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Alkaloids from the Chinese Vine *Gnetum montanum*

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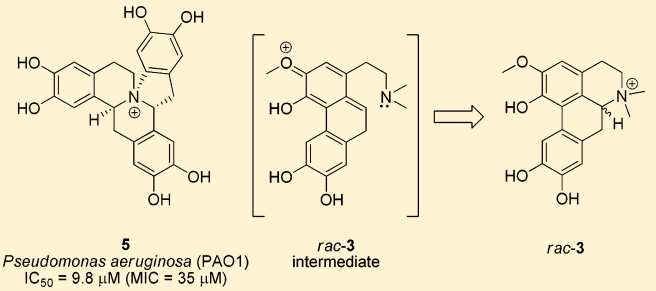
Supporting Information

ABSTRACT: During a high-throughput screening campaign of a prefractionated natural product library, fractions from the Chinese vine *Gnetum montanum* showed in vitro activity against *Pseudomonas aeruginosa* wild-type strain, PAO1. UV-directed isolation of the organic extract from the vine leaves resulted in the purification of the new natural products *N*-methyllaudanosolinium trifluoroacetate (**1**), 3′-hydroxy-*N*,*N*-dimethylcoclaurinium trifluoroacetate (**2**), 1,9,10-trihydroxy-2-methoxy-6-methylaporphinium trifluoroacetate (**3**), and 6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methylaporphinium trifluoroacetate (**4**). Compound **4** is described here for the first time, and this is the first report of compounds **1**−**3** as natural products. Compounds **1**−**3** were found to racemize over time. Starting from commercially available (+)-boldine, through a series of semisynthetic reactions, a mechanism for the racemization of the isolated compounds is proposed. The known natural products (−)-latifolian A (**5**) and magnocurarine (**6**) were also isolated during these studies. The antibacterial activity was explained by the presence of **5**, which displayed an IC_{50} value of 9.8 μM (MIC = 35 μM).



5
Pseudomonas aeruginosa (PAO1)
IC_{50} = 9.8 μM (MIC = 35 μM)

rac-**3**
intermediate

rac-**3**

Species that comprise the group of nonfermenting Gram-negative bacteria pose a major risk for health care, as many possess multidrug resistance.[1] Such species include *Pseudomonas aeruginosa*, *Straphylococcus maltophilia*, and *Acinetobacter* spp., which are opportunistic pathogens and prominent in critically ill, hospitalized patients.[2] Multidrug resistance is genetically acquired by these pathogens and is primarily due to the active transport of drugs out of the cell by efflux pump systems.[3,4] In addition, intrinsic resistance further decreases the efficacy of clinically used treatments, such as ampicillin, cephalosporins, and macrolide antibiotics, mainly due to impermeability.[1,5] Bacterial resistance, particularly among Gram-negative organisms, has increased our reliance upon drugs that have poorer safety profiles.[6] These facts highlight the urgent need to discover new, selective antibiotics with low toxicity and new mechanisms of action. Natural products constitute a promising source of new lead compounds or drugs for antibacterial treatments since most existing antibiotics are based on natural chemotypes.[7]

A high-throughput screening (HTS) campaign was undertaken on the *P. aeruginosa* efflux pump knockout strain, PAO200 (MexAB-OprM deficient mutant), in an effort to increase initial hits by decreasing efflux clearance. HTS hits were retested against the wild-type strain PAO1 and a Gram-positive methicillin-resistant bacterium, *Staph. aureus* (MRSA strain 01A1095),[8] for estimation of Gram selectivity. Three fractions derived from the Chinese vine *Gnetum montanum* showed in vitro activity against *P. aeruginosa* PAO200. These fractions eluted early from an analytical C_{18} HPLC column and displayed prominent UV profiles. UV-directed isolation on the large-scale organic extract from the leaves of *G. montanum* afforded the new benzylisoquinoline alkaloids *N*-methyllaudanosolinium trifluoroacetate (**1**) and 3′-hydroxy-*N*,*N*-dimethylcoclaurinium trifluoroacetate (**2**), the new aporphine alkaloids 1,9,10-trihydroxy-2-methoxy-6-methylaporphinium trifluoroacetate (**3**) and 6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methylaporphinium trifluoroacetate (**4**), and the known alkaloids (−)-latifolian A (**5**) and magnocurarine (**6**). The antibacterial activities of compounds **1**−**6** against *P. aeruginosa* PAO1 and *Staph. aureus* 01A1095 are also reported.

