm) and H-8′ (3.73). All of the observed GHMBC and ROESY correlations were consistent with the tetrahydroberberine skeleton. It is interesting to note that for a number of the *O*-methyl resonances $^4J_{CH}$ GHMBC correlations were observed, facilitating their unequivocal assignment. Within the aporphine portion of the molecule, few ROESY correlations were observed between the 4-, 5-, 6a-, and 7-positions. The lack of any ROESY correlation responses made it very difficult to make stereochemical attributions to the individual resonance assignments. It is possible that the lack of ROESY responses may have been due to either rapid inversion of the *N*-methyl group or possibly the presence of trace quantities of acid resulting in the protonation of the nitrogen. It is interesting to note, however, that ROESY correlations were observed from the 9′- and 10′-*O*-methyl resonances in the tetrahydroberberine subunit to the 10-*O*-methyl resonance in the aporphine portion of the structure, which provides a point of entry into the determination of preferred solution confirmations of the structure. The ^{1}H and ^{13}C NMR chemical shift assignments and GHMBC and ROESY correlations for **1** are contained in Table 1. The resonance assignments are fully consistent with the novel tetrahydroprotoberberine−aporphine structure of thalibealine.



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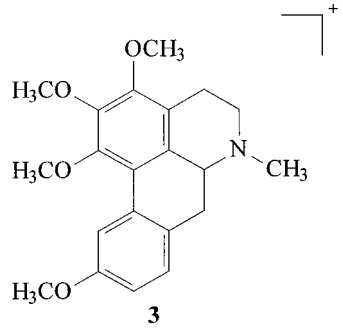

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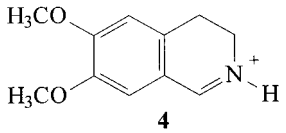

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In summary, thalibealine (**1**) represents the first reported characterization of a tetrahydroprotoberberine−aporphine dimer from nature, and this is the first reported isolation of constituents from *Thalictrum wangii*.

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns hot-stage apparatus and are uncorrected. Specific rotations were measured on a Perkin-Elmer Model 241 automatic polarimeter. UV spectra were

Table 1. Proton and Carbon Chemical Shift Assignments for (−)-Thalibealine (**1**)[a]

position	δ ^{1}H	δ ^{13}C	GHMBC	ROESY
1		149.3		
MeO-1	3.74	60.6	1	MeO-2,11
2		145.2		
MeO-2	3.94	60.9	2	MeO-1, MeO-3
3		149.7		
MeO-3	3.87	60.3	3	MeO-2, 4
3a		122.9		
4	2.80, 2.88	23.5	not observed	MeO-3, 5
5	2.39, 3.07	52.9	not observed	4, MeN-6
MeN-6	2.48	44.0	5, 6a	5, 6a, 7
6a	2.93	62.3	not observed	MeO-3, MeN-6
7	2.40, 2.78	33.4	6a, 8, 11a, 12	MeN-6
7a		128.7		
8	6.40	114.1	7, 7a, 9, 10, 11, 11a, 11b	7, MeO-11′, 13′
	9		146.0	
10		147.4		
MeO-10	4.01	56.0	10, 11	11, MeO-11′
11	8.02	112.0	7a, 8, 9, 10, 11a, 11b	MeO-1, MeO-10
11a		125.8		
11b		122.4		
12		129.3		
1′	6.74	108.5	3′, 4a′, 13a′, 13b′	MeO-2′, 13a′, 13′
2′		147.3		
MeO-2′	3.84	55.8	1′,2′	1′, MeO-3′
3′		147.4		
MeO-3′	3.83	55.9	3′, 4′	MeO-2′, 4′
4′	6.58	111.1	2′, 5′, 13b′	MeO-3′, 5′
4a′		126.4		
5′	2.66, 3.13	28.9	4′, 4a′, 6′, 13b′	4′, 6′
6′	2.64, 3.18	51.4	4a′, 5′, 13a′	8′, 13a′
8′	3.73, 4.04	58.4	6′, 8a′, 9′, 12a′, 13a′	6′, 9′, 13a′
8a′		130.8		
9′	6.59	106.4	8′, 10′, 11′ 12a′, 13′	8′, MeO-10
10′		151.8		
MeO-10′	3.88	56.3	9′, 10′	9′
11′		140.6		
MeO-11′	3.80	61.1	11′	MeO-10
12′		146.4		
12a′		121.3		
13′	2.48, 3.51	30.8	12′, 12a′, 13a′	1′, 13a′
13a′	3.55	59.3	12a′, 13b′	1′, 6′, 8′, 13′