RESULTS AND DISCUSSION

The air-dried and ground leaves of *G. montanum* (Gnetaceae) were sequentially extracted with *n*-hexane, CH_2Cl_2, and MeOH. The *n*-hexane extract was discarded, and the remaining organic extracts were combined and fractionated on polyamide gel using MeOH as eluent. The resulting MeOH wash was subjected to several steps of C_{18} semipreparative HPLC [MeOH/H_2O (0.1% TFA)] to yield **1** (6.1 mg, 0.24% dry wt), **2** (7.2 mg, 0.17% dry wt), **3** (6.3 mg, 0.15% dry wt), **4** (1.3 mg, 0.03% dry wt), **5** (3.6 mg, 0.09% dry wt), and **6** (6.4 mg, 0.15% dry wt). All alkaloids were isolated as their trifluoroacetate salts. Compounds **5** and **6** were assigned to the known compounds (−)-latifolian A and racemic magnocurarine, respectively, following comparison of spectroscopic data.[9,10] (−)-Latifolian A was previously isolated from *G.*

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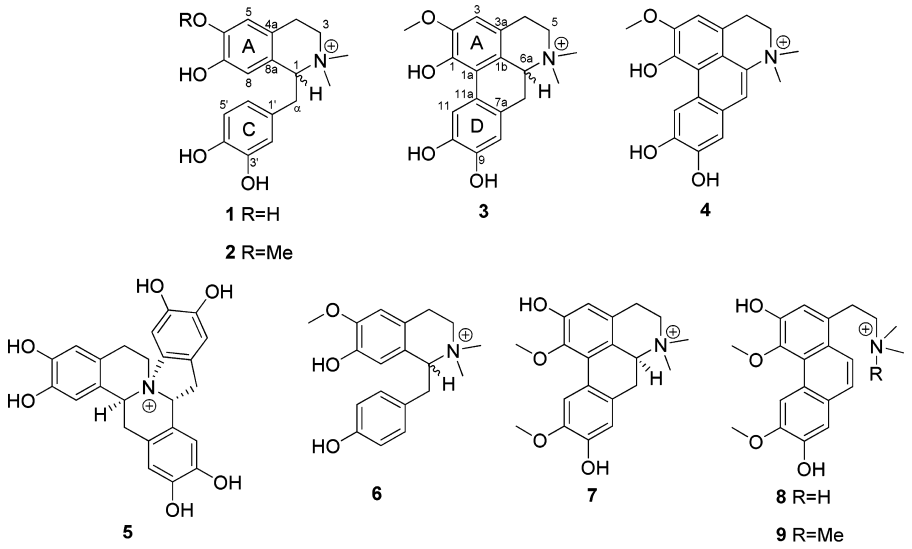
Chart 1



latifolium and exhibited inhibition in an assay against the JNK3 kinase.[10] Magnocurarine has been isolated from a variety of plants and has been reported to display weak antinociceptive activity.[11]

Compound **1** was isolated as a brown gum, and the molecular formula of the quaternary ammonium cation of **1** was determined to be $C_{18}H_{22}NO_4^+$ by HR-ESIMS ([M − TFA]$^+$: m/z 316.1544). Analysis of the ^{1}H NMR and gCOSY spectra (Table 1) revealed the presence of two isolated aromatic singlets at δ_H 6.58 and 5.94, one aromatic ABX system (δ_H 6.65, d, J = 8.0 Hz; δ_H 6.53, d, J = 1.9 Hz; and δ_H 6.35, dd, J = 8.0, 1.9 Hz), two N^+Me groups (δ_H 3.23 and 3.03), two contiguous methylene groups (δ_H 3.68/3.54 and 2.97), and one methine-methylene system (δ_H 4.55/3.44, 2.68). Furthermore, correlations observed in the gHSQC and gHMBC spectra showed the presence of two different substituted aromatic rings (rings A and C), both of which possessed a catechol substructure [δ_C 143.6 and 145.5 (ring A), 145.2 and 144.1 (ring C)]. The two isolated aromatic singlets (δ_H 6.58/δ_C 114.7 and δ_H 5.94/δ_C 114.5) were positioned *para* to each other on ring A on the basis of their overlapping HMBC correlations. The aromatic protons in the ABX system could be assigned to carbons that belonged to ring C (δ_C 115.2, 116.6, and 119.9) following HSQC analysis. The two last carbons belonging to ring A were quaternary (δ_C 118.9 and 121.6). The carbon at δ_C 118.9 showed HMBC correlations with the two contiguous methylenes (δ_C 54.3 and 22.4), while the carbon at δ_C 121.6 displayed HMBC correlations with the methine-methylene system (δ_C 71.3 and 36.5). Moreover the protons in both N^+Me groups showed HMBC correlations with the carbons at δ_C 71.3 and 54.3, to generate a closed ring and deliver an isoquinolinium moiety. Furthermore, correlations between the carbon at δ_C 36.5, present in the methine-methylene system, with protons belonging to ring C confirmed that **1** was a benzylisoquinoline derivative. These two systems were connected through a quaternary carbon at δ_C 127.0. Compound **1** was thus assigned to *N*-methyllaudanosolinium trifluoroacetate. The synthetic preparation of the iodide salt of **1** has previously been reported but was only partially charaterized.[12] We report here the full spectroscopic characterization of **1**.

Table 1. NMR Data for Compounds 1 and 2[a]

	1		2	
position	δ_C mult	δ_H mult (J in Hz)	δ_C mult	δ_H mult (J in Hz)
1	71.3 CH	4.55 dd (9.4, 2.9)	71.2 CH	4.59 dd (9.3, 2.8)
3	54.3 CH_2	α 3.54 m	54.3 CH_2	α 3.58 m
		β 3.68 m		β 3.71 m
4	22.4 CH_2	α 2.97 m	23.8 CH_2	α 3.05 m
		β 2.97 m		β 3.05 m
4a	118.9 C		119.2 C	
5	114.7 CH	6.58 s	113.3 CH	6.79 s
6	143.6 C		147.7 C	
6-OH		9.11 s		
7	145.5 C		145.5 C	
7-OH		8.84 s		8.89 s
8	114.5 CH	5.94 s	114.5 CH	5.95 s
8a	121.6 C		123.3 C	
α	36.5 CH_2	3.44 dd (13.9, 2.9)	36.4 CH_2	3.46 dd (13.9, 2.8)
		2.68 dd (13.9, 9.4)		2.68 dd (13.9, 9.3)
1′	127.0 C		127.0 C	
2′	116.6 CH	6.53 d (1.9)	116.5 CH	6.52 d (1.5)
3′	145.2 C		145.7 C	
3′-OH		8.77 s		8.75 s
4′	144.1 C		143.9 C	
4′-OH		8.92 s		8.91 s
5′	115.2 CH	6.65 d (8.0)	115.2 CH	6.65 d (8.0)
6′	119.9 CH	6.35 dd (8.0, 1.9)	119.9 CH	6.35 dd (8.0, 1.5)
$-N^+Me_2$	50.2 CH_3	3.23 s	50.3 CH_3	3.25 s
	50.0 CH_3	3.03 s	50.0 CH_3	3.04 s
−OMe			55.1 CH_3	3.75 s

[a]Spectra were recorded in DMSO-d_6 at 30 °C.

Comparison of the NMR and UV data of the major metabolite **2** with **1** clearly identified that **2** also belonged to the benzylisoquinoline structure class. The major difference in the NMR spectrum of **1** was that one of the hydroxy moieties had been replaced by a methoxy group in **2** (δ_H 3.75/δ_C 55.1). This was confirmed by HR-ESIMS ([M − TFA]$^+$: m/z

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330.1691), which indicated that the molecular formula of the quaternary ammonium cation of **2** was $C_{19}H_{24}NO_4^+$. The methoxy position was determined following gHMBC analysis. A strong correlation was observed between the methoxy protons and the carbon C-6 (δ_C 147.7). Moreover, the ROESY spectrum showed a strong correlation between the methoxy protons and the aromatic proton H-5 (δ_H 6.79). The structure of compound **2** was therefore assigned as the trifluoroacetate salt of 3′-hydroxy-*N*,*N*-dimethylcoclaurinium. This is the first report of **2** as a natural product. As with compound **1**, the synthesis of **2** has been previously reported.[13] The complete NMR assignment of **2** is presented in Table 1.