[a] Data were internally referenced to $CDCl_3$. GHMBC data correlate the positional proton to the listed carbons. ROESY data correlate the positional proton to the listed protons. The stereochemistry around H-6a could not be determined.

obtained in MeOH on a Hewlett-Packard HP-845 UV−vis spectrophotometer, and the IR spectra were recorded as a thin film on a KBr window using a Nicolet Impact 410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Bruker model WH-300 spectrometer operating at 300 and 75 MHz, respectively. The chemical shifts are reported in δ (ppm) units based on δ TMS = 0. The HSQC and HMBC experiments were performed in $CDCl_3$ and on Varian INOVA spectrometers operating at a proton observation frequency of 399.801 and 599.750 MHz with a Nalorac Micro Dual or Nalorac MIDTG microprobe obtained from Nalorac Cryogenics Corp., Martinez, CA. The EIMS was performed on an Extrel ELQ400 quadrupole instrument equipped with a DCI Probe HP direct probe from Vacumetrics, Inc. or a Hewlett-Packard 5971A. HREIMS were recorded on a Fisons VG Autospec spectrometer or a Fison VG Analytical 70-G spectrometer. Thin-layer chromatography (TLC) was performed using 5 × 20 cm precoated TLC sheets of Si gel 60 F_{254}, 0.2 mm layer thickness (E. Merck). Preparative thin-layer chromatography was performed using 20 × 20 cm glass plates precoated with 1.0 mm of silica gel GF_{254} (Analtech). Column chromatography was performed using silica gel (60−200 Mesh) (Baker Analyzed Reagents). Ion-exchange chromatography was performed using Amberlite IRA-400 (Cl) resin (Aldrich Chemical Co., Inc., Milwaukee, WI). Solvents (Et_2O and CH_2Cl_2) used in fractionation were dried over anhydrous Na_2SO_4 and filtered prior to evaporation. Evaporation of solvents was done at 40 °C under reduced pressure, and all solvents used were analytical grade.

Plant Material. Powdered whole plant of *Thalictrum wangii* Boivin (Ranunculaceae) was obtained by C.y.G. A herbarium sample is on deposit within the Department of Spectral Analysis.

Extraction and Fractionation. Powdered, dried roots (1.65 kg) were extracted via percolation with EtOH (26 L), and the solvent was evaporated to leave a black-brown residue (92.35 g) (fraction A). The extract was treated with aqueous citric acid (1%) (3.3 L) and filtered. The insoluble portion was treated with Et_2O (600 mL) and filtered to leave insoluble material (13.2 g) (fraction B), while the filtrate was added to the citric acid solution and partitioned with Et_2O (500 mL × 6). The Et_2O phase was evaporated to leave a residue (18.3 g) (fraction C) of nonalkaloidal components (Dragendorff negative). The aqueous phase was basified with NH_4OH to pH 8−9 and extracted with Et_2O (500 mL × 6). The Et_2O extracts were combined and evaporated to leave a brown residue (11.4 g) (fraction D). The basic aqueous phase was subsequently extracted with CH_2Cl_2 (500 mL × 6), and the organic layers were pooled and evaporated to leave a dark brown residue (3.51 g) (fraction E). The remaining ammoniacal solution was acidified to pH 3−4 with HCl (1%) and treated with ammonium reineckate (Reinecke salt) solution (1%) until precipitation ceased. The resulting precipitate was filtered via suction, washed with H_2O, dissolved in MeOH (1.6 L), and passed through a column of anion-exchange resin (iodide form) (280 g) in MeOH. The column eluent was evaporated to afford a dark brown residue (11.43 g) (fraction F) (quaternary alkaloid fraction). Fraction D (11.4 g) was dissolved in Et_2O (600 mL) and partitioned with an aqueous solution of NaOH (1%) (500 mL). The Et_2O phase was separated and the alkaline solution partitioned several additional times with Et_2O (600 mL × 5). The Et_2O extracts were combined, washed several times with water, and evaporated to leave a dark brown residue (7.10 g) (fraction G) (nonquaternary nonphenolic alkaloid fraction). Ammonium chloride was added to lower the pH of the remaining basic aqueous solution to pH 8−9. The resulting solution was partitioned with Et_2O (600 mL × 5). The Et_2O extracts were combined and evaporated to leave a brown residue (1.45 g) (fraction H) (nonquaternary phenolic alkaloid fraction).