Compound **3** exhibited a markedly different UV spectrum from that of **1** and **2**. Moreover, the molecular formula of the quaternary ammonium cation of **3** ($C_{19}H_{22}NO_4^+$) obtained from the HR-ESIMS ([M − TFA]$^+$: *m*/*z* 328.1532) indicated that **3** was a didehydro analogue of **2**. Analysis of the ^{1}H NMR spectrum (Table 2) showed three aromatic singlets at δ_H 7.85, 6.78, and 6.71. Signals were still apparent for the two contiguous methylenes (δ_H 3.74/3.65 and 3.18/2.93), the methine-methylene system (δ_H 4.56/3.16, 2.73), the two N^+Me groups (δ_H 3.34 and 2.93), and the methoxy group (δ_H 3.85). These signals are typical for aporphine derivatives[14] and aporphinium salts.[15] The correlations observed in the gHSQC and gHMBC spectra confirmed the structure. The isolated singlet at δ_H 6.78 (δ_C 109.0) was attached to ring A. HMBC correlations were observed from this signal to two oxygenated carbons (δ_C 148.1 and 142.1) and a quaternary aromatic carbon at δ_C 119.5. The carbon at δ_C 119.5 showed HMBC correlations to both contiguous methylene protons (with carbons at δ_C 23.1 and 60.4) as well as the methine-methylene system with carbons at δ_C 68.3 and 28.1. The carbon at δ_C 28.1 also displayed correlations with the two other aromatic proton singlets belonging to ring D. The four remaining carbons of **3** were determined to be two *ortho*-substituted (δ_C 144.6 and 143.7) and quaternary (δ_C 123.4 and 122.5) carbons following HMBC data analysis. Finally the aromatic proton at δ_H 7.85 displayed a correlation with a quaternary carbon at δ_C 120.4, which belongs to ring A, validating the aporphine skeleton. The methoxy group of **3** was positioned at C-2 following 2D NMR data analysis. The methoxy protons (δ_H 3.85) showed a strong HMBC correlation with the C-2 phenolic carbon (δ_C 148.1) and showed a ROESY correlation to the aromatic proton H-3 (δ_H 6.78). Compound **3** was thus assigned as the trifluoroacetate salt of 1,9,10-trihydroxy-2-methoxy-6-methylaporphinium. This is the first report of **3** as a natural product. The synthesis of **3** has been reported by the same group that synthesized **2**.[13] Our data were consistent with the ^{1}H NMR and UV spectra from the literature.

Table 2. NMR Data for Compounds 3 and 4[a]

	3		4	
position	δ_C mult	δ_H mult (*J* in Hz)	δ_C mult	δ_H mult (*J* in Hz)
1	142.1 C		142.9 C	
1-OH		[b]		9.58 s
1a	120.4 C		119.3 C	
1b	119.5 C		115.3 C	
2	148.1 C		147.6 C	
3	109.0 CH	6.78 s	111.3 CH	7.40 s
3a	119.1 C		118.8 C	
4	23.1 CH_2	*α* 2.93 m	23.7 CH_2	3.47 t (5.9)
		β 3.18 m		
5	60.4 CH_2	*α* 3.65 ddd (12.9, 12.9, 4.5)	60.9 CH_2	4.02 t (5.9)
		β 3.74 dd (12.9, 5.8)		
6a	68.3 CH	4.56 dd (13.7, 2.3)	135.7 C	
7	28.1 CH_2	*α* 3.16 dd (13.7, 2.3)	116.3 CH	8.10 s
		β 2.73 dd (13.7, 13.7)		
7a	123.4 C		124.3 C	
8	114.9 CH	6.71 s	112.7 CH	7.30 s
9	143.7 C		143.8 C	
9-OH		[b]		9.73[c]
10	144.6 C		146.5 C	
10-OH		[b]		9.74[c]
11	116.3 CH	7.85 s	113.6 CH	9.22 s
11a	122.5 C		124.1 C	
$-N^+Me_2$	52.9 CH_3	3.34 s	54.5 CH_3	3.64 s
	42.8 CH_3	2.93 s	54.5 CH_3	3.64 s
−OMe	55.9 CH_3	3.85 s	56.4 CH_3	3.99 s

[a]Spectra were recorded in DMSO-d_6 at 30 °C. [b]Signals not observed. [c]Interchangeable signals.

In order to determine the absolute configuration of compounds **1**−**3**, we measured their optical rotation and ECD spectra with the intention to compare against the reported spectra of natural products of known absolute configuration, such as (+)-roefractine,[16] (+)-laudanosine,[17] and (+)-reticuline.[18] We also obtained commercially available (+)-boldine for comparison. The recorded ECD spectra of compounds **1**−**3** all showed an absence of Cotton effects, indicating that the isolated natural products were racemic. Reports of naturally occurring racemates of benzylisoquinoline and aporphine-type alkaloids are not commonly found in the literature. Racemic isococlaurine trifluoroacetate has been reported from *Cimmampelos mucronata*,[19] and the racemic perchlorate salt of xylopinidine has been identified as a constituent of *Xylopia parviflora*.[20] Interestingly, in both publications the authors had also isolated a number of other chiral benzylisoquinoline alkaloids.