Chromatography of Fraction F. Thin-layer chromatograpy of fraction F revealed the presence of two major Dragendorff reagent-positive spots (R_f 0.45 and 0.1) [MeOH−NH_4OH−H_2O (8:1:1)]. Fraction F (11.43 g) was dissolved in MeOH (50 mL), adsorbed onto Si gel (20 g), and chromatographed over a column of Si gel (165 g) in CH_2Cl_2. The polarity was gradually increased via the addition of MeOH, and 200 mL fractions were collected.

Berberine. Rechromatography of the CH_2Cl_2−MeOH (95:5) eluate (1.55 g) over Si gel (50 g) afforded a residue (1.55 g), which was recrystallized from MeOH to afford berberine iodide as yellow needles: mp 260−261 °C; identical by comparison (UV, IR, ^{1}H NMR, EIMS [tetrahydro derivative]) with an authentic sample available in our laboratory.[12]

(+)-Magnoflorine. Rechromatography of the CH_2Cl_2−MeOH (9:1) eluate (1.74 g) over Si gel (55 g) afforded a brown residue (133 mg), which was recrystallized from MeOH to afford magnoflorine iodide as colorless needles: mp 249−250 °C; $[\alpha]^{24}_D$ +208° (*c* 0.5, MeOH); identical by comparison (UV, IR, ^{1}H NMR, EIMS, and specific rotation) with an authentic sample available in our laboratory.[12]

Chromatography of Fraction G. Thin-layer chromatography of fraction G revealed the presence of two Dragendorff

reagent-positive spots (R_f 0.45 and 0.54) [C_6H_6−Me_2CO−MeOH (5.5:4.0:0.1)]. Fraction G (7.10 g) was dissolved in CH_2Cl_2 (15 mL) and chromatographed over a column of Si gel (98 g) in CH_2Cl_2. The polarity was gradually increased via the addition of MeOH, and 200 mL fractions were collected.

(+)-Thalmelatidine. Rechromatography of the CH_2Cl_2−MeOH (95:5) eluate (3.74 g) over Si gel, followed by preparative TLC [C_6H_6−Me_2CO−MeOH−NH_4OH (5.5:4.0:0.5:0.1)] of selected fractions, afforded thalmelatidine as an amorphous residue (33 mg): $[\alpha]^{24}_D$ +31.3° (*c* 0.8, $CHCl_3$) [lit.[2] $[\alpha]^{19}_D$ +47° (*c* 1.0, $CHCl_3$)], identical by comparison (UV, IR, ^{1}H NMR, EIMS, and specific rotation) with literature values.[2,3]

(−)-Thalibealine (1). Rechromatography of the CH_2Cl_2−MeOH (9:1) eluate (1.67 g) over Si gel, followed by preparative TLC [C_6H_6−Me_2CO−MeOH−NH_4OH (5.5:4.0:0.5:0.1)] of selected fractions, afforded thalibealine (**1**) as an amorphous residue (12 mg): $[\alpha]^{24}_D$ −162° (*c* 0.5, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.80), 223 sh (4.69), 281 (4.37), 300 (4.21), 303 sh (4.19), 312 (4.17) nm; FTIR (KBr) ν_{max} 2948, 2851, 1605, 1515, 1465, 1414, 1394, 1361, 1339, 1268, 1252, 1211, 1116, 1088, 1020, 861, 756 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 1; EIMS *m*/*z* 724 [M^+] (70), 533 (25), 518 (36), 502 (91), 369 (29), 354 (20), 192 (100), 176 (54); HREIMS *m*/*z* 724.3334 (70) (calcd for $C_{42}H_{48}N_2O_9$, 724.3359), 533.2432 (25) (calcd for $C_{31}H_{35}NO_7$, 533.2413), 518.2167 (36) (calcd for $C_{30}H_{32}NO_7$, 518.2178), 502.2256 (91) (calcd for $C_{30}H_{32}NO_6$, 502.2229), 192.1010 (100) (calcd for $C_{11}H_{14}NO_2$, 192.1024).

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References and Notes

(1) Named in honor of the memory of Professor Jack L. Beal (deceased), College of Pharmacy, The Ohio State University, who spent his professional life studying the alkaloids of numerous species of the genus *Thalictrum*.
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