With the intent to examine whether the racemic quaternary benzylisoquinoline and aporphine alkaloids identified in this study were artifacts of the isolation procedure, we first reacted commercially available (+)-boldine with an excess of MeI according to a known literature procedure.[21] The crude reaction mixture was chromatographed on a semipreparative C_{18} HPLC column using a gradient from H_2O to MeOH (0.1% TFA) to give *N*-methylboldine trifluoroacetate (**7**) as well as a small amount of *N*-methylsecoboldine trifluoroacetate (**8**) and *N*,*N*-dimethylsecoboldine trifluoroacetate (**9**). Compound **7** initially showed an ECD spectrum comparable to that of (+)-boldine; however, racemization was observed in a MeOH (0.1% TFA) solution over a period of one week, as shown by a complete loss of the ECD Cotton effects (see Supporting Information).

Lee et al. have reported solvolysis of 2-hydroxyaporphines to phenanthrene-type alkaloids via reflux in a 80% solution of propionic acid.[22] The authors proposed a mechanism where the dissociated acid abstracts a proton from the acidic 2-hydroxyaporphine and opens the B ring, followed by deprotonation at C-9 to give a stable phenenthrene product. We propose that the quaternary trifluoroacetates of benzylisoquinoline and aporphine alkaloids isolated in this study can

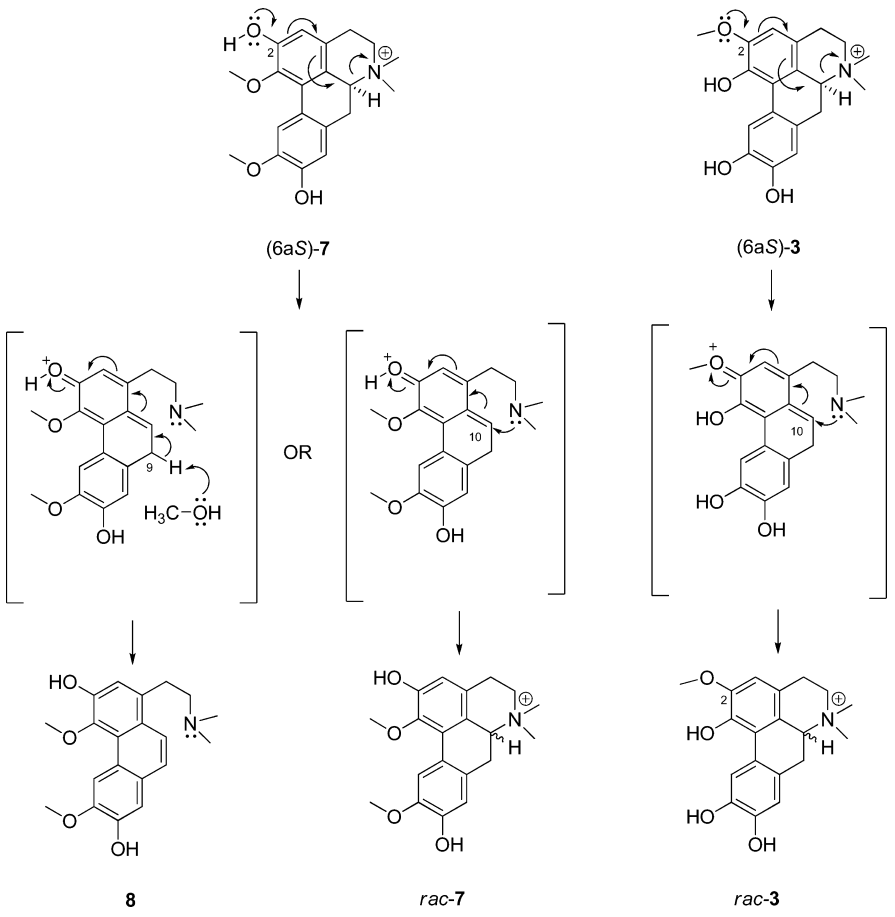

Figure 1. Proposed mechanism for the racemization of **7** and **3**.

rearrange via a similar mechanism as shown in Figure 1. Nucleophilic attack of the nitrogen at C-10 is favored over deprotonation at C-9 in the intermediate and accounts for the racemization of the aporphine alkaloids isolated in this work.

The minor plant metabolite **4** was isolated as an optically inactive gum. The molecular formula of the quaternary ammonium cation of **4** ($C_{19}H_{20}NO_4^+$) was obtained following HR-ESIMS analysis ([M − TFA]$^+$: m/z 326.1382). The molecular formula difference between **4** and **3** was two hydrogen atoms, which indicated that **4** was an oxidized analogue of **3**. Inspection of the ^{1}H NMR spectrum of **4** (Table 2) clearly showed the disappearance of the signals belonging to the methine-methylene system. The aromatic portion of the spectrum also showed major modifications. Four aromatic singlets were detected (δ_H 9.22, 8.10, 7.40, and 7.30), with downfield chemical shifts in comparison with the aromatic proton signals of **3**. These data suggested that **4** belonged to the 6a,7-didehydro-aporphine structure class. The other proton signals of **4** included a N^+Me singlet (δ_H 3.64, 6H), a methoxy singlet (δ_H 3.99, 3H), and a methylene-methylene system (δ_H 4.02 and 3.47). The correlations observed in the gHSQC and gHMBC spectra were in accordance with this structure class.[23] The methoxy group was positioned at C-2 (δ_C 147.6) on the basis of HMBC and ROESY correlations. With the position of the methoxy group established, the structure of **4** was assigned as the trifluoroacetate of 6a,7-didehydro-1,9,10-trihydroxy-2-methoxy-6-methylaporphinium.

Gnetum is currently the only genus belonging to the family Gnetaceae. These gymnosperms have vessel elements in their xylem, a feature more commonly found in the angiosperms. Plants of this genus include trees, shrubs, and vines and are found in Africa, Asia, and Central and South America. The *Gnetum* genus is a well-known source of polyphenols, which are predominantly stilbenoid derivatives.[24] To date, only 10 alkaloids have been reported.[25] *G. montanum* is a vine that can reach more than 10 m in length. It can be found in the forests of southern China, as well as in other South-Asian countries (Bhutan, India, Laos, Myanmar, Thailand, and Vietnam). Fibers from the stem bark have been used for making bags, fishing nets, and ropes. The seeds can be eaten fried and produce an edible oil.[26] This plant has been used as a traditional medicine in China to treat arthritis and bronchitis. *G. montanum* has been the source of stilbenes, dimeric stilbenes, flavonoids, and triterpenes.[27]

Antibacterial screening of compounds **1**−**6** against *P. aeruginosa* PAO1 showed that compound **5** displayed moderate inhibitory activity against *P. aeruginosa* (PAO1), with an IC_{50} value of 9.8 μM (MIC 35 μM). Compounds **3** and **4** displayed weak activities (59% inhibition at 175 μM and 59% inhibition at 87.5 μM, respectively), while **1**, **2**, and **6** were not active at 350 μM. It is worth noting that both aporphine derivatives were slightly active, while the three benzylisoquinolines were inactive. In order to further evaluate the potential antibacterial activity of **1**−**6**, all compounds were tested against the Gram-positive methicillin-resistant bacterium *Staph. aureus* (MRSA strain 01A1095).[8] Compound **5** displayed 55% growth inhibition at 350 μM, while **1**−**4** and **6** showed no activity at 350 μM. These data identified that **5** showed a ∼36-fold

selectivity for *P. aeruginosa* (PAO1) compared to *Staph. aureus* (01A1095).

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were measured on a Jasco P-1020 polarimeter. Circular dichroism spectra were recorded on a Jasco J-715 spectropolarimeter. UV spectra were recorded on a Jasco V650 UV/vis spectrophotometer. NMR spectra were recorded at 30 °C on either a Varian 500 or 600 MHz Unity INOVA spectrometer. The latter spectrometer was equipped with a triple resonance cold probe. The ^{1}H and ^{13}C NMR chemical shifts were referenced to the solvent peaks for DMSO-d_6 at δ_H 2.49 and δ_C 39.5, respectively. LR-ESIMS were recorded on a Mariner time-of-flight spectrometer equipped with a Gilson 215 eight-probe injector. HR-ESIMS were recorded on a Bruker Daltronics Apex III 4.7e Fourier-transform mass spectrometer. A BIOLINE orbital shaker was used for the large-scale extraction of plant material. Machery Nagel Polyamide CC6 (0.05−0.016 mm) was used for tannin/polyphenolic removal. Alltech Davisil 40−60 μm 60 Å C_{18} bonded silica was used for preadsorption work. A Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler were used for HPLC. A Thermo-Electron C_{18} Betasil 143 Å column (5 μm, 21.2 × 150 mm) or a Phenomenex Luna C_{18} column (5 μm, 21.2 × 250 mm) was used for semipreparative HPLC separations. All solvents used for chromatography, UV, and MS were Lab-Scan HPLC grade (RCI Lab-Scan, Bangkok, Thailand), and the H_2O was Millipore Milli-Q PF filtered. (+)-Boldine (cat. # B3916) and ciprofloxacin (cat. # 17850) were purchased from Sigma-Aldrich.

Plant Material. The leaves of *Gnetum montanum* Markgr. (Gnetaceae) were collected by the Zi Yuan Medicine Company in Guangxi Province, China, in January 2000. A voucher specimen (no. 03101699C) has been lodged at the Zi Yuan Medicine Company, China.

Extraction and Isolation. The dried and ground leaves of *G. montanum* (10 g) were sequentially extracted with *n*-hexane (250 mL), CH_2Cl_2 (250 mL), and MeOH (2 × 250 mL). All CH_2Cl_2 and MeOH extracts were combined and dried under reduced pressure to yield a dark green-brown solid (2.88 g). This material was resuspended in MeOH (150 mL), loaded onto MeOH-conditioned polyamide gel (30 g) in a sintered glass column, and washed with MeOH (300 mL) to yield 2.45 g of a tannin-free extract. A portion of this semipurified extract (1 g) was preadsorbed to C_{18}-bonded silica and then packed into a stainless steel HPLC guard cartridge (10 × 30 mm) that was subsequently attached to a C_{18} Betasil HPLC column. Isocratic HPLC conditions of 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for 10 min; then a linear gradient to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min, then analyzed by (+)-LR-ESIMS. Fraction 18 afforded 9.8 mg of **1** (0.24% dry wt). Fraction 20 was purified by HPLC using the same Betasil column as before and a gradient of H_2O (0.1% TFA)/MeOH (0.1% TFA) (80:20 to 50:50 in 60 min) to yield 7.2 mg of **2** (0.17% dry wt). Compound **5** (3.6 mg, 0.09% dry wt) was obtained by separating fraction 21 by HPLC with a Phenomenex Luna C_{18} column using a gradient of H_2O (0.1% TFA)/MeOH (0.1% TFA) (80:20 to 50:50 in 30 min). Fraction 26 was purified by HPLC using a Phenomenex Luna C_{18} column and a gradient of H_2O (0.1% TFA)/MeOH (0.1% TFA) (70:30 to 50:50 in 30 min) to yield 1.3 mg of **4** (0.03% dry wt). Finally, fraction 22 was separated with the Phenomenex Luna C_{18} column using a gradient of H_2O (0.1% TFA)/MeOH (0.1% TFA) (70:30 to 50:50 in 30 min). Thirty fractions (30 × 1 min) were collected from time = 0 min, then analyzed by (+)−LR-ESIMS. Fraction 21 afforded 6.3 mg of **3** (0.15% dry wt), while fraction 23 yielded 6.4 mg of **6** (0.15% dry wt).

***P. aeruginosa* Optical Density (OD_{620}) Viability Assay.** *P. aeruginosa* PAO1 or PAO200 strain (supplied by Pfizer Global Research and Development) cultures were prepared at 3.5 × 10^4 cfu/mL in cation-adjusted Mueller Hinton (caMH) broth (Difco, Detroit, MI, USA), from concentrated frozen stocks of culture. The final bacterial concentration in the assay was 1500 cfu/well. Diluted bacteria (45 μL) were added to a 384-well lidded, sterile clear plate (Becton Dickinson, Franklin Lakes, NJ, USA) containing controls/fractions by a Multidrop liquid handler (Thermo Scientific, Barrington, IL, USA). Plates were incubated at 37 °C in a humidified incubator for 18 h or until the wells reached an optical density (OD_{620}) of between 0.7 and 0.8, then allowed to cool for 30 min. Clear plate seals (Perkin-Elmer, Meriden, CT, USA) were placed over the plate surface before reading on a Multiskan Ascent reader (Thermo Scientific) at 620 nm.

Test fractions, compounds, or control samples (5 μL) were added to the assay plate prior to the addition of bacteria. Samples were prepared by dilution of stock fractions/compounds/controls in DMSO into the assay plate with addition of 0.875 μL of stock and 4.125 μL of autoclaved Milli-Q filtered H_2O with a Minitrak (Perkin-Elmer, Meriden, CT, USA) liquid handler. The final concentration of DMSO in the assay was 1.75%. Each assay plate contained both positive and negative controls in columns 23 and 24, respectively. The positive control, for uninhibited growth, consisted of 5 μL of DMSO/Milli-Q H_2O to a final concentration of 1.75%, and the negative control, or 100% cell death, was comprised of 5 μL of the broad-spectrum antibiotic ciprofloxacin at a final concentration of 5 μg/mL. Whole control plates were included for each assay run, which consisted of duplicate dose−response curves of ciprofloxacin, each in triplicate. Ciprofloxacin was shown to have an IC_{50} value of 0.038 μM against *P. aeruginosa* PAO1.

***Staph. aureus* Optical Density Viability Assay.** The *S. aureus* assay was carried out as per the *P. aeruginosa* assay, with the following modifications: the final bacterial concentration used was 1980 cfu/well, and ciprofloxacin for the internal assay control wells was at 500 μg/mL. Incubation was for 19 h, or until the OD_{620} reached 0.45. Ciprofloxacin was shown to have an IC_{50} value of 125 μM against methicillin-resistant *Staph. aureus* 01A1095.

***N*-Methyllaudanosolinium trifluoroacetate (1):** brown gum; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 0 (0) nm; UV (MeOH) λ_{max} (log ε) 233 sh (3.19), 287 (2.99) nm; ^{1}H and ^{13}C NMR data, see Table 1; (+)-HR-ESIMS *m*/*z* 316.1544 ($C_{18}H_{22}NO_4$: $[M - TFA]^+$, requires 316.1543).

3′-Hydroxy-*N*,*N*-dimethylcoclaurinium trifluoroacetate (2): brown gum; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 0 (0) nm; UV (MeOH) λ_{max} (log ε) 231 sh (3.52), 286 (3.29) nm; ^{1}H and ^{13}C NMR data, see Table 1; (+)-HR-ESIMS *m*/*z* 330.1691 ($C_{19}H_{24}NO_4$: $[M - TFA]^+$, requires 330.1699).

1,9,10-Trihydroxy-2-methoxy-6-methylaporphinium trifluoroacetate (3): brown gum; ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 0 (0) nm; UV (MeOH) λ_{max} (log ε) 229 (3.80), 270 sh (3.28), 281 (3.33), 308 (3.42), 317 sh (3.39) nm; ^{1}H and ^{13}C NMR data, see Table 2; (+)-HR-ESIMS *m*/*z* 328.1532 ($C_{19}H_{22}NO_4$: $[M - TFA]^+$, requires 328.1543).

6a,7-Didehydro-1,9,10-trihydroxy-2-methoxy-6-methylaporphinium trifluoroacetate (4): brown gum; UV (MeOH) λ_{max} (log ε) 222 sh (3.74), 270 (3.88), 288 sh (3.67), 335 (3.35), 353 (3.33), 371 (3.32) nm; ^{1}H and ^{13}C NMR data, see Table 2; (+)-HR-ESIMS *m*/*z* 326.1382 ($C_{19}H_{20}NO_4$: $[M - TFA]^+$, requires 326.1387).

Methylation of (+)-Boldine. Boldine (45 mg, 137 μmol) was dissolved in a mixture of MeCN/MeOH (1:1) to which six molar equivalents of MeI (50 μL, 863 μmol) was added. The reaction mixture was stirred at room temperature for 6 h, after which the solvent was removed under a stream of N_2. The crude reaction mixture was chromatographed on a C_{18} Betasil HPLC column. Isocratic HPLC conditions of 90% H_2O (0.1% TFA)/10% MeOH (0.1% TFA) were initially employed for 10 min; then a linear gradient to MeOH (0.1% TFA) was run over 40 min, followed by isocratic conditions of MeOH (0.1% TFA) for a further 10 min, all at a flow rate of 9 mL/min. Sixty fractions (60 × 1 min) were collected from time = 0 min, then analyzed by (+)-LR-ESIMS. Fractions 24−26 afforded compound **7** as a trifluoroacetate salt (15.8 mg, 51% yield), fractions 30−32 afforded **8** as a trifluoroacetate salt (2.1 mg, 7% yield), and fraction 29 afforded **9** as a trifluoroacetate salt (1.1 mg, 3% yield).

***N*-Methylboldine trifluoroacetate (7):** brown gum; $[\alpha]_D$ +39 (*c* 0.1, MeOH); ECD (MeOH) λ_{max} ($\Delta\varepsilon$) 242 (+28.1) nm; ^{1}H and ^{13}C

NMR data were in good agreement with those reported for the iodide salt;[21] (+)-LR-ESIMS m/z 342.1, $C_{20}H_{24}NO_4$ $[M - TFA]^+$.

***N*-Methylsecoboldine trifluoroacetate (8):** brown gum; ^1H and ^{13}C NMR data were in good agreement with those reported for the iodide salt;[21] (+)-LR-ESIMS m/z 342.1, $C_{21}H_{26}NO_4$ $[M - TFA]^+$.

***N,N*-Dimethylsecoboldine trifluoroacetate (9):** brown gum; ^1H and ^{13}C NMR data were in good agreement with those reported for the iodide salt;[21] (+)-LR-ESIMS m/z 356.1, $C_{20}H_{24}NO_4$ $[M - TFA]^+$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR spectra of compounds **1**−**3**, ^1H and 2D NMR spectra of compound **4**, and the ECD spectra of boldine and compound **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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